KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University)
(Established Under Section 3 of UGC Act, 1956)
Coimbatore – 641 021.

SYLLABUS

DEPARTMENT OF CHEMISTRY

STAFF NAME: Dr. A. THANGAMANI

SUBJECT NAME: ORGANIC CHEMISTRY-III SUB.CODE:18CHP301

SEMESTER: III CLASS: II-M.Sc (CHEMISTRY)

18CHP301 ORGANIC CHEMISTRY- III (NATURAL PRODUCTS) 4H 4C

Instruction Hours/week:L: 4 T:0 P:0 Marks: Internal:40 External: 60 Total:100

Course Objectives

On successful completion of the course the students should have

1. Versatile knowledge about the isolation, synthesis, bio-synthesis and elucidation of various natural products.
2. Learnt the identification of molecular structures.
3. Mastered synthetically important reagents.

Course Outcomes

1. Known the basic classification and role of Terpenoids and alkaloids.
2. Learned the structural elucidation of Zingiberene, Eudesmol, Abietic acid, Caryophyllene and Santonin.
3. Known the basic classification and role of steroids.
4. Learned the structural elucidation of Ergosterol, Vitamin D, Equilenin, Oestrone, Testosterone and Progesterone.
5. Gained knowledge about the synthesis and structure of alkaloids.
6. Understood the isolation and structural determination of alkaloids.
7. Known about the basics of Proteins and enzymes.
8. Known the preparation and synthetic applications of reagents used in organic synthesis.

UNIT-I

Terpenoids: Isolation and classification of terpenoids – general methods of determining structure of terpenoids –structural elucidation and synthesis of Zingiberene, Eudesmol, Abietic acid, Caryophyllene and Santonin-biosynthesis of monoterpenoids**.**

UNIT-II

Steroids: Introduction – structural elucidation and synthesis of Cholesterol (synthesis not necessary), Ergosterol, Vitamin D, Equilenin, Oestrone, Testosterone and Progesterone. Bile acids – biosynthesis of sterols.

UNIT-III

Alkaloids: Definition of an alkaloid-extraction of alkaloids-general properties - general methods of determining structure of alkaloids – structural elucidation and synthesis of Atropine, Morphine and Quinine -biosynthesis of quinoline alkaloids.

UNIT-IV

Proteins: General nature of proteins - classification of proteins – synthesis of peptides – oxytocin- insulin.

Enzymes: Nomenclature and classification - cofactors – specificity of enzyme action-mechanism of enzyme action. Nucleic acids - structures of RNA and DNA and their biological importance.

UNIT- V

Reagents in organic synthesis: Preparations and synthetic applications of DDQ, DBU, Dimethyl sulfoxide, trimethyl silyl iodide, Osmium tetroxide, Selenium dioxide, Dicyclohexylcarbodiimide (DCC), LDA, DIBAL-H and Mercuric acetate.

Text Books:

1. Chatwal, G. R. (2015). *Organic Chemistry of Natural Products Vol. II*. New Delhi: Himalaya Publishing House.
2. Finar, I. L. (2013). *Organic Chemistry Vol. II: Stereochemistry and the Chemistry of Natural Products* (V Edition). New Delhi: Pearson Education, Ltd.
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2. Sanyal, S. N. (2014). *Reactions, Rearrangements and Reagents* (IV Edition). New Delhi: Bharathi Bhawan (Publishers and Distributors).
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LECTURE PLAN
DEPARTMENT OF CHEMISTRY

STAFF NAME: Dr. A. THANGAMANI
SUBJECT NAME: ORGANIC CHEMISTRY-III SUB.CODE:18CHP301
SEMESTER: III CLASS: II-M.Sc (CHEMISTRY)

S.No.	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	**Terpenoids:** Introduction-Isolation and classification of terpenoids.	T1:368-370 T2:1.1-1.5
2	1	General methods of determining structure of terpenoids.	T1:370-372 T2:1.9-1.22
3	1	Structural elucidation and synthesis of Zingiberene.	T1:430-431 T2:1.128-1.132
4	1	Structural elucidation and synthesis of Eudesmol.	T1:438-442
5	1	Structural elucidation and synthesis of Abietic acid.	T1:455-460 T2:1.139-1.146
6	1	Structural elucidation and synthesis of Caryophyllene.	T1:448-450
7	1	Structural elucidation and synthesis of Santonin.	T1:442-446
8	1	Biosynthesis of monoterpenoids.	T1:470-471 T2:9.16-9.18
9	1	Recapitulation and discussion of important questions.	
	Total No of Hours Planned For Unit 1=09		
		UNIT-II	
1	1	**Steroids:** Introduction-Diel's hydrocarbon. Structural elucidation of Cholesterol-structure of the ring system-positions of the hydroxyl group and double bond.	T1:530-537 T2:4.1-4.2 & 4.10-4.16
2	1	Structural elucidation of Cholesterol-nature and position of the side-chain-positions of the two angular methyl groups.	T1:537-542 T2:4.16-4.20

3	1	Structural elucidation and synthesis of Ergosterol.	T1:571-573 T2:4.32-4.35
4	1	Structural elucidation and synthesis of Vitamin D.	T1:573-576
5	1	Structural elucidation and synthesis of Equilenin.	T1:598-600 T2:4.76-4.79
6	1	Structural elucidation and synthesis of Oestrone.	T1:591-595 T2:4.61-4.68
7	1	Structural elucidation and synthesis of Testosterone.	T1:589-591 T2:4.94-4.97
8	1	Structural elucidation and synthesis of Progesterone.	T1: 601-605 T2:4.79-4.87
9	1	Bile acids-biosynthesis of sterols.	T1:581-587 T2:4.35-4.43 & 9.22-9.26
10	1	Recapitulation and discussion of important questions.	
	Total No of Hours Planned For Unit II=10		
		UNIT-III	
1	1	**Alkaloids:** Definition of an alkaloid-extraction of alkaloids-general properties.	T1:710-711 T3:3.1-3.6
2	1	General methods of determining structure of alkaloids.	T1:711-715 T3:3.6-3.19
3	1	Structural elucidation of Atropine. Introduction- constitution-Tropic acid-Tropine.	T1:735-738 T3:3.50-3.57
4	1	Synthesis and stereochemistry of Atropine.	T1:739-741 T3:3.58-3.60
5	1	Structural elucidation of Morphine. Introduction-constitution-Codeine-Thebaine-Codeinone.	T1:762-765 T3:3.109-3.117
6	1	Synthesis and stereochemistry of Morphine.	T1:766-770 T3:3.117-3.122
7	1	Structural elucidation and synthesis of Quinine.	T1:752-755 T3:3.66-3.76
8	1	Biosynthesis of quinoline alkaloids.	T1:780
9	1	Recapitulation and discussion of important questions.	
	Total No of Hours Planned For Unit III=09		
		UNIT-IV	
1	1	**Proteins:** General nature of proteins.	T1:670-672 T3:2.67-2.71

2	1	Classification of proteins.	T1:672-673 T3:2.72-2.74
3	1	Synthesis of peptides-Introduction-benzyloxycarbonyl method- azide method- t-butyloxycarbonyl method.	T1:682-684 T3:2.47-2.51
4	1	Synthesis of peptides-trityl method-phthaloyl method-tosyl method-anhydride method.	T1:684-687 T3:2.51-2.56
5	1	Oxytocin-insulin.	T1:687-690 T3:2.89-2.93 & 2.101-2.105
6	1	**Enzymes:** Nomenclature and classification-cofactors- specificity of enzyme action-mechanism of enzyme action.	T1:697-702
7	1	**Nucleic acids:** Structure of RNA and their biological importance.	T1:834-836 T3:6.25-6.33
8	1	Structure of DNA and their biological importance.	T1:836-838 T3:6.23-6.25
9	1	Recapitulation and discussion of important questions.	
	Total No of Hours Planned For Unit IV=09		
		UNIT-V	
1	1	**Reagents in organic synthesis:** Preparations and synthetic applications of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) and diazobicyclo[5.4.0]undec-7-ene (DBU).	T4:1439,1452, 968-969 & 1297
2	1	Preparations and synthetic applications of dimethyl sulfoxide (DMSO) and trimethylsilyl iodide ($(CH_3)_3SiI$).	T4:1447-1448, 1481-1482 & 1561
3	1	Preparations and synthetic applications of osmium tetroxide (OsO_4) and selenium dioxide (SeO_2).	T4: 992-998, 1440-1441, 1470, 1476 & 1478 R1:224-226 & 237-239
4	1	Preparation and synthetic applications of dicyclohexylcarbodiimide (DCC).	T4:1205-1206 R1:206-209
5	1	Preparation and synthetic applications of lithium diisopropylamide (LDA).	T4:542-544, 1020 & 1287
6	1	Preparation and synthetic applications of di-isobutylaluminium hydride (DIBAL-H).	T4:912, 1523 & 1528
7	1	Preparation and synthetic applications of mercuric acetate ($Hg(OAc)_2$).	T4:885-886 & 1440 R2:412-415
8	1	Recapitulation and discussion of important questions.	

9	1	Discussion of previous ESE question papers.	
10	1	Discussion of previous ESE question papers.	
11	1	Discussion of previous ESE question papers.	
	Total No of Hours Planned for unit V=11		
Total Planned Hours	**48**		

Text Books:

1. Finar, I. L. (2013). *Organic Chemistry Vol. II: Stereochemistry and the Chemistry of Natural Products* (V Edition). New Delhi: Pearson Education, Ltd.

2. Chatwal, G. R. (2015). *Organic Chemistry of Natural Products Vol. II*. New Delhi: Himalaya Publishing House.

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2. Tewari, N. (2011). *Advanced Organic Reaction Mechanism* (III Edition). Kolkata: Books and Allied (P) Ltd.

UNIT-I

SYLLABUS

Terpenoids: Isolation and classification of terpenoids – general methods of determining structure of terpenoids –structural elucidation and synthesis of Zingiberene, Eudesmol, Abietic acid, Caryophyllene and Santonin-biosynthesis of monoterpenoids.

Introduction

The terpenoids form a group of compounds the majority of which occur in the plant kingdom; a few terpenoids have been obtained from other sources. The simpler mono- and sesqui-terpenoids are the chief constituents of the essential oils; these are the volatile oils obtained from the sap and tissues of certain plants and trees. The essential oils have been used in perfumery from the earliest times. The di-and tri-terpenoids which are not steam volatile, are obtained from plant and tree gums and resins. The tetraterpenoids form a group of compounds known as the carotenoids, and it is usual to treat these as a separate group. Rubber is the most important polyterpenoid.

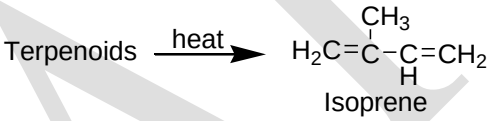
Most natural terpenoid hydrocarbons have the molecular formula $(C_5H_8)_n$ and the value of n is used as a basis for classification.

Number of carbon atoms	Class
(i) 10	Monoterpenoids ($C_{10}H_{16}$)
(ii) 15	Sesquiterpenoids ($C_{15}H_{24}$)
(iii) 20	Diterpenoids ($C_{20}H_{32}$)
(iv) 25	Sesterterpenoids ($C_{25}H_{40}$)
(v) 30	Triterpenoids ($C_{30}H_{48}$)
(vi) 40	Tetraterpenoids (Carotenoids) ($C_{40}H_{64}$)
(vii) >40	Polyterpenoids$(C_5H_8)_n$

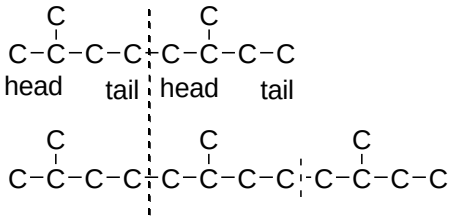
The sesterterpenoids have been discovered recently, and so far only very few are known. In addition to the terpenoid hydrocarbons, there are the oxygenated derivatives of each class which also occur naturally, and these are mainly alcohols, aldehydes or ketones.

The group of compounds discussed in this chapter was originally classified as the 'terpenes', and although this name is still used, there is a tendency to use the more general name 'terpenoids'. This is due to the fact that since the suffix 'ene' signifies unsaturated hydrocarbons, the name 'terpene' is inappropriate to include compounds such as alcohols, aldehydes, ketones, etc. the term 'terpene' is restricted to the hydrocarbons $C_{10}H_{16}$.

The thermal decomposition of almost all terpenoids gives isoprene as one of the products, and this led to the suggestion that the skeleton structures of all naturally occurring terpenoids can be built up of isoprene units; this is known as the isoprene rule, and was first pointed out by Wallach (1887).



Thus the divisibility into isoprene units may be regarded as a necessary condition to be satisfied by the structure of any plant-synthesised terpenoid. Furthermore, Ingold (1925) pointed out that the isoprene units in natural terpenoids were joined 'head to tail' (the head being the branched end of isoprene). This divisibility into isoprene units, and their head to tail union, may conveniently be referred to as the **special isoprene rule**. It should be noted, however, that this rule, which has proved very useful, can only be used as a guiding principle and not as fixed rule. Several exceptions occur, e.g., lavandulol and eremophilone; the carotenoids are joined tail to tail at their centre there are also some terpenoids whose carbon content is not a multiple of five and those whose carbon content is a multiple of five but cannot be divided into isoprene units. The carbon skeletons of open-chain monoterpenoids and sesquiterpenoids are:



Isolation of monoterpenoids and sesquiterpenoids

Plants containing essential oils usually have the greatest concentration at some particular time, e.g., jasmine at sunset. In general, there are four methods of extraction of the terpenoids: (i) expression; (ii) steam distillation; (iii) extraction by means of volatile solvents; (iv) adsorption in purified fats (enfleurage). Method (ii) is the one most widely used; the plant is macerated and then steam distilled. If the compound decomposes under these conditions, it may be extracted with light petrol at 50°C, and the solvent then removed by distillation under reduced pressure. Alternatively, the method of adsorption in fats is used. The fat is warmed to about 50°C, and then the flower petals are spread on the surface of the fat until the latter is saturated. The fat is now digested with ethanol, any fat that dissolves being removed by cooling to 20°C. The essential oils so obtained usually contain a number of terpenoids, and these are separated by fractional distillation. The terpenoid hydrocarbons distil first, and these are followed by the oxygenated derivatives. Distillation of the residue under reduced pressure gives the sesquiterpenoids, and these are separated by fractional distillation. More recently, chromatography (in its various forms) has been used both for isolation and separation of terpenoids. Gas chromatography has been particularly useful for isolating pure configurational forms of a given terpenoid from mixtures produced by synthesis.

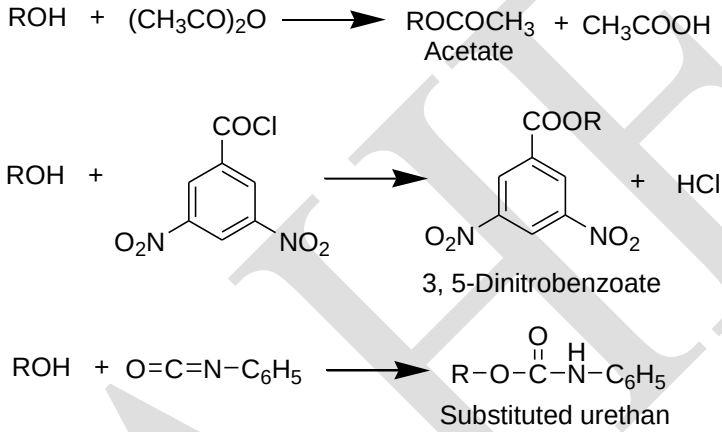
General methods of determining structure

The following brief account gives an indication of the various methods which have been particularly useful (especially oxidative degradation) in elucidating the structures of the terpenoids. Also included are the more modern methods.

(i) A pure specimen is obtained, and the molecular formula is ascertained by the usual methods, and also by means of mass spectrometry. If the terpenoid is optically active, its specific rotation is measured. Optical activity may be used as a means of distinguishing structures.

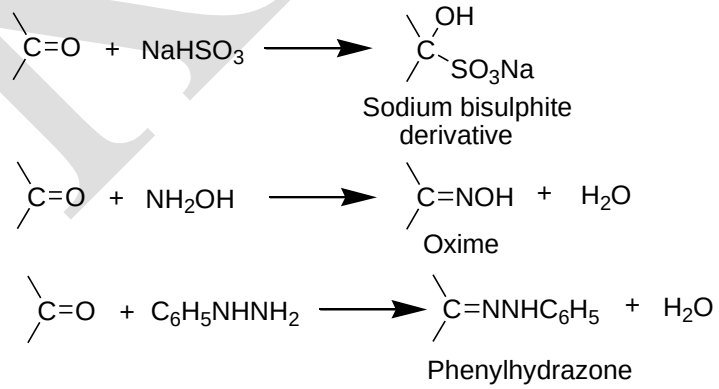
(ii) If oxygen is present in the molecule its functional nature is ascertained, i.e., whether it is present as hydroxyl, aldehyde, ketone, etc.

(a) The hydroxyl groups can be detected by the formation of crystalline acetates with acetic anhydride and benzoates with 3, 5-dinitrobenzoyl chloride. These also yield crystalline substituted urethans with phenyl isocyanate.



Further information about the nature of hydroxyl group is revealed by the rate of esterification. For example, the primary alcohols undergo esterification more readily than secondary and tertiary alcohols. Thus, α-terpineol, a tertiary alcohol, is slowly esterified.

(b) If a terpenoid forms crystalline addition products like bisulphite derivative, oxime and phenylhydrazone, this shows that terpenoid contains a carbonyl group.



The carbonyl group may be present either in the form of aldehydes or keto groups. This can be ascertained by oxidation. The aldehyde on oxidation yields monocarboxylic acid without

loss of carbon atom whereas the ketone on oxidation yields a mixture of lesser number of carbon atoms.

$$RCHO + [O] \longrightarrow RCOOH$$

$$R{-}\overset{\overset{O}{\|}}{C}{-}CH_2R' + [O] \longrightarrow RCOOH + R'COOH$$

Terpenoids having $–CH_2CO-$ groups exhibit special properties. Such terpenoids form oximes with nitrous acid (liberated by action of hydrochloric acid on isoamyl nitrite) and benzylidene derivatives with benzaldehyde in the presence of alkali.

$$\begin{matrix}—CH_2\\ |\\ —C{=}O\end{matrix} + O{=}NOH \xrightarrow[HCl]{C_5H_{11}OH} \begin{matrix}—C{=}N{-}OH\\ |\\ —C{=}O\end{matrix} + H_2O$$

Oxime

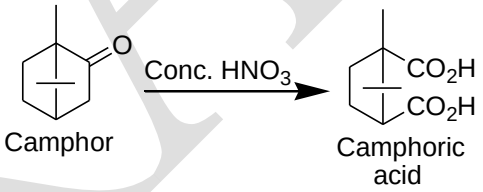
$$\begin{matrix}—CH_2\\ |\\ —C{=}O\end{matrix} + C_6H_5CHO \xrightarrow{NaOH} \begin{matrix}—C{=}CHC_6H_5\\ |\\ —C{=}O\end{matrix} + H_2O$$

Benzylidene derivative

If $–CH_2CO-$ group is present in a ring, the terpenoid on oxidation will yield a dicarboxylic acid without any loss of carbon atom/atoms. For, example,

$$\left\{\begin{matrix}—CH_2\\ |\\ —C{=}O\end{matrix}\right. + [O] \longrightarrow \left\{\begin{matrix}—COOH\\ —COOH\end{matrix}\right.$$

Camphor on oxidation with concentrated nitric acid yields a dicarboxylic acid.



Camphor Camphoric acid

If a terpenoid contains $–COCH_3$ group, it can be detected by **Haloform reaction**, e.g.,

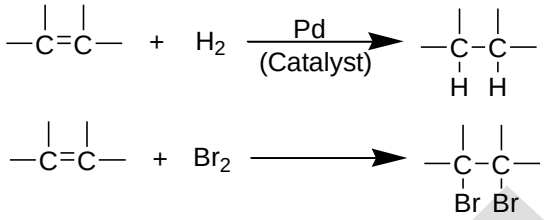
$$—\overset{\overset{O}{\|}}{C}{-}CH_3 + Br_2 \xrightarrow{NaOH} —COONa + CHBr_3$$

On oxidation such a terpenoid will form acetic acid.

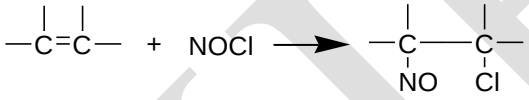
$$—CH_2{-}\overset{\overset{O}{\|}}{C}{-}CH_3 + [O] \longrightarrow —COOH + CH_3COOH$$

(iii)The presence of olefinic bonds is ascertained by means of bromine, and the number of double bonds is determined by analysis of the bromide, or by quantitative hydrogenation, or by

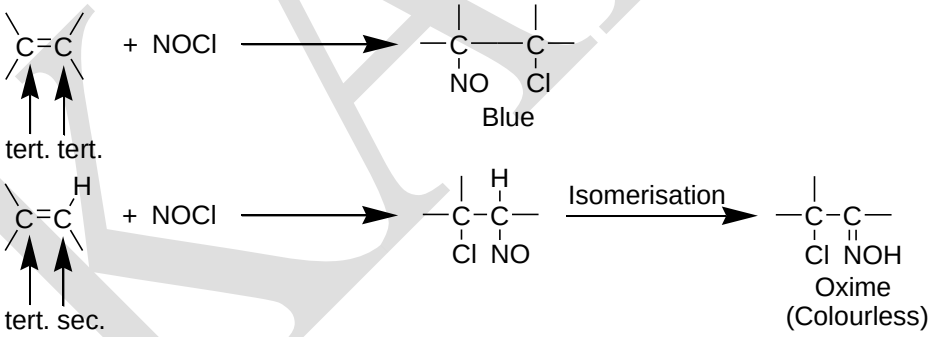
titration with monoperphthalic acid. These facts lead to the molecular formula of the parent hydrocarbon, from which the number of rings present in the structure may be deduced.



(iv) With nitrosyl chloride, terpenoids containing olefinic double bonds form addition compounds which are crystalline products having sharp melting points and may be used for identification and separation of terpenoids.

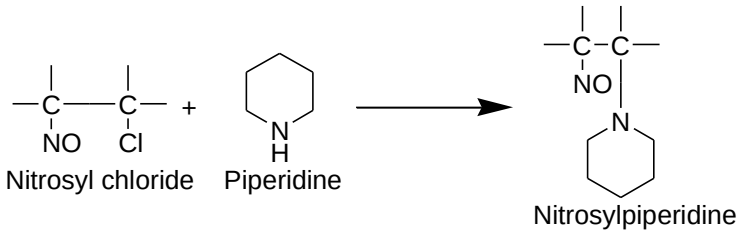


The addition of nitrosyl chloride (**Tilden's reagent**) also gives idea about the nature of the carbon atoms having double bonds in terpenoids. For example, if the two carbon atoms joined by an olefinic linkage are tertiary in nature, then a blue coloured nitroso chloride is obtained. On the other hand, if one of the carbon atoms joined to double bond is tertiary while the other secondary, a colourless nitrosyl chloride, is obtained.



The addition of nitrosyl chloride across a double bond takes place in accordance with the Markownikoff's rule: *the negative part i.e., Cl^- is added to tertiary carbon atom while the posoitive part NO^+ ia added to secondary carbon atom.*

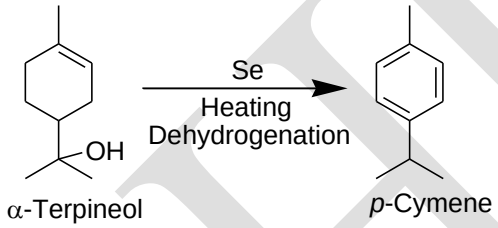
Sometimes, nitrosylpiperines are isolated in place of nitrosyl chloride according to the following steps.



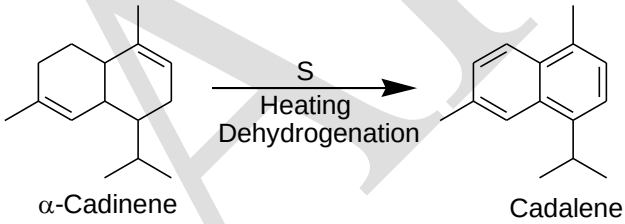
(v) Dehydrogenation of terpenoids with sulphur, selenium, platinum, or palladium, and an examination of the products thereby obtained.

For example,

(a) α-Terpineol when heated with selenium yields *p*-cymene.



(b) The α-camphor when dehydrogenated with sulphur forms cadalene which is 1,6-dimethyl-4-isopropylnaphthalene.



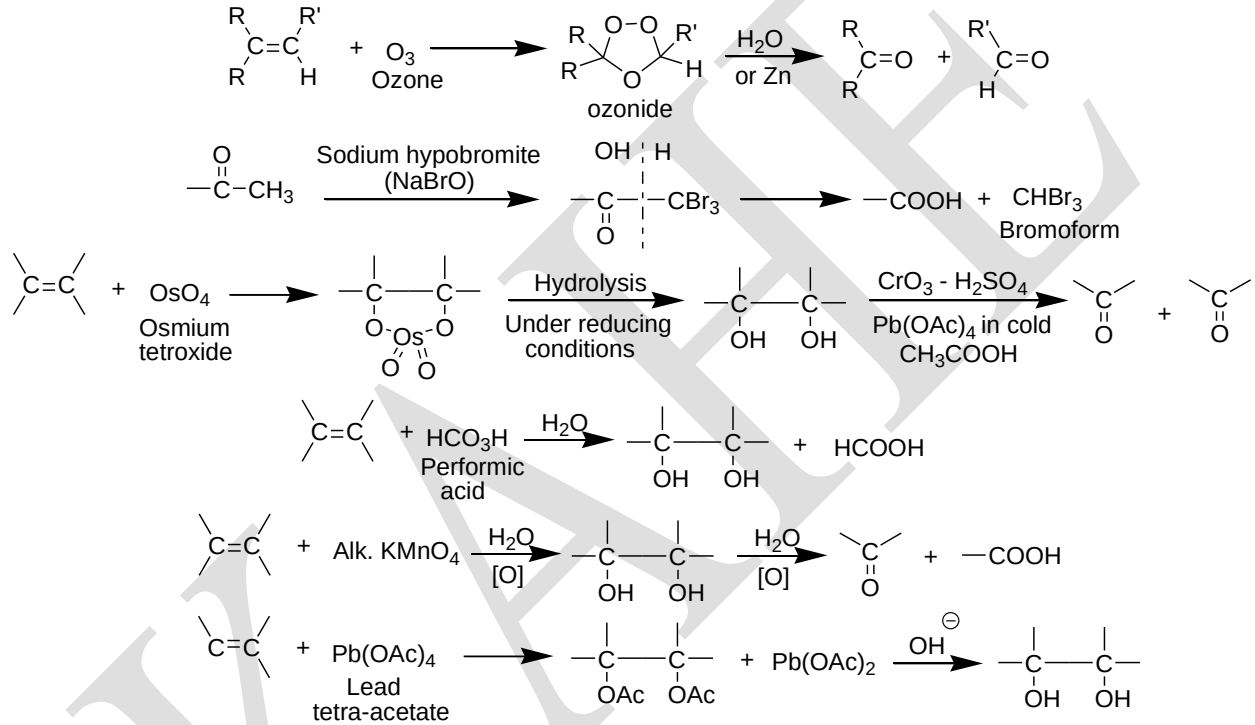
(vi) Measurement of the refractive index leads to a value for the molecular refraction which is calculated according to the following formula.

$$R_M = \frac{n^2-1}{n^2+2} \times \frac{M}{d}$$

Where n is the refractive index, d the density and M the molecular weight of the terpenoid.

From this may be deduced the nature of the carbon skeleton. Also, optical exaltation indicates the presence of double bonds in conjugation.

(vii) Degradative oxidation. The usual reagents used for this purpose are ozone, acid, neutral, or alkaline permanganate, chromic acid and sodium hypobromite. Other reagents are osmium tetroxide, nitric acid, lead tetra-acetate, peroxy-acids, and *N*-bromosuccinimide for allylic bromination. Furthermore, owing to the increased knowledge of the behaviour of oxidising reagents, it is now possible to select a reagent for oxidising a particular group in the molecule. In general, degradative oxidation has been the most powerful tool for elucidating the structures of the terpenoids.



(viii) **Ultraviolet spectroscopy** has been much used in terpenoid chemistry, its main application being the detection of conjugation. In simple acyclic dienes, λ_{max} is 217-228 nm (ε 15000–25000). If the diene is heteroannular (semicyclic), i.e., the conjugated double bonds are not in the same ring, λ_{max} is 230-240 nm (ε 1300 -20000), and if the diene is homoannular, i.e., both double bonds are in the same ring, λ_{max} is 256-265 nm (ε 2500 – 10 000). If an α,β–unsaturated carbonyl system is present, the λ_{max} is 220-250 mn (ε 10000-17 500), and there is also a weak band at λ_{max} 315-330 nm (ε 15-100).

The absorption maximum of a diene system is affected by substituents and Woodward (1942) found that the position of the absorption maximum depends on their number and type. As

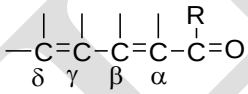
a result, Woodward developed a set of empirical rules (later modified by Fieser, 1948) for calculating λ_{max} from the molecular structure of the compound.

Polyenes

Homoannular dienes (basic value)	253 nm
Heteroannular (and acyclic) dienes (basic value)	214 nm
Increment for each C-substituent	5 nm
Increment for each exocyclic double bond	5 nm
Increment for each doubel bond that extends conjugation	30 nm
λ_{max} (of compound) =	Total

It should be noted that a C-substituent may be an alkyl group or a ring residue.

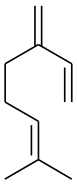
α,β-Unsaturated ketones



R is an alkyl group or a ring residue, and the parent system is C=C –C(R)=O.

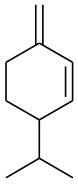
Parent system (basic value)	215 nm
Increment for each C substituent:	
at α-C	10 nm
at β-C	12 nm
at γ-or δ-C	18 nm
Increment for each exocyclic double bond	5 nm
Increment for each double bond that extends conjugation	30 nm
λ_{max} (of compound) =	Total

The following examples illustrate the application of these rules.



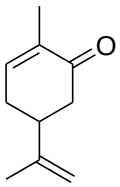
λ_{max} :
Observed, 224 nm.
Calculated (for an acyclic diene with one C-substituent):
214 + 5 = 219 nm.

myrcene



λ_{max} :
Observed, 232 nm.
Calculated (for a heteroannular diene with two C-substituents and one exocyclic double bond):
214 + 2 × 5 + 5 = 229 nm.

β-phellandrene



λ_{max} :
Observed, 235 nm.
Calculated:Parent system 215 nm
C-substituent at α-C 10 nm
C-substituent at β-C 12 nm
λ_{max} = 237 nm

carvone

There is generally good agreement between the calculated and observed values, but notable exceptions are five-membered ring α,β-unsaturated ketones. These have a calculated λ_{max} about 10 nm longer than the observed value.

Allinger *et al.* (1965) have calculated λ_{max} for a number of unsaturated hydrocarbons, and have established a quantitative theoretical basis for Woodward's rules in these compounds.

In addition to their use for detecting conjugation, ultraviolet spectra may be used for detecting the presence of an isolated double bond (175-200 nm), and this is particularly valuable for tetrasubstituted ethylenes, since this grouping cannot be ascertained with certainty in the infrared region. Also, α,β-unsaturated acids, esters, and lactones may often be recognized by their absorption maxima which occur in the region of 220 nm. Conjugated enes and ketones have absorption bands in about the same region, but they may, however, often be distinguished by treating them with a reducing agent, e.g., lithium aluminium hydride. Since conjugated enes are

usually unaffected, their spectra will remain unchanged, but the spectrum of the original conjugated ketone will now be very different (see also infrared spectroscopy below).

(ix) **Infrared spectroscopy** is also useful in terpenoid chemistry, and is very valuable for detecting the presence of a hydroxyl group (3400 cm^{-1}) or an oxo group (saturated: 1750-1700 cm^{-1}; α,β- unsaturated: 1700-1660 cm^{-1}). Examination of Woodward values shows that heteroannular dienes and unsubstituted α,β-unsaturated ketones cannot be distinguished by means of their ultraviolet spectra, but usually can from their infrared spectra (see also above). Also, infrared spectroscopy is particularly useful for detecting the presence of the isopropenyl group, and may often distinguish between *cis*-and *trans*-isomers.

(x) **NMR spectroscopy** has been used to detect and identify double bonds, to determine the nature of end groups and also the number of rings present, and to ascertain the orientation of methyl groups in the molecule. In certain cases, definite structures have been assigned on the basis of NMR spectra.

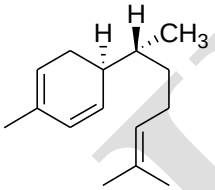
(xi) **Mass spectrometry** is now being increasingly used as a means of elucidating the structure of terpenoids. Thus, it is possible to determine molecular weights, molecular formulae, the nature of various functional groups, and the relative positions of double bonds. Since even simple terpenoids give complicated fragmentation patterns, structural identification of an unknown terpenoid by means of mass spectrometry must be carried out with some caution. It is possible, however, to identify a terpenoid by comparison of its mass spectrum with the reference spectrum of an authentic specimen.

(xiii) **Optical rotation** methods have been successfully applied to the elucidation of the structure of terpenoids, and ORD studies have been used to assign absolute configurations.

(xiii) **X-ray analysis** is very useful, where applicable, for elucidating structure and stereochemistry of terpenoids.

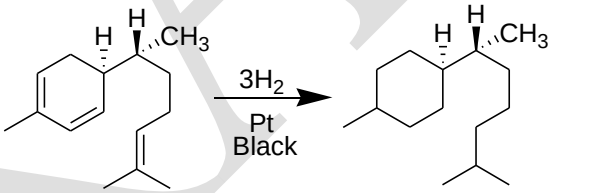
(xiv) After the analytical evidence has led to a tentative structure (or structures), the final proof of structure depends on synthesis. In terpenoid chemistry, many of the syntheses are ambiguous, and in such cases analytical evidence is used in conjunction with the synthesis. Also, because of the introduction of stereoselective syntheses, it is now possible to prepare particular configurational forms of many terpenoids.

Zingiberene



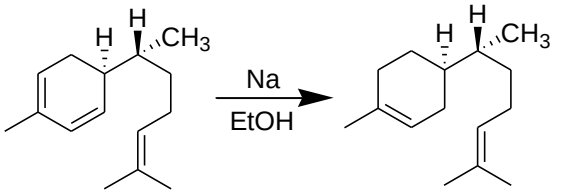
Zingiberene

Molecular formula: $C_{15}H_{24}$, b.p.134ºC/14mm. This occurs in the (-)-form in ginger oil. It forms a dihydrochloride with hydrogen chloride, and thus apparently contains two double bonds. The molecular refraction, however, indicates the presence of three double bonds and, if this be the case, zingiberene is monocyclic. The presence of these three double bonds is conclusively shown by the fact that catalytic hydrogenation (platinum) converts zingiberene into hexahydrozingiberene, $C_{15}H_{30}$.

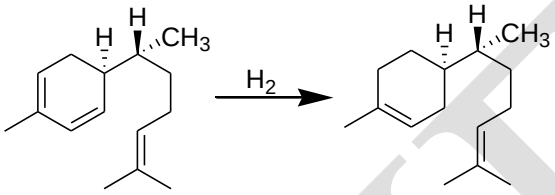


Zingiberene ($C_{15}H_{24}$) Hexahydrozingiberene ($C_{15}H_{30}$)

Zingiberene can be reduced by means of sodium and ethanol to dihydrozingiberene, $C_{15}H_{26;}$ this indicates that two of the double bonds are probably conjugated (Semmler *et al.*, 1913). Further evidence for this conjugation is afforded by the fact that zingiberene shows optical exaltation, whereas dihydrozingiberene does not.



Zingiberene ($C_{15}H_{24}$) Dihydrozingiberene ($C_{15}H_{26}$)

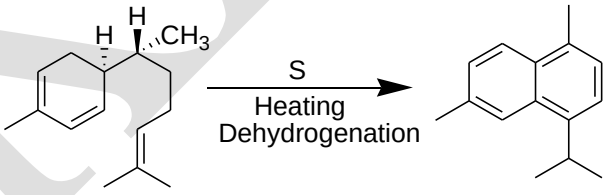


Zingiberene ($C_{15}H_{24}$) (Shows optical exaltation)

Dihydrozingiberene ($C_{15}H_{26}$) (Does not show optical exaltation because it contains two isolated double bonds)

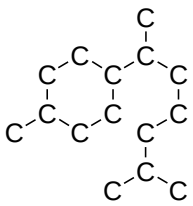
Also, zingiberene forms an adduct with maleic anhydride, and has λ_{max} 260 (ε 2700) nm. The calculated value of λ_{max} on the basis of a homoannular conjugated diene system is 253 + …, whereas the value for a heteroannular system is 214 + …. The conjugated system is therefore almost certainly the former.

When zingiberene is heated with sulphur, it undergoes dehydrogenation to form cadalene. The latter compound is of known structure and has been proved to be 1,6-dimethyl-4-isopropyl naphthalene.

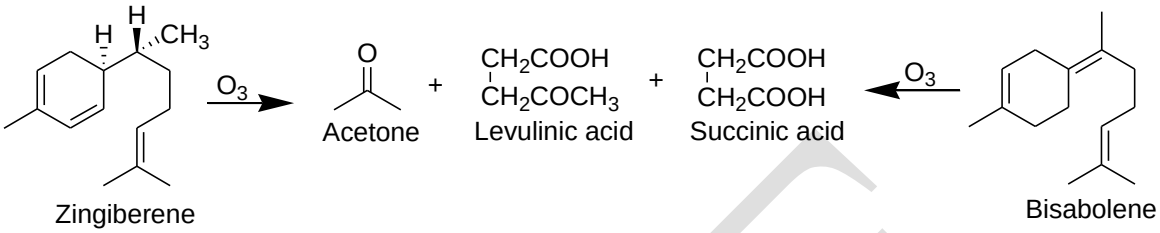


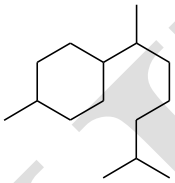
Zingiberene Cadalene

Hence in zingiberene, the following cadalene carbon skeleton must be present.

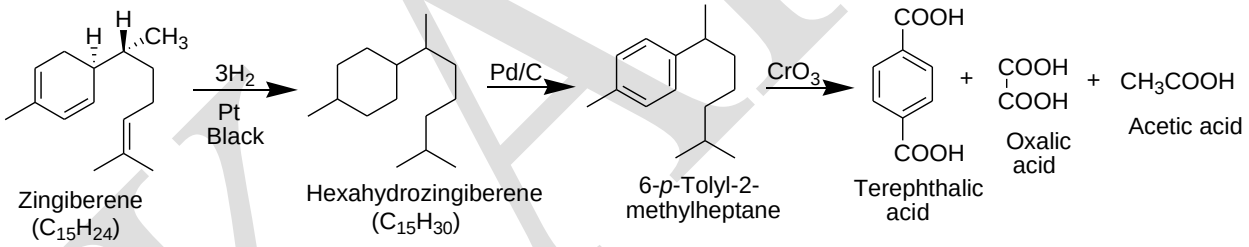


Ozonolysis of zingiberene gives acetone, levulinic acid and succinic acid (Ruzicka *et al.*, 1929). Since these products are also obtained from bisabolene, it appears probable that zingiberene and bisabolene have the same carbon skeleton.

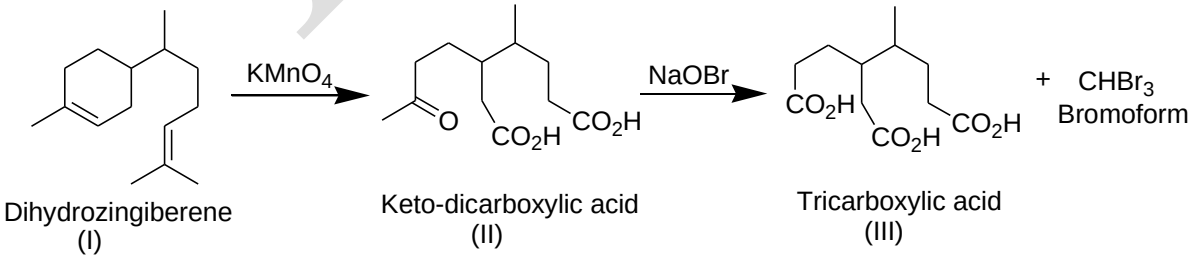




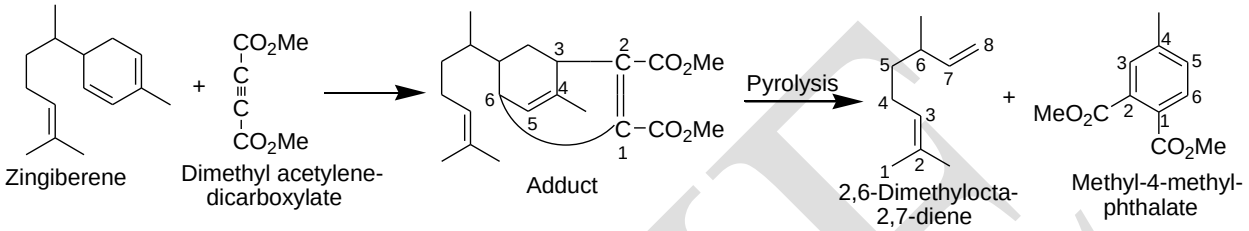
The above carbon skeleton of zingiberene has been confirmed by the fact that hexahydrozingiberene when dehydrogenated over palladised charcoal yields 6-*p*-tolyl-2-methyl heptane which upon oxidation with chromic acid yields acetic acid, oxalic acid and terephthalic acid.



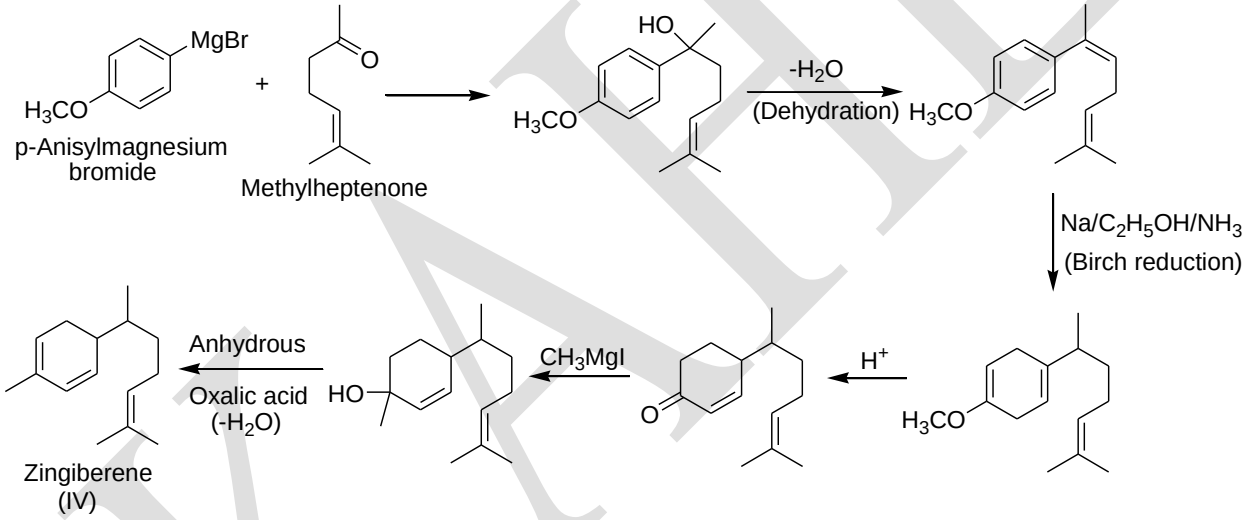
Oxidation of dihydrozingiberene (I) with permanganate gives a keto-dicarboxylic acid, $C_{12}H_{20}O_5$ (II), which on oxidation with sodium hypobromite, forms a tricarboxylic acid, $C_{11}H_{18}O_6$ (III). Thus (II) must contain a methyl ketone group (CH_3CO-) and so, if (I) be assumed as the structure of dihydrozingiberene, the foregoing oxidation reactions may be formulated.



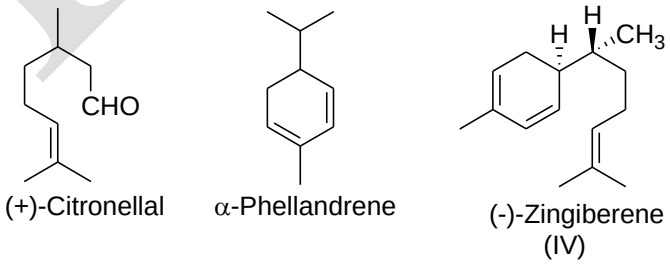
The position of the conjugated system was shown as follows (Eschenmoser *et al.*, 1950). Zingiberene forms an adduct with dimethyl acetylenedicarboxylate, and this adduct (which was not isolated), on pyrolysis, gives 2,6-dimethylocta-2,7-diene and methyl-4-methylphthalate. These reaction can be explained on the assumption that zingiberene has the structure shown below.



The structure of zingiberene has been confirmed by synthesis (Bhattacharya *et al.*, 1950).

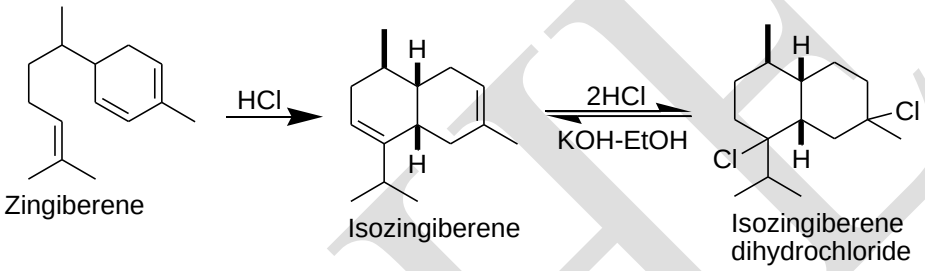


Zingiberene contains two chiral centres. The acyclic chiral centre has been stereochemically related to that in (+) citronellal, and the cyclic chiral centre to that in (-)α-phellandrene. Hence (-) zingiberene has the absolute configuration (IV).



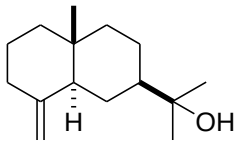
Reactions: The most interesting reaction of zingiberene is its reaction with hydrogen chloride when it forms dihydrochloride, instead of the fact it is having three double bonds. Moreover, the

dihydrochloride on dehydrohalogenation with alcoholic potash never gives back zingiberene but a bicyclic sesquiterpenoid, *isozingiberene*. From these two points it is evident that under the influence of hydrogen chloride, zingiberene itself is first converted into isozingiberene (before any attachment of hydrochloric acid takes place) which in turn being bicyclic is having only two double bonds and thus forms only dihydrochloride. The same has been confirmed by the formation of the identical dihydrochloride by zingiberene and isozingiberene by means of hydrogen chloride.



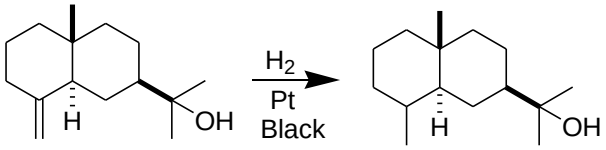
Isozingiberene is also obtained by treating the zingiberene with glacial acetic acid and sulphuric acid for some hours. The presence of two rings (and hence two double bond) in isozingiberene is confirmed by its refractive index 66.50, whilst that calculated for a bicyclic sesquiterpenoid is 66.13. Furthermore, the presence of two double bonds in isozingiberene is further confirmed by its reduction with hydrogen and platinum black to tetrahydro-derivative instead of the hexahydro-compound obtained under similar conditions from zingiberene, which reveals undoubtedly that one double bond of zingiberene has disappeared during its conversion to isozingiberene.

Eudesmol



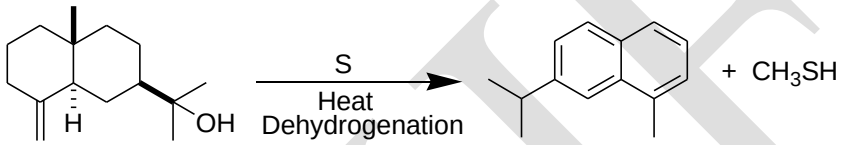
β-Eudesmol

Molecular formula: $C_{15}H_{26}O$. This occurs in eucalyptus oil. Catalytic hydrogenation converts eudesmol into dihydroeudesmol, $C_{15}H_{28}O$.



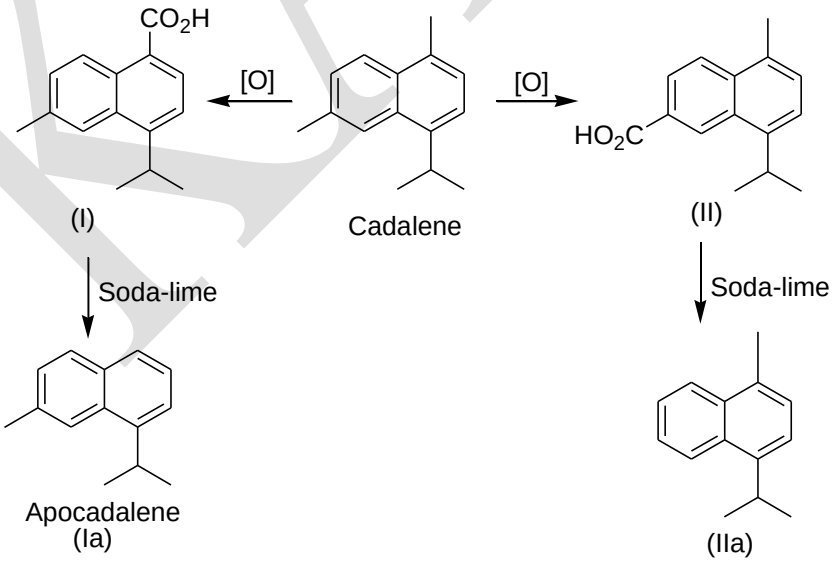
Eudesmol ($C_{15}H_{26}O$) Dihydroeudesmol ($C_{15}H_{28}O$)

Thus one double bond is present in the molecule, and since eudesmol behaves as a tertiary alcohol, the parent hydrocarbon is $C_{15}H_{28} \equiv C_nH_{2n-2}$; eudesmol is therefore bicyclic, when dehydrogenated with sulphur, eudesmol forms eudalene, $C_{14}H_{16}$, and methanethiol (Ruzicka *et al.*, 1922).

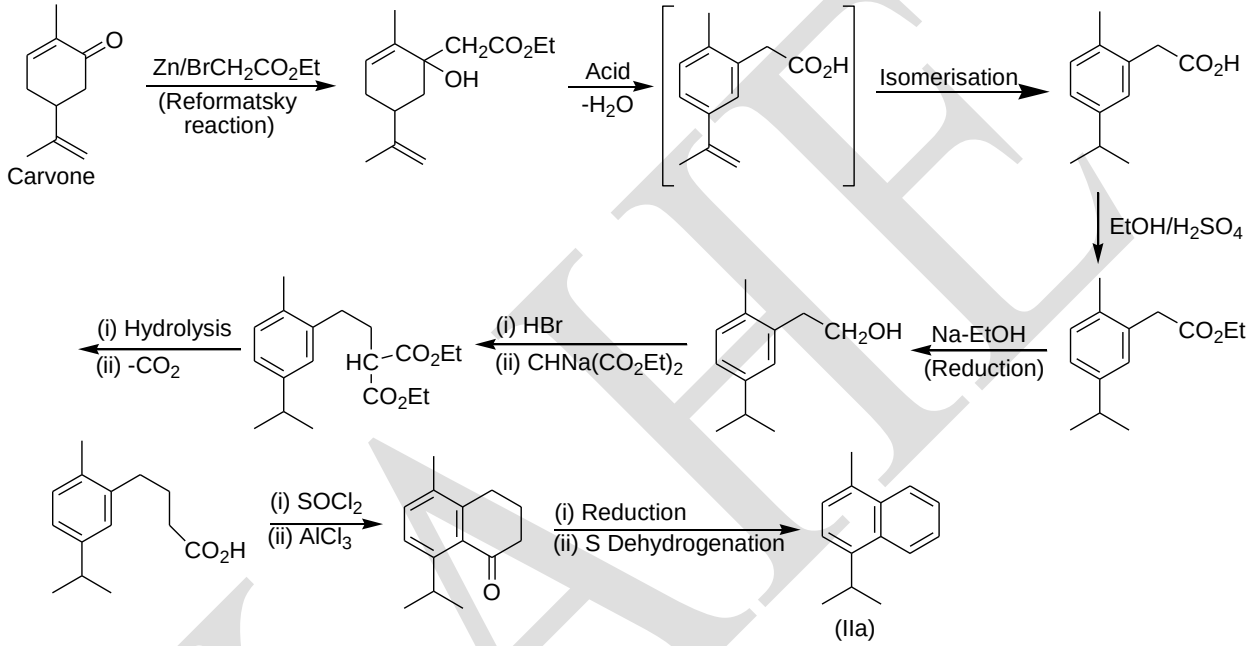


Eudesmol Eudalene

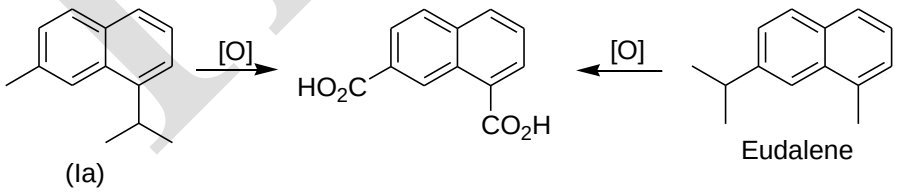
Eudalene behaved as an aromatic compound, and its structure was deduced as follows. Since eudalene was a naphthalene derivative, and since it contained one carbon atom less than cadalene, it was thought to be an apocadalene, i.e., cadalene minus one methyl group. Thus eudalene is either 1-methyl-4-isopropylnaphthalene (IIa) or 7-methyl-1-isopropylnaphthalene (Ia). To test this hypothesis, Ruzicka oxidised cadalene with chromic acid, and thereby obtained a naphthoic acid, $C_{15}H_{16}O_2$, which must be (I) or (II).



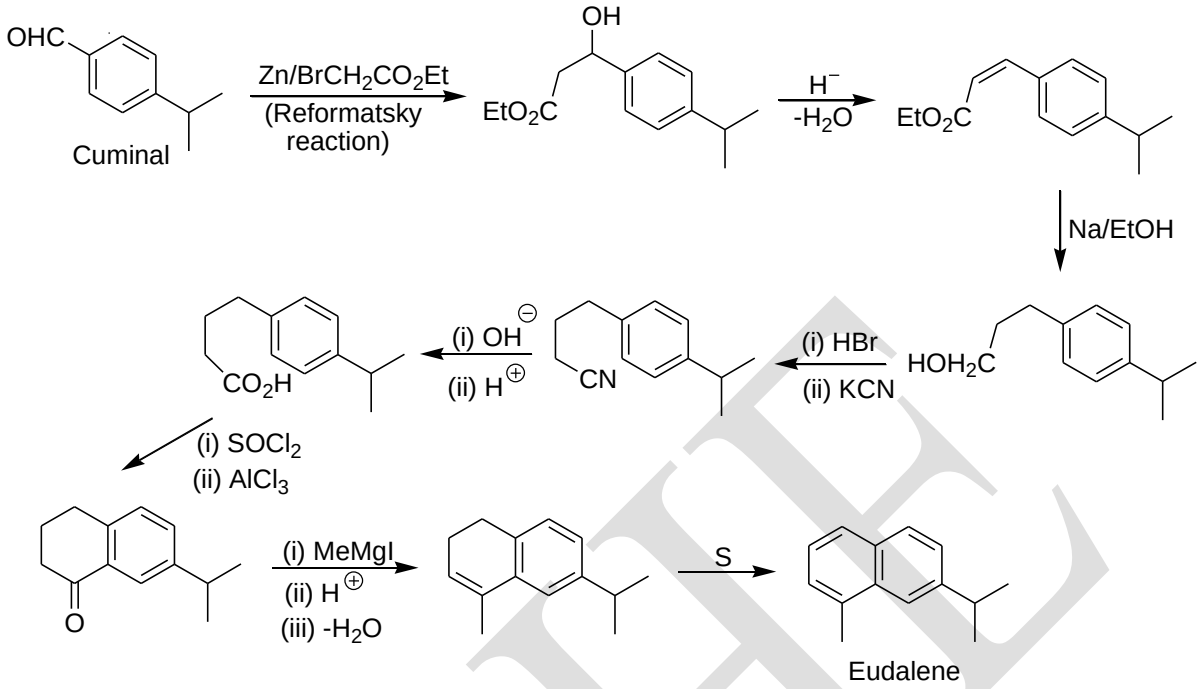
Distillation of this acid with soda-lime gives a methylisopropylnaphthalene which must be (Ia) or (IIa). (IIa) was synthesised from carvone (the synthesis is the same as for cadalene except that ethyl malonate is used instead of ethyl methylmalonate). The synthetic compound (IIa) was found to be different from the hydrocarbon obtained by the distillation of the naphthoic acid from cadalene. Thus the apocadalene obtained must be (Ia) i.e., 7-methyl-1-isopropylnaphthalene.



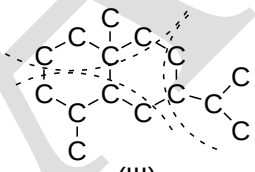
Ruzicka then found that eudalene was not identical with either (Ia) or (IIa). On oxidation, however, eudalene gives the same naphthalenedicarboxylic acid as that which is obtained by the oxidation of (Ia). This is only possible if in eudalene the two side-chains in (Ia) are interchanged, i.e., eudalene is 1-methyl-7-isopropylnaphthalene; thus



This structure for eudalene was proved by synthesis (Ruzicka *et al.*, 1922).

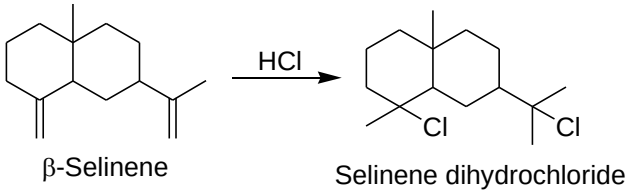


To develop the sesquiterpenoid carbon skeleton from that of eudalene, it is necessary to introduce one carbon atom in such a position that it is eliminated as methanethiol during the sulphur dehydrogenation (see above). If we use the isoprene rule with the units joined head to tail, then there is only one possible structure that fits the requirements, viz., (III).

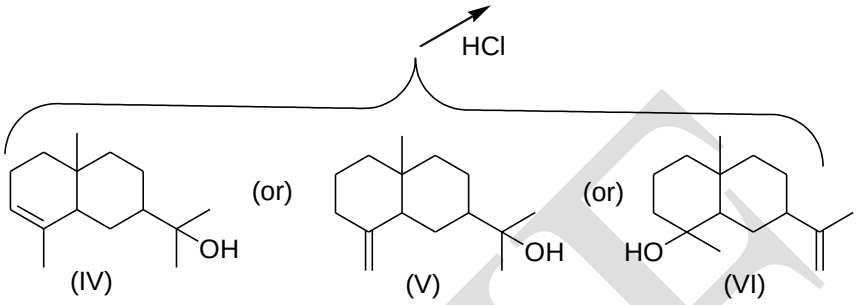


(III)

Now β-selinene combines with hydrogen chloride to form selinene dihydrochloride, which is also obtained by the action of hydrogen chloride on eudesmol (Ruzicka *et al.*, 1927, 1931). Since eudesmol contains one double bond and a tertiary alcohol group, it follows that the double bond must be in the side-chain, and the hydroxyl group in the ring, or *vice versa*, i.e., (IV), (V) or (VI) is the structure of eudesmol.

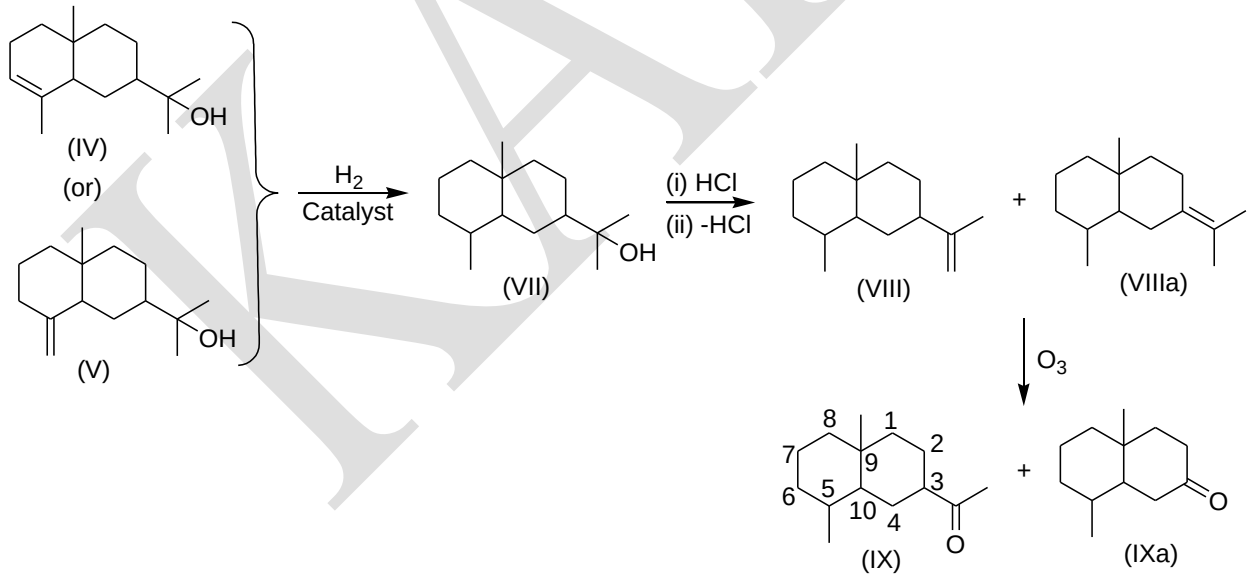


β-Selinene Selinene dihydrochloride



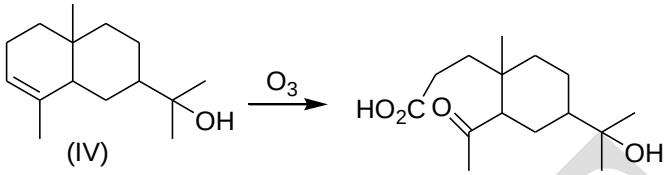
(IV) (V) (VI)

Hydrogenation of eudesmol forms dihydroeudesmol (VII) and this, on treatment with hydrogen chloride followed by boiling with aniline (to remove a molecule of hydrogen chloride), gives dihydroeudesmene (VIII) and (VIIIa). (VIII), on ozonolysis, forms 3-acetyl-5,9-dimethyldecalin (IX) and (VIIIa) forms 5,9-dimethyldecal-3-one (IXa). These results are explained if (IV) or (V) is the structure of eudesmol, but not by (VI). Thus the hydroxyl group is in the isopropyl side-chain.



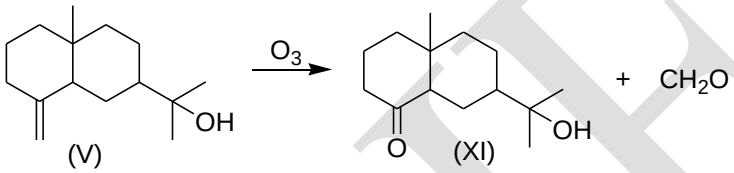
The final problem was to ascertain the position of the double bond in eudesmol, i.e., is the structure (IV) or (V)? Ozonolysis of eudesmol showed that eudesmol is a mixture of (IV) (α-eudesmol) and (V) (β-eudesmol), since two products are obtained: a hydroxyketo-acid (X), with no loss of carbon, and a hydroxyketone (XI), with the loss of one carbon atom. The two

isomers are also clearly distinguished from each other by their infrared absorption spectra. The β-isomer shows a strong band at 889 cm^{-1}; this is characteristic of the alkene $R_2C=CH_2$ (895-885 cm^{-1}). The α-isomer does not show this band.



(IV)
α-Eudesmol

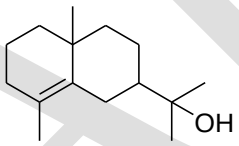
(X)



(V)
β-Eudesmol

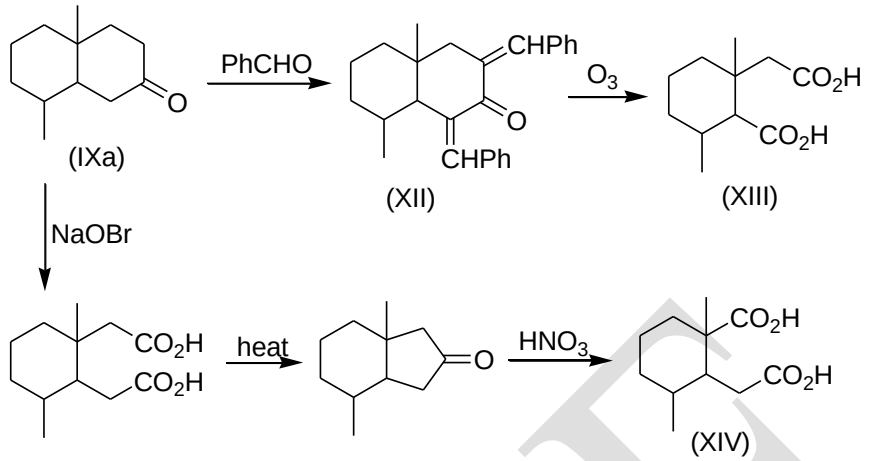
(XI)

The proportions of these two isomers vary with the source, and McQuillin *et al.* (1956) have succeeded in separating them (via their 3,5-dinitrobenzoates), and the same time have characterised a third, synthetic γ-isomer.

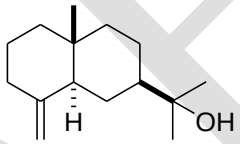


γ-Eudesmol

As described above, the position of the angular methyl group was determined on the basis of the isoprene rule. Since there are exceptions to this rule, it is therefore desirable to confirm its position by other means. This has been done chemically as follows. Ketone (IXa) was converted into its dibenzylidene derivative (XII) and this, on ozonolysis, gave the dicarboxylic acid (XIII). However, there is always the possibility that the dicarboxylic acid produced had structure (XIV). This was therefore also prepared as shown in the Chart.



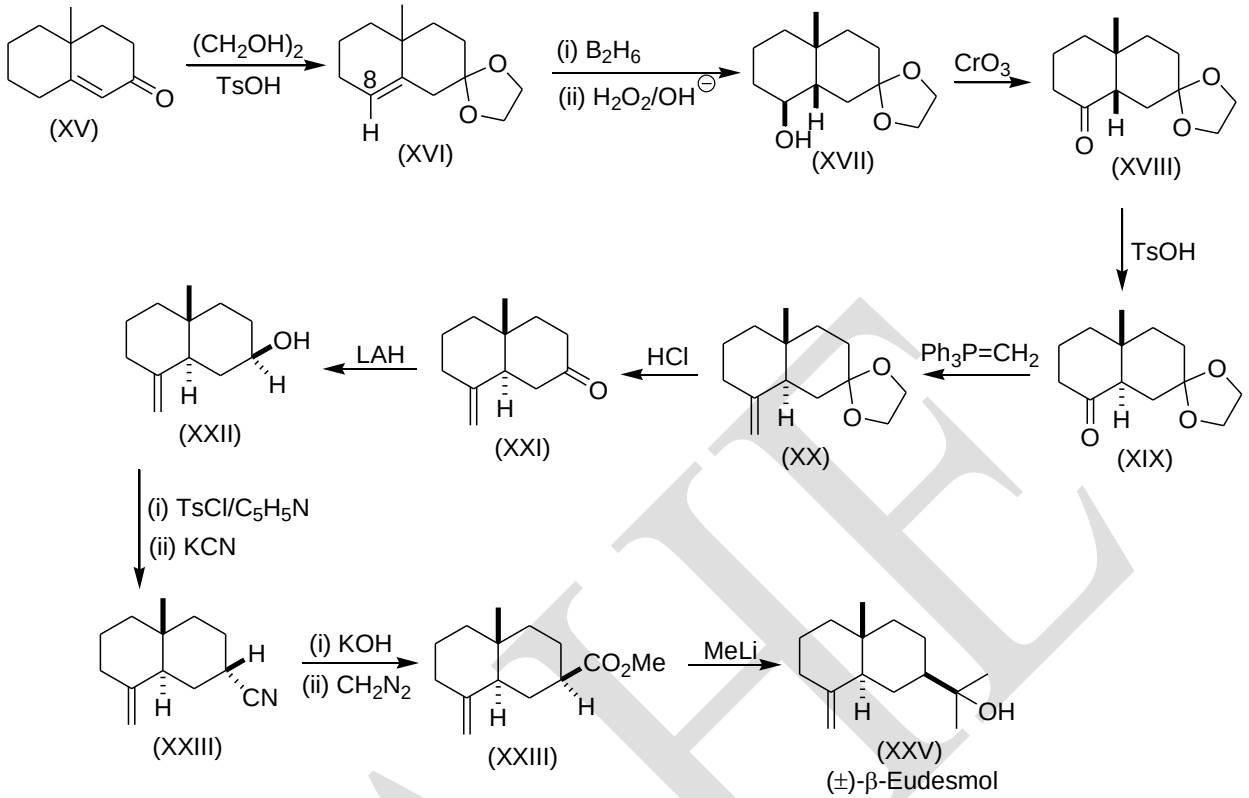
Since only the dicarboxylic acid prepared from the dibenzylidene derivative could be epimerised by boiling in concentrated acid, this indicates that one carboxyl group must be joined to an asymmetric carbon atom attached to a hydrogen atom. Hence this dicarboxylic acid is (XIII) and so confirms the position of the angular methyl group.



β-Eudesmol

Stereochemical considerations have shown that β-eudesmol has the *trans* decalin configuration and that the angular methyl group and the isopropyl group are on the same side (see also the synthesis described below).

Marshall *et al.* (1965) have carried out a stereoselective total synthesis of racemic β-eudesmol as follows.

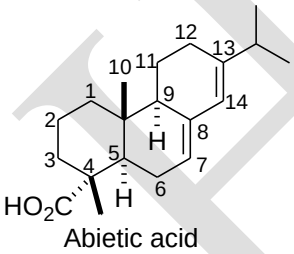


The conversion of (XV) into (XVI), by the use of prescribed conditions, resulted in the formation of the dioxolan (ethylene ketal) (XVI), in which double-bond migration was anticipated by analogy with the behaviour of steroid analogues, but was proved by the fact that the NMR spectrum of (XVI) showed a triplet signal at τ 4.77 for the vinyl proton (H-8). This triplet could only arise by coupling with an adjacent methylene group. Hydroboration of (XVI), followed by oxidation, resulted in hydration of the double bond in the *cis*-manner, and gave (XVII) as the major product. The addition to give *cis*-fustion of the rings (and not the alternative *trans*-fusion) was proved by a separate series of experiments (*cis*-fusion was anticipated by analogy with the behaviour of steroid analogues). Oxidation of (XVII) gave (XVIII) which, on equilibration, gave (XIX) as the predominant isomer (65 per cent). The configurations of the *cis*- and *trans*-isomers were determined from their NMR spectra. (XIX), by means of the Wittig reaction, was converted into (XX) and this, on hydrolysis, gave (XXI), the structure of which was proved by an independent method. Reduction of (XXI) by lithium aluminium hydride gave the alcohol (XXII) and this, on treatment with tosyl chloride, gave the tosyl ester (no inversion) and this with potassium cyanide, gave the inverted cyanide (XXIII). Hydrolysis of this cyanide

now gave the corresponding acid with inversion (at the carbon atom attached to the carboxyl group). The stereochemistry of this acid was proved by an independent method. Finally, this acid was converted into (±)-β-eudesomol as shown. The identity of the natural and the synthetic racemic compound was established by means of their infrared spectra, etc.

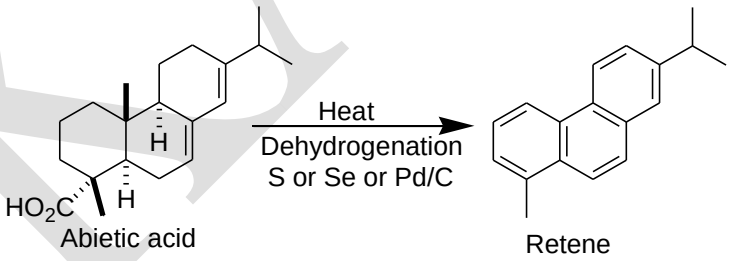
Abietic acid

Molecular formula: $C_{20}H_{30}O_2$, m.p.172-175ºC, is a tricyclic diterpenoid. For our purpose it is useful to have the structure of abietic acid as a reference, and then describe the evidence that led to this structure.



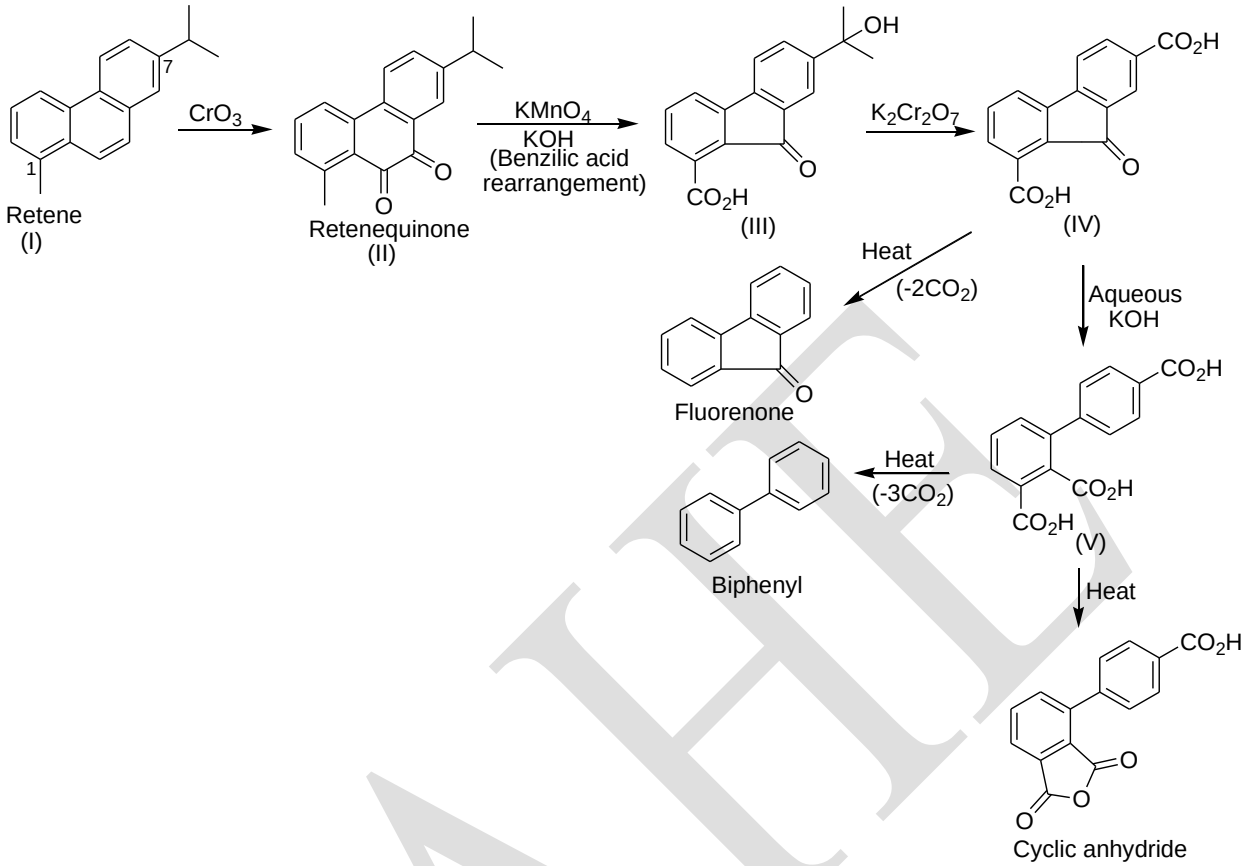
Abietic acid

As abietic acid gives effervescence with sodium bicarbonate, this shows that it contains a carboxylic group. On dehydrogenation with sulphur, abietic acid gives retene (Vesterberg, 1903); better yields of retene are obtained by dehydrogenating with selenium (Diels *et al.*, 1927), or with palladised charcoal (Ruzicka *et al.*, 1933).



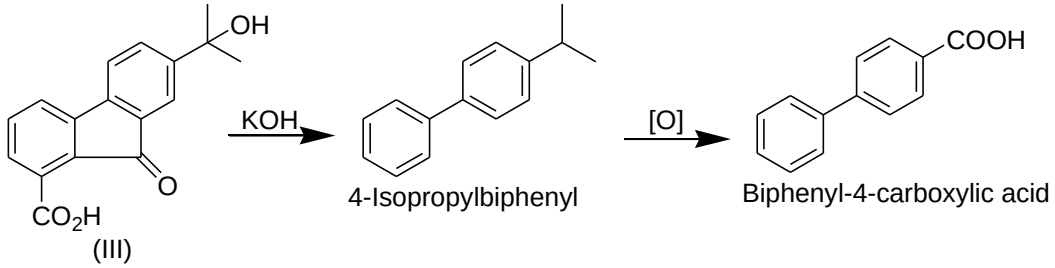
Abietic acid Retene

Retene, $C_{18}H_{18}$, m.p 99ºC, was shown by oxidative degradation to be 1-methyl-7-isopropylphenanthrene (Bucher, 1910). Oxidation of retene (I) gave retenequinone (II) which, on oxidation with alkaline permanganate, gave the key intermediate (II) and this, on oxidation with dichromate, gave (IV). (IV), when heated with concentrated aqueous potassium hydroxide, gave (V).



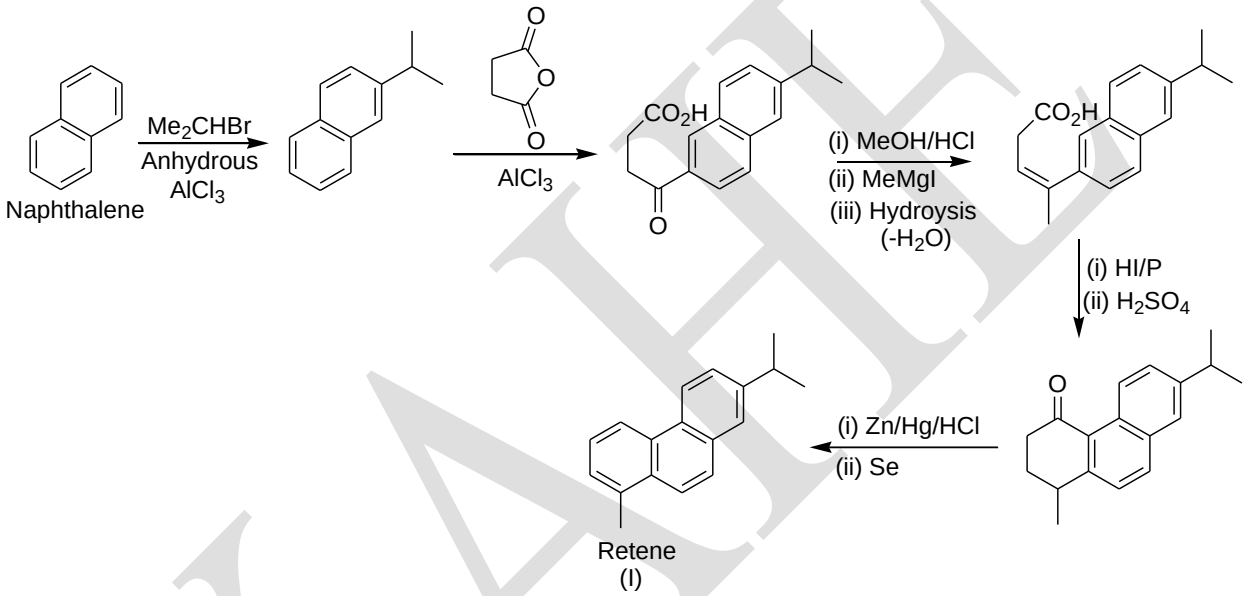
The conversion of (II) into (III) involves a benzilic acid rearrangement. Since (V) formed a cyclic anhydride and (IV) did not, one carboxyl group in (IV) must be ortho to the centre ring. This carboxyl group is derived from an alkyl group in retene and so one alkyl group must be at position 1. Since (III) is formed from (II) by loss of one carbon atom only, this suggests that the carboxyl group is derived from a methyl group (at position 1). On heating, (IV) gave fluorenone and (V) gave biphenyl. Hence the carbon skeletons of (IV) and (V) are established.

The problem now was to locate the position of the isopropyl group in retene (I). This was solved by fusing (III) with potassium hydroxide. The product was shown to be 4-isopropylbiphenyl by oxidation to biphenyl-4-carboxylic acid. Hence retene contains an isopropyl group at position 7.

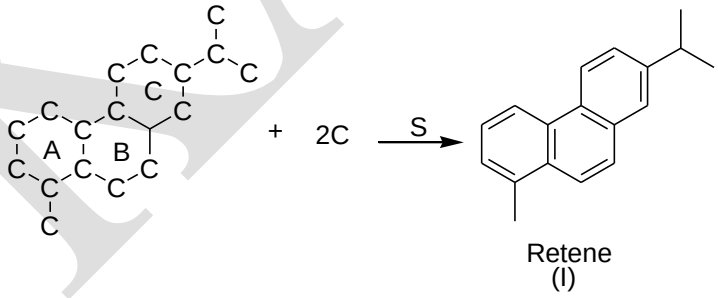


(III)

Structure (I) for retene has been confirmed by synthesis, e.g., that of Haworth *et al.* (1932).



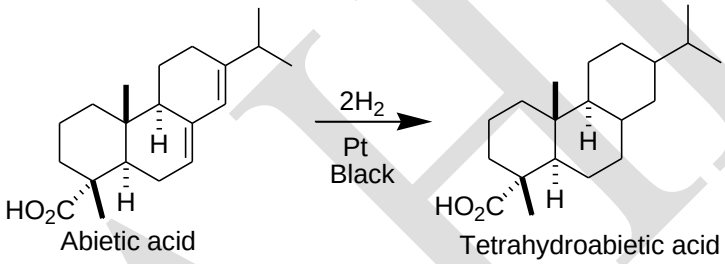
Since two carbon atoms were lost from abietic acid (C_{20}) to form retene (C_{18}), we have;



Now it is known that in sulphur dehydrogenations, carboxyl groups and angular methyl groups can be eliminated. It is therefore possible that the two carbon atoms lost may have been originally the carboxyl group (in abietic acid) and an angular methyl group.

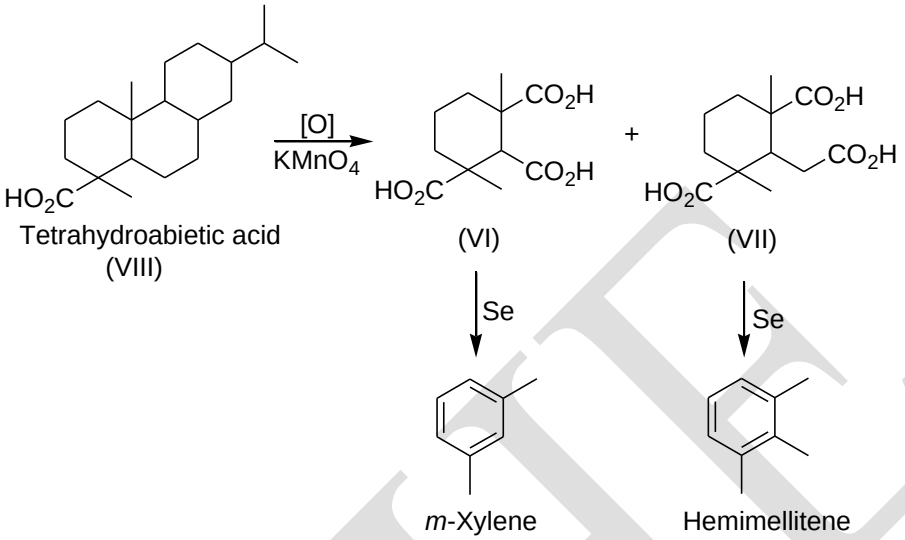
Abietic acid is very difficult to esterify, and since this is characteristic of a carboxyl group attached to a tertiary carbon atom, it suggests that abietic acid contains a carboxyl group in this state. This is supported by the fact that abietic acid evolves carbon monoxide when warmed with concentrated sulphuric acid; this reaction is also characteristic of a carboxyl group attached to a tertiary carbon atom.

Catalytic hydrogenation of abietic acid gives tetrahydroabietic acid, $C_{20}H_{34}O_2$. Thus abietic acid contains two double bonds; also, since the parent hydrocarbon is $C_{19}H_{34}$ (regarding the carboxyl group as a substituent group), abietic acid is tricyclic (parent corresponds to C_nH_{2n-4}), which agrees with the evidence already given.

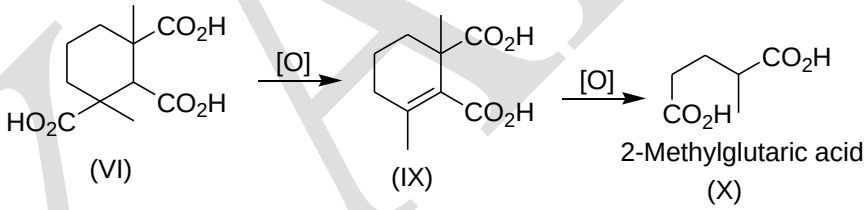


Oxidation of tetrahydroabietic acid (VIII) with potassium permanganate gives a mixture of products among which are two tricarboxylic acids, $C_{11}H_{16}O_6$ (VI), and $C_{12}H_{18}O_6$ (VII) (Ruzicka *et al.*, 1925, 1931). (VI) on dehydrogenation with selenium, forms-*m*-xylene, and (VII) forms hemimellitene (1,2,3-trimethylbenzene) (Ruzicka *et al.*, 1931). In both cases there is a loss of three carbon atoms, and if we assume that these were the three carboxyl groups, then two methyl groups in (VI) and (VII) must be in the meta-position. Furthermore, since (VI) and (VII) each contain the methyl group originally present in abietic acid (position 4), acids VI) and (VII) must contain ring A of abietic acid. This suggests, therefore that there is an angular methyl group at position 10, since it can be expected to be eliminated from this position in sulphur dehydrogenations of abietic acid (this 10-methyl group is *meta* to the 4-methyl group). Vocke (1932) showed that acid (VI) evolves two molecules of carbon monooxide when warmed with concentrated sulphuric acid; this indicates that (VI) contains two carboxyl groups attached to tertiary carbon atoms. These results can be explained by assuming that one carboxyl group in (VI) is that in a abietic acid, and since in both cases this carboxyl group is attached to a tertiary carbon atom, the most likely position of this group is 4 (in abietic acid). Accepting these

assumptions, the oxidation of abietic acid may be formulated as follows, also assuming (VIII) as the carbon skeleton of abietic acid.

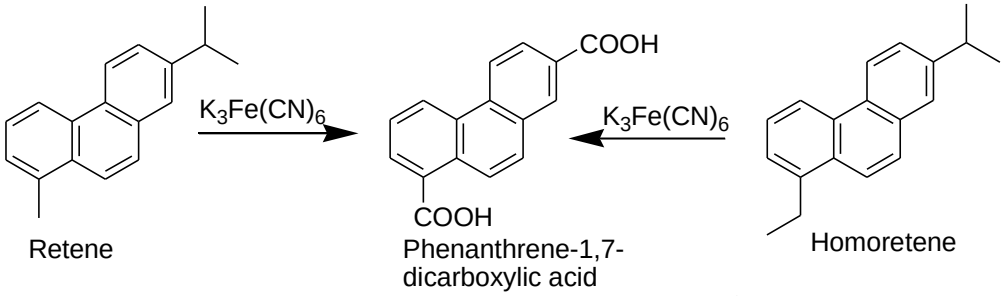


Vocke subjected (VI) to oxidative degradation, and obtained a dicarboxylic acid (IX) which, on further oxidation, gave 2-methylglutaric acid (X). Vocke assumed that (VI) had the structure shown, and formulated the reactions as below, assuming structure (IX) as the best way of explaining the results.

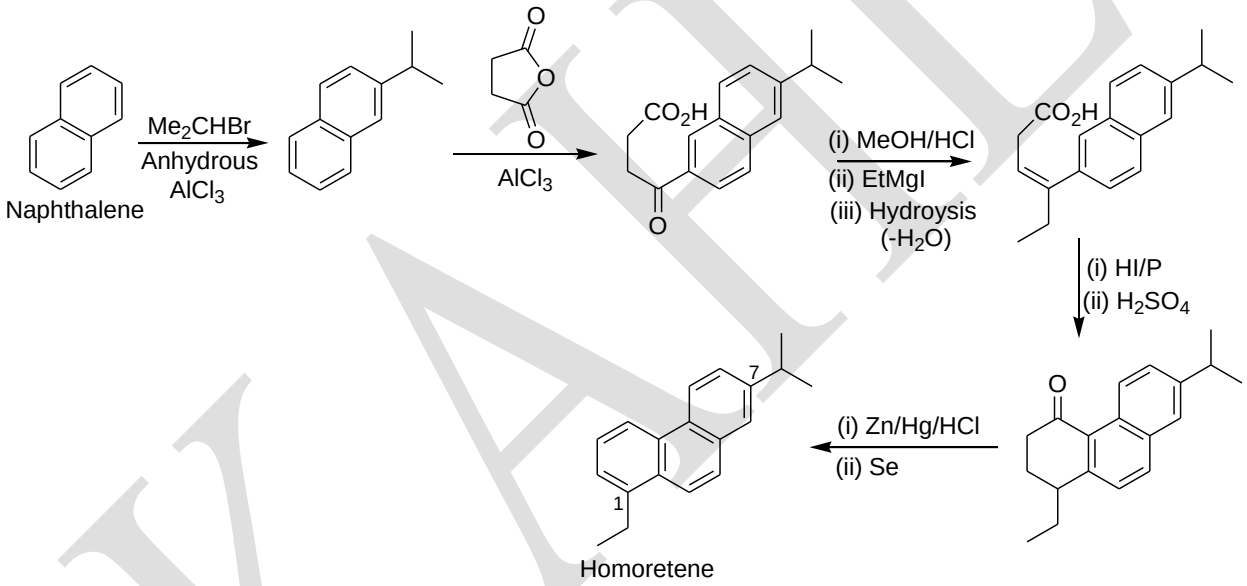


Structure (IX) (assumed by Vocke) has been confirmed by synthesis (Rydon, 1937).

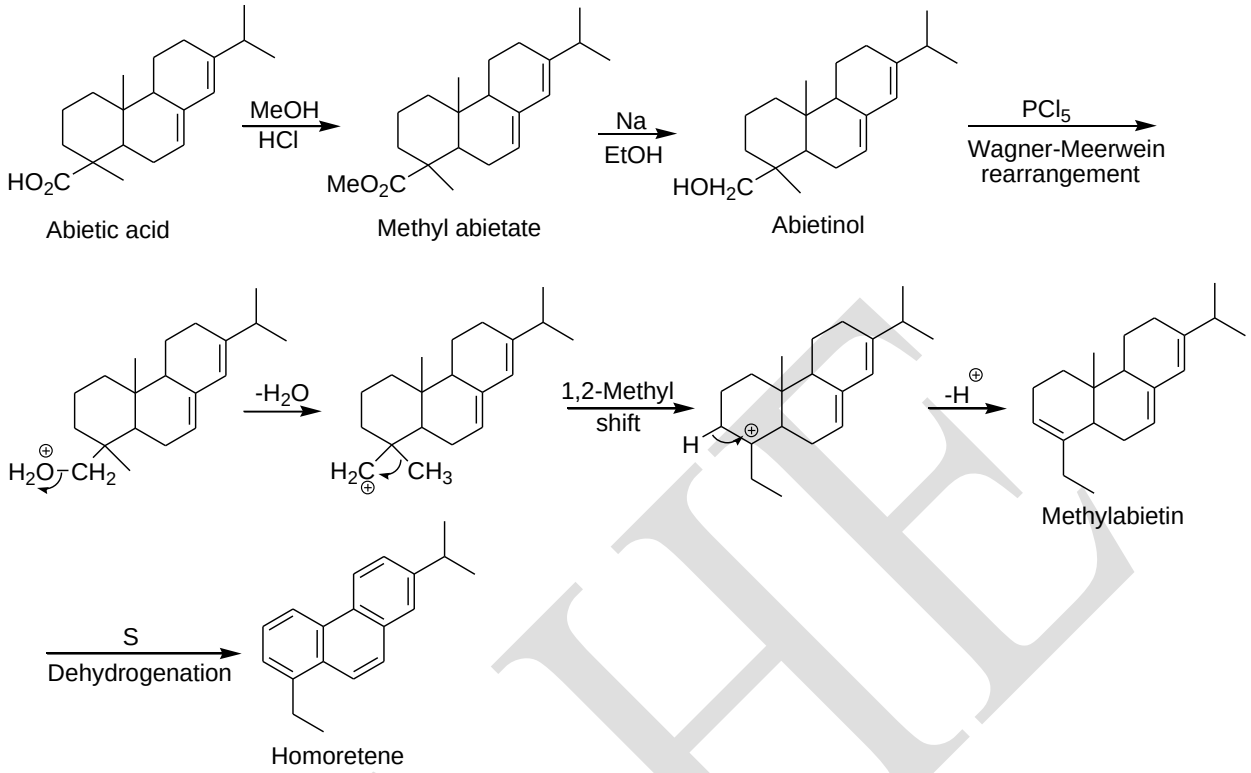
The position of the carboxyl group at position 4 in abietic acid (assumed above) has been confirmed by Ruzicka *et al.* (1922). Methyl abietate, $C_{19}H_{29}CO_2CH_3$ on reduction with sodium and ethanol, forms abietinol, $C_{19}H_{29}CH_2OH$, which, on treatment with phosphorus pentachloride, loses a molecule of water to form "methylabietin", C_{20} H_{30}. This, on distillation with sulphur, forms homoretene, $C_{19}H_{20}$. Homoretene contains one CH_2 group more than retene, and on oxidation with alkaline potassium ferricyanide, gives phenanthrene-1,7-dicarboxylic acid, the identical product obtained from the oxidation of retene under similar conditions (Ruzicka *et al.*, 1932).



These results can only be explained by assuming that homoretene has an ethyl group at position 1 (instead of the methyl group in retene) i.e., homoretene is 1-ethyl-7-isopropylphenanthrene. This has been confirmed by synthesis (Haworth *et al.*, 1932; ethylmagnesium iodide was used instead of methyl magnesium iodide in the synthesis of retene).

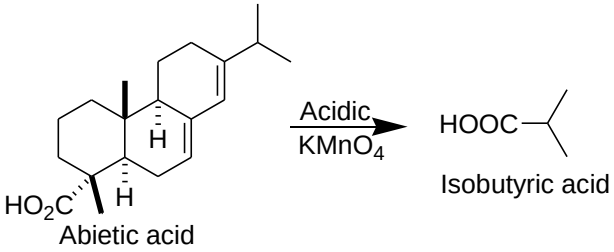


The formation of an ethyl group in homoretene can be explained by assuming that abietinol undergoes a Wagner-Meerwein rearrangement on dehydration Thus:

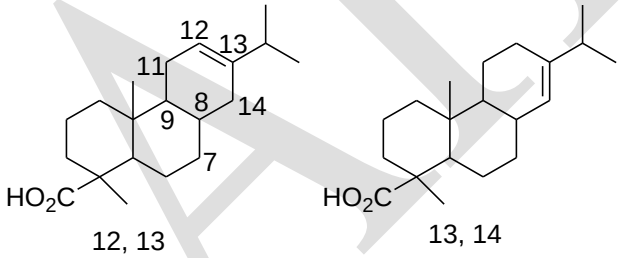


It has already been pointed out that abietic acid has two double bonds. Since abietic acid forms an adduct with maleic anhydride at above 100°C, it was assumed that the two double bonds are conjugated (Ruzika *et al.*, 1932). It was later shown, however, that levopimaric acid also forms the same adduct at room temperature. It thus appears that abietic acid isomerises to levopimaric acid at above 100°C, and then forms the adduct. Thus this reaction cannot be accepted as evidence for conjugation in abietic acid. Abietic acid, however, shows a maximum at 238 (ε 16 000) nm in the ultraviolet region. This indicates that the two double bonds are conjugated, but since the basic value for a homoannular diene system is 253 nm, it may therefore be concluded that the two double bonds are not in the same ring. The calculated value for the structure assigned to abietic acid is 214 + 4 × 5 + 5 = 239 nm. This is supported by the fact that levopimaric acid has $_{max}$ 272.5 (ε 7000) nm, a value in agreement with the two double bonds being in the same ring in this compound (Calculated value for the structure assigned to this acid is 253 + 4 × 5 = 273 nm).

Oxidation of abietic acid with potassium permanganate gives, among other products, isobutyric acid (Ruzicka *et al.*, 1925).



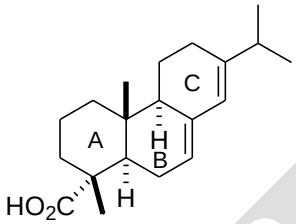
This suggests that one double bond is in ring C and the 12, 13- or 13, 14-position. If the double bond is in the 12,13-position, then the double bond which is conjugated with it, must also be in the same ring (9, 11 or 8, 14); if, 13, 14, then the other double bond could be in the same ring C, but it could also be in ring B. Since, as we have seen, the two double bonds are in different rings, their positions are probably 7, 8 and 13, 14. Further evidence for these positions is afforded by the fact that in the oxidation of abietic acid to give acids (VI) and (VII) (see above), in which ring A is intact, rings B and C are opened, and this can be readily explained only if rings B and C each have a double bond. Oxidative studies on abietic acid by Ruzicka *et al.* (1938-1941) have conclusively confirmed the positions 7,8 and 13,14.



The stereochemistry of abietic acid has been elucidated and has the absolute configuration shown. Since the tricarboxylic acid (VI) is optically inactive, it must possess a plane of symmetry. This is possible only if the meta-carboxyl groups are cis with respect to each other. Barton *et al.* (1948) deduced from the study of the dissociation constants of this acid that the centre carboxyl group was trans to the other two. Their argument was based on the observation that the difference between pK_1 and pK_2 of cycloalkane-1,2-dicarboxylic acids is greater for the cis- than for the trans-acid. Hence rings A and B are fused in a trans manner. This is true only if inversion does not occur in the formation of (VI); this was confirmed by other work.

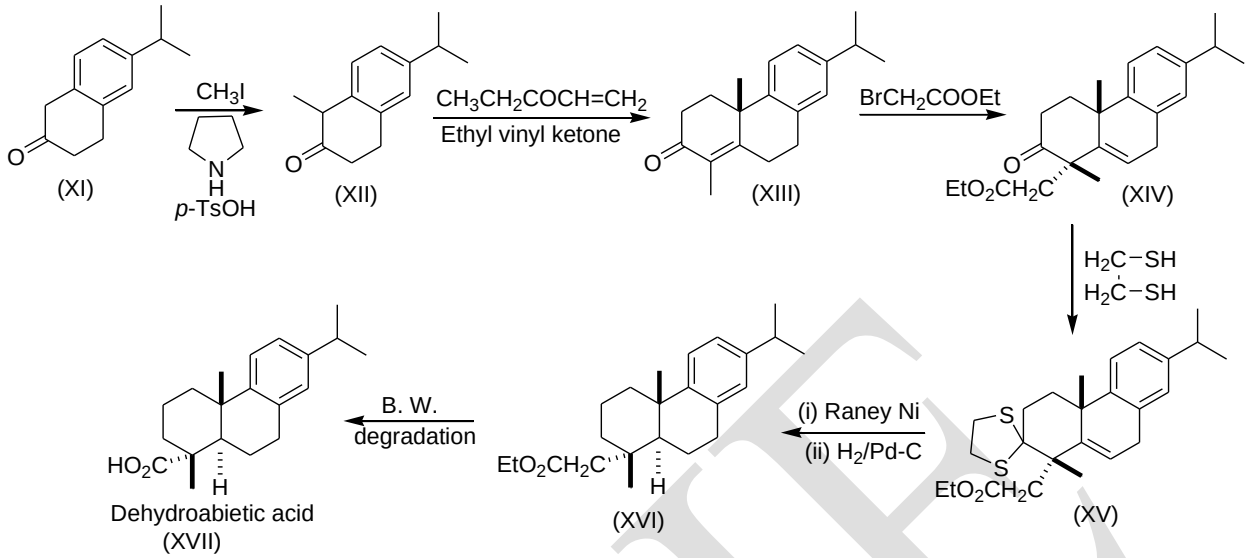
The remaining chiral centre is C-9. Since abietic acid is readily formed from other related acids by acid catalysis which involves double bond migration (see below), it was argued that if

the C_{10}-methyl group and the C_9-hydrogen are trans, this would be the more stable form and so this is the configuration present in abietic acid (Barton, 1949). Klyne (1953) supported this on the basis of his molecular rotation studies and also deduced the absolute configuration shown in the formula of abietic acid.

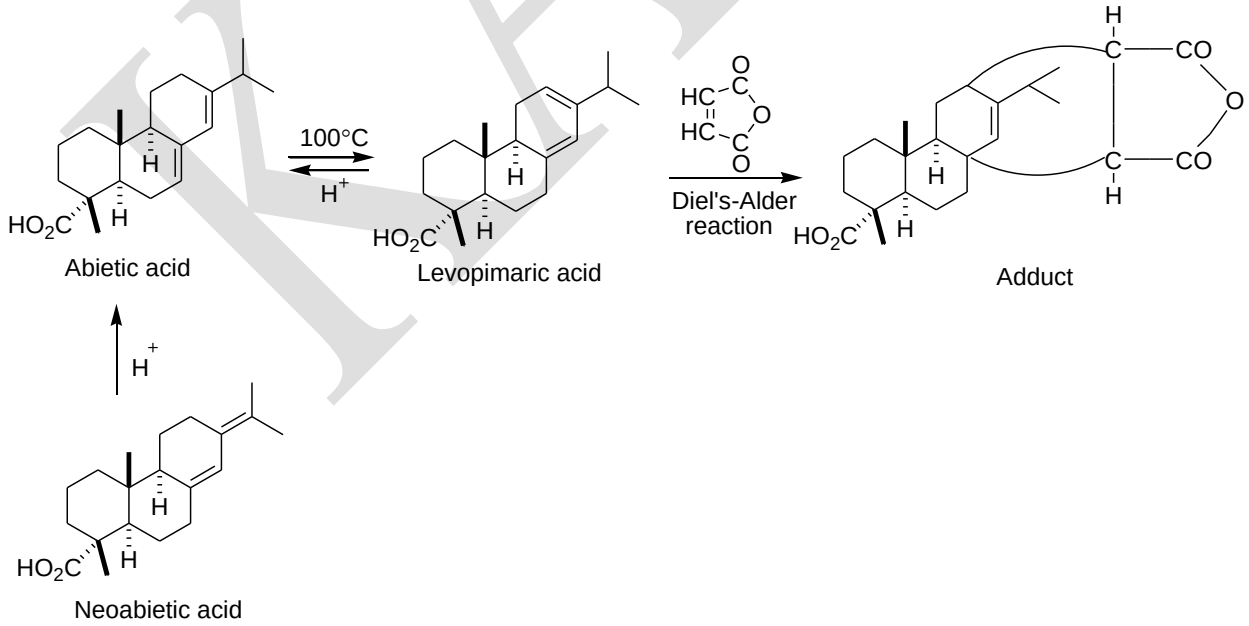


Abietic acid

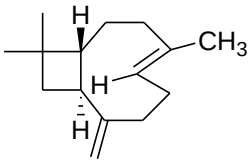
The stereochemistry of abietic acid has been confirmed by Stork *et al.* (1956), who have carried out a stereo specific synthesis of (±)-dehydroabietic acid (XVII). This is shown in the sequence (XI) to (XVII). β-Tetralone (XI)was methylated (MeI) via the pyrrolidine enamine to give (XII) and this, on condensation with ethyl vinyl ketone, gave (XIII). Alkylation of (XIII) with ethyl bromo acetate produced (XIV) in which because of the steric effect of the angular methyl group in (XIII), the acetic ester residue was introduced on the less hindered side of the molecule. (XIV) was converted into its thioketal by ethanedithiol and this, on hydrolysis with alkali, gave (XV) which, on conversion into its methyl ester followed by Raney nickel desulphurization, hydrolysis, and hydrogenation with Pd-C in acetic acid, gave (XVI). The two rings in the tetralin fragment in (XVI) are *trans* fused because addition of hydrogen occurs on the face opposite to the two *cis*-methyl groups. Application of the Barbier-Wieland degradation gave (±)-dehydroabietic acid (XVII). Since this may be prepared from abietic acid and *vice versa*, the stereochemistry of abietic acid is determined.



As mentioned above, abietic acid is apparently produced by the isomerisation of a number of labile precursors present in the oleoresin. These labile precursors are referred to as the primary resin acid, and the two principal ones are levopimaric acid and neoabietic acid. Both acids are readily isomerised to abietic acid in the presence of acid or by heat, and levopimaric and abietic acids form the same adduct with maleic anhydride (see above). Since levopimaric acid has λ_{max} 272.5 nm, this shows that this acid is homoannular diene. We can therefore formulate the Diels-Alder reaction as shown (using as our basis the given structures).

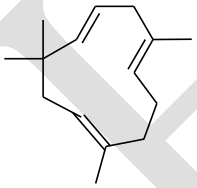


Caryophyllene



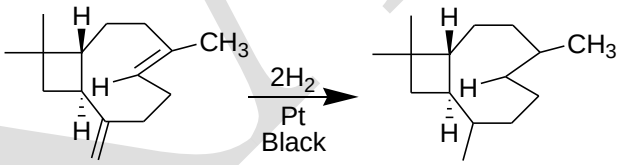
Caryophyllene

B.p.130˚C/14mm. This occurs in oil of cloves together with its geometrical isomer, isocaryophyllene. As will be shown below, it is a macrocyclic sesquiterpenoid. Originally, it was believed that there were three isomers, α-, β- and γ-caryophyllene. However, the α-isomer has now been shown to be identical with humulene; the β-isomer is referred to as caryophyllene and the γ-isomer is isocaryophyllene.



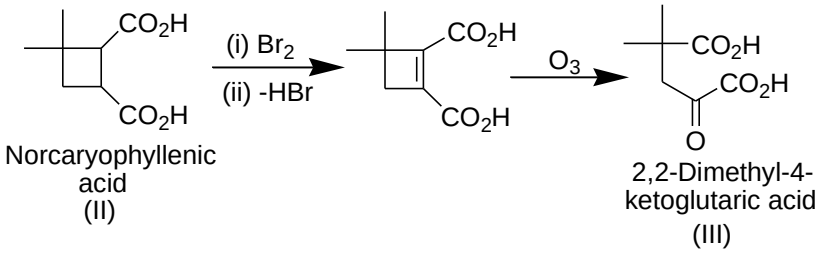
Humulene
or
α-Caryophyllene

The molecular formula of caryophyllene is $C_{15}H_{24}$ and, on catalytic hydrogenation, tetrahydrocaryophyllene, $C_{15}H_{28}$, is formed.



Caryophyllene Tetrahydrocaryophyllene

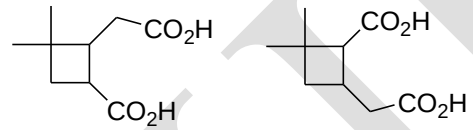
Hence caryophyllene is a bicyclic compound and contains two double bonds. Ozonolysis followed by oxidation with nitric acid converted caryophyllene into a mixture of two dicarboxylic acids, caryophyllenic acid (I), $C_9H_{14}O_4$, and norcaryophyllenic acid (II), $C_8H_{12}O_4$. (II), on bromination, dehydrobromination, and ozonolysis, gave 2,2-dimethyl-4-ketoglutaric acid (III).



Norcaryophyllenic acid (II)

2,2-Dimethyl-4-ketoglutaric acid (III)

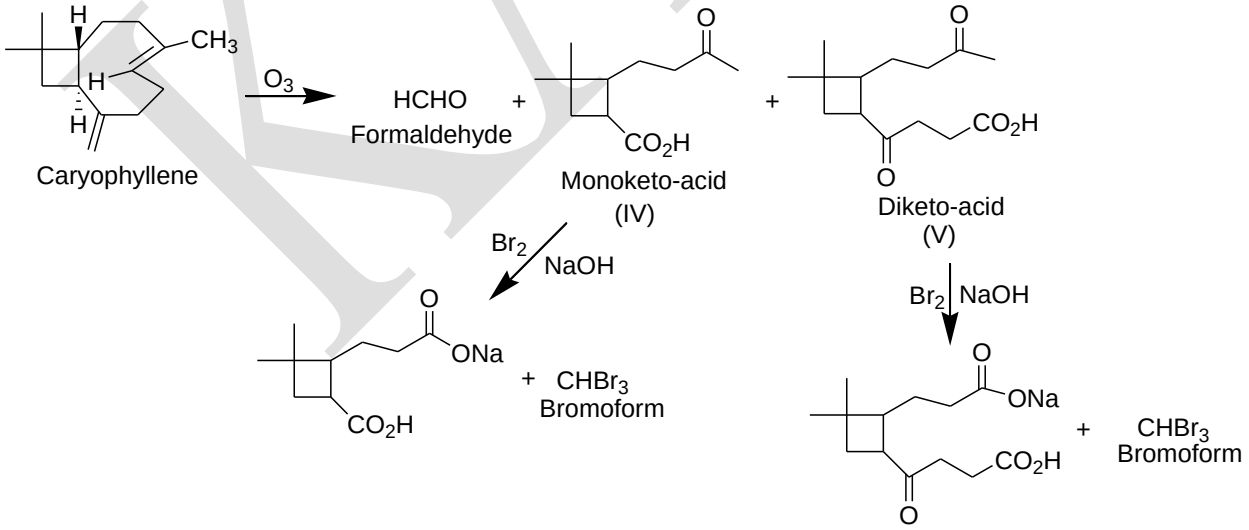
This suggests that (II) is a cyclobutane derivative, and so the reactions may be written as shown. The structure of (II) was confirmed by synthesis. It therefore follows that caryophyllenic acid must be (I) or (Ia), since it can be degraded to (II). Synthesis of these two dicarboxylic acids showed that caryophyllenic acid is (I).



Caryophyllenic acid (I)

(Ia)

The problem now was to elucidate the size of the other ring in caryophyllene. Ozonolysis of caryophyllene gave formaldehyde, a monoketo-acid (IV), $C_{11}H_{18}O_3$, and a diketo-acid (V), $C_{14}H_{22}O_4$. Since both keto acids gave the haloform reaction, both contain an acetyl group. Thus, caryophyllene contains the group-CMe=CH- and an exocyclic methylene group (formaldehyde formation).

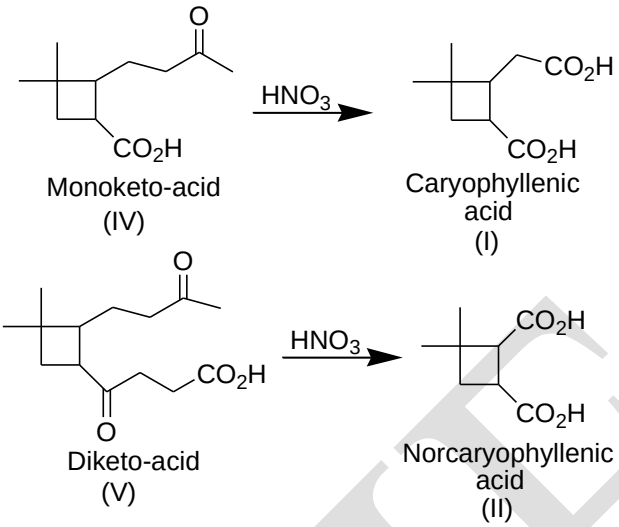


Caryophyllene

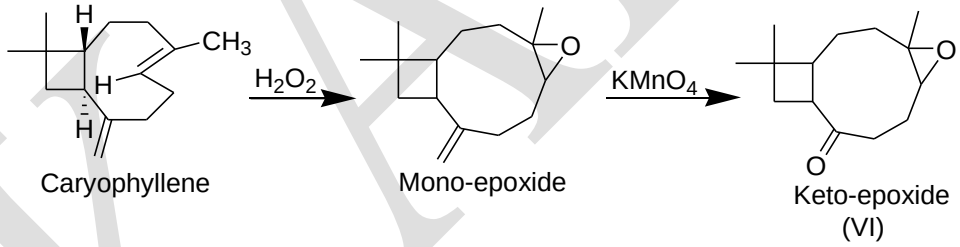
Monoketo-acid (IV)

Diketo-acid (V)

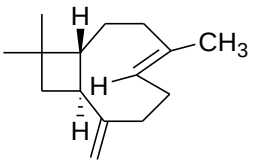
Both (IV) and (V) were oxidised by nitric acid to (I) and (II) and hence (IV) and (V) contain the dimethylcyclobutane system.



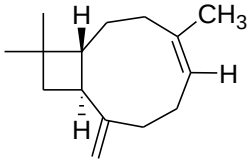
Further work showed that (IV) had the structure shown. Oxidation of caryophyllene with hydrogen peroxide produced a mono-epoxide which, on oxidation with permanganate, gave a keto-epoxide (VI) by removal of the exocyclic methylene carbon atom. Sorm *et al.* (1950) studied the infrared spectra of this keto-epoxide and related compounds, and from the observation of the unusual position of the carbonyl band suggested that a nine-membered ring was present.



On this evidence and that obtained by other workers, structure (VI) was assigned to the keto-epoxide and (VII) to caryophyllene. It therefore follows that (V) is the structure of the diketo-acid (see above). Evidence obtained chemically and by X-ray analysis shows that the ring fusion is *trans* and that the endo cyclic double bond also has the *trans* configuration. Isocaryophyllene (VIII) is the isomer in which the endocyclic double bond has the *cis* configuration.

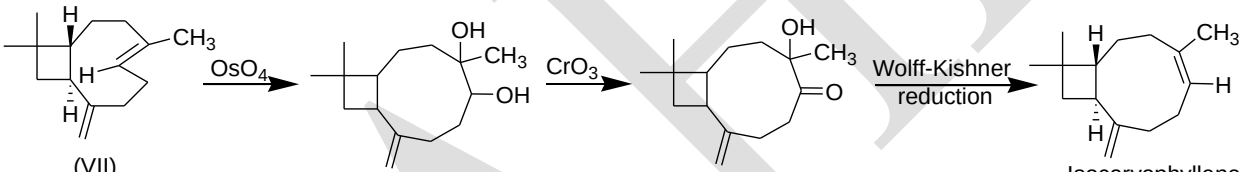


(VII)
Caryophyllene



(VIII)
Isocaryophyllene

(±) Caryophyllene and (±)-isocaryophyllene have now been synthesised by Corey *et al.* (1964). These workers first proved that both isomers contained the same ring fusion (*trans*). Pure caryophyllene was converted into the secondary-tertiary diol, this oxidised (only the secondary alcoholic group) and the resulting ketone subjected to the Wolff-Kishner reduction to give isocaryophyllene.



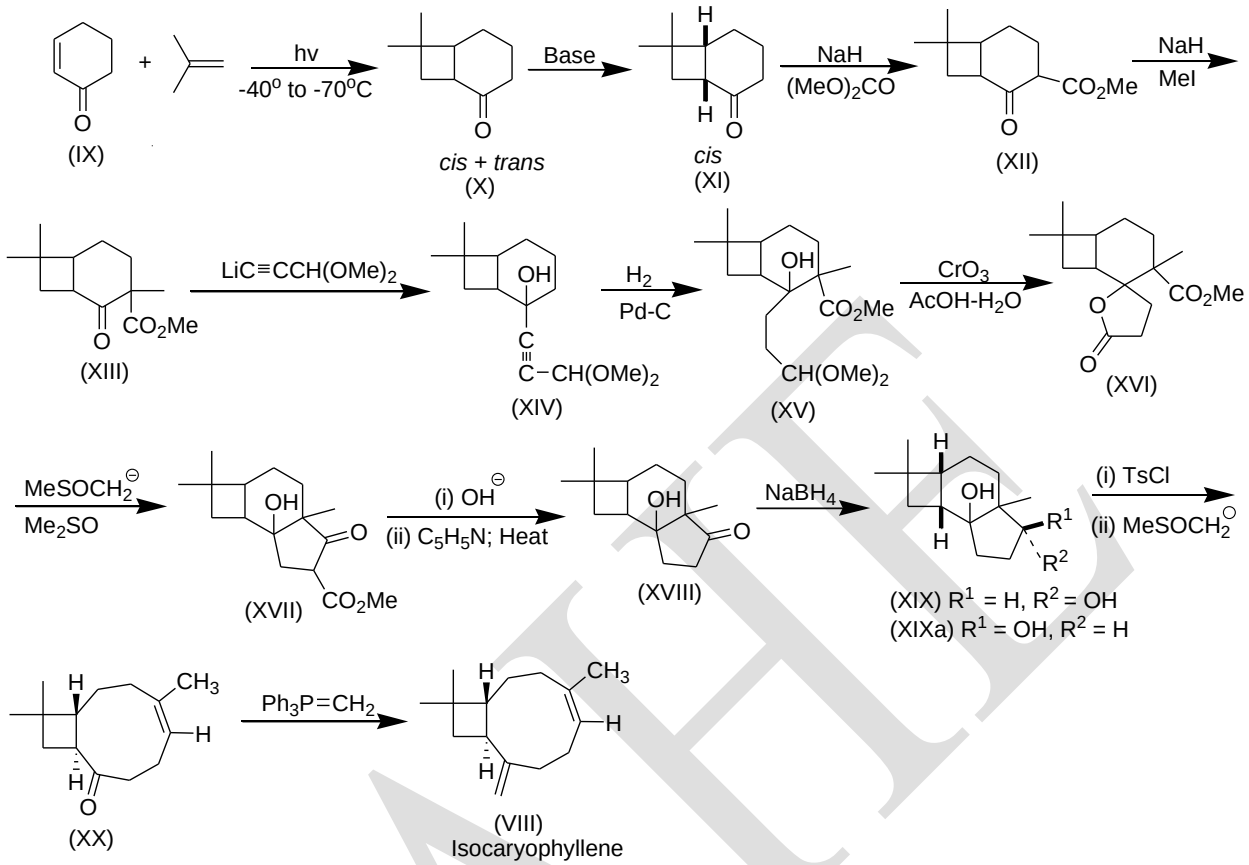
(VII)
Caryophyllene

Secondary-
tertiary diol

Hydroxyl ketone

Isocaryophyllene
(VIII)

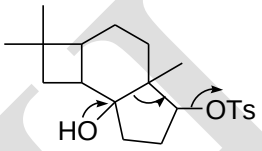
The syntheses were then carried out as shown.



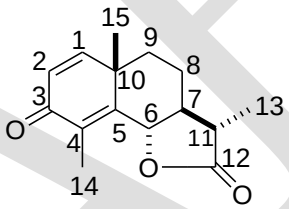
(XVIII) was catalytically hydrogenated (H_2-Raney Ni) to give (XIX) and (XIXa). These were separated by chromatography and treatment of (XIXa) in a similar manner (as described) gave (±)-caryophyllene (VII).

The first point to note is the photochemical addition of isobutene to (IX) to give (X), which was a mixture of *cis*-and *trans*-isomers, and the conversion of the unstable *trans*-isomer, into the stable cis-isomer (XI). The NMR spectrum of (XI) was consistent with this structure and stereochemistry. The NMR analysis of (XII) showed it was a mixture of stereoisomoers. These were not separated, and so (XIV) was also a mixture of stereoisomers. A point of interest to note is that the conversion of (XVI) into (XVII) (a Dieckmann type of reaction) could be effected only by methylsulphinylcarbanion. (XVI) was also a mixture of stereoisomers, whereas the NMR spectrum of (XVIII) showed it to be a pure stereoisomer, but its stereochemistry is not given by these reactions. This, however, does not affect the final result. The essential requirement is the stereochemistry of the internal elimination reaction to give a *cis*- or *trans*-double bond. Corey

et al. argued that if it be assumed that the internal elimination is concerted (i.e., E2) and that the stereoelectronically coplanar mode of elimination operates, the configuration of the alkene formed will be controlled by the relative orientation of the angular methyl group and the vicinal leaving group, the tosyloxy group. When these groups are cis (as in XIXa), the resulting alkene should be trans. Reduction with sodium borohydride gave one stereoisomer only, viz. (XIX; Me and sec –OH *trans*). Treatment of the tosylate with methylsulphinylcarbanion isomerised the *cis*-ring fusion to *trans*, with elimination to give the *cis*-alkenone (XX). This, by means of the Wittig reaction, gave (±)-isocaryophyllene (*cis*-alkene). Starting from (XIXa) gave the *trans*–alkenone, which gave (±)-caryophyllene (*trans*-alkene).



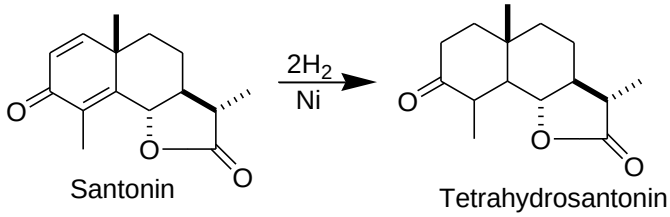
Santonin



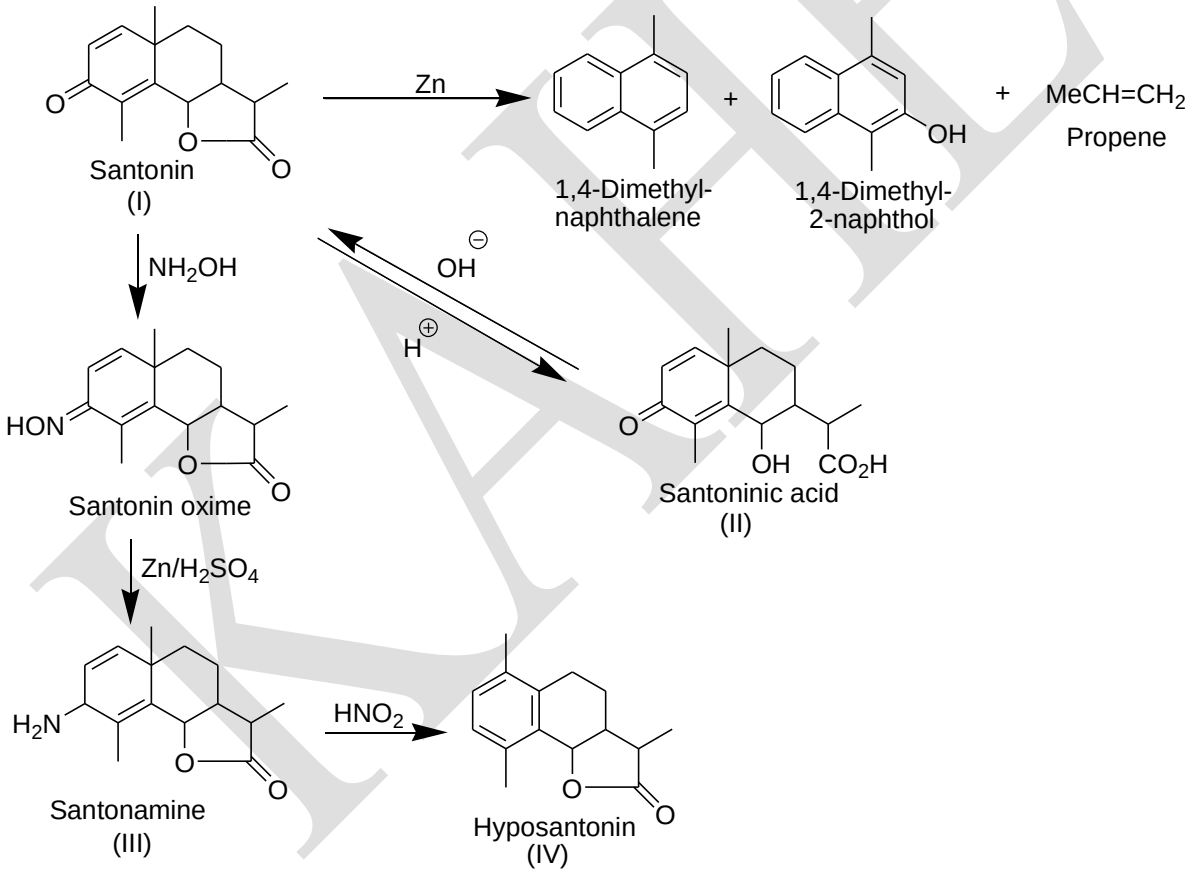
Santonin

Molecular formula: $C_{15}H_{18}O_3$. This occurs in various species of Artemesia (found in Asia). It possesses the eudesmane skeleton but is a sesquiterpenoid lactone. It is widely used in medicine as an anthelmintic (it has the power to expel intestinal worms).

Santonin (I) dissolves in alkali to form the salt of the hydroxy-acid, santoninic acid (II). Hence santonin is a lactone and its infrared spectrum showed it to be a γ-lactone. Santonin contains two double bonds (shown by catalytic hydrogenation) and behaves like an α,β-unsaturated ketone, the presence of this grouping being confirmed by its ultraviolet absorption spectrum (λ_{max} 236 nm, ε 11200).

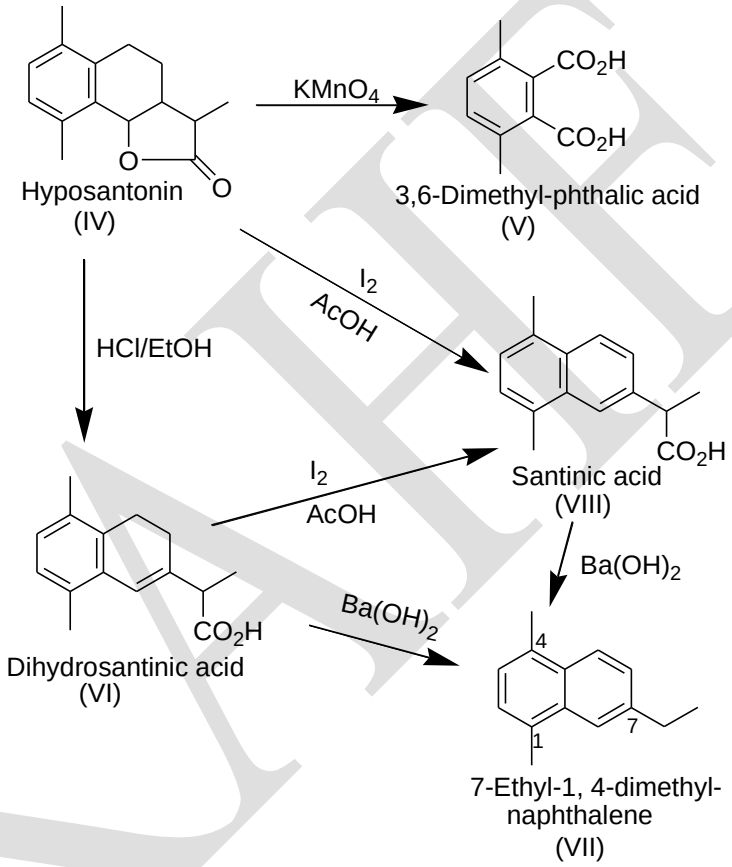


When distilled with zinc dust, santonin gives 1,4-dimethylnaphthalene, propene and a small amount of 1,4-dimethyl-2-naphthol. These products suggest the presence of the naphthalene skeleton. Reduction of santonin oxime produces the amine, santonamine (III) which, with nitrous acid, gives hyposantonin (IV). These reactions may be formulated as shown if we accept the structure of santonin as (I).

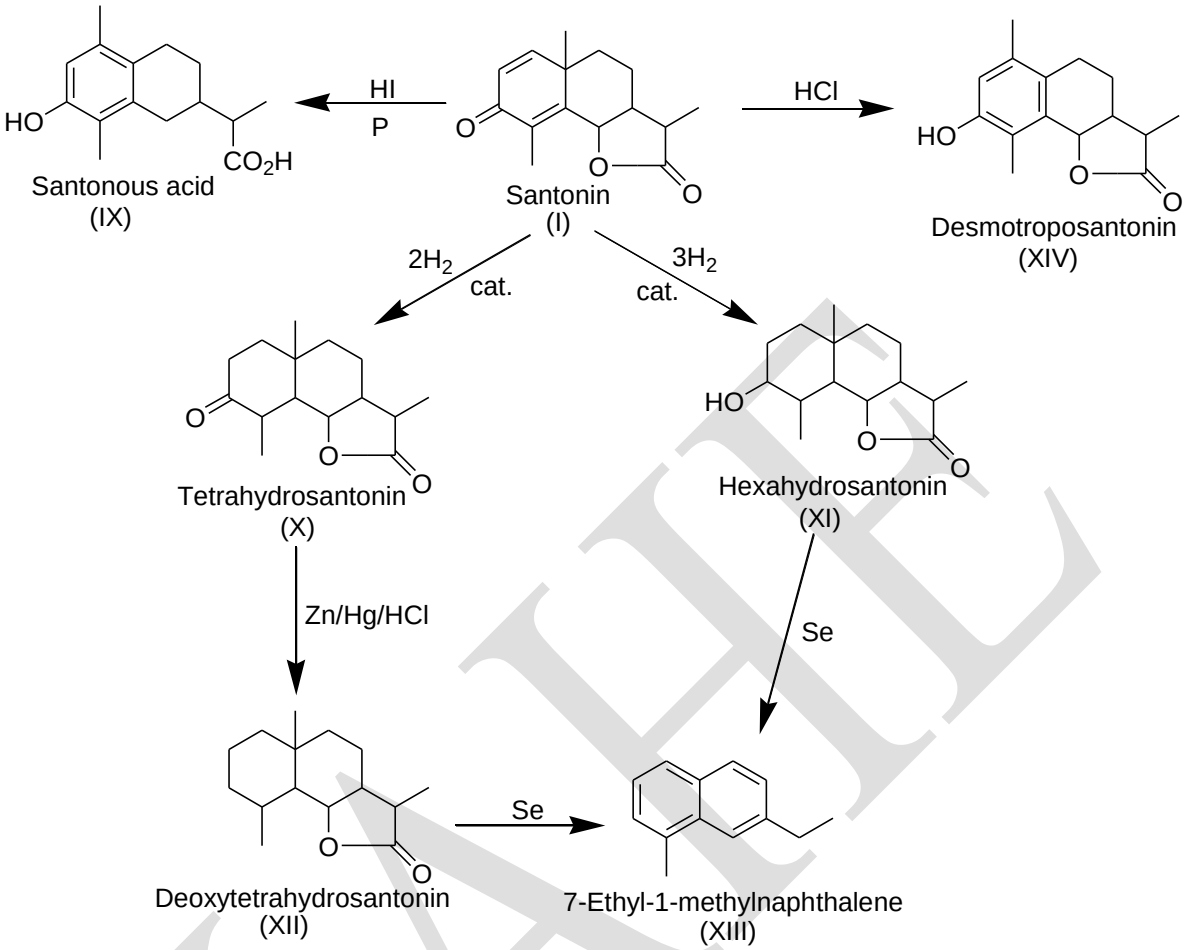


Inspection of the structure of hyposantonin (IV) shows that deamination is accompanied with rearrangement. It was because santonin undergoes facile rearrangements that its structure proved such a difficult problem. Hence it is not surprising that many incorrect structures were proposed for santonin.

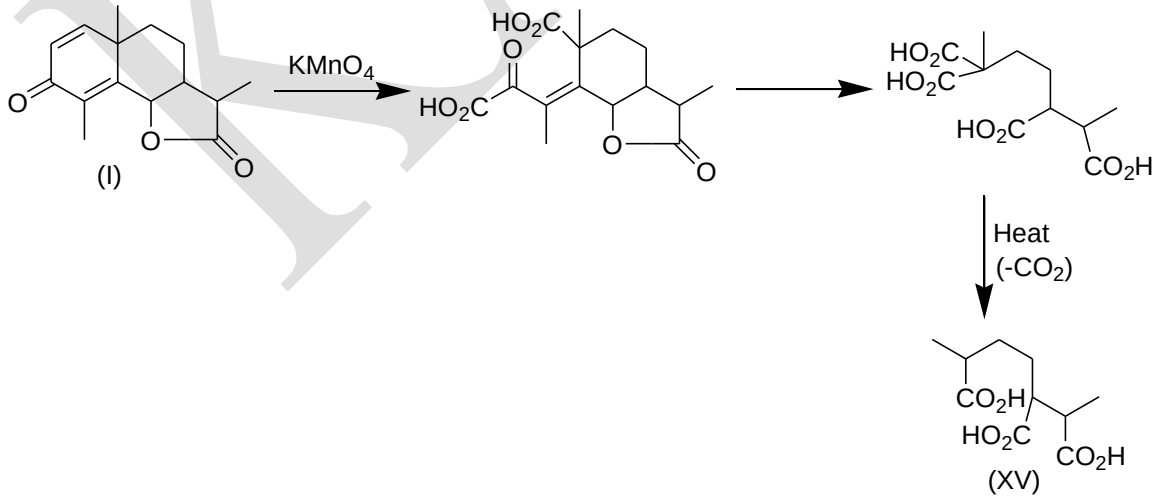
The structure of hyposantonin was elucidated as follows. Oxidation with permanganate gives 3,6-dimethyl-phthalic acid (V), and when heated with ethanolic hydrochloric acid hyposantonin gives a mixture of two isomeric acids, dihydrosantinic acid (VI), which, on heating with barium hydroxide, give the hydrocarbon (VII). Hyposantonin (or VI) on oxidation with iodine in acetic acid gives santinic acid (VIII) which, on heating with barium hydroxide, also gives (VII).



Other reactions carried on santonin (I) were the reduction (HI/P) to santonous acid (IX), catalytic reduction to tetrahydrosantonin (X) and to hexahydrosantonin (XI), (X) by means of the Clemmensen reduction, gave deoxytetrahydrosantonin (XII) and this, on distillation with selenium, gave 7-ethyl-1-methylnaphthalene (XIII), which was also obtained from (XI) by similar treatment. Also, on treatment with cold fuming hydrochloric acid, santonin underwent rearrangement to give desmotroposantonin (XIV).

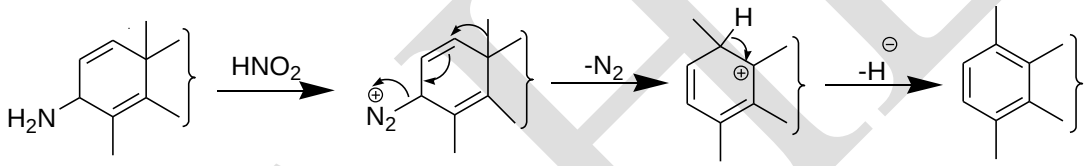


Another set of reactions carried out was the oxidation of santonin with permanganate to give (XV).



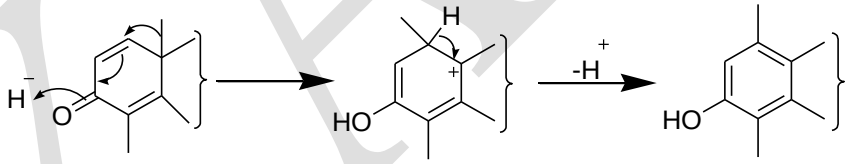
All the early structures proposed for santonin (I) placed the two methyl group in the same ring as the keto-group. In this case, santonin would be a tautomer of a phenol. Santonin, however, has no phenolic properties. Clemo *et al.* (1929, 1930), who carried out most of the foregoing reactions, were the first to propose the correct structure. They argued that if santonin is a sesquiterpenoid, then it could be assumed that it obeyed the isoprene rule. They confirmed this argument by the synthesis of santonous acid (IX) and showed the position of the angular methyl group by the synthesis of (XV). They also established the position of the ether oxygen atom in the lactone ring by the synthesis of desmotroposantonin (XIV). The structure of santonin has been confirmed by synthesis (see below).

The rearrangement of santonamine (III) into hyposantonin can now be explanied on the basis of a 1,2 shift as follows;



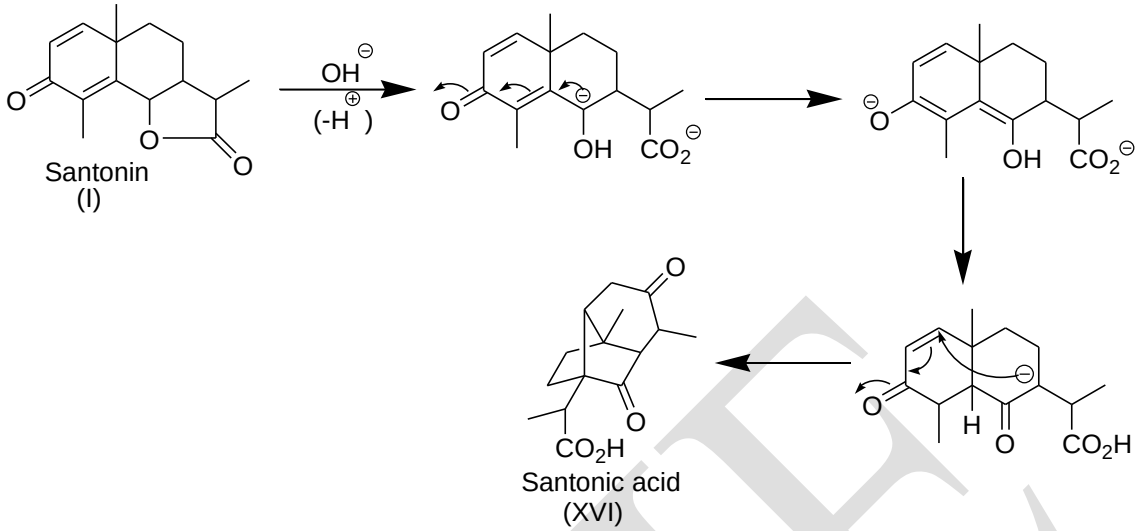
(III) (IV)

The conversion of santonin into desmotroposantonin (XIV) can be explained in a similar way.

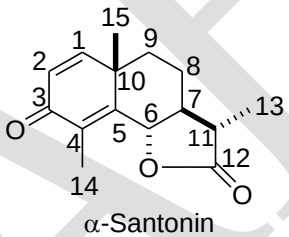


(I) (XIV)

Santonin undergoes many unusual transformations. Here we shall discuss only the conversion of santonin into santonic acid (XVI) by prolonged heating with barium hydroxide solution. Woodward *et al.* (1948) proposed the following mechanism, which involves an internal Michael condensation.

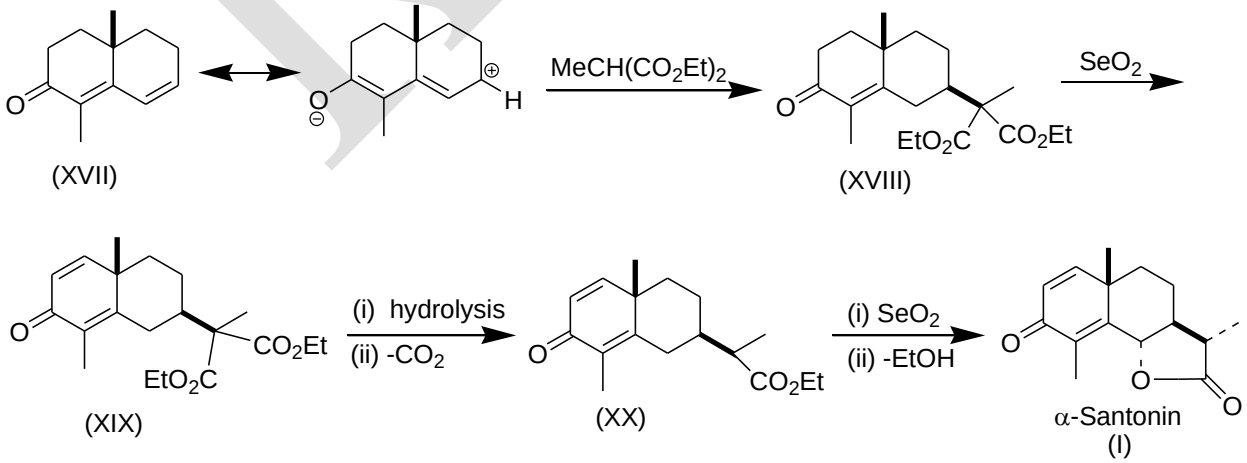


The stereochemistry of santonin has been the subject of extensive investigation, and its absolute configuration is an shown. This is α-santonin; β-santonin, which also occurs naturally, is the C-11 epimer.



Randall *et al.* (1972) have shown that the ^{13}C chemical shifts in α-and β-santonin provide a simple method of determining the stereochemistry of the lactone ring fusion and also the configuration of the methyl group at C-11.

Natural α-and β-santonin have been synthesised, e.g., (Abe *et al.*, 1956).

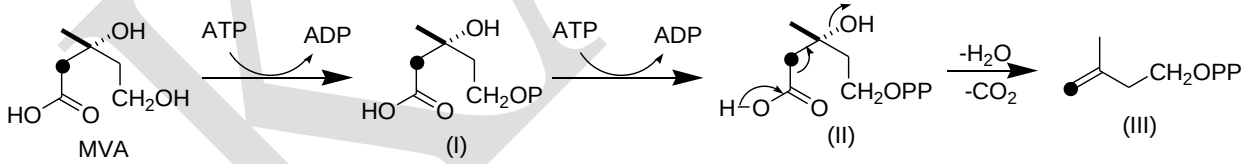


The Michael addition is stereospecific, the malonic ester group taking the more stable equatorial position in (XVIII). Decarboxylation to give (XX) results in the formation of two racemic acids (XX; α-and β). These were separated; the α-acid led to (±)-α–santonin and the β-acid to (±)-β–santonin on oxidation and lactonisation. Since the lactone ring is fused *trans* (e, e; see above stereochemical formula) the selenium dioxide oxidation results in the formation of an equatorial hydroxyl group. Resolution of (±)-(XX) [α-and β] *via* the brucine salts, followed by the same treatment as before, gave the following products: (-) α-and (-) β-(XX) gave respectively (+) α-and (+) β-(XX) gave respectively nature (-) α –and (-) β-santonin.

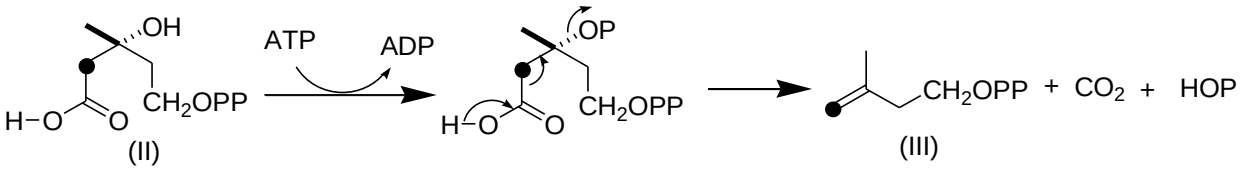
Biosynthesis of terpenoids

The biosynthesis of terpenoids can be subdivided into three definite steps: (i) the formation of a biological isopentane unit from acetate; (ii) the condensation of this unit to form acyclic terpenoids; (iii) the conversion of acyclic into cyclic terpenoids.

Since mevalonic acid (MVA) contains six carbon atoms, one must be lost to form the isopentane unit. By starting with labelled MVA (2-^{14}C), it has been shown that it is the carboxyl group in MVA which is lost. The steps involved in this transformation are believed to be as shown.

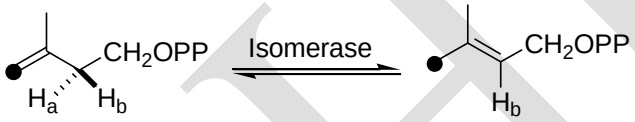


Phosphorylation of MVA first produces MVA 5-phosphate (I) (P = PO_3H_2), and this is followed by a second phosphorylation to give MVA 5–pyrophosphate (II) (PP =$P_2O_6H_3$). (II) now loses a molecule of water to form 3-methylbut-3-enyl (isopentenyl) pyrophosphate (III). The details of this conversion are uncertain, but there is reason to believe it might be (note the *trans* elimination):



(II) (III)

Evidence for this comes the fact that one mole of ATP is converted into one mole of ADP, and one mole of inorganic phosphate is produced. However, the 3-phosphate has not yet been isolated. Thus, the biogenetic isoprene unit is 3-methylbut-3-enyl pyrophosphate, but its participation in the biosynthesis of terpenoids involves its equilibration, in the presence of the appropriate enzyme, with 3-methylbut-2-enyl (β, β-dimethylallyl) pyrophosphate (IV). This isomerisation is stereospecific, H_a being the proton that is eliminated. Also, the newly formed Me group is *trans* to the CH_2OPP group.

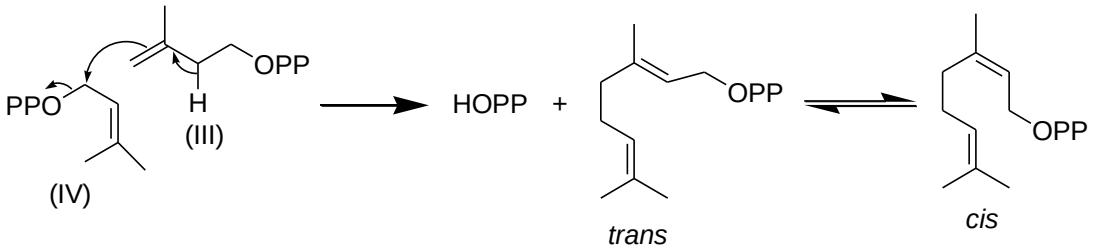


(III) (IV)

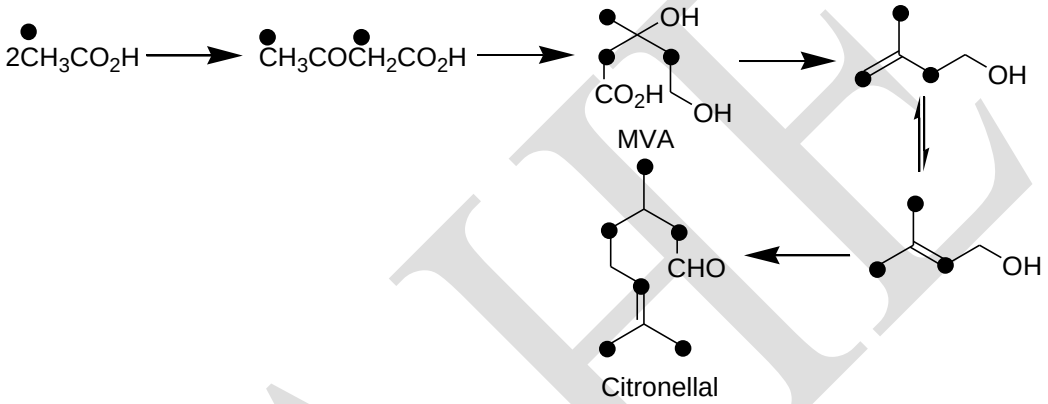
On the basis of the biosynthetic studies carried out, another isoprene rule (in addition to the isoprene and special isoprene rules) has been formulated. This is known as the *biogenetic isoprene rule*, and states that members of the isopentane group should be derivable from simple hypothetical precursors such as geraniol, farnesol and squalene. The biogenetic isoprene rule also includes compounds that originated from regular isoprenoid precursors which, by rearrangement or degradation, give products that no longer obey the isoprene rule, e.g., gibberellins.

The monoterpenoids

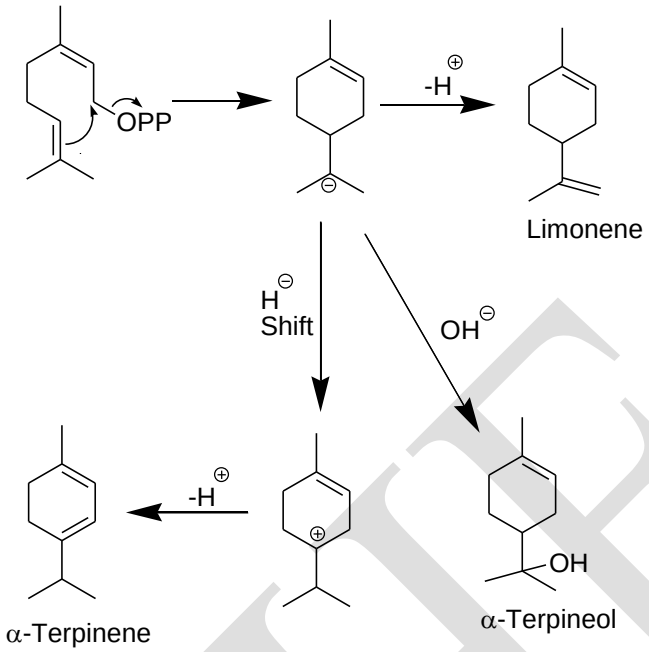
All the experimental evidence supports the view that units (III) and (IV) combine to form geranyl pyrophosphate (*trans* isomer), (III) acting as the nucleophilic reagent and (IV) as the electrophilic reagent (to give head to tail union). The steps involved are not yet clear (an enzyme may be involved as an intermediate complex). The reaction is therefore shown in its simplest form:



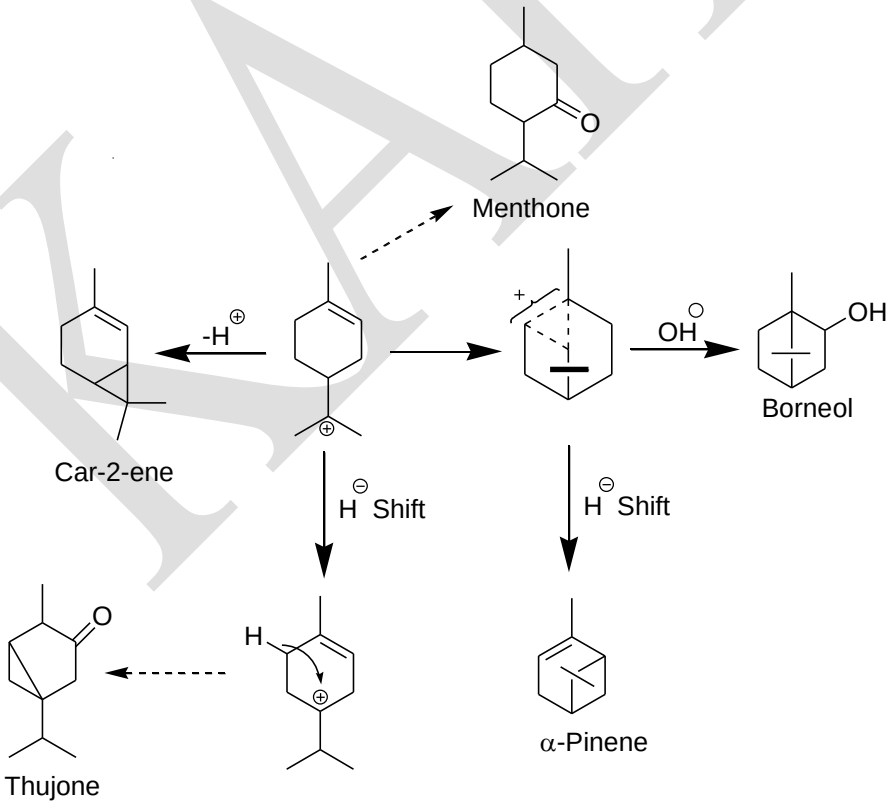
This route (and *via* MVA) is supported by the fact that biosynthetic experiments with labelled acetate lead to citronellal labelled in accord with the acetate-MVA pathway.



Geranyl pyrophosphate now serves as the precursor for the monocyclic monoterpenoids *via* the *cis*-isomer (nerol). The mechanisms involved in ring-closure are not certain, but a favoured one is *via* ionic intermediates (see the acid-catalysed cyclisation of geraniol into α-terpineol); e.g.,



It is then reasonable to extend these arguments to the formation of bicyclic monoterpenoids, e.g.,



There is however, some evidence obtained from biosynthetic experiments that is not in accord with the labelling of the products based on the mechanisms given.

Text Books:

1. Finar, I. L. (2013). *Organic Chemistry Vol. II: Stereochemistry and the Chemistry of Natural Products* (V Edition). New Delhi: Pearson Education, Ltd.

2. Chatwal, G. R. (2015). *Organic Chemistry of Natural Products Vol. II*. New Delhi: Himalaya Publishing House.

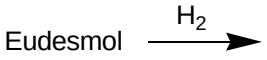
POSSIBLE QUESTIONS

PART- A – Multiple Choice Questions
(Each Question Carry One Mark) (Online Examinations)

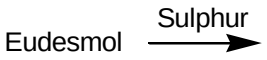
PART-B (Each Question Carry Two Marks)

1. Write the structures of three isomers of Eudesmol.
2. Explain how ultraviolet spectroscopy is useful in terpenoid chemistry?
3. Draw the structures of Zingiberene, Eudesmol, Abietic acid and Caryophyllene.
4. Write the structure of the expected product in each of the following reactions.

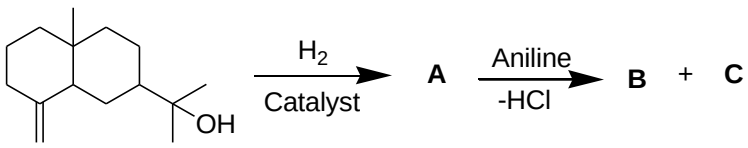
(i)



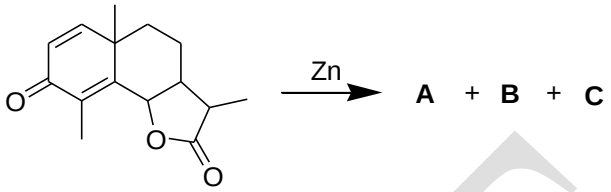
(ii)



5. Identify the products in the following reactions.



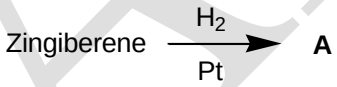
6. Explain the synthesis of retene?
7. Why abietic acid is very difficult to esterify?
8. Write the structure of the expected products in the following reaction?



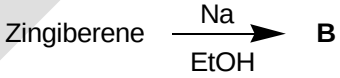
PART-C (Each Question Carry Six Marks)

1. (i) How are terpenes classified?
   (ii) Elucidate the structure of Zingiberene.
2. (i) Explain the biosynthesis of monoterpenoids.
   (ii) Explain the conversion of Santonin into santonic acid.
3. (i) Explain the isolation of monoterpenoids and sesquiterpenoids.
   (ii) Identify the products **A**, **B**, **C**, **D** & **E** in the following transformations.

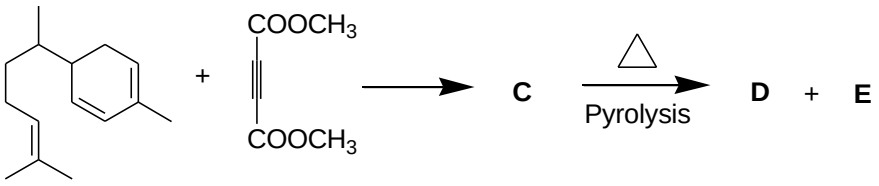
(a)



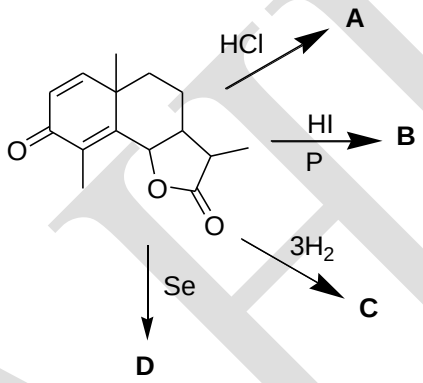
(b)



(c)



4. Explain the structural elucidation and synthesis of Eudesmol.
5. (i) Write the stereospecific synthesis of (±) dehydroabiteic acid.
   (ii) Outline the synthesis of Eudalene.
6. Explain the general methods of determining structure of Terpenoids.
7. (i) Outline the synthesis of Caryophyllene.
   (ii) Explain the position of double bond in Abietic acid.
8. Explain the structural elucidation and synthesis of Santonin.
9. Identify the products **A**, **B**, **C** & **D** in the following transformations.

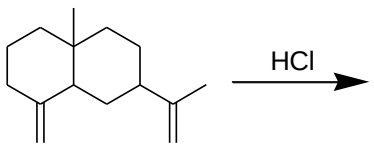


10. How will you determine the position of the carboxylic acid group and position of angular methyl group in Abietic acid?

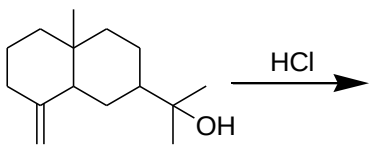
PART-D (Each Question Carry Ten Marks)

1. (a) Write the structure of the expected product in each of the following reactions.

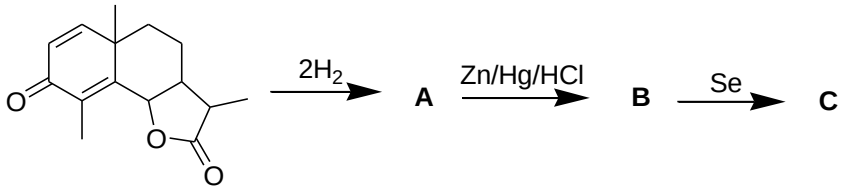
(i)



(ii)



(b) Identify the products **A**, **B** and **C** in the following transformations.

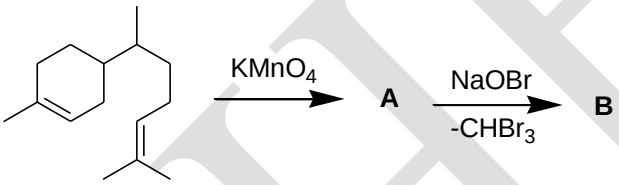


(c) Explain the synthesis of Zingiberene.

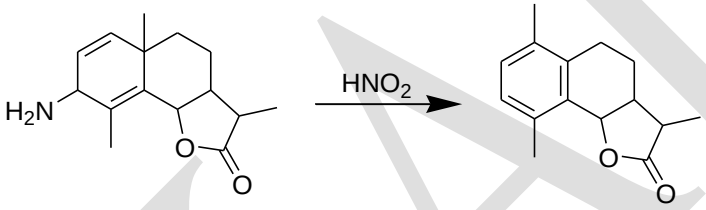
2. (i) Write the structures of three isomers of Eudesmol.

(ii) Draw the structures of Zingiberene, Eudesmol, Abietic acid and Caryophyllene.

(iii) Identify the compounds **A** and **B** in the following transformations.



(iv) Write the mechanism of the following transformation?



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DEPARTMENT OF CHEMISTRY

UNIT-I

TERPENOIDS

PART-A–Multiple Choice Questions

(Each Question Carry One Mark) (Online Examinations)

1. The molecular formula for zingiberene is

a) $C_{14}H_{30}$ b) $C_{15}H_{26}$ **c) $C_{15}H_{24}$** d) $C_{20}H_{40}$

2. Isoprene units have the general molecular formula

a) $C_{10}H_{16}$ **b) C_5H_8** c) C_5H_{16} d) $C_{10}H_8$

3. Ultraviolet spectroscopy mainly used in terpenoid chemistry

a) detecting of functional group b) detecting of non-functional group

c) detecting of conjugation d) detecting of non-conjugation

4. Catalytic hydrogenation of zingiberene yields

a) dihydozingiberene **b) hexahydrozingiberne**

c) trihydrozingiberene d) tetrahydrozingiberene

5. The number of double bonds present in zingiberene is

a) 2 b) 1 c) 5 **d) 3**

6. The λ_{max} value of zingiberene is

a) 253 nm b) 230 nm **c) 260 nm** d) 214 nm

7. The molecular formula for sesquiterpenoids is

a) $C_{10}H_{16}$ b) C_5H_8 c) C_5H_{16} **d) $C_{15}H_{24}$**

8. Ozonolysis of zingiberene gives

a) laevulic acid b) acetic acid c) bromoform d) ketodicarboxylic acid

9. Zingiberene can be reduced by means of sodium in ethanol to gives

a) dihydozingiberene b) hexahydrozingiberne

c) trihydrozingiberene d) tetrahydrozingiberene

10. The acyclic chiral centre of zingiberene is stereochemically related to that in

a) (+)–citronellal b) (-)-α-phellandrene c) (-)–citronellal d) (+)-α-phellandrene

11. Santonin on prolonged heating with barium hydroxide gives

a) santonous acid b) santinic acid c) santoninic acid **d) santonic acid**

12. How many chiral centers present in zingiberene

a) 1 b) 3 **c) 2** d) 5

13. $C_{30}H_{48}$ represents as

a) monoterpenoids **b) triterpenoids** c) diterpenoids d) sesquiterpenoids

14. Adsorption in purified fats method is also known as

a) enfleurage b) distillation c) extraction d) expression

15. Zingiberene forms an adduct with

a) acetic anhydride b) propionic anhydride c) phthalic anhydride **d) maleic anhydride**

16. Eudesmol behaves as a

a) tertiary alcohol b) primary alcohol c) secondary alcohol d) ditertiary alcohol

17. Eudalene behaved as an

a) aliphatic compound **b) aromatic compound**

c) heterocyclic compound d) unsaturated compound

18. The molecular formula for abietic acid is

a) $C_{30}H_{20}O_2$ b) $C_{20}H_{20}O_3$ c) $C_{30}H_{20}O_3$ **d) $C_{20}H_{30}O_2$**

19. Abietic acid when warmed with concentrated sulphuric acid evolves

a) CO b) CO_2 c) SO_2 d) H_2

20. Catalytic hydrogenation of abietic acid gives

a) dihydroabietic acid **b) tetrahydroabietic acid** c) retene d) biphenyl

21. The parent hydrocarbon of abietic acid in the form of

a) C_nH_{2n} b) C_nH_{2n-2} **c) C_nH_{2n-4}** d) C_nH_{2n-6}

22. Abietic acid is an

a) monocyclic compound b) bicyclic compound **c) tricyclic compound**

d) tetracyclic compound

23. Sulphur dehydrogenation of Abietic acid gives

a) cadalene b) bisabolene c) homoretene **d) retene**

24. $C_{18}H_{18}$ represents as

a) retene b) bisabolene c) homoretene d) cadalene

25. Retene when oxidized with CrO_3 yields

a) biphenyl **b) retenequinone** c) flurenone d) phenanthrene

26. Zingiberene occurs in

a) eucalyptus oil b) oil of cloves **c) ginger oil** d) various species of Artemesia

27. Zingiberene is an

a) bicyclic sesquiterpenoids b) tricyclic sesquiterpenoids

c) tetracyclic sesquiterpenoids **d) monocyclic sesquiterpenoids**

28. The molecular formula for santonin is

a) $C_{15}H_{20}O_3$ b) $C_{15}H_{28}O_3$ **c) $C_{15}H_{18}O_3$** d) $C_{20}H_{20}O_3$

29. Santonin dissolves in alkali to form the salt of the hydroxy acid is known as

a) santonic acid b) santinic acid c) santonous acid **d) santoninic acid**

30. The λ_{max} value for santonin is

a) 236 nm b) 246 nm c) 230 nm d) 270 nm

31. The number of double bonds present in santonin is

a) 1 **b) 2** c) 5 d) 3

32. Reaction of santonamine with nitrous acid gives

a) santonic acid b) santoninic acid **c) hyposantonin** d) santinic acid

33. Hyposantonin on oxidation with iodine in acetic acid gives

a) santoninic acid b) santonic acid c) santonous acid **d) santinic acid**

34. Santonin on treatment with fuming HCl gives

a) desmotroposantonin b) santonic acid c) santinic acid d) santonous acid

35. The molecular formula for caryophyllene is

a) $C_{15}H_{20}$ **b) $C_{15}H_{24}$** c) $C_{18}H_{20}$ d) $C_{15}H_{28}$

36. Catalytic hydrogenation of caryophyllene gives

a) caryophyllenic acid b) norcaryophyllenic acid **c) tetrahydrocaryophyllene**

d) dihydrocaryophyllene

37. The molecular formula for norcaryophyllenic acid is

a) $C_9H_{14}O$ b) $C_{15}H_{24}O$ c) $C_{15}H_{28}$ **d) $C_8H_{12}O_4$**

38. Abietic acid when methylated yields

a) methyl abietate b) abitenol c) methyl abietin d) homoretene

39. The λ_{max} value for abietic acid is

a) 230 nm **b) 238 nm** c) 256 nm d) 265 nm

40. Molecular formula for eudesmol is

a) $C_{20}H_{14}O$ b) $C_{15}H_{28}O$ **c) $C_{15}H_{26}O$** d) $C_{15}H_{12}O_4$

41. Catalytic hydrogenation of eudesmol gives

a) eudalene b) tetrahydoeudesmol c) trihydroeudesmol **d) dihydroeudesmol**

42. Eudesmol is an

a) bicyclic compound b) monocyclic compound

c) tricyclic compound d) tetracyclic compound

43. Sulphur dehydrogenation of eudesmol gives

a) cadalene b) retene c) apocadalene **d) eudalene**

44. The molecular formula for eudalene is

a) $C_{15}H_{20}$ b) $C_{15}H_{24}$ **c) $C_{14}H_{16}$** d) $C_{15}H_{28}$

45. β-Eudesmol has the

a) β-selinene configuration b) α- selinene configuration

c) trans-decalin configuration d) cis–decalin configuration

46. The ring fusion in caryophyllene is

a) cis configuration b) cis-decalin configuration

c) trans-decalin configuration **d) trans configuration**

47. The ring fusion in isocaryophyllene is

a) cis configuration b) cis-decalin configuration

c) trans configuration d) trans-decalin configuration

48. The endocyclic double bond in caryophyllene is

a) cis configuration b) cis-decalin configuration

c) trans-decalin configuration **d) trans configuration**

49. The endocyclic double bond in isocaryophellene is

a) cis configuration b) cis-decalin configuration

c) trans-decalin configuration d) trans configuration

50. The α-isomer of caryophyllene structure identical with

a) isocaryophyllene **b) humulene** c) tetrahydrocaryophyllene d) caryophyllenic acid

51. 1-methyl-7-isopropylnaphthalene is known as

a) cadalene b) apocadalene **c) eudalene** d) eudesmol

52. Selenium dehydrogenation of hexahydrosantonin yields

a) cadalene b) apocadalene c) 1-methyl-4-isopropylnaphthalene

d) 7-ethyl-1-methylnapthalene

53. Clemmensen reduction of tetrahydrosantonin yields

a) deoxytetrahydrosantonin b) desmotroposantonin

c) 1-methyl-4-isopropylnaphthalene d) 7-ethyl-1-methylnaphthalene

54. The oxidation of hyposantonin with permanganate gives

a) desmotroposantonin **b) 3,6-dimethyl phthalic acid**

c) deoxytetrahydrosantonin d) santinic acid

55. Hyposantonin when heated with ethanolic hydrochloric acid gives

a) desmotroposantonin b) 3,6-dimethyl phthalic acid

c) deoxytetrahydrosantonin **d) dihydrosantinic acid**

56. Abietic acid is very difficult to esterify because of

a) carboxyl group attached to a tertiary carbon atom b) carboxyl group attached to a secondary carbon atom c) carboxyl group attached to a primary carbon atom d) carboxyl group attached to a quaternary carbon atom

57. 1-Ethyl-7-isopropylphenanthrene is known as

a) cadalene b) apocadalene **c) homoretene** d) retene

58. Sulphur distillation of methylabietin gives

a) abietinol b) methyl abietate c) retene **d) homoretene**

59. The conversion of abietinol to methylabietin is an example for

a) Wanger-Meerwein rearrangement b) Fries rearrangement

c) Benzilic acid rearrangement d) Wolff rearrangement

60. β-isomer of caryophyllene is also called as

a) humulene **b) caryophyllene** c) isocaryophyllene d) tetrahydrocaryophyllene

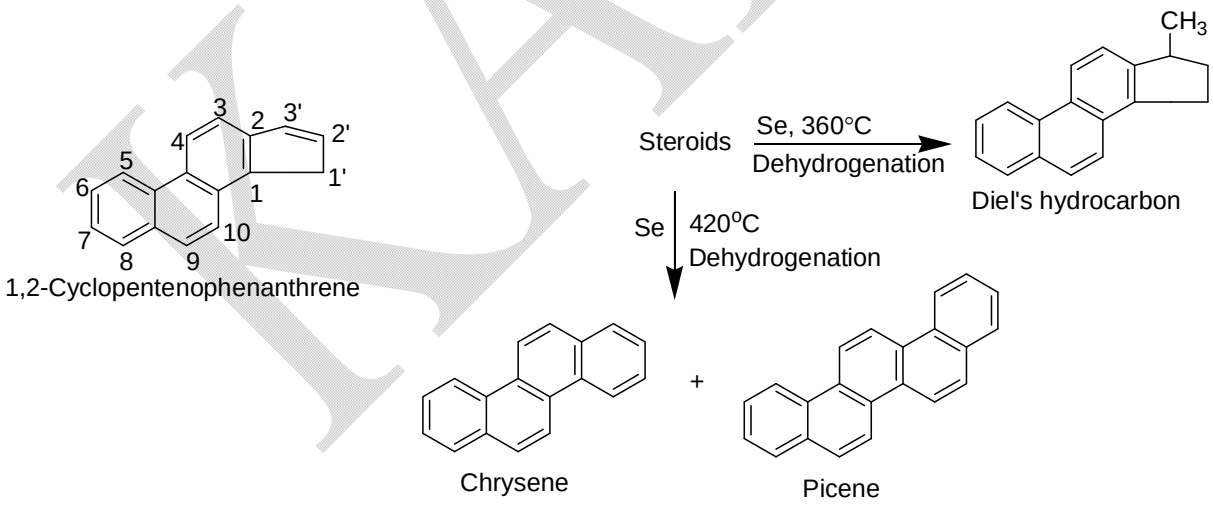
UNIT-II

SYLLABUS

Steroids: Introduction – structural elucidation and synthesis of Cholesterol (synthesis not necessary), Ergosterol, Vitamin D, Equilenin, Oestrone, Testosterone and Progesterone. Bile acids – biosynthesis of sterols.

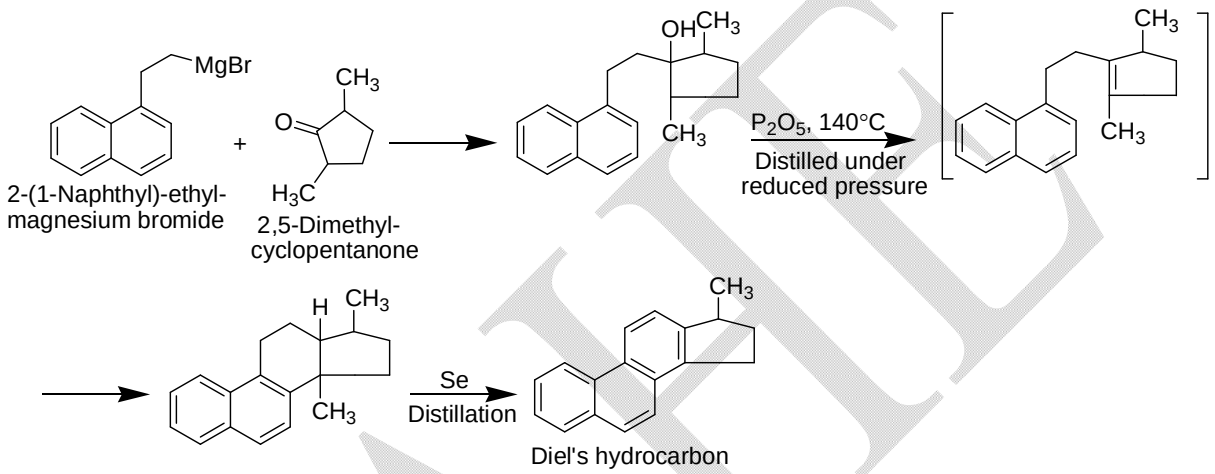
Introduction

The steroids form a group of structurally related compounds which are widely distributed in animals and plants. Included in the steroids are the sterols (from which the name steroids is derived), vitamin D, the bile acids, a number of sex hormones, the adrenal cortex hormones, some carcinogenic hydrocarbons, certain sapogenins, etc. the structures of the steroids are based on the 1,2-cyclopentenophenanthrene skeleton (Rosenheim and King, 1932; Wieland and Dane, 1932). All the steroids give, among other products, Diels' hydrocarbon on dehydrogenation with selenium at 360°C (Diels, 1927). In fact, a steroid could be defined as and compound which give Diel's hydrocarbon when distilled with selenium. When the distillation with selenium is carried out at 420°C, the steroids give mainly chrysene and a small amount of picene.



In the earlier work, the various steroids were designated by trivial names, but the tendency now is to discard these in favour of systematic names, which may be applied when the structure is known.

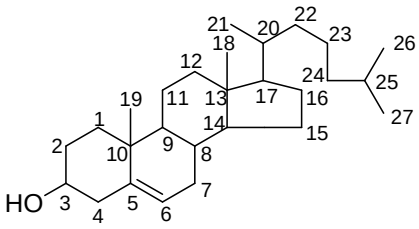
Diels hydrocarbon is a solid, m.p.126-127°C. Its molecular formula is $C_{18}H_{16}$, and the results of oxidation experiments, X-ray crystal analysis and absorption spectrum measurements showed that the hydrocarbon is probably 3'-methyl-1,2-cyclopentenophenanthrene. This structure was definitely established by synthesis, e.g., that of Harper, Kon and Ruzicka (1934), who used the Bogert-Cook method, starting from 2-(1-naphthyl)-ethylmagnesium bromide and 2,5-dimethylcyclopentanone.



Sterols

Sterols occur in animal and plant oils and fats. They are crystalline compounds, and contain an alcoholic group; they occur free or as esters of the higher fatty acids, and are isolated from the unsaponifiable portion of oils and fats. Cholesterol, 5α-cholestan-3β-ol (cholestanol) and 5β-cholestan-3β-ol (coprostanol) are the animal sterols; ergosterol and stigmasterol are the principal plants sterols. The sterols that are obtained from animal sources are often referred to as the *zoosterols*, and those obtained from plant sources as the *phytosterols*. A third group of sterols, which are obtained from yeast and fungi, are referred to as the *mycosterols*. This classification, however, is not rigid, since some sterols are obtained from more than one of these groups.

Cholesterol



Cholesterol

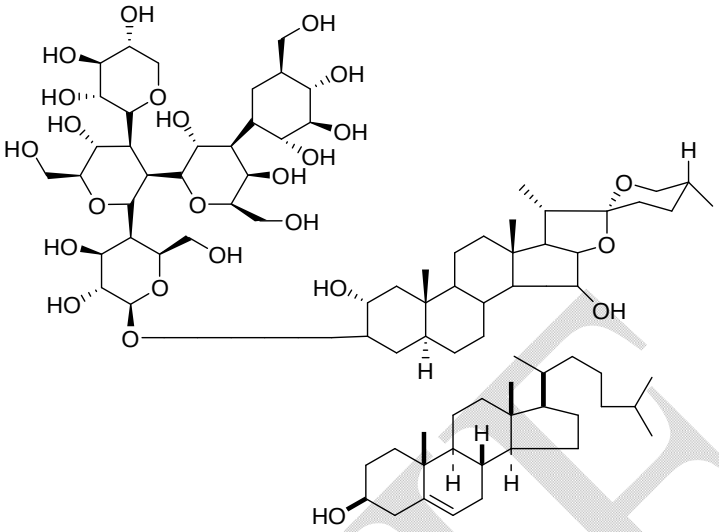
Molecular formula: $C_{27}H_{46}O$, m.p. 149°C. This is the sterol of the higher animals, occurring free or as fatty esters in all animal cells, particularly in the brain and spinal cord. Cholesterol was first isolated from human gallstones (these consist almost entirely of cholesterol). The main sources of cholesterol are the fish-liver oils and the brain and spinal cord of cattle. Lanoline, the fat from wool, is a mixture of cholesteryl palmitate, stearate and oleate.

Cholesterol is a white crystalline solid which is optically active, ($[\alpha]_D$ 39°). Cholesterol (and other sterols) gives many colour reactions, e.g.,

(i) **The Salkowski reaction (1908)**. When concentrated sulphuric acid is added to a solution of cholesterol in chloroform, a red colour is produced in the chloroform layer.

(ii) **The Liebermann-Burchard reaction (1885, 1890)**. A greenish colour is developed when a solution of cholesterol in chloroform is treated with concentrated sulphuric acid and acetic anhydride.

When an ethanolic solution of cholesterol is treated with an ethanolic solution of digitonin, a large white precipitate of cholesterol digitonide is formed. This is a molecular complex containing one molecule of cholesterol and one digitonin, from which the components may be recovered by dissolving the complex in pyridine (which brings about complete dissociation) and then adding ether (the cholesterol remains in solution and the digitonin is precipitated). An alternative method is to dissolve the digitonide in dimethyl sulphoxide and heat on a steam bath. Dissociation occurs, and on cooling only the sterol is precipitated (Issidorides *et al.*, 1962). Digitonide formation is used for the estimation of cholesterol. An interesting point in this connection is that 3β-hydroxysteriods usually form complexes with digitonin, whereas the corresponding 3α-compounds do not.



Cholesterol digitonide

Isolation of cholesterol:

Source of cholesterol + Ethanol

↓

Ethanolic solution of cholesterol + Ethanolic solution of digitonin

↓

White precipitate of cholesterol digitonide

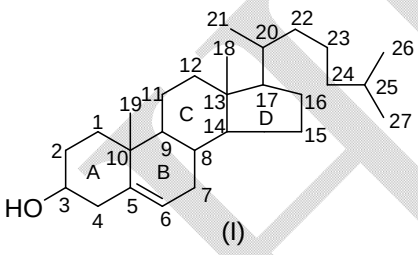
Pyridine / Dimethyl sulfoxide/Heating

Breakdown of cholesterol and digitonin (Pyridine) → Ether → Ether solution + Digitonin precipitate

Ether solution → Evaporation → Cholesterol

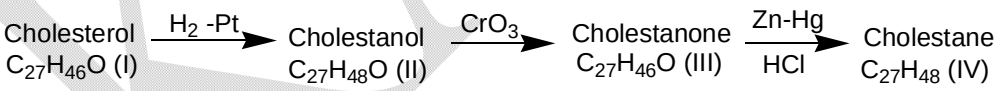
Breakdown of cholesterol and digitonin (Dimethyl sulfoxide/Heating) → Cooling → Cholesterol is deposited

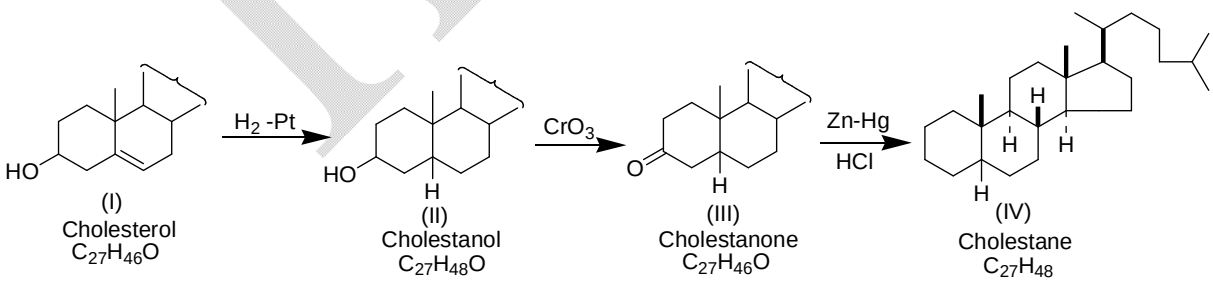
The structure of cholesterol was elucidated only after a tremendous amount of work was done, particularly by Wieland, Windaus and their coworkers (1903-1932). Only a very bare outline is given here, and in order to appreciate the evidence that is going to be described, it is necessary to have the established structure of cholesterol at the beginning of our discussion. (I) is the structure of cholesterol and shows the method of numbering. The molecule consists of a side-chain and a nucleus which is composed of four rings; these rings are usually designated A, B, C and D (or (I), (II), (III) and (IV), beginning from the six-membered ring on the left (see also (iii) below). It should be noted that the nucleus contains two angular methyl groups, one at C-10 and the other at C-13.



(i) Structure of the ring system. Under this heading we shall deal with the nature of the ring system present in cholesterol; the problem of the angular methyl groups is dealt with later [see (iv)].

The usual tests for functional groups showed that cholesterol contains one double bond and one hydroxyl group. Now let us consider the following set of reactions.

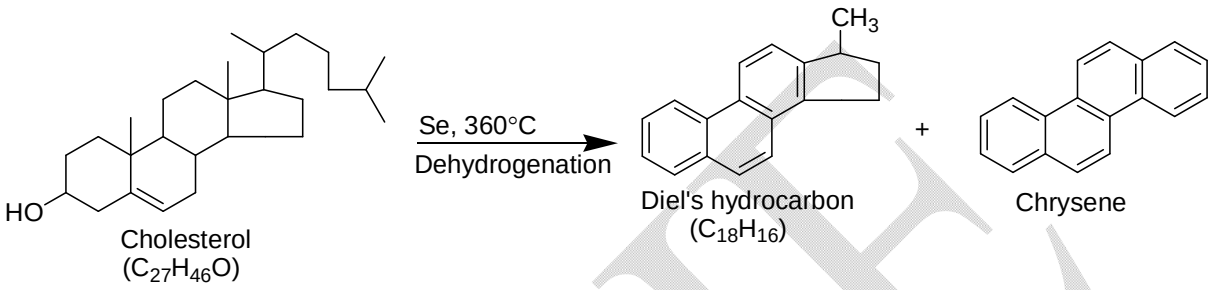




The conversion of cholesterol into cholesterol (II) shows the presence of one double bond in (I) and the oxidation (II) to the ketone cholestanone (III) shows that cholesterol is a secondary

alcohol. Cholestane (IV) is a saturated hydrocarbon, and corresponds to the general formula C_nH_{2n-6}, and consequently is tetracyclic; thus cholesterol is tetracyclic. [D.B.E. of cholestane is 27 + 1 – 48/2 = 4].

When cholesterol is distilled with selenium at 360°C, Diel's hydrocarbon is obtained.



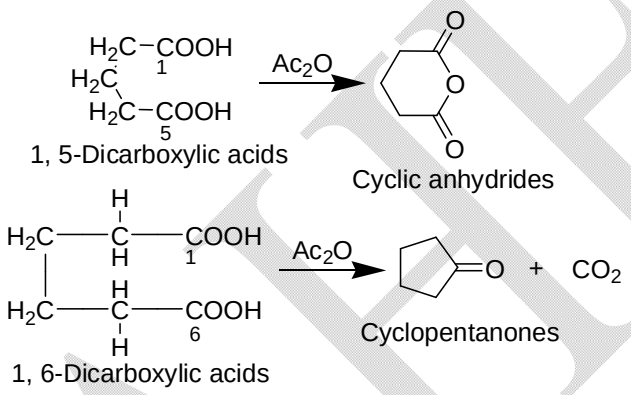
The formation of this compound could be explained by assuming that this nucleus is present in cholesterol. The yield of this hydrocarbon, however, is always poor and other products are always formed at the same time, particularly chrysene. Thus, on the basis of this dehydrogenation, the presence of the cyclopentenophenanthrene nucleus must be accepted with reserve. Rosenheim and King (1932) thought that chrysene was the normal product of the selenium dehydrogenation, and so proposed (on this basis and also on some information obtained from X-ray analysis work of Bernal, 1932) that the steroids contained the chrysene skeleton. Within a few months, however, Rosenheim and King (1932) modified this suggestion, as did also Wieland and Dane (1932). These two groups of workers proposed that the cyclopentenophenanthrene nucleus is the one present in cholesterol (i.e., in steroids in general). This structure fits far better all the evidence that has been obtained from a detailed investigation of the oxidation products of the sterols and bile acids and has now been confirmed by the synthesis of cholesterol.

(a) The nature of the nucleus in sterols and bile acids was shown to be the same, since 5β-cholanic acid (cholanic acid) or 5α-cholanic acid (allocholanic acid) is one of the oxidation products.

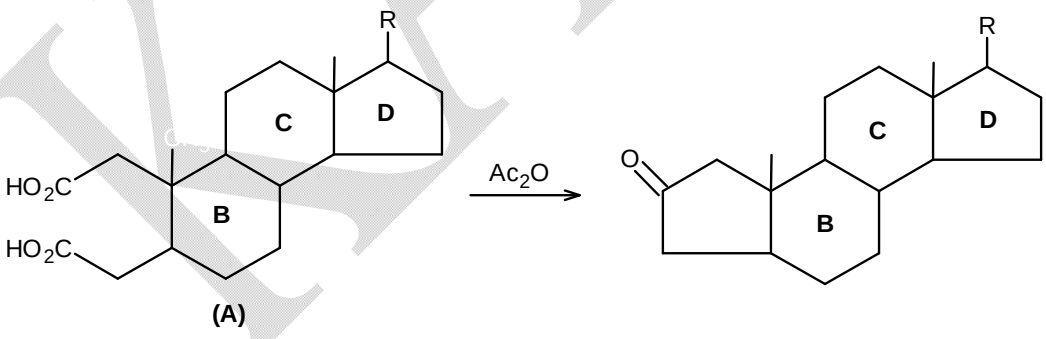
(b) The oxidation of the bile acids led to the formation of products in which various rings were opened. The examination of these products showed that the positions of the hydroxyl

groups were limited mainly to three positions 3, 7 and 12 and further work showed that the hydroxyl groups behaved differently towards a given reagent.

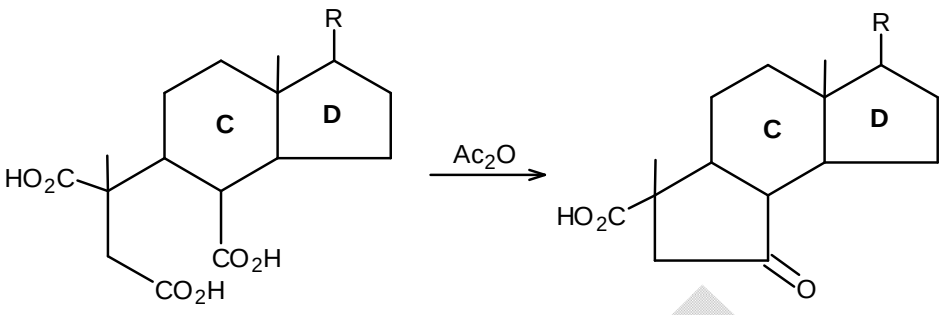
(c) The rings in the steroid nucleus were opened to give a dicarboxylic acid and the relative positions of the two carboxyl groups with respect to each other were determined by the application of Blanc's rule: on heating with acetic anhydride, 1,5-dicarboxylic acids form cyclic anhydrides, and 1,6-dicarboxylic acids form cyclopentanones with elimination of carbon dioxide.



Ring A. Cholesterol and the cholic acids were converted into the dicarboxylic acid (A) which gave a cyclopentanone, and so ring A is six-membered (R is the appropriate side-chain).

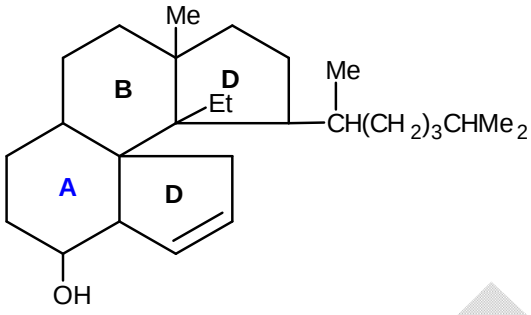


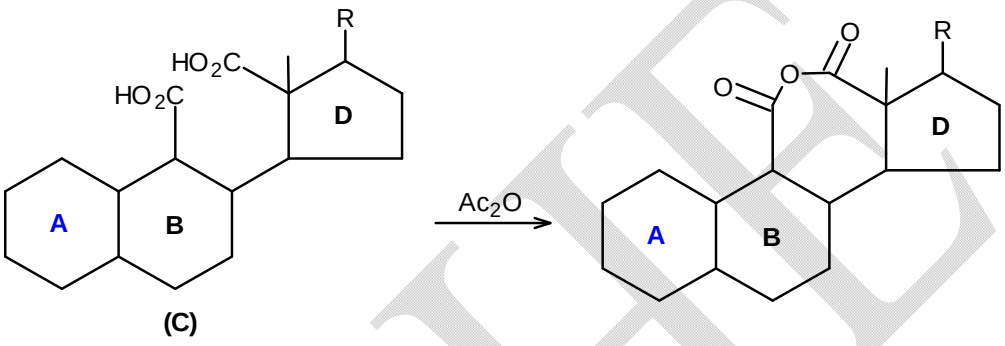
Ring B. Cholesterol was converted into the tricarboxylic acid (B) which gave the cyclopentanone derivative shown. Hence ring **B** is six-membered.



(B)

Ring C. Deoxycholic acid was converted into a dicarboxylic acid which gave a cyclic anhydride. It was therefore assumed that ring C was five-membered, and this led Windaus and Wieland (1928) to propose the following formula for cholesterol, and the uncertain point (at that time) was the nature of the two extra carbon atoms these were assumed to be present as an ethyl group at position 10, but Wieland *et al.* (1930) finally proved that there was no ethyl group at this position. These two 'homeless' carbon atoms were not placed until Rosenheim and King first proposed that steroids contained the chrysene nucleus and then proposed the cyclopentenophenanthrene nucleus. Bernal (1932) also showed, from the X-ray analysis of cholesterol, ergosterol, etc., that the molecule was thin, whereas the above structure for the steroid nucleus would be rather thick.

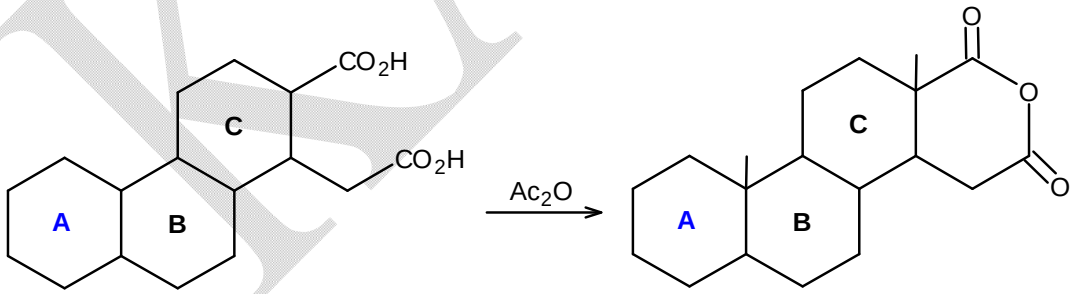




(C)

If we use the correct structure of cholesterol, the cyclisation reaction results in the formation of a **seven-membered cyclic anhydride**. Thus, in this case (and in some others), the Blanc rule fails and leads to erroneous conclusions.

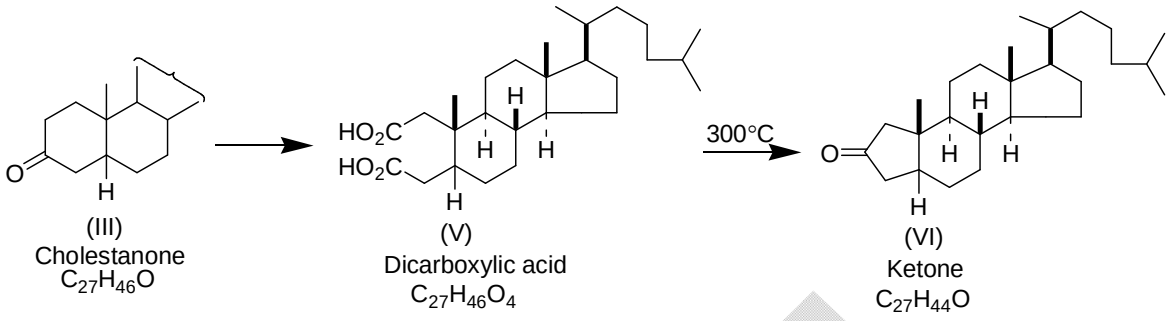
Ring D. 5β-cholestane (Coprostane) was converted into etiobilianic acid (see (iii), below) and this gave a cyclic anhydride. Hence ring **D** is five-membered.



(D)

(ii) Positions of hydroxyl group and double bond. Let us consider the following reactions:

Cholestanone $C_{27}H_{46}O$ (III) $\xrightarrow{HNO_3}$ Dicarboxylic acid $C_{27}H_{46}O_4$ (V) $\xrightarrow{300°C}$ Ketone $C_{27}H_{44}O$ (VI)

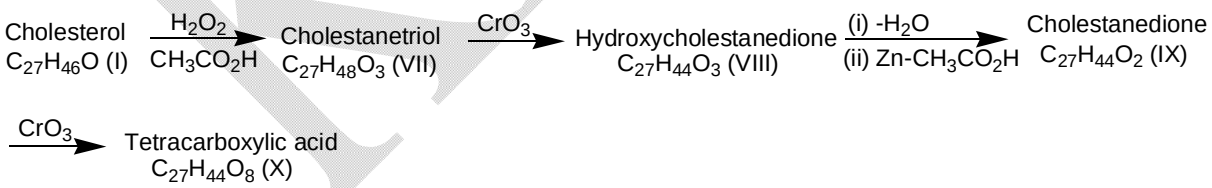


(III) Cholestanone $C_{27}H_{46}O$ — (V) Dicarboxylic acid $C_{27}H_{46}O_4$ — (VI) Ketone $C_{27}H_{44}O$

Since the dicarboxylic acid (V) contains the same number of carbon atoms as the ketone (III) from which it is derived, the keto group in (III) must therefore be in a ring. Also, since pyrolysis of the dicarboxylic acid (V) produces a ketone with the loss of one carbon atom, it therefore follows from Blanc's rule that (V) is either a 1,6- or 1,7-dicarboxylic acid. Now we have seen that the nucleus contains three six-membered rings and one five-membered ring. Thus the dicarboxylic acid (V) must be obtained by the opening of ring A, B or C and consequently it follows that the hydroxyl group in cholesterol (which was converted into the keto group in cholestanone; see (i) above) is in ring A, B or C.

Actually two isomeric dicarboxylic acids are obtained when cholestanone is oxidised. The formation of these two acids indicates that the keto group in cholestanone is flanked on either side by a methylene group, i.e., group $–CH_2COCH_2–$ is present in cholestanone. Examination of the reference structure (I) of cholesterol shows that such an arrangement is possible only if the hydroxyl group is in ring A.

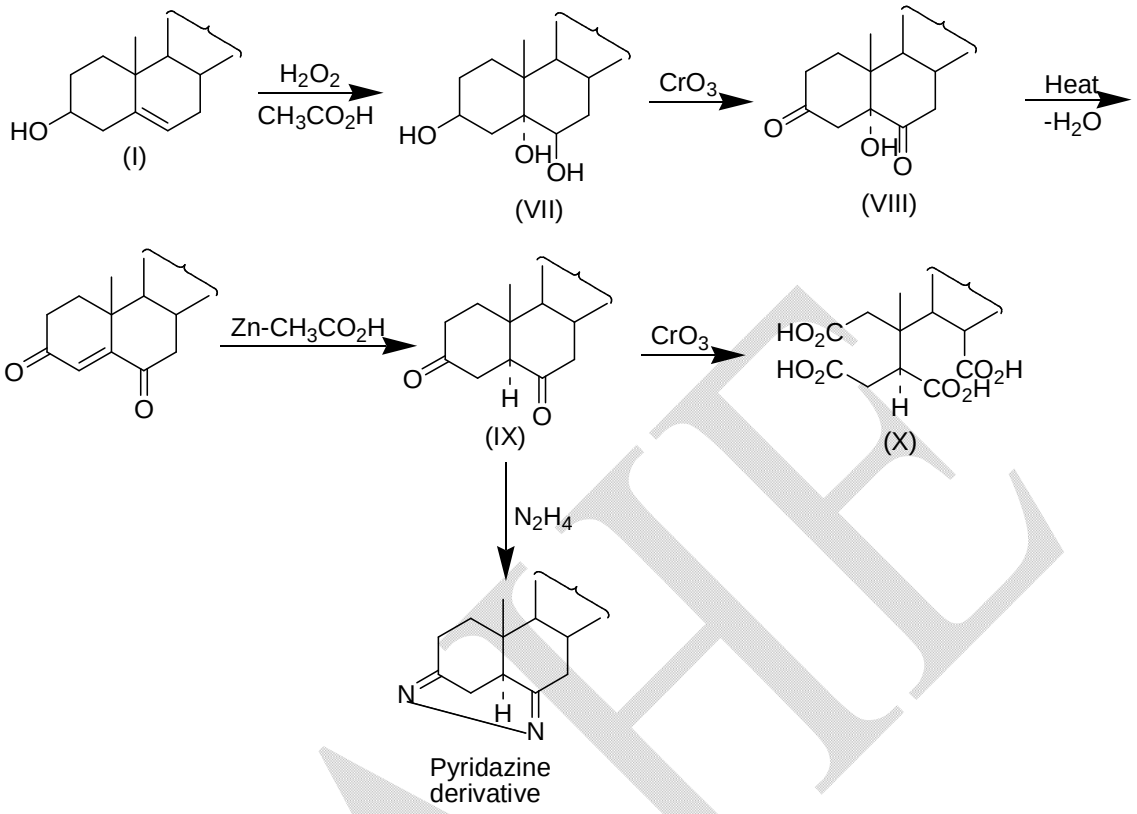
Now let us consider the further set of reactions:



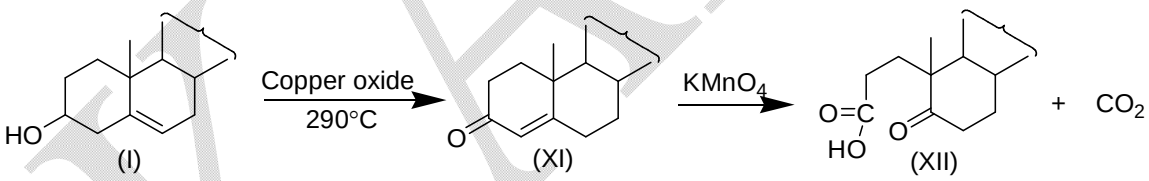
In the conversion of (I) into (VII), the double bond in (I) is hydroxylated. Since only two of the three hydroxyl groups in (VII) are oxidised to produce (VIII), these two groups are secondary alcoholic groups (one of these being the secondary alcoholic group in cholesterol), and the third, being resistant to oxidation, is probably a tertiary alcoholic group. Dehydration of (VIII) (by heating in *vacuo*) and subsequent reduction of the double bond forms (IX), and this,

on oxidation, gives a tetracarboxylic acid **without loss of carbon atoms**. Thus the two keto groups in (IX) must be in different rings; had they been in the same ring, then carbon would have been lost and (X) not obtained. It therefore follows that the hydroxyl group and double bond in cholesterol must be in different rings. Furthermore, since (IX) form a pyridazine derivative with hydrazine, (IX) is a γ-diketone. Since we have already tentatively placed the hydroxyl group in ring A, the above reaction can be readily explained if we place the hydroxyl group at position 3, and the double bond between 5 and 6. In the following equations only ring A and B are drawn; this is an accepted convention of focusing attention on any part of the steroid molecule that is under consideration (also note that full lines represent groups lying above the plane, and broken lines group lying below the plane). Noller (1939) has shown that the pyridazine derivative is a polymer, and so the interpretation that (IX) is a γ-diketone is rendered uncertain. Supporting evidence, however, for the above interpretation is afforded by the fact that when cholesterol is heated with copper oxide at 290°C, cholestenone (XI) is produced and this on oxidation with permanganate forms a keto-acid (XII) with the loss of one carbon atom. The formation of (XII) indicates that the keto group and the double bond in cholestenone are in the same ring. The ultraviolet absorption spectrum of cholestenone, λ_{max} 240 nm, shows that the keto group and the double bond are conjugated (Menschick *et al.*, 1932). These results can be explained if we assume that the double bond in cholesterol migrates in the formation of cholestenone, the simplest explanation being that the hydroxyl group is in position 3 and the double bond between 5 and 6, position 5 being common to both rings A and B. Thus:

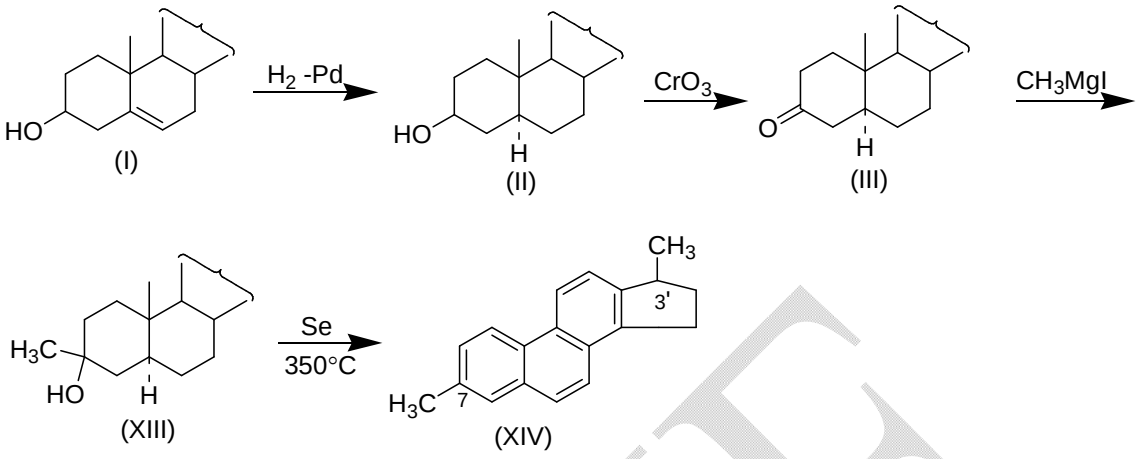
(i)



(ii)

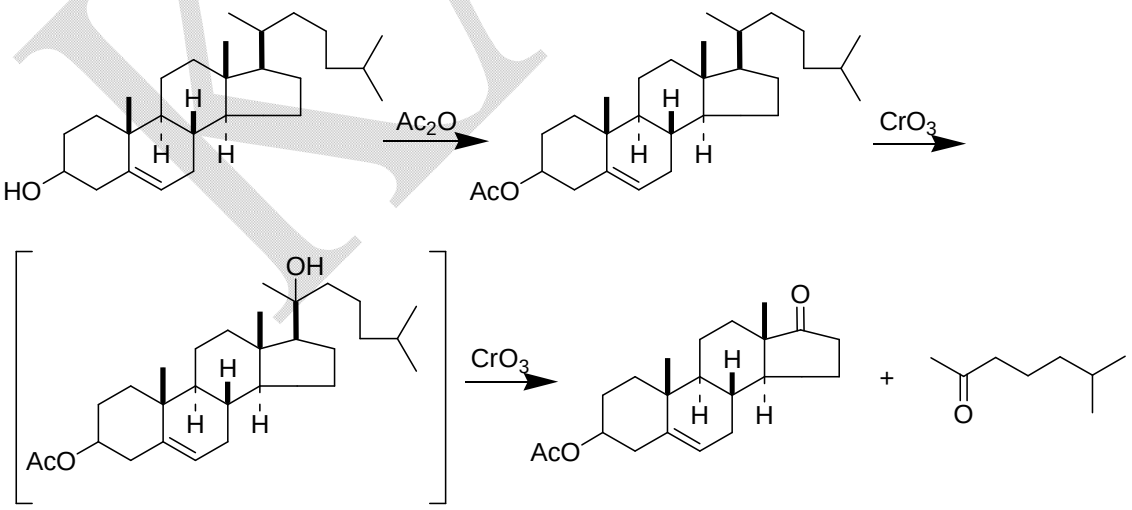


The position of the hydroxyl group at position 3 is definitely proved by the experiments of Kon *et al.* (1937, 1939). These authors reduced cholesterol (I) to cholestanol (II), oxidised this to cholestanone (III), treated this with methylmagnesium iodide and dehydrogenated the product, a tertiary alcohol (XIII), to 3′,7-dimethyl-cyclopentenophenanthrene (XIV) by means of selenium. The structure of (XIV) was proved by synthesis, and so the reactions may be formulated as follows, with the hydroxyl at position 3.



The stereochemistry of the various reactions given above is discussed.

(iii) Nature and position of the side-chain. Acetylation of cholesterol produces cholesteryl acetate and this, on oxidation with chromium trioxide, forms a steam-volatile ketone and the acetate of a hydroxyketone (which is not steam volatile). The ketone was shown to be isohexyl methyl ketone, $CH_3CO(CH_2)_3\,CH(CH_3)_2$. Thus this ketone is the side-chain of cholesterol, the point of attachment of the side-chain being at the carbon of the keto group. These results do not show where the side-chain is attached to the nucleus of cholesterol, but if we accept that the position is at 17, then we may formulate the reactions as follows:



The nature of the side-chain has also been shown by the application of the Barbier-Wieland degradation. Since this method also leads to evidence that shows which ring of the nucleus is attached to the side-chain, we shall consider the problem of the nature of the side-chain again.

The Barbier-Wieland degradation offers a means of 'stepping down' an acid one carbon atom at a time as follows:

$$RCH_2CO_2H \xrightarrow[HCl]{CH_3OH} RCH_2CO_2CH_3 \xrightarrow{2C_6H_5MgBr} RCH_2C(OH)(C_6H_5)_2 \xrightarrow{-H_2O} RCH{=}C(C_6H_5)_2$$

$$\xrightarrow{CrO_3} RCO_2H + (C_6H_5)_2CO$$

Methylmagnesium bromide may be used instead of phenylmagnesium bromide, and the alcohol so obtained may be directly oxidised:

$$RCH_2C(OH)(CH_3)_2 \xrightarrow{CrO_3} RCO_2H + (CH_3)_2CO$$

In the following account, only phenylmagnesium bromide will be used to demonstrate the application of the method to the steroids.

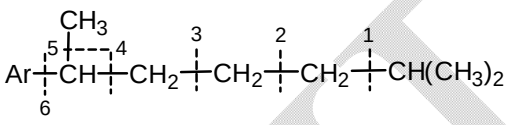
Cholesterol was first converted into 5β-cholestane (coprostane). If we represent the nucleus of 5β-cholestane as Ar and the side-chain as C_n, then we may formulate the degradation of 5β-cholestane as follows (B-W represents a Barbier-Wieland degradation):

$$\underset{Ar-C_n}{\text{5β-Cholestane}} \xrightarrow{CrO_3} CH_3COCH_3 + \underset{Ar-C_{n-3}}{\text{5β-Cholanic acid}} \xrightarrow{B\text{-}W} (C_6H_5)_2CO + \underset{Ar-C_{n-4}}{\text{Nor-5β-Cholanic acid}} \xrightarrow{B\text{-}W}$$

$$(C_6H_5)_2CO + \underset{Ar-C_{n-5}}{\text{Bisnor-5β-Cholanic acid}} \xrightarrow{B\text{-}W} (C_6H_5)_2CO + \underset{Ar-C_{n-6}}{\text{Etiocholyl methyl ketone}} \xrightarrow{CrO_3} \underset{Ar-C_{n-7}}{\text{5β-Etianic acid}}$$

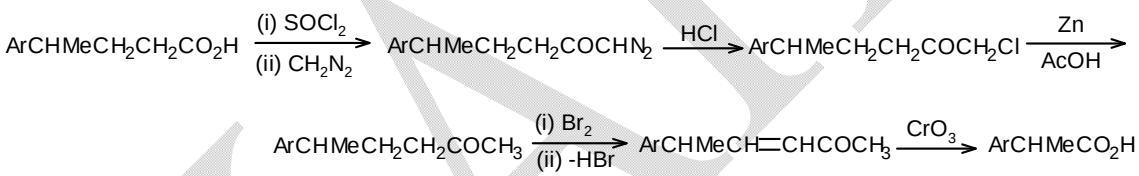
The formation of acetone from 5β-cholestane indicates that the side-chain terminates in an isopropyl group. The conversion of bisnor-5β-cholanic acid into a ketone shows that there is an alkyl group on the α-carbon atom in the former compound. Furthermore, since the ketone is oxidised to 5β-etianic acid (formerly known as aetiocholanic acid) with the loss of one carbon atom, the ketone must be a methyl ketone, and so the alkyl group on the α-carbon atom in bisnor-5β-cholanic acid is a methyl group.

Now the carboxyl group in etianic acid is directly attached to the nucleus; this is shown by the following fact. When etianic acid is subjected to one more Barbier-Wieland degradation, a ketone, etiocholanone, is obtained and this, on oxidation with nitric acid, gives a dicarboxylic acid, etiobilianic acid, without loss of any carbon atoms. Thus etiocholanone must be a cyclic ketone, and so it follows that there are eight carbon atoms in the side-chain, which must have the following structure in order to account for the foregoing degradations (see also the end of this section (iii):

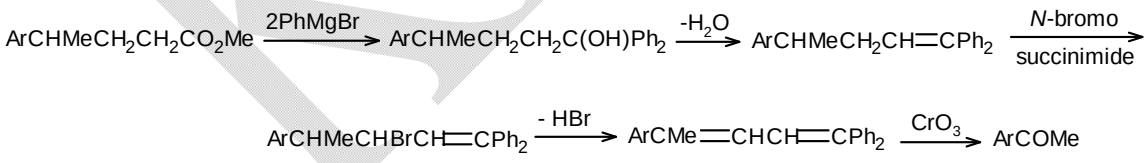


In addition to the Barbier-Wieland degradation, there are also other methods for degrading the side-chain.

(i) Gallagher *et al.* (1946) have introduced a method to eliminate two carbon atoms at a time:



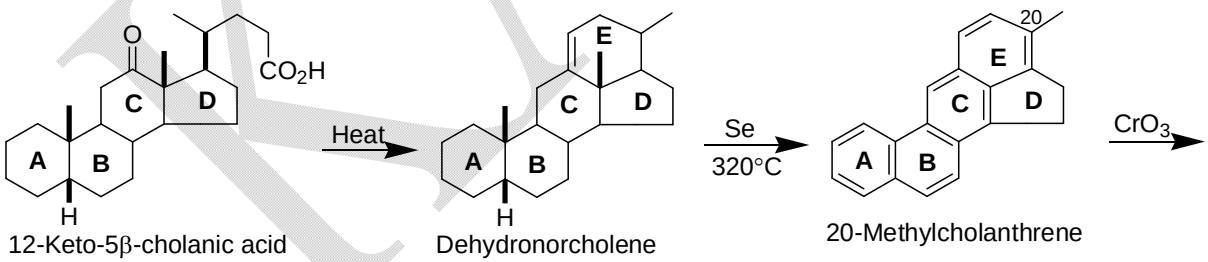
(ii) Miescher *et al.* (1944) have introduced a method to eliminate three carbon atoms at a time:



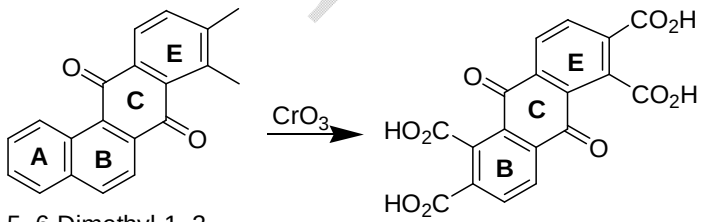
(iii) Jones *et al.* (1958) have carried out the fission of a steroid side-chain with an acid catalyst and have then subjected volatile products to chromatography. This method has been used with as little as 30 mg of material

The problem now is: where is the position of this side-chain? This is partly answered by the following observation. The dicarboxylic acid, etiobilianic acid, forms an anhydride when

heated with acetic anhydride. Thus the ketone (etiocholanone) is probably a five-membered ring ketone (in accordance with Blanc's rule) and therefore the side-chain is attached to the five-membered ring D. The actual point of attachment to this ring, however, is not shown by this work. The formation of Diel's hydrocarbon from cholesterol suggests that the side-chain is at position 17, since selenium dehydrogenations may degrade a side-chain to a methyl group. Position 17 is also supported by evidence obtained from X-ray photographs and surface film measurements. Finally, the following chemical evidence may be cited to show that the position of the side-chain is 17. As we have been above, 5β-cholanic acid may be obtained by the oxidation of 5β-cholestane. 5β-Cholanic acid may also be obtained by the oxidation of deoxycholic acid (a bile acid) followed by a Clemmensen reduction. Thus the side-chains in cholesterol and deoxycholic acid are in the same position. Now deoxycholic acid can also be converted into 12-keto-5β-cholanic acid which, on heating to 320°C, loses water and carbon dioxide to form dehydronorcholene (Wieland *et al.*, 1930). This, when distilled with selenium, forms 20-methylcholanthrene, the structure of which is indicted by its oxidation to 5, 6-dimethyl-1, 2-benzanthraquinone which, in turn, gives on further oxidation, anthraquinone-1,2,5,6-tetracarboxylic acid (Cook, 1933). Finally, the structure of 20-methylcholanthrene has been confirmed by synthesis. The foregoing facts can be explained only if the side-chain in cholesterol in position 17; thus:



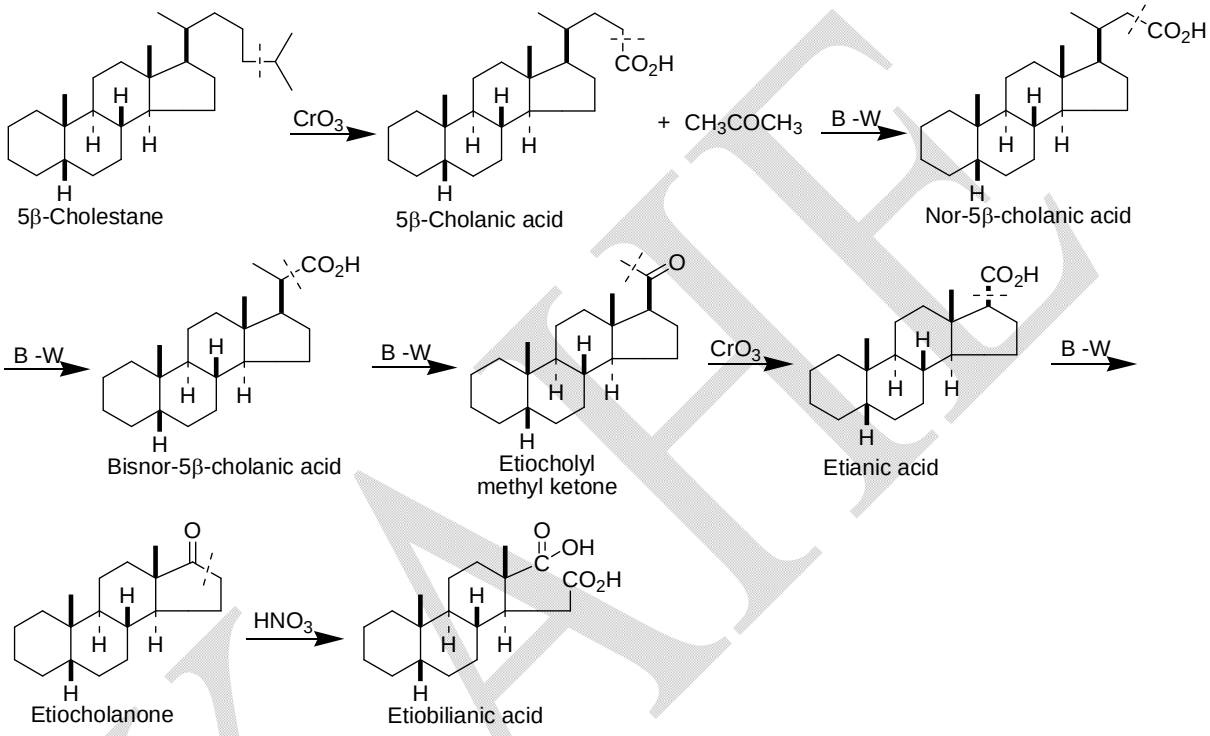
12-Keto-5β-cholanic acid Dehydronorcholene 20-Methylcholanthrene



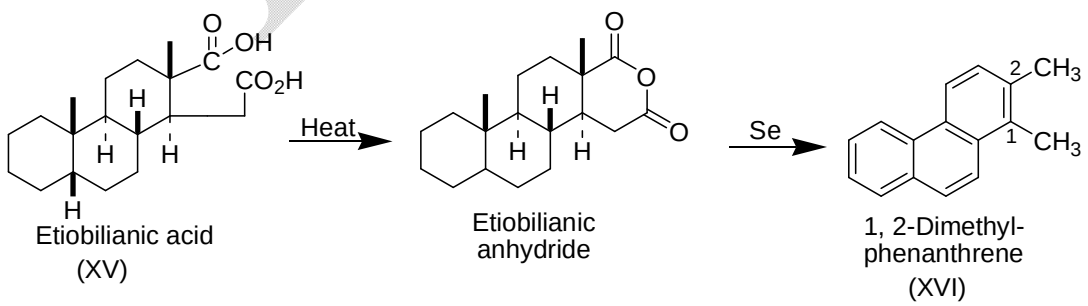
5, 6-Dimethyl-1, 2-benzanthraquinone Anthraquinone-1, 2, 5,6-tetracarboxylic acid

It should be noted that the isolation of methylcholanthrene affords additional evidence for the presence of the cyclopentenophenanthrene nucleus in cholesterol.

Thus, now that we know the nature and position of the side-chain, we can formulate the conversion of 5β-cholestane into etiobilianic acid as follows:

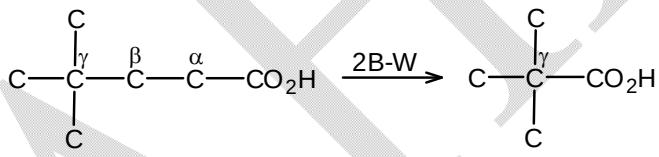


A point of interest in this connection is that when the anhydride of etiobilianic acid is distilled with selenium, 1,2-dimethylphenanthrene is obtained (Butenandt *et al.*, 1933). This also provides proof for the presence of the phenanthrene nucleus in cholesterol, and also evidence for the position of the C-13 angular methyl group (see iv).

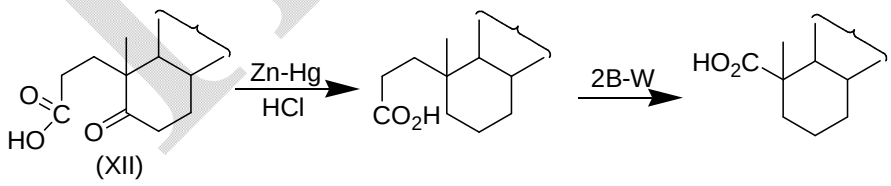


(iv) Positions of the two angular methyl groups. The cyclopentenophenanthrene nucleus of cholesterol accounts for seventeen carbon atoms and the side-chain for eight. Thus twenty-five carbon atoms in all have accounted for, but since the molecular formula of cholesterol is $C_{27}H_{46}O$, two more carbon atoms must be fitted into the structure. These two carbon atoms have been shown to be angular methyl groups.

In elucidating the positions of the hydroxyl group and double bond, one of the compounds obtained was the keto-acid (XII). This compound, when subjected to the Clemmensen reduction and followed by two Barbier-Wieland degradations, gives an acid which is very difficult to esterify, and evolves carbon monoxide when warmed with concentrated sulphuric acid (Tschesche, 1932). Since these reactions are characteristic of an acid containing a carboxyl group attached to a tertiary carbon atom, the side-chain in (XII) must be of the type.

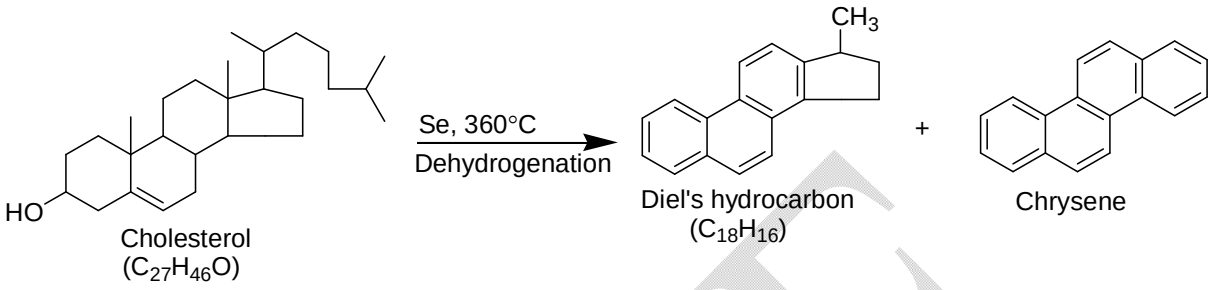


Thus there must be an alkyl group at position 10 in (XII). This could be an ethyl group (as originally believed by Windaus and Wieland) or a methyl group, provided that in the latter case the second 'missing' carbon atom can be accounted for. As we shall see later, there is also a methyl group at position 13 and so the alkyl group at position 10 must be a methyl group. On this basis, the degradation of (XII) may be formulated.

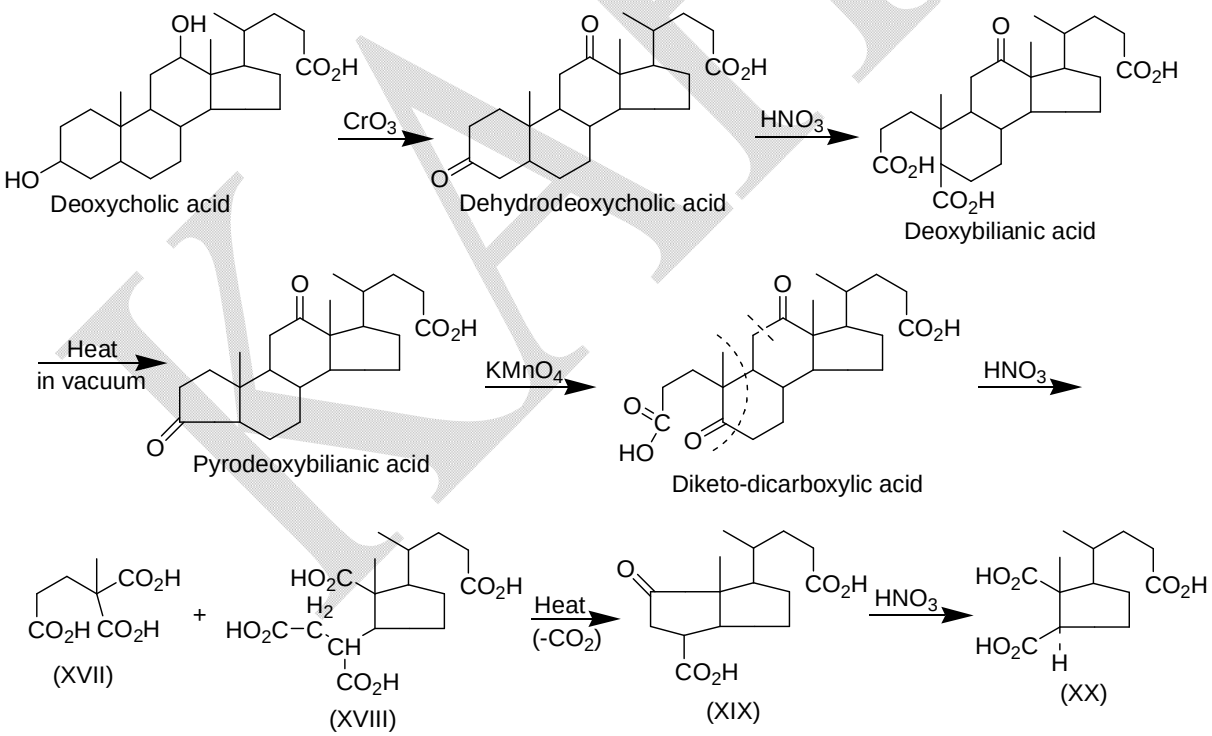


The position of the other angular methyl group is indicated by the following evidence. When cholesterol is distilled with selenium, chrysene is obtained as well as Diel's hydrocarbon. How, then, is the former produced if the latter is the ring skeleton of cholesterol? One possible explanation is that there is an angular methyl group at position 13, and on selenium

dehydrogenation, this methyl group enters the five-membered ring D to form a six-membered ring; thus:



This evidence, however, is not conclusive, since ring expansion could have taken place had the angular methyl group been at position 14. Further support for the positions of the two angular methyl groups is given by the following degradative experiments (Wieland *et al.*, 1924, 1928, 1933):



(XVII) was shown to be butane-2,2,4-tricarboxylic acid; thus there is a methyl group at position 10. (XVIII) was shown to be a tetracarboxylic acid containing a cyclopentane ring with a side-chain.

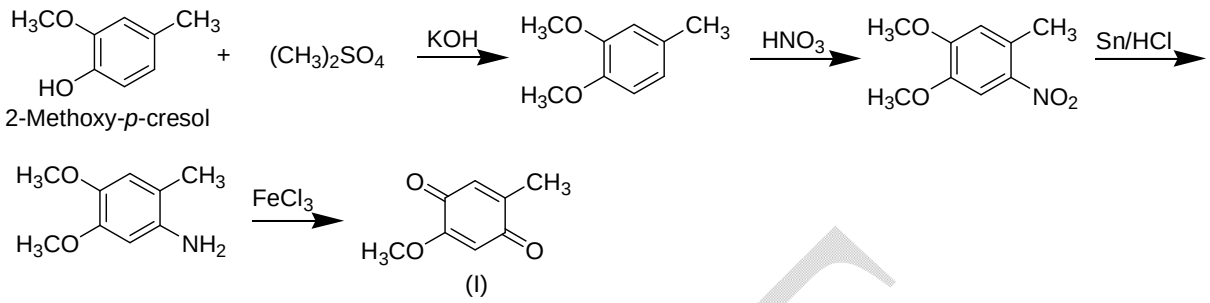
$$-CH(CH_3)CH_2CH_2CO_2H.$$

Thus this compound is derived from ring D. (XX) was also shown to be a tricarboxylic acid containing a cyclopentane ring. Furthermore, one carboxyl group in (XX) was shown to be attached to a tertiary carbon atom, and so it follows that there is a methyl group at 13 or 14. (XX) was then shown to have the *trans* configuration, i.e., the two carboxyl groups are *trans*. Thus its precursor (XIX) must have its two rings in the *trans* configuration (the methyl group and hydrogen atom at the junction of the rings are thus *trans*). Theoretical considerations of the strain involved in the *cis*- and *trans*-forms of (XIX) suggest that the *cis*-form of (XIX) would have been obtained had the methyl group been at position 14. Thus the position of this angular methyl group appears (from this evidence) to be 13 and this is supported by the fact that etiobilianic acid [(XV), section (iii)] gives 1,2-dimethylphenanthrene (XVI) on dehydrogenation with selenium. Had the angular methyl group been at position 14, 1-methylphenanthrene would most likely have been obtained.

Synthesis

Two groups of workers, viz., Robinson et al. (1951) and Woodward et al (1951), have synthesised cholesterol. One of the outstanding difficulties in the synthesis of steroids is the stereochemical problem. The cholesterol nucleus contains eight chiral centres and so 256 optical isomers are possible. Thus every step in the synthesis which produced a new chiral centre had to result in the formation of some (the more the better) of the desired stereoisomer, and at the same time resolution of racemic modifications also had to be practicable. Another difficulty was attacking a particular point in the molecule without affecting the other parts. This problem led to the development of specific reagents. The following is an outline of the Woodward synthesis. Some steps are not stereospecific or even stereoselective. Later syntheses of various steroids are superior in this respect. The synthesis of cholesterol described here is of the type: C → CD →BCD → ABCD.

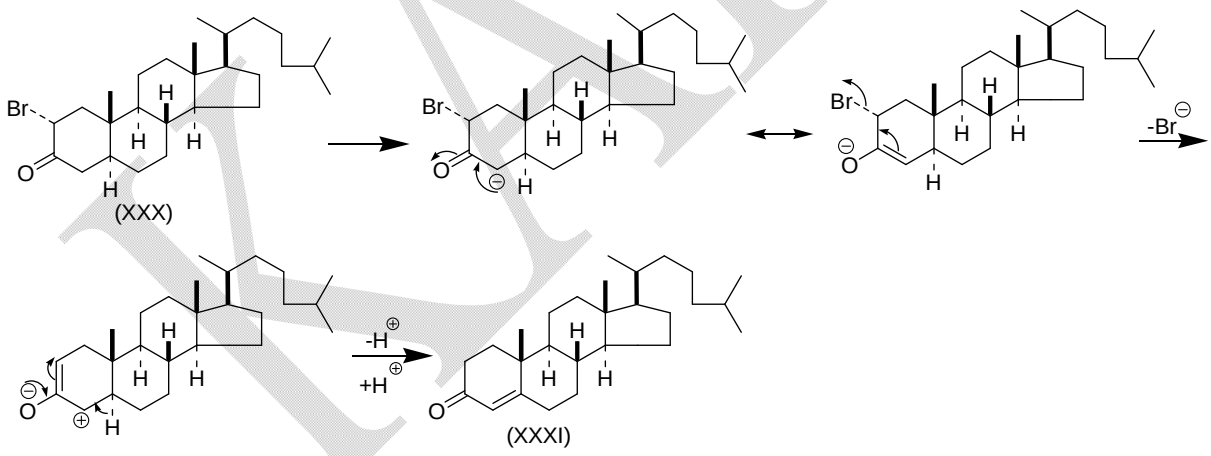
4-methoxy-2,5-toluquinone (I) was prepared form 2-methoxy-*p*-cresol as follows:



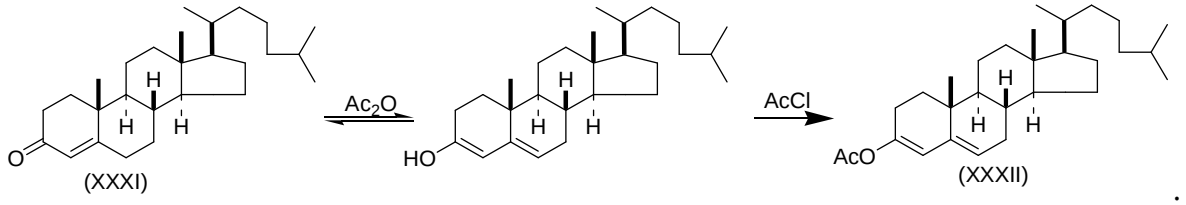
(I) was condensed with butadiene (Diels-Alder-reaction) to give (II). This has the cis configuration and was isomerised (quantitatively) to the *trans*-isomer (III) by dissolving in a aqueous alkali, adding a seed crystal of the trans-isomer and then acidifying. Isomerisation occurs via the enolate to give the more stable *trans*-isomer. (III), on reduction with lithium aluminium hydride, gave (IV). (VI) is a vinylether of glycol which, on treatment with aqueous acid, undergoes hydrolysis (demethylation) to give a β-hydroxyketone which is readily dehydrated to (V) in acid solution. Conversion of (V) to (VI) by removal of the hydroxyl group was carried out by a new technique: (V) was acetylated and the product, the ketol acetate, was heated with zinc in acetic anhydride to give (VI) (reduction with metal and acid usually reduces, α, β-unsaturated bonds in ketones). (VI) on treatment with ethyl formate in the presence of sodium methoxide, gave the hydroxymethylene ketone (VII) (Claisen condensation). When this was treated with ethyl vinyl ketone in the presence of potassium *t*-butoxide, (VIII) was formed (Michael condensation). The object of the double bond in the ketone ring in (VI) is to prevent formylation occurring on that side of the keto group, and the purpose of the formyl group is to produce an active methylene group (this is now flanked on both sides by carbonyl groups). The necessity for this 'activation' lies in the fact that ethyl vinyl ketone tends to self-condense, and consequently decrease the yield of (VIII). Both operations are examples of the introduction of regiospecific control elements. (VIII) was now cyclised quantitatively by means of potassium hydroxide in aqueous dioxane to the single product (IX). This is the desired compound; the other possible isomer ((IX) with the two hydrogens cis instead of *trans* as shown) is not formed since the *cis*-isomer is less stable than the *trans* due to greater steric interactions in the former, i.e., the cyclisation is stereospecific (steric effect control). Also, the cyclisation occurs by an intramolecular aldol condensation followed by dehyaration. (IX) was then treated with osmium

tetroxide to give two *cis*-glycols of structure (X) (one is *cis* the respect to the angular methyl group and the other is *trans*). Glycol formation occurs readily at the isolated double bond (the other two double bonds are conjugated and so have less double bond character than an isolated double bond; the reaction with osmium tetroxide is very sensitive to this change). These glycols were separated and the desired isomer (the one insoluble in benzene) was treated with acetone in the presence of anhydrous copper sulphate to give the isopropylidene derivative (XI). This, on catalytic reduction (H_2-Pd/$SrCO_3$) gave (XII) which was condensed with ethyl formate in the presence of sodium methoxide to give (XIII), and this was then converted into (XIV) by means of methylaniline. The purpose of this treatment was to block undesired condensation reactions on this side of the keto group (at this position 3); this is another example of a regiospecific control element. When (XIV) was condensed with vinyl cyanide (cyanoethylation) and the product hydrolysed with alkali, the product was a mixture of two keto acids. These were separated and the stereoisomer (XV) [methyl group in front and propionic acid group behind the plane of the rings] was converted into the enol lactone (XVI) which, on treatment with methylmagnesiuum bromide, gave (XVII), and this, on ring closure by means of alkali, gave (XVIII). When this was oxidised with periodic acid in aqueous dioxane, the dialdehyde (XIX) was obtained (via hydrolysis of the diol), and this, when heated in benzene solution in the presence of a small amount of piperidine acetate, gave (XX) (and a small amount of isomer). This cyclisation occurs by an intramolecular aldol condensation under the influence of the base, piperidine acetate. Since either aldehyde group can be involved in the condensation, two products are possible. In (XIX), the upper methylene group is *cis* to the hydrogen atom at C-14, whereas the lower methylene group is cis to the 18-methyl group. Hence, the upper methylene group experiences less steric hindrance than the lower one and consequently it is the former that loses a proton to form the carbanion. Therefore (XX) is the predominant isomer. (XX) was oxidised to the corresponding acid which was then converted into the methylester (XXI) with diazomethane. (XXI), a racemate, was resolved by reduction of the keto group with sodium borohydride to the hydroxy esters ((±)-3α- and (±)-3β). The (+)–form of the 3β-alcohol was preferentially precipitated by digitonin, and this stereoisomer was now oxidised (Oppenauer oxidation) to give the desired stereoisomer (+)-(XXI). This was catalytically reduced (H_2-Pt) to (XXII), which was then oxidised to (XXIII) which was now a mixture of stereoisomers (from the mixture of (XXII); H at

17 behind and in front). These were separated, reduced (sodium borohydride), and hydrolysed. The β-isomer, (XXIV), was converted into the methyl ketone by first acetylating, then treating with thionyl chloride and finally with dimethylcadmium. This acetylated hydroxyketone, (XXV), on treatment with isohexylmagnesium bromide, gave (XXVI). This was a mixture of isomers (a new chiral centre has been introduced at position 20) (XXVI), on dehydration, gave one product, (XXVII), and this on catalytic hydrogenation (H_2-Pt), gave a mixture of 5α-cholestanyl acetates (the chiral C-20 has been re-introduced). These acetates were separated and the desired isomer, on hydrolysis, gave 5α-cholestan-3β-ol, (XXVIII), which was identical with natural cholesterol. The conversion of cholestanol into cholesterol (XXXIII) is then carried out by a series of reactions introduced by various workers. Bromination of (XXIX) in acetic acid in the presence of hydrogen bromide (as catalyst) gives the 2α-bromo-derivative (XXX). (XXX), on treatment with pyridine, gives (XXXI). The mechanism of this elimination is uncertain. A possibility is that because the equatorial bromine is difficult to remove by the E2 mechanism, a 1,4-elimination occurs by removal of a proton from position 4 by the base (the methylene group in this position is activated by the adjacent oxo group; however, the bromination of acetone).

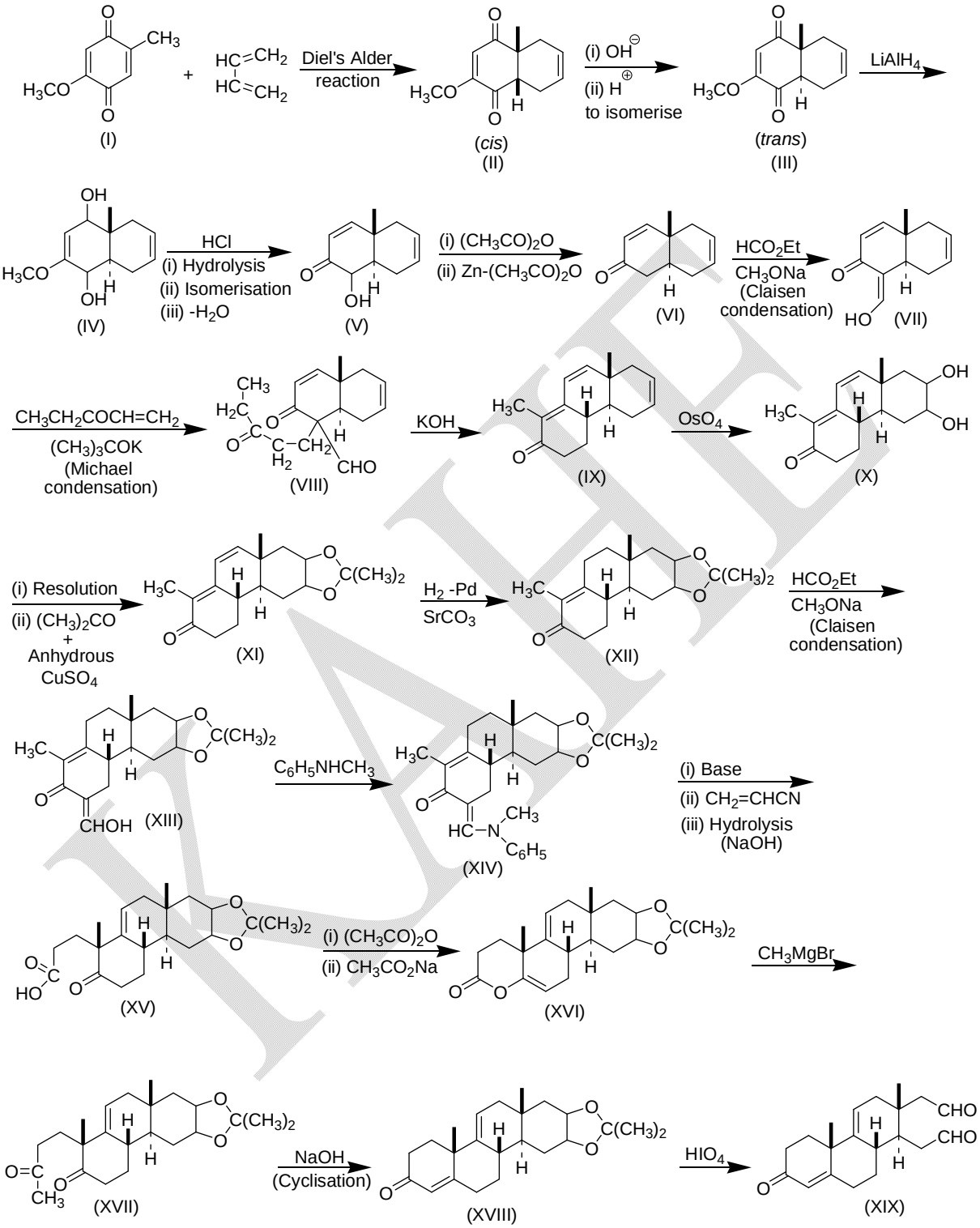


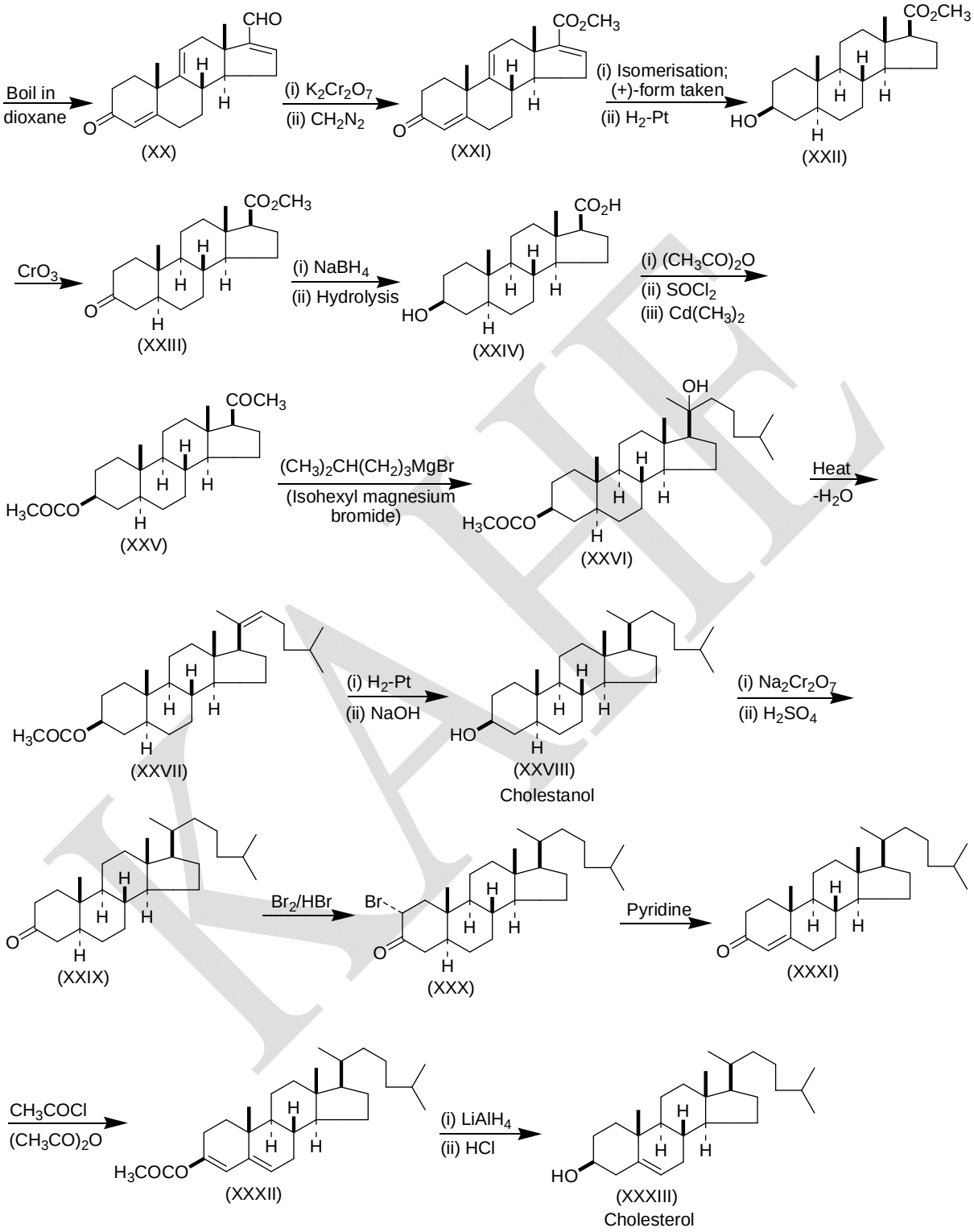
Heating (XXXI) with acetyl chloride in the presence of acetic anhydride gives the enol acetate (XXXII) which, on reduction with lithium aluminium hydride followed by acidification, gives cholesterol (XXXIII). The mechanism of this reaction is uncertain



(XXXI) (XXXII)

Possibly the electron-attracting effect of the acetoxy-group activates the 3,4-double bond to hydride transfer from the lithium aluminium hydride.

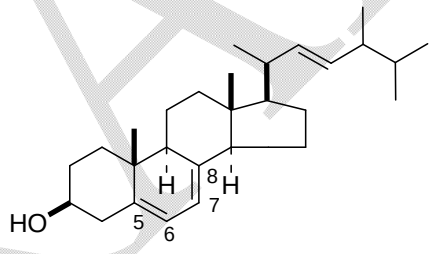

(I)
Diel's Alder reaction
(cis)
(II)
(i) OH⊖
(ii) H⊕
to isomerise
(trans)
(III)
LiAlH4
(IV)
HCl
(i) Hydrolysis
(ii) Isomerisation
(iii) -H2O
(V)
(i) (CH3CO)2O
(ii) Zn-(CH3CO)2O
(VI)
HCO2Et
CH3ONa
(Claisen condensation)
(VII)
CH3CH2COCH=CH2
(CH3)3COK
(Michael condensation)
(VIII)
KOH
(IX)
OsO4
(X)
(i) Resolution
(ii) (CH3)2CO + Anhydrous CuSO4
(XI)
H2 -Pd
SrCO3
(XII)
HCO2Et
CH3ONa
(Claisen condensation)
(XIII)
C6H5NHCH3
(XIV)
(i) Base
(ii) CH2=CHCN
(iii) Hydrolysis (NaOH)
(XV)
(i) (CH3CO)2O
(ii) CH3CO2Na
(XVI)
CH3MgBr
(XVII)
NaOH
(Cyclisation)
(XVIII)
HIO4
(XIX)


Boil in dioxane
(XX)
(i) $K_2Cr_2O_7$
(ii) CH_2N_2
(XXI)
(i) Isomerisation; (+)-form taken
(ii) H_2-Pt
(XXII)
CrO_3
(XXIII)
(i) $NaBH_4$
(ii) Hydrolysis
(XXIV)
(i) $(CH_3CO)_2O$
(ii) $SOCl_2$
(iii) $Cd(CH_3)_2$
$(CH_3)_2CH(CH_2)_3MgBr$
(Isohexyl magnesium bromide)
(XXV)
(XXVI)
Heat
$-H_2O$
(i) H_2-Pt
(ii) NaOH
(XXVII)
(XXVIII)
Cholestanol
(i) $Na_2Cr_2O_7$
(ii) H_2SO_4
Br_2/HBr
(XXIX)
(XXX)
Pyridine
(XXXI)
CH_3COCl
$(CH_3CO)_2O$
(XXXII)
(i) $LiAlH_4$
(ii) HCl
(XXXIII)
Cholesterol

An important point to note is that this total synthesis has involved a very large number of steps, and in most cases of this type the overall yield is very small. It may vary from about 4 to about 0.0005 per cent, depending on the number of steps involved. Thus, these syntheses cannot be expected to be a commercial source of these compounds. However, once a total synthesis has been accomplished, other syntheses of the compound may be carried out by starting from any particular intermediate prepared in the sequence. Such a compound may actually occur naturally or be a degradation product of the desired final product. In many cases, the starting material may be a natural compound that can be efficiently converted into the desired product. In such cases, the synthesis of the desired product is referred to as a partial synthesis. In general, most complex molecules have been prepared by partial syntheses before total syntheses have been achieved. Partial syntheses can be commercially important, but complete confirmation of structure is always necessary, and this is usually achieved by total synthesis.

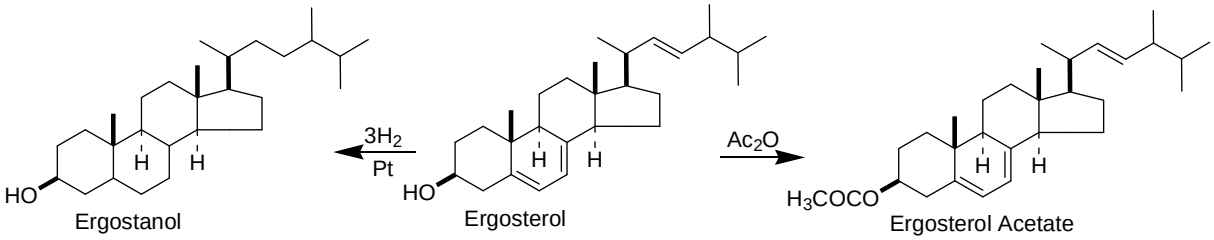
A more recent total synthesis of (±)-cholesterol has been carried out by Johnson *et al.* (1966) using the hydrochrysene approach.

Ergosterol

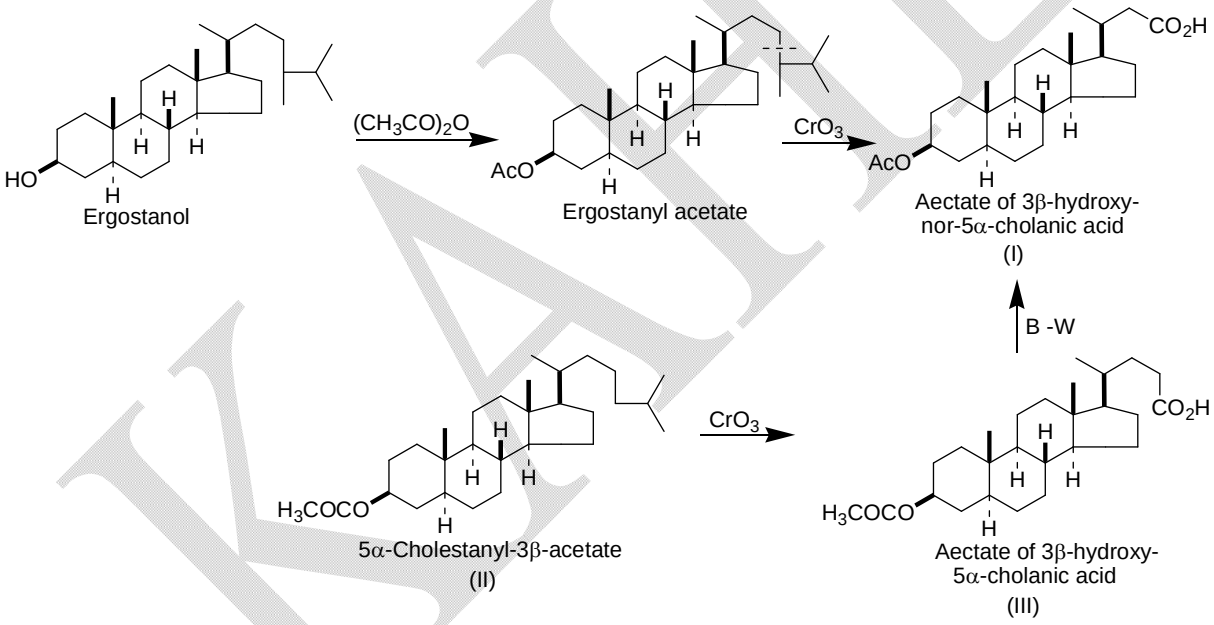


Ergosterol

Molecular formula: $C_{28}H_{44}O$, m.p. 165ºC, $[\alpha]_D$-135º, λ_{max} 282 nm. This occurs in yeast. Erogosterol forms esters, e.g., an acetate with acetic anhydride; thus there is a hydroxyl group present in ergosterol. Catalytic hydrogenation (platinum) of ergosterol produces ergostanol, $C_{28}H_{50}O$; hence there are three double bonds in ergosterol.

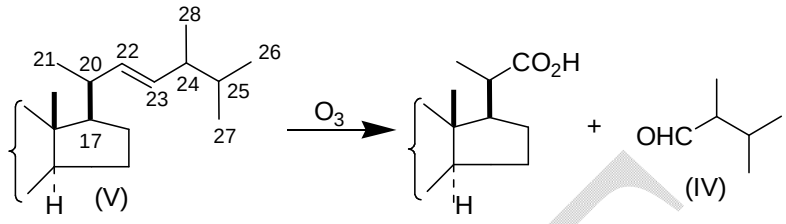


When ergostanol is acetylated and the product then oxidised, the acetate of 3β-hydroxynor-5α-cholanic acid, (I) is obtained (Fernholz *et al.*, 1934). The identity of (I) is established by the fact that 5α-cholestanyl 3β-acetate (II) (a compound of known structure), gives, on oxidation, the acetate of 3β-hydroxy-5α-cholanic acid (III) and this, after one Barbier-Wieland degradation, gives (I); thus:



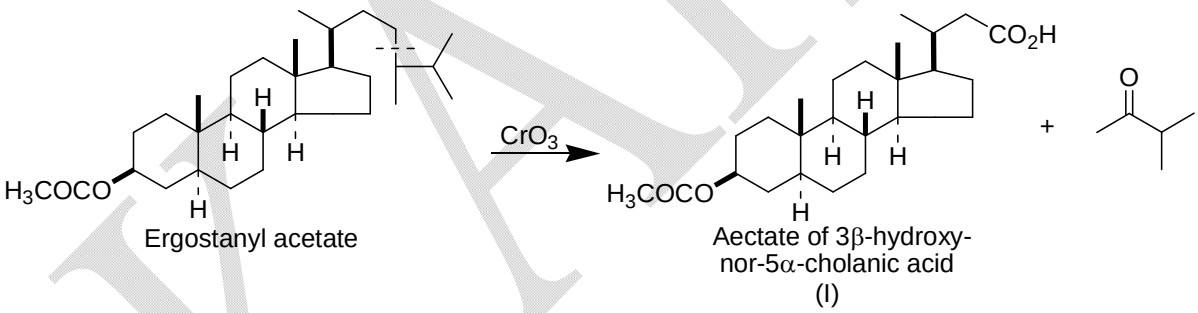
Thus ergostanol and 5α-cholestan-3β-ol have identical nuclei, the same position of the hydroxyl group and the same position of the side-chain. The only difference must be the nature of the side chain, and hence it follows that ergosterol contains one more carbon atom in its side-chain than cholesterol (the former compound is $C_{28}H_{44}O$ and the latter $C_{27}H_{46}O$). Ozonolysis of ergosterol gives, among other products, methylisopropylacetaldehyde (IV). This can be accounted for if the side-chain of ergosterol is as shown in (V) (Windaus et al., 1932). Also since

the infrared spectrum of ergosterol showed a band at 970 cm^{-1}, the 22, 23-double bond has the trans-configuration.



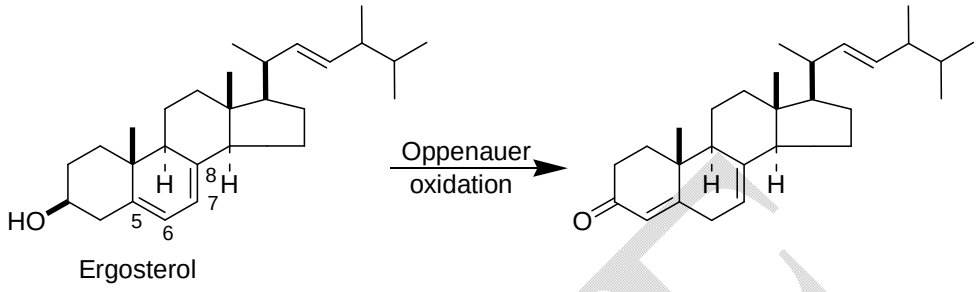
On this basis, the oxidation of ergostanyl acetate to the acetate of 3β-hydroxynor-5α-cholanic acid (I) is readily explained.

We have now accounted for all the structural features of ergosterol except the positions of the three double bonds. The position of one of these is actually shown in the above account; it is C_{22}-C_{23}. The side-chain must contain only one double bond, since if more than one were present, more than one fragment (IV) would have been removed on ozonolysis.



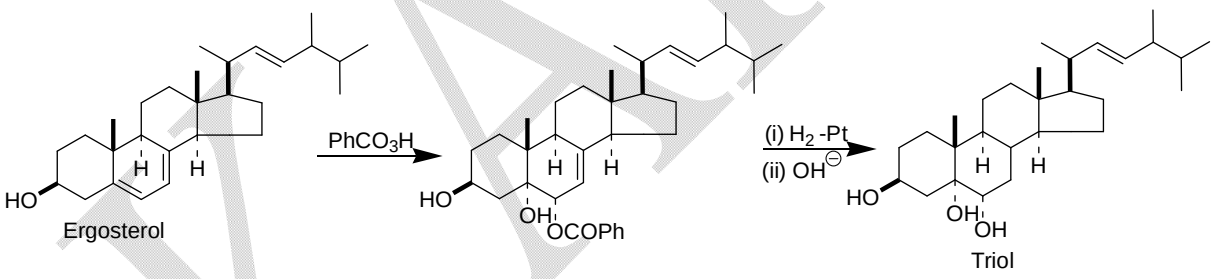
Thus the other two double bonds must be in the nucleus. When heated with maleic anhydride at 135˚C, ergosterol forms an adduct, and so it follows that the two double bonds (in the nucleus) are conjugated (Windaus *et al.*, 1931). Now ergosterol has an absorption maximum at 282 nm. Conjugated acyclic dienes absorb in the region of 220-250 nm, but if the diene is in a ring system, then the absorption is shifted to the region 260-290 nm. Thus the two double bonds in the nucleus of ergosterol are in one of the rings (Dimroth *et al.*, 1936). When ergosterol is subjected to the Oppenauer oxidation (aluminium t-butoxide and acetone), the product is an α, β-unsaturated ketone (λ_{max} 235 nm). This can only be explained by assuming that one of the double bonds is in the 5,6-position, and moves to the 4,5-position during the oxidation. The other double

bond is therefore 7,8 in order to be conjugated with the one that is 5,6. Hence the conjugated system is in ring B and the oxidation is explained as follows:



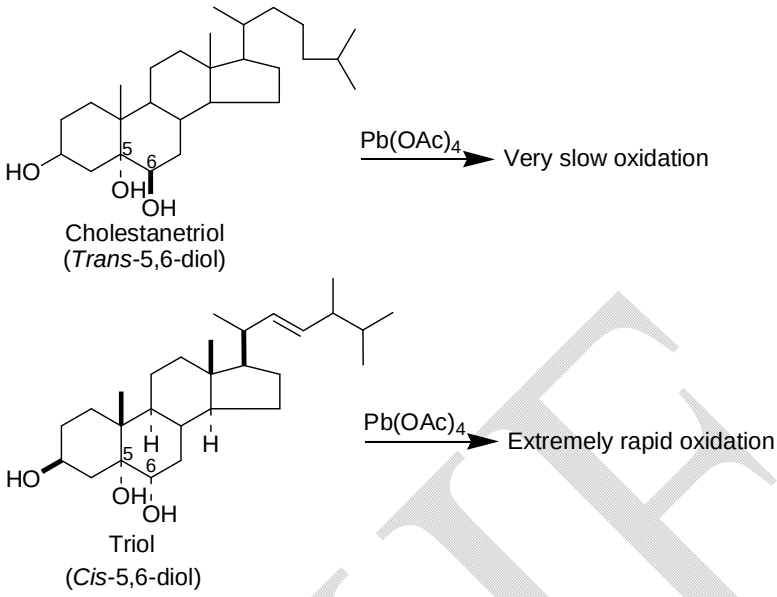
Ergosterol

This is supported by the oxidation of ergosterol with perbenzoic acid to give the monobenzoate of a triol. This on catalytic hydrogenation followed by hydrolysis, gave a saturated triol which under-went fission when treated with lead tetra-acetate. Hence, two hydroxyl groups must be in the vicinal position and also, since the triol formed only a diacetate, one hydroxyl group is therefore tertiary. These results are readily explained on the basis that one double bond is in the 5,6-position.



Ergosterol Triol

An interesting point about this triol is that it is a 5α, 6α-derivative, whereas it might have been expected to have been the 5α, 6β-compound. That it was the *cis*-5,6-diol was shown by the fact that it was oxidised by lead tetra-acetate extremely rapidly when compared to the rate of oxidation of cholestanetriol, which is a *trans*-5,6-diol. With ergosterol, the 5,6-epoxide (α-configuration) is probably formed as expected, but because of the 7,8-double bond which is allylic with C-6, this epoxide is readily opened by benzoic acid (from the per-acid) to give the 6-benzoate with retention at this position, *i.e.*, the *cis*-1,2-glycol).



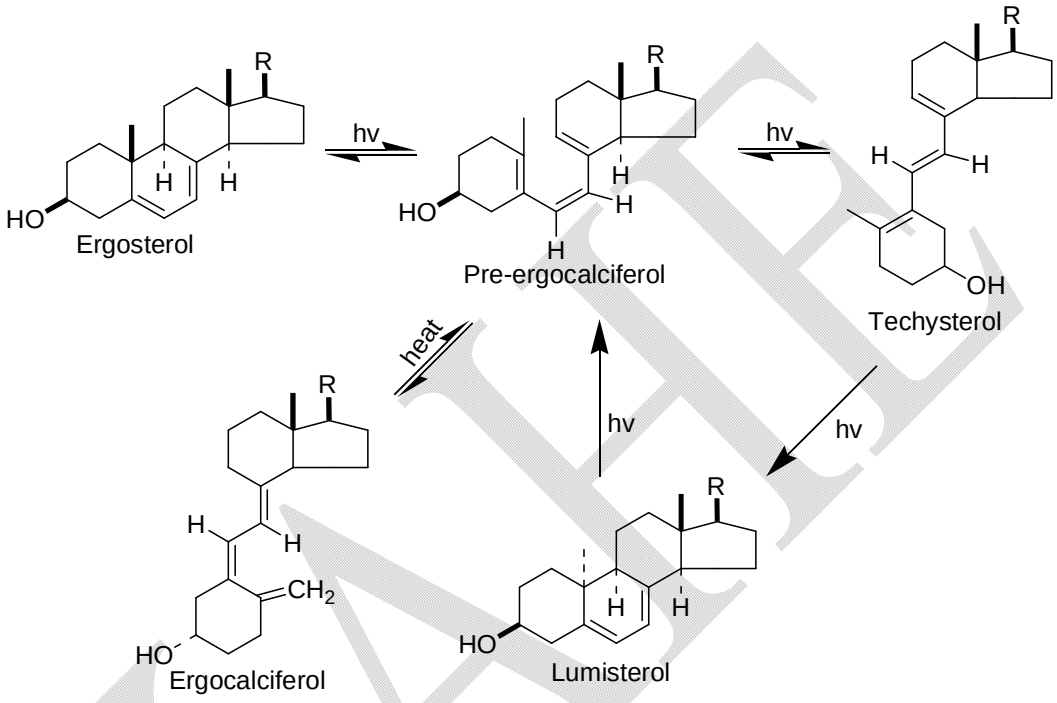
Vitamin D

This vitamin is the antirachitic vitamin; it is essential for bone formation, its function being the control of calcium and phosphorus metabolism.

Steenbock *et al.* (1924) showed that when various foods were irradiated with ultraviolet light, they acquired antirachitic properties. This was then followed by the discovery that the active compound was in the unsaponifiable fraction (the sterol fraction). At first, it was believed that the precursor of the active compound was cholesterol, but subsequently the precursor was shown to be some 'impurity' that was in the cholesterol fraction (e.g., by Heilbron *et al.* 1926). The ultraviolet absorption spectrum of this 'impure cholesterol' indicated the presence of a small amount of some substance that was more unsaturated than cholesterol. This led to the suggestion that ergosterol was the provitamin D in the impure cholesterol', and the investigation of the effect of ultraviolet light on ergosterol resulted in the isolation from the irradiated product of a compound which had very strong antirachitic properties. This compound was named **calciferol** by the Medical Research Council (1931), and **vitamin D_1,** by Windaus (1931). This potent crystalline compound, however, was subsequently shown to be a molecular compound of calciferol and lumisterol (one molecule of each). Windaus (1932) therefore renamed the pure

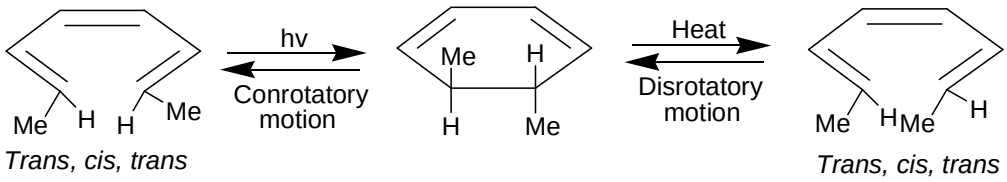
potent compound as **vitamin D_2**, but the M.R.C. retained the original name calciferol. The Chemical Society (1951) has proposed the name **ergocalciferol** for this pure compound.

A detailed study of the irradiation of ergosterol with ultraviolet light (280 nm) has led to the proposal that the series of changes is as follows (R = C_9H_{17}):



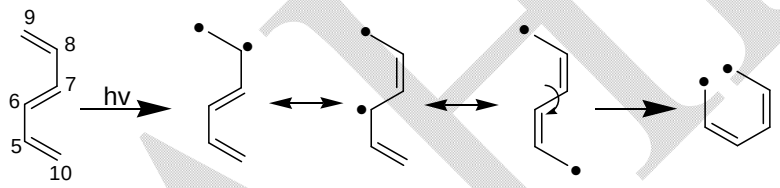
λ_{max} and ε: Ergosterol, 282 nm (11,750): pre-ergocalciferol, 262 nm (8910); tachysterol, 281 nm (24, 550), ergocalciferol, 265 nm (18,333); lumisterol, 280 nm (8500).

The course of these changes can now be explained in terms of the Woodward-Hoffmann selection rules for electrocyclic reactions. The primary reaction is the opening of the 1,3-diene ring B in ergosterol to give an equilibrium mixture with the acyclic triene, pre-ergocalciferol. Under the influence of light, this occurs by a conrotatory motion, whereas by means of heat the ring-opening occurs by a disrotatory motion, e.g., the opening of *trans*-5,6-dimethylcyclohexa-1,3-diene to give octa-2,4,6-triene.

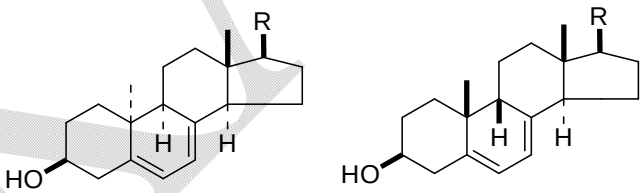


Trans, cis, trans *Trans, cis, trans*

Thus, ergosterol undergoes photochemical ring opening and by a conrotatory motion to give pre-ergocalciferol, in which the centre double bond 6,7 is *cis*. When this is irradiated, isomerisation about the 6,7-double bond (to *trans*) now occurs to give tachysterol. When this is further irradiated, ring-closure occurs to give lumisterol in which Me-19 and H-9 are still *trans*, but now Me-19 has the α-configuration and H-9 the β. This is believed to occurs as follows. The *trans*-6,7-double bond acquires a large amount of single-bond character and this permits rotation so that carbon atoms 9 and 10 can reform the σ-bond (only the conjugated system has been drawn).



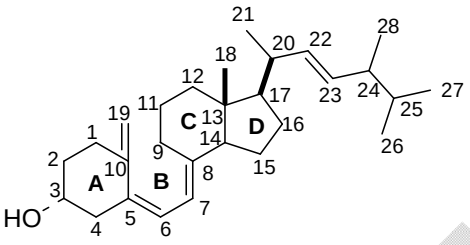
When heated, pre-ergocalciferol forms an equilibrium mixture with ergocalciferol. Further heating of either of these two compounds results in the formation of a mixture of pyrocalciferol and isopyrocalciferol.



Pyrocalciferol Isopyrocalciferol

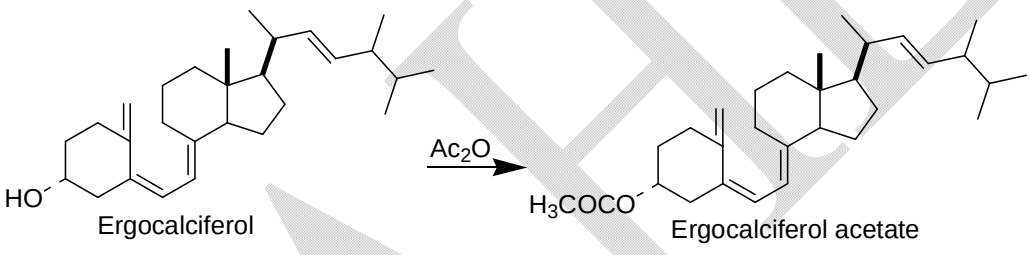
Both of these have the Me-19 and H-9 in the *cis*-position. In this case ring-closure occurs by a disrotatory motion, resulting in the formation of either '*cis*-product', depending on the direction of motion (both clockwise or both anticlockwise; see the cyclohexadiene example, above). It should also be noted that since pre-ergocalciferol is converted into ergosterol photochemically, ring-closure occurs by a conrotatory motion (see also above). Thus the product will have the *trans*-configuration. This could produce ergosterol and lumisterol but, as we have been, only the former is the actual product. The reason for this is uncertain.

Ergocalciferol (Calciferol, Vitamin D_2)

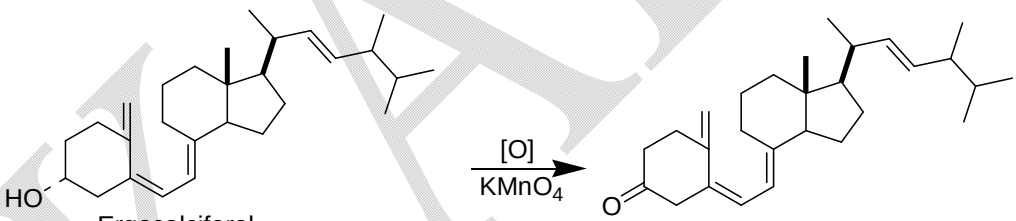


Vitamin D_2

It is a crystalline solid, m.p.115-117ºC, $[\alpha]_D$ + 130º. Its molecular formula is $C_{28}H_{44}O$, and since it forms esters, the oxygen is present as a hydroxyl group. Furthermore, since ergocalciferol gives a ketone on oxidation, this hydroxyl group is a secondary alcoholic group.

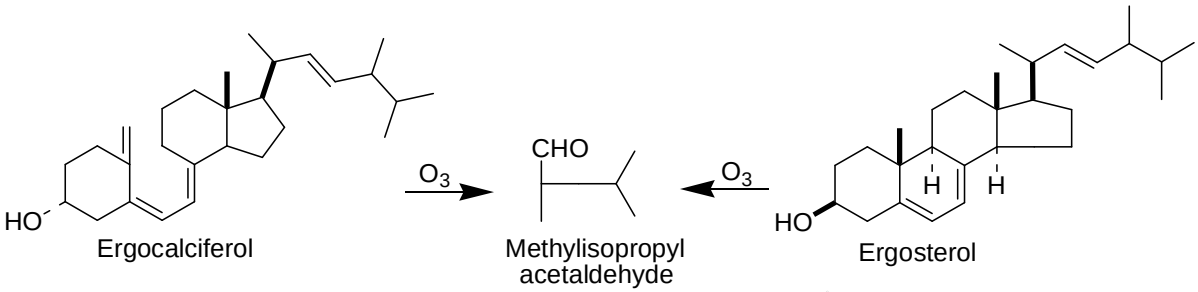


Ergocalciferol Ergocalciferol acetate

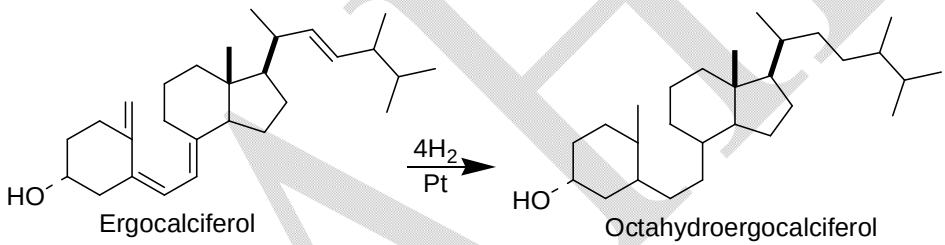


Ergocalciferol Ketone

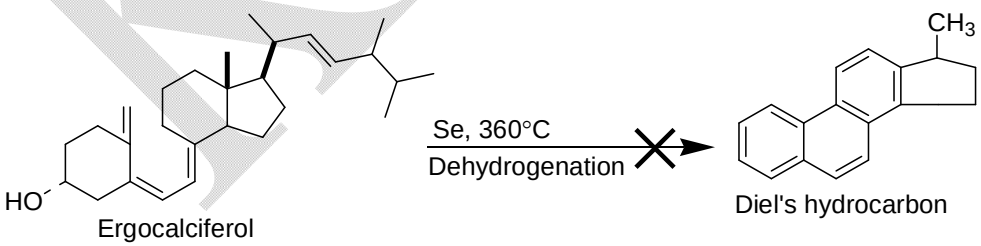
Ozonolysis of ergocalciferol produces, among other products, methylisopropylacetaldehyde. Thus the side-chain in ergocalciferol is the same as that in ergosterol.



Catalytic hydrogenation converts ergocalciferol into the fully saturated compound octahydroergocalciferol, $C_{28}H_{52}O$. This shows that there are four double bonds present, and since one is in the side-chain, three are therefore in the nucleus. The parent hydrocarbon of ergocalciferol is $C_{28}H_{52}$ and since this corresponds to the general formula C_nH_{2n-4}, the molecule therefore is tricyclic (D.B.E = 28+1–52/2 = 3; therefore three rings are present).



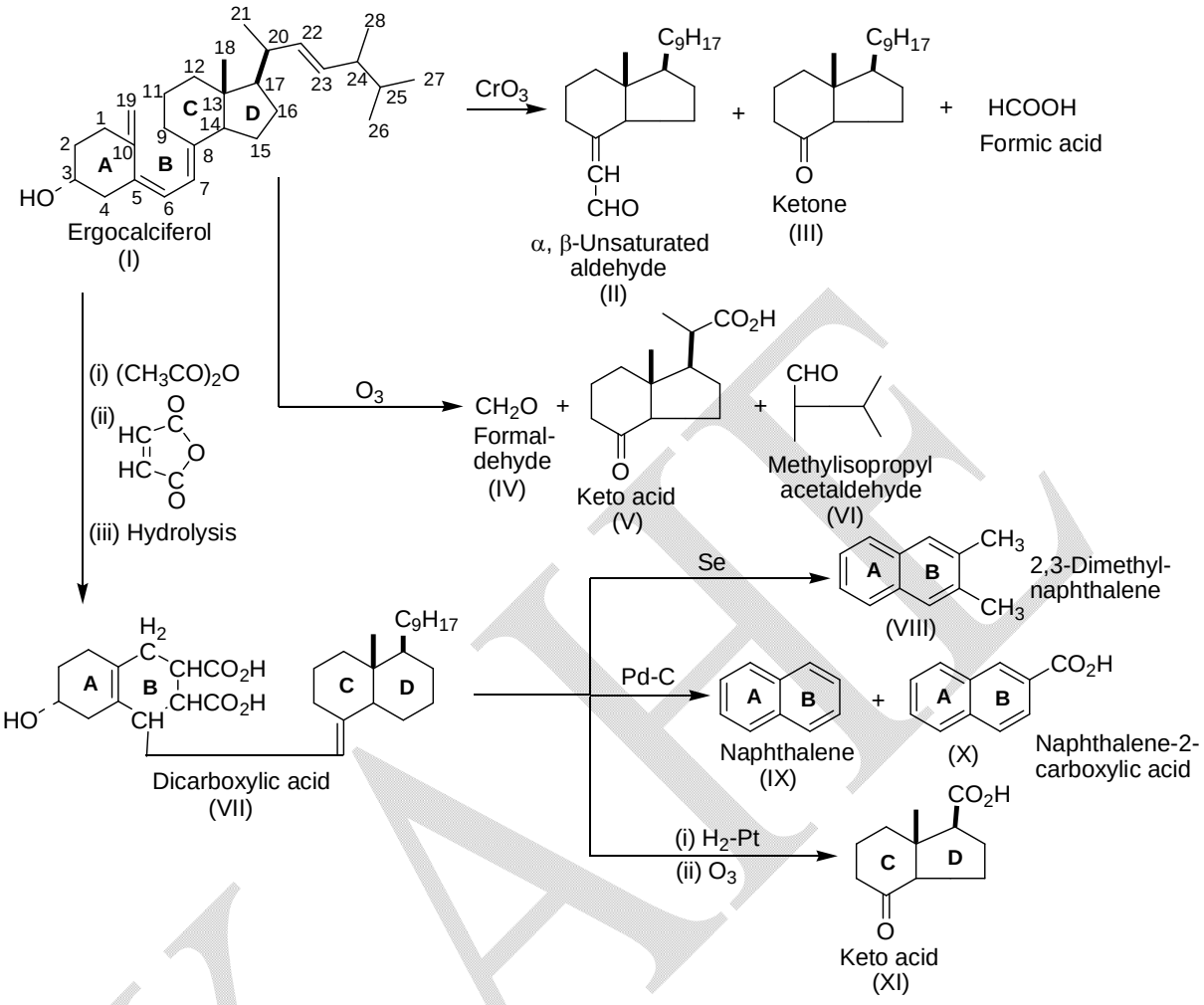
Furthermore, ergocalciferol does not give Diels' hydrocarbon when distilled with selenium. These facts indicate that ergocalciferol does not contain the four-ring system of ergosterol.



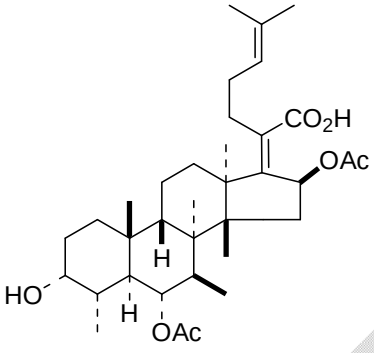
The problem is therefore to ascertain which of the rings in ergosterol has been opened in the formation of ergocalciferol. The following reactions of ergocalciferol are readily explained on the assumption that its structure is (I). The absorption spectrum of the semicarbazone of (II) ($C_{21}H_{34}O$) was shown to be characteristic of α,β-unsaturated aldehdes (λ_{max} 275 nm). The

absence of the hydroxyl group and the carbon content of (II) indicate the absence of ring A. These facts suggest that in ergocalciferol 'ring B' is open between C-9 and C-10, and that (II) arises by scission of the molecule at a double bond in position 5,6 and can be an α,β-unsaturated aldehyde only if there is a double bond at 7,8 (these double bonds are also present in ergosterol). The isolation of the ketone (III) ($C_{19}H_{32}O$) confirms the presence of the double bond at 7,8 (Heilbron *et al.*, 1935).

The isolation of formaldehyde (IV) shows the presence of an exocyclic methylene group, and the presence of this group at C-10 is in keeping with the opening of ring B at 9, 10. The formation of (V) ($C_{13}H_{20}O_3$), a keto-acid, suggests that ring B is open at 9,10 and that there are double bonds at 7,8 and 22,23. The position of the latter double bond is confirmed by the isolation of methylisopropylacetaldehyde (VI) (Heilbron *et al.*, 1936).

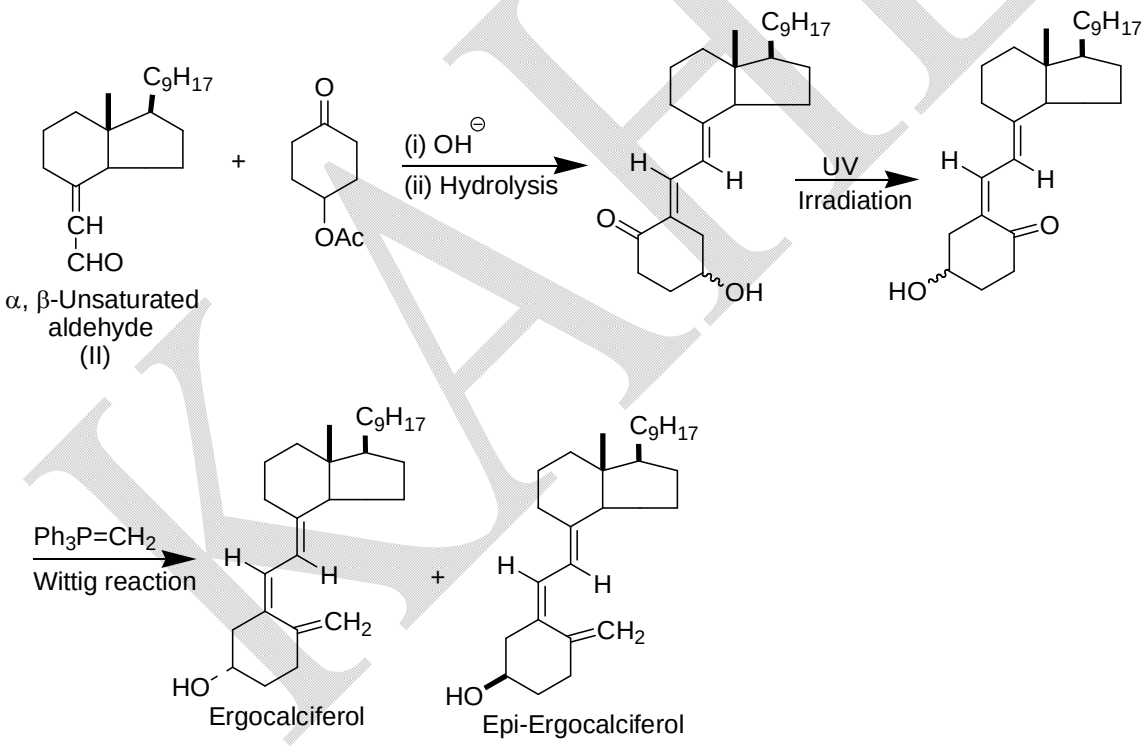


Structure (I) for ergocalciferol is also supported by the formation of (VII), the structure of which is shown by the products (VIII), (IX), (X) and (XI) (Windaus *et al.*, 1936). The production of 2,3-dimethylnaphthalene (VIII) is in keeping with the fact that carboxyl groups sometimes give rise hydroxyl and acetoxyl groups in ring B of (IV). Further work by Chou *et al.* (1967) have confirmed (IV) as the structure of cephalosporin P_1.



Cephalosporin P_1
(IV)

Finally, the structure of vitamin D_2 has been confirmed by its partial synthesis carried out by Lythgoe *et al.* (1958) from the aldehyde (II) as follows.

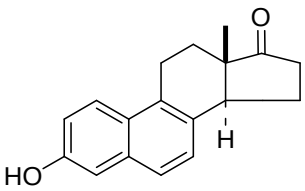


α, β-Unsaturated aldehyde
(II)

Ergocalciferol

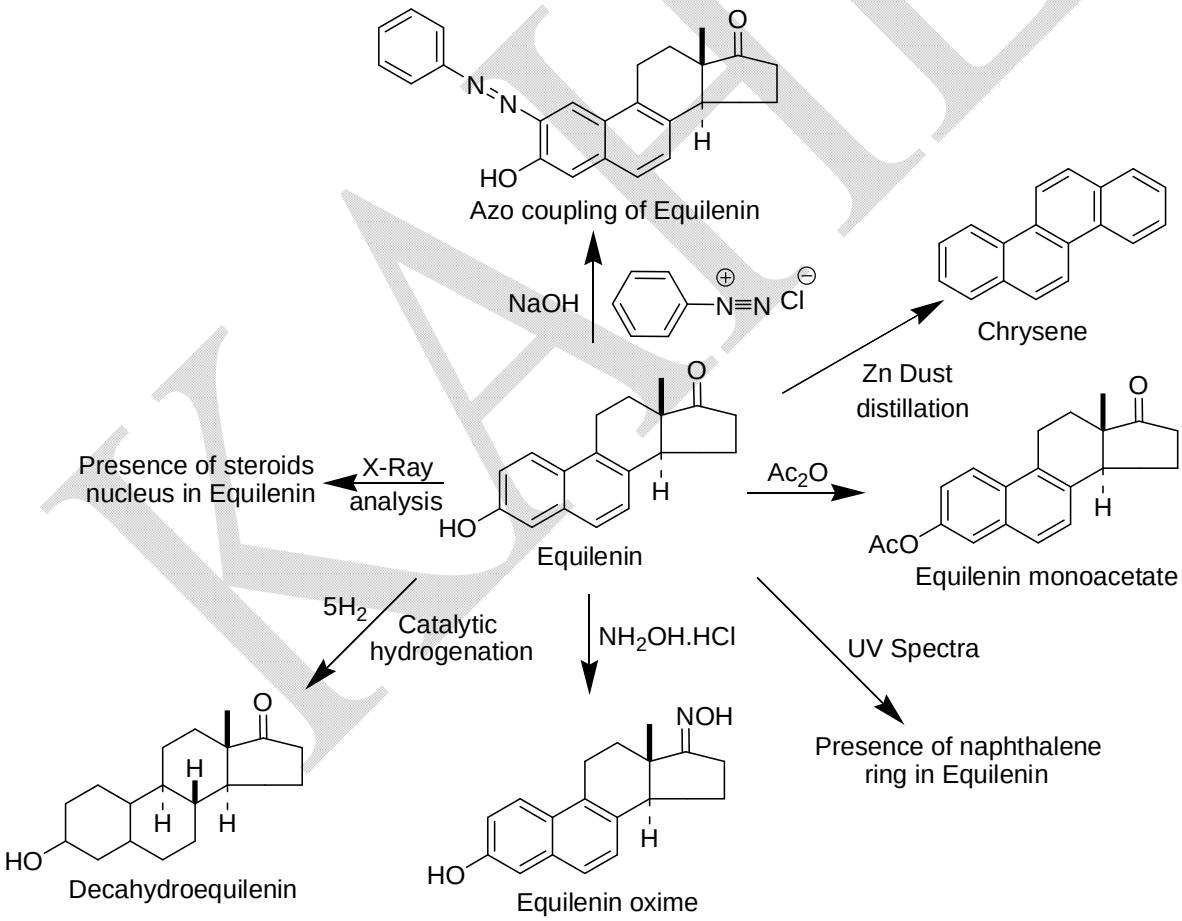
Epi-Ergocalciferol

(+)-Equilenin



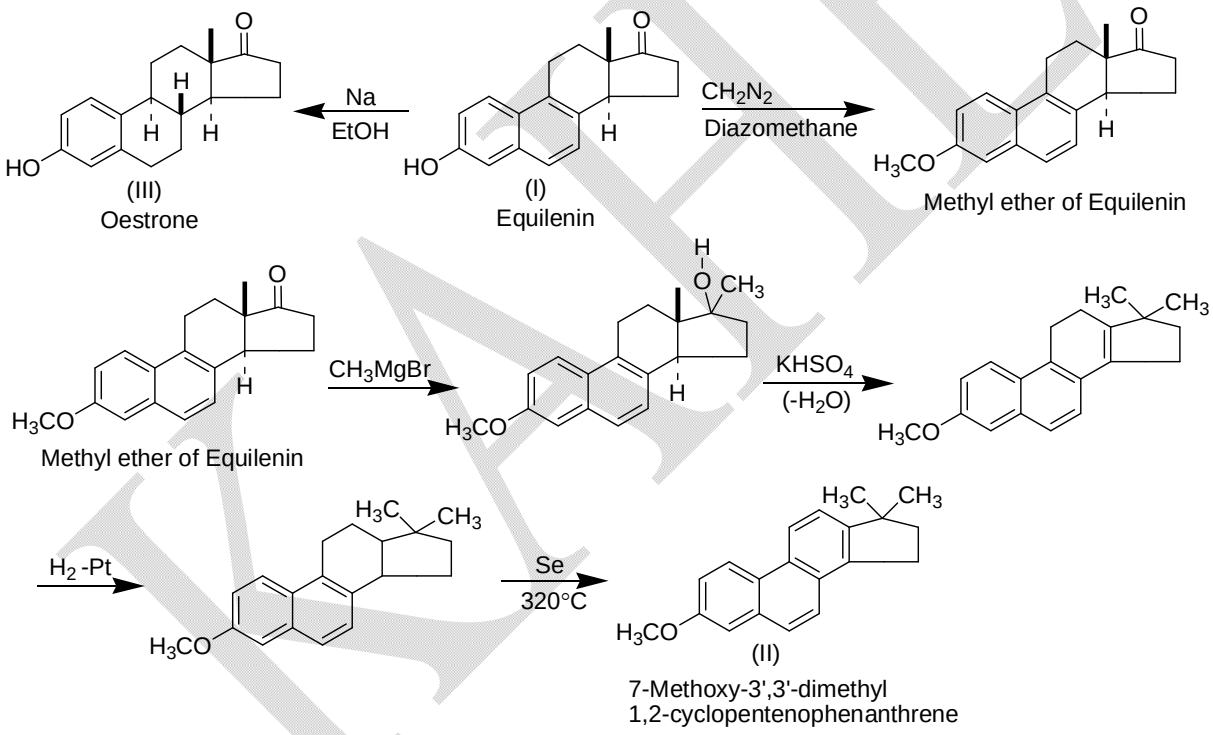
Equilenin

Molecular formula: $C_{18}H_{18}O_2$, m.p. 258-259ºC. $[\alpha]_D$ +87º. This has been isolated from the urine of pregnant mares by Girard *et al.* (1932); it is not a very potent oestrogen. The reactions of equilenin show that a phenolic hydroxyl group and a ketonic group are present, and also that the molecule contains five double bonds.



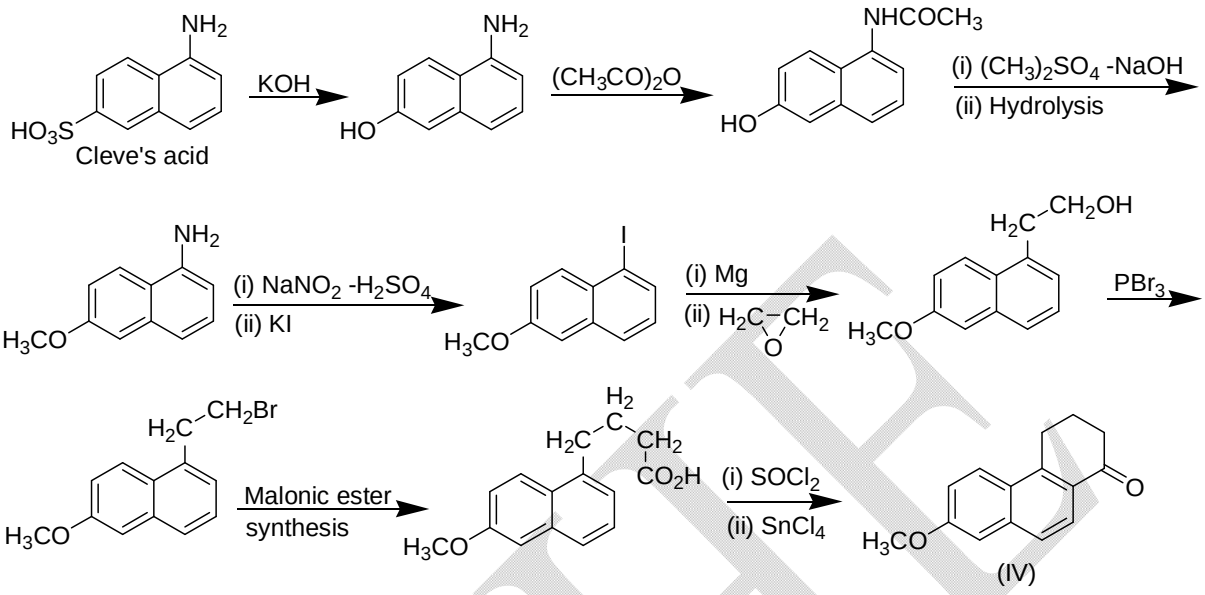
When the methyl ether of equilenin is treated with methylmagnesium iodide, then the alcohol dehydrated, catalytically reduced and then dehydrogenated with selenium, the product is

7-methoxy-3, 3-dimethyl -1,2 cyclopentenophenanthrene (II). Thus the structure of equilenin is the same as that of oestrone, except that the former has two more double bonds than the latter (Cook *et al.*, 1935). Now the absorption spectrum of equilenin shows that it is a naphthalene derivative. Thus, since ring A in oestrone is benzenoid, it appears probable that ring B in equilenin is also benzenoid, i.e., ring A and B form the naphthalene nucleus in equilenin. All the foregoing reactions of equilenin may be readily explained by assuming that (I) is its structure, and further evidence that has been given to support this is the claim by Marker *et al.* (1938) that equilenin may be reduced to oestrone (III) by sodium and ethanol. This reduction, however, has apparently never been substantiated (Dauben *et al.*, 1956).

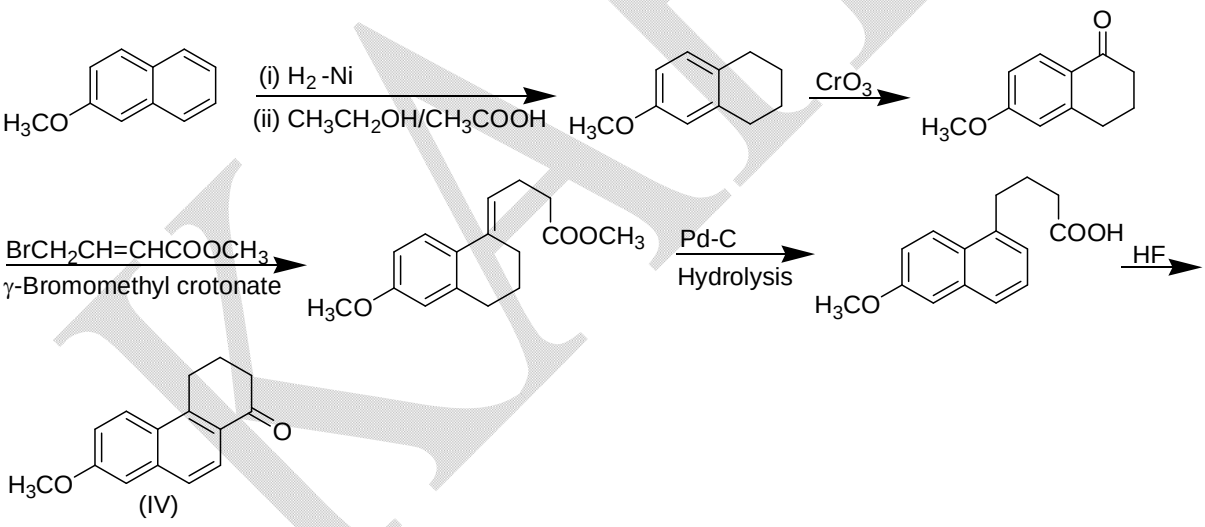


The structure of equilenin has been confirmed by synthesis. The first synthesis was by Bachmann *et al.* (1940), but was somewhat improved by Johnson *et al.* (1947). In the following chart, compound (IV) is synthesised by the method of Bachmann, and the rest of the synthesis is that of Johnson, who started with compound (IV) (Johnson's synthesis involves fewer steps than Bachmann's).

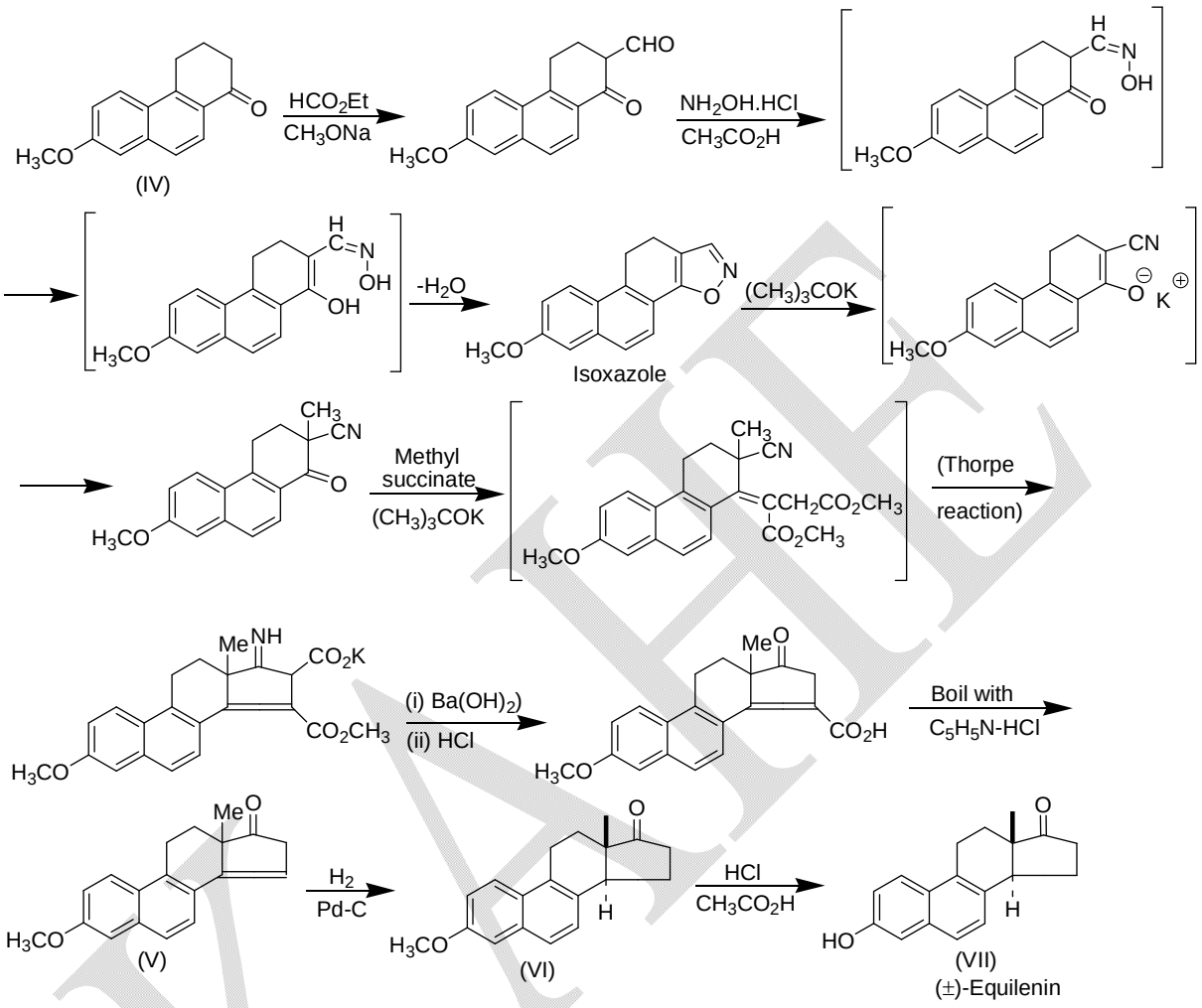
(a) Synthesis of (IV) by Bachmann's synthesis



(b) Synthesis of (IV) from β-methoxynaphthalene by Stork's synthesis

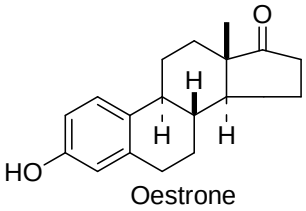


(c) Synthesis of equilenin from (IV) by Johnson's synthesis



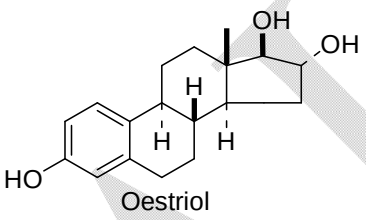
Reduction of (V) gives a mixture of (±)-equilenin methyl ether (VI) (rings C/D *trans*), and isoequilenin methyl ether (rings C/D *cis*); these are separated by fractional crystallisation from acetone methanol, the equilenin derivative being the less soluble isomer. Product (VII) is (±)-equilenin, and is resolved via the menthoxyacetic ester. The (+)-equilenin so obtained is identical with the natural product. It should be noted here that equilenin contains only two chiral centres, and so the stereochemical problems involved are far simpler than those for cholesterol and oestrone.

Oestrone (Estrone)

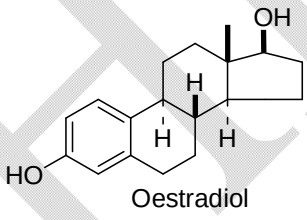


Oestrone

It has been known for a long time that there are hormones which control the uterine cycle, but it was not until 1929 that Butenandt and Doisy independently isolated the active substance oestrone from the urine of pregnant women. Oestrone is the first known member of the sex hormones, and soon after its discovery two other hormones were isolated, oestriol and oestradiol.



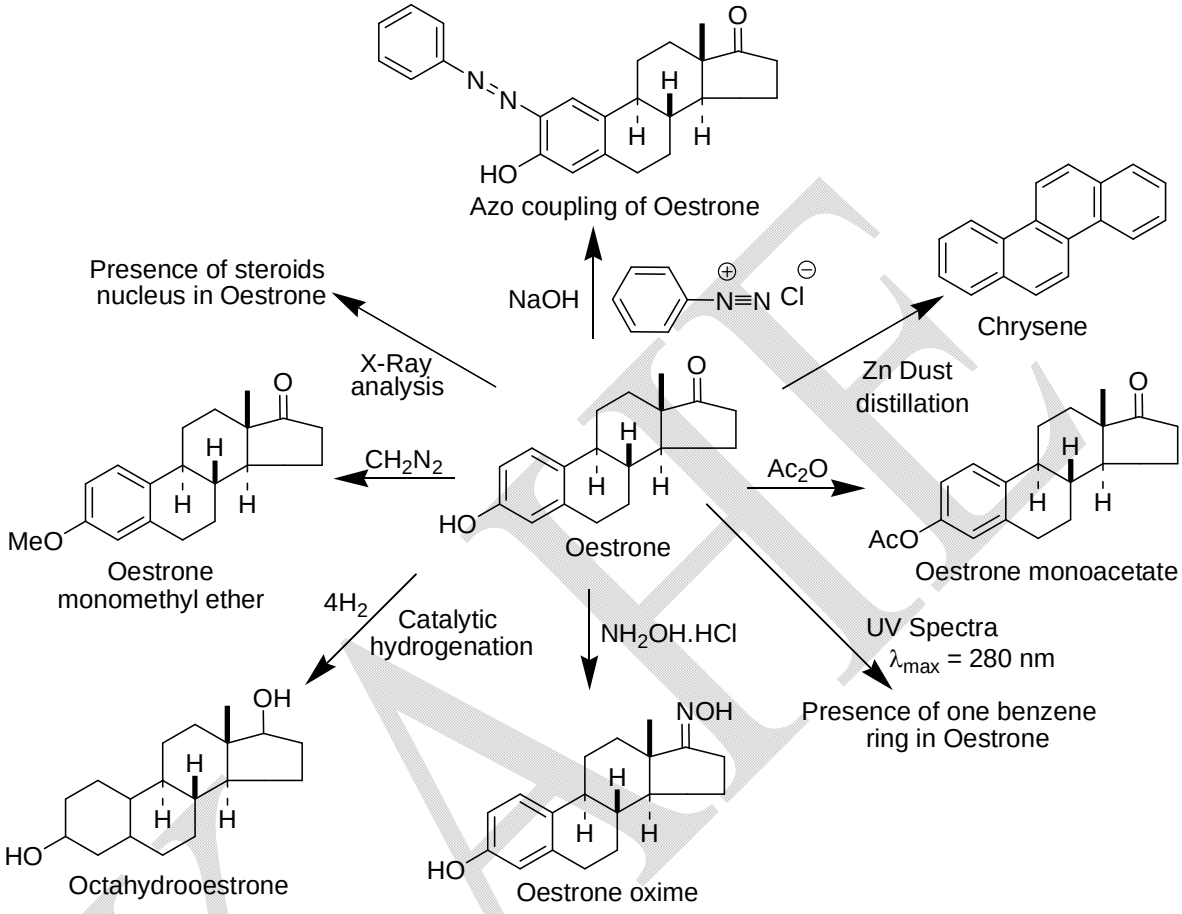
Oestriol



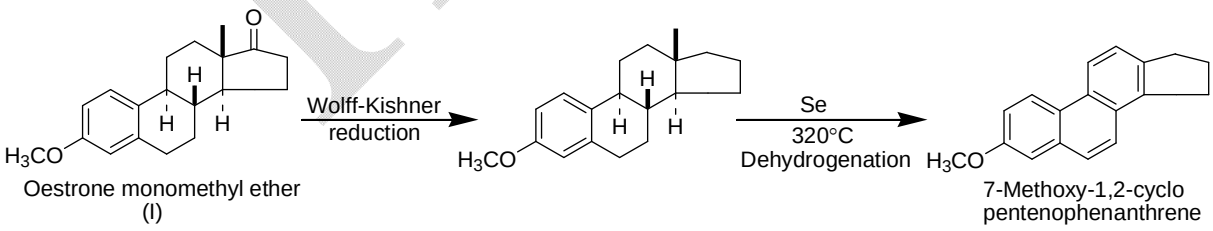
Oestradiol

(+)-Oestrone, m.p. 259°C, $[\alpha]_D$ + 170°, has the molecular formula $C_{18}H_{22}O_2$. It behaves as a ketone (forms an oxime, etc) and contains one hydroxyl group (it forms a monoacetate and a monomethyl ether). Furthermore, this hydroxyl group is **phenolic**, since oestrone couples with diazonium salts in alkaline solution (this reaction is typical of phenols). When distilled with zinc dust, oestrone forms chrysene; this led to the suggestion that oestrone is related to the steroids. The X-ray analysis of oestrone also indicates the presence of the steroid nucleus and at the same time showed that the keto group and the hydroxyl group are at the opposite ends of the molecule (Bernal, 1932). On catalytic hydrogenation, oestrone forms octahydrooestrone, $C_{18}H_{30}O_2$. This compound contains two hydroxyl groups (two hydrogen atoms are used for converting the keto group to an alcoholic group), and so six hydrogen atoms are used to saturate **three** double bonds. If these three double bonds are in one ring, i.e., there is a benzenoid ring present, then the phenolic hydroxyl group bonds are in one ring, i.e., there is a benzenoid ring present, then the phenolic hydroxyl group can be accounted for. The presence of one benzene ring in the structure

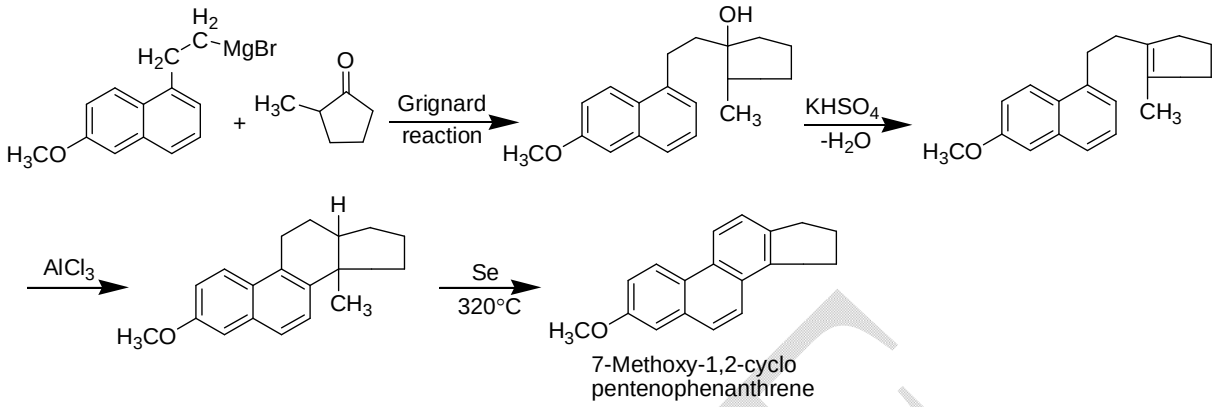
of oestrone is supported by measurements of the molecular refraction and the ultraviolet absorption spectrum (λ_{max} 280 nm).



When the methyl ether of oestrone is subjected to the Wolff-Kishner reduction, and the product distilled with selenium, 7-methoxy-1,2-cyclopentenophenanthrene is formed.

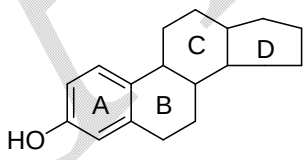


The structure of this compound was established by the following synthesis (Cook *et al.*, 1934).

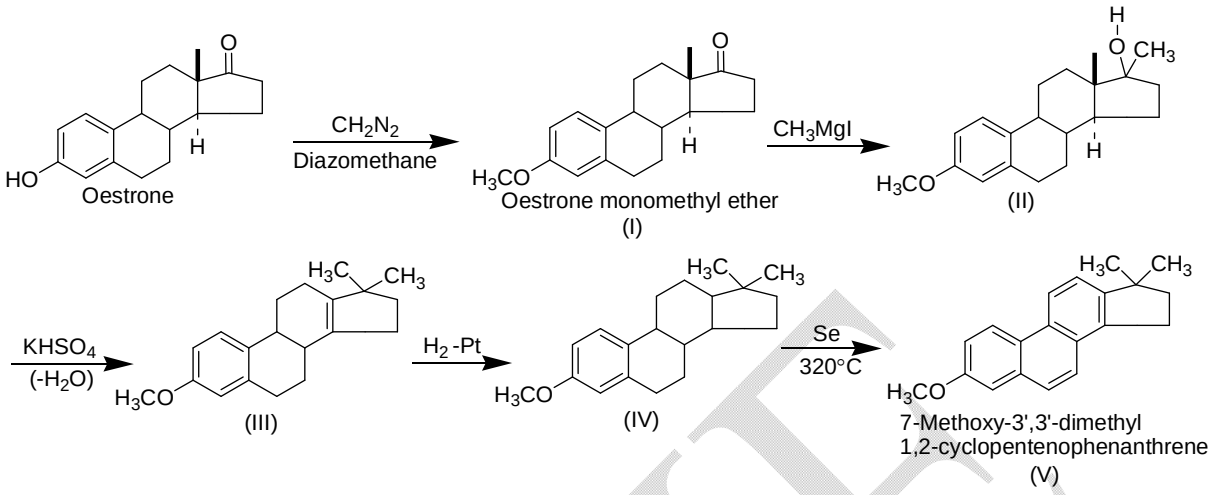


7-Methoxy-1,2-cyclo pentenophenanthrene

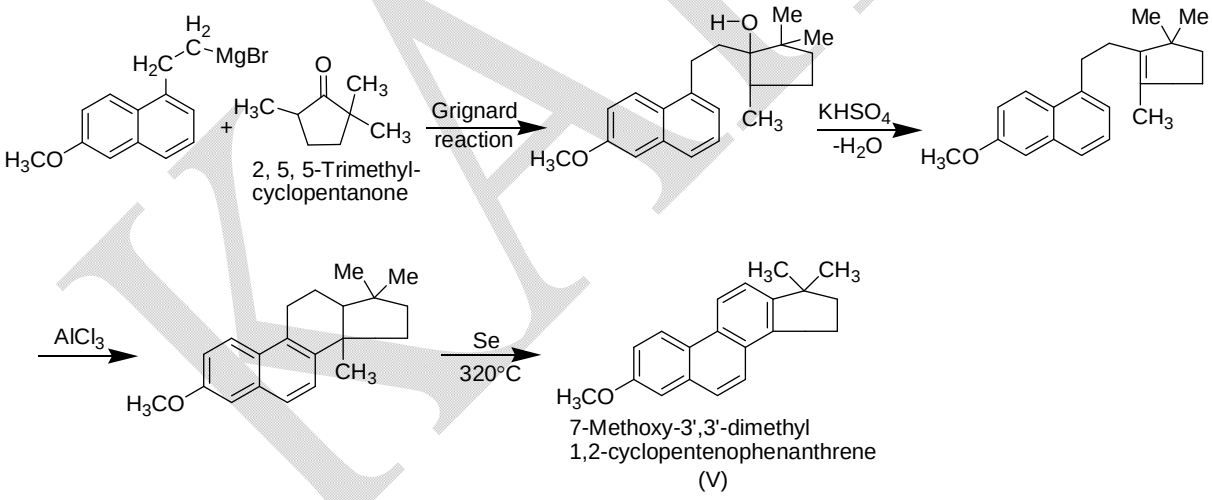
Thus the benzene ring in oestrone is ring A, and the (phenolic) hydroxyl group is at position 3; hence the skeleton of oestrone is as shown. Into this skeleton we must fit the keto group, and since this skeleton contains only 17 carbon atoms, another carbon atom must also be placed. The position of the keto group was shown to be at 17, and the extra carbon atom was shown to be an angular methyl group at position 13, as follows (Cook *et al.*, 1935). When the methyl ether of oestrone (I) is treated with methylmagnesium iodide, compound (II) is obtained. When (II) is dehydrated with potassium hydrogen sulphate to (III), this catalytically reduced to (IV) and then (IV) distilled with selenium, the product is 7-methoxy-3′,3′-dimethyl-1,2-cyclopentenophenanthrene (V).



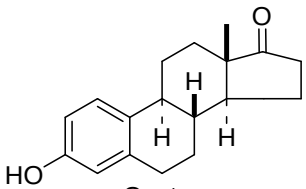
The formation of (V) can be explained only if there is a keto group at position 17 and an angular methyl group at position 13. It should be noted that in the given equations, the dehydration is accompanied by the migration of the angular methyl group; this assumption is based on the analogy with known examples in which this occurs. Furthermore, this migration of a methyl group is characteristic of *trans*-fused hydrindanols of type (II), and so the configuration of rings C/D is *trans* (*cis*-C/D fusion leads to dehydration without rearrangement). In the *trans*-C/D fusion, the CH_3-18 group is in the axial position and so satisfies the stereoelectronic requirements for the 1,2-migration with loss of the hydroxyl group at C-17.



The structure of (V) has been confirmed by synthesis (Cook *et al.*, 1935). The synthesis is same as that of 7-methoxy-1,2-cyclopentenophenanthrene except that the reacting ketone in this case is 2, 5, 5-trimethylcyclopentanone instead of 2-methylcyclopentanone.Thus the structure of oestrone is an shown (see also below).

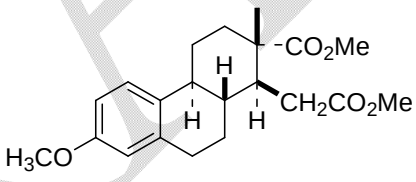


This has been confirmed by the total synthesis of Anner and Miescher (1948). These authors started with the phenanthrene derivative (VI) which had been prepared previously by Robinson *et al.* (1938), and by Bachmann *et al.* (1942). The first step of the Anner-Miescher synthesis involves the Reformatsky reaction and a later one the Arndt-Eistert synthesis.



Oestrone

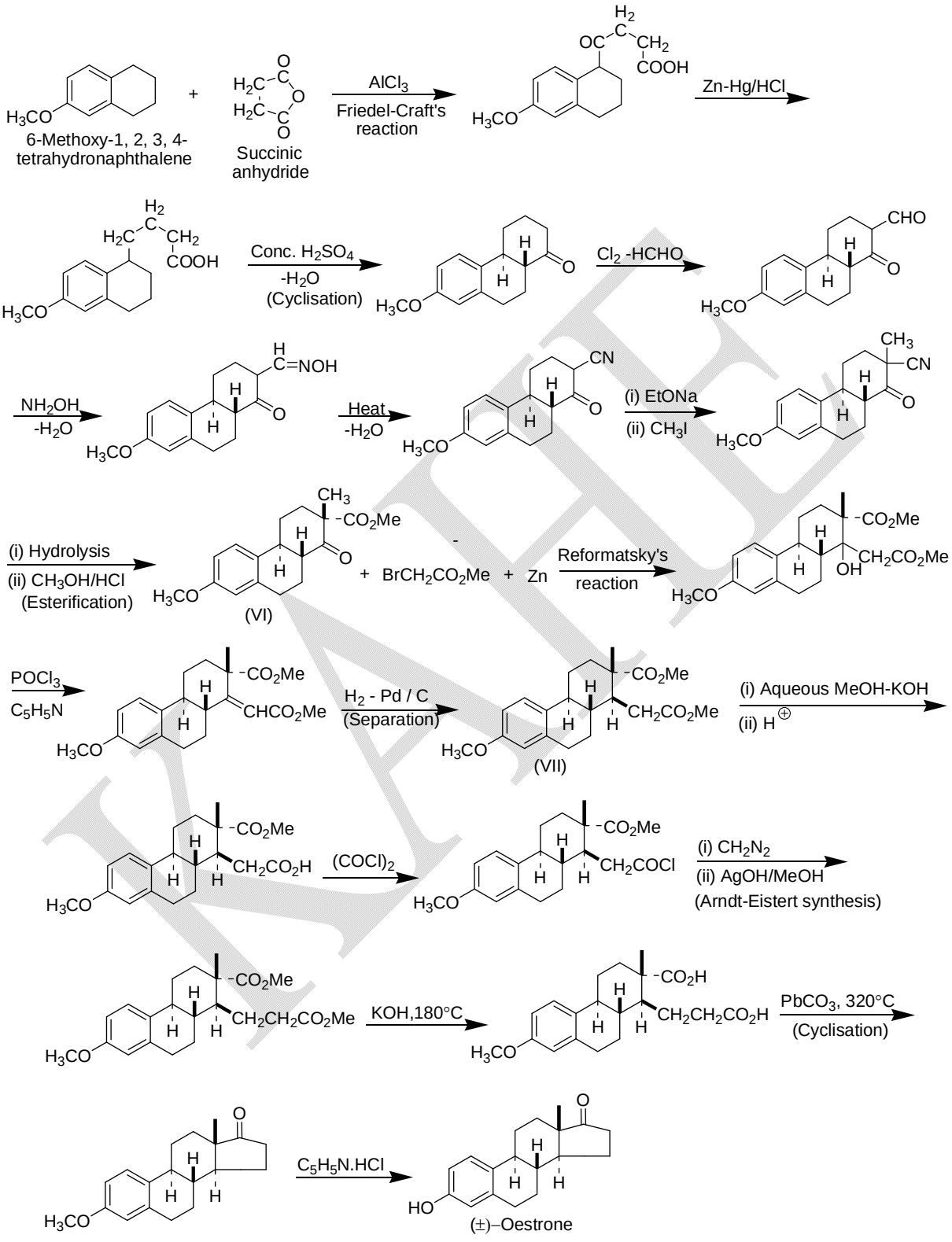
The stereochemical problems involved in the synthesis of oestrone are not so complicated as in cholesterol, since only four chiral centres are present in the hormone. (VI) contains 3 chiral centres are present and so complicated in the hormone. Three have been isolated by Anner and Miescher, and one of these was converted into (±)-oestrone (C/D *trans*) and the stereoisomer (C/D *cis*), (±)-iso-oestrone. These were separated and the (±)-oestrone resolved with (-)-menthoxyacetic acid. The (+)-enantiomer that was obtained was shown to be identical with the natural compound. The *trans*-B/C fusion of the racemate used (for the oestrone synthesis) was deduced from other synthetic work, and the β-configuration of the CH_3-18 had already been established. The catalytic reduction step produced a mixture of stereoisomers (dimethyl esters). These were separated by fractional crystallization and the one chosen for the oestrone synthesis, (VII), was that which was identical with the methyl ether dimethyl ester of 'natural' (+)-*trans*-marrianolic acid.



Methyl ether dimethyl ester of
(+)-*trans*-marrianolic acid

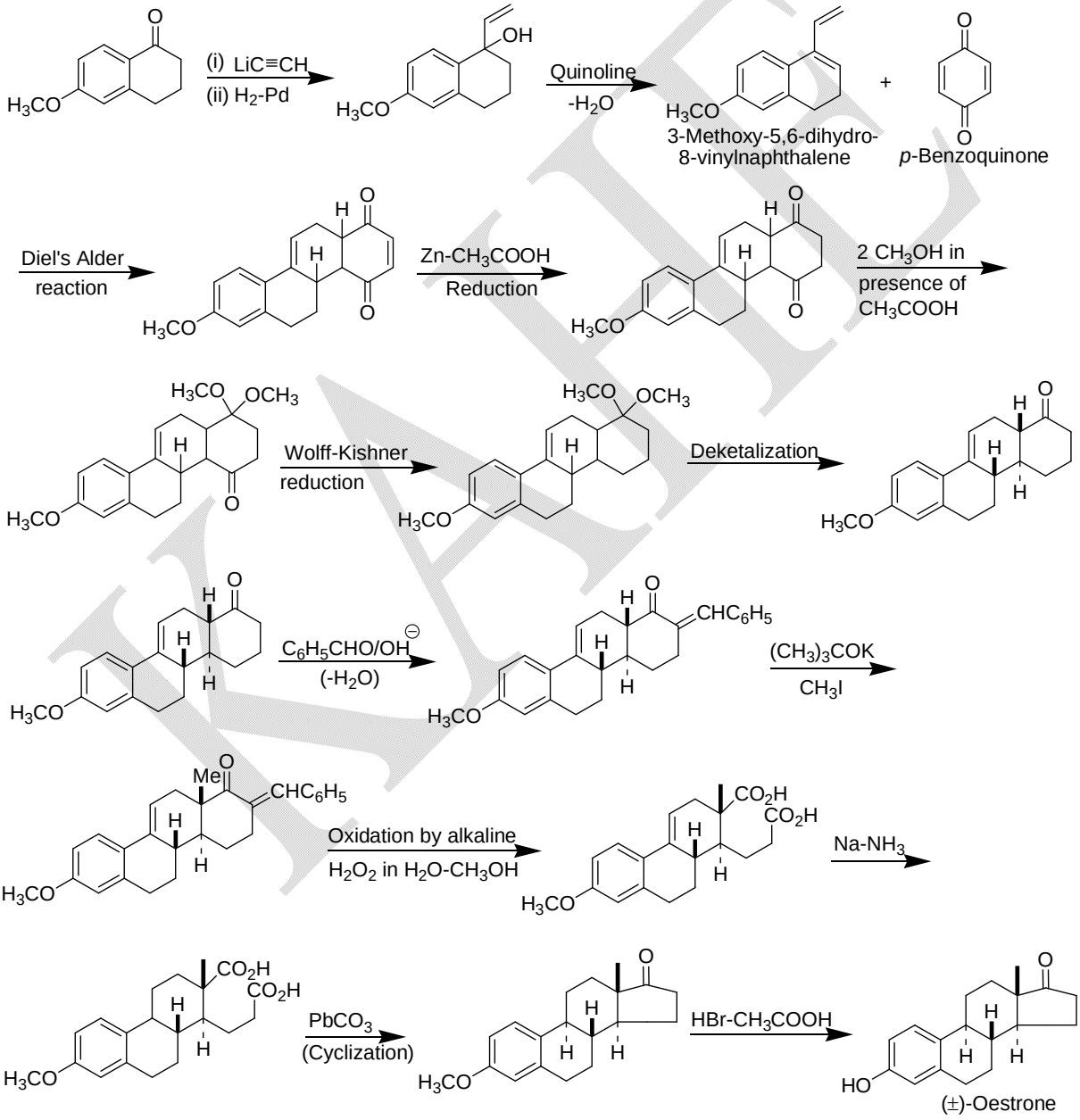
Miescher and Anner have also prepared various isomers of oestrone by using other stereoisomers of (VI) and (VII), e.g., (±)-iso-oestrone (C/D *cis*).

(a) Miescher and Anner synthesis of oestrone

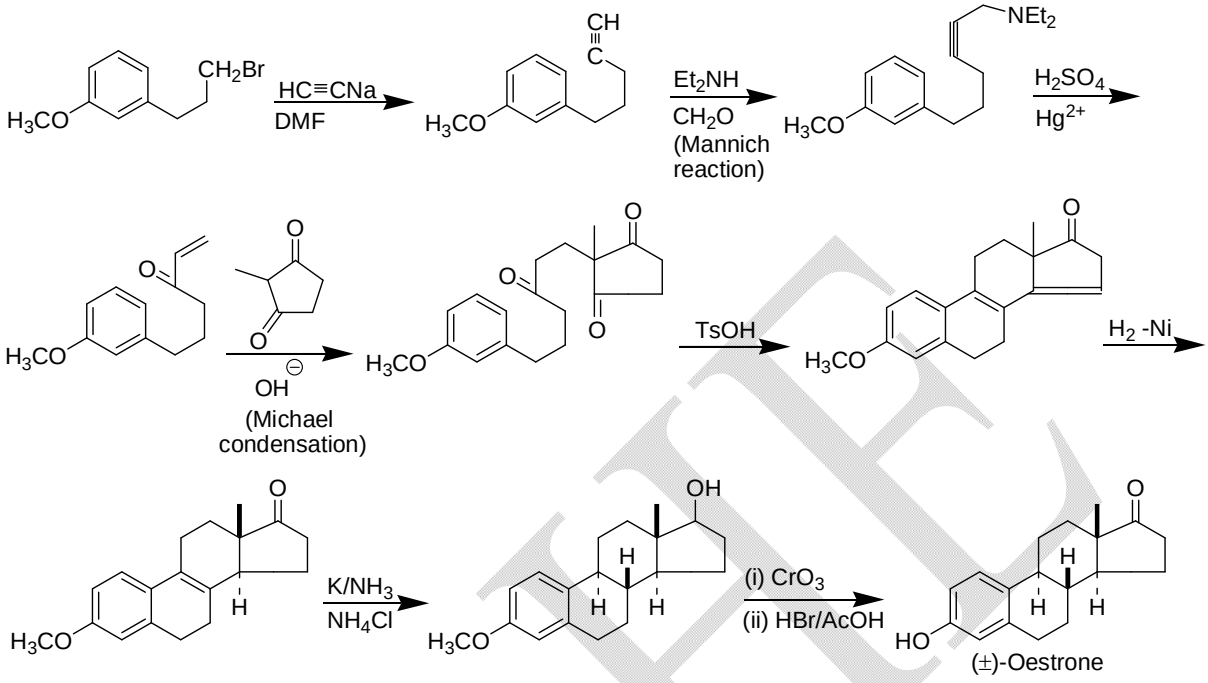

6-Methoxy-1, 2, 3, 4-tetrahydronaphthalene
Succinic anhydride
AlCl3
Friedel-Craft's reaction
Zn-Hg/HCl
Conc. H2SO4
-H2O
(Cyclisation)
Cl2 -HCHO
NH2OH
-H2O
Heat
-H2O
(i) EtONa
(ii) CH3I
(i) Hydrolysis
(ii) CH3OH/HCl
(Esterification)
(VI)
Reformatsky's reaction
POCl3
C5H5N
H2 - Pd / C
(Separation)
(VII)
(i) Aqueous MeOH-KOH
(ii) H⊕
(COCl)2
(i) CH2N2
(ii) AgOH/MeOH
(Arndt-Eistert synthesis)
KOH,180°C
PbCO3, 320°C
(Cyclisation)
C5H5N.HCl
(±)–Oestrone

Johnson *et al.* (1958, 1962) have also carried out a total synthesis of oestrone; each step in their synthesis was stereospecific, but Hughes *et al.* (1960) have described total synthesis of oestrone which appear to be simpler than any previous method and just as efficient. The better method is as follows and involves a Mannich reaction and a Micheal condensation.

(b) Johnson *et al.* synthesis of oestrone

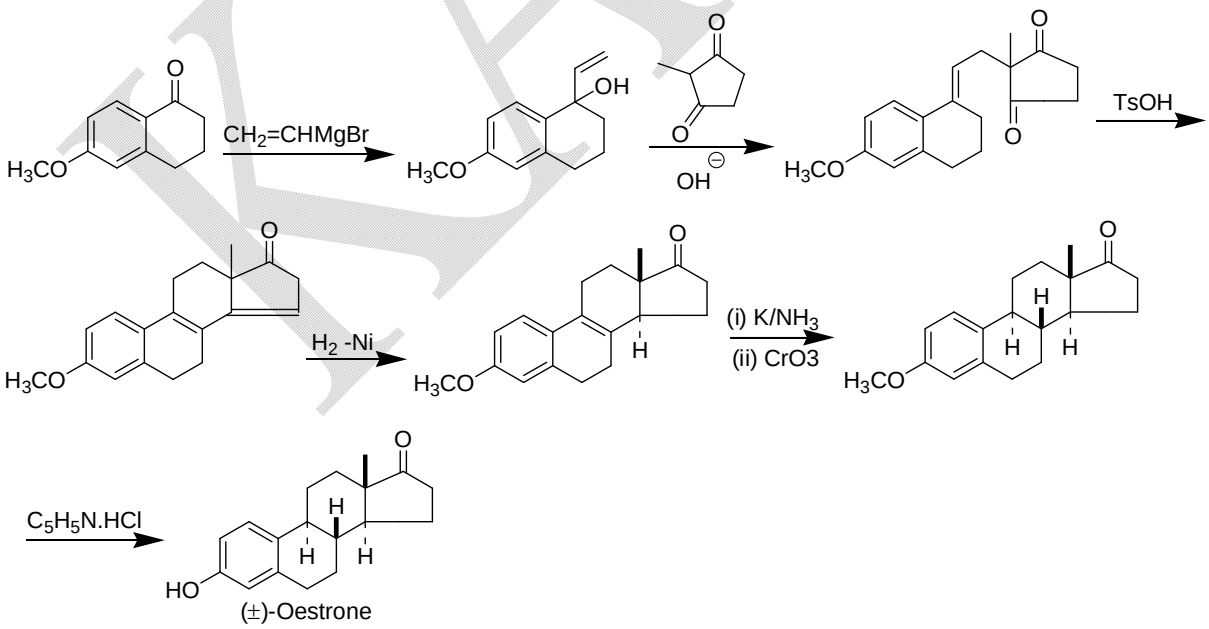


(c) Hughes *et al.* synthesis of oestrone

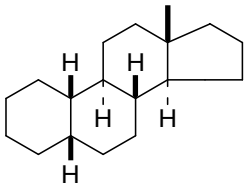


(d) Torgov *et al.* synthesis of oestrone

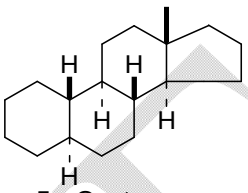
On the other hand, Torgov *et al.* (1960-1962) have synthesized oestrone as follows:



The parent hydrocarbon with a methyl group at C-13 and without a side chain at C-17 is now named *oestrane*, and unsaturation is indicated by the usual systematic terminations, but ambiguity in the numbering is avoided by inclusion of a number of brackets, e.g., oestrone is oestra-1,3,5-(10)-triene-17-one, and oestriol is oestra-1,3,5(10)-triene-3, 16α, 17β-triol.

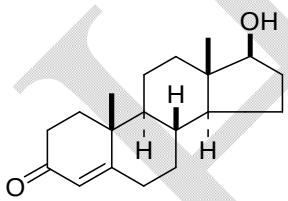


5β-Oestrane



5α-Oestrane

Testosterone



Testosterone

Introduction: There are at least 5 steroidal hormones which exhibit a typical androgenic activity. All are derivatives of androstane. However, the most potent so far is testosterone. Of the other compounds which approach the activity of testosterone, androsterone is the most potent, having approximately $1/7^{th}$ of the activity of testosterone.

Isolation: Testosterone was isolated for the first time by E. Laqueur *et al.* (1935) from testes. From 100 kg of testes they obtained 10 mg of testosterone. The earlier attempts of isolating testosterone failed due to its instability in presence of alkali.

Properties: It melts at 155ºC. It is optically active; $[\alpha]_D = +109^o$. Its λ_{max} is 240 nm.

Physiological action: Testosterone appears to be the real male sex hormone, others are metabolic products of it. The various functions of testosterone are as follows.

(i) This stimulates the development of the secondary male sex characteristics.

(ii) This assists in bringing about the descent of the testes in cryptorchidism.

(iii) This inhibits the secretion of the anterior pituitary gonadotropins (Moore, 1935).

(iv) Testosterone and its derivatives have been found useful in the treatment of advanced metastatic carcinoma of the breast.

(v) Occasionally, testosterone may produce Jaundice.

(vi) Testosterone is used in the treatment of the menopausal syndrome, combined with estrogens.

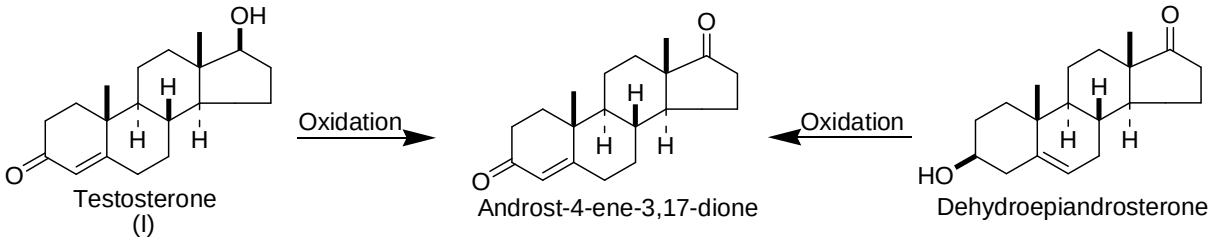
Constitution of Testosterone: This has been eatablished on the basis of following facts:

1. *Molecular formula*. From usual analytical data, it was concluded that the molecular formula of testosterone is $C_{19}H_{28}O_2$

2. *Presence of a tetracyclic system*. The usual tests reveal that testosterone contains one double bond, one ketonic group and one secondary alcoholic group. Further the molecular formula of the parent hydrocarbon corresponds to the general formula having a tetracyclic system.

3. *Presence of α, β-unsaturated ketone group.* As testosterone is very sensitive to alkali, this reveals that it contains α, β-unsaturated ketonic group. The presence of this group has been further proved by its UV spectrum (λ_{max} = 240 nm). The UV spectrum and its sensitivity alkali strongly supported the fact that testosterone is structurally related to progesterone.

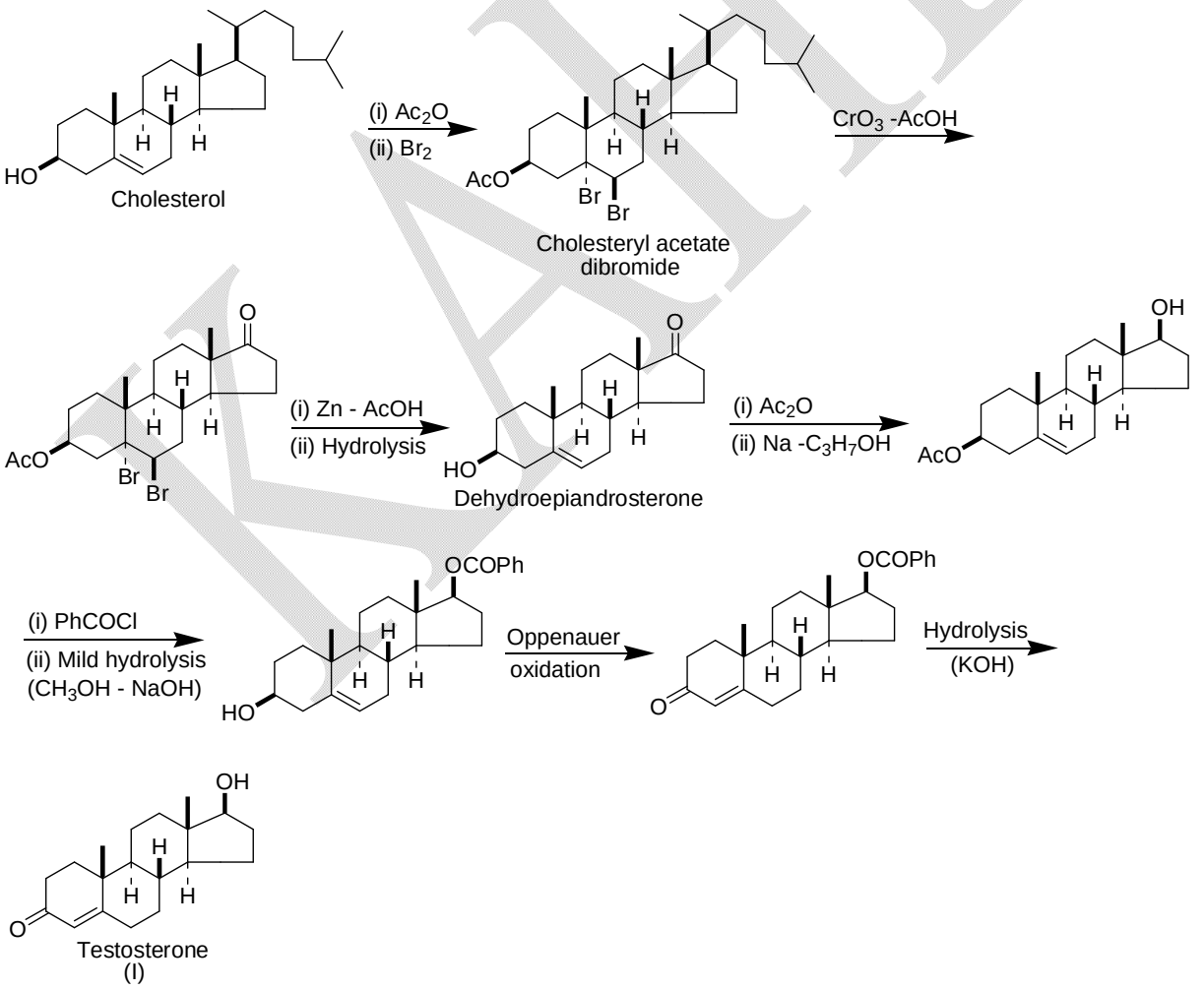
4. *Oxidation*. In 1935, K. David oxidised testosterone to a diketone, androst-4-ene-3,17-dione, a compound of known structure. The latter compound is also obtained by the Oppenauer oxidation of dehydroepiandrosterone. The formation of this diketone compound could only be explained if (I) is the structure of testosterone.



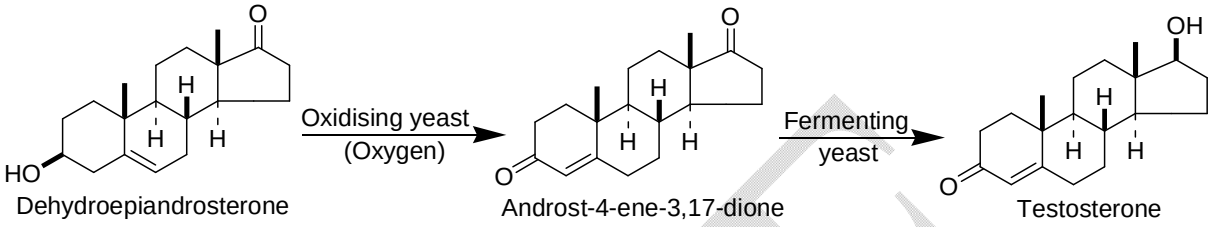
Testosterone (I) — Androst-4-ene-3,17-dione — Dehydroepiandrosterone

5. *Synthesis*. The structure of (I) has been confirmed by its synthesis from cholesterol by Butenandt (1935) and Ruzicka (1935). The Oppenauer oxidation step in this method was introduced by Oppenauer (1937).

This preparation of testosterone establishes the structure of this hormone which had been shown to contain one hydroxyl group and an α, β-unsaturated ketone group.



This method has been improved by Mamoli (1938), who converted dehydroepiandrosterone into testosterone by means of micro-organisms; the first stage uses an oxidising yeast in the presence of oxygen, and the second stage fermenting yeast.

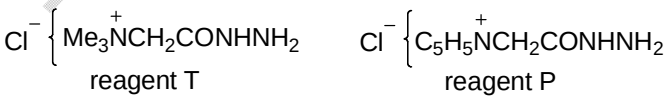


Dehydroepiandrosterone Androst-4-ene-3,17-dione Testosterone

Elisberg *et al.* (1952) have shown that sodium borohydride selectively reduces the 3-keto group in the presence of others at 11, 12, 17 or 20. On the other hand, Norymberski *et al.* (1954) have shown that if there is a double bond in position 4,5 then the keto group at 17 or 20 is preferentially reduced to that at 3. Thus androst-4-ene-3,17-dione is reduced to testosterone by sodium borohydride. Johnson *et al.* (1960) have adapted Johnson's synthesis of equilenin to provide an improved synthesis of testosterone.

6. *Stereochemistry*. The stereochemistry of testosterone, except for the configuration of the hydroxyl group at C-17, is established by its preparation from cholesterol. The C-17 hydroxyl group was shown to have the β-configuration by molecular rotation measurements and by the examination of the rates of hydrolysis of various testosterone esters.

It appears that testosterone is the real male sex hormone in the body; the others are metabolic products of testosterone. The ketonic steroids are separated from the non-ketonic steroids (all from urine) by means of Girard's reagents (P and T); the ketonic compounds form soluble derivatives, and may be regenerated by hydrolysis. Many other hormones have also been isolated from urine.

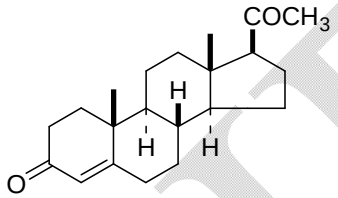


Many commercial preparations are now carried out by means of microbiological transformations. The more important ones in steroid chemistry include oxidations (oxidation of alcohols, hydroxylation, epoxidation, dehydrogenation); reductions (carbonyl to hydroxyl,

saturation of an ethylenic double bond); esterification and hydrolysis; isomerisations; resolution of (±)-modifications.

Mamoli's method described above has now been replaced by more efficient non-microbiological methods.

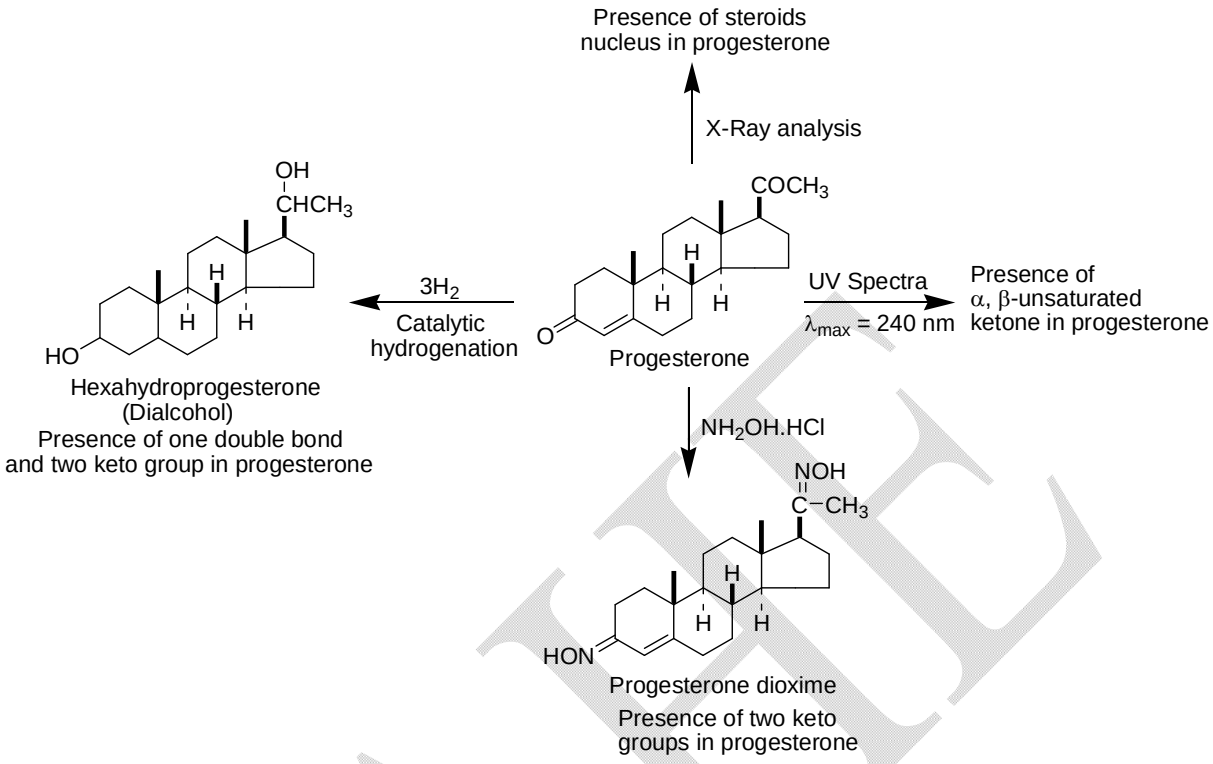
Progesterone



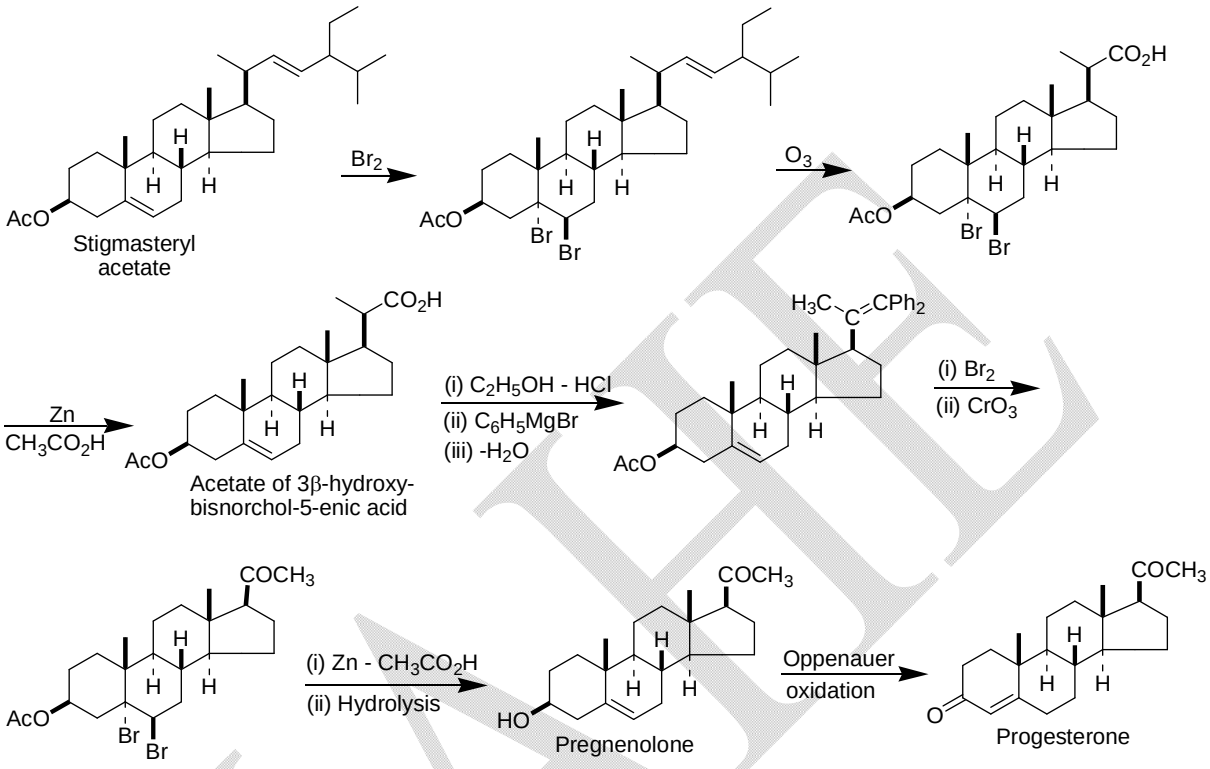
Progesterone

Molecular formula: $C_{21}H_{30}O_2$, m.p.128ºC, $[\alpha]_D$ +192º. This was first isolated in a pure form by Butenandt *et al.* (1934) from the *corpora lutea* of pregnant sows.

The chemical reactions of progesterone show that there are two keto groups present, and since on catalytic reduction three molecules of hydrogen are added to form the dialcohol $C_{21}H_{36}O_2$, it therefore follows that progesterone contains one double bond (four hydrogen atoms are used to convert the two keto groups to alcoholic groups). Thus the parent hydrocarbon of progesterone is $C_{21}H_{36}$, and since this corresponds to the general formula C_nH_{2n-6}, progesterone is therefore tetracyclic (D.B.E of $C_{21}H_{36}O_2$ is 21 + 1 – 36/2 =4 rings). Furthermore, X-ray studies have shown that progesterone contains the steroid nucleus, and this is further supported by the fact that progesterone may be prepared from e.g., stigmasterol and cholesterol. These preparations also show the structure of progesterone, but do not provide conclusive evidence for the position of the double bond in progesterone, since the results can be interpreted equally well on the assumption that the double bond is 4,5 or 5,6. The absorption spectrum of progesterone, however, shows that it is an α,β-unsaturated ketone (λ_{max} 240 nm), and this suggests that the position of the double bond is 4,5 (see below). Finally, progesterone has also been synthesised from diosgenin and from pregnanediol, and the preparation from the latter, taken in conjunction with the others, definitely shows that the position of the double bond in progesterone is 4,5.

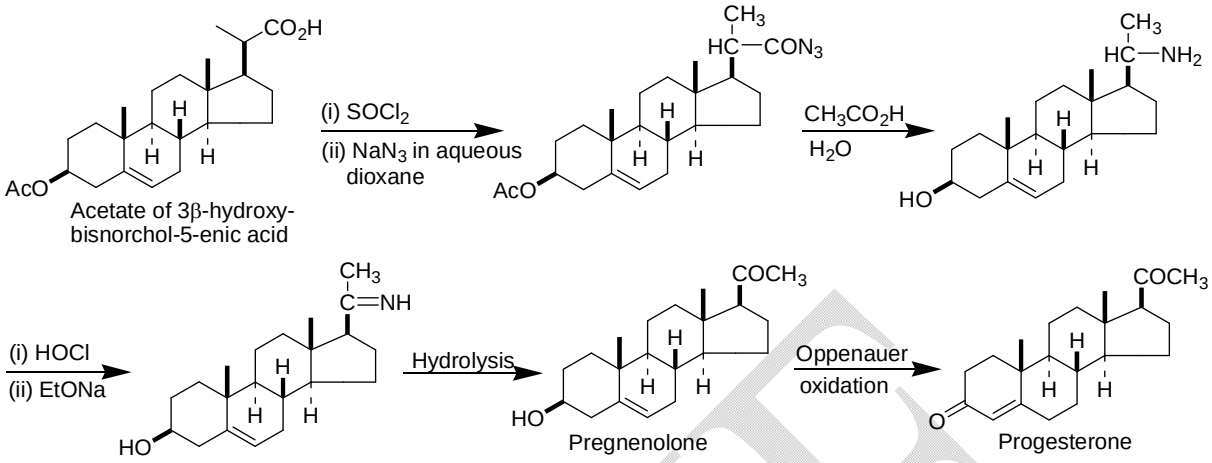

Presence of steroids nucleus in progesterone
X-Ray analysis
COCH3
OH
CHCH3
3H2
Catalytic hydrogenation
UV Spectra
λmax = 240 nm
Presence of α, β-unsaturated ketone in progesterone
Progesterone
Hexahydroprogesterone (Dialcohol)
Presence of one double bond and two keto group in progesterone
NH2OH.HCl
NOH
C−CH3
HON
Progesterone dioxime
Presence of two keto groups in progesterone

(i) **Progesterone from stigmasterol** (Butenandt *et al.*, 1934, with improvements by other workers)

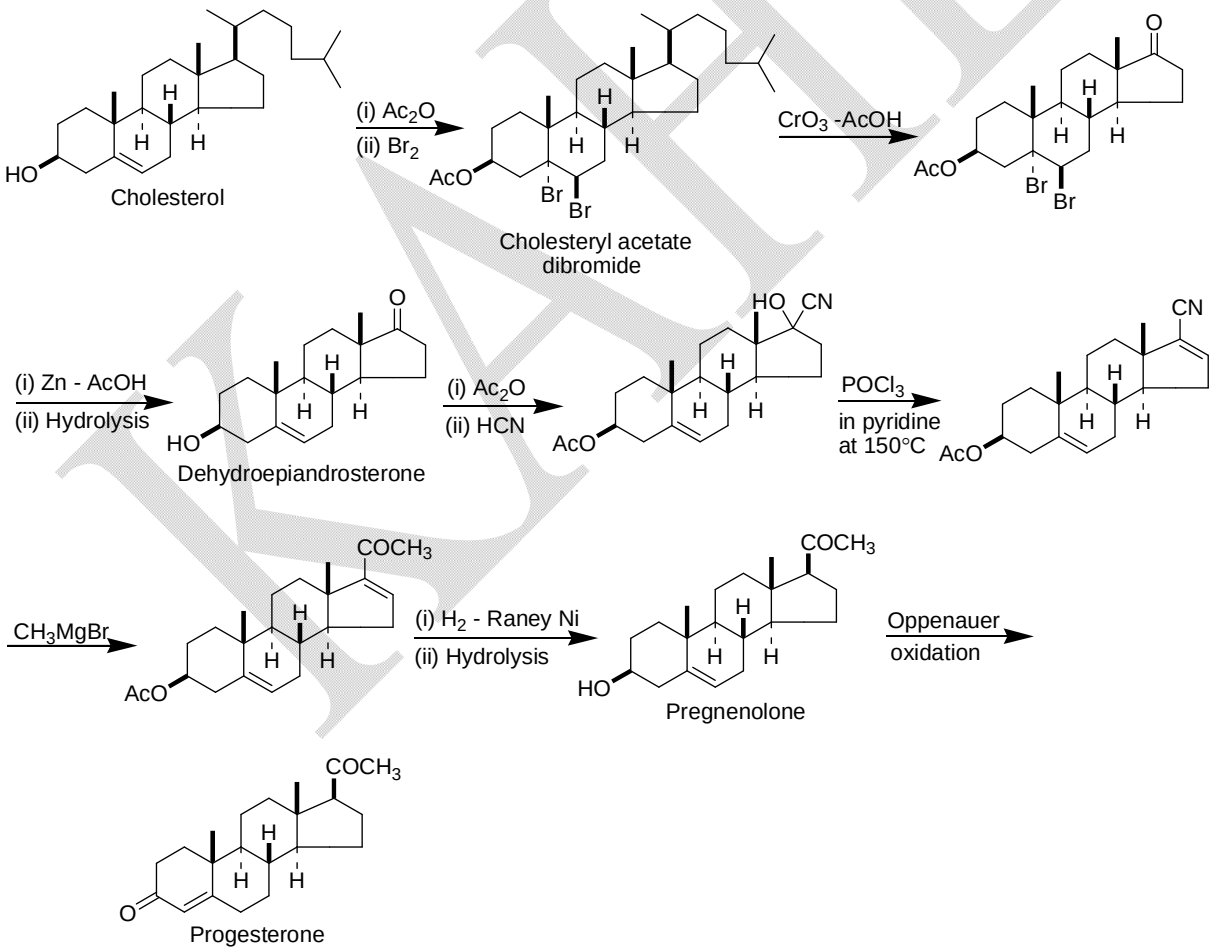


Pregnenolone has also been isolated from the *corpus luteum.*

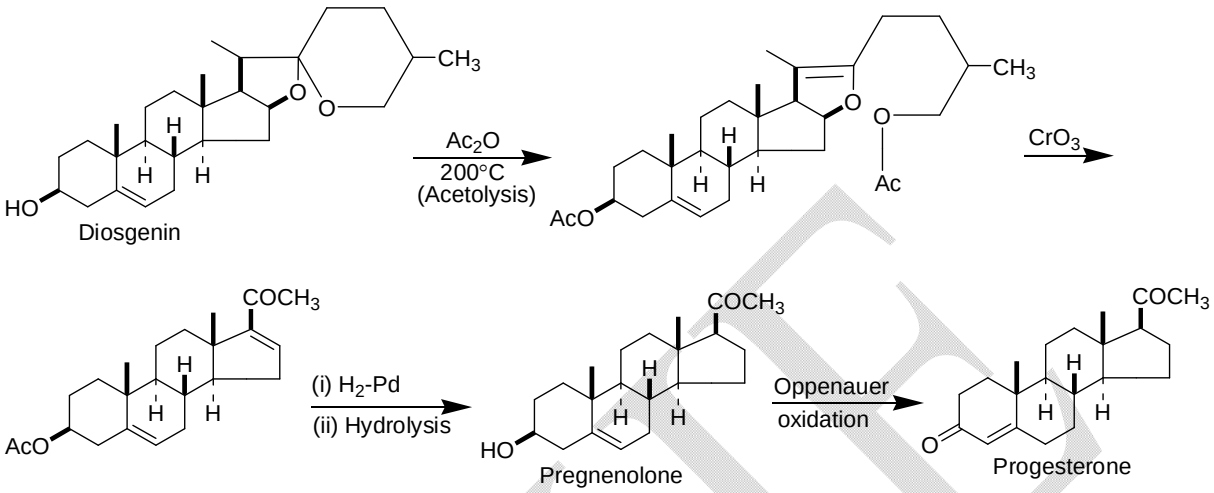
Acetate of 3β-hydroxybisnorchol-5-enic acid obtained in step (i) is converted into pregnenolone by a modified Curtius degradation. The overall yield is in the order of 50-55%. Finally, pregnenolone when subjected to Oppenauer oxidation yields Progesterone.



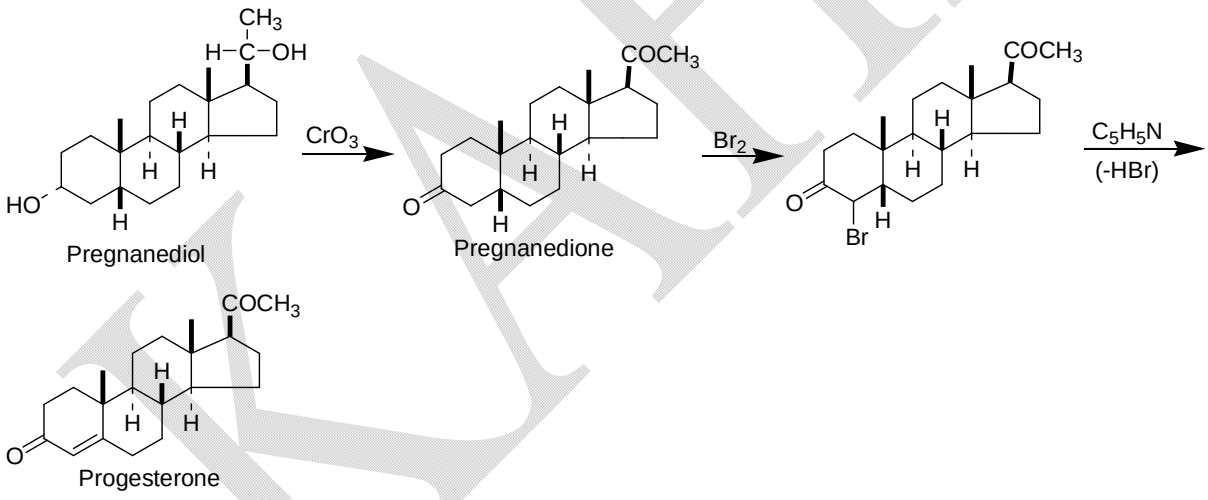
(ii) Progesterone form cholesterol (Butenandt *et al.,* 1939). Cholesterol is converted into dehydroepiandrosterone, and then as follows:



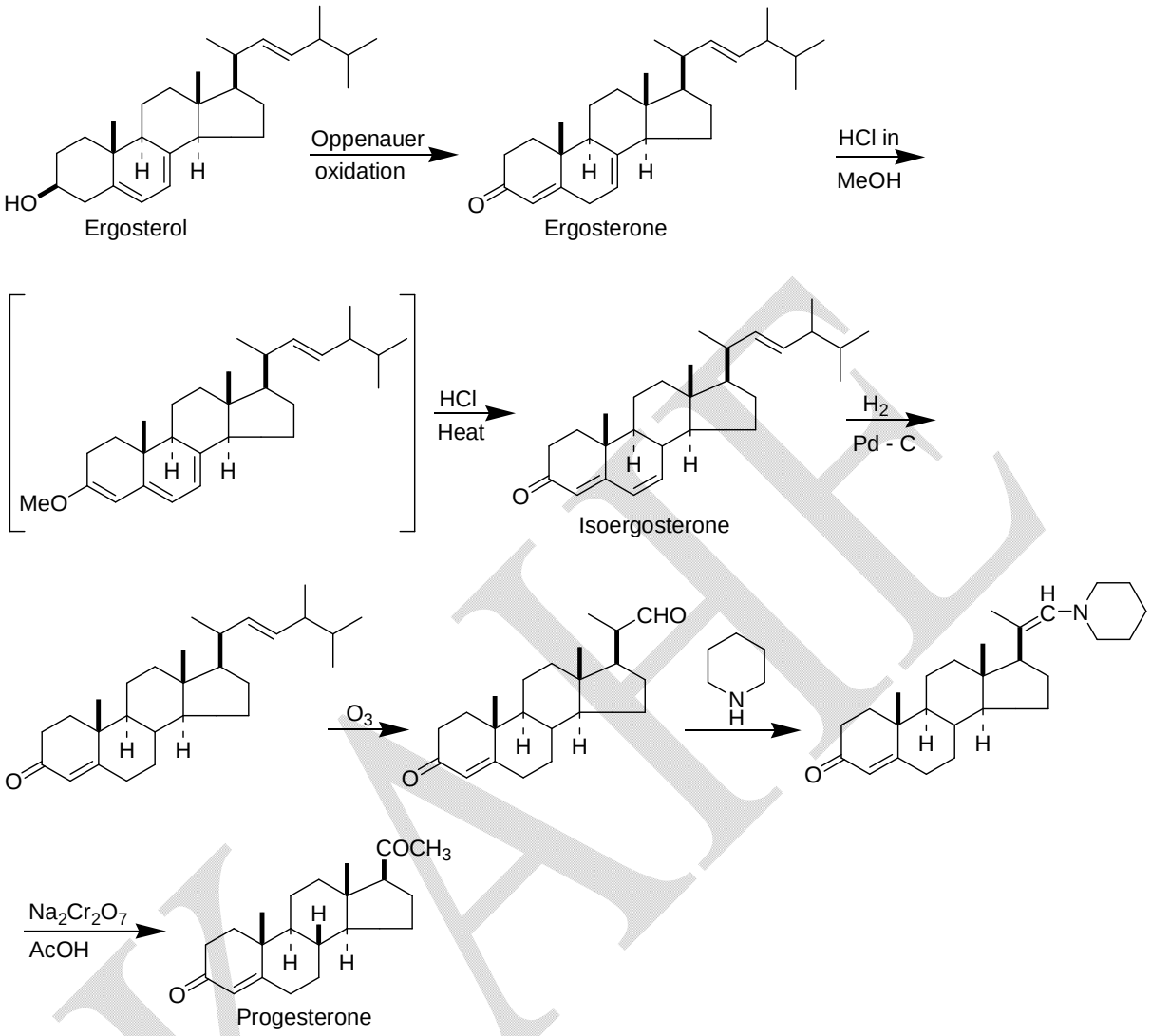
(iii) Progesterone from diosgenin (Marker *et al.,* 1940, 1941). Diosgenin (a sapogenin) occurs as a glycoside in the root of *Trillium erectum*.



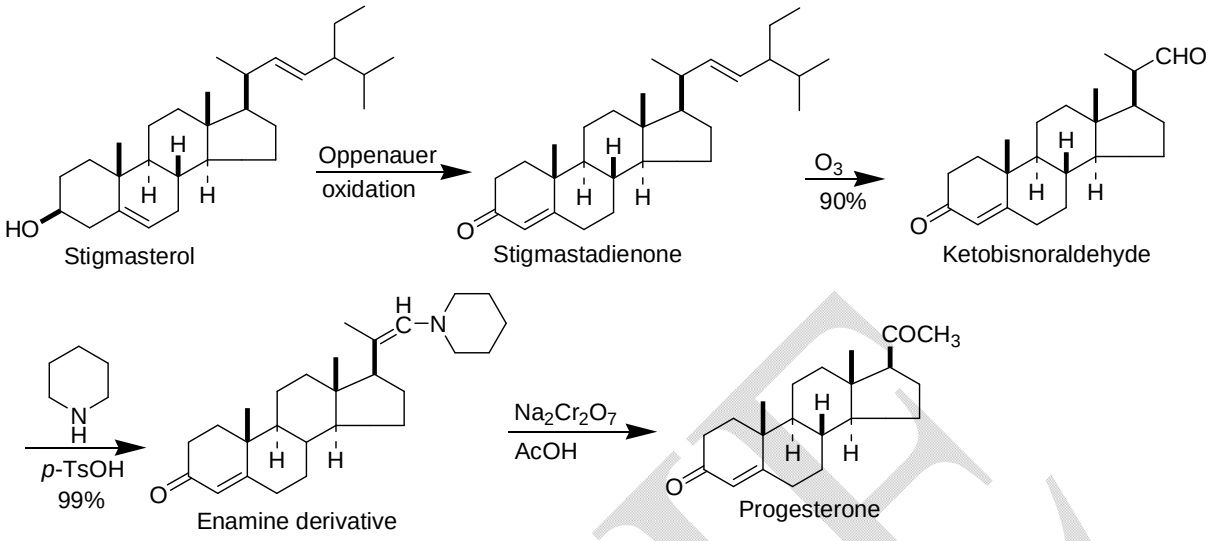
(iv) Progesterone from pregnanediol (Butenandt *et al.,* 1934).



(v) Progesterone from ergosterol (shepherd *et al.,* 1955). This appears to be the most practical synthesis (note the enamine step).



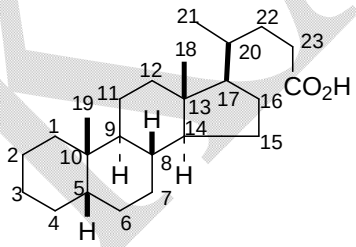
(vi) Progesterone from stigmastadienone. Stigmasterol is readily obtained from the abundant soyabean oil. Stigmasterol on oxidation yields stigmastadienone. The synthesis of progesterone from stigmastadienone involves the following steps.



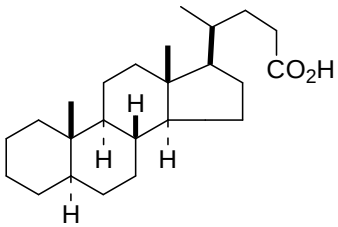
Bile acids

Introduction

The bile acids occur in bile (a secretion of the liver which is stored in the gall-bladder) of most animals combined as amides with either glycine ($NH_2CH_2CO_2H$) or taurine ($NH_2CH_2CH_2SO_3H$). e.g., glycocholic acid (= glycine + cholic acid), taurocholic acid (= taurine + cholic acid). The bile acids are present as sodium salts, and they functions as emulsifying agents in the intestinal tract, e.g., fats, which are insoluble in water, are rendered 'soluble', and so may be absorbed in the intestine.



5β-Cholanic acid
(Cholanic acid)



5α-Cholanic acid
(Allocholanic acid)

Most of the bile acids are hydroxy-derivatives of either 5β-cholanic acid or 5α-cholanic acid. Dehydration of a bile acid by heating in a vacuum, followed by catalytic reduction, gives either 5β-cholanic or 5α-cholanic acid.

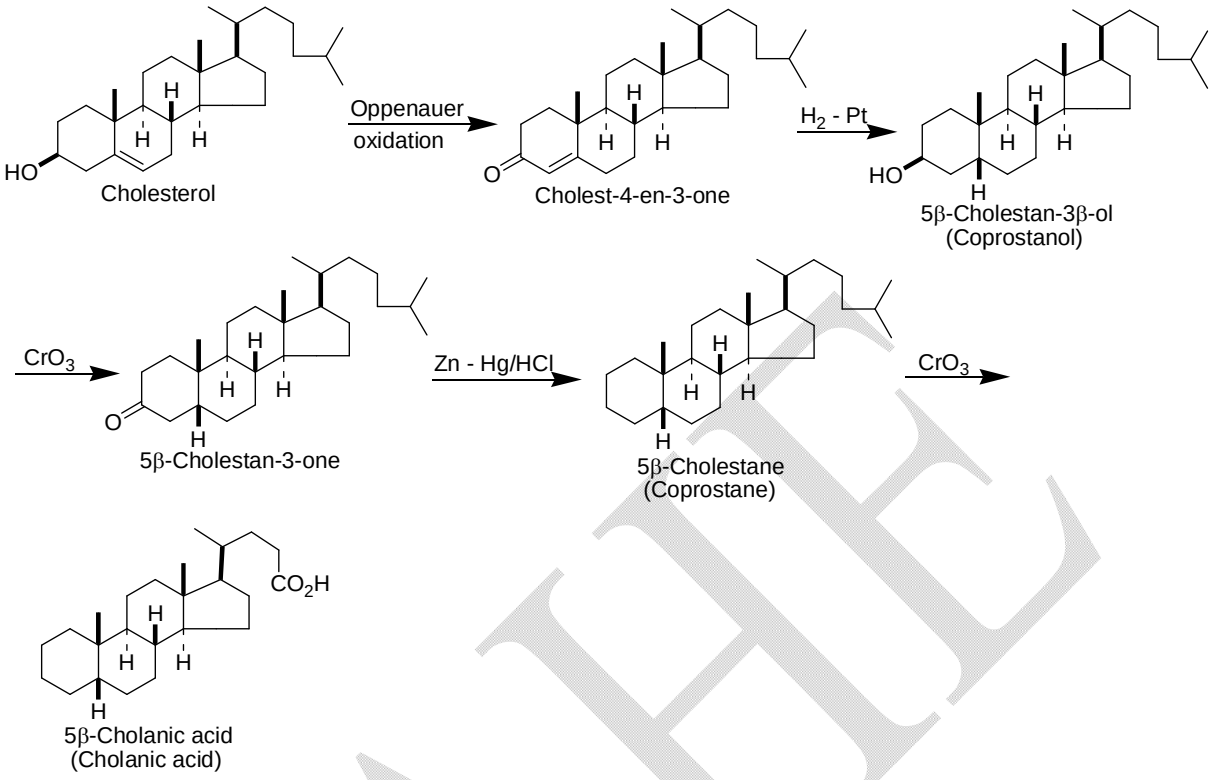
About twenty natural bile acids have been characterised, and many others are synthetic. The positions of the hydroxyl groups are any of the following; 3, 6, 7, 11, 12 and 23, and in almost all of the natural bile acids the configurations of the hydroxyl groups are α. Some of the more important natural bile acids are:

Name	M.P. ºC	Hydroxyl groups	Source	$[\alpha]_D^o$
Cholic acid	195	3α, 7α, 12α	Man, ox	+37
Deoxycholic acid	172	3α, 12α	Man, ox	+53
Lithocholic acid	186	3α	Man, ox	+32
Chenodeoxycholic acid	140	3α, 7α,	Man, ox, hen	+11
α-Hyodeoxycholic acid	197	3α, 6α,	Pig	+8

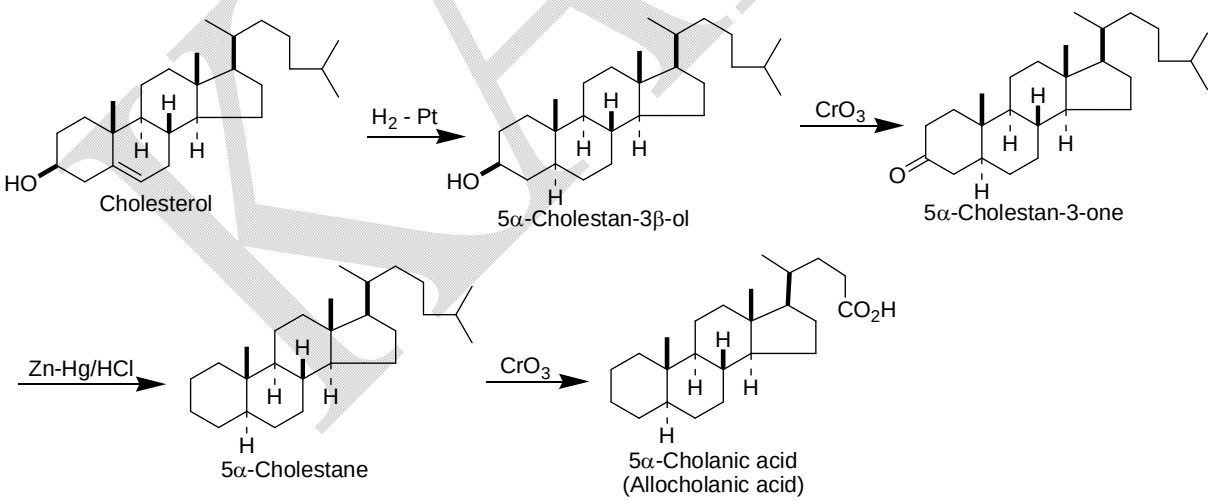
The structures of 5β-cholanic acid (cholanic acid) and 5α-cholanic acid (allocholanic acid)

These acids may be derived from 5β-cholestane (coprostane) and 5α-cholestane, respectively, as follows. At the same time, these reactions show the relationship between the bile acid and the sterols (Windaus, 1919).

5β-Cholanic acid, m.p. 164°C, $[\alpha]_D$ +22°

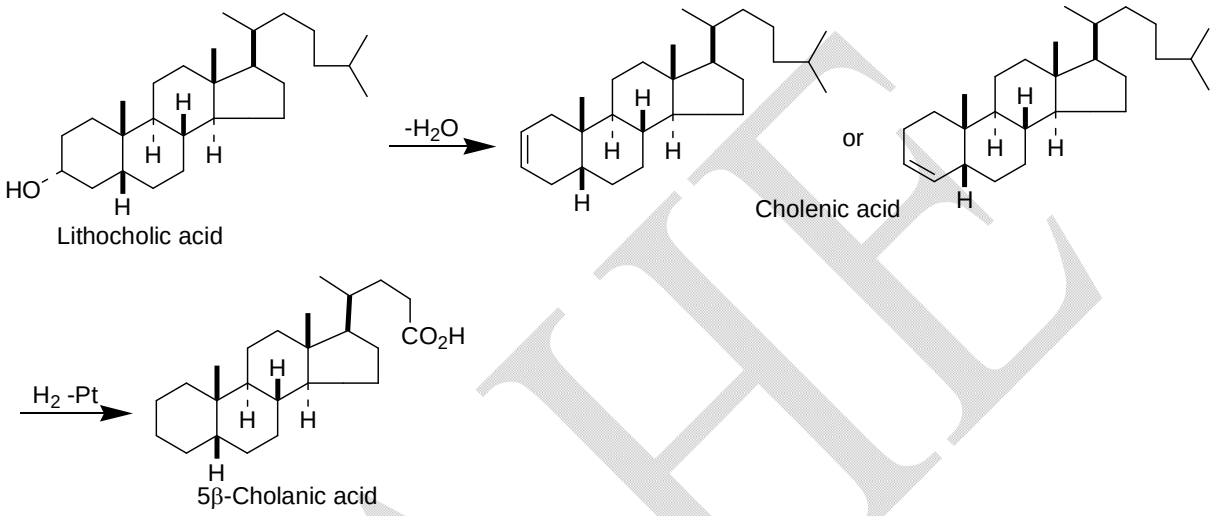


5α-Cholanic acid, m.p. 173°C, $[\alpha]_D$ +22°



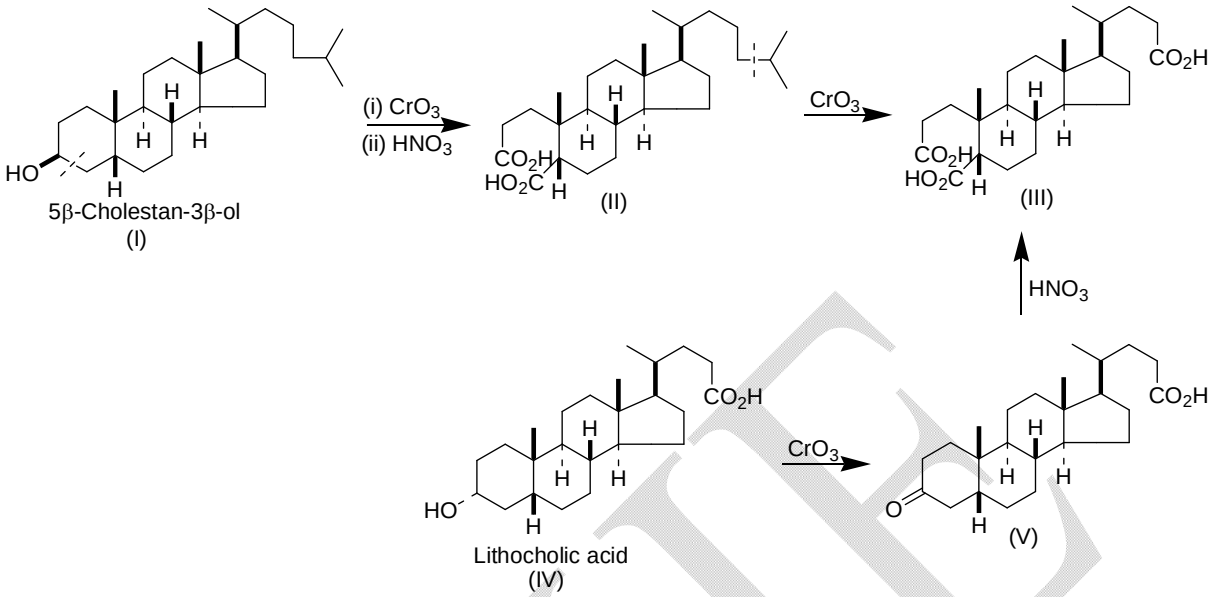
Structure of the bile acids

Since all the bile acids can be converted into either of the cholanic acids, the former are therefore hydroxy-derivatives of the latter, e.g., lithocholic acid can be converted into 5β-cholanic acid as follows:

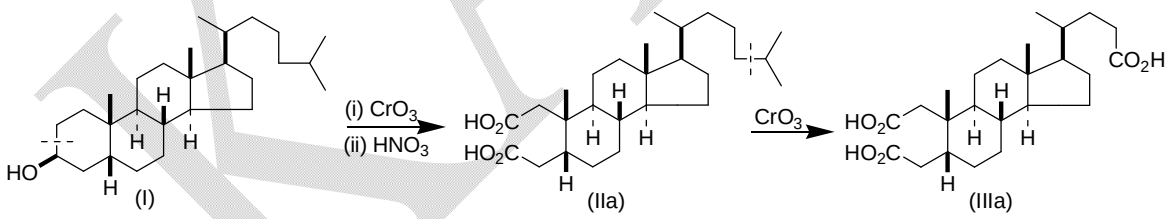


According to Fieser *et al.* (1955), cholenic acid is a mixture of the two compounds shown, the chol-3-enic acid being the main constituent.

The positions of the hydroxyl groups in the bile acids have been determined by means of oxidative degradation, e.g., the position of the hydroxyl group in lithocholic acids is shown to be at 3 as follows. Cholesterol can be converted into 5β-cholestan-3β-ol (I) which, on oxidation with chromium trioxide, forms a ketone and this, when oxidised with nitric acid, gives a dicarboxylic acid (II). (II) On further oxidation with nitric acid, produces the tricarboxylic acid, lithobilianic acid (III). Lithocholic acid (IV), on oxidation with chromium trioxide, forms dehydrolithocholic acid (V) and this, when oxidised with nitric acid, forms (III). It therefore follows that the hydroxyl group in lithocholic acid is probably in the same position as in 5β-cholestan-3β-ol, viz., position 3. Thus:



The above evidence is not conclusive, since had the hydroxyl group in lithocholic acid been at position 4, (III) could still have been obtained. In practice, however, the oxidation of (I) produces two isomeric acids for (II), one being (II) as shown, and the other (IIa) in which the ring A is opened between C-2 and C-3; this acid, on further oxidation, gives isolithobilianic acid (IIIa). Since the oxidation of lithocholic acid (IV) also produces a mixture of the same two acids, (III) and (IIIa), there can be no doubt that the hydroxyl group is at position 3.



The configuration of the hydroxyl group in lithocholic acid has been shown to be α by, e.g., the oxidative degradation of the acetates of lithocholic acid and 5β-cholestan-3α-ol (epicoprostanol) to 5β-androsterone (5-isoandrosterone). Since all of the natural bile acids except one ('β' hyodeoxycholic acid) can be converted into lithocholic acid, all have therefore the α-configuration for the hydroxyl group at C-3.

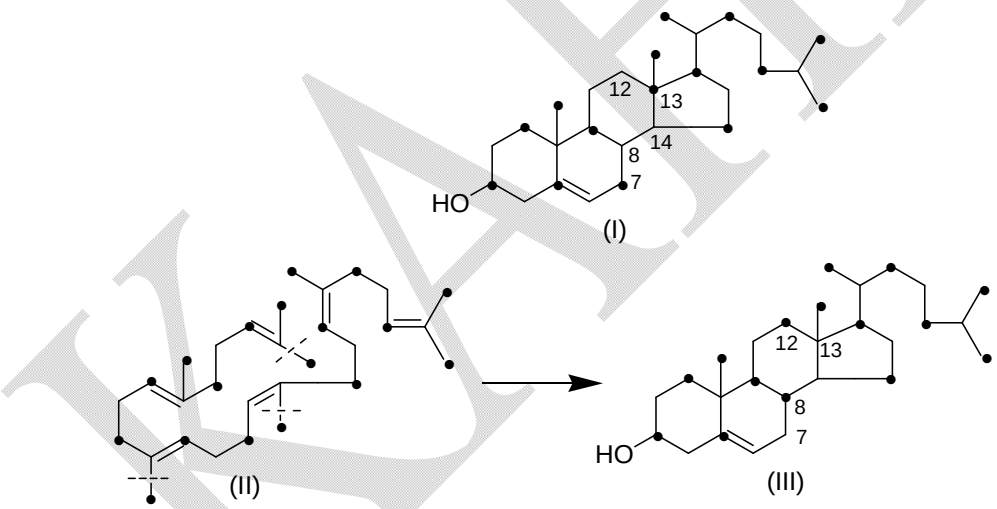
The bile acids form molecular compounds with various substances. Cholic acid, in particular, forms these molecular compounds with such compounds as fatty acids, esters, alcohols, etc; these are known as the choleic acids. These choleic acids are of the channel complex type.

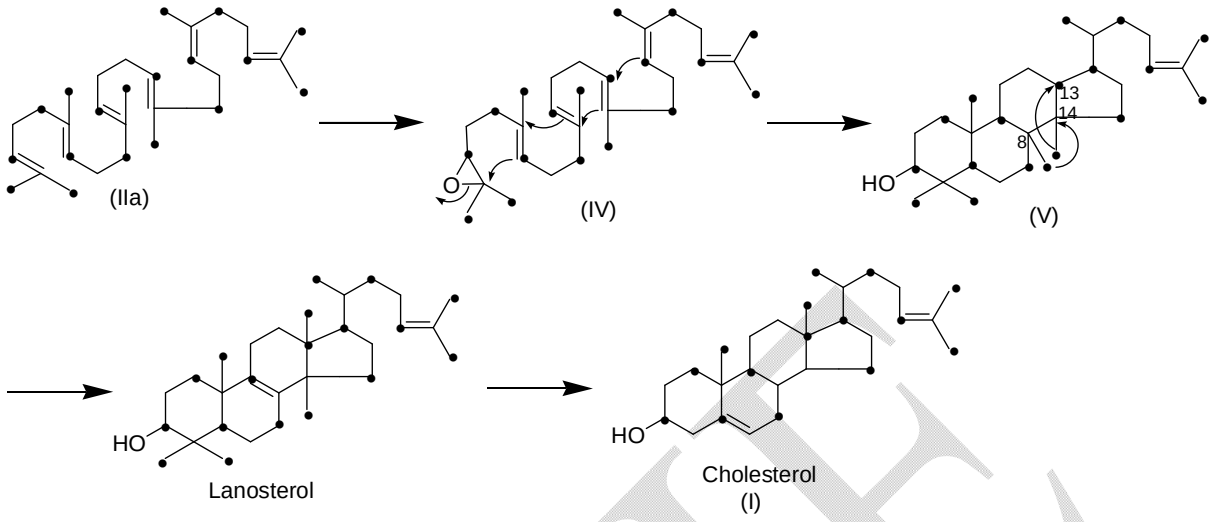
The bile acids discussed in the foregoing account are all derivatives of 5β-cholanic or 5α-cholanic acid. There are however, some bile acids which are not derivatives of the cholanic acids, e.g., in the bile of crocodiles there is the bile acid 3α,7α,12α-trihydroxycoprostanic acid, $C_{27}H_{46}O_5$.

Biosynthesis of sterols

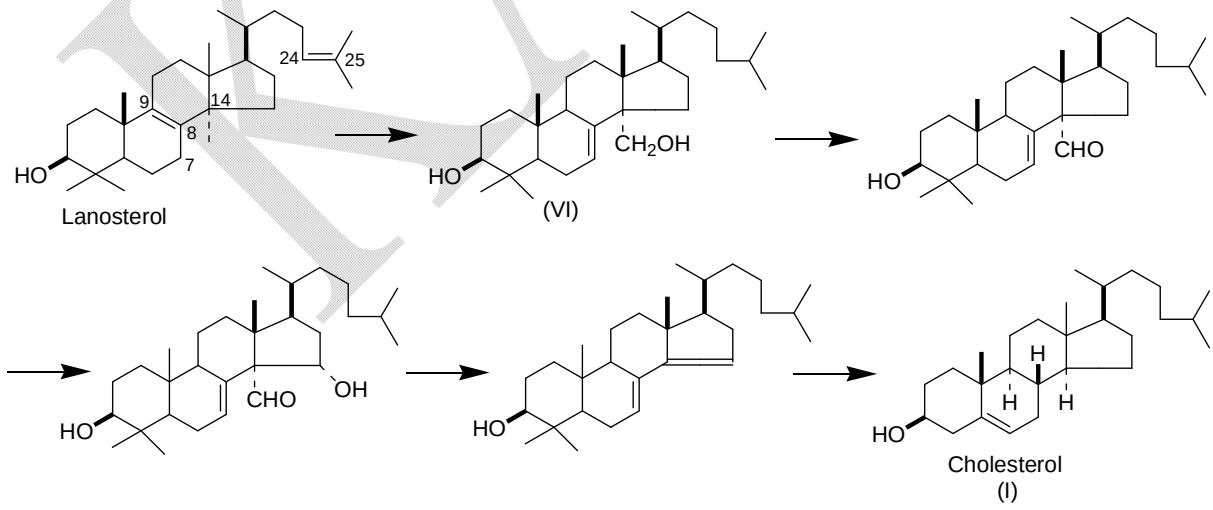
It has long been known that animals can synthesise cholesterol, but the possible pathways were unknown until biosynthetic cholesterol was prepared from acetic acid labelled isotopically (with ^{14}C) in either the methyl or the carboxyl group, or labelled in both groups ($^{13}CH_3{}^{14}CO_2H$). These tracer studies were carried out mainly by Bloch *et al.* (1942) and by Cornforth *et al.* (1953), and the results established that the distribution of the carbon atoms is as shown in (I), in which carbon atoms derived from the methyl group of acetic acid are indicated by dots. Thus acetic acid can be regarded as the fundamental unit. Evidence was also obtained that isovaleric acid can serve as a precursor for cholesterol, and then Tavormina *et al.* (1956), using labelled mevalonic acid (MVA), showed that this is converted almost completely into cholesterol by rat liver; the route from acetic acid to MVA. The problem now is to discover the route whereby MVA is converted into cholesterol. As far back as 1926 Heilbron *et al.* suggested that squalene is a precursor of cholesterol, and Robinson (1934) proposed a scheme for the cyclisation of the squalene molecule with the loss of three methyl groups. Biosynthetic experiments have established that squalene is produced by the linkage of two farnesyl residues joined tail to tail and that the methyl group distribution is as shown in (II). Cyclisation with loss of the three methyl groups (indicated by broken lines) proposed by Robinson (before the labelled distribution in cholesterol was known) was formulated as (II) → (III). Comparison of formula (III) with (I) shows that the former is incorrectly labelled at C-7, C-8, C-12 and C-13. Furthermore, since Bloch *et al.* (1952) showed experimentally that squalene is a precursor of cholesterol, the Robinson scheme of cyclisation is untenable. Woodward *et al.* (1953), however, suggested that

squalene is first cyclised to lanosterol, and then this loses three methyl groups to give cholesterol. Furthermore, Bloch *et al.* (1955) showed that lanosterol is converted into cholesterol in rats, and in 1956 carried out the biosynthesis of lanosterol from labelled acetate. Thus we have evidence for the suggested route from squalene to cholesterol. As mentioned above, Woodward *et al.* (1953) suggested that squalene ring-closes to form lanosterol, and proposed a 1,3-shift of the methyl group at C-8 to C-13. On the other hand, Ruzicka *et al.* (1955) and Block *et al.* (1957) proposed a 1,2 shift of the methyl group from C-14 to C-13 and another 1,2-shift from C-8 to C-14. Further work by Bloch *et al.* (1958) showed that the 1,2 shifts were correct; this is supported by the work of Cornforth *et al.* (1958). Also, van Tamelen *et al.* (1966, 1967) and Corey *et al.* (1967) have now shown that 2,3-epoxy-squalene is an intermediate in the conversion of squalene into lanosterol. The various steps (under the influence of the appropriate enzymes) are believed to be as shown.





In the conversion of lanosterol into cholesterol, the methyl groups at C-4', 4', and 14' are removed. Bloch *et al.* (1957) assumed these were eliminated as carbon dioxide via oxidation to carboxyl groups, the C-14 methyl group being removed first. There is now a great deal of evidence to support this sequence and for the removal of the C-4' and 4' methyl groups, but Barton *et al.* (1971, 1972) have shown that the C-14 methyl group is removed as formic acid via oxidation to the aldehyde. It is believed that (VI) is formed lanosterol; this involves migration of the double bond from 8,9 to 7,8 oxidation of the CH_3 at C-14 to CH_2OH, and saturation of the double bond at 24, 25 (R = -$CHMe(CH_2)_3CHMe_2$).



The biosynthesis of ergosterol from acetate has been carried out by Bloch *et al.* (1951), and the distribution pattern corresponds to that of cholesterol. Hanahan *et al.* (1953) showed that, except for CH_3-28, the carbon skeleton of ergosterol appears to be formed from the cyclisation of squalene. The carbon atom that produces CH_3-28, however, arises by an independent route. It has been found that formate and, better still, methionine (an amino-acid) are sources of CH_3-28.

Text Books:

1. Finar, I. L. (2013). *Organic Chemistry Vol. II: Stereochemistry and the Chemistry of Natural Products* (V Edition). New Delhi: Pearson Education, Ltd.

2. Chatwal, G. R. (2015). *Organic Chemistry of Natural Products Vol. II*. New Delhi: Himalaya Publishing House.

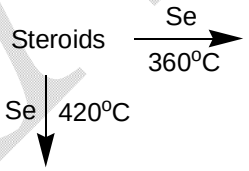
POSSIBLE QUESTIONS

PART- A – Multiple Choice Questions

(Each Question Carry One Mark) (Online Examinations)

PART-B (Each Question Carry Two Marks)

1. Write the structure of the expected product in the following reaction.



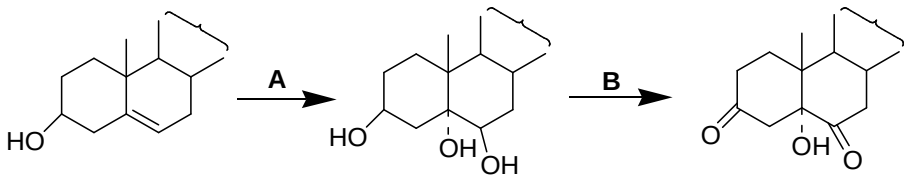
2. Explain what is Salkowski reaction?
3. Explain the Libermann-Burchard reaction?
4. Define Blanc's rule?
5. Write the structures of bile acid?
6. Explain the synthesis of 7-methyl-1, 2-cyclopentenophenathrene?
7. Write the conversion of Pregnanediol to Progesterone?
8. Write the structure of Equilenin and Oestrone?

PART-C (Each Question Carry Six Marks)

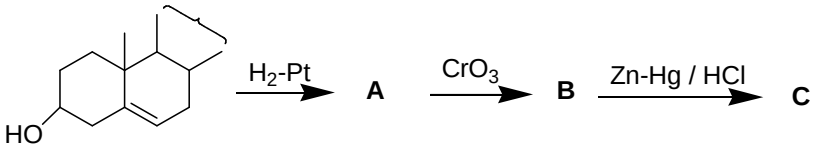
1. ((i)Explain the nature of side chain and position of double bond in Ergosterol.
   (ii) How will you convert Cholesterol into Progesterone?
2. Explain the positions of the hydroxyl group and double bond in Cholesterol.
3. (i) Write the conversion of Ergosterol into Progesterone.
   (ii) Outline the conversion of cholesterol into 5β-cholanic acid.
4. Detail the evidence that led to the structural elucidation of Oestrone.
5. (i)How will you bring about the following conversions?
   a. Diosgenin to Progesterone
   b. Stigmasterol to Progesterone
   (ii) Write notes on bile acids.
6. Explain the structural elucidation and synthesis of Ergosterol.
7. (i) Write the conversion of Cholesterol into Testosterone.
   (ii) Write any one of the synthesis of Oestrone.
8. Explain the structural elucidation and synthesis of Equilenin.
9. (i) Explain the nature and position of side chain in Cholesterol.
   (ii) Write the Mamoli's synthesis of Testosterone.
10. Give a note on Vitamin D.

PART-D (Each Question Carry Ten Marks)

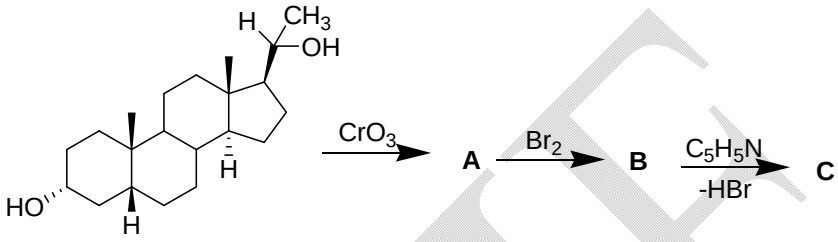
1. (a) Explain the synthesis of Diel's hydrocarbon?
   (b) How to prove that the ring A in Cholesterol is six-membered?
   (c) Explain the synthesis of 5β-Cholanic acid?
   (d) How 5α-Cholanic acid is synthesised?
2. (i) Complete the following sequence of reactions giving missing reagents **A** and **B**.



(ii) Complete the following sequence of reactions giving missing products **A**, **B** and **C**.



(iii) Identify the products in the following reactions.



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DEPARTMENT OF CHEMISTRY

UNIT-II

STEROIDS

PART-A–Multiple Choice Questions

(Each Question Carry One Mark) (Online Examinations)

1. Steroids have the

a) 1,2–cyclopentenophenanthrene ring b) 1,3-cyclopentenophenanthrene ring

c) phenanthrene ring d) anthracene ring

2. Selenium dehydrogenation of steroids at 360ºC yields

a) chrysene b) picene c) phenanthrene **d) Diel's hydrocarbon**

3. Selenium dehydrogenation of steroids at 420ºC yields

a) chrysene b) picene c) phenanthrene d) Diel's hydrocarbon

4. 3'-methyl-1,2-cyclopentenophenanthrene is known as

a) chrycene **b) Diel's hydrocarbon** c) phenanthrene d) picene

5. The sterols that are obtained from animal sources are referred as

a) phytosterols b) mycosterols **c) zoosterols** d) myosterols

6. The sterols that are obtained from plant materials are referred as

a) phytosterols b) mycosterols c) zoosterols d) myosterols

7. The sterols that are obtained from yeast and fungi are referred as

a) phytosterols **b) mycosterols** c) zoosterols d) myosterols

8. The example for zoosterol is

a) cholesterol b) ergosterol c) fungi d) stigmasterol

9. Cholesterol was first isolated from

a) fish liver oils b) brain **c) human gallstones** d) spinal card of the cattle

10. The melting point of cholesterol is

a) 194ºC b**) 149ºC** c) 139ºC d) 140ºC

11. The main source of cholesterol is

a) fish liver oils b) egg c) human gallstones d) wood

12. The specific rotation of cholesterol is

a) 29º b) 30º **c) 39º** d) 24º

13. The reaction which produces red colour while chloroform solution of cholesterol reacting with sulphuric acid

a) Liebermann-Burchard reaction b) Liebermann reaction

c) dye test **d) Salkowski reaction**

14. Digitonide formation is used for estimation of

a) alkaloids **b) steroids** c) terpeniods d) proteins

15. The reaction which produces green colour while chloroform solution of cholesterol reacting with sulphuric acid and acetic anhydride

a) Liebermann-Burchard reaction b) Liebermann reaction

c) dye test d) Salkowski reaction

16. The molecular formula for cholesterol is

a) $C_{20}H_{42}O$ b) $C_{22}H_{42}O$ **c) $C_{27}H_{46}O$** d) $C_{28}H_{48}O$

17. The number of hydroxyl group present in cholesterol is

a) 1 b) 2 c) 3 d) 4

18. The number of double bonds present in cholesterol is

a) 2 **b) 1** c) 3 d) 4

19. Cholesterol on reduction with H_2-Pt yields

a) cholestanone b) cholestane **c) cholestanol** d) cholestanetriol

20. Cholesterol when distilled with selenium at 360ºC, yields Diels hydrocarbon and

a) chrysene b) ergosterol c) phenanthrene d) picene

21. The molecular formula for ergosterol is

a) $C_{20}H_{42}O$ b) $C_{22}H_{44}O$ **c) $C_{28}H_{44}O$** d) $C_{28}H_{42}O$

22. The λ_{max} value for ergosterol is

a) 238 nm **b) 282 nm** c) 245 nm d) 254nm

23. The number of hydroxyl group present in ergosterol is

a) 3 b) 5 c) 2 **d) 1**

24. The number of double bonds present in ergosterol is

a) 3 b) 5 c) 2 d) 1

25. The infrared spectrum of ergosterol showed a band at 970 cm^{-1} indicates

a) hydroxyl group **b) trans-alkene group** c) cis-alkene group d) nitro group

26. The molecular formula for Ergostanol is

a) $C_{20}H_{42}O$ b) $C_{22}H_{44}O$ c) $C_{28}H_{44}O$ **d) $C_{28}H_{50}O$**

27. Oppenauer oxidation of ergosterol yields

a) methyl ketone **b) α,β-unsaturated ketone** c) saturated ketone d) acyclic ketone

28. When bile acid is dehydrated by heating in a vacuum followed by catalytic reduction gives

a) 5α-cholanic acid b) cholestane c) cholestanol d) cholesterol

29. Progesterone forms oxime with

a) semicarbazone **b) hydroxylamine** c) phenylhydrazine d) hydrazine

30. Anhydride of etiobilianic acid when distilled with selenium yields

a) 1,4 dimethylphenanthrene b) 3,3 dimethylphenanthrene

c) 1,3 dimethylphenanthrene **d) 1,2 dimethylphenanthrene**

31. Ergosterol under the influence of light gives

a) pre-ergocalciferol b) cholesterol c) oestrone d) equilenin

32. The molecular formula for oestrone

a) $C_{22}H_{18}O$ **b) $C_{18}H_{22}O_2$** c) $C_{22}H_{44}O$ d) $C_{22}H_{48}O_2$

33. Oesterone when treated with oxime yields

a) trioxime b) dioxime c) dihydroprogesterone **d) monooxime**

34. The number of hydroxyl group present in oesterone is

a) 3 b) 5 c) 2 **d) 1**

35. The number of double bonds present in oesterone is

a) 2 b) 5 **c) 3** d) 5

36. Oesterone when distilled with zinc dust yields

a) Diels hydrocarbon **b) chrysene** c) phenanthrene d) picene

37. The molecular formula for equilenin is

a) $C_{18}H_{18}O$ b) $C_{22}H_{18}O_2$ c) $C_{18}H_{22}O_2$ d) $C_{22}H_{48}O_2$

38. The number of double bonds present in equilenin

a) 5 b) 2 c) 3 c)1

39. The molecular formula for progesterone is

a) $C_{18}H_{18}O_2$ **b) $C_{21}H_{30}O_2$** c) $C_{18}H_{22}O_2$ d) $C_{22}H_{48}O_2$

40. The λ_{max} value for testosterone is

a) 231 nm b) 243nm **c) 240 nm** d) 256 nm

41. The molecular formula for testosterone is

a) $C_{21}H_{30}O_2$ b) $C_{18}H_{18}O_2$ c) $C_{22}H_{48}O_2$ **d) $C_{19}H_{28}O_2$**

42. Testosterone is prepared from

a) cholesterol b) ergosterol c) progesterone d) oestrone

43. The number of double bonds present in testosterone is

a) 2 **b) 1** c) 3 d) 4

44. Lithocholic acid when subjected to vacuum distillation yields

a) cholesterol b) cholanic acid **c) cholenic acid** d) coprostanol

45. Which one is α,β-unsaturated ketone?

a) cholesterol b) oestrone c) equilenin **d) progestrone**

46. The λ_{max} value for oesterone is

a) 280 nm b) 276 nm c) 240 nm d) 260 nm

47. The oesterone λ_{max} value is 280 nm indicate that

a) presence of ketone group b**) presence of one benzene ring**

c) presence of two benzene ring d) presence of α,β-unsaturated ketone

48. Molecular formula for ergocalciferol is

a) $C_{28}H_{40}O$ b) $C_{22}H_{44}O$ **c) $C_{28}H_{44}O$** d) $C_{20}H_{40}O$

49. The catalytic hydrogenation of ergocalciferol yields

a) dihydroergocalciferol b) trihydroergocalciferol

c) tetrahydroergocalciferol **d) octahydroergocalciferol**

50. Ergocalciferol is an

a) monocyclic compound b) bicyclic compound

c**) tricyclic compound** d) tetracyclic compound

51. The parent hydrocarbon of ergocalciferol is

a) $C_{29}H_{52}$ **b) $C_{28}H_{52}$** c) $C_{28}H_{44}$ d) $C_{20}H_{46}$

52. Ozonolysis of ergocalciferol yields formaldehyde indicate that

a) exocycic methylene group b) endocyclic methylene group

c) keto group d) aldehyde group

53. The number of hydroxyl groups in α-hyodeoxycholic acid is

a) 1 **b) 2** c) 3 d) 4

54. The number of hydroxyl groups in chenodeoxycholic acid is

a) 1 b) 4 c) 3 **d) 2**

55. The number of keto groups in progesterone is

a) 1 b) 4 **c) 2** d) 3

56. Oppenauer oxidation of cholesterol followed by reduction yields

a) cholanic acid b) cholenic acid c) coprostane **d) coprostanol**

57. The number of hydroxyl groups present in lithocholic acid is

a) 1 b) 2 c) 3 d) 4

58. Cholic acid contains

a) 1 hydroxyl group b) 2 hydroxyl group

c) 3 hydroxyl groups d) 4 hydroxyl groups

59. The cholesterol is treated with hydrogen peroxide and acetic acid gives

a) cholestanone b) cholestane c) cholestanol **d) cholestanetriol**

60. Coprostanol is

a) 5α-cholestan-3β-ol **b) 5β-cholestan-3β-ol**

c) 5β-cholestan-5β-ol d) 5α-cholestan-3β-ol

UNIT-III

SYLLABUS

Alkaloids: Definition of an alkaloid-extraction of alkaloids-general properties - general methods of determining structure of alkaloids – structural elucidation and synthesis of Atropine, Morphine and Quinine -biosynthesis of quinoline alkaloids.

Definition of an alkaloid

Originally the name **alkaloid** (which means alkali-like) was given to all organic bases isolated from plants. This definition covers an extraordinary wide variety of compounds, and as the study of 'alkaloids' progressed, so the definition changed. Konigs (1880) suggested that alkaloids should be defined as naturally occurring organic bases which contain a pyridine ring. This definition, however, embraces only a limited number of compounds, and so the definition was again modified a little later by Ladenburg, who proposed to define alkaloids as natural plant compounds having a basic character and containing at least one nitrogen atom in a heterocyclic ring. Ladenburg's definition excludes any synthetic compounds and any compounds obtained from animal sources. One must admit that even today it is still difficult to define an alkaloid. The term is generally limited to organic bases formed in plants. Not all authors do this, and so they specify those alkaloids obtained from plants as *plant alkaloids* (or *vegetable alkaloids*). On the whole, alkaloids are very poisonous, but are used medicinally in very small quantities. Thus we find that the basic properties, (usually) complex structures, physiological action and plant origin are the main characters which define plant alkaloids. Even so, the class of compounds known as the *purines*, which possess the above characters, are not usually included under the heading of alkaloids (some purines are also obtained from animal sources).

It is interesting to note in this connection that Serturner (1806) isolated a basic compound from opium. Up to that time it was believed that plants produced only acids or neutral compounds.

Extraction of alkaloids

Alkaloids are usually found in the seeds, root, leaves, or bark of the plant, and generally occur as salts of various plant acids, e.g, acetic, oxalic, citric, malic, tartaric acid, etc. A common method of isolation of alkaloids is as follows. The plant is dried, then finely powdered and extracted with boiling methanol. The solvent is distilled off, and the residues treated with inorganic acids, where upon the bases are extracted as their soluble salts. The free bases are liberated by the addition of sodium carbonate and extracted with various solvents, e.g., ether, chloroform, etc. The mixtures of bases thus obtained are separated by various methods into the individual compounds. More recent methods of separation involve the use of chromatography. Lee (1960) has converted plant alkaloids into their reineckates, dissolved these in acetone, and passed this solution through an ion exchange column, and thereby obtained the alkaloids in a high state of purity. (Reinecke's solution is $NH_4[Cr(NCS)_4(NH_3)_2]$. Most alkaloids are obtained from natural sources, but a few are synthesised commercially, e.g., ephedrine and papaverine.

General properties

The alkaloids are usually colourless, crystalline, non-volatile solids which are insoluble in water, but are soluble in ethanol, ether, chloroform, etc. Some alkaloids are liquids which are soluble in water, e.g. coniine and nicotine, and a few are coloured, e.g., berberine is yellow. Most alkaloids have a bitter taste and are optically active (laevorotatory). They are generally tertiary nitrogen compounds and contain one or two nitrogen atoms usually in the tertiary state in a ring system; most of the alkaloids also contain oxygen. The optically active alkaloids are very useful for resolving racemic acids. The alkaloids form insoluble precipitates with solutions of phosphotungstic acid, phosphomolybdic acid, picric acid, potassium mercuri-iodide, etc. Many of these precipitates have definite crystalline shapes and so may be used to help in the identification of an alkaloid. Some of these reagents are also used as a means of detecting alkaloids in paper and thin layer chromatography.

General methods for determining structure

As we have seen in earlier chapters, structure determination involves the use of a variety of chemical and physical methods. Many of the following chemical methods, although part of the

general approach in structure determination, are those which have been particularly useful in alkaloid chemistry.

(i) After a pure specimen has been obtained it is subjected to qualitative analysis (invariably the alkaloid contains (carbon), hydrogen and nitrogen; most alkaloids also contain oxygen). This is then followed by quantitative analysis and thus the empirical formula is obtained; determination of the molecular weight finally leads to the molecular formula. If the alkaloid is optically active, its specific rotation is also measured.

(ii) When an alkaloid contains oxygen, the functional nature of this element is determined:

(a) *Hydroxyl group*. The presence of this group may be ascertained by the action of acetic anhydride, acetyl chloride or benzoyl chloride on the alkaloid (acylation must usually be considered in conjunction with the nature of the nitrogen also present in the molecule).

$$ROH + (CH_3CO)_2O \longrightarrow \underset{\text{Acetate}}{ROCOCH_3} + CH_3COOH$$

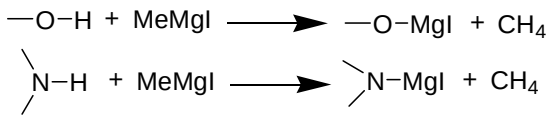
$$ROH + CH_3COCl \longrightarrow ROCOCH_3 + HCl$$

$$ROH + C_6H_5COCl \longrightarrow \underset{\text{Benzoate}}{ROCOC_6H_5} + HCl$$

However, the above test for oxygen should be applied carefully because amines if present in an alkaloid also yield acetyl and benzoyl derivatives. Then the number of hydroxyl groups is determined by *acetylation* or *Zerewitnoff's method*. In the former method, the number of hydroxyl groups is determined by acetylating the alkaloid and hydrolysing the acetyl derivative with a known volume of 1N NaOH.

$$ROH + CH_3COCl \longrightarrow ROCOCH_3 \xrightarrow{NaOH} ROH + CH_3COONa$$

The excess of alkali is estimated by titration with a standard solution of HCl acid. The number of acetyl groups or hydroxyl groups can be calculated from the volume of alkali used for hydrolysis. In the latter method, hydroxyl groups (and any N-H groups) can be detected and quantitatively estimated by treatment with methylmagnesium iodide.



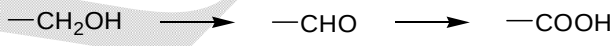
In this method, CH_4 is obtained quantitatively and therefore can be estimated volumetrically, giving the confirmation about the number of –OH and >N-H groups. Thus,

1 –OH group = 1 >N-H group = 22.4 litres of N_2 at S. T. P.

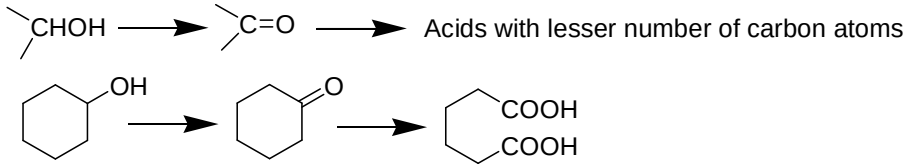
The next problem is to decide whether the hydroxyl group is alcoholic or phenolic. It is phenolic if the alkaloid is soluble in sodium hydroxide and reprecipitated by carbon dioxide; also a coloration with ferric chloride will indicate the presence of a phenolic group. If the compound does not behave as a phenol, the hydroxyl group may be assumed to be alcoholic, and this assumption may be verified by the action of dehydrating agents (most alkaloids containing and alcoholic group are readily dehydrated by sulphuric acid or phosphorus pentoxide). The behaviour of the compound towards oxidising agents will also disclose the presence of an alcoholic group.

If alcoholic hydroxyl group is present, then the nature of the alcoholic group. i.e., primary, secondary or tertiary is determined by oxidation or by dehydration to unsaturated compound.

(i) Primary alcoholic group (-CH_2OH) on oxidation yields first an aldehyde (-CHO) and then acid having the same number of carbon atoms as the parent alcohol.

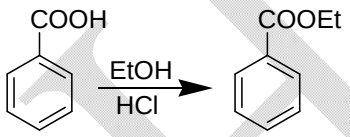


(ii) On oxidation, secondary alcohol (>CHOH) first yields ketone having the same number of carbon atoms and then acid having the lesser number of carbon atoms. However, if the secondary alcoholic group is attached to cyclic carbon atom, then the compound gets oxidized to open chain dicarboxylic acid having the same number of carbon atoms.



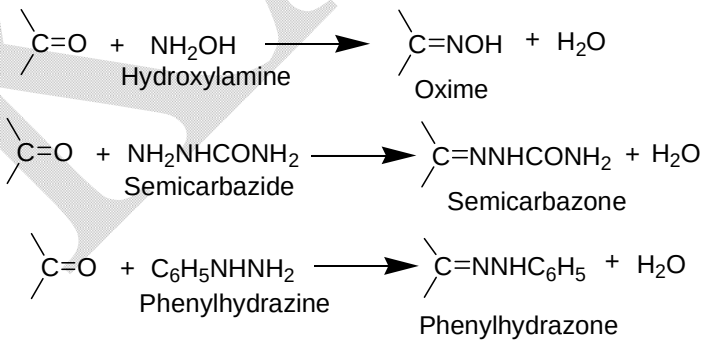
(iii) Tertiary alcohols on oxidation yield ketone and acid having the lesser number of carbon atoms.

(b) *Carboxyl group*. The solubility of alkaloid in aqueous sodium carbonate or ammonia indicates the presence of a carboxyl group. The formation of ester also shows the presence of a carboxyl group.



The number of carboxylic groups may be determined volumetrically by titration against a standard barium hydroxide solution using phenolphthalein as an indicator or gravimetrically by silver salt method.

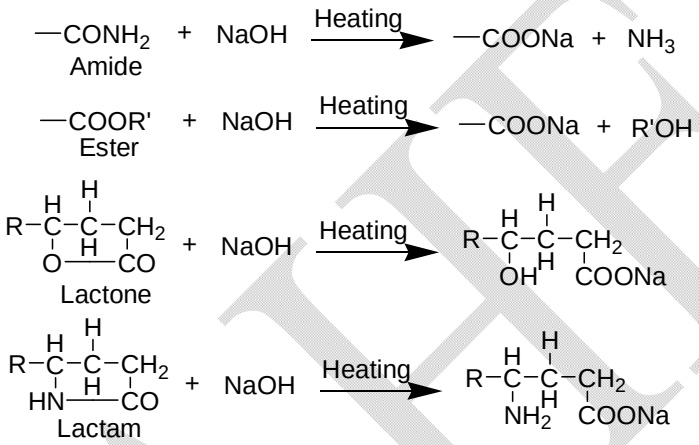
(c) *Oxo group*. The presence of an oxo group is readily ascertained by the formation of an oxime, semicarbazone and phenylhydrazone.



Distinction between an aldehyde and a ketone can be made on the basis of reduction and oxidation reactions.

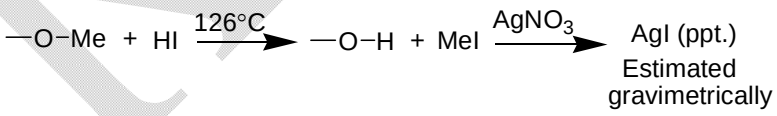
The presence of an oxo group and distinction between an aldehyde and a ketone may be further confirmed by several physical methods such as as infra-red, ultraviolet and NMR techniques.

(d) Hydrolysis of the alkaloid and an examination of the products led to information that the compound is an ester, lactone, amide, lactam or a betaine.

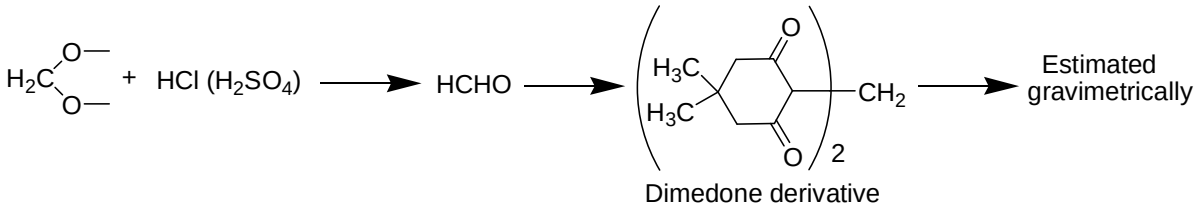


(e) The Zerewitinoff active hydrogen determination may be applied to the alkaloid.

(f) *Methoxyl group*. The presence of methoxyl groups and their number may be determined by the *Zeisel method*. The alkaloid is heated with concentrated hydriodic acid at its boiling point (126°C); the methoxyl groups are thereby converted into methyl iodide, which is then absorbed by ethanolic silver nitrate and the silver iodide is weighed.

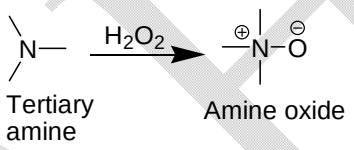


(g) *Methylenedioxyl group* (-OCH_2O-). The presence of this group is indicated by the formation of formaldehyde when the alkaloid is heated with hydrochloric or sulphuric acid.

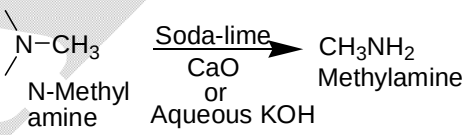


(iii) *The functional nature of the nitrogen*.

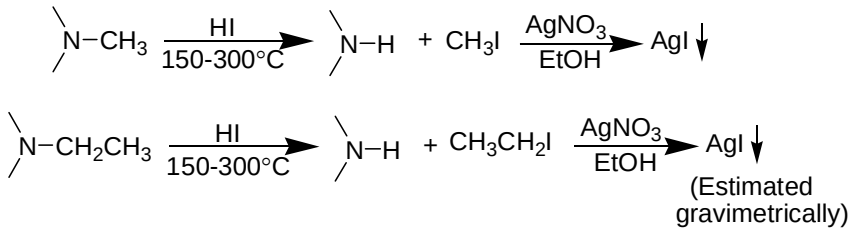
(a) The general reactions of the alkaloid with acetic anhydride, methyl iodide and nitrous acid often show the nature of the nitrogen, e.g., if all the reactions are negative, then the nitrogen is most probably tertiary. The difficulty here is that some alkaloids may undergo ring fission, the product being an *N*-acylated derivative. If the alkaloid forms an amine oxide with 30 per cent hydrogen peroxide, then the nitrogen atom is tertiary.



(b) Distillation of an alkaloid with aqueous potassium hydroxide usually leads to information regarding the nature and number of alkyl groups attached to nitrogen. The formation (in the volatile products) of methylamine, dimethylamine or trimethylamine indicates respectively the attachment of one, two or three methyl groups to a nitrogen atom; the formation of ammonia shows the presence of an amino group.

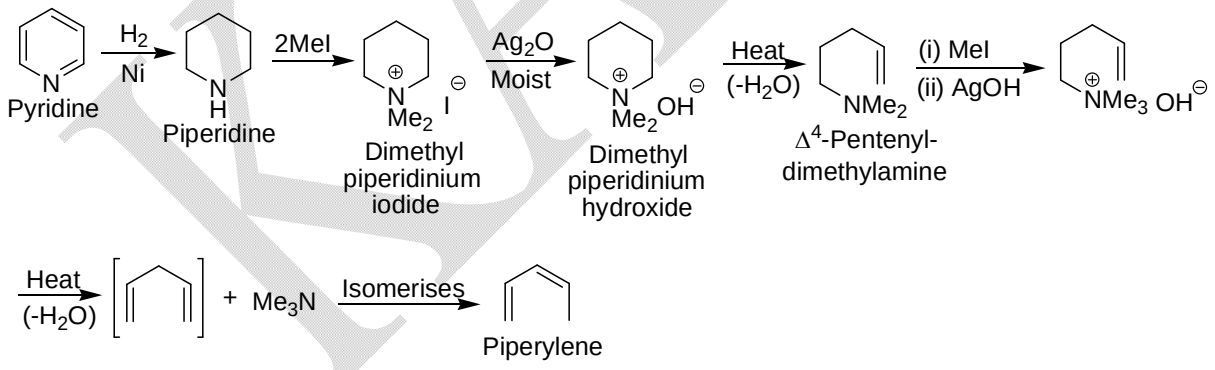


(c) The presence of *N*-methyl groups and their number may be determined by means of the *Herzig-Meyer method*. When the alkaloid is heated with hydriodic acid at 150-300°C under pressure, *N*-methyl groups are converted into methyl iodide.



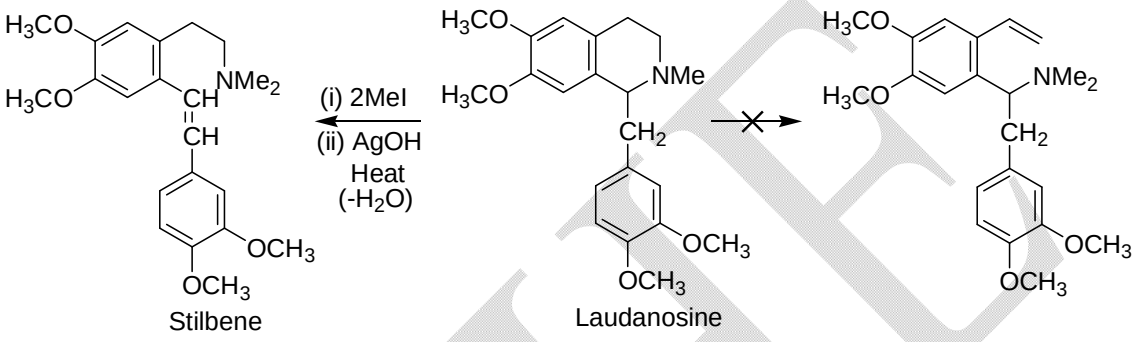
(d) The results of hydrolysis will show the presence of an amide, lactam or betaine.

(e) *Hofmann's exhaustive methylation* method (1883) is a very important process in alkaloid chemistry, since by its means heterocyclic rings are opened with the elimination of nitrogen, and the nature of the carbon skeleton is thereby obtained. The general procedure is to hydrogenate the heterocyclic ring (if this is unsaturated), convert this compound to the quaternary methylammonium hydroxide which is then heated. In this last stage a molecule of water is eliminated, a hydrogen atom in the *β–position* with respect to the nitrogen atom combining with the hydroxyl group, and the ring is opened at the nitrogen atom on the same side as the β-hydrogen atom eliminated. The process is repeated on the product; this result in the complete removal of the nitrogen atom from the molecule, leaving an unsaturated hydrocarbon which, in general, isomerises to a conjugated diene; e.g., pyridine gives piperylene.

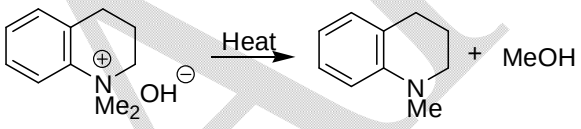


Although the general procedure for exhaustive methylation is to heat the quaternary hydroxide at about 200°C, in a number of cases the reaction may be carried out by refluxing an aqueous or ethanolic solution of potassium hydroxide containing the methiodide or methosulphate of the base. This procedure is usually satisfactory for bases which contain a benzene ring in the β-position to the nitrogen atom. This may be explained on the basis that

benzylic hydrogen has an increased acidity (and so is more readily removed) because of stabilisation of the transition state by conjugation (with the benzene ring). An interesting example of this is the case of laudanosine. When either β-hydrogen atom is eliminated, a styrene derivative is formed, but in one there is more extended conjugation than in the other, and so the former is the product.

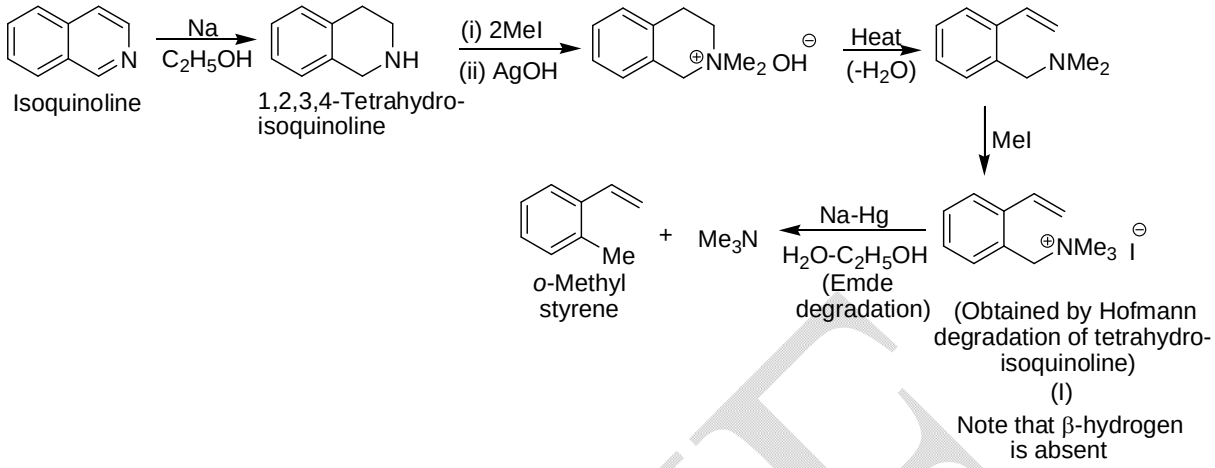


Even though the compound contains a β-hydrogen atom, the exhaustive methylation method may fail, e.g., tetrahydroquinoline.



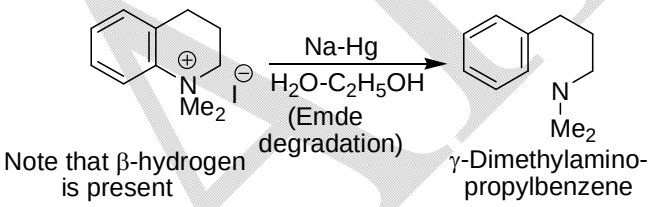
Alcohols are often obtained as a by-product in this elimination reaction, and in some cases no alkene is obtained at all (as in the above example).

When the base does not contain a β-hydrogen atom, the exhaustive methylation method fails. In such cases the Emde modification (1909, 1912) may be used. In this method the quaternary ammonium halide is reduced with sodium amalgam in aqueous ethanol or with sodium in liquid ammonia, or is catalytically hydrogenated, e.g.,

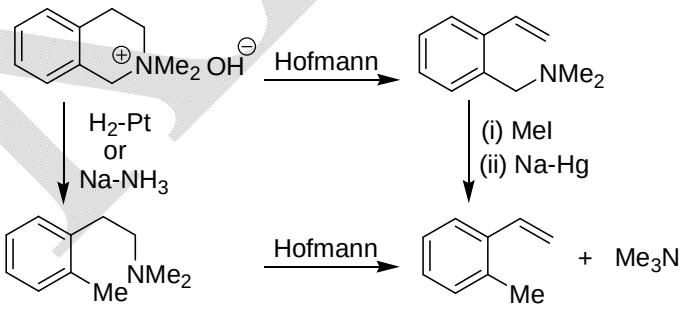


Examination of (I) shows that β-hydrogen is absent; hence Hofmann's method cannot be used.

It has been mentioned above that exhaustive methylation fails with tetrahydroquinoline. The heterocyclic ring, however, is opened by the Emde degradation.

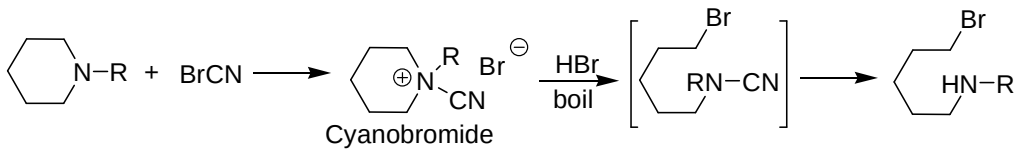


The Emde degradation on tetrahydroisoquinoline is also interesting:

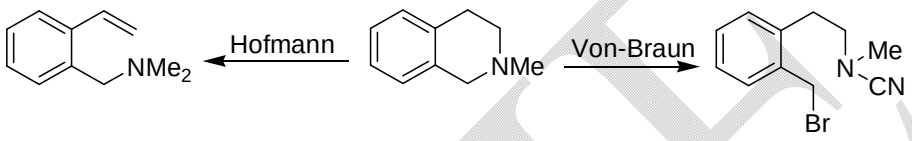


Other methods for opening heterocyclic rings containing nitrogen are:

(i) Von Braun's method for *tertiary* cyclic amines; e.g.,

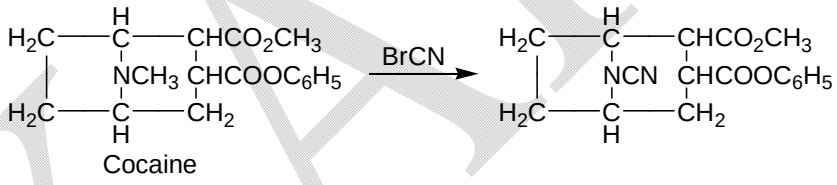


A point of interest about the cyanogen bromide method is that it is often successful with compounds that fail with the Hofmann method. Furthermore, where both methods are applicable, ring-opening occurs at different points of the ring, e.g.,

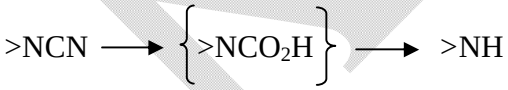


In general, fission of unsymmetrical amines by cyanogen bromide occurs to give the bromide of the 'shorter' bromide (see example given).

In the above examples, the ring is opened, but in other cases dealkylation occurs with formation of the cyclic *N*-cyano derivative, e.g., cocaine:

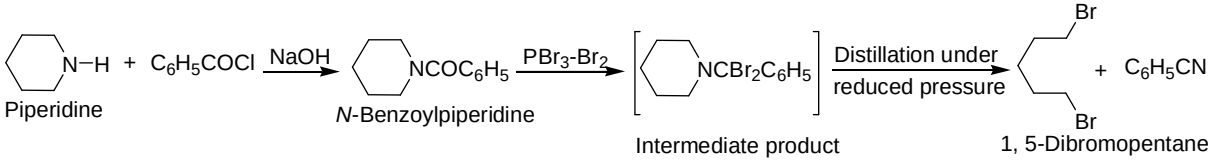


Hydrolysis of the cyano compound with hydrochloric acid brings about the following changes:

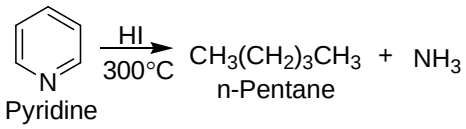


Thus, the final result is the removal of the *N*-methyl group without opening of the ring.

(ii) *Von Braun's method* for *secondary* cyclic amines; e.g.,



(iii) In a number of cases the ring may be opened by heating with hydriodic acid at 300°C, e.g.,



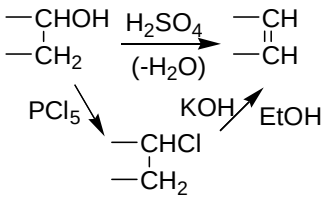
(iv) The presence of unsaturation in an alkaloid may be ascertained by the addition of bromine or halogen acids, or by the ability to be hydroxylated with dilute alkaline permanganate. Reduction by means of sodium amalgam, sodium and ethanol, tin and hydrochloric acid, hydriodic acid et., also may be used to show the presence of unsaturation. In some cases, reduction may decompose the molecule. This often happens when catalytic reduction is used (ring cleavage occurs), and hence milder methods of reduction are desirable. Two particularly mild reducing reagents are lithium aluminium hydride and sodium borohydride. Sodium in liquid ammonia gives the Emde type of degradations.

(v) *Oxidation*. This is one of the most valuable means of determining the structure of alkaloids. By varying the 'strength' of the oxidising agent, it is possible to obtain a variety of products:

(a) Mild oxidation is usually effected with hydrogen peroxide, ozone, iodine in ethanolic solution, or alkaline potassium ferricyanide.

(b) Moderate oxidation may be carried out by means of acid or alkaline potassium permanganate, or chromium trioxide in acetic acid.

(c) Vigorous oxidation is usually effected by potassium dichromate-sulphuric acid, chromium trioxide-sulphuric acid, concentrated nitric acid, or manganese dioxide-sulphuric acid.



This classification is by no means rigid; the 'strength' of an oxidising agent depends to some extent on the nature of the compound being oxidised. In those cases where it can be done, better results are sometimes achieved by first dehydrating the compound and then oxidising the unsaturated compound thus obtained; oxidation is readily effected at a double bond. More recently, mercuric acetate has been used to dehydrogenate certain alkaloids, thereby introducing olefinic bonds.

(vi) Fusion of an alkaloid with solid potassium hydroxide often produces relatively simple fragments, the nature of which will give information on the type of nuclei present in the molecule.

(vii) Zinc dust distillation. This usually gives the same products as (vi) except that when the alkaloid contains oxygen this is removed.

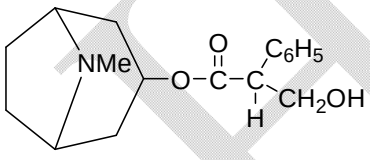
(viii) Physical methods are now being used, in conjunction with chemical methods, to elucidate structure, e.g., infrared spectra studies are used to identify many functional groups; ultraviolet spectra are used to indicate the likely type of structure present; and X-ray analysis has offered a means of distinguishing between alternative structures that appear to fit equally well the alkaloid in question. Owing to the introduction of computers, it is now possible to quickly perform the calculations from X-ray data, and so the complete stereochemical structure can be obtained from a single crystal. A very good example is that of thelepogine, $C_{20}H_{31}NO$, the structure of which has been determined by X-ray analysis; no chemical work was carried out (Fridrichsons *et al.*, 1960).

NMR spectroscopy is a more recent method for detecting many functional groups, e.g., olefinic protons, N-, O-, and C-methyl groups, and heterocyclic rings such as pyridine, pyrrole, indole, etc. More recently still, mass spectrometry has been used for structure elucidation of various groups of alkaloids. It is often possible to determine the type of nucleus-aromatic and heterocyclic-and the size and structure of side-chains. Mass spectrometry may also used on the products formed from, e.g., zinc dust distillation.

The stereochemistry of alkaloids has been solved by classical methods, X-ray analysis, and more recently by means of optical rotatory dispersion and circular dichroism where these are applicable (i.e., only with optically active alkaloids).

(ix) *Synthesis*. The foregoing analytical work will ultimately lead to the proposal of a tentative structure (or structures) for the alkaloid under consideration. Because of the increasing value of physical methods in elucidating structure, synthesis of the compound as a means of final proof of structure is less important than it used to be. Nevertheless, synthesis will always give additional evidence for the structure assigned, and may also provide much better way of obtaining a particular alkaloid (than from natural sources).

Atropine



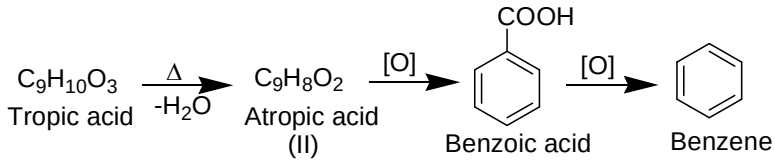
Atropine

Molecular formula: $C_{17}H_{23}NO_3$, m.p.118°C, occurs in deadly nightshade (*Atropa belladonna*) together with hyoscyamine. Hyoscyamine is optically active $[\alpha]_D$ -22°, but readily racemises to atropine when warmed in an ethanolic alkaline solution; thus atropine is (±)-hyoscyamine.

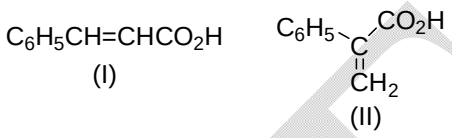
When warmed with barium hydroxide solution, atropine is hydrolysed to (±)–tropic acid and tropine (an alcohol); thus atropine is the tropine ester of tropic acid. When (-)-hyoscyamine is hydrolysed with cold water, tropine and (-)-tropic acid are obtained.

$$\underset{\text{Atropine}}{C_{17}H_{23}NO_3} + H_2O \xrightarrow[\Delta]{Ba(OH)_2} \underset{\text{(±)-Tropic acid}}{C_9H_{10}O_3} + \underset{\text{Tropine}}{C_8H_{15}NO} \xleftarrow[H_2O]{\text{Cold}} \text{(-)-Hyoscyamine}$$

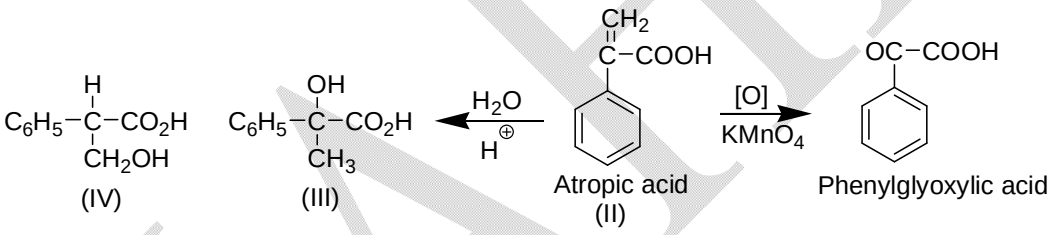
(±)-Tropic acid. $C_9H_{10}O_3$, m.p. 117°C $[\alpha]_D$ ± 81.5°, is a saturated compound (it does not add on bromine); the usual tests show that it contains one carboxyl group and one alcoholic group. When heated strongly, tropic acid loses a molecule of water to form atropic acid, $C_9H_8O_2$, and this, on oxidation, gives benzoic acid. Thus tropic and atropic acids contain a benzene ring with one side-chain.



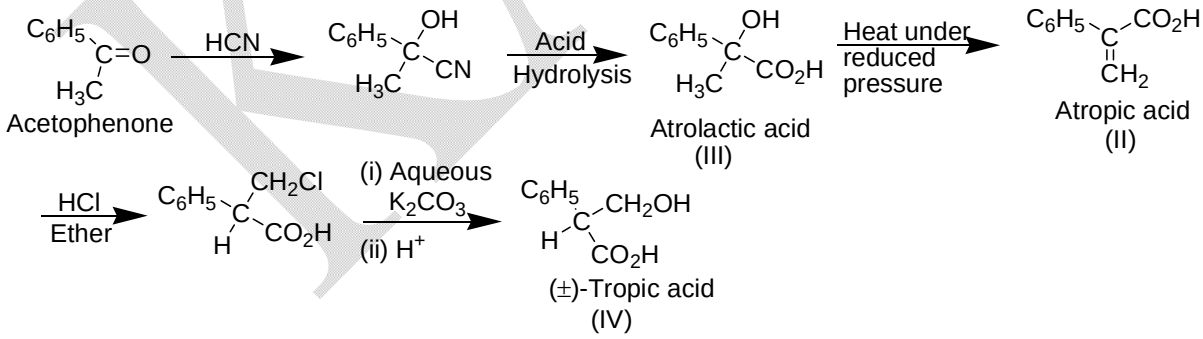
It therefore follows that atropic acid could be either (I) or (II). Since, however, (I) is known to be cinnamic acid (II) must be atropic acid.



This is supported by the fact that oxidation of atropic acid with permanganate gives phenylglyoxylic acid ($PhCOCO_2H$). Addition of a molecule of water to (II) would therefore give tropic acid which, consequently, must be either (III) or (IV).



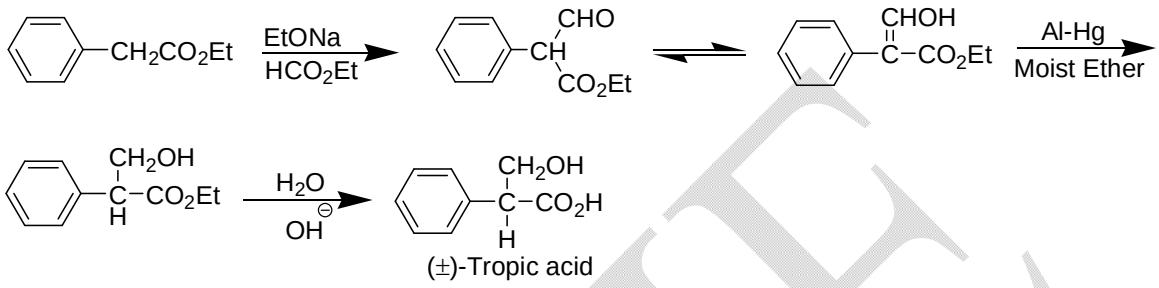
Tropic acid has been shown to be (IV) by synthesis, e.g., Mackenzie and Wood (1919), starting from acetophenone.



(III) is atrolactic acid, and it s dehydration to (II) confirms the structure of atropic acid. It should also be noted that the addition of hydrogen chloride takes place contrary to Markownikoff's rule; had the addition been in accordance with the rule, then atrolactic acid would have again been obtained. It is tropic acid that contains the chiral centre which gives rise

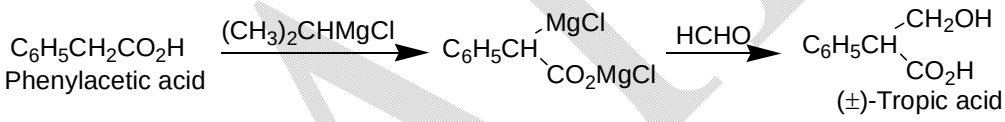
to the optically active hyoscyamine. The above synthesis results in (±)–tropic acid, and this has been resolved by means of quinine.

Muller and Wislicenus (1918) also synthesized (±)–tropic acid.



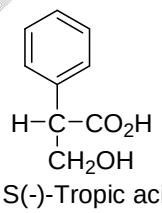
(±)-Tropic acid

Blicke *et al.* (1952) have synthesised tropic acid by boiling phenylacetic acid with isopropylmagnesium chloride in ethereal solution, and then treating the product, a Grignard reagent, with formaldehyde.



(±)-Tropic acid

Fodor *et al.* (1961) have established the absolute configuration of (-)-tropic acid by its correlation with (-)-alanine. According to the Cahn-Ingold-Prelog convention, natural tropic acid is (S) -(-)-tropic acid.

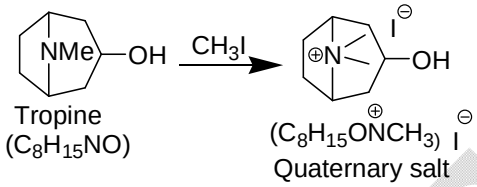


S(-)-Tropic acid

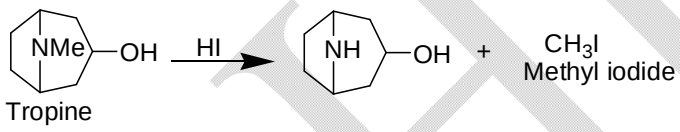
Tropine (Tropanol). Its structure is established as follows:

(a) Its molecular formula has been found to be $C_8H_{15}NO$. Its melt at 63°C.

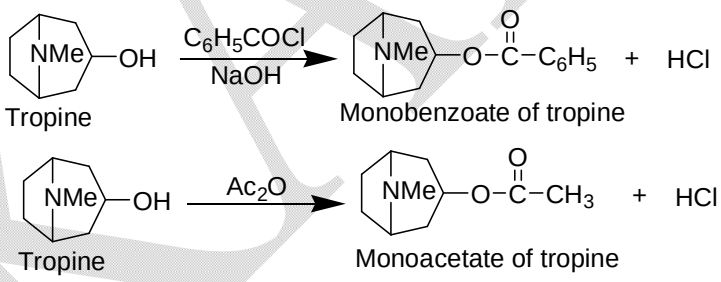
(b) When tropine is treated with methyl iodide, it yields a crystalline additive compound. This reaction reveals that the nitrogen atom in tropine is tertiary.



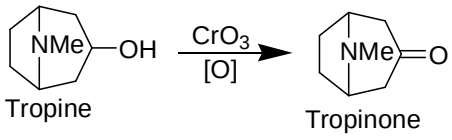
(c) When fused with alkali, tropine yields methyl amine, indicating that it must contain a *N*-methyl, *i.e.*, >N-CH_3 group. This is also confirmed by the fact that tropine when heated with hydrogen iodide at 150ºC (Herzig-Meyer method) yields one mole of methyl iodide.



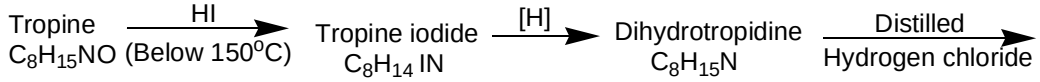
(d) As tropine forms a monoacetate and a monobenzoate, this indicates that it must contain a hydroxyl group.

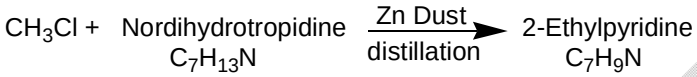


(e) When tropine is oxidised with chromic acid, it yields a tropinone, $C_8H_{13}NO$, which gives characteristic reactions of a ketone. Therefore, the hydroxyl group must be a secondary alcoholic (-CHOH-) group.

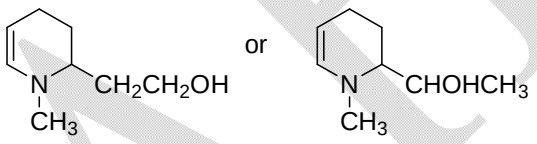


(f) The structure of tropine was investigated by Ladenburg (1883, 1887), who showed that the molecule contained a reduced pyridine nucleus:

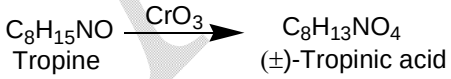




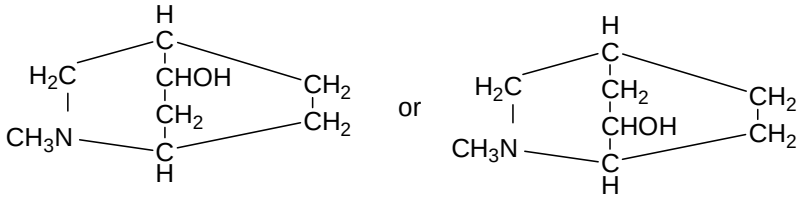
Tropine iodide is formed by the replacement of the alcoholic group in tropine by an iodine atom, which is then replaced by hydrogen to form dihydrotropidine (tropane). The formation of methyl chloride indicates the presence of an *N*-methyl group and the isolation of 2-ethylpyridine shows the presence of this nucleus (in a reduced form). Largely on this evidence, Ladenburg was led to suggest the following alternative formulae for tropine:



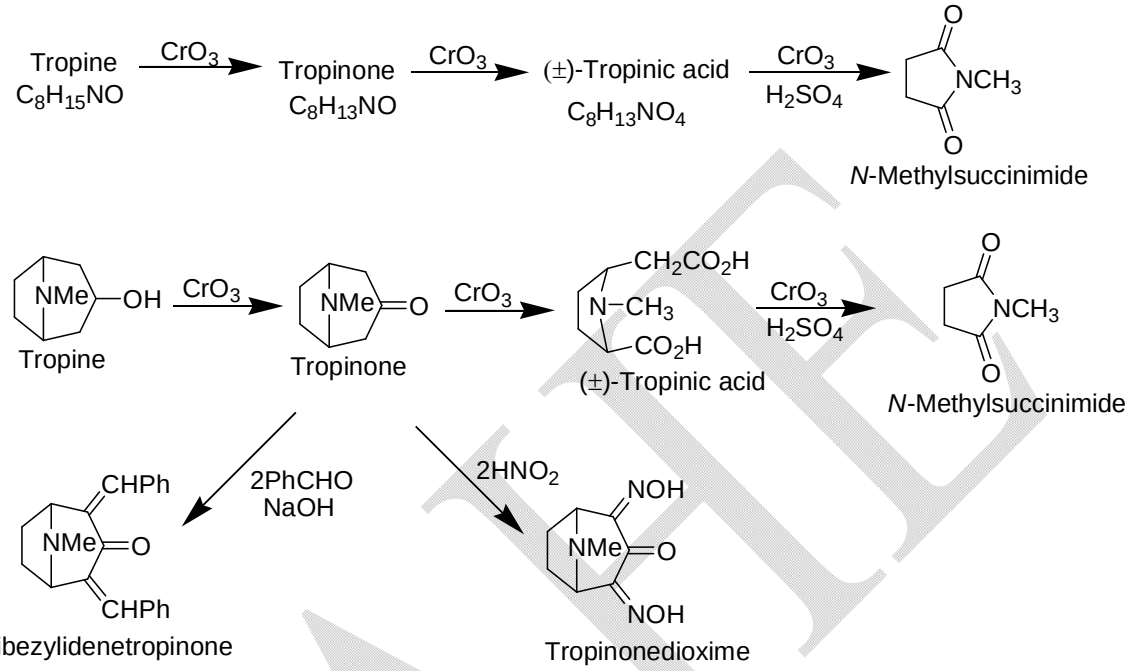
Merling (1891), by the oxidation of tropine with chromium trioxide, obtained (±)-tropinic acid.



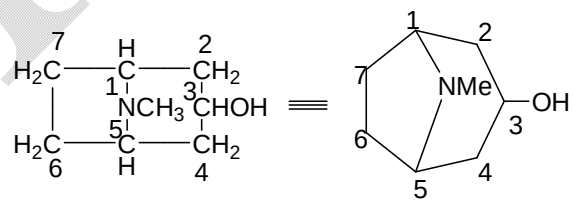
Tropinic acid is a dicarboxylic acid, and since there is no loss of carbon in its formation, the hydroxyl group in tropine must therefore be in a ring system. Thus Ladenburg's formula is untenable, and so Merling proposed the following structures for tropine:



Willstatter (1895-1901) then examined the oxidation products of tropine obtained as follows:



Tropinone behaved as a ketone; thus tropine is a secondary alcohol. Willstatter (1897) also showed that tropinone forms a dibenzylidene derivative with benzaldehyde, and a di-oximino derivative when treated with amyl nitrite and hydrochloric acid. Thus tropinone contains the CH_2COCH_2 grouping, and so it follows that Merling's formula is also untenable. Willstatter therefore proposed three possible structures for tropine, but eliminated two by the consideration of various reactions of tropine, and was left with the following (which contains a pyridine and a pyrrole nucleus with the nitrogen atom common to both):

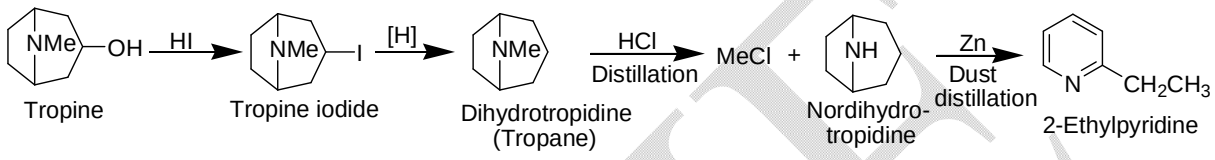


Not only did this fit the facts best, but it was also supported by the following evidence: (i) Exhaustive methylation of tropine give tropilidene (cycloheptatriene), C_7H_8. (ii) Exhaustive

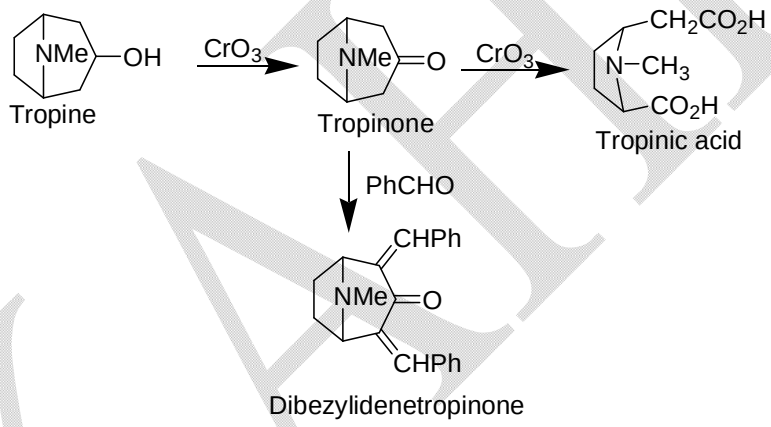
methylation of tropinic acid gives an unsaturated dicarboxylic acid which, on reduction, forms pimelic acid. (iii) Tropinone, on oxidation with CrO_3-H_2SO_4, gives *N*-methylsuccinimide. This indicates the presence of a pyrrolidine ring in tropinone.

All the foregoing reactions of tropine can be readily explained on the Willstatter formula.

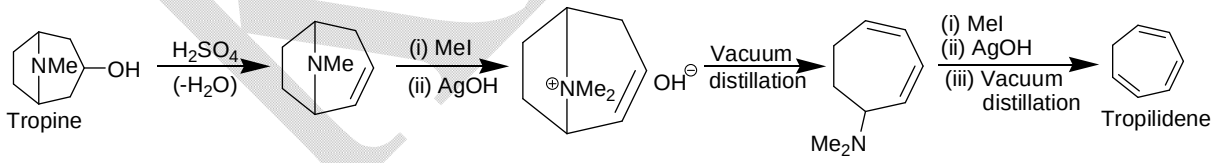
Formation of 2-ethylpyridine from tropine



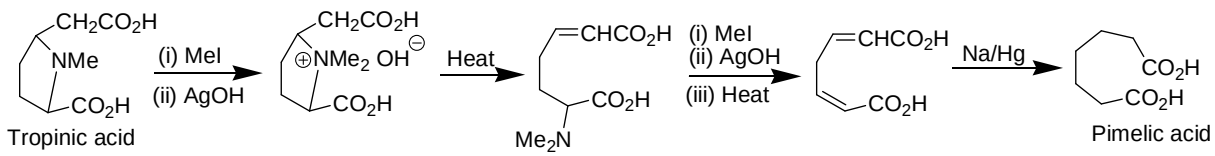
Formation of tropinone and tropinic acid from tropine



Formation of tropilidene (cycloheptatriene) form tropine

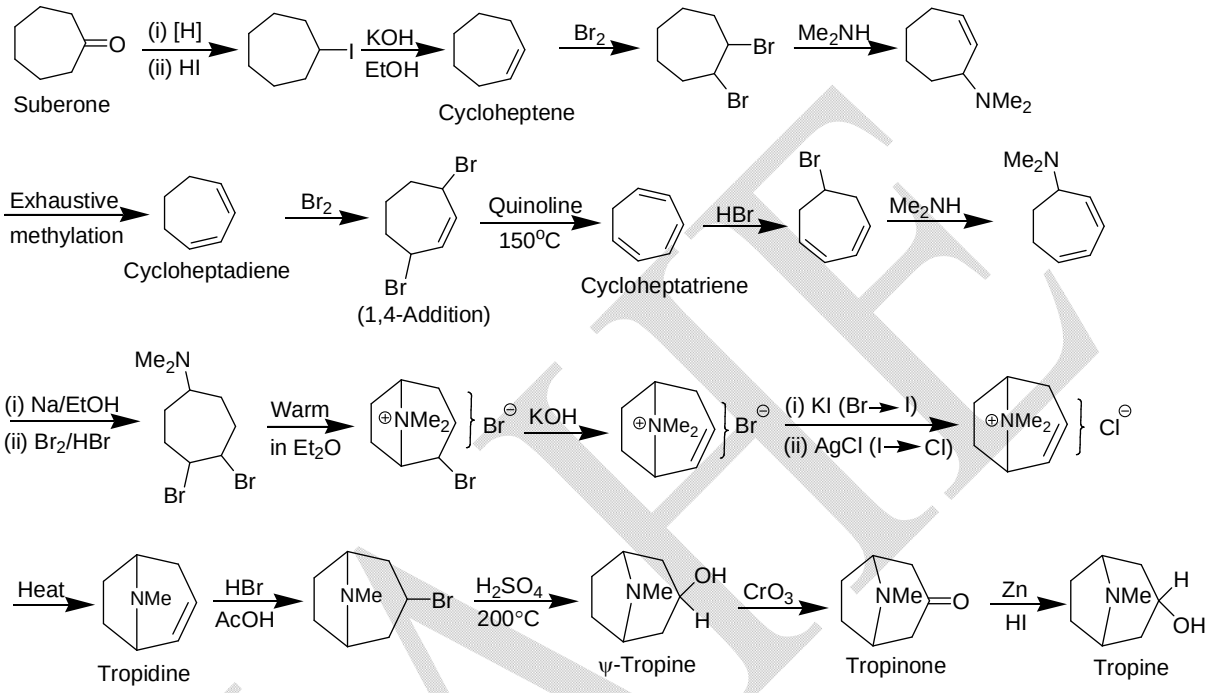


Formation of pimelic acid from tropinic acid



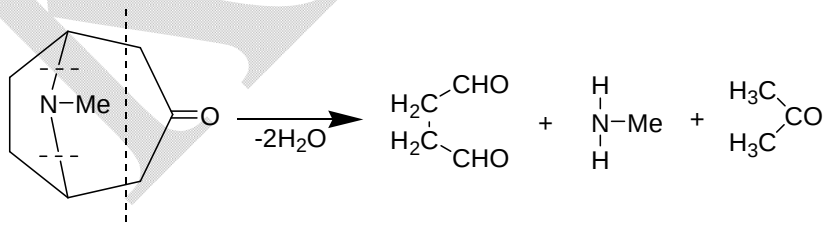
The structure of tropine has been confirmed by synthesis, one by Willstatter (1900-1903) and the other by Robinson (1917).

Willstatter's synthesis

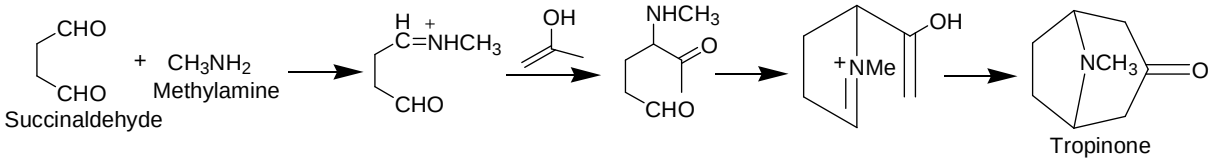


Robinson's synthesis

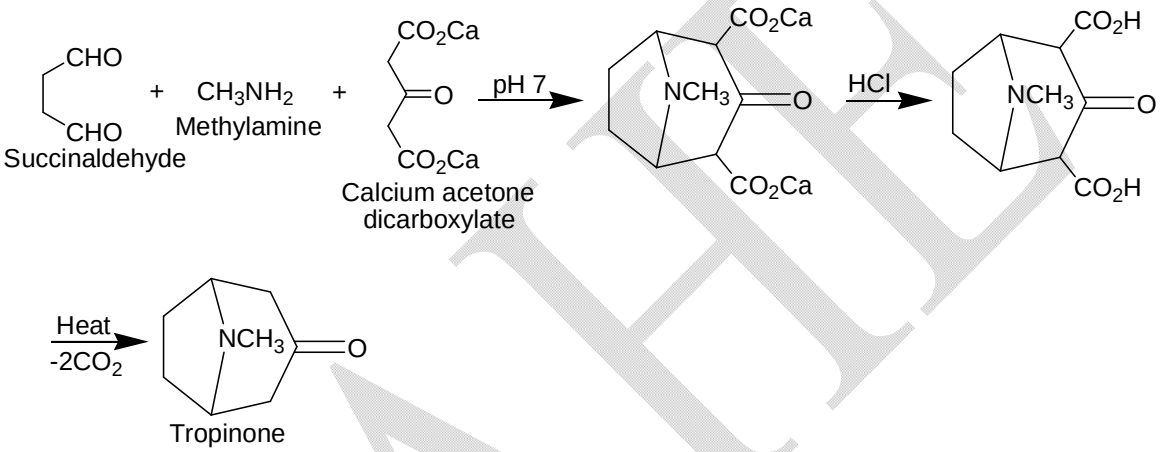
Robinson imagined that the skeleton of tropinone could, by means of hydrolysis, be broken down into the three units; succindialdehyde, methylamine and acetone.



Furthermore, Robinson though that these three units could be joined by means of double Mannich reaction to form tropinone in one step. When this mixture was allowed to stand in water for thirty minutes, tropinone was produced in very small yield. The reaction may be formulated as shown.

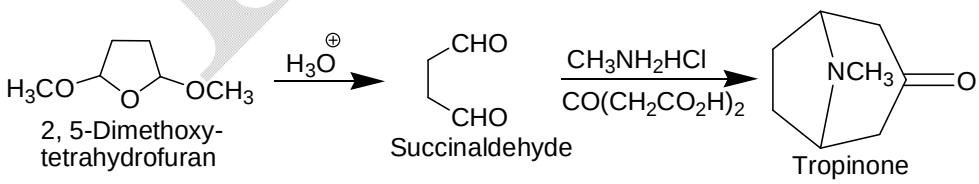


A much better yield (40 per cent) is obtained by using calcium acetonedicarboxylate or ethyl acetonedicarboxylate instead of acetone; the calcium salt or ester so produced is converted into tropinone by warming with hydrochloric acid, e.g., (ca = Ca/2):



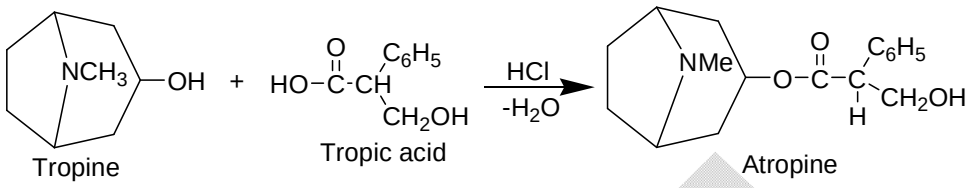
In acetonedicarboxylic acid, because each methylene group is flanked by two carbonyl groups, there is a greater amount of the enol form.

Schopf *et al.* (1935) have obtained a yield of 70-85 per cent by carrying out Robinson's synthesis at a pH of 7. Elming *et al.* (1958) have also synthesised tropinone using methylamine hydrochloride, acetonedicarboxylic acid and generating succinaldehyde in situ by the action of acid on 2,5-dimethoxytetrahydrofuran.



The yield was 81 per cent, but in this case 'physiological conditions' were not necessary.

The final problem is to combine tropine with tropic acid; this has been done by heating the two together in the presence of hydrogen chloride.



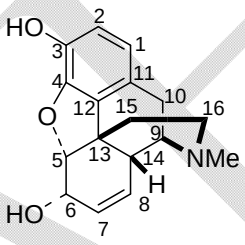
Tropine Tropic acid Atropine

If (+) – or (-) –tropic acid is used, the product is (+)- or (-)-hyoscyamine, respectively.

Morphine, codeine and thebaine

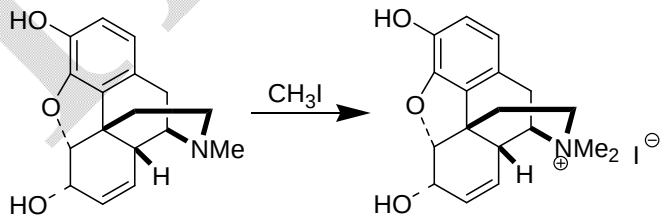
These are three important opium alkaloids which contain the phenanthrene nucleus.

(-)-Morphine

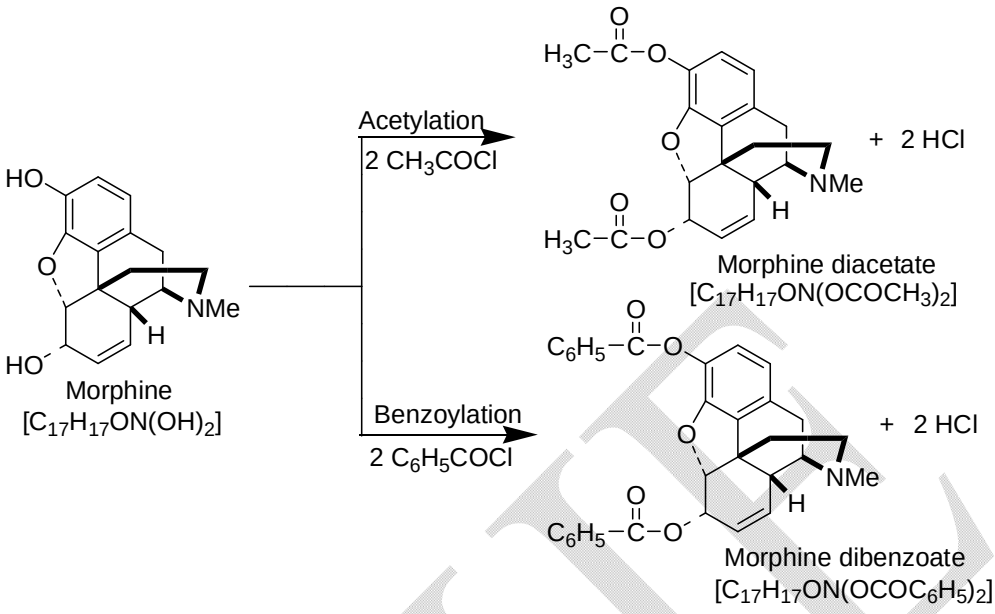


Morphine

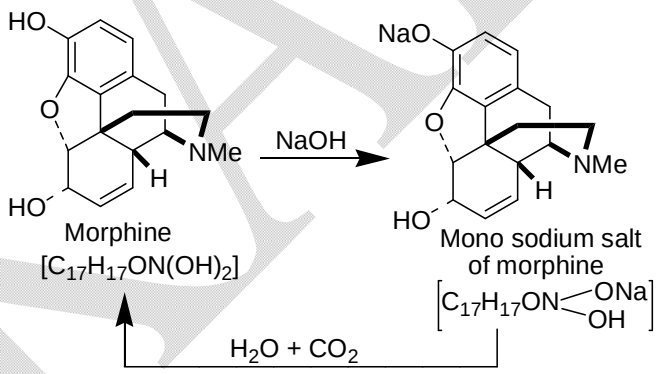
Molecular formula: $C_{17}H_{19}NO_3$, m.p. 254°C, $[\alpha]_D$ -131°, is the chief alkaloid in opium, and was the first alkaloid to be isolated (Serturner, 1806). The usual tests show that the nitrogen atom is in the tertiary state, and since morphine forms a diacetate (known as **heroin)** and a dibenzoate, two hydroxyl groups are therefore present in the molecule.



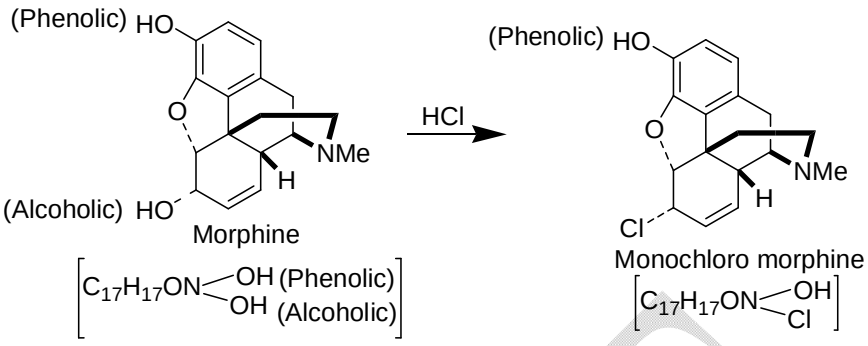
Morphine ($C_{17}H_{19}NO_3$) Morphine methiodide ($C_{17}H_{19}O_3N.CH_3I$)



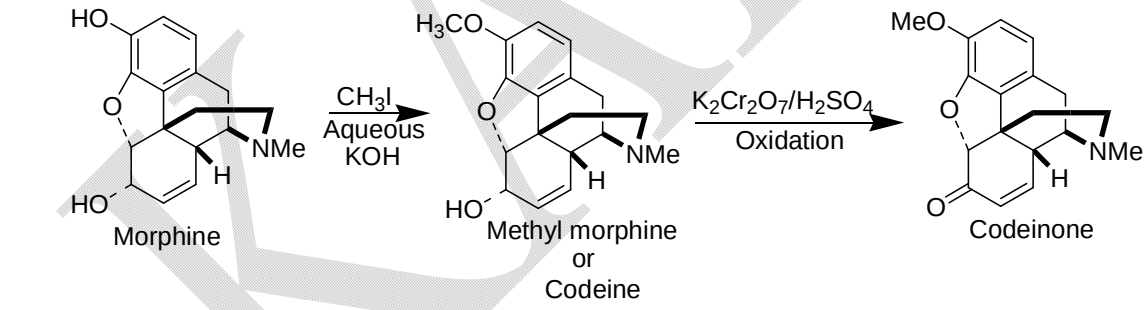
Morphine gives the ferric chloride test for phenols, and dissolves in aqueous sodium hydroxide to form a monosodium salt, and this is reconverted into morphine by the action of carbon dioxide; thus one of the hydroxyl groups is phenolic (Matthiessen *et al.*, 1869).

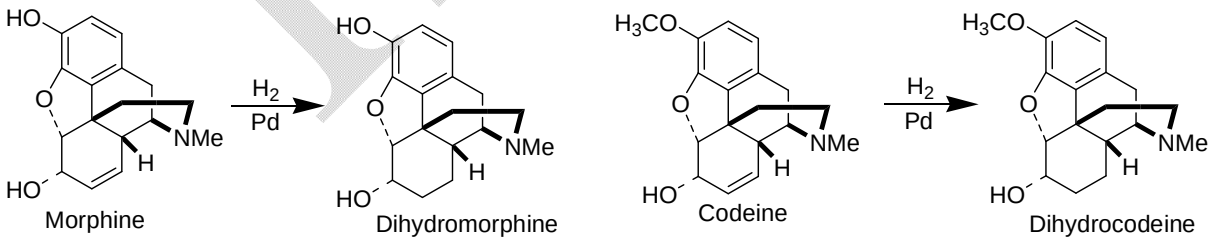


The second hydroxyl group is secondary alcoholic, as is shown by the following reactions. Halogen acids convert morphine into a monohalogeno derivative, one hydroxyl group being replaced by a halogen atom.



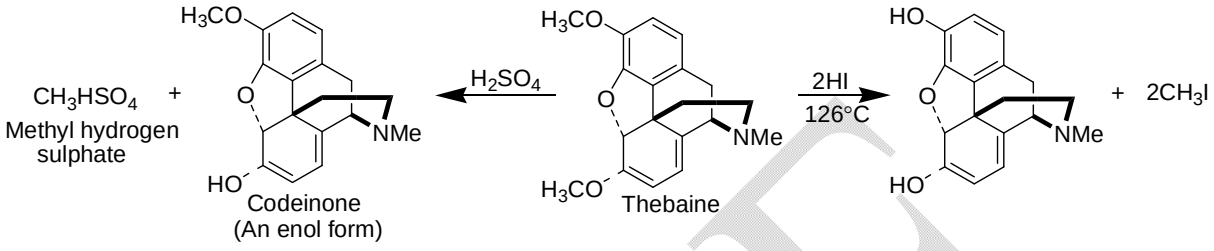
When heated with methyl iodide in the presence of aqueous potassium hydroxide, morphine is methylated to give (-) **codeine**, $C_{18}H_{21}NO_3$, m.p. 155°C $[\alpha]_D$ –135° (Grimaux, 1881). Since codeine is no longer soluble in alkalis, it therefore follows that it is only the phenolic hydroxyl group in morphine that has been methylated. Furthermore, codeine can be oxidised by chromic acid to codeinone, a ketone (Hesse, 1884). Thus the hydroxyl group in codeine (and this one in morphine) is secondary alcoholic, and so codeine is the monomethyl (phenolic) ether of morphine. Also, codeine absorbs one molecule of hydrogen on catalytic reduction (Pd), and therefore both codeine and morphine contain one ethylenic bond.



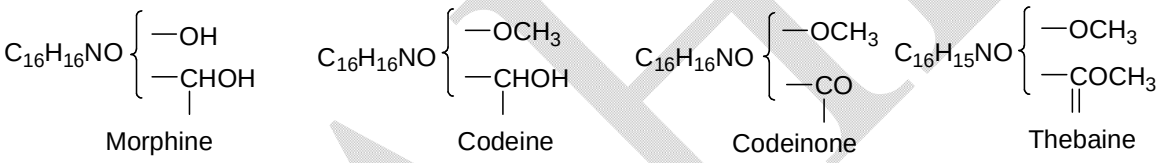


(-)–Thebaine, $C_{19}H_{21}NO_3$, m.p.193°C, $[\alpha]_D$ -219°, produces two molecules of methyl iodide when heated with hydriodic acid (Zeisel method); hence thebaine is a dimethoxy

derivative. When heated with sulphuric acid, thebaine eliminates one methyl group as methyl hydrogen sulphate, and forms codeinone (Knorr, 1906). The formation of a ketone led Knorr to suggest that thebaine is the methyl ether of the enolic form of codeinone.

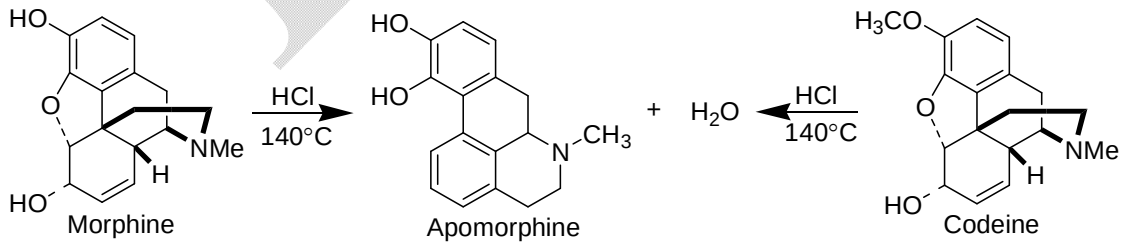


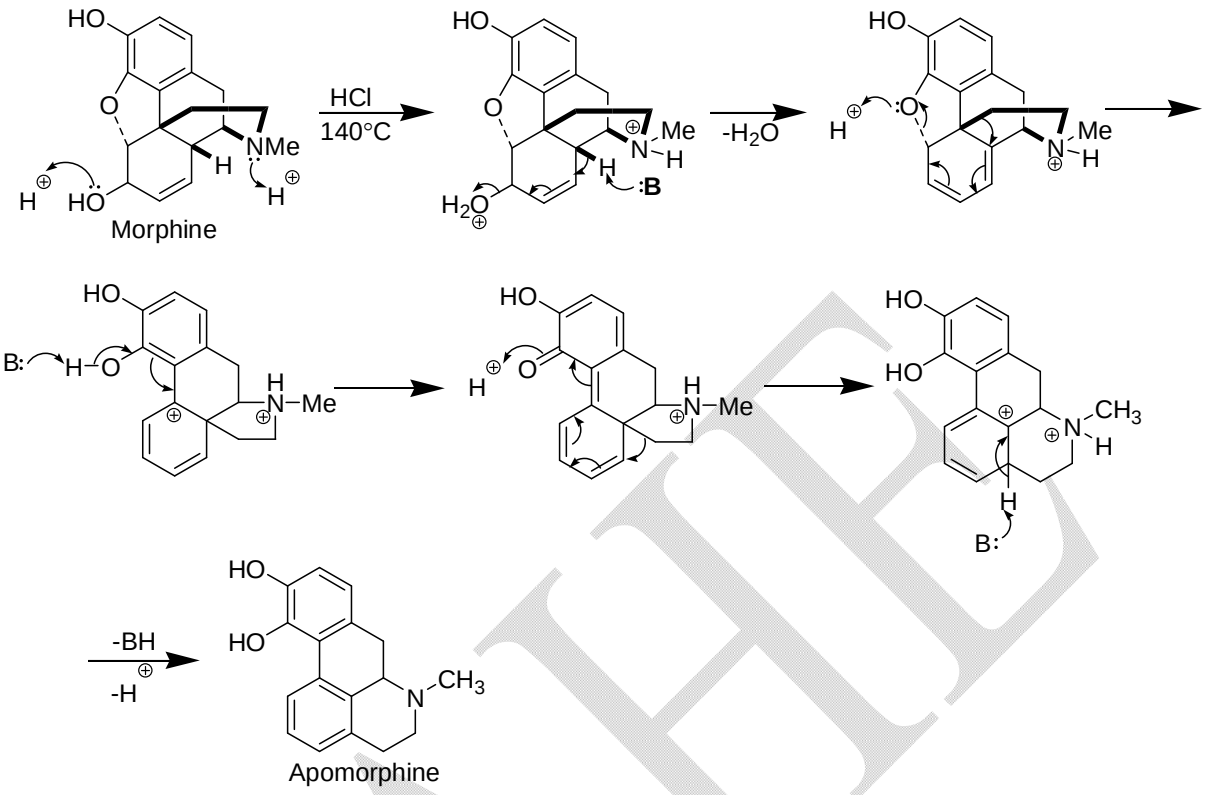
The foregoing work can thus be summarised by assigning the following formulae to the compounds described:

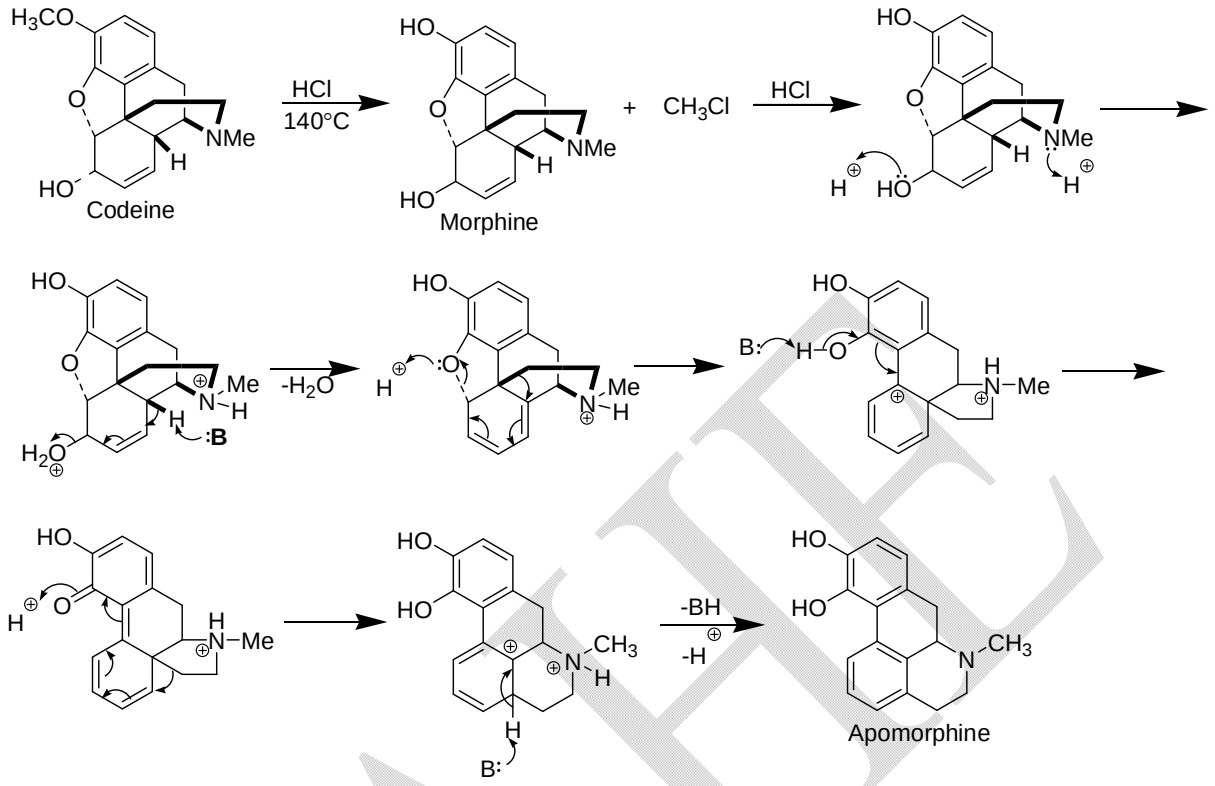


So far, we have accounted for the functional nature of two of the oxygen atoms; the unreactivity of the third oxygen atom suggests that it is probably of the ether type (Vongerichten, 1881).

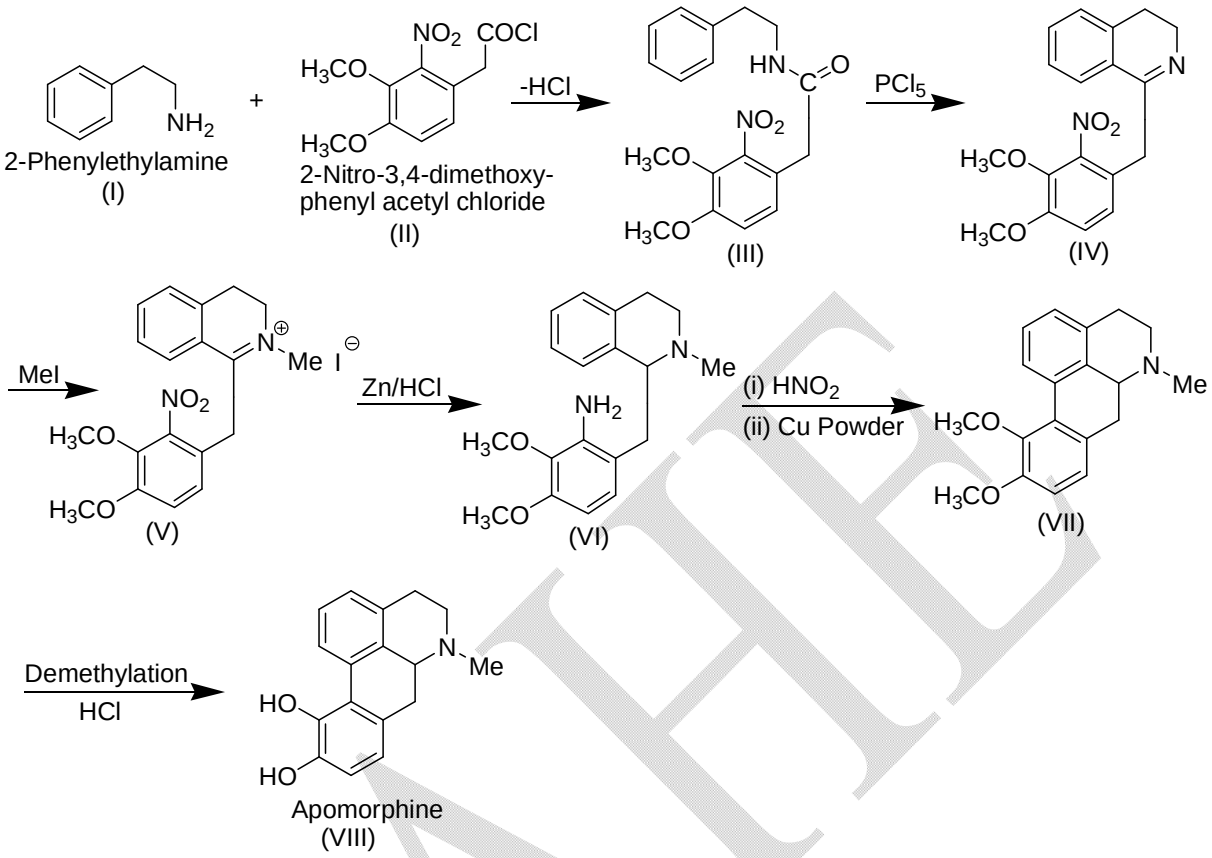
All three alkaloids are tertiary bases (each combines with one molecule of methyl iodide to form a methiodide). When heated with hydrochloric acid at 140°C under pressure morphine loses one molecule of water to form apomorphine, $C_{17}H_{17}NO_2$. Codeine, under the same conditions, also gives apomorphine (and some other products).



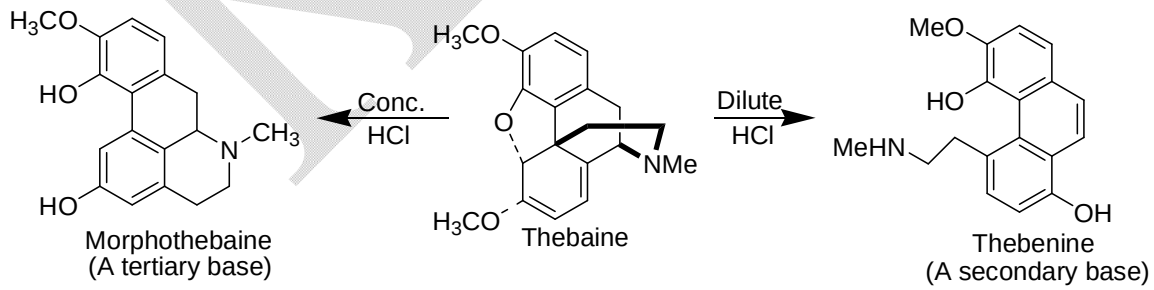

HO
O
NMe
H
H
HO
H
Morphine
HCl
140°C
HO
O
N
Me
H
H
:B
H2O
-H2O
HO
H
:O
N
Me
H
HO
B:
H
O
H
N
Me
HO
H
O
H
N
Me
HO
HO
N
CH3
H
H
B:
-BH
-H
HO
HO
N
CH3
Apomorphine

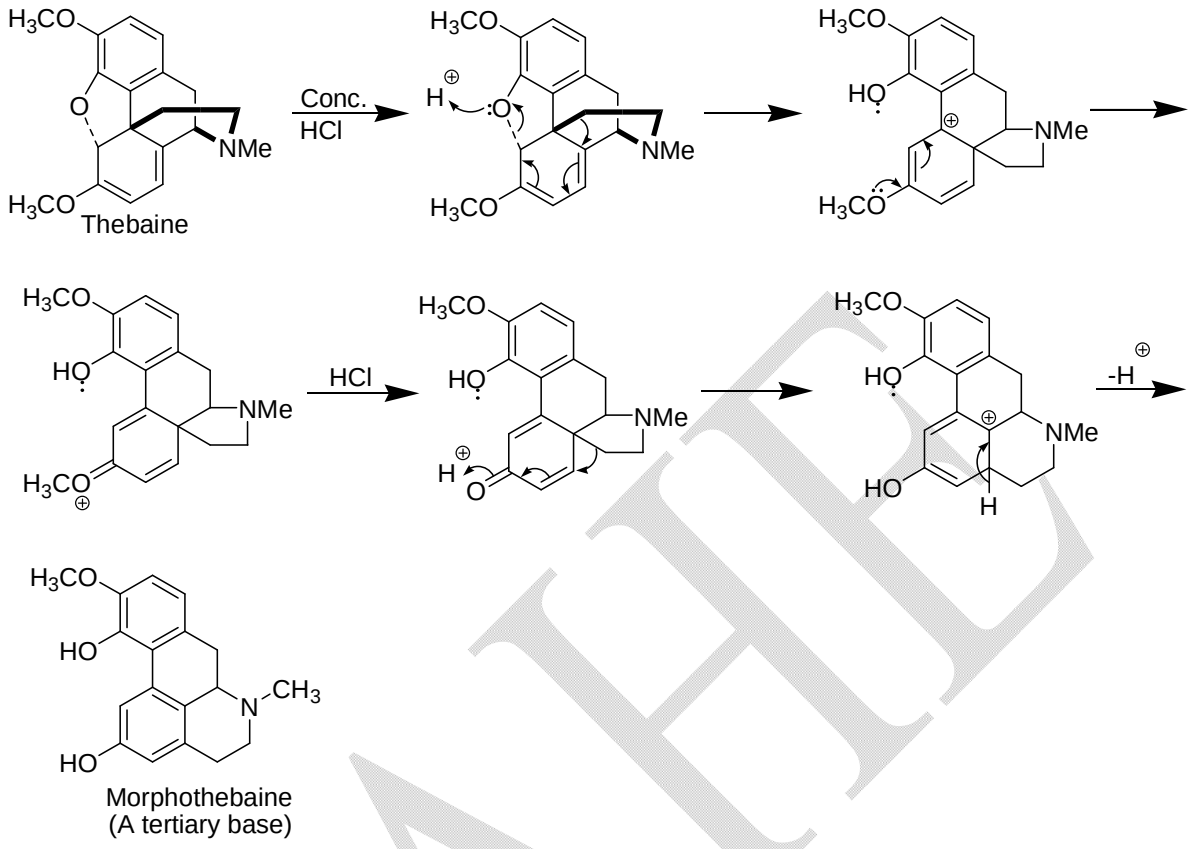


The structure of apomorphine (VIII) has been confirmed synthesis by William S. Burroughs. The amide (III), prepared from the 2-phenylethylamine (I) and the 2-nitro-3,4-dimethoxyphenyl acetyl chloride (II), on Bischler-Napieralski isoquinoline ring closure yields the nitro base (IV), the methiodide of which (V) on reduction is converted into the amino-base (VI), which gives the aporphine (VII) on diazotization and cyclisation by heating with copper powder. The last stage of this process is analogous to the Pschorr phenanthrene cyclisation, developed during earlier work on morphine and thebaine. The demethylation of (VII) yields the apomorphine (VIII).

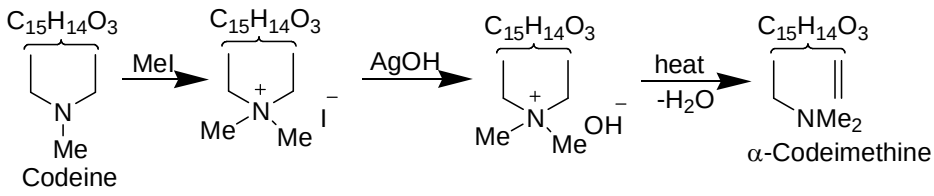


Thebaine, however, when heated with dilute hydrochloric acid, forms thebenine, $C_{18}H_{19}NO_3$ (a secondary base), and with concentrated hydrochloric acid, morphothebaine, $C_{18}H_{19}NO_3$ (a tertiary base). Thus in the formation of thebenine from thebaine, a tertiary nitrogen atom is converted into a secondary one.

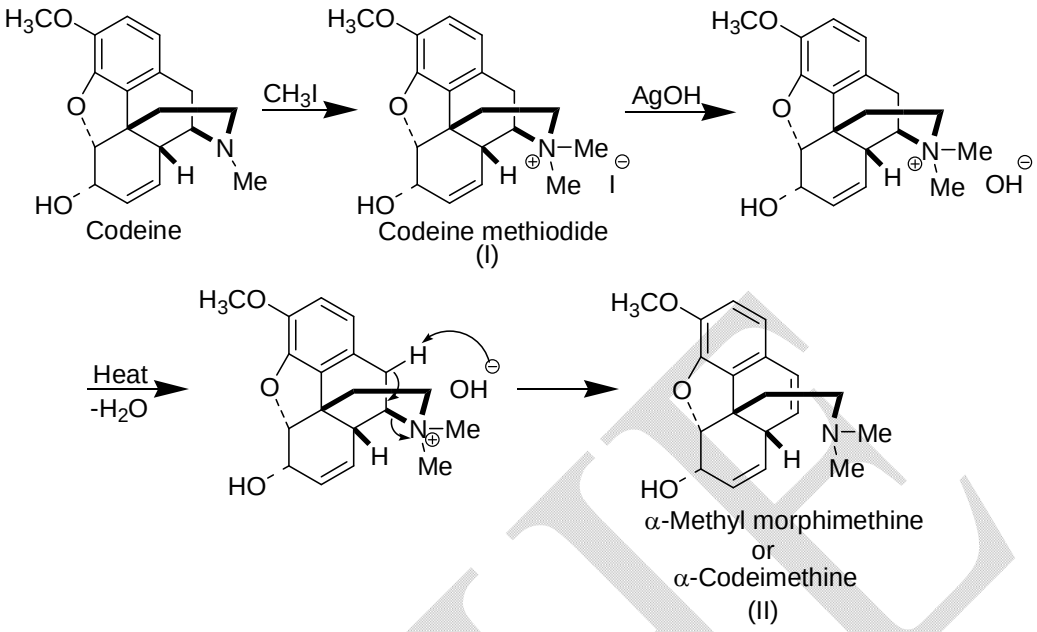




For this change to occur, the tertiary nitrogen must be of the type >NR, where the nitrogen is in a ring system; had the nitrogen been in the group $–NR_2$, then the formation of a primary base could be expected. The presence of a cyclic tertiary base system is supported by the fact that codeine, when subjected to exhaustive methylation, produces α-codeimethine, the formula of which contains one more CH_2 than codeine itself, and the nitrogen atom is not lost. If codeine contains an acyclic *t*-amine system, then the product would contain fewer carbon atoms and loss of nitrogen would occur. If codeine contains a *t*-cyclic base system, the results are then readily explained:

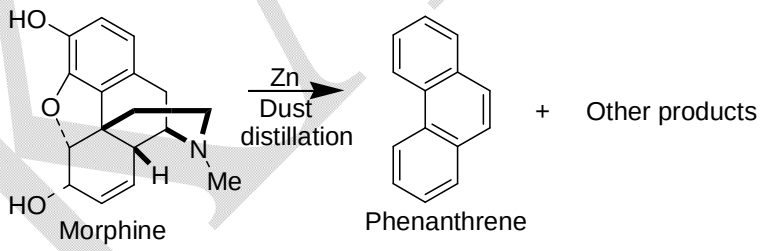


(Or)

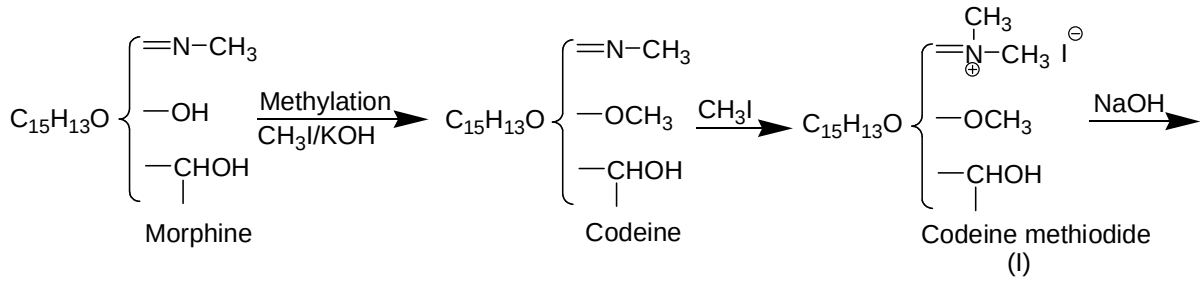


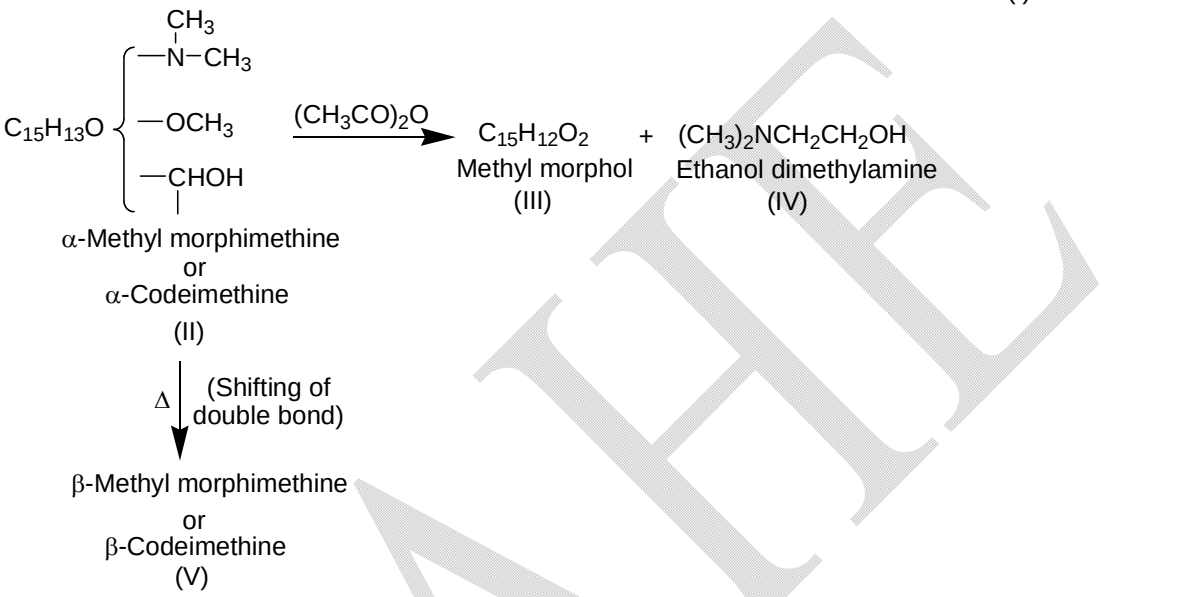
Evidence for the *N*-methyl group is given later, and it should be noted that α–codeimethine and its β-isomer are identical with α- and β-methylmorphimethine, respectively (see below).

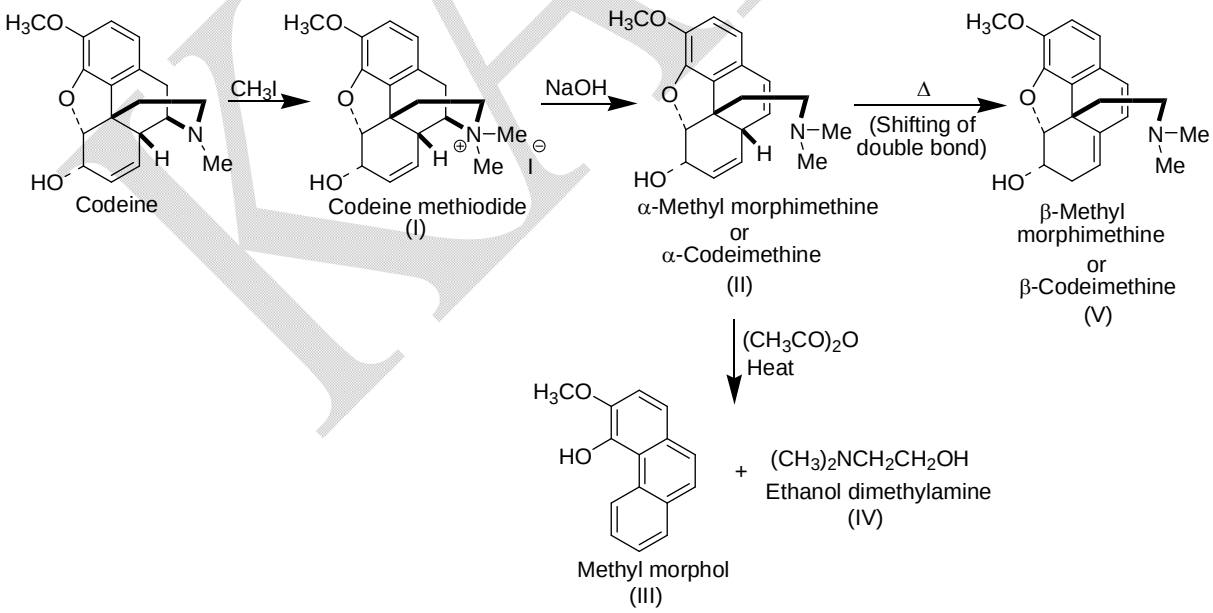
When morphine is distilled with zinc dust, phenanthrene and a number of bases are produced (Vongerichten *et al.,* 1869).



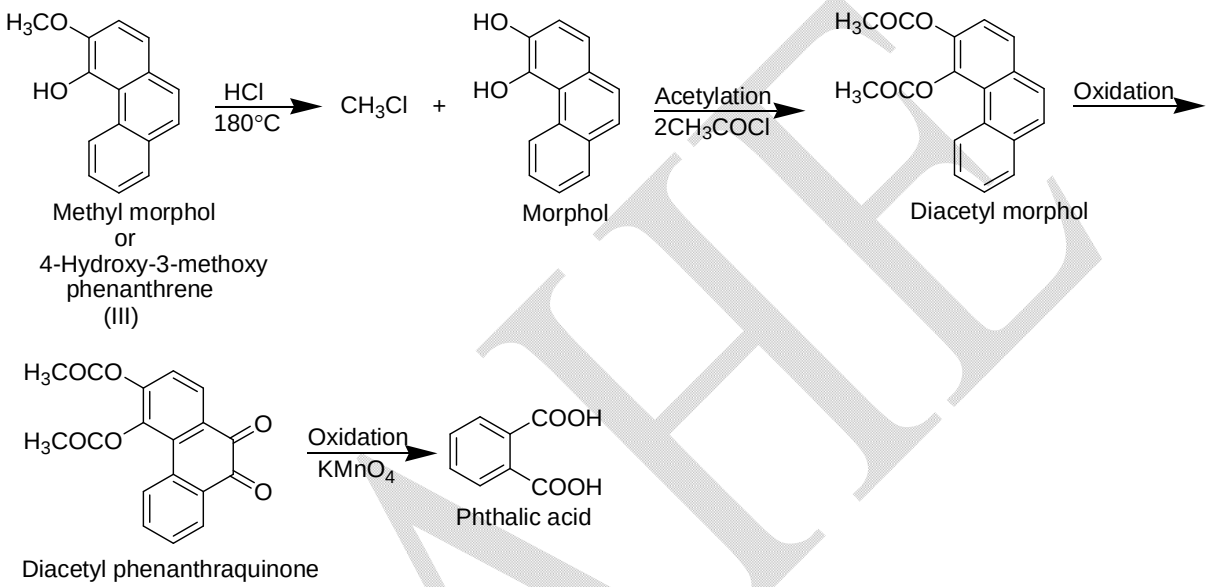
This suggests that a phenanthrene nucleus is probably present, and this has been confirmed as follows. When codeine methiodide (I) is boiled with sodium hydroxide solution, α-methylmorphimethine (II) is obtained and this, on heating with acetic anhydride, forms methyl morphol (III) and ethanoldimethylamine (IV) [some of (II) isomerises to β-methylmorphimethine].


C15H13O
=N−CH3
—OH
—CHOH
Methylation
CH3I/KOH
Morphine
=N−CH3
—OCH3
—CHOH
CH3I
Codeine
CH3
=N−CH3 I
—OCH3
—CHOH
NaOH
Codeine methiodide
(I)

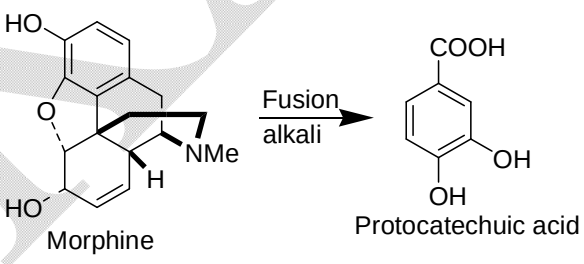

CH3
—N−CH3
C15H13O
—OCH3
—CHOH
α-Methyl morphimethine
or
α-Codeimethine
(II)
(CH3CO)2O
C15H12O2
Methyl morphol
(III)
+
(CH3)2NCH2CH2OH
Ethanol dimethylamine
(IV)
Δ
(Shifting of
double bond)
β-Methyl morphimethine
or
β-Codeimethine
(V)


H3CO
O
N
H
Me
HO
Codeine
CH3I
N−Me
I
Codeine methiodide
(I)
NaOH
N−Me
Me
α-Methyl morphimethine
or
α-Codeimethine
(II)
Δ
(Shifting of
double bond)
β-Methyl
morphimethine
or
β-Codeimethine
(V)
(CH3CO)2O
Heat
+
(CH3)2NCH2CH2OH
Ethanol dimethylamine
(IV)
Methyl morphol
(III)

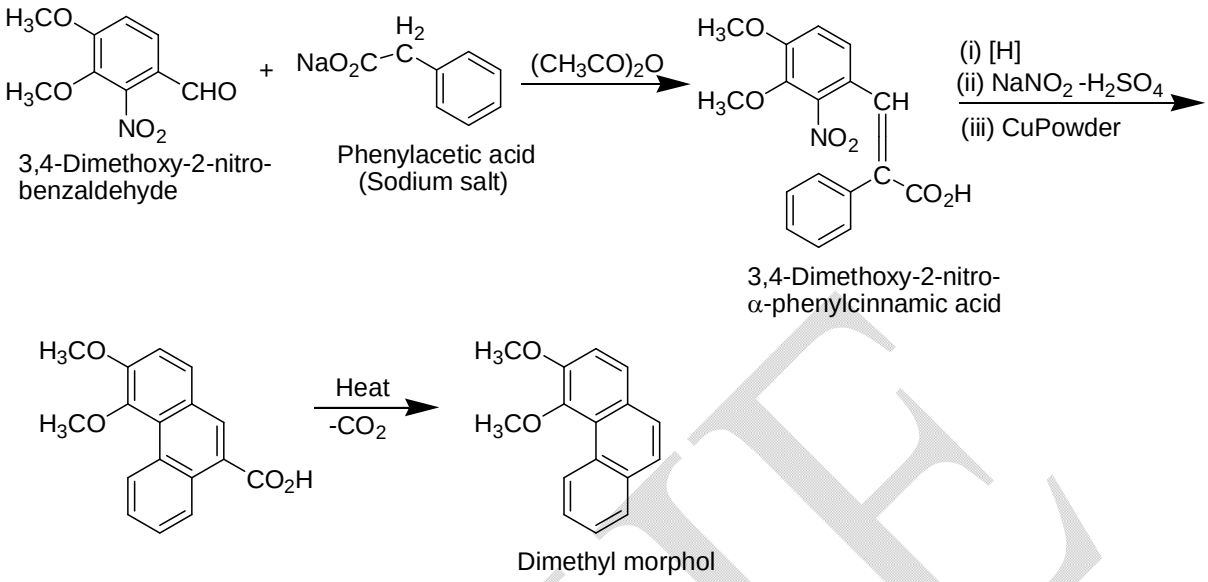
The structure of methyl morphol (III) was ascertained by heating it with hydrochloric acid at 180°C, under pressure; methyl chloride and a dihydroxyphenanthrene, morphol, were obtained. Oxidation of diacetylmorphol gives a diacetylphenanthraquinone; thus positions 9 and 10 are free. On further oxidation (permanganate), the quinone is converted into phthalic acid; therefore the two hydroxyl groups are in the same ring.



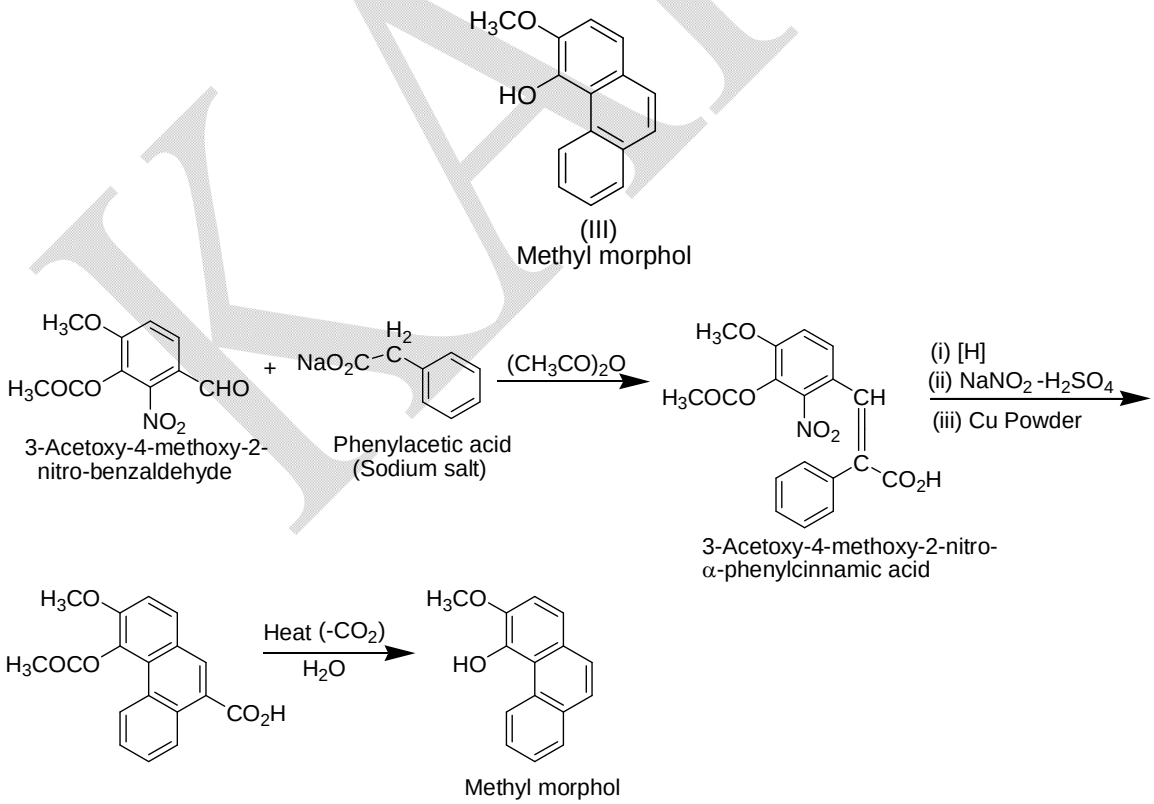
Since the fusion of morphine with alkali gives protocatechuic acid, this shows that both hydroxyl groups in morphol are in the ortho-position.



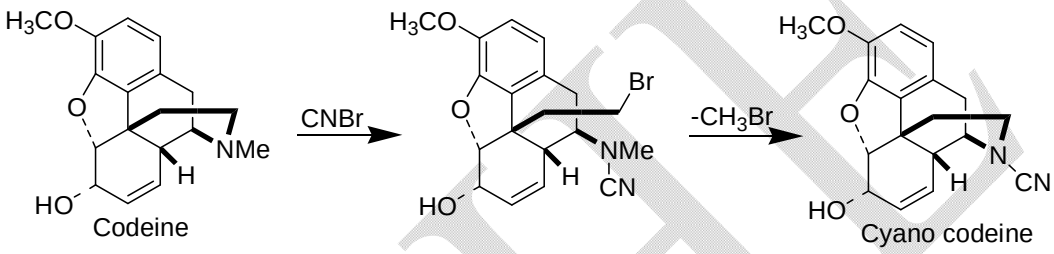
Finally, Pschorr *et al.* (1900) showed by synthesis that dimethyl morphol is 3,4-dimethoxyphenanthrene.



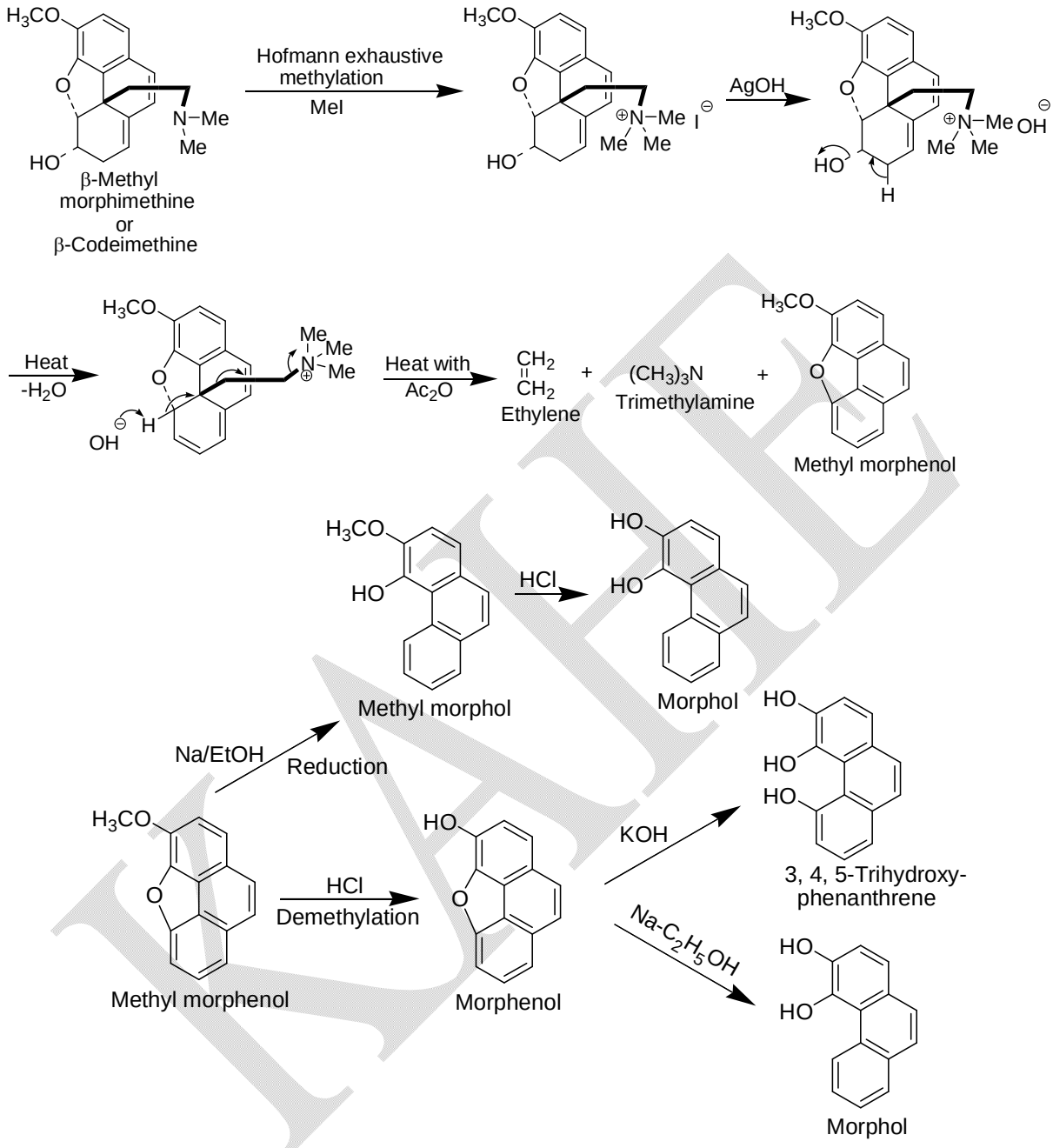
Then Pschorr *et al.* (1902) synthesised methyl morphol (III), and showed it to be 4-hydroxy-3-methoxyphenanthrene (in this synthesis Pschorr used 3-acetoxy-4-methoxy-2-nitrobenzaldehyde).



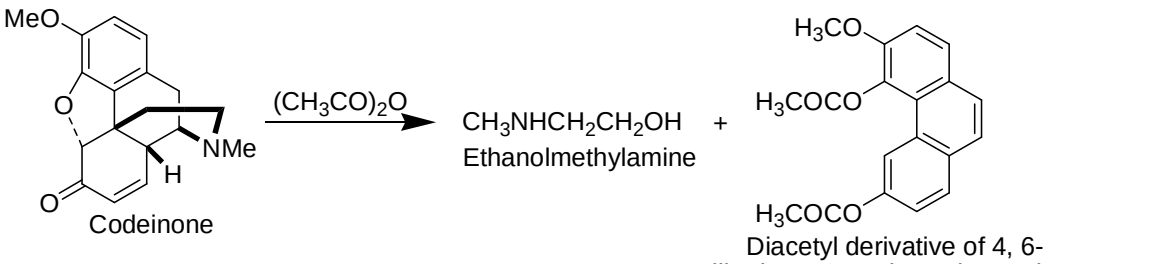
The formation of ethanoldimethylamine (IV) from α-methylmorphimethine indicates that there is a $>NCH_3$ group in codeine (only one methyl iodide molecule adds to codeine to form codeine to form codeine methiodide; it has also been shown above that this nitrogen is in a heterocyclic ring). This is confirmed by the following evidence. When codeine is subjected to the von Braun degradation, three hydrogen atoms are lost and one nitrogen atom is added. This can readily be interpreted by the conversion of $>NCH_3$ into >NCN, and so it follows that all three alkaloids contain an *N*-methyl group.



When β-methylmorphimethine is heated with water, the products obtained are trimethylamine, ethylene and methyl morphenol (Vogerichten, 1896). Demethylation of this compound with hydrochloric acid produces morphenol, a compound which contains one phenolic hydroxyl group and an inert oxygen atom. On fusion with potassium hydroxide, morphenol gives 3,4,5-trihydroxyphenanthrene (Vongerichten *et al.,* 1906). The structure of this compound was shown by the synthesis of 3,4,5-trimethoxyphenanthrene, which was found to be identical with the product obtained by methylating the trihydroxyphenanthrene obtained from morphenol (Pschorr *et al.,* 1912). Furthermore, the reduction of morphenol with sodium and ethanol gives morphol (Vongerichten, 1898). These results can be explained by assuming that morphenol has a structure containing an ether linkage in positions 4,5 (of the phenanthrene nucleus).



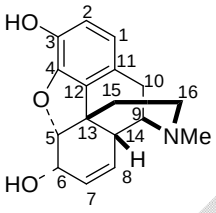
Codeinone, on heating with acetic anhydride, gives ethanolmethylamine and the diacetyl derivative of 4,6-dihydroxy-3-methoxyphenanthrene.



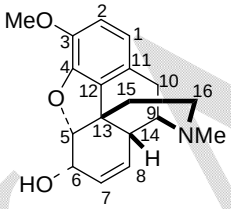
Diacetyl derivative of 4, 6-dihydroxy-3-methoxyphenanthrene
or
3-Methoxy-4,6-diacetoxyphenanthrene

The position 3 of the methoxyl group and the position 4 of the hydroxyl group have already been accounted for the hydroxyl group in the 6-position must therefore be produced from the oxygen of the keto group in codeinone.

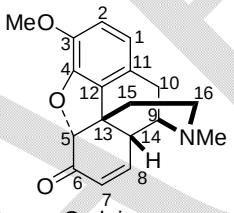
Based on the foregoing evidence, and a large amount of other experimental work, Gulland and Robinson (1923, 1925) proposed the following structures; these have been written with the configurations assigned by later workers (See below).



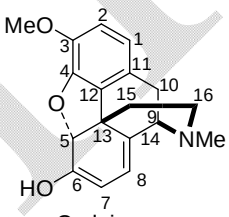
Morphine



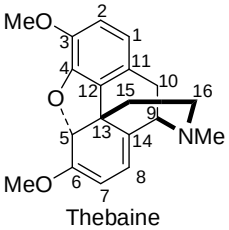
Codeine



Codeinone



Codeinone
(an enol form)

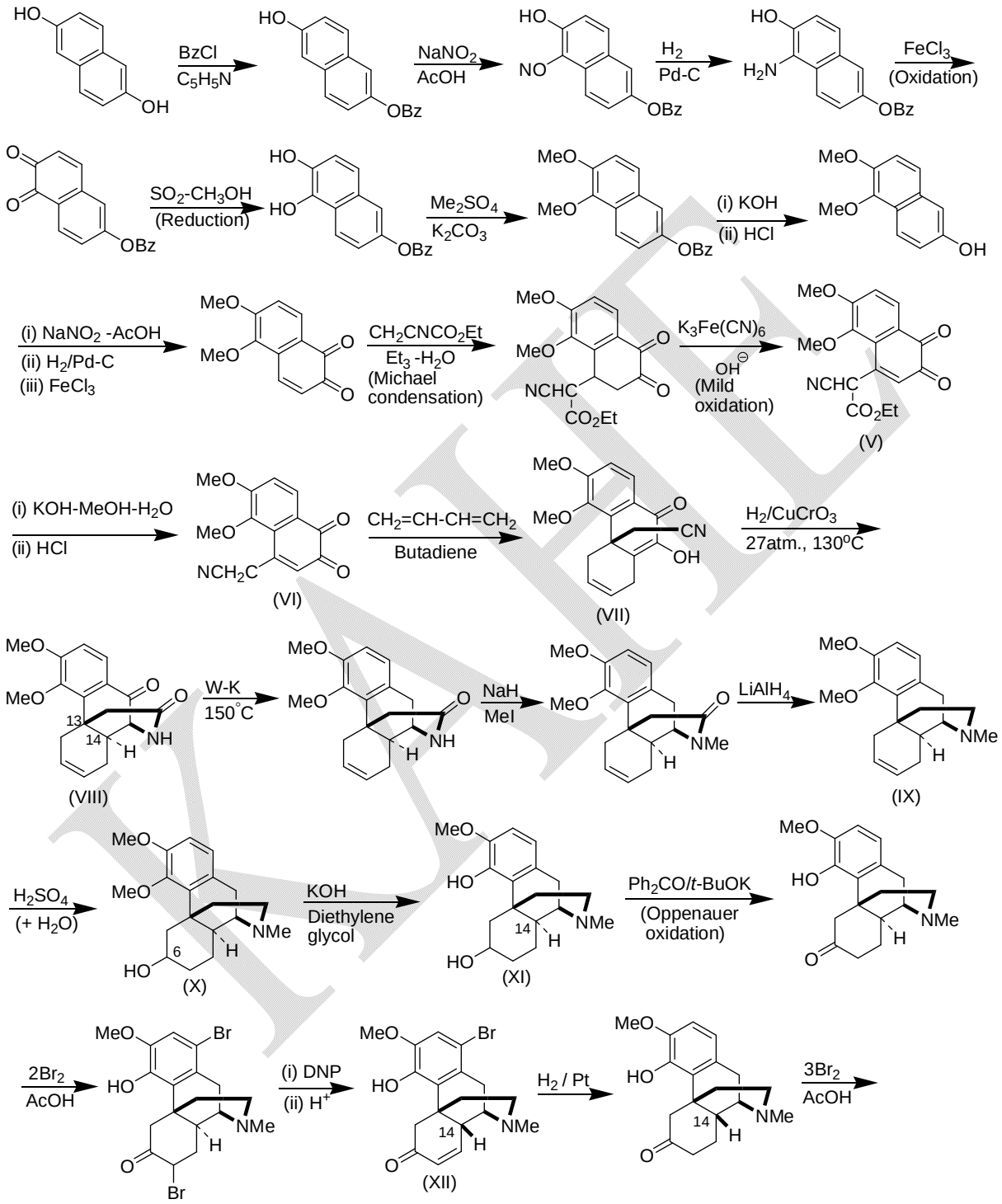


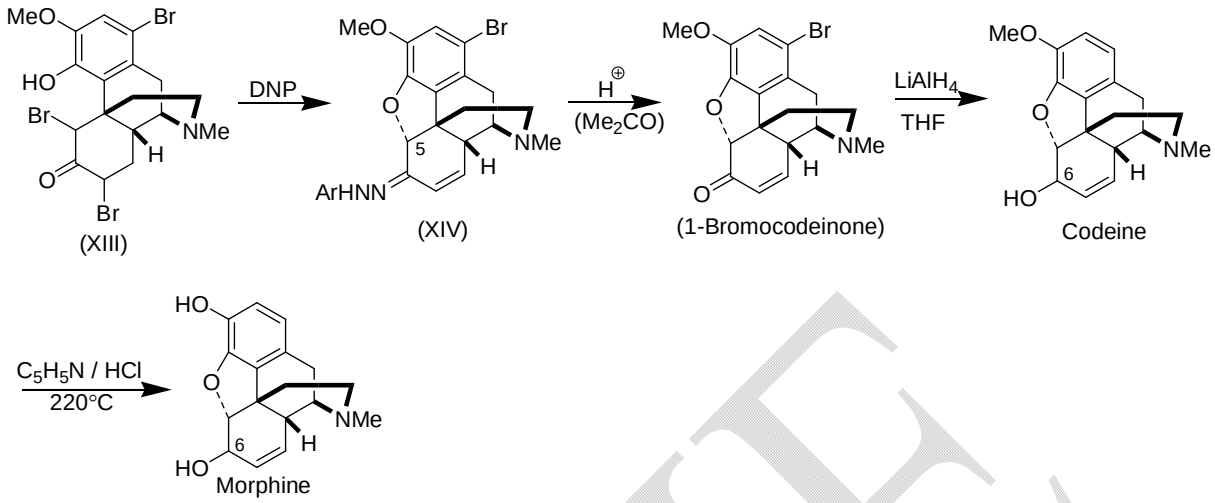
Thebaine

One piece of evidence used by Gulland and Robinson was that it had previously been shown that the nitrogen atom must be attached to C-9 or C-10. These workers therefore proposed that the nitrogen-carbon side-chain must be attached to C-13 or C-14. This was based on the argument that aromatisation of the hydrogenated phenanthrene nucleus must occur with loss or migration of that side-chain. Hence, the side-chain must be attached at an angular carbon atom. Of the two possibilities, C-13 and C-14, C-13 was chosen since, on this basis, it was possible to explain some of the rearrangements undergone by various members of this group of alkaloids. The correctness of this assignment was later demonstrated experimentally (Rapoport *et al.*, 1947), but the attachment of the nitrogen atom to C-9 was proved only by the synthesis of morphine and codeine.

Morphine has now been synthesised in different ways; the following synthesis is that of Gates *et al.* (1956) [Bz =PhCO; W-K = Wolff-Kishner reaction; DNP = 2,4-dintrophenylhdrazine].

(a) Gate's synthesis



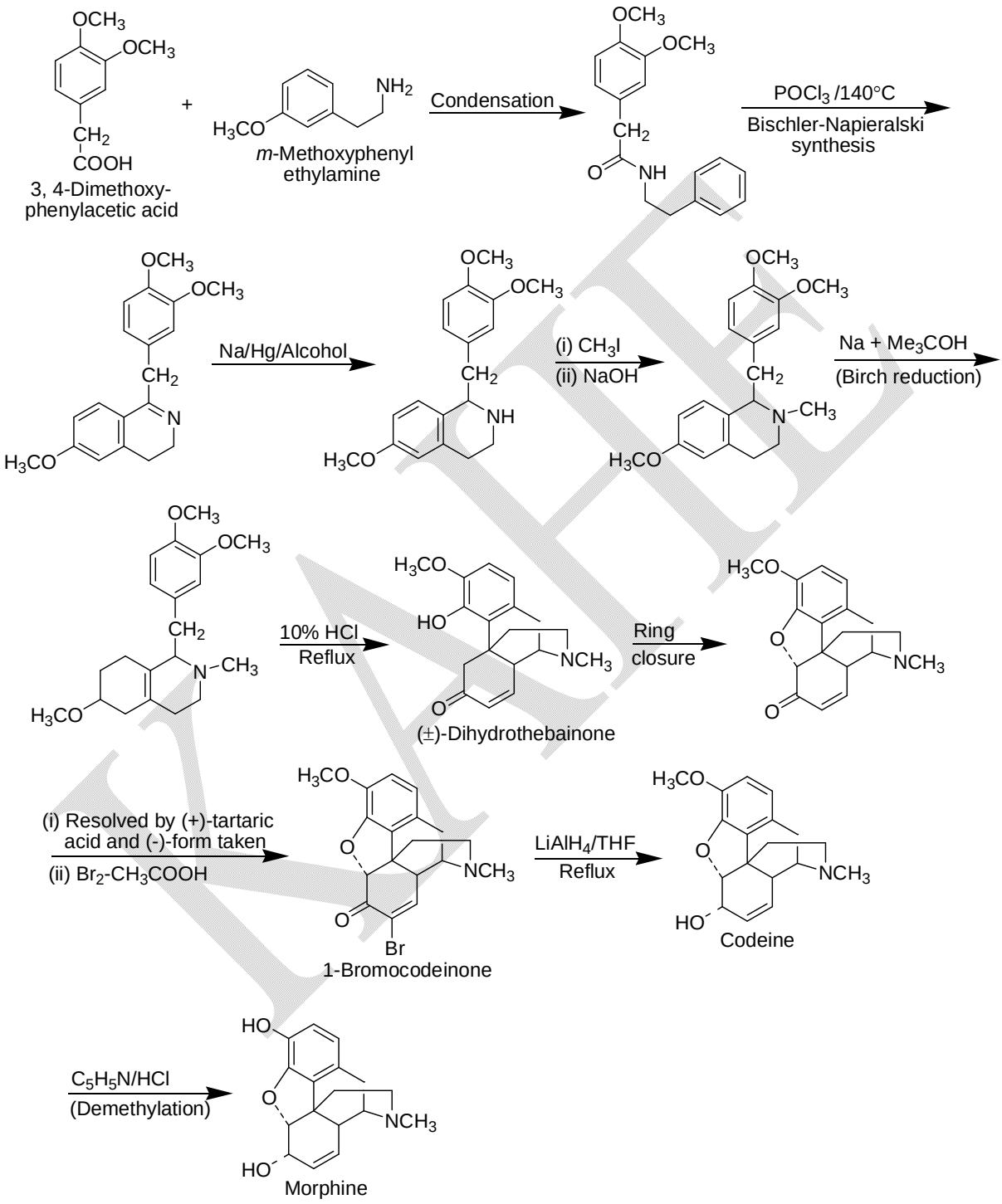


The approach adopted by Gates was the synthesis of the hydrophenanthrene precursor. Since the Schotten Baumann method of benzoylation produced the dibenzoate of 2,6- dihydroxy-naphthalene, the conditions for monobenzoylation had to be worked out. The object of this protection of one hydroxyl group was to permit the carrying out of the desired sequence of reactions at one part of the molecule at a time. Nitrosation gave the 1-nitroso- compound and oxidation of the 1-amino-2- hydroxyl–derivative gave the 1, 2- quinone which was readily reduced by sulphur dioxide. These two hyrdroxyl groups were protected by methylation, the protecting benzoyl group removed and this part of the molecule was subjected to the previous sequence of reactions to give the 1,2-quinone as shown. This quinone was condensed with ethyl cyanoacetate (Michael condensation) and the product was oxidised under mild conditions to regenerate the quinone (V). Selective hydrolysis of (V) gave the salt of the α-cyanoacid which, on acidification, readily underwent decarboxylation to give (VI). This loss of carbon dioxide may be explained by the principle of vinylogy applied to a β-keto acid. It must be admitted, however, that the cyclic state would be highly strained. (VI) underwent the Diels–Alder reaction on treatment with butadiene to give the enol form ((VII); probably formed from the diketo precursor). The result is that the cis-stereospecificity addition of the Diels–Alder reaction (to give a cis-hydrogen) has been lost. Catalytic reduction of (VII) gave (VIII), in which the ethanamine bridge at C-13 was trans to the hydrogen atom at C-14. This was the 'wrong' orientation for the hydrogen atom at C-14. Also, the reduction resulted in cyclisation to form the lactam (VIII), the structure of which was proved by infrared spectroscopy (the mechanism of this

cyclisation is uncertain). Since (IX) could be obtained from natural sources (by degradation of thebaine), further synthetic steps could be carried out on the 'natural' compound ((IX) is said to be a synthetic 'relay). Furthermore, since synthetic (IX) was the (±)-form and 'natural' (IX) was optically active-the (+)-form-the latter was used in the subsequent synthetic steps. Hydration of (IX) gave (X), the desired product (6-OH) and some isomeric 7-OH compound. It can now be seen that Diels-Alder reaction has led to a cyanomethyl group at C-13 in the correct orientation for further steps leading to cyclisation to form the ethanamine bridge, and also to the 6,7-double bond which was to act as a means of introducing the 6-OH group. Demethylation of (X) gave (XI) in which the hydroxyl group was in the correct position (the reason for this selectivity is uncertain). At this stage, the inversion of the chiral centre at C-14 was carried out to give the correct orientation in (XII). Dehydrobromination of an α-bromoketone with the formation of the DNP derivative is a standard reaction. The mechanism of this inversion can be explained on the basis that the C-14 hydrogen atom is in the vinylogous α-position with respect to the C=N group at C-6 (i.e., C_6 = NNHAr). This is the imine-enamine tautomeric system:

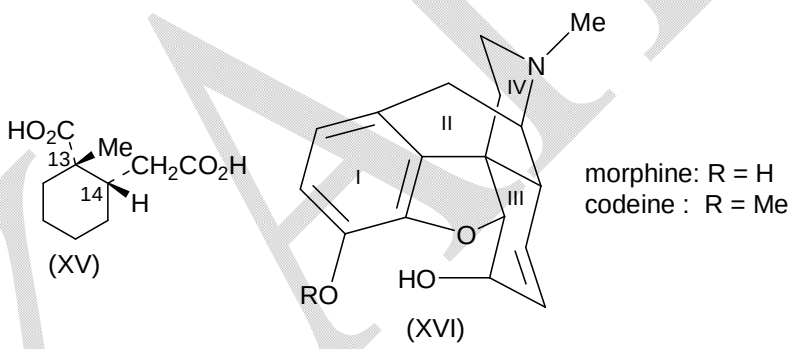
The steps leading from (XII) to (XIII) resulted in the correct orientation of the oxide bridge in morphine. The reason is not certain. A possibility is as follows. The 6-OH group in codeine and morphine has been shown to be axial, and since it has also been established that the oxygen at C-5 is cis with respect to the OH at C-6, the oxygen atom is equatorial. Hence, if the bromine atom at C-5 in (XIII) is axial, then attack by the C-4 hydroxyl group can readily occur by an S_N2 mechanism. As we have seen, bromination of steroid ketones produces the α-axial bromoderivative (at first). Reduction of codeinone by lithium aluminium hydride caused the removal of the bromine atom (this is unusual for an aromatic compound) and the formation of the correct alcohol epimer (axial), codeine. This stereospecificity has been attributed to the steric hindrance caused by the benzene ring.

(b) Morrison, Waite and Shavel Jr., Tetrahedron Letters Vol. No. 41, P. 4055, 1967.



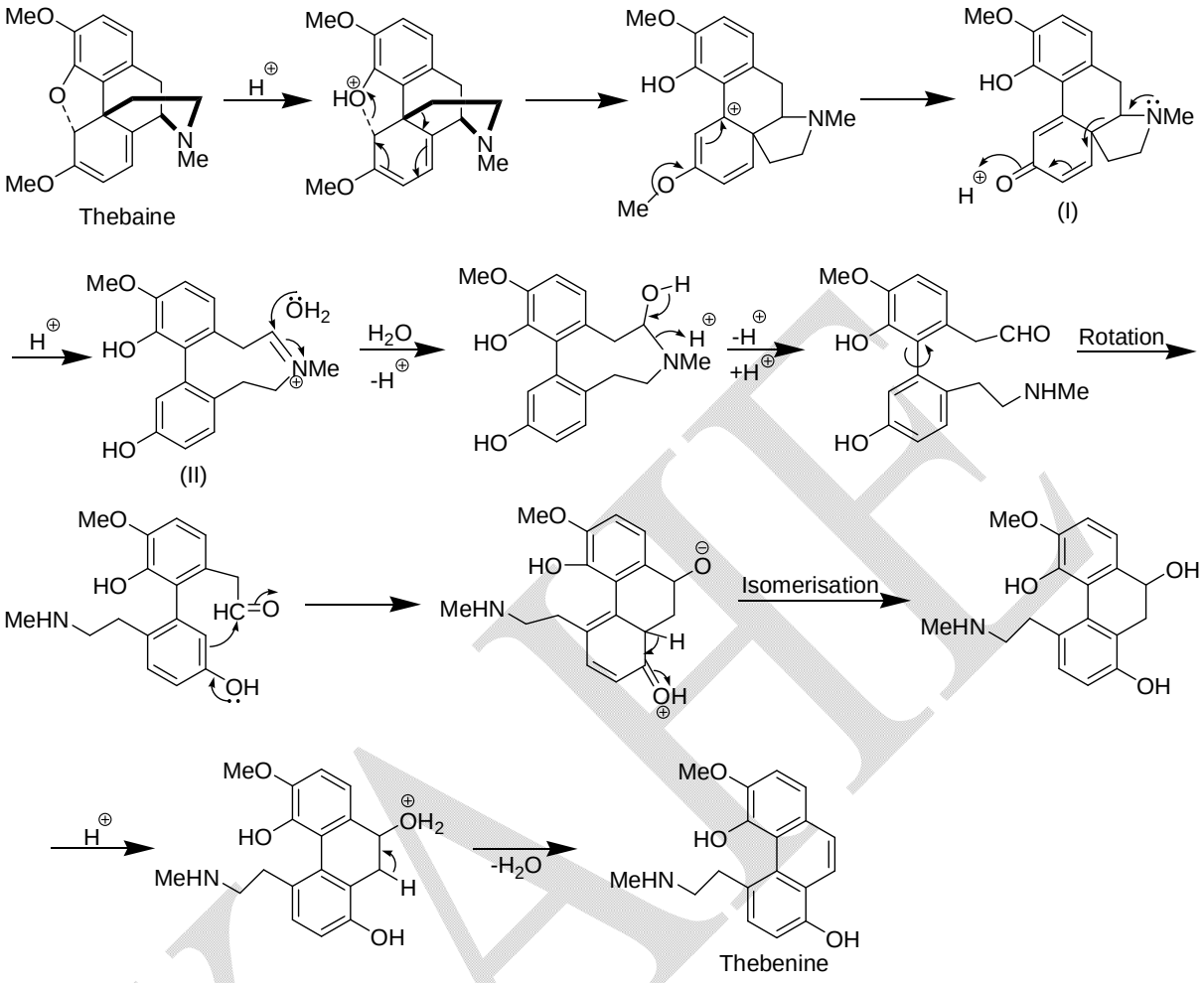
Stereochemistry of morphine and codeine

Each of these compounds contains five chiral centres (5,6,9,13 and 14) but since the bridged ring system across positions 9, 13 must be cis, eight pairs of enantiomers are possible for each compound. A great deal of chemical work has been carried out to deduce the stereochemistry of codeine and has led to the configuration given above, i.e., the hydrogen atoms at C-5, C-6, and C-14 are all cis, and the bridge at C-9 and C-13 is also cis. This stereochemistry has been confirmed by X-ray analysis (Mackay et al., 1955), but it was not possible, however, to determine the absolute configuration by this method. This has been done as follows. Degradation of thebaine gave the dicarboxylic acid (XV), thereby establishing the absolute stereochemistry at C-13 and C-14 (Kalvoda *et al.*, 1955). The conformational formulae of morphine and codeine may be written as (XVI). The chair form has been used for the cyclohexene ring and rings I, II and the oxide bridge lie approximately in the plane of the paper and rings III and IV are approximately perpendicular to the plane of the paper.

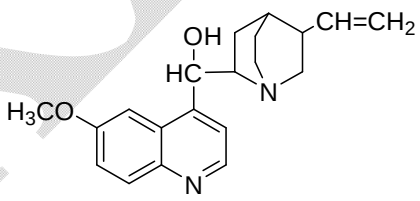


Molecular rearrangements

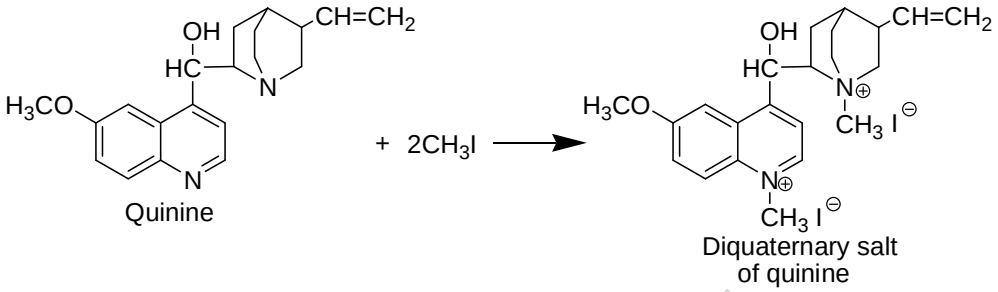
Thebaine and its derivatives undergo many types of rearrangement, most of which occur under the influence of acid, e.g., when heated with dilute hydrochloric acid, thebaine rapidly undergoes rearrangement to form thebenine. One suggestion is that the Joshi *et al.*, (1968) confirmed structure (III) by synthesis. The used the Vilsmeier-Haack aldehyde synthesis on 2-hydroxycarbazole (V). This gave a mixture of the 3-(VI) and the 1-aldehyde (VII). These were separated chromatographically and (VI) on prolonged shaking with 3,3-dimethyl-allyl bromide in the presence of aqueous potassium hydroxide, gave heptaphylline (III).



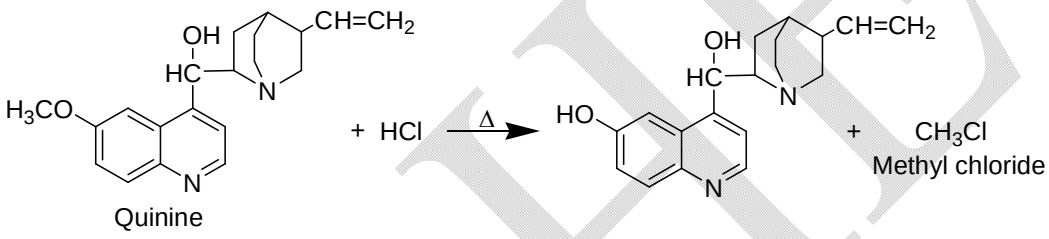
(-)-Quinine



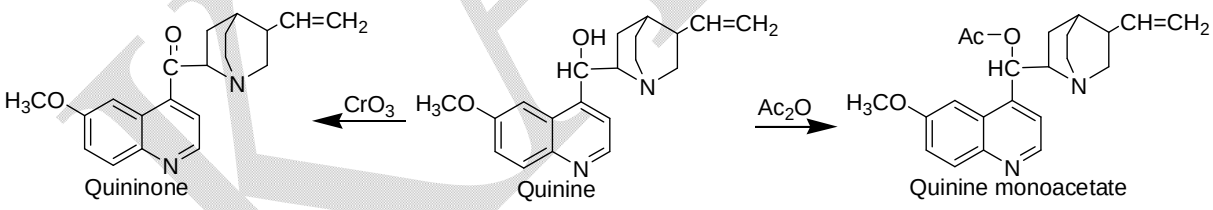
Molecular formula: $C_{20}H_{24}N_2O_2$, m.p. 177°C, is used as a febrifuge and as an antimalarial. Since quinine adds on two molecules of methyl iodide to form a diquaternary salt, it is therefore a ditertiary base.



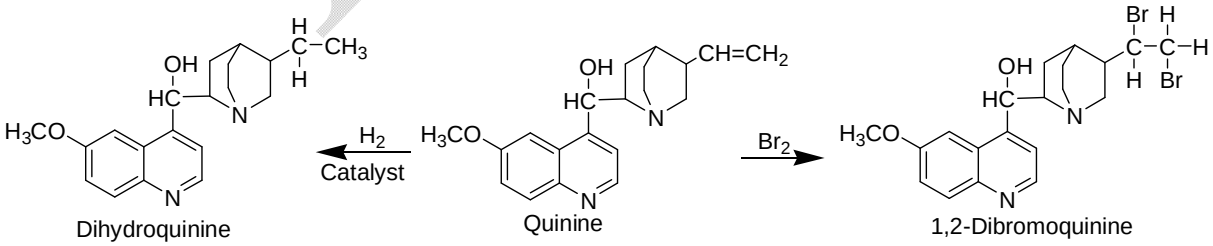
When heated with hydrochloric acid, quinine eliminates one carbon atom as methyl chloride; therefore there is one methoxyl group present in the molecule.



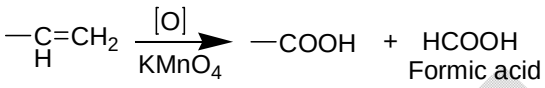
Since quinine forms a monoacetate and a monobenzoate, one hydroxyl group must be present, and that this is secondary alcoholic is shown by the fact that oxidation of quinine with chromium trioxide produces quininone, a ketone.



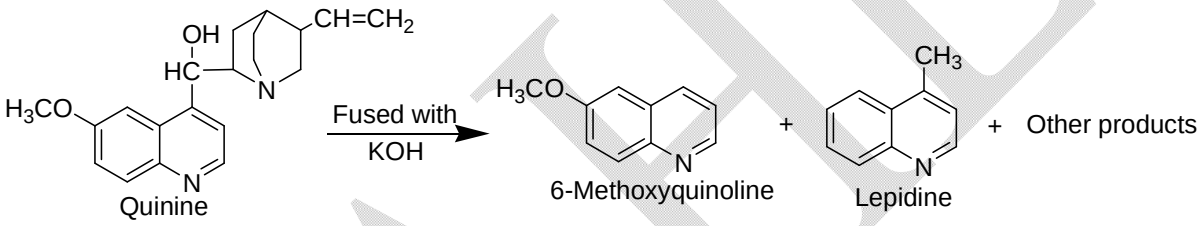
Quinine also contains one ethylenic double bond, as is shown by the fact that it adds on one molecule of bromine, etc.



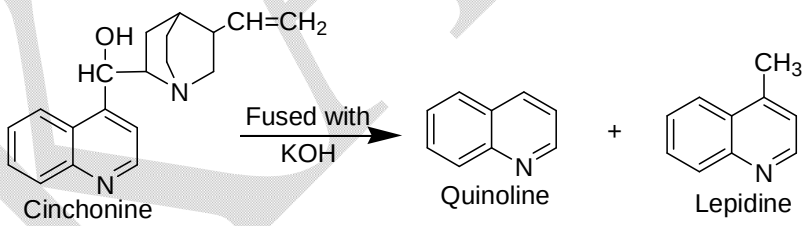
When controlled oxidation of quinine is done with $KMnO_4$, it yields a monocarboxylic acid along with formic acid. This reaction reveals that a vinyl group is present in quinine.



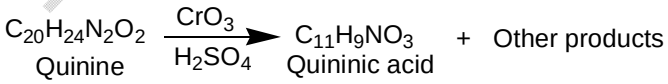
When quinine is fused with concentrated potassium hydroxide, it yields a mixture of 6-methoxyquinoline and lepidine (4-methylquinoline) along with other products. These products prove that a quinoline nucleus is present in quinine.



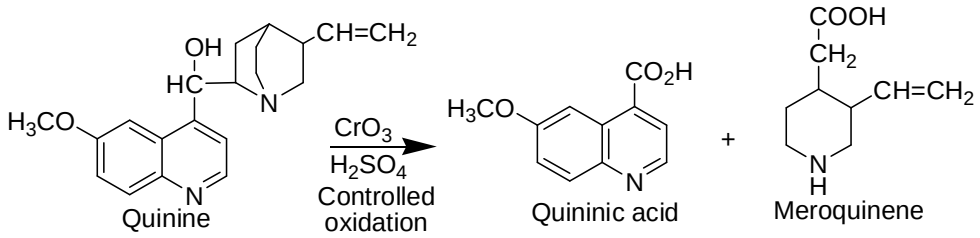
Cinchonine when fused with potassium hydroxide under the same conditions yields quinoline and lepidine, indicating that quinine is methoxycinchonine.



Oxidation of quinine with chromic acid produces, among other products, quininic acid.

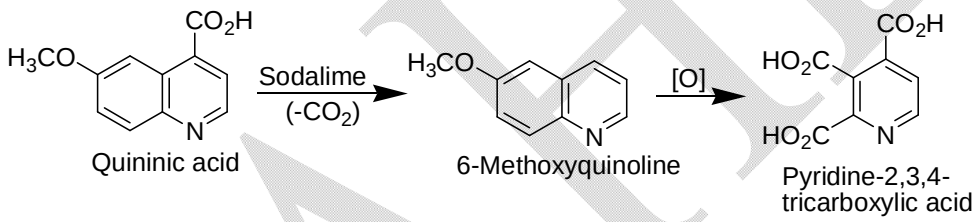


On the other hand, controlled oxidation of quinine with chromic acid gives quininic acid and meroquinene. Thus the 'second-half' in both quinine and cinchonine is the same, and so the problem is to elucidate the structure of quininic acid.

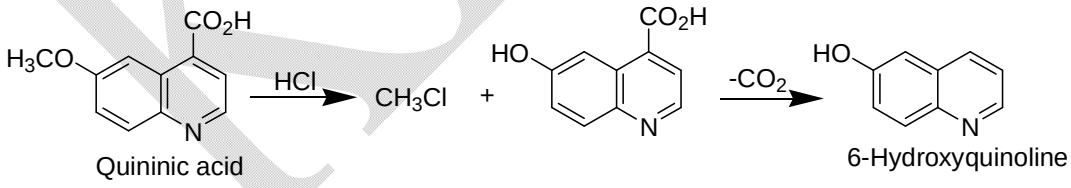


Structure of Quininic acid. This is established as follows:

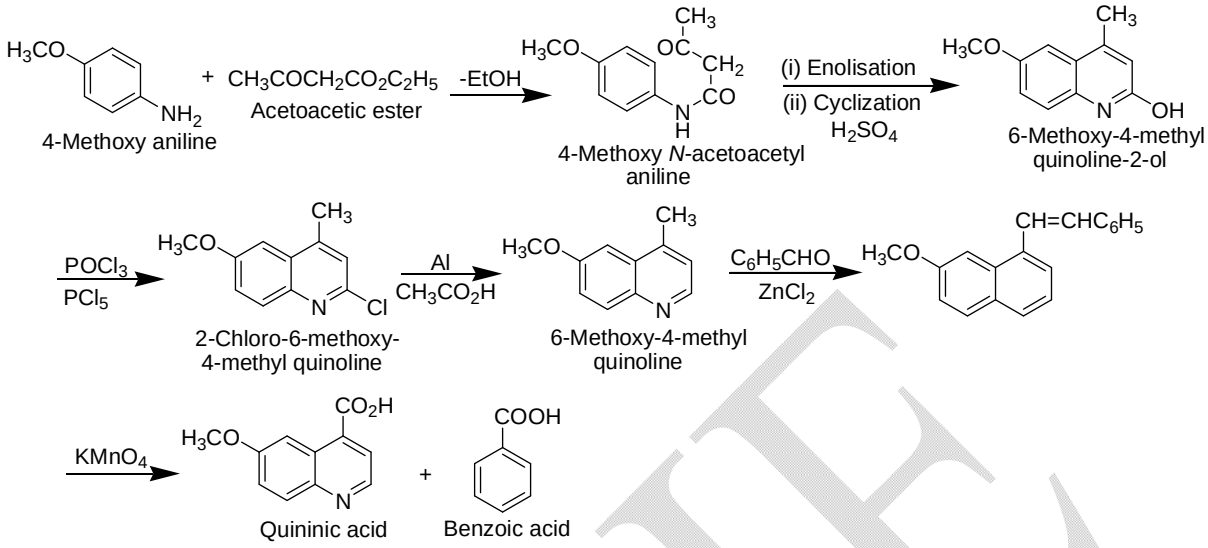
When heated with soda-lime, quininic acid is decarboxylated to a methoxyquinoline, and since, on oxidation with chromic acid, quininic acid forms pyridine-2,3,4-tricarboxylic acid, the methoxyl group must be a substituent in the benzene ring (of quinoline), and the carboxyl group at position 4 (Skraup, 1881).



The position of the methoxyl group was ascertained by heating quininic acid with hydrochloric acid and decarboxylating the demethylated product; 6-hydroxyquinoline (a known compound) was obtained. Thus quininic acid is 6-mehoxycinchoninic acid.



This structure for quininic acid has been confirmed by synthesis (Rabe *et al.*, 1931)



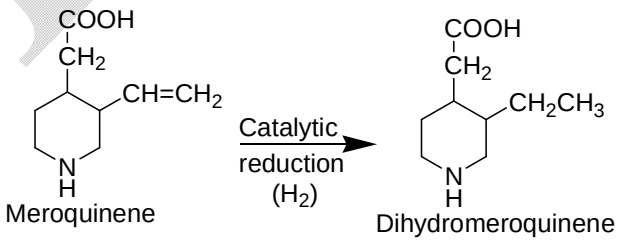
The direct oxidation of 6-methoxy-4-methylquinoline to quininic acid is extremely difficult; oxidation of the methyl group is accompanied by the oxidation of the benzene ring, the final product being pyridine-2,3,4-tricarboxylic acid.

Structure of meroquinene

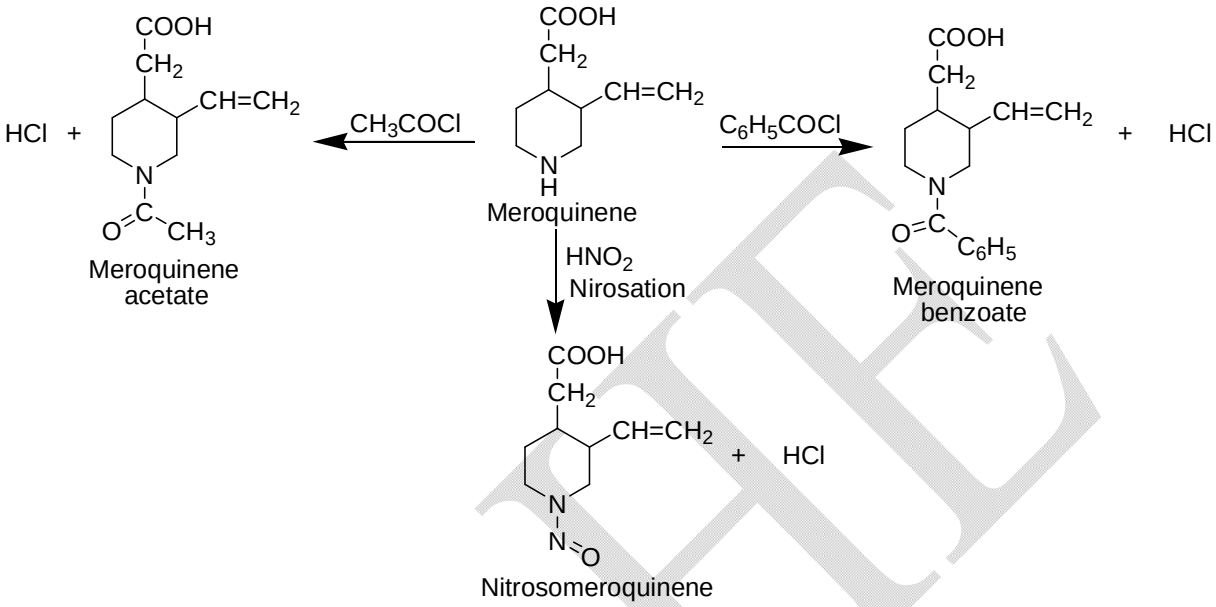
(a) Its molecular formula has been found to be $C_9H_{15}NO_2$.

(b) As it forms a monosodium salt as well as an ester, it reveals the presence of a carboxylic group (-COOH).

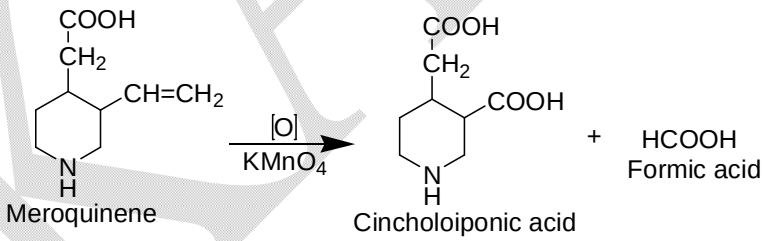
(c) When meroquinene is reduced with hydrogen, it takes up one molecule of hydrogen, suggesting that a ethylene double bond is present in it. The presence of ethylenic double bond indicates that –CH=CH_2, i. e., side-chain is still present in meroquinene.



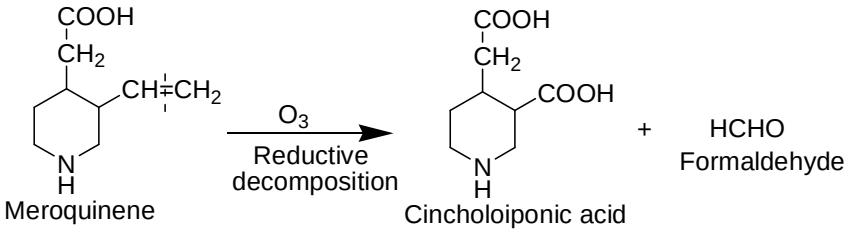
(d) As meroquinene can be benzoylated, acetylated and nitrosated (forms nitroso derivative with HNO_2), it means that a secondary amino group is present in meroquinene.



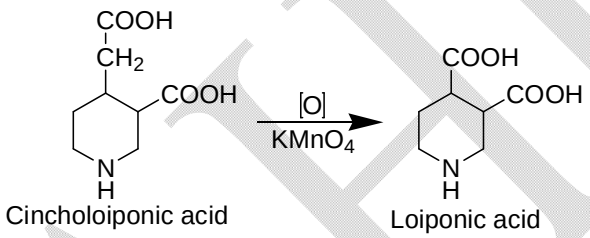
(e) When meroquinene is oxidised with cold acidified $KMnO_4$, it yields a cincholoiponic acid (a dicarboxylic acid) and formic acid.



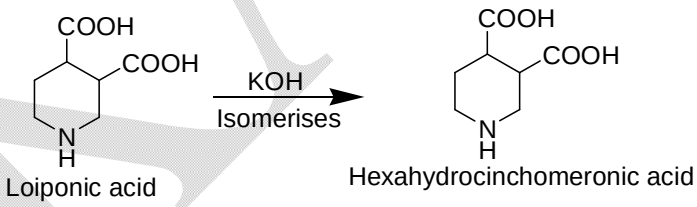
The formation of formic acid reveals the presence of $-CH=CH_2$ (vinyl group) side-chain in meroquinene. The presence of this group is further confirmed by the ozonolysis of meroquinene followed by its reduction when formaldehyde is produced.



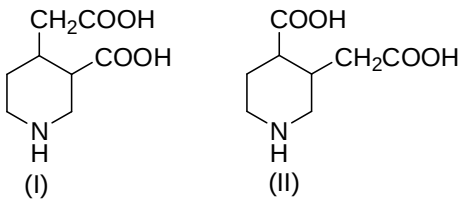
(f) When cincholoiponic acid is oxidised with acid permanganate, it yields loiponic acid, $C_7H_{11}NO_4$. As loiponic acid is a dicarboxylic acid and contains one methylene group than its precursor cincholoiponic acid, this means that the latter contains at least a side-chain $–CH_2COOH$.



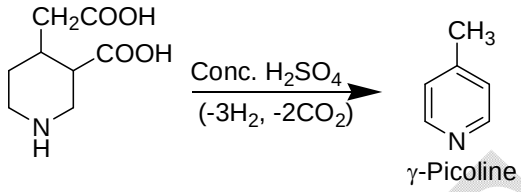
(g) Loiponic acid has been somewhat less stable and isomerises to more stable hexahydrocinchomeronic acid, $C_7H_{11}NO_4$ (piperidine-3,4-dicarboxylic acid) on treatment with KOH at about 200ºC; hence loiponic acid should also be piperidine-3,4-dicarboxylic acid.



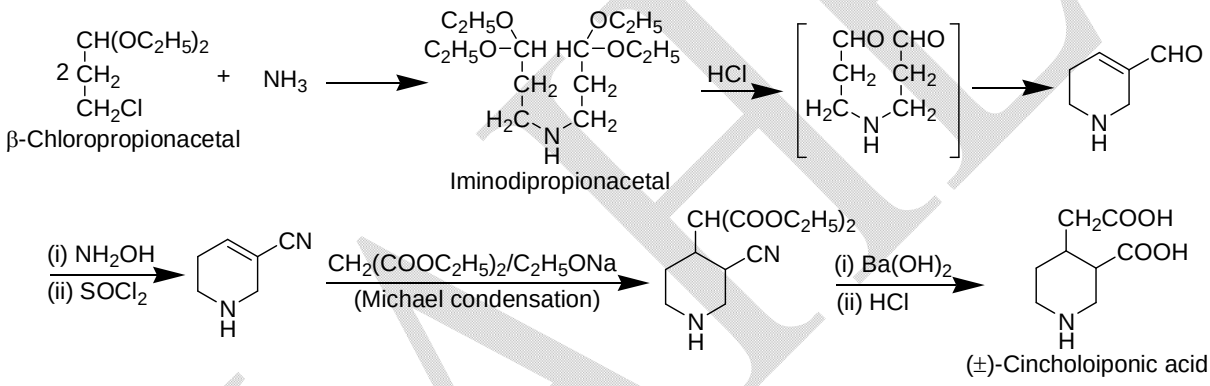
(h) Now as cincholoiponic acid is having one more $–CH_2-$ than the loiponic acid; it must be either (I) or (II).



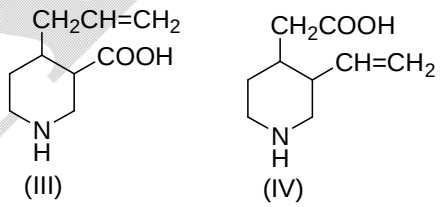
Cincholoiponic acid has been found to be (I) because on heating with Conc. H_2SO_4 it gives γ-picoline.



The structure (I) for cincholoiponic acid has been further proved by its synthesis (Wohl *et al.*, 1907).

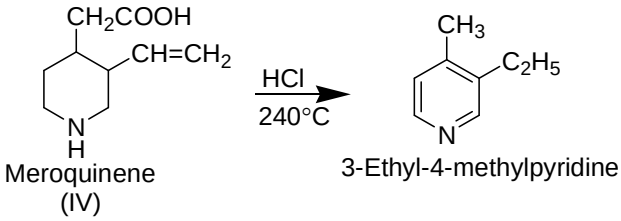


(i) It is known that cincholoiponic acid is obtained along with HCOOH by the oxidation of meroquinene (a monocarboxylic acid), the latter must be having a grouping of the type –CH=CH_2. Hence, meroquinene may be either (III) or (IV).



But structure (IV) has been found to be correct due to the following observations:

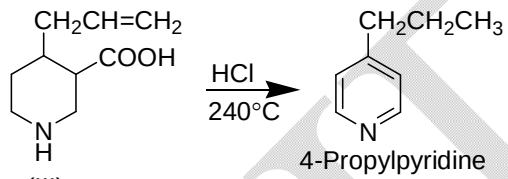
(a) Meroquinene on heating with hydrochloric acid at about 240ºC yields 3-ethyl-4-methylpyridine.



Meroquinene
(IV)

3-Ethyl-4-methylpyridine

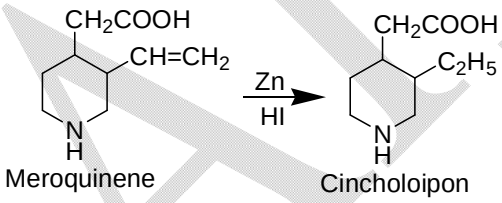
Had the structure been III, 4-propylpyridine would have been formed.



(III)

4-Propylpyridine

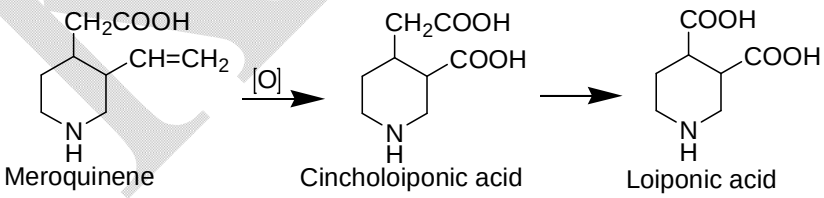
(b) Meroquinene on reduction with zinc and hydriodic acid yields cincholoipon, $C_9H_{17}NO_2$, which is having a carboxyl group and an ethyl group.



Meroquinene

Cincholoipon

Had the structure been (III) propyl group would have been present at position 4. Hence the oxidation of meroquinene and its degraded products may be depicted as follows:



Meroquinene

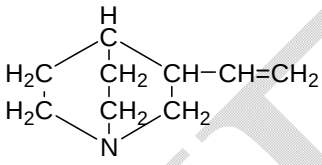
Cincholoiponic acid

Loiponic acid

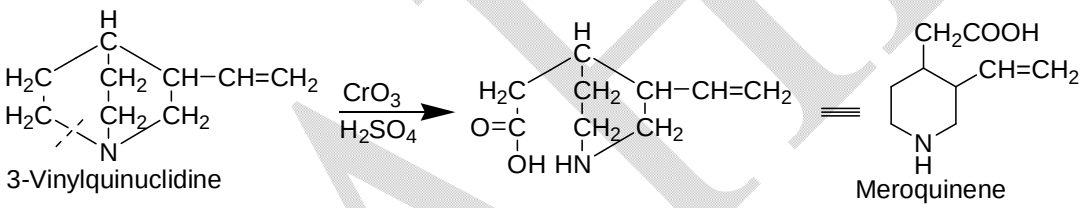
Point of linking between quininic acid and meroquinene in quinine

In the quinine molecule carboxylic groups are not present. But its both oxidation products, i.e., quininic acid and meroquinene, possess free carboxylic groups indicating that the two fragments are linked with each other by means of the carboxylic carbon atoms. Further,

quinine is a ditertiary base whereas meroquinene possesses a secondary nitrogen atom, thus indicating that in the formation of quinine a tertiary nitrogen is converted into a secondary nitrogen atom and at the same time a –COOH group is produced. This behavior is possible only if nitrogen forms a part of a condensed ring system. A possible explanation is that the precursor of meroquinene is having the following structure:



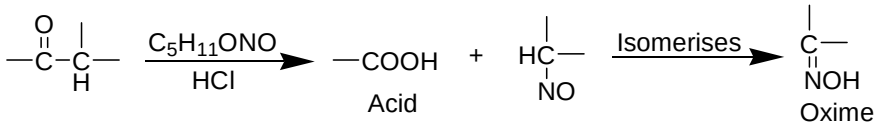
When 3-vinyl quinuclidine is oxidised with CrO_3, one C-N bond is ruptured during the oxidation, thus producing a secondary N-atom from a tertiary one and –COOH group also.



The above structure of 3-vinylquinuclidine is confirmed by the synthesis of 3-ethyl-quinuclidine. From the above facts, we can draw the conclusion that in quinine molecule, the quinoline fraction is joined at position 4 to the quinuclidine at position 8.

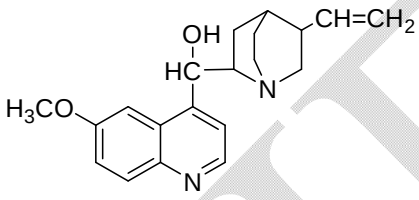
Position of the secondary alcoholic group

Now the last question arises: What is the position of the secondary alcoholic group in the *second half* of the quinine. This was demonstrated by Rabe. Rabe oxidised quinine to quininone by gentle oxidation with CrO_3. The quininone when treated with amyl nitrite and hydrochloric acid yields quininic acid and an oxime. The formation of an acid and oxime reveals the presence of the group -CO-CH<, i. e., a methine group adjacent to a carbonyl group.



The structure of the oxime obtained in the above reaction is ascertained by its hydrolysis to meroquinene and hydroxylamine. Thus, it follows that the quinoline and quinuclidine units are linked through the –CHOH- group.

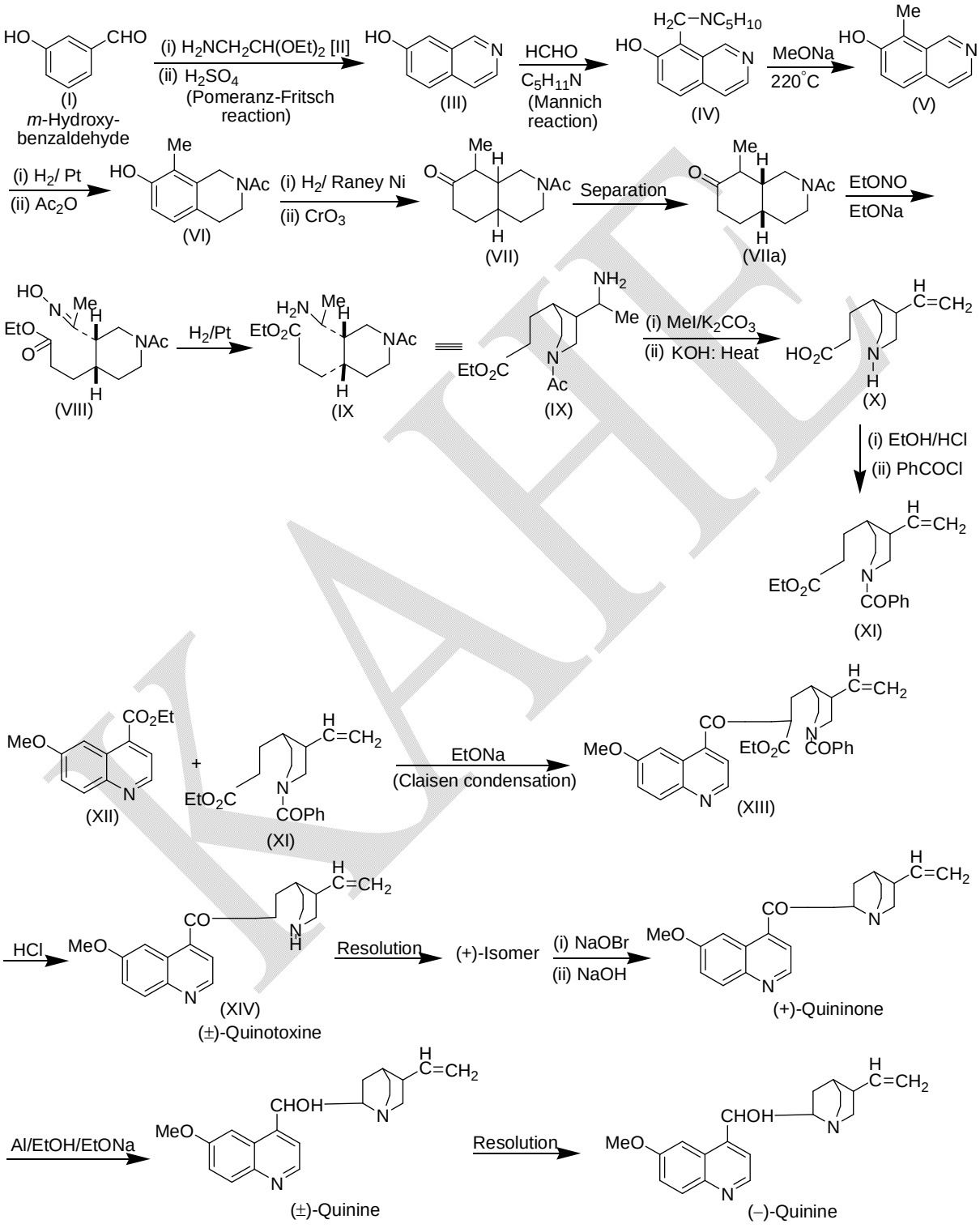
On the basis of all the foregoing facts the structure of quinine may be represented as follows:



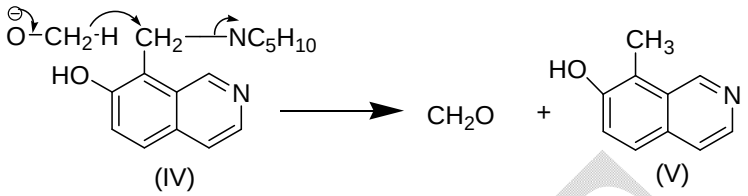
Quinine

Rabe *et al.* (1918) carried out a partial synthesis of quinine starting from quinotoxine, which was prepared by heating quinine in acetic acid. Woodward and Doering (1944) have synthesised (+)-quinotoxine, and so we now have a total synthesis of quinine. The following is Woodward and Doering's work up to (+)-quinotoxine, and from this to quinine is Rabe's work. m-Hydroxybenzaldehyde (I) was condensed with aminoacetal (II) and the product cyclised with sulphuric acid to give 7-hydroxyisoquinoline (III); this is an example of the Pomeranz-Fritsch reaction. This was treated with formaldehyde in methanol solution containing piperidine (Mannich reaction). The complex formed (IV) was converted into7-hydroxy-8-methyl-isoquinoline (V) by heating with methanolic sodium methoxide at 200°C. (V), on catalytic reduction (platinum) followed by acetylation, gave *N*-acetyl-7-hydroxy-8-methyl-1,2,3,4-tetrahydroisoquinoline (VI), which on further catalytic reduction by heating with a Raney nickel catalyst under pressure and then followed by oxidation with chromium trioxide was converted into *N*-acetyl-7-keto-8-methyldecahydroisoquinoline (VII). (VII) was a mixture of cis- and trans-isomers; these were separated (via their crystalline hydrates) and the cis-isomer (VIIa) then treated with ethyl nitrite in the presence of sodium ethoxide to give the homomeroquinene derivative (VIII). This, on reduction, gave (IX), which may now be written more conveniently as shown. Exhaustive methylation of (IX), followed by hydrolysis, gave cis-(±)-homomeroquinene (X). (X), after esterification and benzoylation, gave (XI) which, on condensation with ethyl quininate (XII), produced (XIII) (a β-keto-ester). This, on heating with 16 per cent hydrochloric

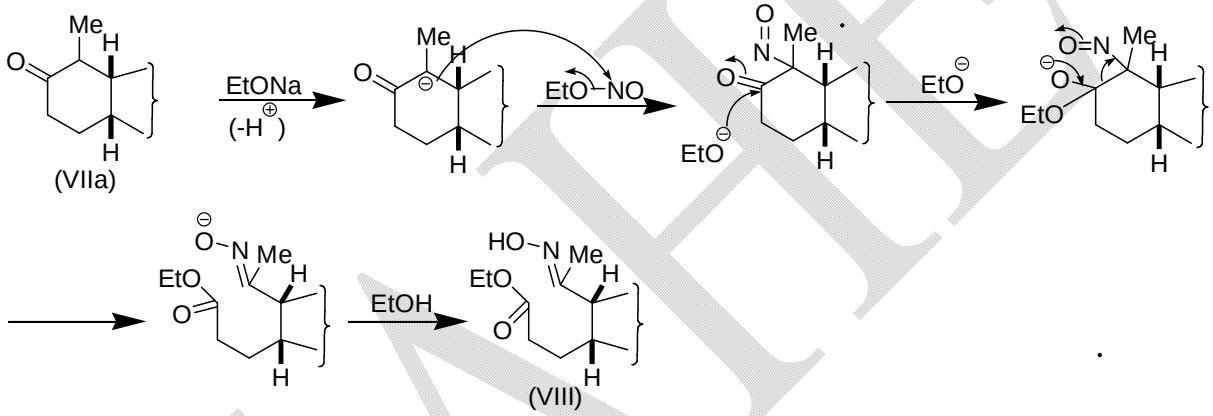
acid, was hydrolysed and decarboxylated to (±) quinotoxine (XIV). This was resolved via its dibenzoyl-tartrate (tartaric acid proved unsuccessful for resolution).



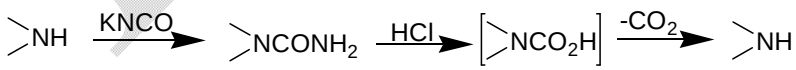
Conversion of (IV) into (V) failed with hydrogenolysis (H_2 + catalyst). The mechanism of the reaction with methoxide ion probably occurs by hydride ion transfer.



The conversion of (VIIa) to (VIII) possibly occurs as follows (the tertiary hydrogen atom is removed in preference to the secondary):



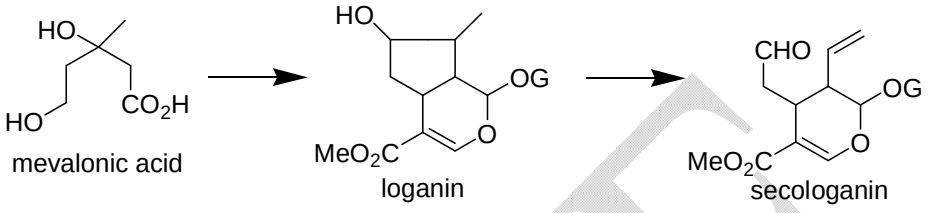
(IX) contains a new chiral centre, but this is lost when the amino-acid side-chain is converted into the vinyl group. The exhaustive methylation step was carried out by heating (IX) with ethaolic methyl iodide in the presence of potassium carbonate, followed by heating the quaternary salt with 60 per cent potassium hydroxide solution at 140°C (note the formation of the Hofmann product). (X) proved difficult to isolate and so it was treated with potassium cyanate, followed by hydrolysis of the ureide:



Enamine structure and, on protonation, forms an iminium salt which undergoes an intramolecular nucleophilic cyclisation.

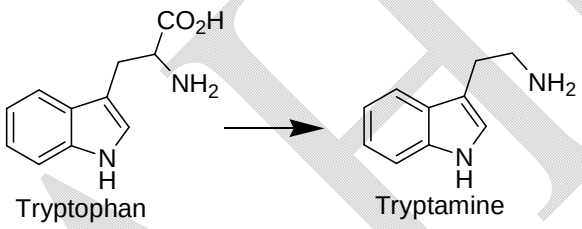
Biosynthesis of Quinoline alkaloids

The biosynthesis of the cinchona alkaloids has been shown to proceed from tryptophan as the precursor. Another precursor is believed to be **secologanin,** which is derived form **loganin**, a natural terpenoid of the iridoid group, and has been shown to be derived from mevalonic acid. Thus (G = glucose):

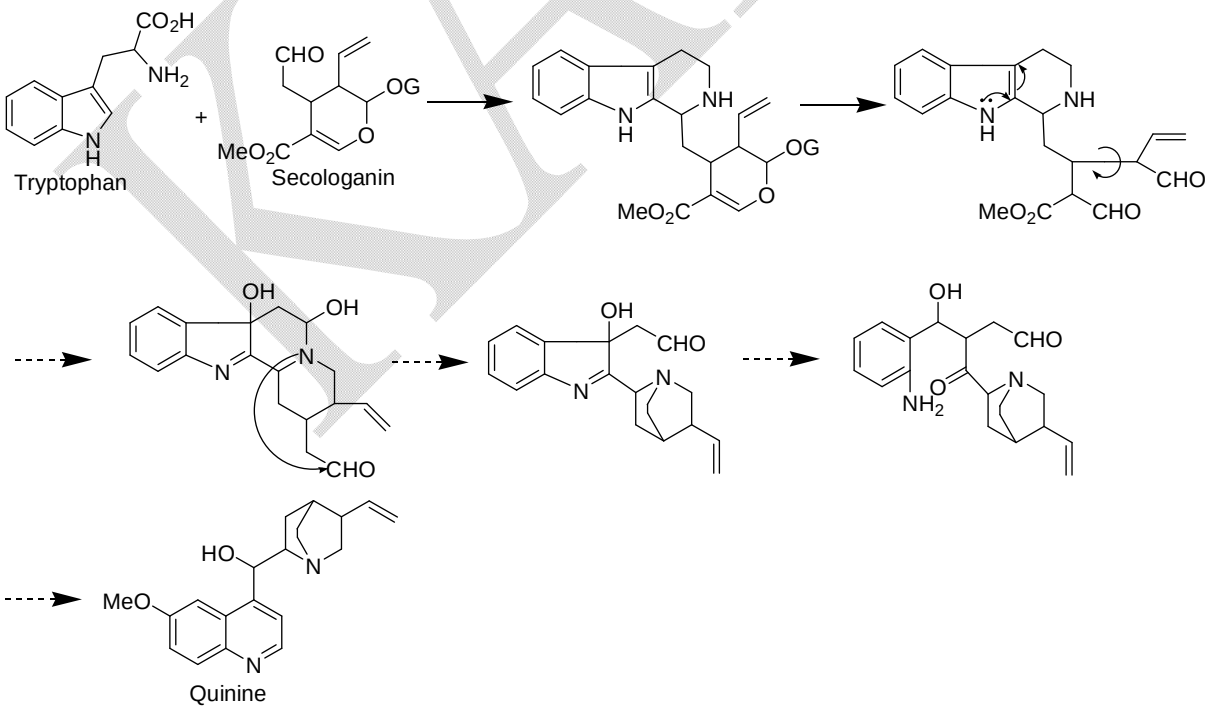


We may now give some of the steps involved in the biosynthesis of quinine.

(i)



(ii)



Text Books:

1. Finar, I. L. (2013). *Organic Chemistry Vol. II: Stereochemistry and the Chemistry of Natural Products* (V Edition). New Delhi: Pearson Education, Ltd.

2. Chatwal, G. R. (2015). *Organic Chemistry of Natural Products Vol. I.* New Delhi: Himalaya Publishing House.

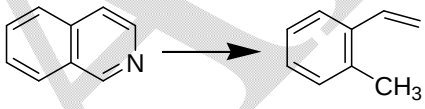
POSSIBLE QUESTIONS

PART- A – Multiple Choice Questions

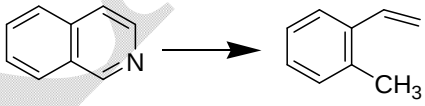
(Each Question Carry One Mark) (Online Examinations)

PART-B (Each Question Carry Two Marks)

1. How the presence of hydroxyl group is confirmed in the alkaloids?
2. How will you confirm the carboxyl group in alkaloids?
3. What is Zeisel method?
4. Explain the mechanism of the below reaction.



5. Explain the mechanism of the reaction below.



6. Draw the conversion of tropine into 2-ethylpyridine.
7. Write the synthesis of quininic acid?
8. Draw the structures of quininic acid, 6-hydroxy quinoline and quinine.

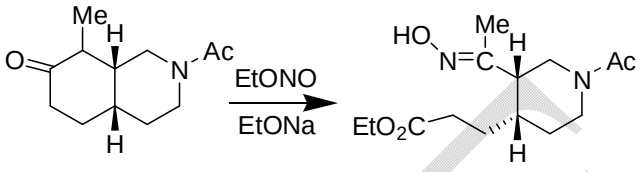
PART-C (Each Question Carry Six Marks)

1. (i) Explain the synthesis of Morphine.

(ii) How will you establish the following?

(a) position of $-OCH_3$ in Quinine. (b) position of phenolic –OH group in Morphine.

2. Explain the structural elucidation and synthesis of Quinine.
3. (i) Explain the mechanism of the reaction below



(ii) How quininic acid is synthesized.

4. (i) Explain the conversion of Thebaine into Thebenine.

(ii) Explain the synthesis of Tropine.

5. (i) Explain the stereochemistry of Morphine.

(ii) Explain the synthesis of Quinine.

6. Explain the biosynthesis of Quinoline alkaloids.
7. (i) Write the conversion of 3, 4-dimethoxy-2-nitro-benzaldehyde into dimethylmorphol.

(ii) Write notes on extraction of alkaloids.

8. (i) Explain the structural elucidation of Tropic acid.

(ii) Write note on Robinson's synthesis of Tropinone.

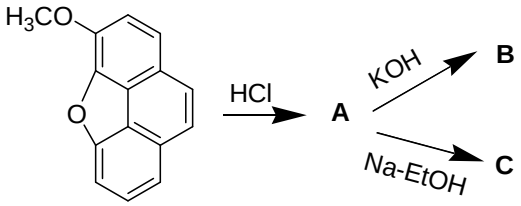
9. (i) Explain Von Braun's method of determining the structure of alkaloids.

(ii) Explain Emde's degradation with suitable example.

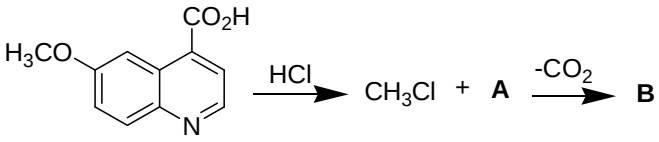
10. Explain the structural elucidation of Tropine.

PART-D (Each Question Carry Ten Marks)

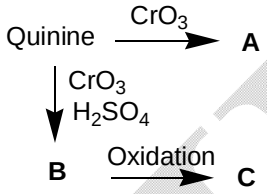
1. (a) Identify the compounds **A**, **B** and **C** in the following transformation?



(b) Identify the compounds **A** and **B** in the following transformation?

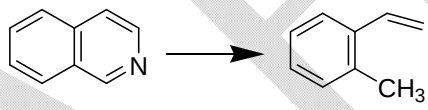


(c) Identify the compounds **A**, **B** and **C** in the following transformation?



(d) Explain the conversion of quininic acid to 6-hydroxyquinoline?

2. (i) Explain the mechanism of the below reaction.



(ii) What is Herzig-Meyer method?

(iii) How the presence of hydroxyl group is confirmed in the alkaloids?

(iv) Explain Lanenburg's definition of alkaloids.

(v) How the presence of methoxy group is confirmed in the alkaloids?

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DEPARTMENT OF CHEMISTRY

UNIT-III

ALKALOIDS

PART-A–Multiple Choice Questions

(Each Question Carry One Mark) (Online Examinations)

1. The naturally plant compounds having a basic character and containing at least one nitrogen in a heterocyclic ring is known as

a) alkaloids b) steroids c) terpeniods d) proteins

2. Morphine is an example for

a) tropane alkaloid **b) phenantherne alkaloid** c) indole alkaloid d) quinoline alkaloid

3. The example for commercially synthesized alkaloid is

a) morphine b) quinine **c) papaverine** d) atropine

4. The example for water soluble alkaloid is

a) morphine b) quinine c) papaverine **d) nicotine**

5. The most of the alkaloids are

a) optically active b) meso c) racemic mixture d) optically inactive

6. The example for coloured alkaloid is

a) morphine **b) berberine** c) papaverine d) quinine

7. Oxidation of tropinic acid gives

a) N-methyl succinic acid b) pimelic acid **c) N-methylsuccinimide** d) 2-ethyl pyridine

8. Atropine is the racemic form of

a) (±)-hyoscyamine b) (±)-tropic acid c) tropine d) tropinic acid

9. The molecular formula for atropine is

a) $C_{21}H_{30}NO_2$ b) $C_{18}H_{28}NO_2$ **c) $C_{17}H_{23}NO_3$** d) $C_{33}H_{40}NO_9$

10. Hydrolysis of atropine with barium hydroxide yields

a) (±)-tropic acid b) tropinone c) tropinic acid d) tropane

11. The molecular formula for tropic acid is

a) $C_9H_{30}O_2$ b) $C_8H_8O_2$ c) $C_7H_{20}O_3$ **d) $C_9H_{10}O_3$**

12. The number of hydroxyl groups present in tropic acid is

a) 2 b) 3 **c) 1** d) 4

13. Tropic acid on strong heating yields

a) benzoic acid **b) atropic acid** c) atrolactic acid d) tropine

14. The molecular formula for tropine is

a) $C_9H_{30}NO$ b) $C_8H_{15}NO$ **c) $C_6H_{15}NO$** d) $C_9H_{10}NO$

15. Oxidation of tropine with chromic acid yields

a) tropic acid b) tropinone c) **(±)-tropinic acid** d) tropane

16. Tropinic acid on Hofmann exhaustive methylation followed by reduction yields

a) tropidine b) tropilidine **c) pimelic acid** d) tropinone

17. The molecular formula for quinine is

a) $C_{18}H_{24}N_2O_4$ **b) $C_{20}H_{24}N_2O_2$** c) $C_8H_{26}N_2O_4$ d) $C_9H_{24}N_2O_8$

18. Oxidation of quinine with chromic acid produces

a) 6-methoxy quinoline b) meroquinene c) quininic acid **d) quininone**

19. Controlled oxidation of quinine with chromic acid yields

a) 6-methoxy quinoline b) quinoline **c) quininic acid** d) quininone

20. Oxidation of quininic acid yields

a) 6-methoxy quinoline **b) pyridine-2,3,4-tricarboxylic acid**

c) 6-methoxy-4-methylquinoline d) meroquinene

21. The molecular formula for quininic acid is

a) $C_{11}H_9NO_3$ b) $C_{20}H_{24}N_2O_2$ c) $C_8H_{26}N_2O_4$ d) $C_9H_{24}N_2O_8$

22. Quininic acid is

a) 6-hydroxyquinoline **b) 6-methoxycinchoninic acid**

c) 6-methoxy-4-methylquinoline d) pyridine-2,3,4-tricarboxylic acid

23. The number of methoxyl group in quinine is

a) 3 b) 4 **c) 1** d) 2

24. The number of hydroxyl group in quinine is

a) 3 b) 4 c) 2 **d) 1**

25. The molecular formula for morphine is

a) $C_9H_{24}N_2O_8$ b) $C_9H_{15}NO_2$ **c) $C_{17}H_{19}NO_3$** d) $C_{11}H_9NO_3$

26. The number of tertiary nitrogen atom present in morphine is

a) 1 b) 3 c) 2 d) 4

27. The number of hydroxyl groups present in morphine is

a) 1 b) 3 **c) 2** d) 4

28. The number of double bonds present in morphine is

a) 1 b) 3 c) 2 d) 4

29. The molecular formula for methylmorphol is

a) $C_{11}H_9O_3$ b) $C_9H_{15}O_2$ c) $C_{17}H_{19}O_3$ **d) $C_{15}H_{12}O_2$**

30. Methylmorphenol when demethylated with HCl yields

a) morphol **b) morphenol** c) trihydroxymorphine d) β-mehtylmorphimethine

31. Morphenol on reduction with sodium and ethanol yields

a) morphol b) methylmorphenol c) trihydroxymorphine d) β-mehtylmorphimethine

32. The absolute configuration of (-)-tropic acid by its correlation with

a) glycine b) L-proline **c) (-)-alanine** d) (+)-alanine

33. The Cahn-Ingold-Prelog notation of natural tropic acid is

a) (R)-(-)-tropic acid b) (R)-(+)-tropic acid

c) (S)-(-)-tropic acid d) (S)-(+)-tropic acid

34. The number of methoxyl groups present in an alkaloid is determined by

a) Emde degradation **b) Ziesel method** c) Von Braun's method d) Hofmann degradation

35. The Ziesel method the alkaloids is heated with

a) HCl b) HBr **c) HI** d) H_2SO_4

36. The Ziesel method the methoxyl group is converted into

a) $CHBr_3$ b) CH_3Br c) $CHCl_3$ **d) CH_3I**

37. The presence of carboxyl group in alkaloids is confirmed by

a) sodium carbonate test b) dye test c) nitration test d) ignition test

38. The alkaloid is heated with hydrochloric acid gives formaldehyde indicated by

a) methoxyl group **b) methylenedioxyl group** c) N-methyl group d) carboxyl group

39. If the alkaloid forms an amine oxide with 30% hydrogen peroxide then the nitrogen atom is

a) primary b) secondary **c) tertiary** d) quaternary

40. The presence of alkyl groups in alkaloids is confirmed by

a) sodium carbonate test b) NaOH test **c) KOH test** d) nitration test

41. The example for quinoline alkaloid is

a) hygrine b) piperine c) nicotine **d) quinine**

42. In alkaloid the presence of N-methyl groups and their number is determined by

a**) Herzig-Meyer method** b) Ziesel method

c) Von Braun's method d) Hofmann degradation

43. The Hofmann's exhaustive methylation method the pyridine converted into

a) piperidine **b) piperylene** c) morpholine d) thiomorpholine

44. Which methods pyridine converted into piperylene?

a) Herzig-Meyer method b) Ziesel method

c) Hofmann's exhaustive methylation method d) Von Braun's method

45. Which compound is fail for Hofmann's exhaustive methylation method?

a) pyridine b) laudanosine c) tetrahydroisoquinoline **d) tetrahydroquinoline**

46. The sodium amalgam in aqueous ethanol is known as

a) Emde degradation b) Ziesel method

c) Hofmann's exhaustive methylation method d) Von Braun's method

47. The Von Braun's method is applicable for

a) primary cyclic amines **b) tertiary cyclic amines**

c) primary cyclic ketones d) quaternary cyclic amines

48. The cyanogens bromide is used for

a) Emde degradation b) Ziesel method

c) Hofmann's exhaustive methylation method **d) Von Braun's method**

49. The Von Braun's method the tertiary cyclic amines is converted into

a) N-cyano derivative b) N-methyl derivative

c) N-nitroso derivative d) N-ethyl derivative

50. Which reagent is used for Von Braun's method for secondary cyclic amines?

a) acetyl chloride **b) benzoyl chloride** c) acetic anhydride d) DDQ

51. The example for mild oxidant is

a) alkaline potassium permanganate b) concentrated nitric acid **c) ozone**

d) chromium trioxide

52. The example for moderate oxidant is

a) hydrogen peroxide b) concentrated nitric acid c) ozone **d) chromium trioxide**

53. The example for vigorous oxidant is

a) concentrated nitric acid b) hydrogen peroxide c) ozone d) chromium trioxide

54. The atrolactic acid is

a) optically inactive compound **b) optically active** compound c) racemic compound

d) meso compound

55. Tropine behaves as a

a) optically inactive compound b) unsaturated compound **c) saturated compound**

d) meso compound

56. Tropine molecule contains an

a) keto group b) alkene group c) phenolic group **d) alcoholic group**

57. Tropine behaves as

a) secondary alcohol b) primary alcohol c) phenolic compound d) tertiary alcohol

58. Dihydrotropidine is also known as

a) tropic acid b) tropinone c) (±)-tropinic acid **d) tropane**

59. Cycloheptatriene is also known as

a) dihydrotropidine b) nordihydrotropidine **c) tropilidene** d) tropane

60. Which reaction is responsible for synthesis of tropinone?

a) Michael addition **b) double Mannich reaction** c) Aldol reaction

d) Robinson annulation

UNIT-IV

SYLLABUS

Proteins and Enzymes: Proteins: General nature of proteins - classification of proteins – synthesis of peptides – oxytocin- insulin.
Enzymes: Nomenclature and classification - cofactors – specificity of enzyme action- mechanism of enzyme action. Nucleic acids - structures of RNA and DNA and their biological importance.

General nature of proteins

The name *protein* was introduced by Mulder (1839), who derived it from the Greek word proteios (meaning *first*). Proteins are nitrogenous substances which occur in the protoplasm of all animal and plant cells. Their composition varies with the source: carbon, 46-55 per cent; hydrogen, 6-9-per cent; oxygen, 12-30 per cent; nitrogen, 10-32 per cent; sulphur, 0.2-0.3 per cent. Other elements may also be present, e.g., phosphorus (nucleoproteins), iron (haemoglobin).

As we have seen, proteins can be broken down into smaller and smaller fragments until the final products are the amino-acids. This sequence may be written as:

Protein → polypeptides → peptides → amino-acids

There is no sharp dividing line between peptides, polypeptides and proteins. One arbitrary convention designates proteins as those molecules with a molecular weight above ~10000 and peptides (polypeptides) as those molecules with a molecular weight below ~10000. In general, proteins and peptides differ in physical and chemical properties which can be correlated with the differences in molecular size. Both groups often exhibit physiological activity, behaving as, e.g., enzymes, hormones, growth factors, etc.

Synthetic peptides of very high molecular weight are often referred to as polypeptides, and their methods of preparation and the study of their properties have provided a great deal of information on the structure and properties of proteins.

Proteins are amphoteric, their behaviour as an anion or a cation depending on the pH of the solution. At some definite pH, characteristic for each protein, the positive and negative

charges are exactly balanced, i.e., there is no net charge on the protein molecule, and the molecules will not migrate in an electric field. In this condition the protein is said to be at its isoelectric point, and at this pH the protein has its least solubility, i.e., it is most readily precipitated. The osmotic pressure and viscosity of the protein solution are also a minimum at the isoelectric point. The amphoteric nature of proteins is due to the presence of a large number of free acidic and basic groups arising from the amino-acid units in the molecule. These groups can be titrated with alkali or acid, and by this means it has been possible to identify acidic and basic groups belonging to the various amino-acid units.

All proteins are optically active, and may be coagulated and precipitated from aqueous solution by heat, the addition of acids, alkalis, salts, organic solvents miscible with water, etc. Proteins in this precipitated state are said to be **denatured**, and the process of reaching this state, **denaturation**, occurs most readily near the isoelectric point. Denaturation is now believed to be the result of changes in *conformation* or *unfolding* of the protein molecule. Associated with denaturation are changes in optical rotation and (usually) the loss of biological activity, e.g., enzymes (all are proteins) become inactive when denatured.

Denaturation is generally irreversible, but many examples are now known where the process has been reversed. This reversal of denaturation has been called **renaturation** or **refolding.** When denaturation is effected by heat, renaturation does not usually result on rapid cooling. If, however, cooling is carried out very slowly, renaturation often occurs. In these circumstances the process of renaturation has been referred to as **annealing.**

Proteins exhibit a variety of colour reactions, e.g.,

(i) **Biuret reaction**. Addition of a very dilute solution of copper sulphate to an alkaline solution of a protein produces a red or violet colour. This reaction is due to the presence of the grouping –CO-NH-CHR-CO-NH-. At least two peptide linkages (-CONH-) must be present (dipeptides do not give the test).

(ii) **Xanthoproteic reaction**. Proteins usually produce a yellow colour when warmed with concentrated nitric acid, and the colour becomes orange when the solution is made alkaline. This reaction is due to the nitration of the benzene ring in phenylalanine, tyrosine and tryptophan.

(iii) **Millon's reaction**. Millon's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) usually produces on addition to a protein solution a white precipitate which turns red on heating. This reaction is characteristic of phenols, and so is given by proteins containing tyrosine (this is the only phenolic amino-acid that occurs in proteins).

(iv) **Ninhydrin test**. Proteins (and peptides) give this test, but the colours are different from that of the amino-acids.

The molecular weights of proteins have been determined by means of ultracentrifugal sedimentation, osmotic pressure measurements, X-ray diffraction, light scattering effects, molecular sieves (gel filtration), and by chemical analysis. Chemical methods are based on the estimation of a particular amino-acid. Thus, suppose the percentage composition of amino-acids in a protein has been determined. From these values it is possible to calculate the mole proportions of each amino-acid by dividing its percentage weight by its molecular weight. We now choose the amino-acid present in the least molar amount and on the assumption that only one of these amino-acid residues is present in the protein, the molecular weight, M_1 of the protein, is give by

$$M_1 = \frac{100}{x} \times m \quad \text{or} \quad x = \frac{m}{M_1} \times 100$$

where x is the percentage weight and m is the molecular weight of the amino-acid. If two molecules of the amino-acid are present per molecule of protein, the percentage weight is still x, but now we have

$$x = \frac{2m}{M_2} \times 100 = \frac{2m}{2M_1} \times 100$$

i.e., the molecular weight M_2 is $2M_1$. Hence, if n molecules (where n must be an integral number) of the amino-acid are present, the molecular weight of the protein is nM_1 . Therefore M_1 is the *minimum molecular weight* of the protein and nM_1 is its true molecular weight.

As an example, let us consider the protein bovine insulin. The amino-acid that occurs in the smallest molar amount is threonine: 2 per cent, m=119.

$$\therefore M_1 = \frac{100}{2} \times 119 = 5950$$

Now, bovine insulin has been shown to contain one molecule of threonine and hence its true molecular weight is also 5950.

Since the modern methods of estimating amino-acids have a high degree of accuracy, a knowledge of the minimum molecular weight is extremely valuable. This is because many of the methods used for the determination of molecular weights of proteins (and peptides) are apparently accurate only within the limits of about 3-5 per cent. It should be noted that elemental analysis as a means of obtaining molecular formulae of proteins is unsatisfactory because of their very high molecular weights.

The average molecular weight of the common amino-acids is about 141.5, and since one molecule of water is lost in the formation of the peptide bond, a peptide containing n amino-acid residues has an approximate molecular weight 141.5n - 18n = 123.5n. Hence n= *M*/123.5. For the purpose of simplifying the calculation (and with little effect on the approximation), we may replace 123.5 by 125.

The values of molecular weight recorded for proteins vary considerably, ranging from about 5000 to many millions.

One of the difficulties in protein chemistry (including peptides) is to be able to decide whether the specimen being investigated is pure. Although many proteins (and peptides) have been obtained crystalline, these have no characteristic melting points. Various criteria are therefore used to show homogeneity, e.g., constant solubility, chromatography (column, paper, and ion-exchange), paper electrophoresis, etc.

Since the solubility of a protein depends on pH, the presence or absence of salts, etc. by controlling these factors it is possible to separate proteins. Thus, for example by adjusting the pH of a solution containing a mixture of proteins (or peptides) to the isoelectric point of each protein in turn, each of these will be precipitated in turn. Alternatively, the *salting out* method may be used to separate proteins. The solubility of many proteins is increased in the presence of small

concentrations of various neutral salts. This is referred to as *salting in*, and bivalent cations are more effective than univalent cations. As the concentration of the ion is increased, the solubility of the protein passes through a maximum, then begins to decrease and at a sufficiently high concentration (of ion) the protein is precipitated, i.e., salted out. Not only can cations precipitate proteins (as salts), but so can suitable anions, e.g., tungstic acid, phosphotungstic acid, trichloroacetic acid, etc.

A third method of separation of proteins based on solubility is the controlled precipitation by organic solvents miscible with water.

From the foregoing account it can be seen that, in general, methods used for isolating proteins are also used for their separation and purification.

Many proteins are not composed of a single peptide chain but consist of a number of subunits. Furthermore, these subunits may or may not be identical.

Peptides have been classified as **homeomeric** when the products of hydrolysis are amino-acids only, and as **heteromeric** when other products in addition to amino-acids are obtained. A large number of peptides are linear but many are cyclic. Cyclic peptides have been classified as **homodetic** when their structures contain only peptide linkages. On the other hand, when the rings contain both amide (peptide) and other types of linkages, e.g., disulphide, the cyclic peptides are classified as **heterodetic**, e.g., oxytocin. This latter class has also been called **cyclodepsipeptides**, but apparently some authors restrict this term to those heteromeric cyclic peptides composed of amino-acids and hydroxyacids linked by amide and ester bonds. In this case, the compounds have been referred to as the **peptolides**. They have been isolated from bacteria, fungi, etc., and many show biological activity.

Classification of proteins

Several arbitrary classifications of the proteins are in use. One method divides the proteins into two groups, *fibrous proteins*, which are insoluble in common solvents, but are soluble in concentrated acids and alkalis, and *globular proteins*, which are soluble in water and in dilute acids, alkalis and salts.

A more common method of classification is the division of proteins into the three main groups; simple, conjugated, and derived proteins. Each group is subdivided into a number of classes designated by general names. Each class contains sub-classes of proteins of similar but not identical physical and chemical properties, e.g., in A(i), below, one sub-class of albumin is serum albumin. The term serum indicates that this group of albumins occurs in the blood serum of vertebrates, e.g., man, horse, sheep, dog, etc. However, all these serum albumins differ from each other.

A. Simple proteins. These give only amino-acids or their derivatives on hydrolysis.

(i) *Albumins*. These are soluble in water (and in acids and alkalis), and are coagulated by heat. They are precipitated by saturating their solutions with ammonium sulphate.

Albumins are usually low or deficient in glycine; some albumins are serum albumin, egg albumin and lactalbumin.

(ii) *Globulins*. These are insoluble in water, but are soluble in dilute salt solution and in dilute solutions of strong inorganic acids and alkalis. They are precipitated by half saturating their solutions with ammonium sulphate, and they are coagulated by heat.

Globulins usually contain glycine; some typical globulins are serum globulin, tissue globulin and vegetable globulin.

(iii) *Prolamins*. These are insoluble in water or salt solution, but are soluble in dilute acids and alkalis, and in 70-90 per cent ethanol.

Prolamins are deficient in lysine, and contain large amounts of proline; some prolamins are zein (from maize), gliadin (from wheat) and hordein (from barley).

(iv) *Glutelins*. These are insoluble in water or dilute salt solution, but are soluble in dilute acids and alkalis; they are coagulated by heat. They are comparatively rich in arginine, proline and glutamic acid.

Some glutelins are glutenin (from wheat) and oyrzenin (from rice).

(v) *Scleroproteins* (*albuminoids*). These are insoluble in water or salt solution, but are soluble in concentrated acids or alkalis.

Examples: Keratin (from hair, hoof), fibroin (from silk); these are not attacked by enzymes.

Sub members of the scleroproteins are:

(a) *Collagens* (in skin, tendons and bones); these form gelatin (a water-soluble protein) when boiled with water. Collagens are attacked by pepsin or trypsin.

(b) *Elastins* (in tendons and arteries); these are not converted into gelatin, and are attacked slowly by trypsin.

(vi) *Basic proteins*. These are strongly basic, and fall into two groups.

(a) *Histones*. These are soluble in water or dilute acids, but are insoluble in dilute ammonia. They are not coagulated by heat, and contain large amounts of histidine and arginine, but contain no tryptophan and very little cystine or methionine; they are hydrolysed by pepsin and trypsin. Histones are the proteins of the nucleic acids, haemoglobin, etc.

(b) *Protamins*. These are more basic than the histones and have a simpler structure. They are soluble in water, dilute acids and dilute ammonia; they are not coagulated by heat, and are precipitated from solution by ethanol. They contain large amounts of arginine, and occur in various nucleic acids. They do not contain sulphur, and are hydrolysed by various enzymes, e.g., trypsin, papain, but not by pepsin.

B. Conjugated proteins. These are proteins which contain a non-protein group (i.e., a compound not containing amino-acid residues) attached to the protein part. The non-protein group is known as the *prosthetic group*, and it may be separated from the protein part by careful hydrolysis.

(i) *Nucleoproteins*. The prosthetic group is a nucleic acid.

(ii) *Chromoproteins*. These are characterised by the presence of a coloured prosthetic group. Examples: chlorophyll and haemoglobin. These examples contain metals (see also (vi), below), but in many cases the prosthetic group is organic, e.g., in visual purple the prosthetic group is a carotenoid pigment.

(iii) *Glycoproteins*. In these the prosthetic group contains a carbohydrate or a derivative of the carbohydrates. They are also known as *mucoproteins*.

(iv) *Phosphoproteins.* These are conjugated proteins in which the prosthetic group contains phosphoric acid in some form other than in the nucleic acids or in the lipoproteins.

(v) *Lipoproteins*. In these the prosthetic group is lecithin, kephalin, etc.

(vi) *Metalloproteins.* These all contain a metal which is an integral part of the structure. Many metals occur, e.g., iron, magnesium, copper, manganese. Examples are haemoglobin and chlorophyll which, as we have seen, may also be classed as chromoproteins (see (ii), above).

C. Derived proteins are degradation products obtained by the action of acid, alkalis or enzymes on proteins.

Protein→ Denatured proteins; insoluble proteins formed by the action of heat, etc., on proteins

↓

Primary proteoses: (*metaproteins*): insoluble in water or dilute salt solution, but are soluble in

↓ acids or alkalis. They are precipitated by half-saturation with ammonium sulphate.

Secondary proteoses: soluble in water, not coagulated by heat, and are precipitated by saturation

↓ with ammonium sulphate.

Peptones

↓

Polypeptides

↓

Simple peptides

↓

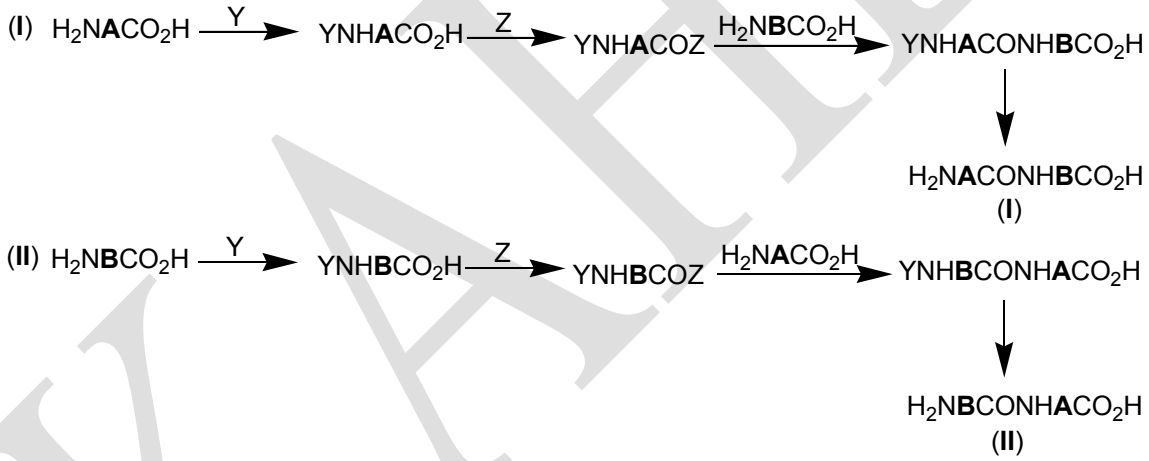
Amino-acids

These are soluble in water, not coagulated by heat, and are not precipitated by saturation with ammonium sulphate.

Synthesis of peptides

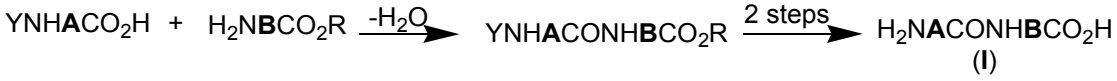
The general principles may be illustrated by consideration of the synthesis of a dipeptide. As we have seen, two different amino-acids, L-**A** and L-**B** may be combine in two different ways (2!):

To prepare (**I**), the amino-group of **A** must be protected and the carboxyl group of **A** must be activated so that it readily reacts with the free amino-group of **B**. similarly, to prepare (**II**), the amino-group of **B** must be protected and the carboxyl group of **B** must be activated. Hence, if **Y** is the protecting group and **Z** is the activating group, we have:



In each case, the final step involves the removal of the protecting group **Y** to give the dipeptide.

Other routes to dipeptides are as follows. The amino-group of the amino-acid which is to be *N*-terminal is protected and so is the carboxyl group of the amino-acid which is to be *C*-terminal. These two protected amino-acids may then be combined directly by means of a suitable reagent to give a dipeptide protected at both its *N*-and *C*-terminals. Thus (**I**) may be synthesised as follows (R is the carboxyl protecting group):



Alternatively, the carboxyl group of the *N*-terminal protected amino-acid is converted into an activated group and, after combination of the two amino-acid derivatives, a dipeptide is obtained which again has both its N-and C-terminals protected, e.g., the synthesis of (**II**):

To extend the length of the peptide chain, one of the protecting groups in the dipeptide is selectively removed and the peptide is built up from this end. Thus, a peptide chain may be extended, one amino-acid residue at a time, from either end of its precursor. On the other hand, a number of suitable simple peptides may be synthesised, these then linked together to give the required protected peptide (or protein), from which the protecting groups are finally removed.

One other point that requires consideration is that if the amino-acid side-chain contains reactive groups, these must be protected. Such reactive groups are, e.g., amino (lysine), carboxyl (aspartic acid), hydroxyl (tyrosine), thiol (cysteine).

Many protecting groups have been introduced and their number is continually increasing. Fischer (1901-1907) introduced methods which, although they led to the synthesis of an octadeca-peptide, are no longer used.

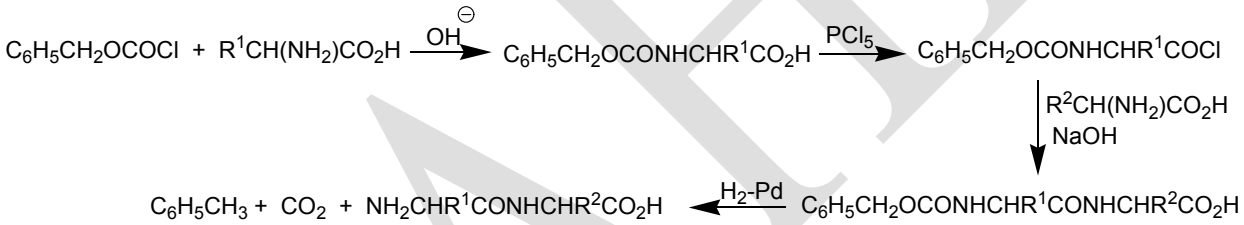
Since peptide synthesis involves protecting *N*-and *C*-terminals (and also reactive side-chains), it is necessary to use protecting groups which can be selectively removed one at a time. It is also important that protecting groups should be easily introduced and should be removable under sufficiently mild conditions that the peptide bond is not hydrolysed and that no racemisation or rearrangements occur.

Five useful amino protecting groups are: benzyloxycarbonyl (carbobenzyloxy), t-butyloxy-carbonyl (Boc; carbo-t-butyloxy), trityl (triphenylmethyl), phthaloyl, and tosyl (Ts; *p*-toluene-sulphonyl). The usual method of protecting a carboxyl group is esterification, the common esters being methyl, ethyl, benzyl, and t-butyl. Reactive side-chain protecting groups are, e.g., benzyl for thiol and hydroxyl, acetyl for hydroxyl, etc. Activation of the carboxyl group has been carried out in various ways, e.g., by conversion into the acid chloride, acid azide, or

p-nitrophenyl ester. Finally, the direct combination between an end amino-group and an end carboxyl group is effected by means of dicyclohexylcarbodi-imide (DCC) in an organic solvent (methylene dichloride, THF, etc.,). The following account illustrates the applications of these techniques.

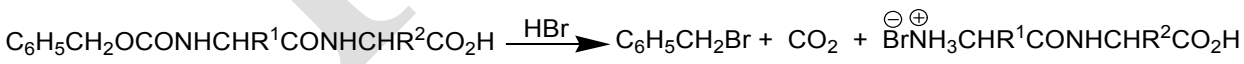
Bergmann (1932) introduced benzyloxycarbonyl chloride (also known as carbobenzyloxy chloride) as an amino protecting group, and this appears to be the most widely used method of protection. It is readily prepared by the action of carbonyl chloride on benzyl alcohol in toluene solution.

The procedure is then as follows:



If the amino-acid contains sulphur, then catalytic reduction cannot be used, since the sulphur poisons the catalyst; the removal of the blocking group, however, may be successfully accomplished by means of sodium in liquid ammonia.

A later method of removing this group is to treat the derivative with hydrogen bromide in acetic acid or nitromethane (Ben-Ishai *et al.,* 1952; Anderson *et al.,* 1952):

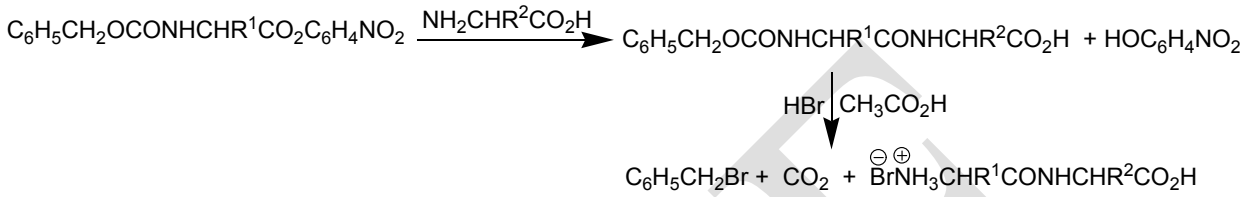


The use of *N*-benzyloxycarbonyl derivatives causes no appreciable racemisation.

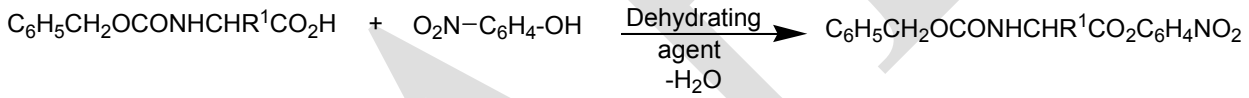
Modification of Bergmann's method. These are discussed as follows:

(i) The success of benzyloxycarbonyl group as an amino protecting group has led to the development of a number of modified versions. For example, *p*-nitrobenzyloxycarbonyl

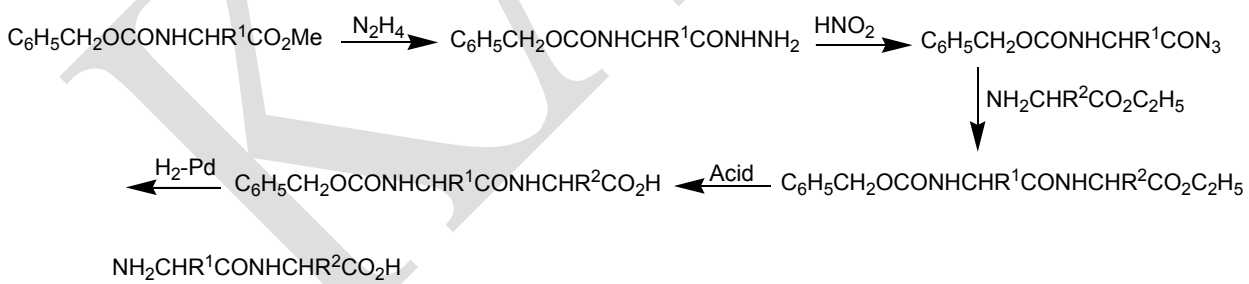
substitution yields substances which are more readily crystallised than benzyloxycarbonyl derivatives themselves. However, the *p*-nitrobenzyloxycarbonyl group is less readily cleaved by hydrogen bromide solution. The preparation of peptide involving this type of group is illustrated as follows:



p-Nitrophenyl esters of amino acids required for the above reactions are prepared in good yield by adding a dehydrating agent like dicyclohexylcarbodi-imide ($C_6H_{11}N{=}C{=}NC_6H_{11}$), to a solution of the benzyloxycarbonyl derivatives of the amino-acid and *p*-nitrophenol in ethyl acetate (du Vigneaud *et al.,* 1959).

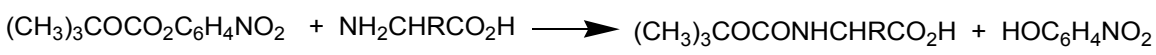


(ii) Another modification of Bergmann's method is to use azide of acid chlorides or esters. This is illustrated as follows.



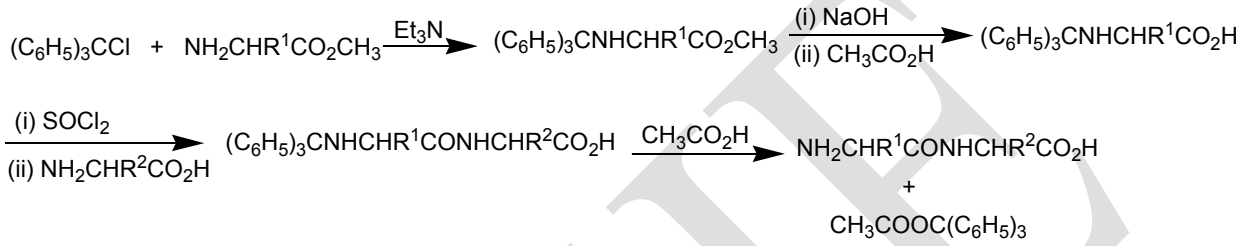
The azide synthesis is not accompanied by racemisation.

The t-butyloxycarbonyl reagent is not used at its chloride, since this is unstable, but is used as its p-nitrophenyl ester:

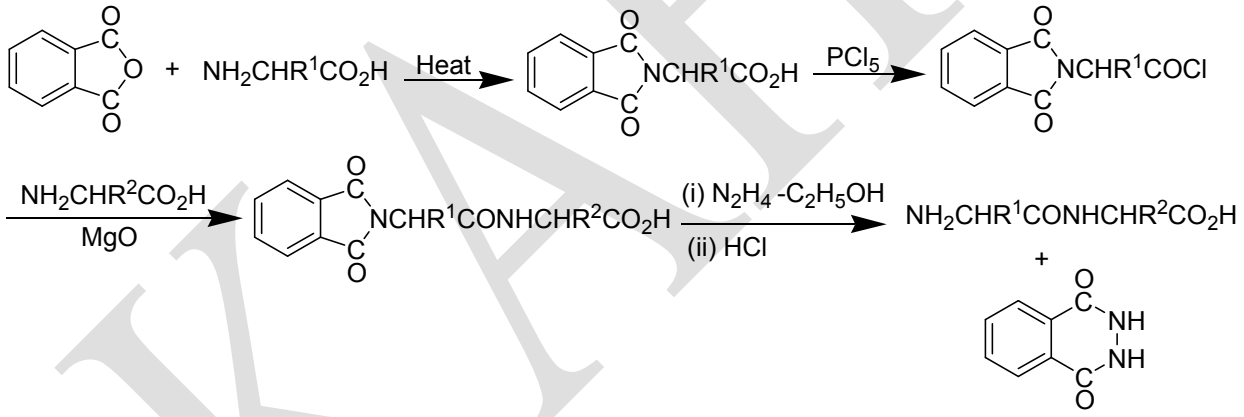


This group is readily removed by HBr-CH_3CO_2H, and also by HCl-CH_3CO_2H, the latter being particularly useful in that the benzyloxycarbonyl group is not removed by this reagent. The best reagent for removing the t-butyloxycarbonyl group appears to be trifluoroacetic acid.

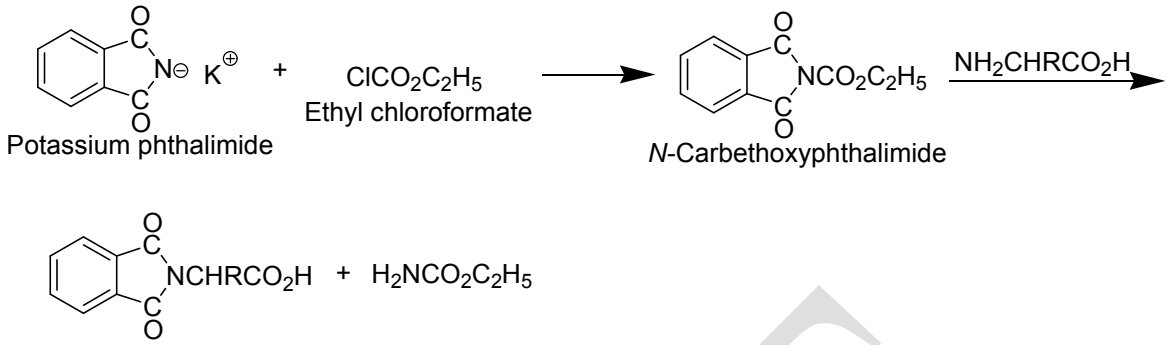
The trityl reagent is simple to use, and may be removed by heating in acetic acid or catalytically (H_2-Pd), e.g.,



Sheehan *et al.* (1940) have used the phthaloyl group as a means of protecting an end amino-group.

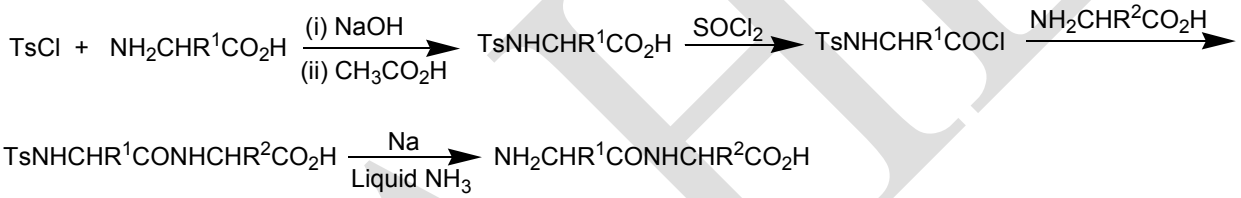


Phthaloylation occurs without racemisation provided the temperature does not exceed about 150°C. On the other hand, Nefkens (1960) has introduced a much milder method of phthaloylation starting from *N*-carbethoxyphthalimide (prepared from potassium phthalimide and ethyl chloroformate). This reagent reacts with amino-acids in aqueous sodium hydrogen carbonate solution at room temperature to form the optically pure phthaloyl derivative in excellent yield.



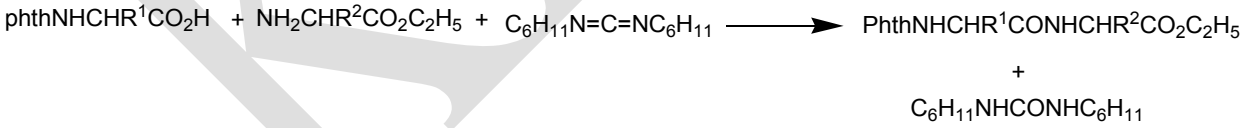
Weygand *et al.* (1961) have also prepared phthaloyl derivatives of amino-acids (without racemisation) by heating the acid with diethyl phthalate and triethylamine in phenol.

An example of the use of tosyl chloride as a protecting reagent is (Fischer, 1915):

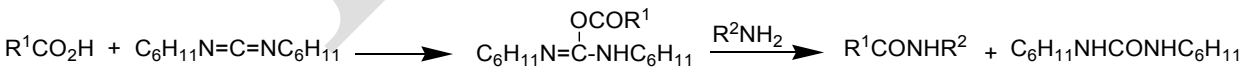


No racemisation occurs in this synthesis.

Sheehan *et al.* (1956) showed that a protected *N*-amino-acid combines directly with an amino-acid ester in the presence of dicyclohexylcarbodi-imide in an inert solvent (methylene chloride, THF, etc: Phth = phthaloyl group; see also above):



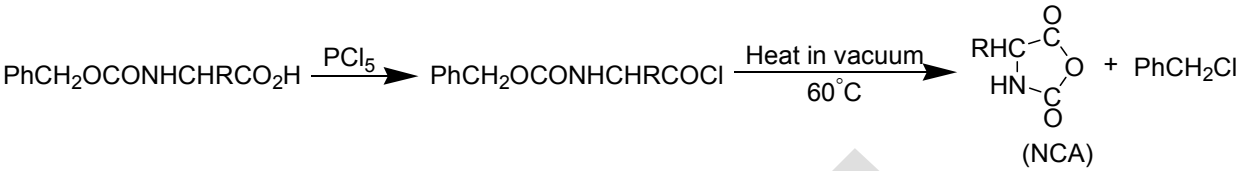
The mechanism of this reaction is believed to be:



This reaction occurs with very little racemisation, but there are usually side-reactions which make difficult the purification of the desired product.

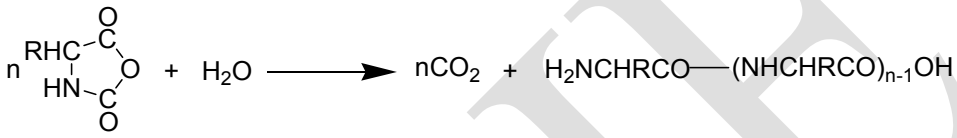
A different approach to peptide synthesis is the **anhydride method**. One application is as follows, the cyclic anhydride, N-carboxyanhydride (NCA) being the unit for polymerisation. The

NCA derivative may be prepared in a number of ways. A common method is to convert the amino-acid into its N-benzyloxycarbonyl derivative and then to proceed as shown.



(NCA)

Polymerisation is effected by heating the NCA derivative in an organic solvent (dimethylformamide, dioxane, etc.) in the presence of a catalyst, e.g., water, amines, etc.,

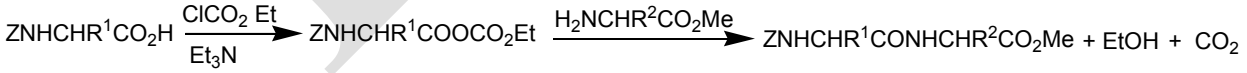


If a mixture of different anhydrides is used, the product is a polymer containing different residues in random distribution.

The NCA derivative can also be used to build up a peptide chain, one amino-acid residue at a time. NCA combines with an amino-acid in alkaline solution (pH10), and after acidification the product is a dipeptide.

This dipeptide may then be coupled with another NCA derivative, and so on.

A different approach to the anhydride method makes use of a mixed anhydride derived from ethyl chloroformate as follows.



Examples have been given above where the carboxyl group has been protected as the methyl or ethyl ester. A difficulty here is that alkaline hydrolysis of the peptide ester may cause racemisation. This difficulty may be avoided by use of benzyl esters, since these can be split by catalytic hydrogenolysis (H_2-Pd) to give toluene.

$$RCO_2CH_2Ph \xrightarrow{H_2\text{-}Pd} RCO_2H + PhCH_3$$

The thiol group (in cysteine) may be protected by S-benzylation (benzyl chloride in the presence of aqueous ethanolic sodium hydroxide). This group is not removed by HBr-AcOH but is by sodium in liquid ammonia. Benzylation may also be used to protect hydroxyl groups, e.g., in tyrosine, and is removed by HBr-AcOH.

t-Butyl esters are also useful since they may readily be prepared by the action of isobutene on the amino-acid in the presence of a small amount of concentrated sulphuric acid. Furthermore, the t-butyl group is easily removed by treating the ester with anhydrous trifluoroacetic acid or with dry hydrogen chloride.

Since racemisation is always possible in some of the methods described for peptide synthesis, it is desirable to be able to ascertain whether this has happened. One way is to attempt hydrolysis of the synthetic peptide with enzymes (which are highly stereospecific). It may be possible to separate mixtures of diastereoisomeric peptides by paper and thin-layer chromatography, etc. Weinstein *et al.* (1972) have used NMR spectroscopy to determine the amount of racemisation.

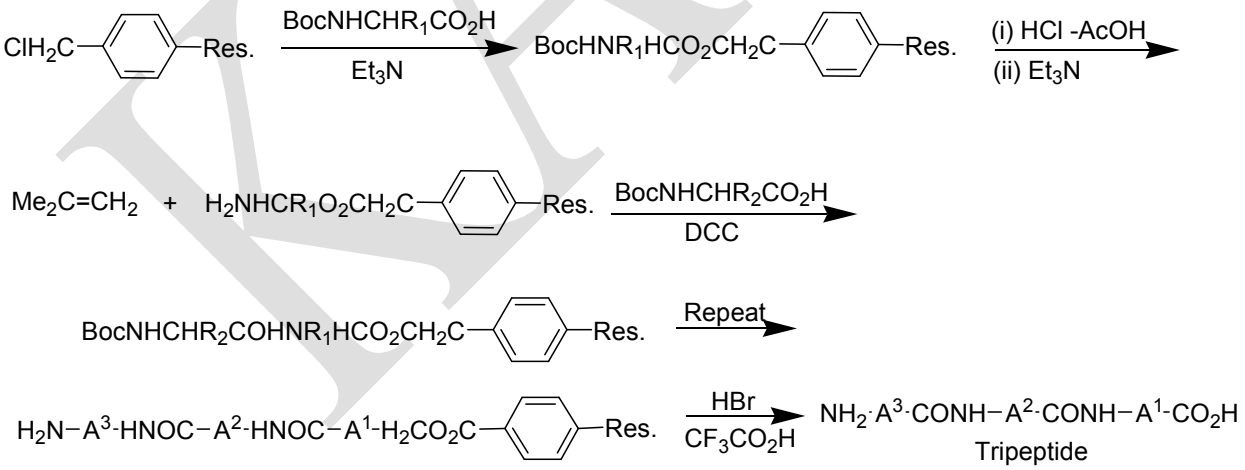
Cyclic peptides may be synthesised in various ways. Small peptides may be cyclodimerised and relatively long peptides cyclised (by self condensation) in dilute solution. A common method starts with peptide active esters. Relatively large rings may be formed by cyclising the peptide under the influence of dicyclohexylcarbodi-imide. The mixed anhydride method has also been used.

Solid-phase peptide synthesis

Merrifield (1964) has introduced the 'solid phase' method in which an amino-acid or a peptide is bound chemically to an insoluble synthetic resin and then the chain is built up, one amino-acid residue at a time, at the free end. When the desired peptide has been synthesised, it is liberated from the solid support. The principles used for the peptide synthesis are those which have been described above. The method has been automated, i.e., each addition of the appropriate amino-acid is carried out automatically at a predetermined time. Some outstanding

advantages of this solid phase method are: (i) because of the use of the insoluble solid support, purification of products is not necessary, excess of reagents being removed by through washing with suitable solvents; (ii) high yields; (iii) the time has been considerably shortened for synthesizing peptides (and proteins).

The method may be illustrated with the following example. The resin, which is a copolymer of styrene and divinylbenzene, is chloromethylated. This results in the formation of 'benzyl chloride groups' through which the 'first' amino-acid becomes attached as the benzyl ester. The 'first' amino-acid, which is to be the *C*-terminal end of the peptide, is protected at its amino-group by, e.g., the t-butyloxycarbonyl group, is heated with the resin in the presence of triethylamine in a suitable solvent. The protecting group is selectively removed by HCl-AcOH and the hydrochloride of the amino-group is converted into the free amino-group by the addition of excess of triethylamine. This benzyl ester of the 'first' amino-acid residue is now coupled with the *N*-t-butyloxycarbonyl derivative of the 'second' amino-acid by means of dicyclohexylcarbodi-imide. The cycle is then repeated with the *N*-protected 'third' amino-acid, and so on. When the desired peptide has been synthesised, the ester bond linking it to the resin may be split by dry hydrogen bromide in trifluoroacetic acid.



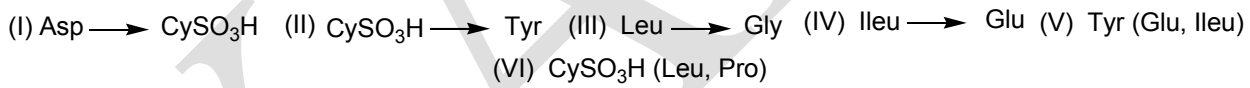
Oxytocin

As an illustration of the principles involved in the determination of the sequence of amino-acids, we shall first consider **oxytocin**, the hormone which occurs in the posterior pituitary gland and is responsible for uterine contraction. The structure was established

independently by du Vigneaud *et al.* (1953, 1954) and Tuppy *et al.* (1953). Oxytocin is extracted from the gland by acetic acid and is purified by chromatography or electrophoresis.

The isoelectric point of oxytocin is 7.7, a value which suggests the presence of a free amino group and no free carboxyl group. Complete hydrolysis with acid and the quantitative estimation of the amino-acids (chromatography on starch) showed the presence of an equimolecular mixture of eight acids: cystine, glycine, leucine, isoleucine, proline, aspartic acid, glutamic acid and tyrosine. Ammonia was also obtained, the ratio of this to any one amino-acid being 3:1. The production of ammonia in this proportion suggests the presence of three carbonamido groups. Also the molecular weight of oxytocin (determined by physical methods) was about 1000, a value which indicates that the molecule is an octapeptide.

Tuppy's procedure was as follows. Since oxidation of oxytocin with performic acid gives disulphonic acid with a molecular weight corresponding to an oxytocin disulphonic acid, this suggestes that oxytocin is a ring compound, the ring therefore including the S-S bond of cystine. Controlled hydrolysis of oxidised oxytocin with hydrochloric acid, four dipeptides and two peptides were isolated, together with two molecules of cysteic acid.



The sequence in each dipeptide (I)-(IV) was established by the DNP method, i.e., treatment of the dipeptide with FDNB followed by hydrolysis with acid, and identification of the dinitrophenyl derivative (the 'end group') by chromatography. End-group analysis of (V) showed tyrosine was the amino-terminal residue, and it therefore follows from the sequence in (IV) that the sequence in (V) is Tyr → Ileu → Glu (this must be so, since only one amino-acid residue of each kind is presence in oxytocin). Furthermore, from the sequence in (II) it follows that inoxytocin the sequence of four amino acids is $CySO_3H$ → Tyr → Ileu → Glu. Also, from the sequence in (III), the sequence in (VI) must be $CySO_3H$ → Pro → Leu, since Leu must be the terminal residue in order that (III) may be obtained. Hence the sequences of these four amino-acids are $CySO_3H$ → Pro → Leu → Gly.

Partial hydrolysis of oxidised oxytocin with the proteinase isolated form *Bacillus subtilies* gave glycine amide and tetrapeptides (VII) and (VIII), the amino-acids in which were identified by

(VII) $CySO_3H$ (Glu, Tyr, Ileu) (VIII) Asp ($CySO_3H$, Leu, Pro)

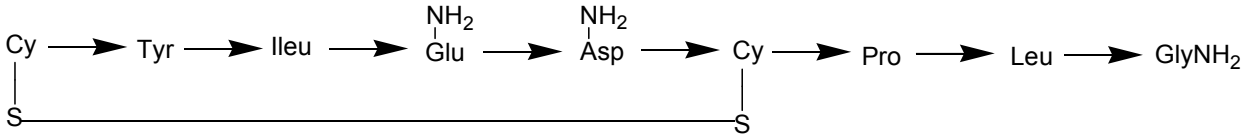
hydrolysis and chromatography, and also the end-group was determined. The sequence in (VII) has already been established to be $CySO_3H$ → Tyr → Ileu → Glu (see above), and since the addition of Asp to (VI) gives (VIII), it follows that the sequence in (VIIII) is Asp → $CySO_3H$ → Pro → Leu (Leu shown to be the end-group).

Isolation of glycine amide (form the enzyme hydrolysis) shows that it is an end-group, and from (III) and (VIII) it follows that there is the following sequence:

Since the amino-terminal group in (VII) is $CySO_3H$, combining the two sequences now established, the sequence in oxidised oxytocin that accounts for all the facts is:

The carbonamido groups have been placed as shown because (α) oxytocin contains three such groups (see above); (b) the terminal glycine amide accounts for one (see above); (c) glutamic and aspartic acids are the only two acids which each possess two carboxyl groups, and since in the others, all monocarboxylic acids, the carboxyl group must be involved in the peptide link, then only these two dicarboxylic acids can have carbonamido groups. There is however, the problem of deciding which carboxyl group is the carbonamido group, i.e., whether it is the α or γ one of Glu and the α or β one of Asp.

Finally, since oxidised oxytocin is produced without chain fission, it suggests the presence of the S-S ring (see above). Assuming that α-carboxyl groups (of Glu and Asp) are involved in the peptide linkages, then the structure of oxytocin is:



Du Vigneadu's procedure is different from Tuppy's in that the structure of oxytocin was determined mainly as the result of the examination of many fragments obtained by the partial hydrolysis of oxytocin, performic acid-oxidised oxytocin oxidised with bromine-water, and desulphurised oxytocin. The resulting peptides were separated into acidic and neutral components by means of ion-exchange resins, and were then further separated by paper chromatography. It was shown that oxidised oxytocin had only one N-terminal group (DNP method) and that this was cystine. When oxidised oxytocin was treated with bromine-water, a dibromopeptide and a heptapeptide were obtained. Hydrolysis and end-group analysis of the dipeptide showed it to be $CySO_3H$ →$TyrBr_2$ (3,5–dibromo derivative). Hydrolysis of the heptapeptide gave $CySO_3H$, Leu, Ileu, Pro, Glu, Asp, Gly and ammonia, and end-group analysis showed that the N-terminal residue was isoleucine. Since oxytocin has only one terminal amino group (see above), the amino group in isoleucine must have formed the peptide link with tyrosine. Thus, the sequence of three residues is established: $CySO_3H$ → Tyr → Ileu.

Controlled hydrolysis of the heptapeptide produced four fragments, (XIII)-(XVII), and hydrolysis of desulphurised oxytocin (by means of Raney nickel) gave four fragments, (XVIII)-(XXI).

(IX)Asp, $CySO_3H$ (X) $CySO_3H$, Pro (XI) $CySO_3H$, Pro, Leu (XIII) $CySO_3H$, Pro, Leu, Gly (XIII) $CySO_3H$, Asp, Glu (XIV) Leu, Gly, Pro (XV) CySSCy, Asp, Glu (XVI) Tyr, CySSCy, Asp, Glu (XVII) Tyr, CySSCy, Asp, Glu, Leu, Ileu (XVIII) Ala, Asp (XIX) Glu, Ileu (XX) Ala, Asp, Glu (XXI) Ala, Asp, Glu, Leu, Ileu

In peptides (XVII) and (XXI), differentiation between Leu and Ileu was not made, i.e., these peptides contain only one of these acids, but which one was not determined (both acids appeared together on the chromatogram).

Application of the DNP method to (IX) showed that its sequence was Asp → $CySO_3H$. Consideration of the acids in (IX)-(XII) shows that the sequence of five residues in oxidised oxytocin is therefore (XXII):

(XXII) Asp → $CySO_3H$ → Pro → Leu → Gly

This accounts for (XIV) and at the same time shows its sequence. On the other hand, since (XIII) contains (IX), it follows that Glu may be added to form the sequence (XXIII).

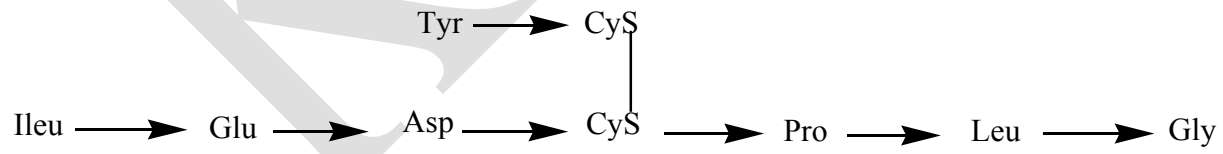
(XXIII) Glu → Asp → $CySO_3H$ → Pro → Leu → Gly

Now, in desulphurisation, the $–CH_2S$-group is converted into the $-CH_3$ group. Thus, instead of cystine, two molecules of alanine (which is not present in oxytocin) will be produced. Hence, (XVIII) corresponds to (IX) and (XX) to (XIII). Also, the isolation of (XIX) shows that Glu is linked to Ileu, and since Glu is linked to Asp as shown in (XXIII), Ileu must be in the sequence (XXIV).

(XXIV) Ileu → Glu → Asp → $CySO_3H$ → Pro → Leu → Gly

Since Ileu is now assigned, it follows that (XVII) is Tyr, CySSCy, Asp, Glu, Ileu, and (XXI) is Ala, Asp, Glu, Ileu.

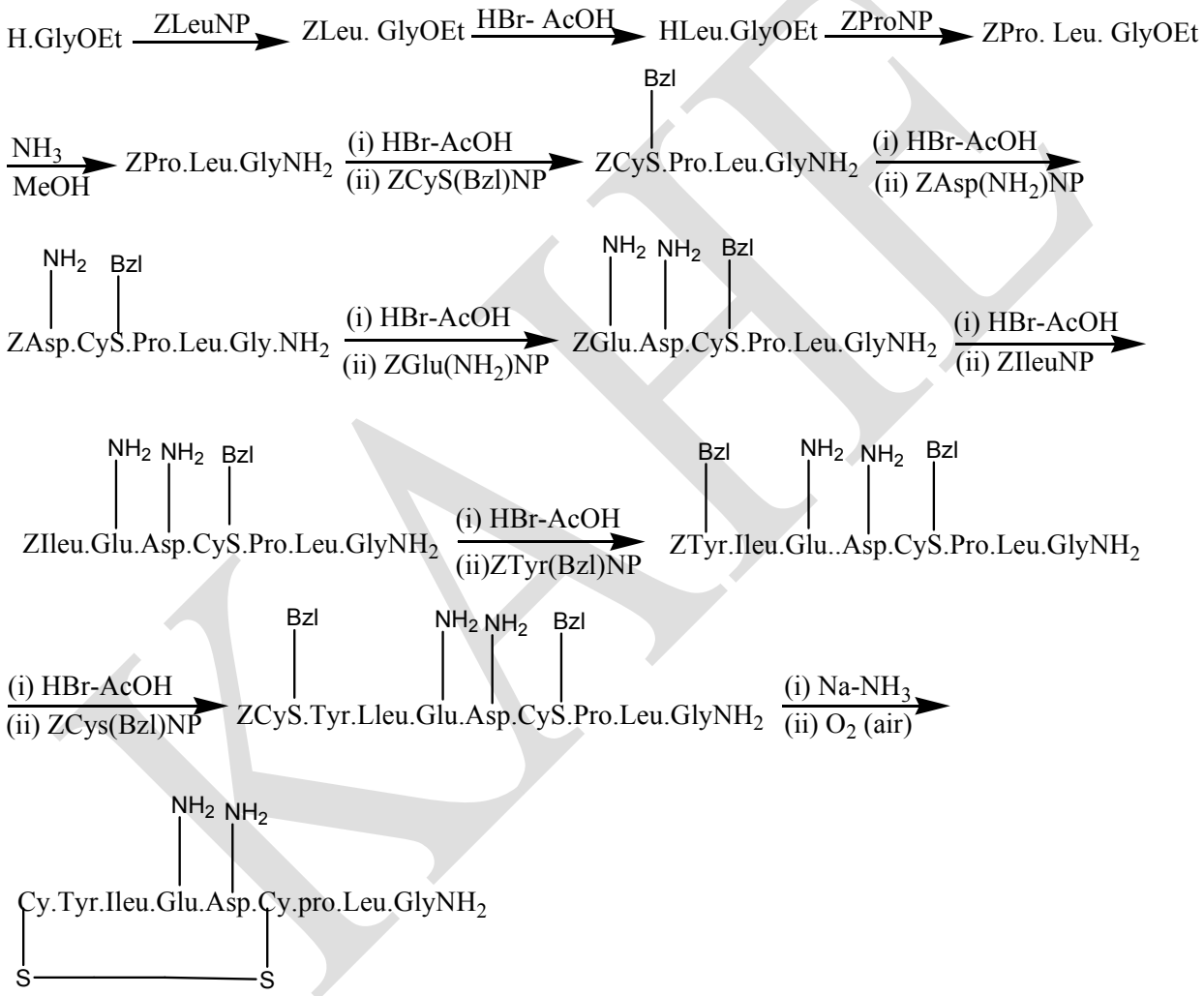
If Try is joined to one half of the cystine residue, with Asp joined to the other half, then (XVI) is accounted for, i.e., oxytocin contains the sequence.



This accounts for the eight amino-acids, and since the only free amino group present is in cystine (see above), and since oxidation does not bring about fission, oxytocin must be cyclic, and this is satisfied by joining Tyr to Ileu. The Gly end is not satisfactory, since this residue is present as carbon amide. This was confirmed by application of the Edman method of end-group analysis to oxidised oxytocin. The first four acids were removed, the order of removal being: $CySO_3H$, Tyr, Ile and Glu.

The carbonamido groups were placed as described above (in Tuppy's method), and the structure for oxytocin was therefore the same as that established by Tuppy.

The structure of oxytocin has been confirmed by a number of syntheses. The one described here is that of du Vigneaud *et al.* (1959). In the following equations, the symbols used are OEt = ethyl ester, NP = p-nitrophenyl ester, Bzl =benzyl, Z=benzyloxycarbonyl:



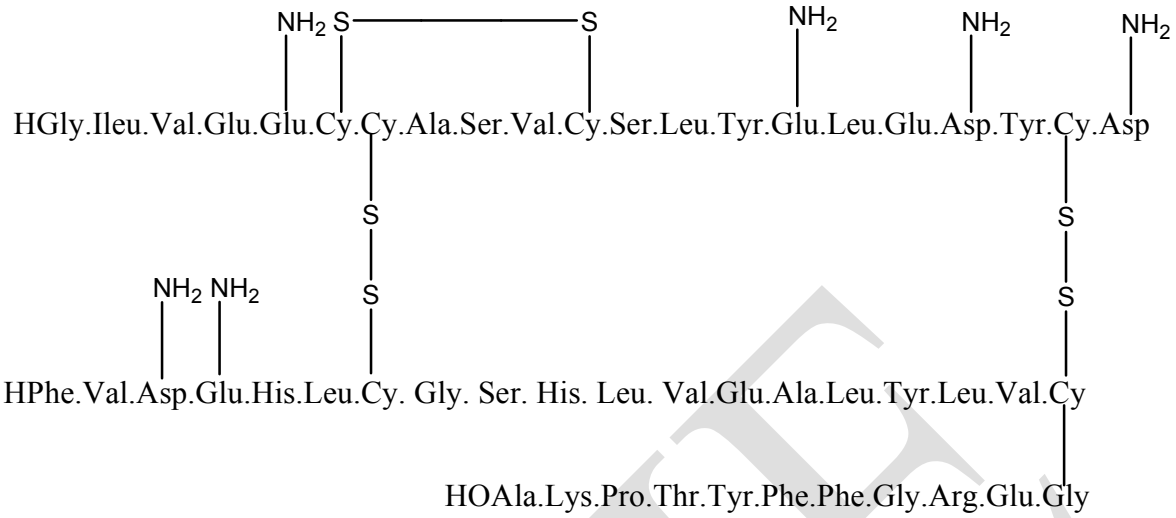
Insulin

This is the hormone which occurs in the pancreas and was first protein whose amino–acids sequence was worked out (Sanger *et al.,* 1951–1955). Measurement of the molecular weight of insulin gave values which varied with the concentration. The values were multiples of 12 000, viz. 12 000, 24 000 and 36 000. The minimum molecular weight of insulin, determined

from amino-acid analysis, was about 6000. It was originally believed that 12000 was the true molecular weight, but later work based on osmotic pressure and sedimentation measurements in organic solvents showed that the 'monomer' of insulin actually did have the molecular weight 6000.

N-Terminal amino-acid determination (DNP method) showed the presence of one glycine residue and one phenylalanine residue. Hence, insulin contains two peptide chains linked together. Since amino-acid analysis had shown the presence of cystine, it was assumed that the two chains were joined by disulphide bonds. Insulin was therefore oxidised with perfomic acid. This produced two peptides, which were separated (by electrophoresis or by chromatography) and examined individually. The peptide with the N-terminal glycine residue was called the A-chain and that with the N-terminal phenylalanine residue was called the B-chain. Each chain was subjected to partial hydrolysis with acids and with enzymes. The A-chain gave about 35 fragments with acid hydrolysis and about 10 fragments with enzymic hydrolysis; the B-chain gave respectively about 50 and about 10. The fragments were separated (by electrophoresis or by chromatography) and examined by end-group analysis (DNP method) and for amino-acid residues. Then, by means of the overlapping procedure, the primary structure of each chain was deduced. The A-chain was shown to contain 21 amino-acid residues and the B-chain 30. The A-chain contained four cysteic acid residues and the B-chain two. Hence, the A-chain contains a disulphide ring and is linked to the B-chain by two disulphide bonds.

Insulins from different sources, e.g., cattle, sheep, horses, etc., differ slightly, but all show identical hormonal activity. The formula shown is that of bovine insulin. It will be seen that there is a small ring system containing cystine, alanine, serine and valine. The differences in the insulins from various sources appear to concern this ring only. Thus, the sequence Ala → Ser → Val in bovine insulin is replaced by Ala → Gly → Val in sheep insulin, and by Thr → Gly → Ileu in horse insulin (Brown et al., 1955; Harris et al., 1956). Katsoyannis *et al.* (1966) have synthesised human and sheep insulins; Merrifield *et al.* (1968) have synthesised the A-and B-chains by the solid phase method (S10).



Enzymes

General nature of enzymes

Enzymes are biological catalysts which bring about chemical reactions in living cells. They are produced by the living organism, and are usually present in only very small amounts in the various cells (about 0.01 percent). They can also exhibit their activity even when they have been extracted from their source. All enzymes are globular proteins, many have been identified and a large number have been obtained in crystalline form.

Nomenclature and classification

A common method of naming enzymes is to add the suffix ase to the name of the substrate, i.e., the substance being acted upon, e.g., esterase acts on esters, amylase on starch (amylum), protease on proteins, urease on urea, etc. some enzymes, however, have retained their trivial names, e.g., emulsin, pepsin, trypsin, etc. Names are also used for particular enzymes, e.g., urease, amylase, or as general names for groups of enzymes, e.g., esterases, proteases, etc.

The above nomenclature is still widely used, but it has led to difficulties as more and more enzymes have been isolated. Because of this, the International Commission of Enzymes (1961) has recommended a systematic method of nomenclature and classification. According to this system, enzymes are divided into six main groups according to the nature of the reaction that is catalysed, and each main group is given a code number. The main groups are:

1. **Oxidoreductases.** These enzymes catalyse oxidation-reduction reactions, and include oxidases (direct oxidation with molecular oxygen), dehydrogenases (removal of hydrogen from substrates), etc.

2. **Transferases.** This groups of enzymes catalyses the transfer of various functional groups, e.g., transaminase.

3. Hydrolases. These catalyse hydrolytic reactions, e.g., proteases (proteins), esterases (esters,) etc.

4. Lyases. There are two types of lyases, one which catalyses addition to double bonds and the other which catalyses removal of groups and leaves double bonds.

5. Isomerases. These catalyse various types of isomerisation, e.g., racemases, epimerases, etc.

6. Ligases. These enzymes catalyse the formation of a bond between two molecules and are accompanied by the breaking of a pyrophosphate bond of ATP or similar triphosphate (see, e.g., S15). Each of these main groups is divided into subgroups which take the number of their main group followed by another number which specifies the type of group in the substrate that undergoes reaction. The subgroups are also divided into sub-subgroups. These are indicated by a third figure which gives more detailed information on the groups involved in the reaction. Finally, a fourth figure indicates the serial number of the enzyme in its sub-subgroup. Thus, an enzyme is specified by four numbers (separated by points), e.g., 1.1.1.1 is the oxidoreductase which is involved in hydrogen transfer from a CHOH group to NAD^+ or $NADP^+$ as acceptor. The trivial name of this enzyme is alcohol dehydrogenase.

The systematic names of enzymes consist of two parts, the first part specifying the substrate (or substrates) and the second part, which ends in 'ase' indicates the nature of the reaction that is catalysed. For example, let us consider the reaction:

L-alanine + 2-oxoglutarate → pyruvate + L-glutamate

This reaction is catalysed by the enzyme transaminase. Since this is a subgroup of the main group of enzymes, the transferases, the common name transaminase has been changed to the more systematic name aminotransferase. Thus, this enzyme is named as L-alanine: 2-

oxoglutarate aminotransferase; its Enzyme Commission number is 2.6.1.2. The trivial name of this enzyme is alanine aminotransferase, and was formerly called glutamic-pyruvic transaminase.

Cofactors

Many enzymes require the presence of non-protein compounds in order to perform their catalytic action. These compounds are collectively known as **cofactors** or **activators,** and fall into three main groups. **Coenzymes** are organic molecules which may be separated from the enzyme by, e.g., dialysis. On the other hand, some cofactors are bound to the enzyme and then referred to as the **prosthetic group** of its enzyme. Finally, cofactors may be inorganic ions. In some cases the metal is tightly bound to the enzyme which is then referred to as a **metalloenzyme.** In other cases the enzymes are 'metal-activated'. Metalactivators are uni- or bi-valent metal cations, e.g., Na^+, K^+, Mg^{2+}, Zn^{2+}, Ca^{2+}.

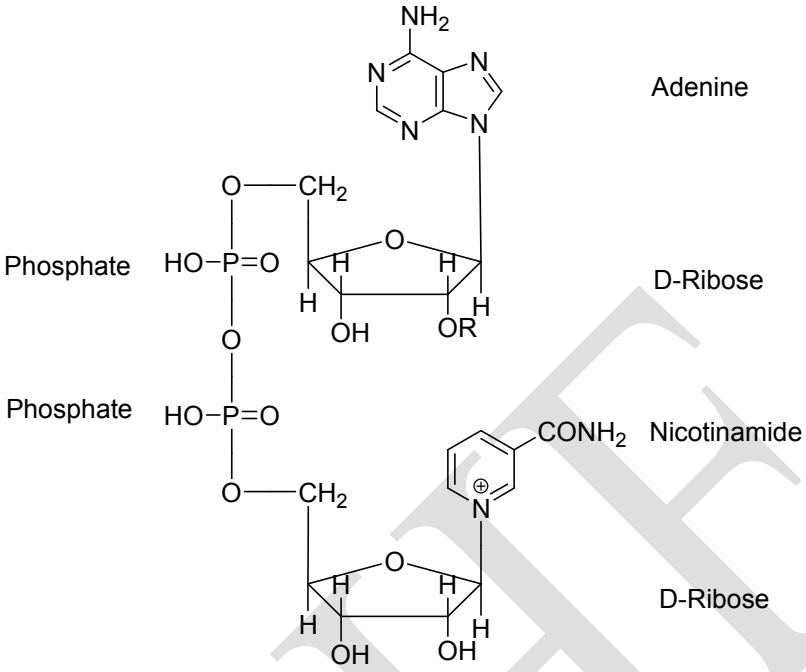
The complex, enzyme-cofactor, is known as a **holoenzyme,** and when the cofactor has been removed the protein that remains is known as an apoenzyme. This has no enzymic activity.

Some enzymes are synthesised in the organism in an inactive form; this is known as a **zymogen**. Thus, e.g., the enzyme pepsin is synthesised as its zymogen, pepsinogen. This is converted into pepsin in the presence of hydrochloric acid.

Coenzymes and prosthetic groups generally act as carriers of specific functional groups or specific atoms. In order to act in this manner, these cofactors must exist in two forms, one form being converted into the other during a catalysed reaction, and the latter being reconverted into the former by a coupled reaction. These two reactions may, or may not, follow each other. Here, we shall discuss three coenzymes which are nucleotides.

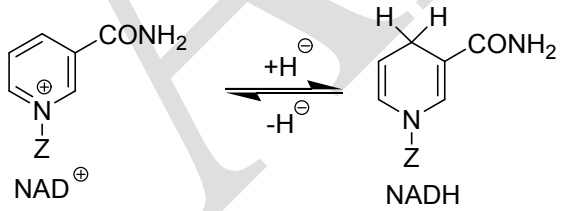
Nicotinamide-adenine dinucleotide (NAD^+)

This was formerly known as diphosphopyridine nucleotide (DPN) and has the structure shown.



NAD^+: R = H; $NADP^+$: R = PO_3H_2

This coenzyme functions as an acceptor of hydrogen atoms and electrons in the presence of dehydro-genases and is thereby converted into the reduced form **NADH**. Since only the nicotinamide moiety is involved in this transfer, the reaction may be written as shown.

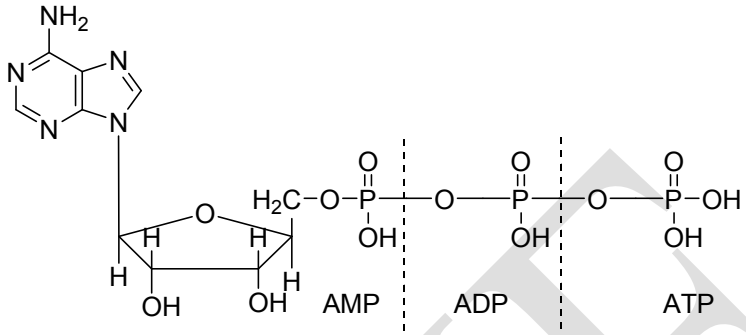


Nicotinamide-adenine dinucleotide phosphate ($NADP^+$)

This was formerly known as triphosphopyridine nucleotide (TPN) and has the structure shown (see above). This also behaves as an acceptor of hydrogen atoms and electrons, thereby being converted into the reduced form, **NADPH**. It appears that **NAD^+** and **NADH** are usually involved in degradative processes, whereas **$NADP^+$** and **NADPH** are usually involved in synthetic processes.

Adenosine triphosphate (ATP) has the structure shown. It is involved in enzyme-catalysed transphosphorylation reactions, transferring one phosphate group to the substrate, itself

being converted into **adenosine diphosphate (ADP)**. This, in turn, can also transfer a phosphate group and is thereby converted into **adenosine monophosphate (AMP)**.



For chemical reactions to proceed, energy must be supplied to overcome the energy barriers. In biosynthetic processes, this energy is supplied by ATP when it is involved in transphosphorylation reactions in the presence of a suitable enzyme, e.g.,

$$ROH + ATP \rightarrow R\text{-}OPO(OH_2) + ADP$$

ADP also behave as a phosphorylating agent, e.g.,

$$ROH + ADP \rightarrow R\text{-}OPO(OH_2) + AMP$$

A less usual reaction of ATP is pyrophosphorylation, e.g.,

$$ROH + ATP \rightarrow R\text{-}OPO(OH)\text{-}O\text{-}PO(OH_2) + AMP$$

Inspection of their structural formulae (see above) shows that the phosphate group in AMP is linked by the normal ester bond. On the other hand, the terminal phosphate groups in ADP and ATP are linked to a phosphate group by an acid anhydride bond. In hydrolytic reactions, the free energy change (heat of reaction) of an ester bond is ~ -4.0 to – 12.5 kJ mol^{-1}, whereas that for the acid anhydride bond is ~33.5 kJ mol^{-1}. Hence in transphosphorylation reactions by ATP or ADP, there is a net free energy change of ~ -29.5 to ~ - 21.0 kJ mol^{-1}. It is this energy which is used to 'drive' coupled reactions. These acid anhydride bonds have been referred to as 'energy-rich' bonds, and are sometimes represented by the symbol ~, e.g., ATP has been written as:

adenine-ribose-O-PO(OH) ~ O- PO(OH) ~ O - $PO(OH)_2$

Specificity of enzyme action

One of the most characteristic properties of enzymes is their specificity of action. This specificity may be manifested in one of three ways:

(i) An enzyme may catalyse a particular type of reaction, e.g., esterases hydrolyse only esters. Such enzymes are said to be *reaction specific*. On the other hand, an enzyme may be specific for a particular compound or class of compounds. These enzymes are *substrate specific*, e.g., urease hydrolyses only urea; phosphatases hydrolyse only phosphate esters.

(ii) Many enzymes exhibit a *kinetic specificity*, e.g., esterases, although hydrolysing all esters, hydrolyse the various esters at different rates; pepsin hydrolyses the peptide link, but is most active for those links in which, among other things, the amino group belongs to an aromatic amino-acid and the carboxyl group is one of a dicarboxylic amino-acid.

(iii) Many enzymes are *stereospecific*, e.g., maltase hydrolyses α-glycosides but not β-glycosides, whereas emulsin hydrolyses the latter but not the former.

It should be noted, however, that a given enzyme can exhibit more than one of the specificities, e.g., esterases, while hydrolysing only esters, may also hydrolyse one enantiomer (of an optically active ester) more rapidly than the other.

Mechanism of enzyme action

It has been shown that the rate of enzyme-catalysed reactions depends on a number of factors. The pH of the solution has a great effect on enzyme activity, and it has been found that an enzyme behaves efficiently as a catalyst over a narrow range of pH. This optimum pH is characteristic of a particular enzyme and is determined experimentally; it is usually between pH 5 and pH 9. As we have seen, extremes of pH denature proteins and so it is reasonable to suppose that the spatial arrangement of the molecular structure plays a part in enzymic activity.

Like all chemical reactions, enzyme-catalysed reactions are affected by changes in temperature, the rate being increased as the temperature rises. However, since enzymes can be

denatured by heat, too high a temperature destroys the activity of the enzyme. Many enzymes have an optimum temperature between 40° and 50°C, but the range may be higher, particularly for plant enzymes.

The rate of an enzyme-catalysed reaction depends on the concentration of the substrate and that of the enzyme. If the substrate is in excess, the rate is directly proportional to the concentration of the enzyme. On the other hand, if the enzyme concentration is kept constant, then the rate increases rapidly as the substrate concentration increases slowly. However, as the substrate concentration increases further, the rate increases much more slowly and finally reaches a maximum at a high substrate concentration (the rate versus substrate concentration gives a hyperbolic curve). This behaviour has been interpreted as follows. The substrate 'combines' with a particular region on the enzyme surface to form a complex. These regions are the active sites, and the complex is known as the **Michaelis complex** (1913). An enzyme may have one or more active sites. When all of these sites are occupied, the enzyme is now 'saturated' and consequently no further rate increase is possible. The substrate concentration (of the hyperbolic curve) corresponding to half the maximum rate is called the **Michaelis constant**, K_m. Its reciprocal ($1/K_m$) is a measure of the affinity of an enzyme for the substrate, e.g., if K_m is large, $1/K_m$ is small; this indicates that the substrate concentration must be large in order to achieve half the maximum rate.

The general belief is that enzyme-catalysed reactions proceed through a number of steps. If we represent the enzyme (together with its cofactor) as E, the substrate as S, and the products as P, the reaction may be written (in simple terms) as:

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

The existence of these intermediates has been established by various means, e.g., their isolation in some cases, spectroscopic studies, isotopic labelling experiments, etc. the nature of the interactions between enzyme and substrate can be of various types: hydrogen bonds, electrostatic forces, hydrophobic bonds, and chemical bonds.

Like 'chemical' catalysts, enzymes lower the energy of activation (E) of the reactions which they catalyse but they are far more efficient than the former, i.e., they lower the energy of activation to a much greater extent, e.g., the decomposition of hydrogen peroxide:

$$H_2O_2 \xrightarrow{\text{Catalyst}} H_2O + 1/2\ O_2$$

When platinum is the catalyst, E is ~ 50.2 kJ mol-1, whereas for the enzyme catalase (as catalyst), E is ~ 2.5 KJ mol^{-1}.

The mechanism whereby enzymes effect these large rate accelerations is still uncertain. It is generally accepted, however, that mechanisms in which enzymes participate involve the usual types of reactions, i.e., nucleophilic, electrophilic, homolytic, rearrangements, etc. Several contributing factors have been suggested to account for the high efficiency of enzyme-catalysed reactions.

(i) *Proximity effect*. Binding of the reactant molecules (substrate and cofactor) to the enzyme results in an 'increased concentration' of the reactant molecules.

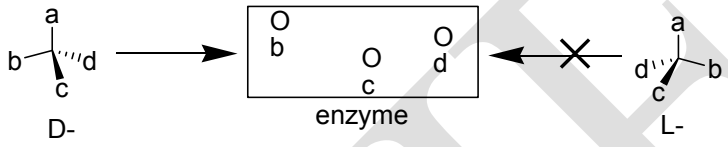
(ii) Binding causes the reactant molecules to be correctly oriented and consequently the transition state is reached more readily.

(iii) Binding produces a strain effect in the reactant molecules and consequently the bonds to be broken are 'deformed', thereby being brought to a state close to those existing in the transition state. Thus, the energy of activation of the reaction is lowered.

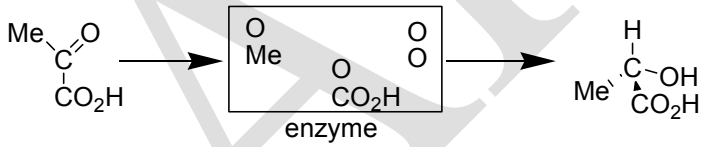
It is well established that the catalytic effects of enzymes are due to their three-dimensional structure (see also above). X-ray studies have shown that certain amino-acids, which are not necessarily adjacent in the primary structure, are 'brought together' through folding, thereby producing an active site. Since the mode of folding is dependent on the sequence of the amino-acids (primary structure), the latter must be one factor that contributes to the specificity of enzyme action, i.e., there is a steric relationship between the enzyme and the substrate. This was the basis of the 'lock-and-key' theory proposed by Fischer (1894) to explain enzyme specificity.

According to this theory, the geometry of the enzyme, the 'lock', is complementary to that of the substrate, the 'key', the result being that the latter fits into the former as a key fits into a lock.

The stereospecificity of an enzyme may be explained on the lock-and-key theory as follows. If we assume that an optically active compound can be bound to the enzyme through a minimum of three points (Bergmann *et al.,* 1935), then the 'fit' will occur with either the D or L-enantiomer, but

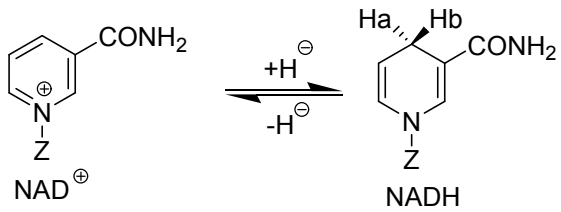


not with both, e.g., if the D-enantiomer fits, the L will not (and *vice versa*). Similarly, reduction of e.g., pyruvic acid to lactic acid, will occur on one side (enantiotopic or prochiral faces) to produce one enantiomer of lactic acid. The pyruvic acid molecule fits into the enzyme in one way



only and consequently hydrogen transfer must occur to one face only, thereby resulting in the formation of only one enantiomer of lactic acid.

Now let us consider the cofactor NAD^+. This has a pair of enantiotopic (prochiral) faces and when a hydride ion is accepted, NADH is formed and this contains enantiotopic (prochiral) hydrogens at the 4-position:



Experimental work has shown that the NAD^+ -enzyme complex is usually stereospecific, only one hydrogen (H_a or H_b) reacting exclusively. Which face of NAD^+ is attacked and which hydride ion from NADH is transferred depends on the nature of the enzyme.

Ribonucleic acids

These are polymers of ribonucleotides, and hydrolysis by alkali or by certain enzymes results in a mixture of ribonucleotides. Hydrogen-ion titration on purified RNAs showed that secondary phosphate ionisations are absent. This suggests that the individual ribonucleotides are linked together by phosphodiester bonds. As we have seen, the attachment of the phosphate is at the 3'-position in the ribose molecule. Hence possible internucleotide bond are 2'-3' and 3'-5'. The answer has been obtained by various means, the most important being the use of enzymes which are known to hydrolyse specific ester bonds in nucleotides. Thus, it has been shown that: (a) the enzyme *spleen phosphodiesterase* (specific for the C-5'-OP bond) converts RNAs into a mixture of ribonucleoside 3'-Phosphates: *(b)* snake venom phosphodiesterase (Specific for the C-3'-OP bond) hydrolyses RNAs to mixture of ribonucleoside 5'-phosphates. Hence, RNAs have a linear structure of unit linked by 3'-5' bonds. There appears to be little, if any, branched chains.

As we have seen, the common based in RNAs are adenine, guanine, uracil, and cytosine. Early work on the base composition of nucleic acid led to the conclusion that the four bases were present in equimolar proportions. Subsequent work, as a result of accurate method of analysis has shown that the molar proportions of the bases vary considerably according to the source of the nucleic acid: ribosomal (r) and transfer (t) RNAs (17) and messenger (m) RNAs. The less common bases are widespread in tRNAs. It has also been shown that the keto-bases (guanine and Uracil) and the amino-bases (adenine and cytosine) are present in all RNAs in roughly equal amounts.

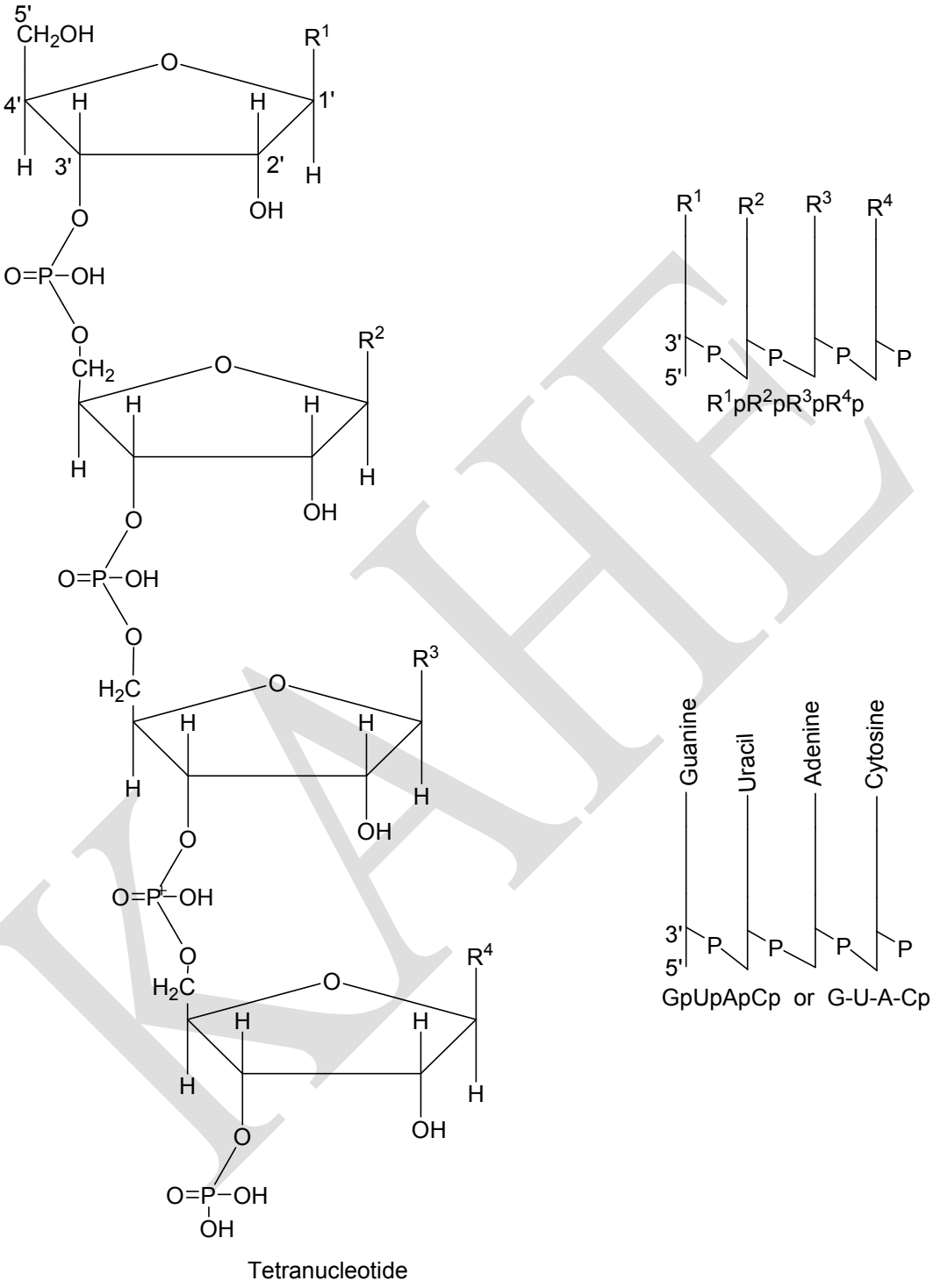


Fig. 1

A great deal of work has been done to elucidate the sequence of the bases in RNAs and methods are, in principle, similar to those used in the determination of the primary structure of

proteins. End groups have been determined by enzyme hydrolysis of the RNA with snake venom phosphodiesterase (see above). Among the nucleotide (mainly nucleoside 5'-phosphates) will be some nucleoside (R^1-end in Fig. 1) and some nucleotides 3'.5'-diphosphates (R^4-end in Fig. 1). These can be identified and estimated by means of chromatographic methods. Hence, the end groups are determined and the length of the polynucleotide chain can be estimated.

The nucleotide sequence has been determined for some of the relatively short RNAs by the use of different enzymes, end-group analysis, and the application of the overlapping method. A point of interest in this connection is that RNAs are synthesised in association with DNAs. Hence it can be expected that there will be some correspondence in the base sequence between the DNA and its complementary RNA.

On the evidence discussed above, the primary structure of RNAs may be written as shown in Fig.1. The abbreviated forms are also given; in these the letters refer to the nucleoside, e.g., G= Guanosine; U= Uridine: etc.

Various method have been used to determine the molecular weight of purified nucleic acids, e.g., end-group assay (see above), ultracentrifugation, light scattering, etc. Values obtained for RNAs range from about 2×10^4 to 2×10^6.

The secondary structure of RNAs has also been investigated. The results (mainly from X-ray analysis) appear to indicate that RNAs exist as single strands which contain helical segments stabilized by hydrogen bonding. There are, however, some examples of RNAs which exist as double strands (double helical structure).

Deoxyribonucleic acids

These are polymer of the deoxyribonucleotides and hydrolysis by certain enzymes results in a mixture of the monomers. Hydrogen-ion titration on purified DNAs showed the presence of *phosphodiester bonds*. Alkaline hydrolysis of DNAs is very slow; this is due to the absence of the 2'-hydroxyl group in deoxyribose, thereby preventing the formation of the cyclic 2',3'-phosphate which is readily formed with RNAs. This difference towards alkaline hydrolysis is used as a means of separating RNAs from DNAs. The nature of the internucleotide bonds was

established by means of enzymic hydrolysis. *Pancreatic deoxyribonuclease* converts DNAs into a mixture of oligonucleotides (average of about four nucleotide units) which contain a 5'-phosphate residue (the 3'-hydroxyl group is free). This mixture of oligonucleotides may then be subjected to the action of *spleen phosodiesterase* (deoxyribonuclease II). This results in the formation of a mixture of deoxyribonucleoside 3'-phosphates. These experiments have led to the conclusion that DNAs have linear structure of unit linked by 3'-5' bonds. Also, as for the RNAs, there appears to be no branching. Hence, the structure of DNAs may be represented by Fig. 1 (replace ribose by deoxyribose, i.e., 2'-OH by H).

The common bases in DNAs are adenine (A) guanine (G), thymine (T) and Cytosine (C). As with RNAs, the molar proportions of these bases vary considerably according to the source of DNA. There are, however, some important difference between RNAs and DNAs. The following regularities (with very few exceptions) in the composition of DNAs have been observed:

(a) A =T; (b) G=C.

From this it follows that:

(c) A+G =T+C; (d) A+C =G+T.

With DNAs, the sum of the keto-bases (G +T) is equal to the sum of the amino bases (A+C), and not roughly equal as in RNAs. The equivalence of A and T and of G and C are of paramount importance in connection with the secondary structure of DNAs.

The nucleotide sequence in DNAs has been investigated by controlled degradation with enzymes, acid etc.

Khorana *et al*. (1970) have now synthesized a gene.

The molecular weights of DNAs have been determined by various physical methods; the values obtained range from about10^6 to 10^9.

Now let us consider the secondary structure of DNAs Wilkins *et al.* (1953), from their X-ray studies, showed that the DNA molecule has helical form, and suggested the helix contain two intertwined strands. Watson and Crick (1953), however, proposed that the secondary structure was two DNA chain, wound as right-handed helices round a common axis but heading

in opposite directions (Fig. 2a). Furthermore, the two chains are wound in such a manner that pyrimidine and purine bases point towards each other and it is hydrogen bonding between pairs of bases that holds the helices together. Also, the extremely important point made, based on steric considerations, is that pairing of bases can occur only between a pyrimidine and purine, and that a given pyrimidine can pair only with its complementary purine. Such complementary pairs are A-T (Fig. 2b) and G-C (Fig. 2c). The A-T pair is held together by two hydrogen bonds and the G-C pair by three hydrogen bonds. The ring-planes of each pair of bases lie in the same plane and are perpendicular to the axis of the helix. The 'backbone' of each DNA strand consists of deoxyribose-phosphate units. This double helix accounts for the equivalence of A and T and of G and C (see above).

This Watson-Crick Model of DNA has been confirmed, with slight corrections, by later work. X-ray studies have shown that the pairs are planar and that the hydrogen bonds are almost collinear, their lengths lying between 2.8 and 2.9Å. Each turn of helix contains 10 nucleotide pairs, and the diameter of the helix is about 20Å. The spacing between adjacent pairs in 3.4Å, it can be seen from this arrangement of the two helices that the two DNA chains must be complementary to each other, *i.e.,* a chain with a given sequence of bases can pair only with another chain which has the complementary sequence of bases.

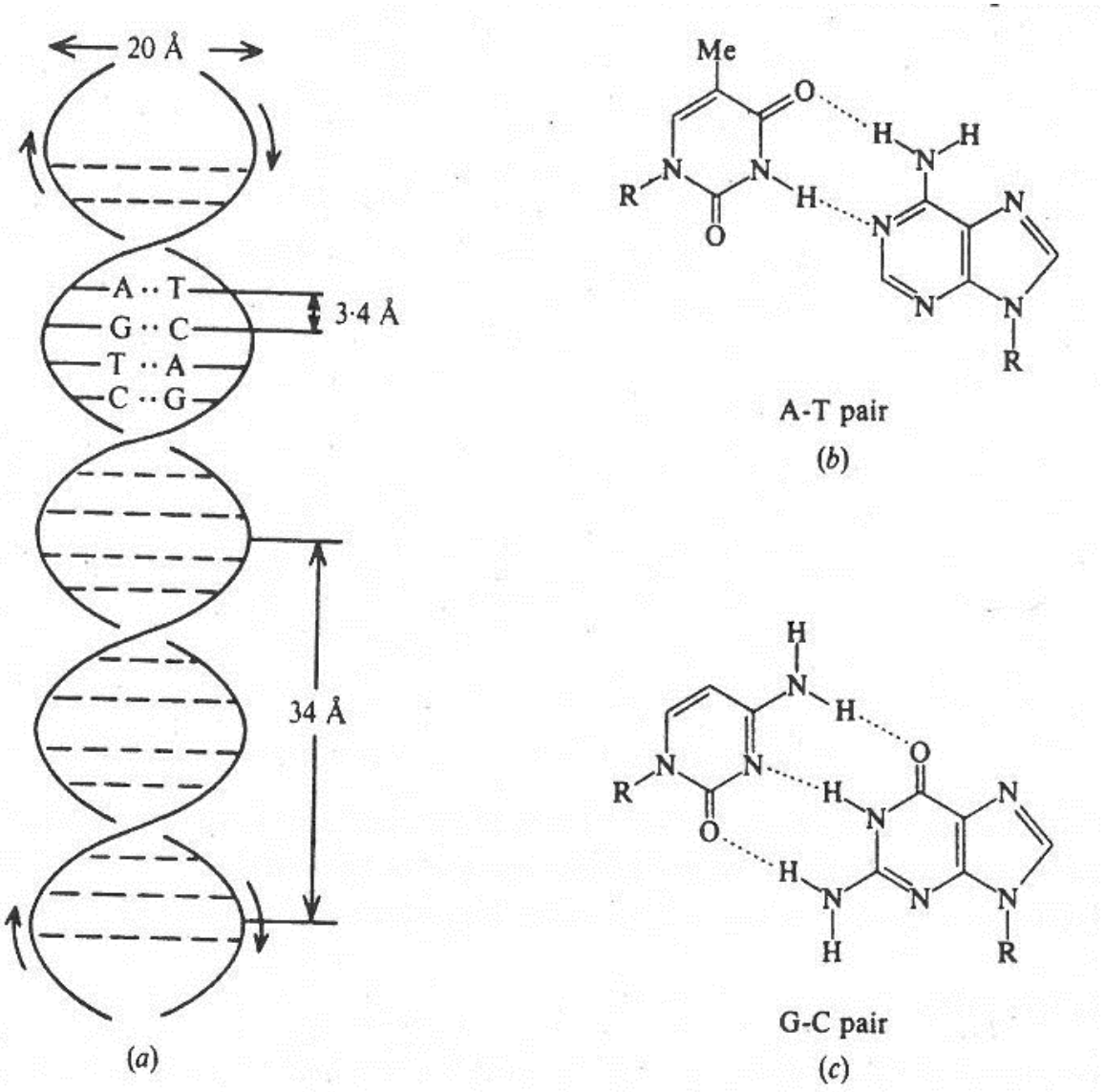


Fig. 2

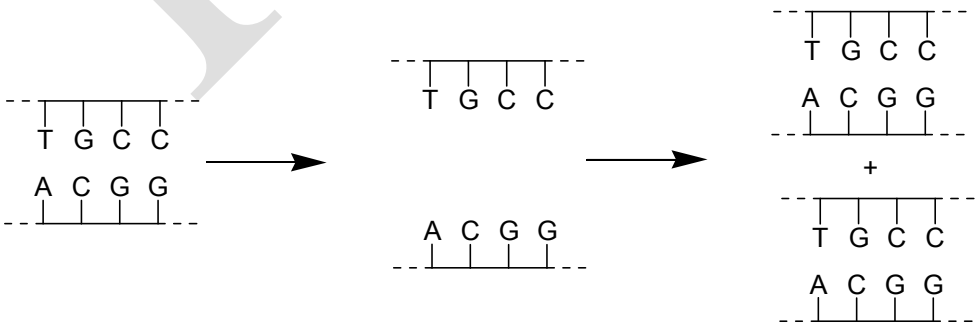
X-ray analysis has also shown that the crystalline shape of the double helix is dependent on the amount of water present. When the water content is about 40 per cent, X-ray analysis shows the presence of a regular three-dimensional crystalline structure (the A structure; repeat unit along the axis: 28 Å). On the other hand, at higher water content (70 per cent), the X-ray pattern shows that the double helices are parallel and packed side by side, but not in a regular manner (the (b) structure; repeat unit along the axis: 34 Å).

From 1959 on words, it has been found that DNAs can exist as cyclic strands, *i.e.,* as rings. Double helical DNAs have also been isolated in the form of a ring. These are example of naturally occurring catenanes, the two rings of which are interlocked by a topological bond having a very large winding number.

DNAs, like proteins, undergo change in helical content under certain conditions. These changes have been studied by the methods used in protein chemistry. Thus, when DNAs are heated in dilute aqueous solution, they undergo helix-random coil transitions. *i.e.,* they undergo thermal denaturation. The double helix separates into two separate strands. If the solution is cooled rapidly the two strands remain separate, but if cooled slowly the original double helix is often formed (anneling, renaturation). Extremes of pH also bring about denaturation (irreversible). Single-stranded ring DNAs are extremely resistant to denaturation. DNAs in the form of catenanes, by suitable treatment, can undergo a single break in one of the strands. This broken strand can be made to unwind and to separate from intact strand by careful denaturation. The single-stranded ring can be isolated.

Replication of DNAs.

Heredity is the term applied to the transmission of the potential characteristic of parents to their offspring. Genes are 'units' of heredity, and are arranged in linear sequence along the chromosomes. Chromosome are composed of deoxyribonucleoproteins, but the gene themselves consist of DNAs. As we have seen, DNAs exist as complementary pairs, and hence, if a pair split longitudinally, each chain will pair with bases from the medium, the final result being that each chain forms two paired chain which are *replicas* of the *original* pairs.



A difficulty with this hypothesis is the mechanism whereby the double helix unwinds to form two single strands. Several explanations have been proposed, but none is certain. Nevertheless, whatever may be the mechanism, it is widely accepted that each strand retains its structure on replication.

It is the particular sequence of bases in each DNA which determined the genetic properties of the chromosome, and these DNAs control the sequence of the bases in the RNAs which, in turn, control the sequence of amino-acids in the proteins.

Text Books:

1. Finar, I. L. (2013). *Organic Chemistry Vol. II: Stereochemistry and the Chemistry of Natural Products* (V Edition). New Delhi: Pearson Education, Ltd.
2. Chatwal, G. R. (2015). *Organic Chemistry of Natural Products Vol. I*. New Delhi: Himalaya Publishing House.

POSSIBLE QUESTIONS

PART- A – Multiple Choice Questions
(Each Question Carry One Mark) (Online Examinations)

PART-B (Each Question Carry Two Marks)

1. Define denaturation of proteins?
2. What is meant by annealing?
3. What is million's reaction?
4. Explain Xanthoproteic reaction?
5. What is C-terminal amino acid?
6. Explain Bergmann method for amino protecting group?
7. Explain how tosyl chloride acts as a protecting reagent for amino acid?
8. Explain how anhydride acts as a protecting reagent for amino acid?
9. Define "refolding"?
10. Define protamins with suitable examples?

PART-C (Each Question Carry Six Marks)

1. Discuss the biological significance of DNA and RNA.
2. (i) Explain the mechanism of an enzyme action.
   (ii) Explain the solid-phase peptide synthesis.
3. (i) Explain the classification of proteins.
   (ii) Explain how the following act as amino protecting groups in the synthesis of peptide
   (a) Benzyloxy carbonyl group (b) phthaloyl group
4. Explain the synthesis of peptide.
5. (i) Write notes on cofactors.
   (ii) Explain the specificity of enzyme action.
6. Write briefly on mechanism of an enzyme action.
7. (i) Explain the colour reactions of proteins.
   (ii) Write notes on conjugated proteins.
8. Explain the structure of RNA and their biological importance.
9. (i) Explain the utility of carbobenzyloxy chloride & phthalic anhydride in synthesis of Polypeptides
   (ii) Write the Bergmann's method of protecting amino group in polypeptides.
10. Explain the chemistry of oxytocin.

PART-D (Each Question Carry Ten Marks)

1. (i) Explain Biuret reaction to test proteins?
   (ii) Explain how phthaloyl group is used to protect an end amino-group (Gabriel phthalimide synthesis).
   (iii) Define mucoproteins?
   (iv) Define metalloproteins with suitable examples?

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DEPARTMENT OF CHEMISTRY

UNIT-IV

PROTEINS AND ENZYMES

PART-A–Multiple Choice Questions

(Each Question Carry One Mark) (Online Examinations)

1. Among the following which protein is soluble in water?

a) albumins b) globulins c) prolamins d) glutelins

2. The name protein was introduced by

a) Bergmann **b) Mulder** c) Meerifield d) Weinstein

3. Proteins are

a) calcium substance b) phosphate substance c) sulphate substance **d) nitrogenous substance**

4. Which one element present in nucleoprotein

a) phosphorus b) calcium c) magnesium d) sulphur

5. Which the metal present in hemoglobin

a) Co **b) Fe** c) Mn d) Mo

6. The proteins having molecular weight

a) below 10,000 b) 5,000 **c) above 10,000** d) 4,000

7. The peptides having molecular weight

a) 4,000 b) 5,000 c) above 10,000 **d) below 10,000**

8. Synthetic peptides of very high molecular weight are often referred to as

a) polypeptides b) peptides c) amino-acids d) nucleoprotein

9. The proteins and peptides are differ in

a) physical properties only **b) physical and chemical properties**

c) chemical properties only d) biological properties

10. Proteins are

a) acidic b) basic **c) amphoteric** d) neutral

11. All proteins are

a) optically inactive b) meso **c) optically active** d) racemic

12. Denaturation of protein molecule which result the changes in

a) folding b) configuration c) hydrogen bonding **d) conformation**

13. Denaturation of protein molecule which loss the

a) physical properties b) physical and chemical properties

c) chemical properties **d) biological activity**

14. The reversible of denaturation is known as

a) refolding b) unfolding c) folding d) conformation

15. Biuret reaction is responsible for

a) carbohydrates **b) proteins** c) alkaloids d) steroids

16. The biuret reaction proteins usually produce a

a) blue colour b) pink colour **c) red colour** d) white colour

17. Which one amino acid present in benzene ring

a) glycine b) alanine c) sarcosine **d) phenylalanine**

18. Xanthoproteic reaction is responsible for

a) glycine b) alanine **c) phenylalanine** d) sarcosine

19. Which one is phenolic amino acid?

a) glycine **b) tyrosine** c) phenylalanine d) sarcosine

20. Millons reaction is characteristic for

a) phenols b) amines c) acids d) amides

21. Millons reagent is

a) mercuric nitrate in nitric acid containing a trace of nitric acid

b) mercuric nitrate in nitric acid containing a trace of nitrous acid

c) mercuric nitrite in nitric acid containing a trace of nitrous acid

d) mercuric nitrite in nitric acid containing a trace of nitric acid

22. Benzyloxycarbonyl chloride is used as a

a) side-chain protecting group b) carboxyl protecting group

c) amino protecting group d) activation of carboxyl group

23. Trityl reagent is used as an amino protecting group in

a) azide method b) tosyl method c) phthaloyl method **d) trityl method**

24. Triphenyl chloromethane is also called as

a) trityl reagent b) tosyl reagent c) t-butyloxycarbonyl reagent d) DCC

25. Phthaloyl group is used as an amino protecting group in

a) Bergmann method **b) Sheehan method** c) Fischer method d) Merrifield method

26. Tosyl chloride is used as an amino protecting group in

a) Bergmann method b) Sheehan method **c) Fischer method** d) Merrifield method

27. Which one method is used to protect the carboxylic acid group in polypeptides?

a) oxidation b) reduction c) hydrolysis **d) esterification**

28. Activation of carboxyl group in polypeptides are done by converting the group into

a) acid chloride b) aldehyde c) ketone d) alcohol

29. Which one reagent is used to activate the carboxyl group in polypeptides?

a) DDQ **b) DCC** c) DBU d) Ozone

30. How much percentage of carbon present in proteins

a) 10-32 b) 0.2-0.3 **c) 46-55** d) 12-30

31. How much percentage of nitrogen present in proteins

a) 10-32 b) 0.2-0.3 c) 46-55 d) 12-30

32. How much percentage of sulphur present in proteins

a) 10-32 **b) 0.2-0.3** c) 46-55 d) 12-30

33. How much percentage of hydrogen present in proteins

a) 10-32 b) 0.2-0.3 **c) 6-9** d) 12-30

34. Proteins constitute the chief structural unit is

a) protoplasm b) enzymes c) mytoconteria d) myoglobin

35. Proteins do not migrate at a particular pH is known as

a) neutral b) acidic **c) isoelectric point** d) basic

36. Albumins are usually deficient in

a) glycine b) alanine c) sarcosine d) tyrosine

37. The -CO-NH-CHR-CO-NH-group in proteins is responsible for

a) Xanthoprotic reaction **b) Biuret reaction** c) Millon reaction d) Ninhydrin reaction

38. Enzymes are

a) physical catalysts b) chemical catalysts **c) biological catalysts** d) organo catalysts

39. The enzymes which catalyses oxidation-reduction reactions are

a) oxidoreductases b) hydrolases c) transferases d) ligases

40. The enzymes which catalyses hydrolytic reactions are

a) oxidoreductases **b) hydrolases** c) transferases d) ligases

41. The enzymes which catalyse racemases are

a) oxidoreductases b) hydrolases **c) isomerases** d) ligases

42. The non protein compounds which are required by enzymes to perform catalytic action are known as

a) proteins **b) cofactors** c) amino acids d) coenzymes

43. When the cofactor has been removed the protein that remains is known as

a) coenzymes b) holoenzyme c) metalloenzyme **d) apoenzyme**

44. The enzymes which are synthesized in the organism in an inactive form is known as

a) zymogen b) holoenzyme c) metalloenzyme d) apoenzyme

45. An enzyme may catalyse a particular type of reaction, such enzyme is said to be

a) kinetic specificity **b) reaction specificity** c) stereospecificity

d) thermal specificity

46. The isoelectric point of oxytocin is

a) 9 b) 4.7 **c) 7.7** d) 0

47. The disease caused by the deficiency of insulin is

a) beri-beri **b) diabetes** c) cancer d) rickets

48. When insulin hydrolysed it yields

a) 32 amino acid residues b) 40 amino acid residues c) 50 amino acid residues

d) 51 amino acid residues

49. The length of hydrogen bonds in DNA is lying between

a) 3.4 & 3.4 Å b) 3 & 4 Å **c) 2.8 & 2.9 Å** d) 5 & 5.1 Å

50. The diameter of helix in DNA is

a) 12 Å b) 19 Å c) 24 Å **d) 20 Å**

51. The acid hydrolysis of oxytocin yields

a) 4 amino acids b) 10 amino acids **c) 8 amino acids** d) 6 amino acids

52. The oxidation of oxytocin with performic acid gives

a) oxytocine diamide **b) oxytocin disulphonic acid**

c) oxytocin chloride d) oxytocin dicarboxylic acid

53. The controlled hydrolysis of oxidized oxytocin with hydrochloric acid gives two moles of

a) dipeptide b) tetrapeptide c) sulphonic acid d) cysteic acid

54. Partial hydrolysis of oxidized oxytocin with the enzyme proteinase yields

a) glycine amide b) dipeptide c) tripeptide d) cysteic acid

55. Example for fibrous protein is

a) albumins **b) prolamins** c) oxygen carrying proteins d) silk

56. Example for globular proteins is

a) albumins b) oxygen carrying proteins c) prolamins d) Silk

57. The metal is tightly bound to the enzyme which is then referred to as

a) zymogen b) holoenzyme **c) metalloenzyme** d) apoenzyme

58. The trityl reagent may be removed by heating with

a) acetic acid b) HBr c) sodium in liquid ammonia d) HCl

59. Benzyloxycarbonyl chloride is readily prepared by the action of carbonyl chloride on

a) acetic acid **b) benzyl alcohol** c) benzyl azide d) benzyl chloride

60. The non-protein group is known as the

a) prosthetic group b) non-prosthetic group c) protoplasm d) heme

UNIT-V

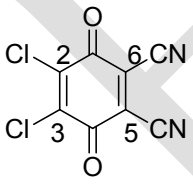
SYLLABUS

Reagents in organic synthesis: Preparations and synthetic applications of DDQ, DBU, Dimethyl sulfoxide, trimethyl silyl iodide, Osmium tetroxide, Selenium dioxide, Dicyclohexylcarbodiimide (DCC), LDA, DIBAL-H and Mercuric acetate.

Reagent Definition

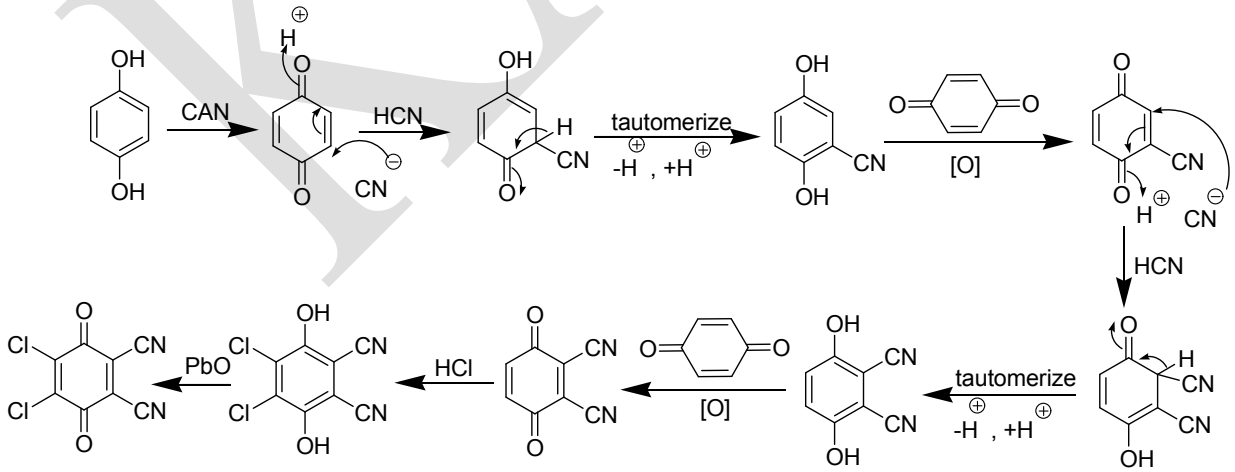
A reagent is a "substance or compound that is added to a system in order to bring about a chemical reaction, or added to see if a reaction occurs.

2,3-DICHLORO-5,6-DICYANO-*p*-BENZOQUINONE (DDQ)



Preparation from hydroquinone

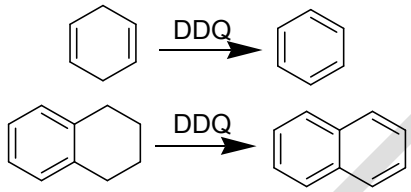
DDQ is prepared by the oxidation of hydroquinone followed by Michael addition of HCN and HCl and repetition of these steps.



Uses

Mainly used for dehydrogenation leading to aromatization or more stable systems.

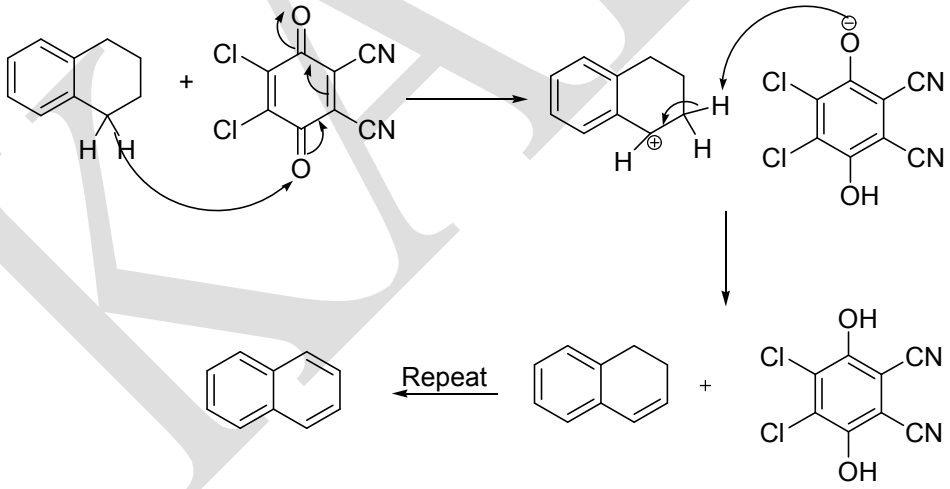
Examples:



Performing reaction with DDQ

- Normally the reaction is performed in benzene. In benzene it is red in colour due to the charge transfer complex.
- As the reaction proceeds, DDQ is reduced to hydroquinone which is insoluble in reaction medium and gets precipitated as yellow solid.
- From this the progress of the reaction can be monitored.

Mechanism

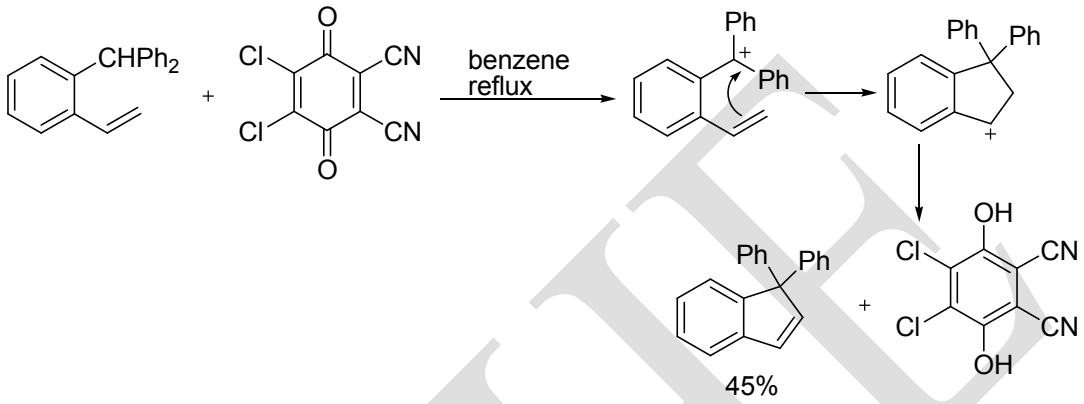


- The reaction probably proceeds via an initial hydride ion transfer and subsequent proton transfer to DDQ.
- Removal of two moles of hydrogen requires two moles of DDQ.

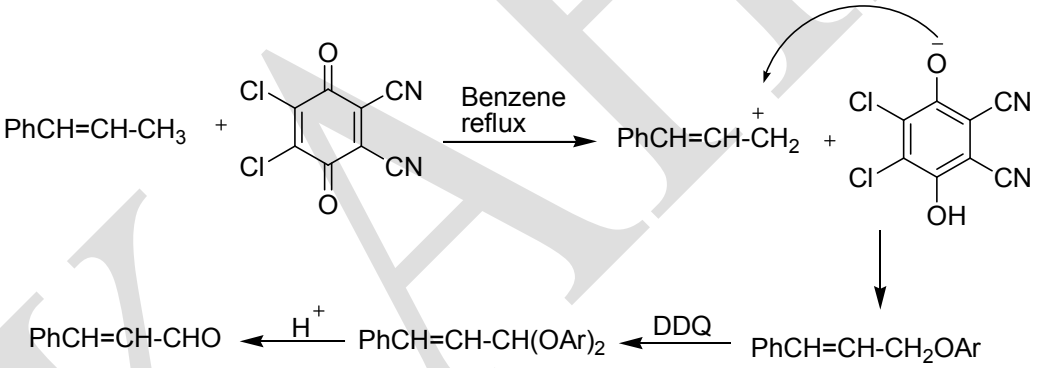
Evidence for the formation carbocations in these reactions.

The carbocations have been trapped.

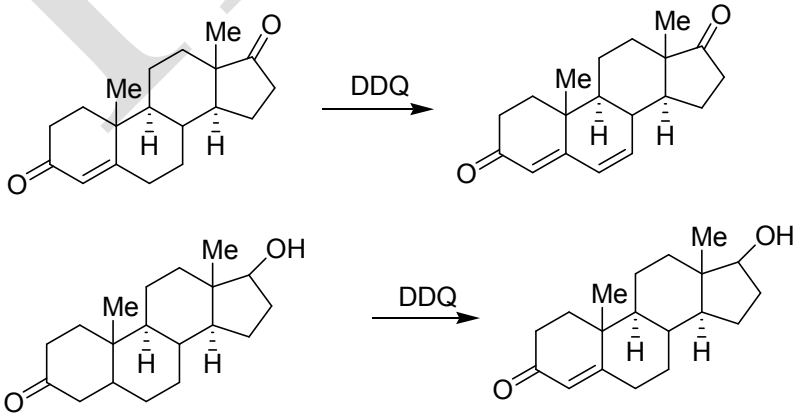
Intramolecular trapping of the carbocation by the double bond.



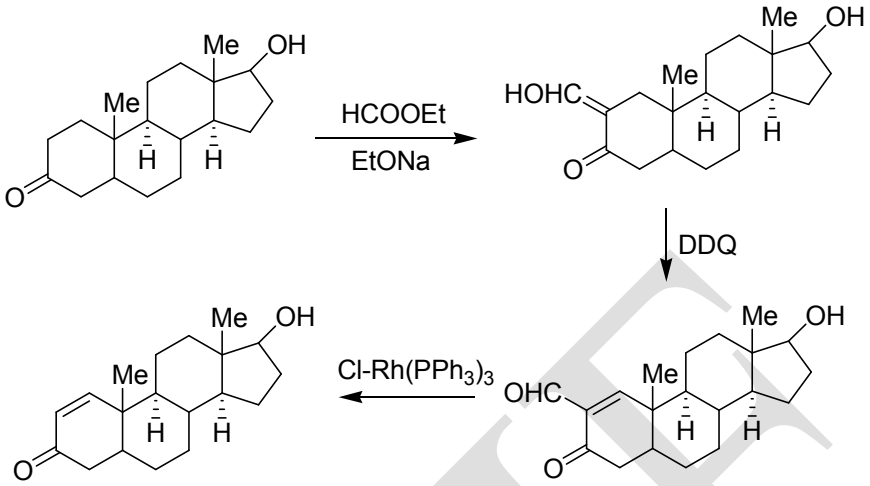
Trapping by intermolecular process



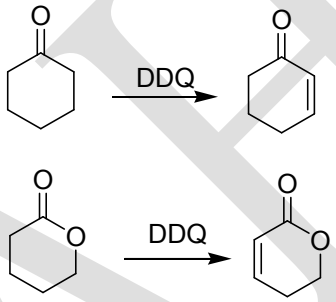
Dehydrogenation of α, β-unsaturated ketones



Preparation of another regioisomer

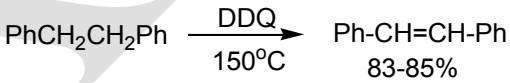


Dehydrogenation of cyclic ketones and lactones



Dehydrogenation of acyclic system

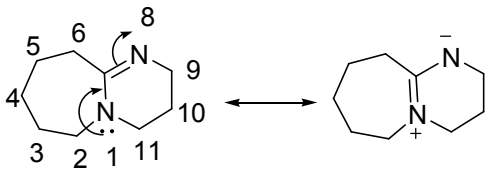
- DDQ effects the dehydrogenation of 1,2- diphenylethane while chloranil (2,3,5,6-tetrachloro-parabenzoquinone) fails, as DDQ is more reactive than chloranil.



Restrictions for the use of DDQ

- DDQ oxidizes amines and allylic alcohols.
- DDQ also undergoes Diels-Alder reaction with unhindered 1,3-dienes.

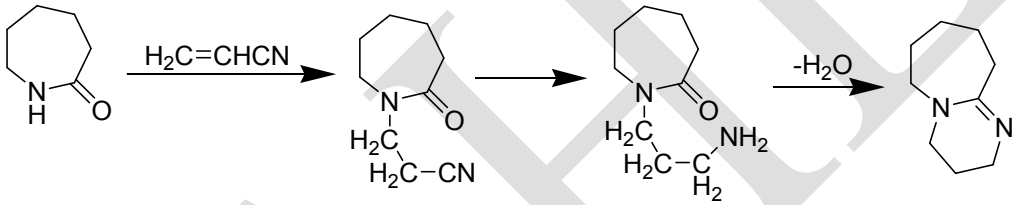
1,8-DIAZABICYCLO[5.4.0]UNDEC-7-ENE (DBU)



- This is an organic base useful in a variety of base mediated organic transformations such as **elimination reactions, isomerization, esterifications, amidations, etherifications, condensations, carboxylations and halogenations** under mild conditions.

Preparation

- From caprolactam and acrylonitrile.

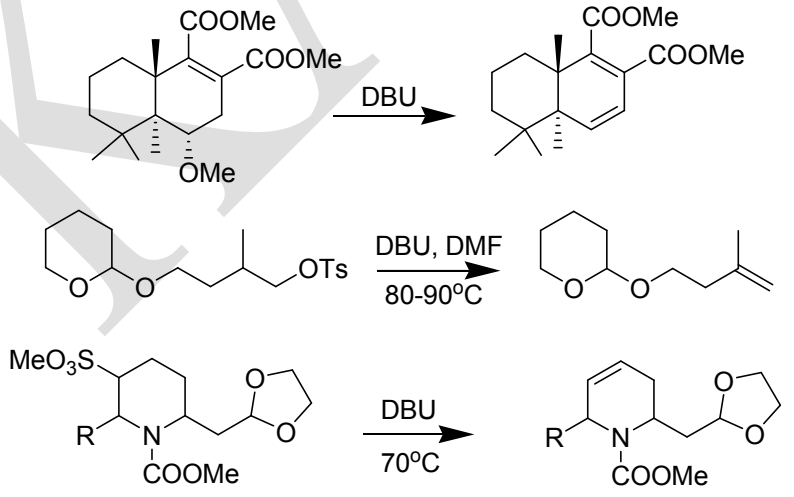


Elimination reactions

- This is a good reagent for difficult cases of dehydrohalogenation.

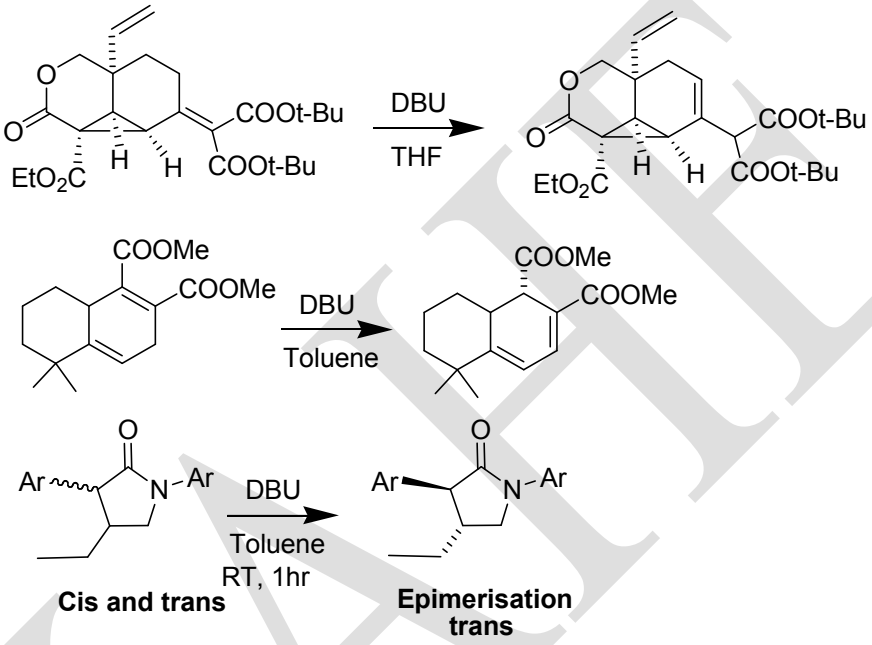
Examples

As a base in E_2 elimination

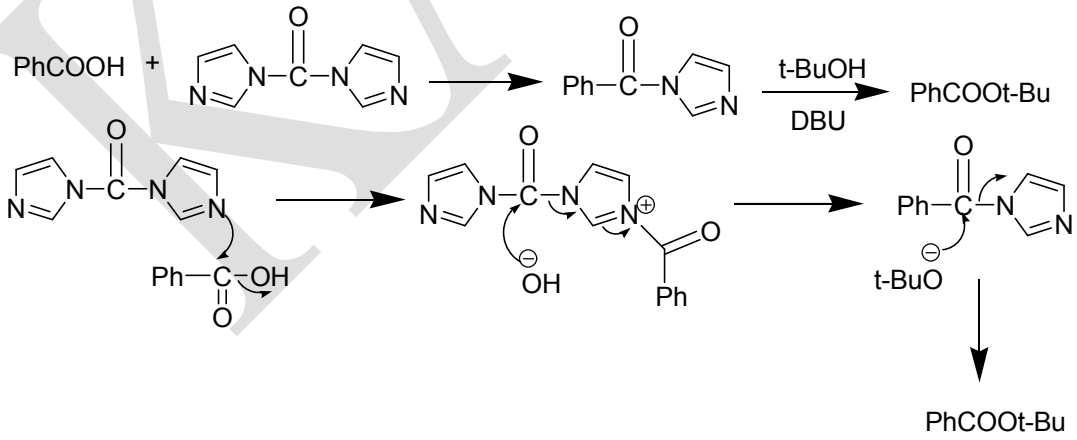


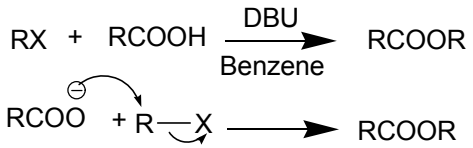
As a base in isomerizations

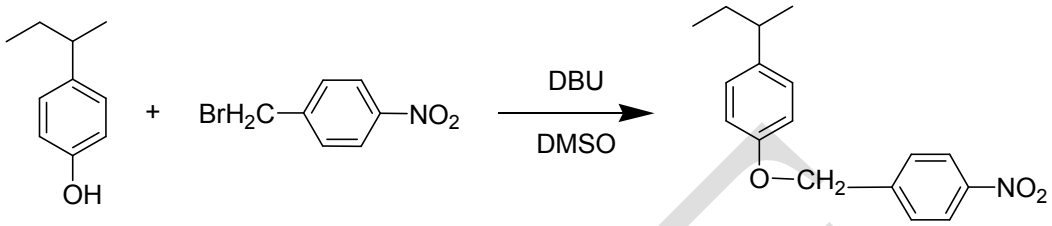
- DBU is used for base-mediated double bond migrations and epimerization.
- Isomerizations generally require proton abstraction at a carbon α to a carbonyl group or other –R group and are thermodynamically controlled.



As a base to facilitate ester and ether formation

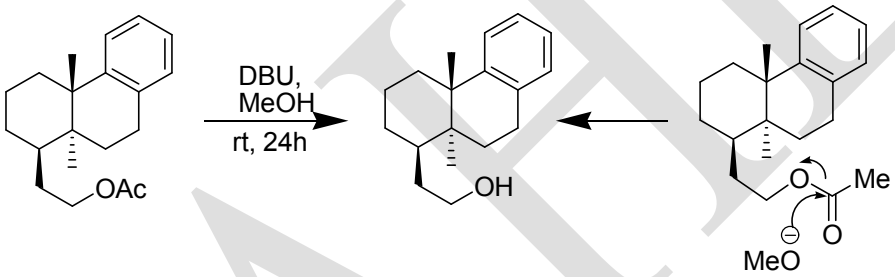




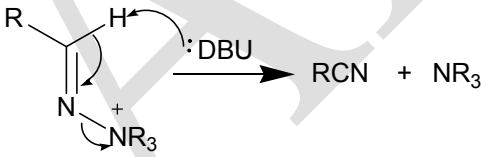


Desterification

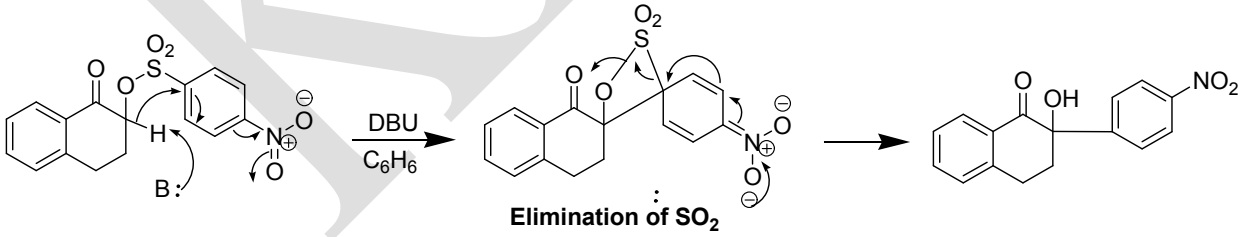
- DBU has also been employed in certain desterification.



As a base in nitrile formation

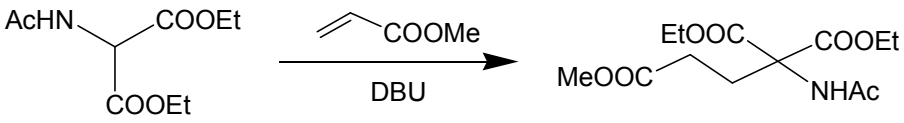


As a base leading to rearrangement



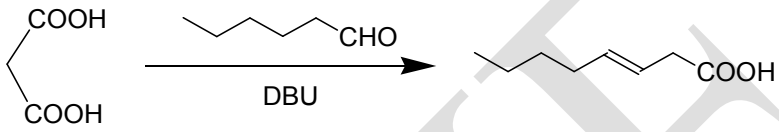
As a base in Michael addition

- DBU was used as a base in the Michael addition of diethyl acetamidomalonate with methyl acrylate in the synthesis of glutamic acid.



As a base in Knoevenagel condensation

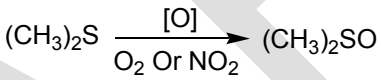
- DBU was used as a base in the Knoevenagel condensation of malonic acid with hexanal resulting in the formation of β,γ-unsaturated ester.



DIMETHYL SULFOXIDE [$(CH_3)_2SO$]

Preparation

It is industrially produced by oxidation of dimethyl sulfide with oxygen or nitrogen dioxide.

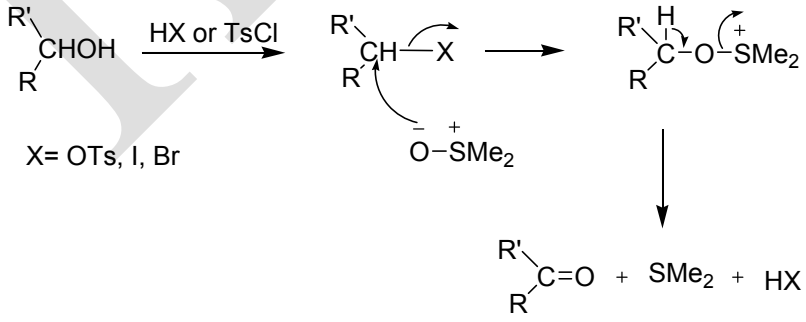


MAJOR USES

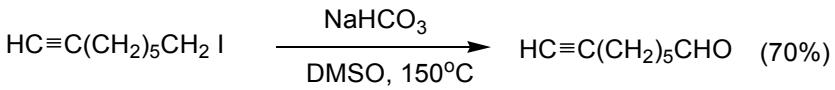
- Solvent
- Oxidant

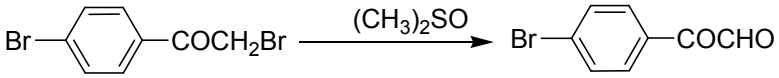
Oxidations

- Oxidation of tosylates and iodides/ bromides



Examples





(84%)

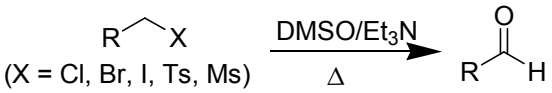
Kornblum Oxidation

1. General description of the reaction

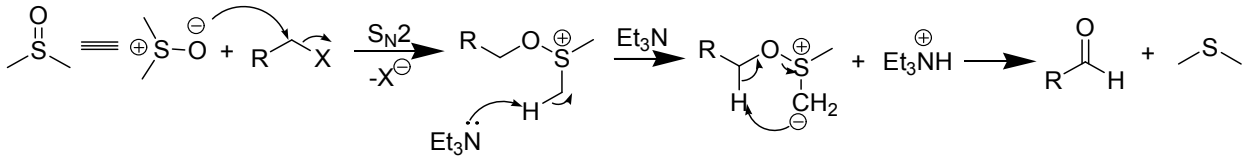
It is the formation of aldehydes by treatment of primary alkyl halides with dimethyl sulfoxide and a hydrogen acceptor. Thus it is often known as the Kornblum oxidation. Occasionally, it is also referred to as the Kornblum reaction. In fact, this is the first mild oxidation leading to a carbonyl compound using DMSO. In this reaction, bases such as triethyl amine and sodium bicarbonate are usually used as the proton acceptor; however, the oxidation of 4-chloro-3-methyl-2-buten-1-ol acetate does not proceed well when sodium bicarbonate is used as the proton scavenger, thus a sodium or potassium phosphate dibasic (Na_2HPO4 or K_2HPO4) is used.

Generally, this reaction works well for active alkyl halides, such as benzylic or allylic halides or alkyl iodide, because it initially involves the nucleophilic substitution of a halide into an alkoxysulphonium ion that transforms into an aldehyde when treated with a base. Thus those non-active alkyl halides are often converted into corresponding tosylates and are then treated with DMSO and a base. On the other hand, it has been found that a non-nucleophilic silver salt such as $AgBF_4$ or zinc sulfide can assist the nucleophilic substitution that is added to the reaction system. Unfortunately, this reaction is less selective or is uncontrollable for the oxidation of multibenzylic halides and always requires high temperature treatments.

2. General reaction scheme



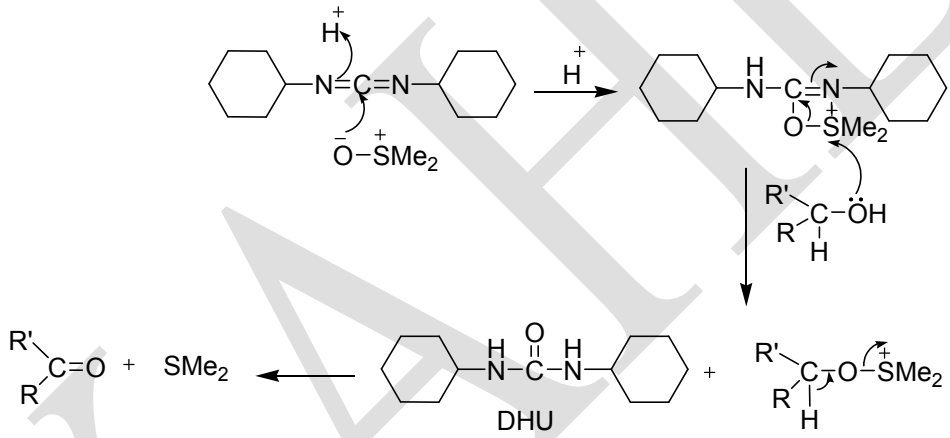
3. Proposed mechanism



4. Applications

This reaction is useful for converting primary alkyl halides into the corresponding aldehydes.

- Direct oxidation of alcohols by DMSO in presence of DCC in a single step without conversion of OH groups into as better leaving group.



- Since the use of DCC as a condensing reagent in this oxidation procedure results in the production of DHU whose removal maybe difficult from the reaction mixture, other have been examined.
- Among these are ketimines, mercuric acetate, acetic anhydride or other anhydrides pyridine-sulfur trioxide complex.

Pfitzner-Moffatt Oxidation

1. General description of the reaction

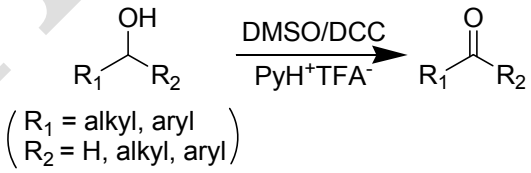
It is the oxidation of primary and secondary alcohols to the corresponding aldehydes and ketones under mild and almost neutral conditions by the combination of dimethyl sulfoxide (DMSO) and dicyclohexylcarbodiimide (DCC) in the presence of a proton source, such as phosphoric acid or pyridinium trifluoroacetate. This method was initially known as the **Pfitzner-**

Moffatt technique, and is now referred in general to as the **Pfitzner-Moffatt oxidation**. Occasionally, it is called the **Moffatt oxidation**, and the combination of DMSO and DCC is known as the **Pfitzner-Moffatt reagent**.

This reaction is applicable to primary and secondary hydroxyl groups in a variety of compounds, including alkaloids, steroids, and carbohydrates, in which other functional groups such as olefin, amine, tosylate, and tertiary hydroxyl groups are not affected. However, this reaction also has some drawbacks. For example, the equatorial OHs in steroids are readily oxidized, whereas some axial OHs are inert to the Pfitzner-Moffatt reagent.

In addition, the oxidation of nucleotides with free 3′-hydroxyl groups such as thymidine 5′-phosphate and free 3′-hydroxyl containing nucleosides in the presence of anhydrous phosphoric acid, leads to the cleavage of the *N*-glycosidic bond and the release of the nitrogenous bases. In this reaction, the amount of DMSO can be 10–100% of solvent, in combination with another inert molecule, such as benzene, as the co-solvent. Strong mineral acids such as H_2SO_4, HCl, and $HClO_4$ are not good activators for DMSO. It has been proposed that this reaction involves the activation of DMSO by DCC through an acid-catalyzed process, followed by an alcohol attack to form dimethylalkoxysulfonium salt and *N*,*N*-dicyclohexylurea, and the formation of carbonyl compounds via proton transfers and the evolution of dimethylsulfide. Sometimes, this reaction also yields by-products of $R_2CHO\text{-}CH_2SCH_3$ arising from the rearrangement.

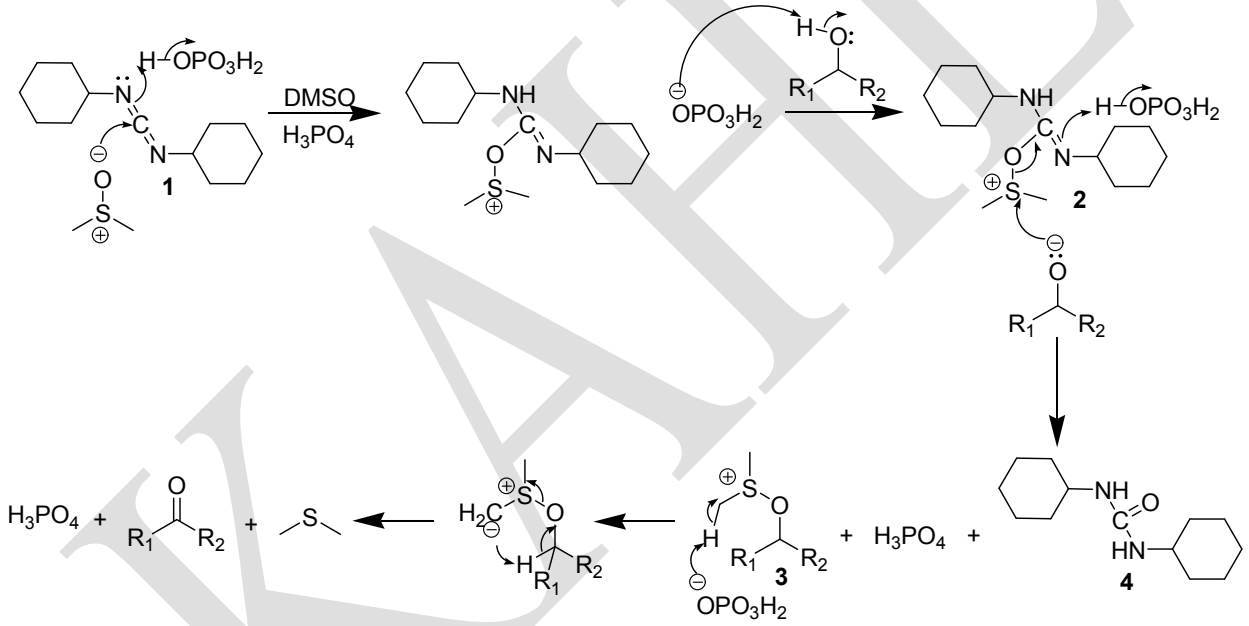
2. General reaction scheme



3. Proposed mechanisms

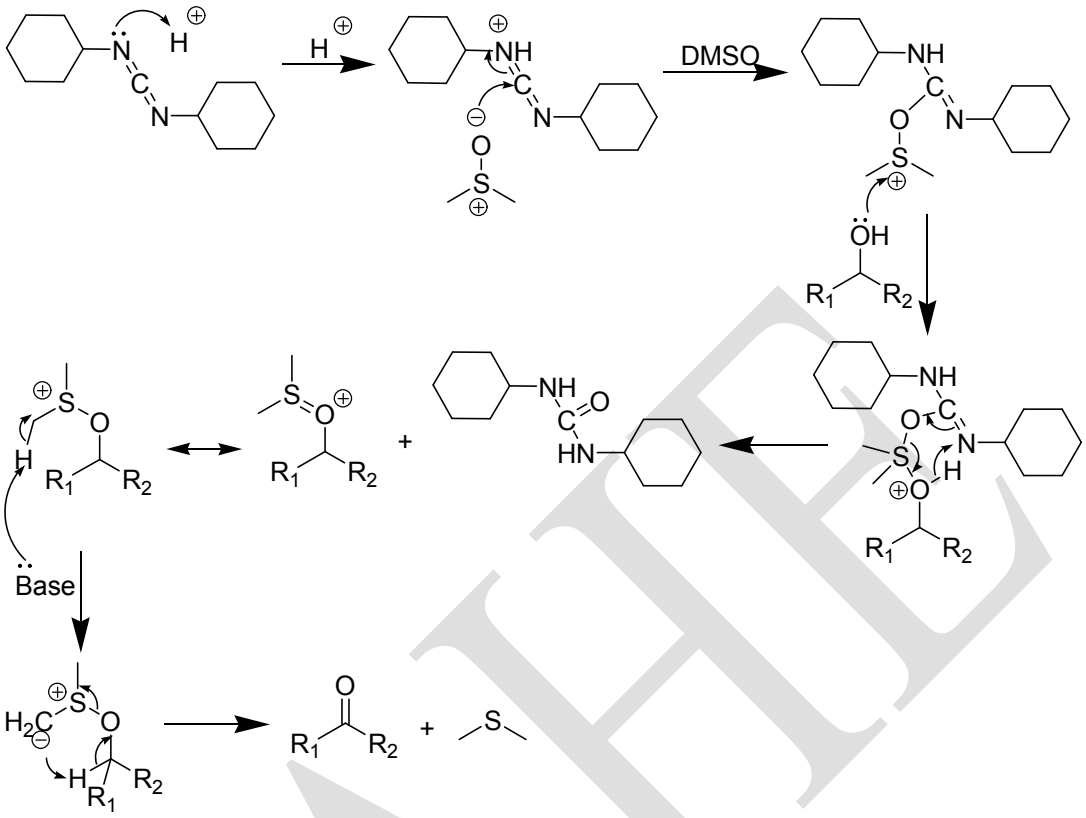
Mechanism 1

The activator is prepared by protonation of dicyclohexyl carbodiimide (**1**, DCC), which is attacked by dimethyl sulfoxide. Intermediate **2** is again protonated to facilitate addition of the alcohol oxygen on the sulfur atom. Stable dicyclohexyl urea **4** is formed along with sulfenate salt **3**. This species suffers collapse to the carbonyl compound under the influence of the dihydrogenphosphate anion. Although phosphoric acid is an effective acid catalyst for this reaction, sulfuric acid, hydrogen chloride and trifluoroacetic acid are not. However, pyridinium trifluoroacetate is an effective catalyst. It is critical that the conjugate base of the acid is basic enough to effect the last step of the reaction.



Mechanism 2

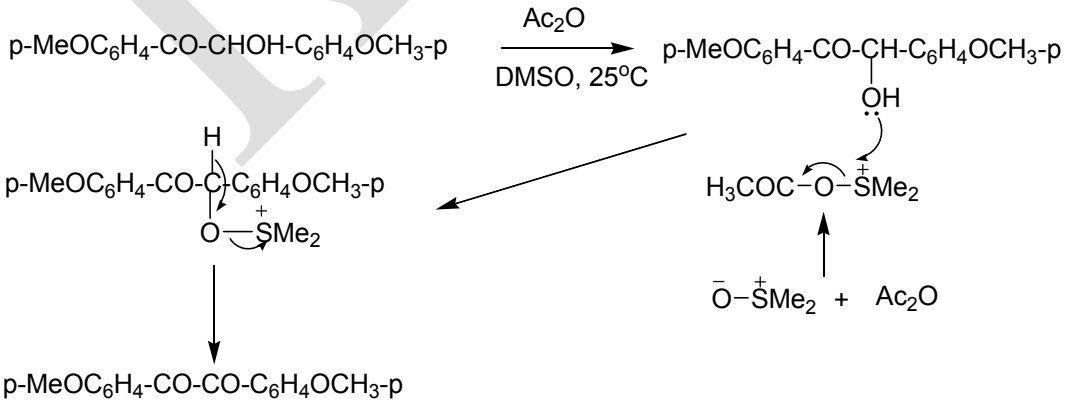
Displayed below is the mechanism for Pfitzner-Moffatt oxidation in the presence of both an acid and base.



4. Applications

This reaction has general application in the oxidation of primary and secondary alcohols to corresponding aldehydes and ketones.

- Acetic anhydride is used for only oxidation of alcohols, since primary alcohols will be transformed into acetate in a competing reaction before reaction with dimethyl sulfoxide reducing the yield of the carbonyl compound.

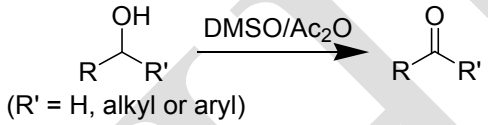


Albright-Goldman Oxidation

1. General description of the reaction

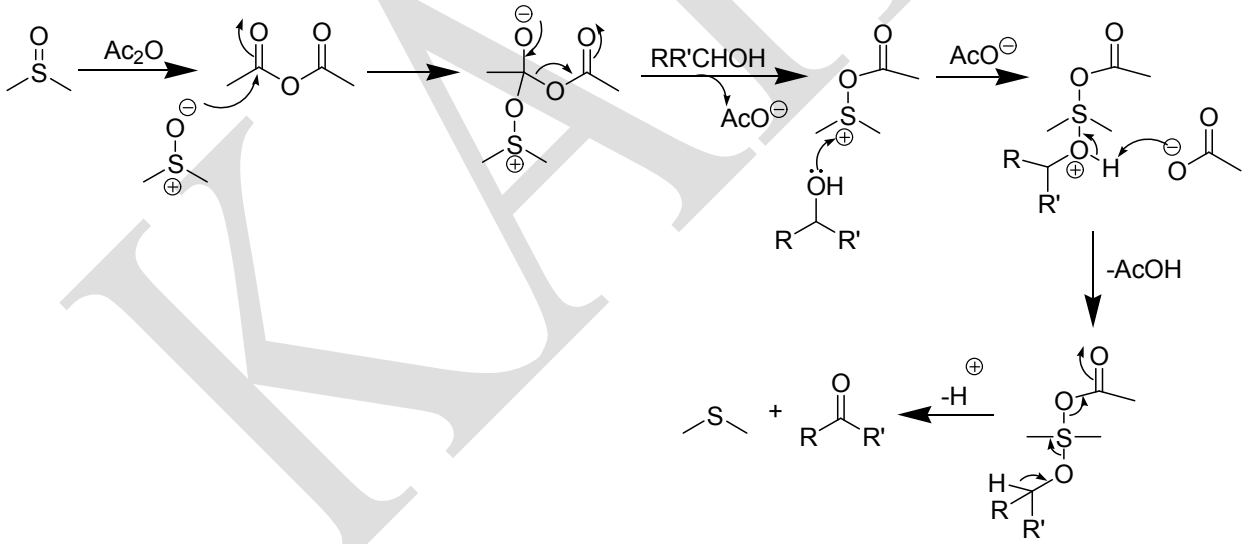
It is a mild conversion of primary and secondary alcohols into corresponding aldehydes and ketones using the mixture of dimethyl sulfoxide and acetic anhydride as the oxidant. This reaction is particularly useful for the oxidation of the sterically hindered hydroxyl groups. In general, the oxidation is carried out by allowing a mixture of 1 mmol primary or secondary alcohol, 3 mL DMSO, and 2 mL (20 mmol excess) acetic anhydride to stand at room temperature for 18–24 h.

2. General reaction scheme



3. Proposed mechanism

Displayed here is a simple illustration of this reaction.



4. Applications

This reaction has been used to convert primary and secondary alcohols into corresponding aldehydes and ketones, especially for the sterically hindered alcohols. This reaction has been commonly used in carbohydrate transformation. However, for the oxidation of phenols with DMSO/Ac_2O, the thiomethoxymethylation of the corresponding phenols occurs.

- When **oxalyl chloride** is used instead of acetic anhydride, the method is known as **swern oxidation**.

Moffatt-Swern Oxidation

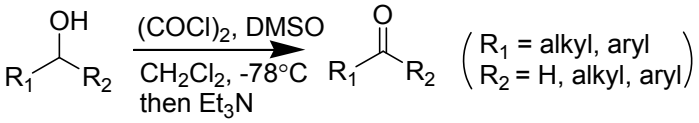
1. General description of the reaction

It is the oxidation of primary and secondary alcohols to carbonyl compounds (aldehydes and ketones) from dimethyl sulfoxide (DMSO) in combination with oxalyl chloride and triethylamine under anhydrous conditions. Therefore, it is generally known as the **Moffatt-Swern oxidation** or simply the **Swern oxidation**. Occasionally, it is also referred to as the **Swern-Moffatt oxidation**, or **Moffatt oxidation**. The combination of oxalyl chloride, DMSO, and triethylamine is called the **Swern-Moffatt reagent** or **Moffatt reagent**. It is known that this reaction involves the formation of an ylide through an alkoxydimethylsulfonium ion; if it is impossible to form the ylide, then there would be no such oxidation.

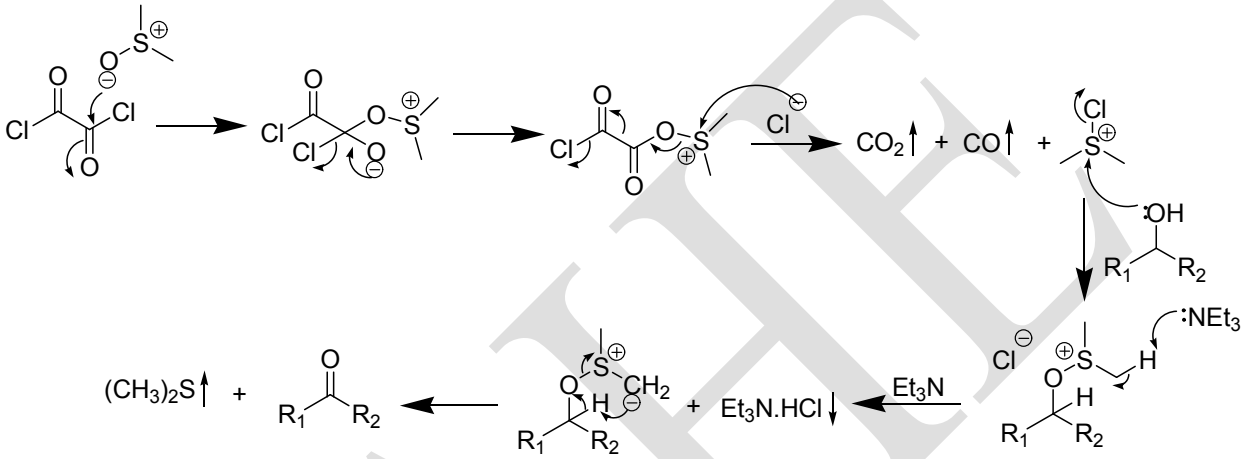
Compared to chromium-based oxidation reagents, such as PCC and PDC, the Swern-Moffatt reagent is less toxic. In addition, this reaction is also superior to the PCC and *Dess-Martin Periodinane Oxidation* in some cases. On the other hand, this reaction does not cleave the adjacent di-carbonyl functionality. For example, the Moffatt-Swern oxidation converts terminal 1,2-diol into α-hydroxy aldehyde but not into an α-keto aldehyde, whereas the modification using the combination of DMSO/EDC/Cl_2CHCO_2H can transform α-hydroxyamide to α-ketoamide.

Because this reaction is very mild and selective, it has been widely used in organic synthesis, even for the preparation of some very unstable aldehydes, which are subjected to subsequent transformation directly. For ketones that are sensitive to triethylamine, they can be prepared by substituting triethylamine with the Hunig's base. However, this reaction also has some flaws. For example, it must be carried out under anhydrous conditions because of oxalyl chloride; the reaction temperature is often very low to avoid the *Pummerer Rearrangement*. In addition, this reaction may leave trace impurities that cannot be detected by spectroscopy or combustion analysis but could affect some subsequent reactions, such as palladium catalyzed reactions. Moreover, this reaction may cause the racemization of chiral molecules.

2. General reaction scheme



3. Proposed mechanism



4. Applications

This reaction has wide application in the preparation of ketones and aldehydes.

Parikh-Doering Oxidation

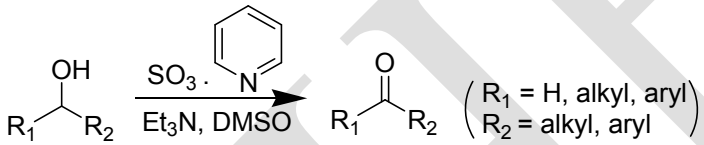
1. General description of the reaction

It is to oxidize primary and secondary alcohols into aldehydes and ketones at room temperature using sulfur trioxide-pyridine complex in combination with dimethylsulfoxide and triethylamine. Therefore, this reaction is called the **Parikh-Doering oxidation**. In addition, this reaction is also known as the **Parikh-Doering protocol** or **Doering oxidation**.

The attractive features of this oxidation include the fast reaction rate (usually completed within minutes), convenient working conditions (room temperature instead of cryogenic reaction temperature), functional group tolerance, negligible side products (methyl thiomethyl ether derivative of alcohol), easy-to-handle reagents, and flexibility to charge with more reagent if necessary.

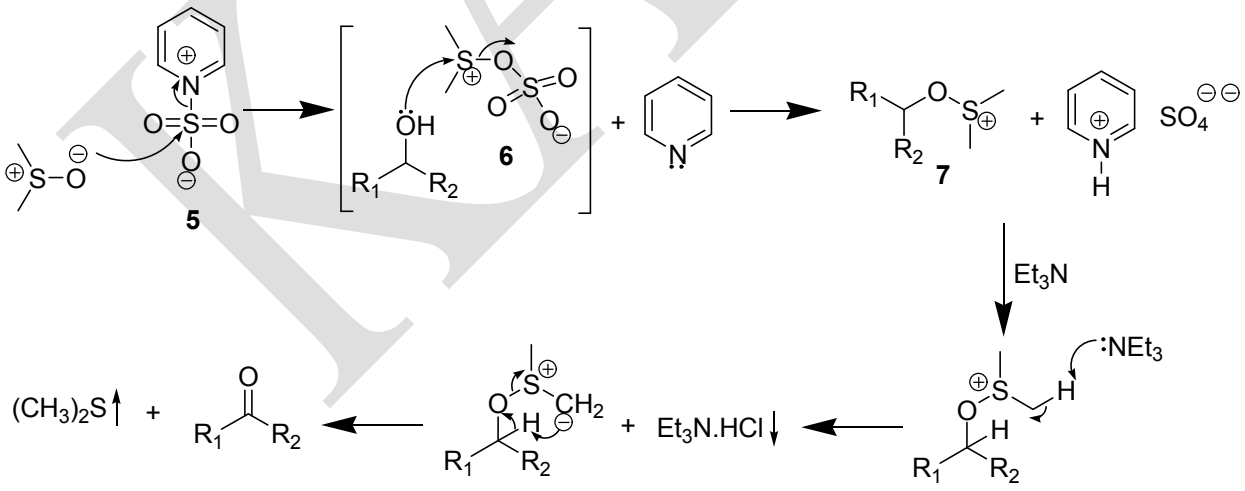
The functional group tolerance is clearly demonstrated by the fact that double bond, epoxide, acetals, etc. are not affected, even under the conditions for oxidizing alcohol in tosylate salt. The **Parikh-Doering oxidation** is especially feasible for the oxidation of alcohols mounted onto polymer and in the preparation of *α,β*-unsaturated carbonyl compounds, by which the former situation has often been recognized as problematic under other conditions. The oxidation has been proven to be superior to TPAP/NMO oxidation but not as good as the *Moffatt-Swern Oxidation* and *Corey-Suggs Oxidation* for the oxidation of long-chain alcohols. In addition, this reaction is not good for the highly strained alcohols.

2. General reaction scheme



3. Proposed mechanism

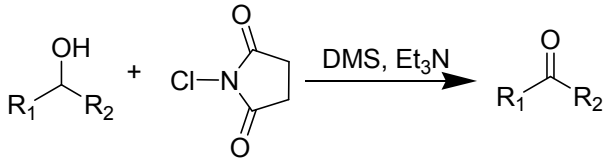
This oxidation utilizes the pyridine sulfur trioxide complex (**5**) as the activator of dimethyl sulfoxide. Sulfate is the leaving group in the displacement by the alcohol (primary or secondary) in intermediate **6**. Sulfenate **7** is decomposed by the intramolecular mechanism (vide supra) to yield an aldehyde or ketone.



4. Applications

This reaction is generally useful for the conversion of primary and secondary alcohols into carbonyl compounds.

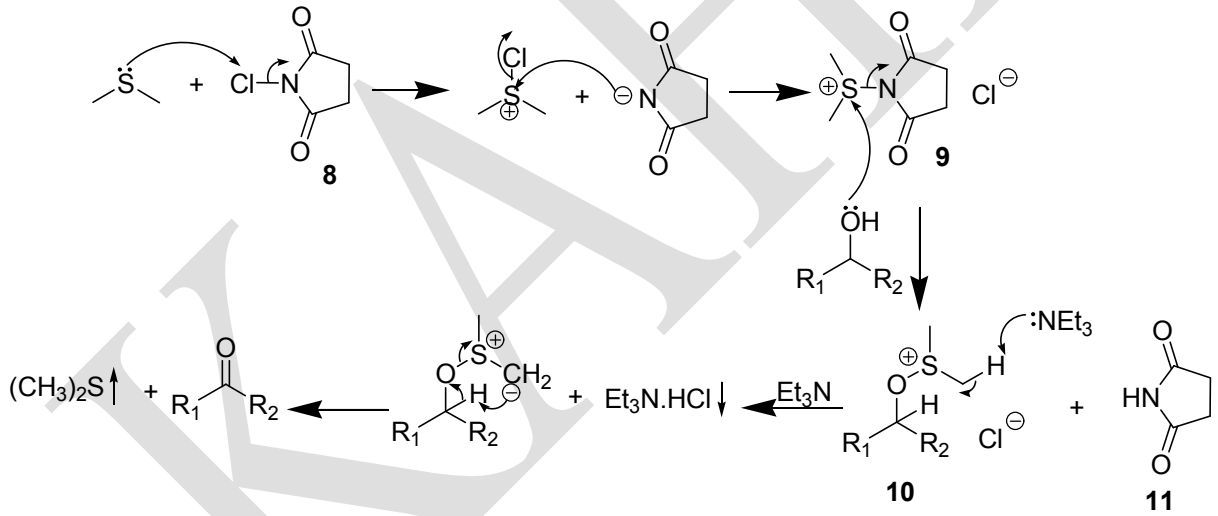
Corey-Kim Oxidation



Proposed mechanism

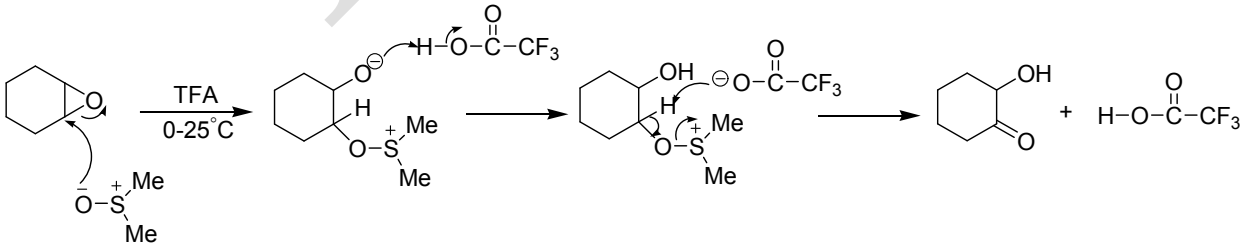
In this procedure dimethyl sulfide is activated (oxidized) with *N*-chlorosuccinimide (**8**) to provide reagent **9**. In the reaction of this species with an alcohol, the succinimidyl group functions as a leaving group. The usual sulfenate intermediate **10** collapses by the intramolecular mechanism upon the addition of triethylamine.

Proposed mechanism



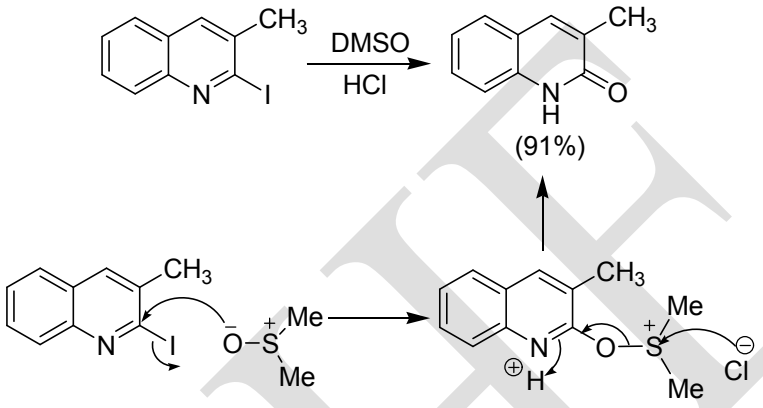
Reaction of epoxides

- Epoxides with DMSO give the corresponding α-hydroxy ketones or aldehydes.

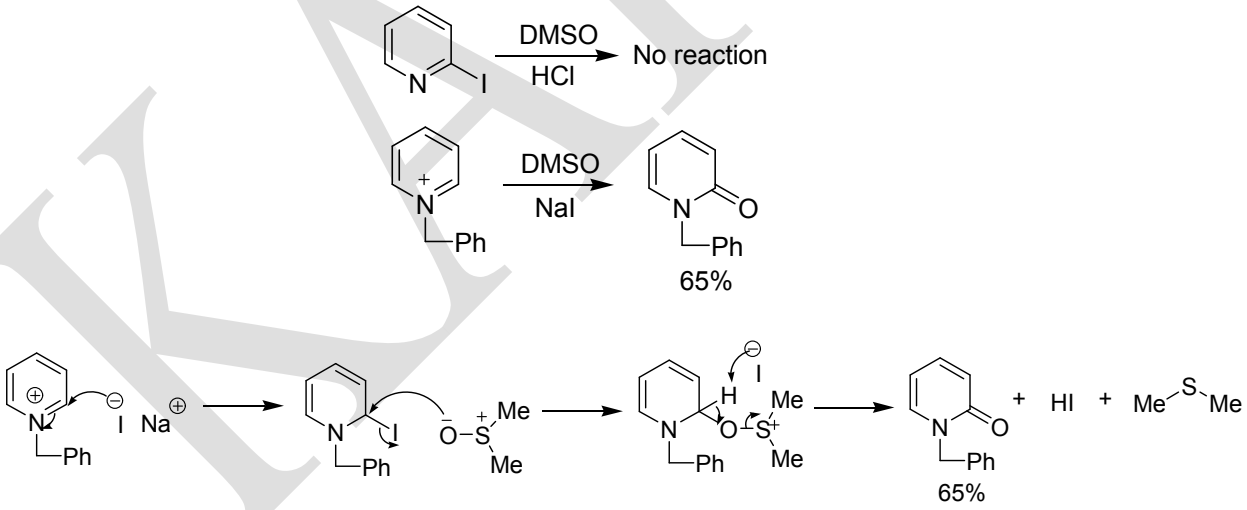


Aromatic nucleophilic substitution

- 2-Iodo-3-methylquinoline is converted into 3-methyl-2-quinolone in 91% yield by treatment with DMSO and a catalytic amount of HCl at 100°C for 24 hr. 2-Bromo- and 2-chloroquinolines are converted into quinolones if sodium iodide to the reaction mixture.



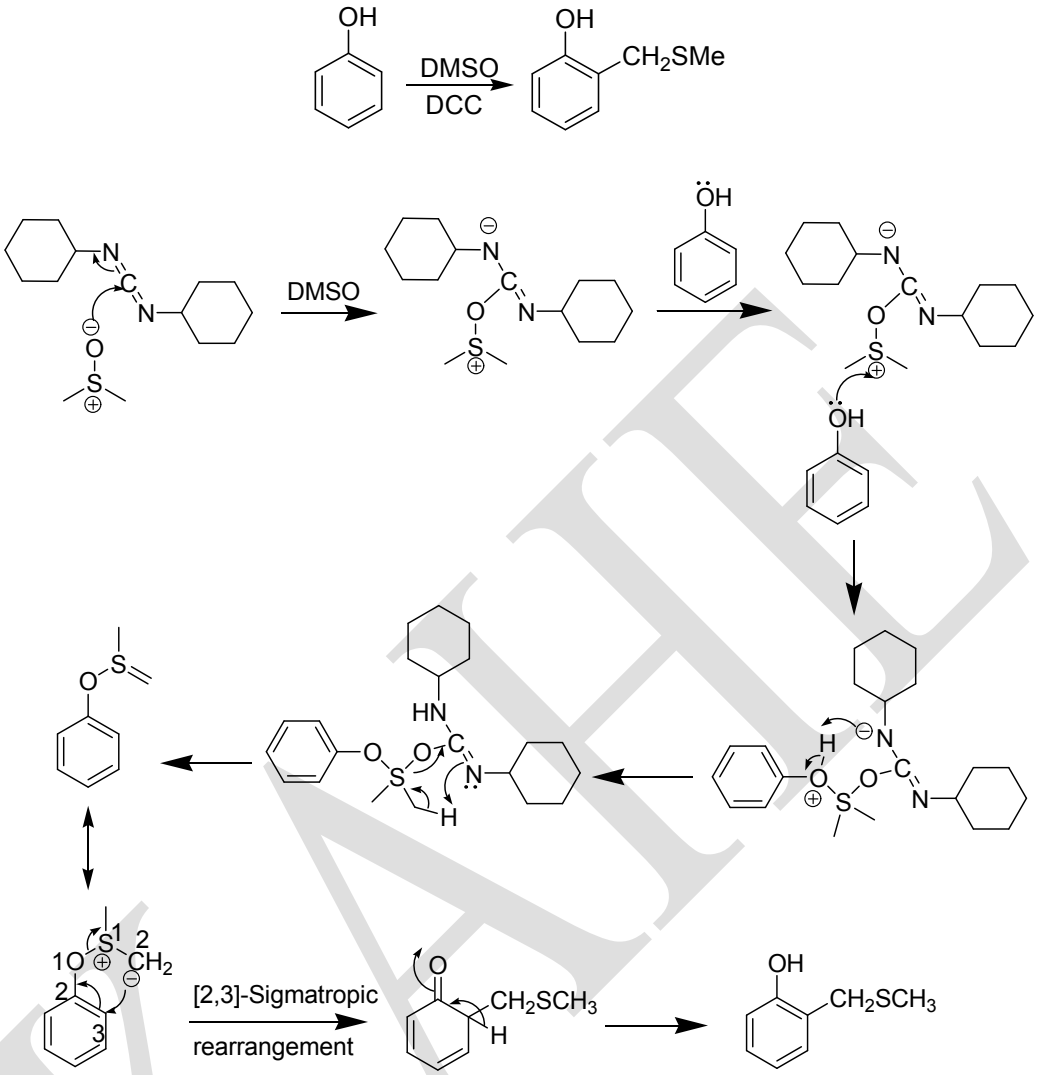
- 2-Iodopyridine does not react under these conditions, however pyridinium salts such as the one shown below are converted into pyridones.



These reactions probably involve oxidation and reduction of a sulfoxium intermediate.

Alkylthioalkylation of phenols

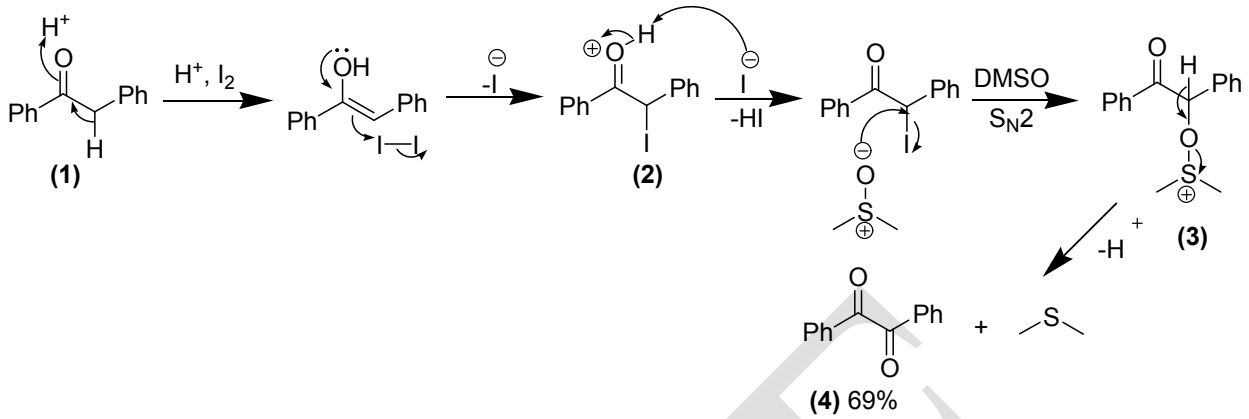
- A methylthiomethyl group can be inserted into the ortho position of phenols by heating with DMSO and DCC.
- It is possible to convert the CH_2SMe into either a CHO or Me and hence it is an indirect for the introduction of a CHO or Me group ortho to an OH or amino group.



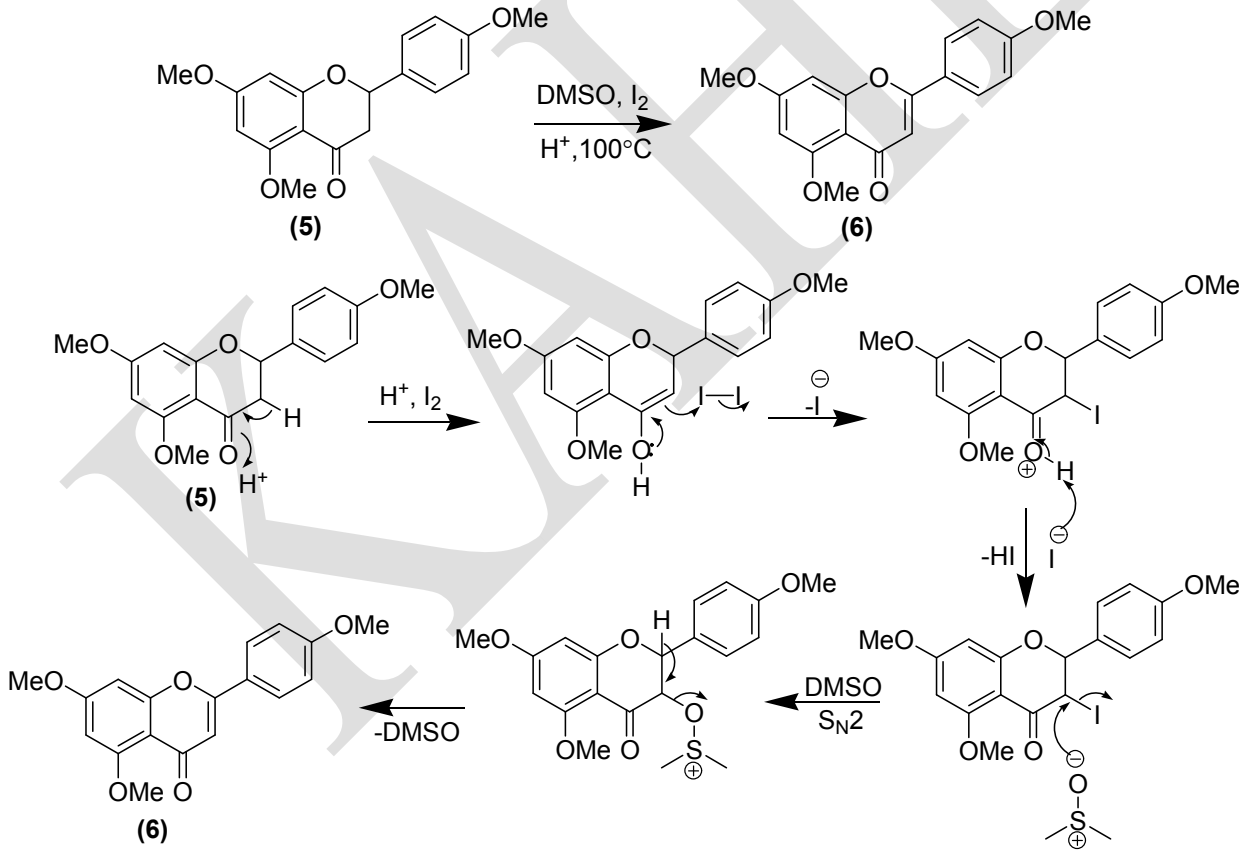
Dimethyl Sulfoxide-Iodine (DMSO + I_2)

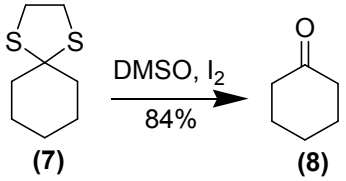
Oxidant with diverse applicability, including the conversion of benzoyl methylene groups to di- and triketones, thioacetals to carbonyl compounds, and ketones to α,β-unsaturated ketones.

The formation of dicarbonyl compounds by this reagent is a variation of the Kornblum oxidation and presumably involves acid-catalyzed iodination of the carbonyl compound **(1)** to give an *α*-iodo ketone **(2)** which undergoes displacement by DMSO to give an alkoxysulfonium ion **(3);** this gives a dicarbonyl compound **(4)** in a 1,2-elimination with assistance by base.

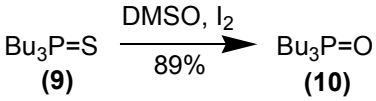


Reaction of the flavanone **(5)** to give **(6)** presumably involves an α-iodo ketone which undergoes elimination.The ethylene thioacetal of cyclohexanone **(7)** gives cyclohexanone **(8)** and phosphine sulfides **(9)** and selenides give the oxo analogs **(10)**.





(7) (8)



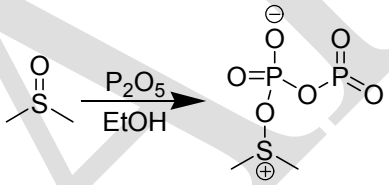
(9) (10)

Dimethyl Sulfoxide-Phosphorus Pentoxide (DMSO + P_2O_5) (Onodera Oxidation)

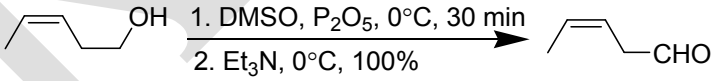
Oxidant for the conversion of primary and secondary alcohols to aldehydes and ketones, respectively; avoids overoxidation to carboxylic acids; modified procedure gives very good yields with short reaction times at 0°C with minimal formation of byproducts; inexpensive.

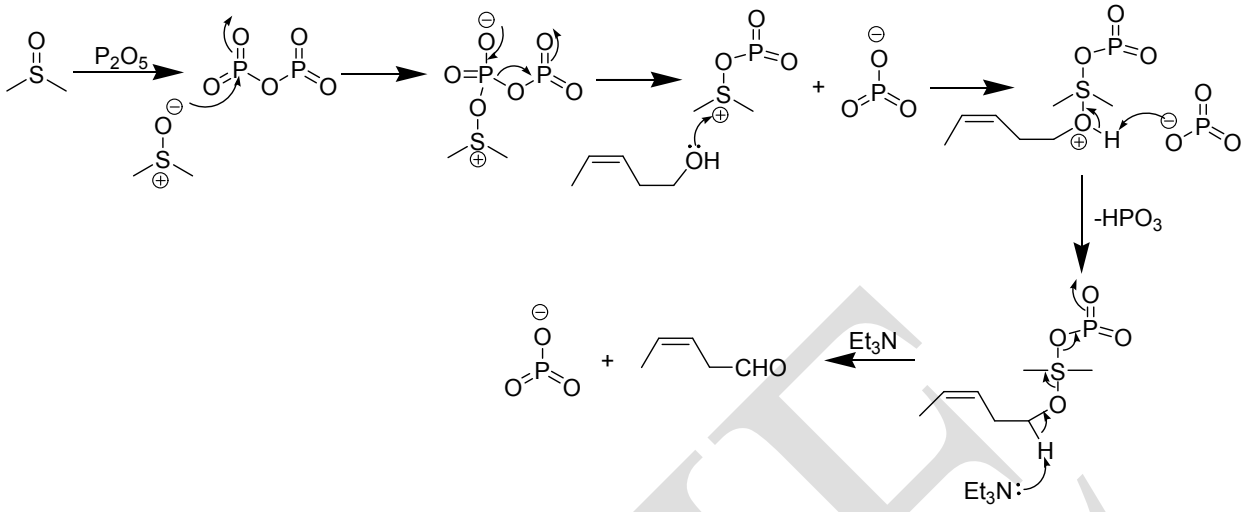
Preparation

The active oxidant, formulated as $Me_2{}^+SO(P_2O_5)^-$, is generated in situ from the reaction of DMSO and P_2O_5 in the presence of the alcohol.



The DMSO and P_2O_5 react to form $Me_2{}^+SO(P_2O_5)^-$, which reacts *in situ* with the alcohol ROH to give the alkoxysulfonium ion $Me_2{}^+SOR^-$ common to most oxidations by activated DMSO.





TRIMETHYLSILYL IODIDE (Me_3SiI)

Preparation

(i) Trimethylsilyl chloride may be prepared by the reaction of silicone tetrachloride with methylmagnesium bromide in diethyl ether. Trimethylsilyl chloride with sodium iodide in acetonitrile is the most economical and facile. For Trimethylsilyl iodide (TMS-I) it appears that the simplest method really is best.

$$SiCl_4 + 3CH_3MgBr \xrightarrow{Et_2O} (CH_3)_3SiCl + MgBrCl \xrightarrow[CH_3CN]{NaI} (CH_3)_3SiI + NaCl$$

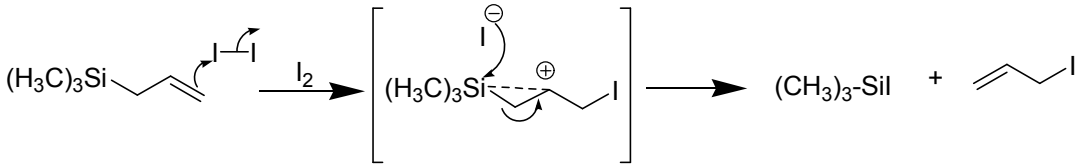
(ii) TMS-I has been prepared by reaction of hexamethyldisiloxane with iodine and aluminum powder. However, hexamethyldisilane is not easily prepared except by reaction of TMS-CI with sodium-potassium alloy.

$$(CH_3)_3SiCl \xrightarrow{Na\,/\,K} (CH_3)_3SiSi(CH_3)_3 \xrightarrow[Al\ powder]{I_2} 2(CH_3)_3\text{-}Si\text{-}I$$

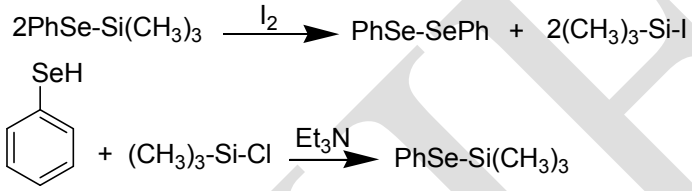
(iii) TMS-I has been prepared by several *in-situ* reactions. Among these are the reactions of trimethylphenylsilane with iodine at 120°C. This reaction may be catalyzed by aluminium iodide.

$$Ph\text{-}Si(CH_3)_3 \xrightarrow[AlI_3]{I_2} Ph\text{-}I + (CH_3)_3\text{-}Si\text{-}I$$

(iv) TMS-I results from *in-situ* reaction of allyltrimethylsilane with iodine. A problem is that allyl iodide is itself a reactive electrophile which may alkylate nucleophilic centers in the substrate or product. Further, only half the iodine atoms are productively utilized.



(v) TMS-I has also been generated *in-situ* by the reaction of iodine with phenylseleno-trimethylsilane. Phenylseleno-trimethylsilane (bp 110-115°C/18 mm) has been prepared by reaction of phenylselenol, TMS-CI and triethylamine or by the reaction of sodium phenylselenide and TMS-CI in THF.

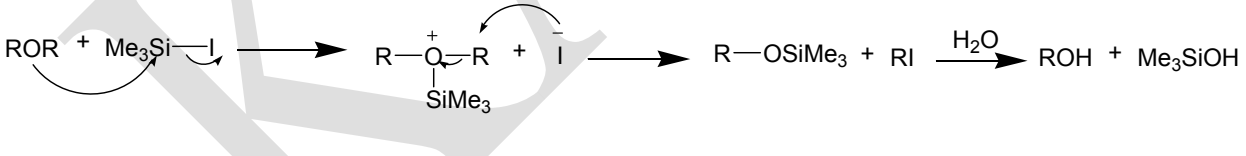


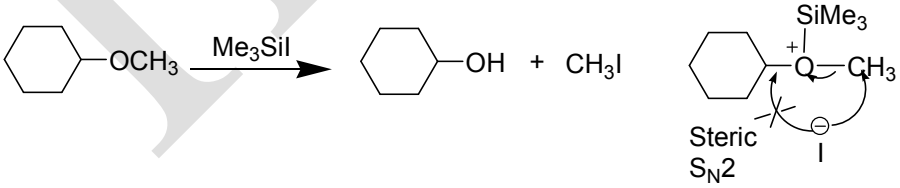
Uses

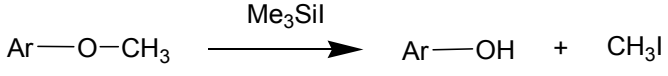
- Mainly used to cleave ethers and esters.
- Me_3SiI cleaves methyl ethers in a period of a few hours at room temperature.
- Benzyl and t-butyl ethers are cleaved very rapidly.

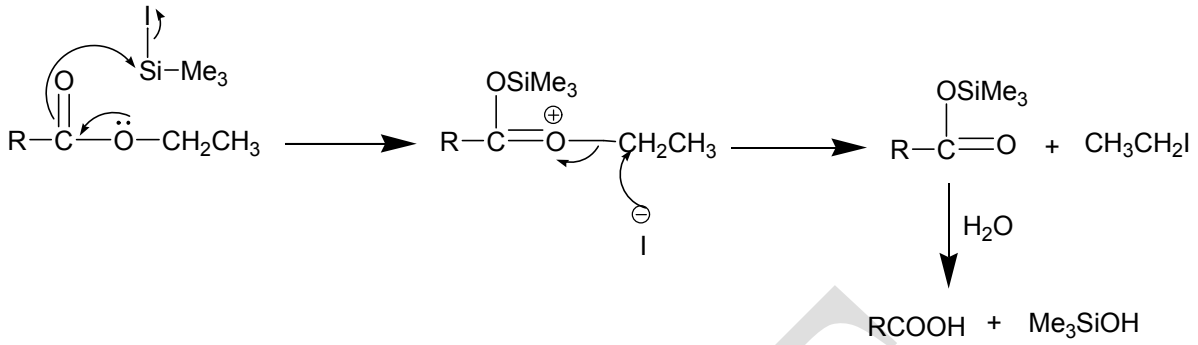
Mechanism

- The reaction presumably proceeds via an initially formed silyl oxonium ion:



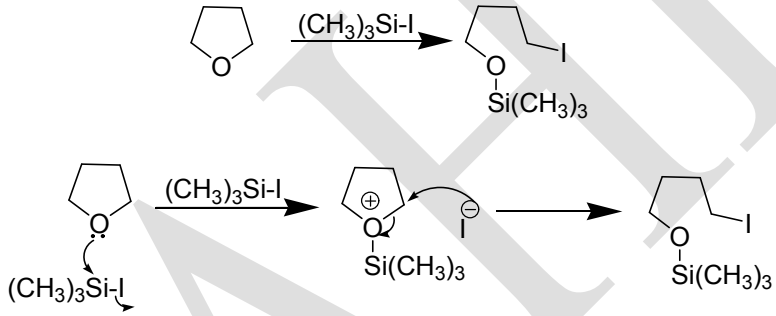




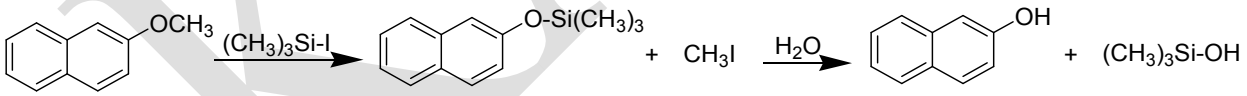


Cleavage of Ethers

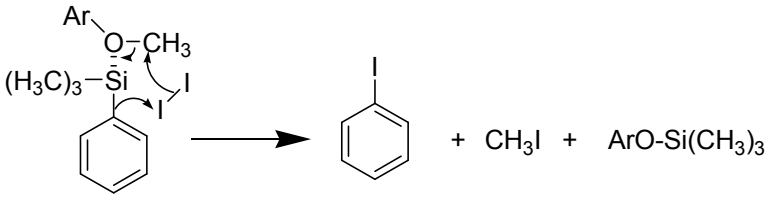
Voronkov found that tetrahydropyran reacts with TMS-I to yield 1-iodo-5-trimethylsilyloxypentane.



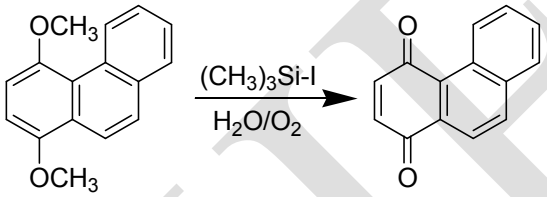
Both Olah and Voronkov reported that TMS-I would cleave aryl methyl ethers under neutral conditions to yield methyl iodide and aryloxytrimethylsilanes. The latter could be easily hydrolyzed to phenols.



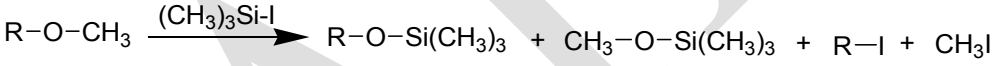
TMS-I, generated *in-situ* by reaction of trimethylphenylsilane with a 10% molar excess of iodine at 110°C, reacts with aryl methyl ethers to give high yields (90%) of cleavage products. In the proposed cyclic six-membered ring transition state the hard acid silicon interacts with the hard oxygen of the ether, while simultaneously, the soft iodine interacts with carbon. Free TMS-I may not be involved.



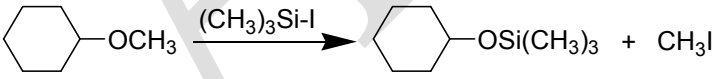
This reaction is general. For example, TMS-I cleaves sterically congested aryl methyl ethers, such as 1,4-dimethoxyphenanthrene, to yield 1,4-dihydroxyphenanthrene which is oxidized during work-up to l,4-phenanthraquinone.

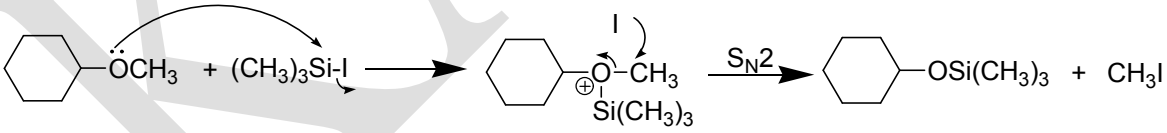


Jung found that TMS-I would cleave trityl, benzyl, and *t*-butyl ethers much faster than methyl, ethyl, isopropyl or cyclohexyl ethers. The cleavage of unsymmetrical dialkyl ethers is often not regioselective.

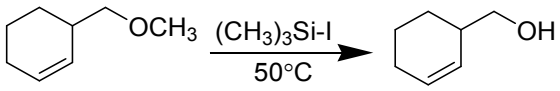


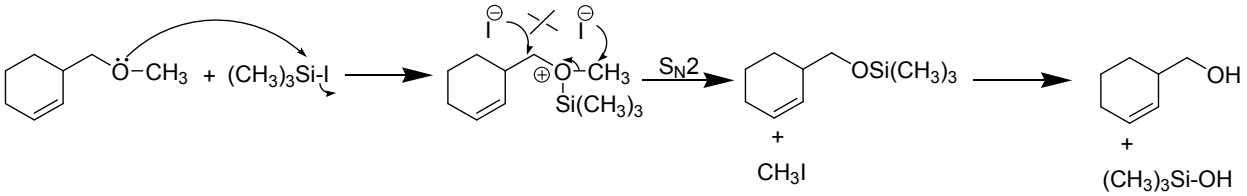
However, methyl cyclohexyl ether reacts with TMS-I to yield predominantly methyl iodide and cyclohexanoxytrimethylsilane.



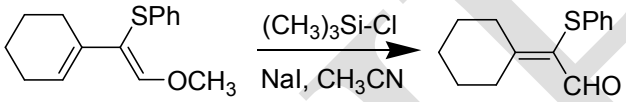


An alternative mechanism has been proposed to account for this specificity. Transfer of a trimethylsilyl group to the ether oxygen may form a dialkyltrimethylsilyloxonium/iodide ion pair. S_N2 nucleophilic attack by iodide on a methyl carbon would be favored over attack on a cyclohexyl carbon. C-C double and triple bonds, ketone carbonyls, and aryl halides are stable to the reaction conditions.



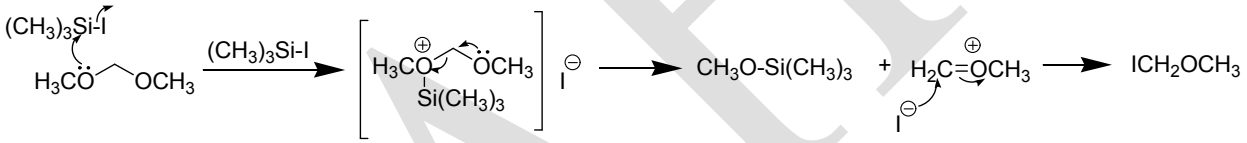


TMS-I generated *in-situ* by reaction of TMS-CI and sodium iodide in acetonitrile cleaves enol and dienol methyl ethers. Aqueous work-up provides aldehydes or ketones in quantitative yield. This is noteworthy since such dienol methyl ethers are susceptible to acid catalyzed polymerization.

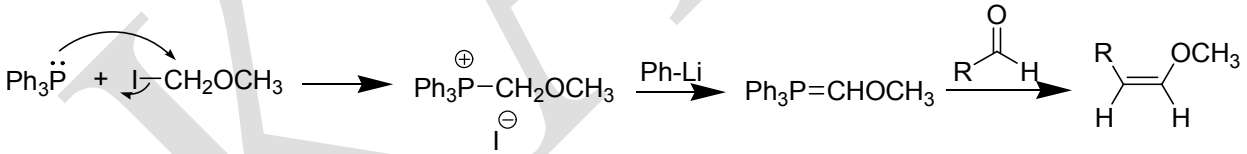


Cleavage of Acetals and Ketals

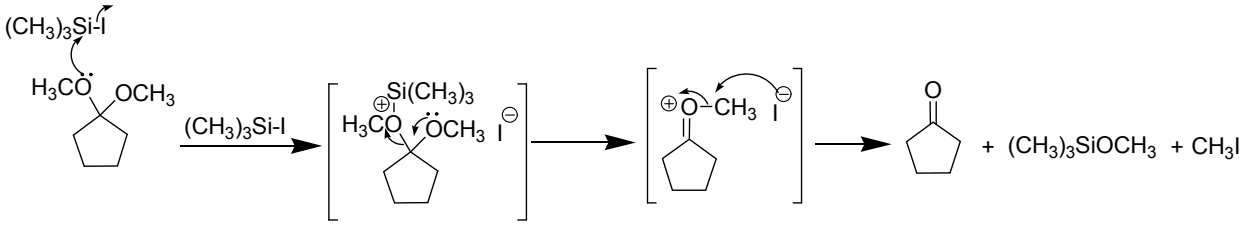
TMS-I reacts with dimethoxymethane to yield iodomethyl methyl ether.



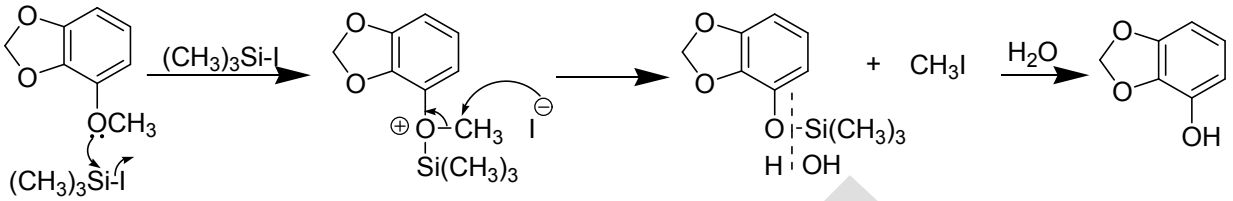
Iodomethyl methyl ether is not only a viable substitute for chloromethyl methyl ether, a restricted carcinogen, but also a valuable synthetic intermediate.



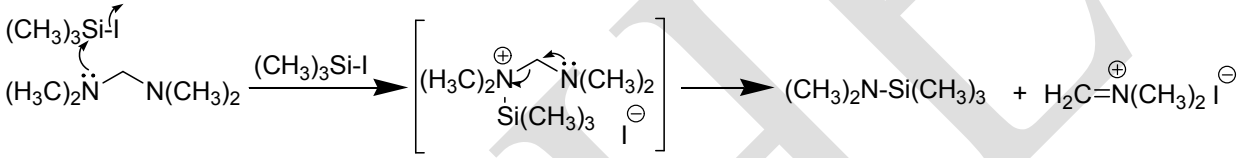
Ketones and aldehydes are frequently protected by conversion to dimethyl or diethyl acetals and ketals. Deprotection and regeneration of the ketone or aldehyde functionality is normally carried out by treatment with aq. acid. Jung has found that both dimethyl and diethyl acetals and ketals can be converted back to aldehydes and ketones under neutral conditions by treatment with TMS-I in chloroform or CCl_4.



The methyl ether of sesamol methyl ether is selectively cleaved by reaction with TMS-I in quinoline.

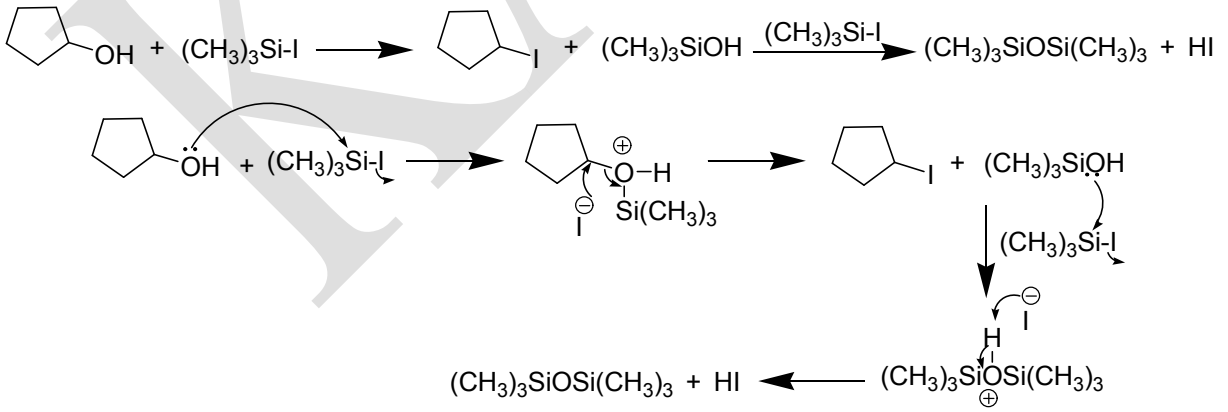


TMS-I reacts with bis(dimethylamino)methane to yield dimethyl(methylene)ammonium iodide, a valuable synthetic intermediate. Other bis(dialkylamino)methanes react in a similar manner.

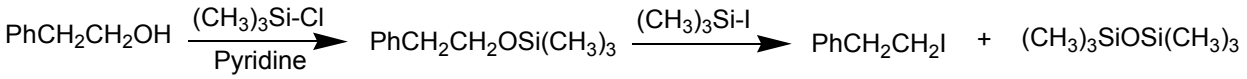


Conversion of Alcohols and Alkoxytrimethylsilanes to Alkyl Iodides

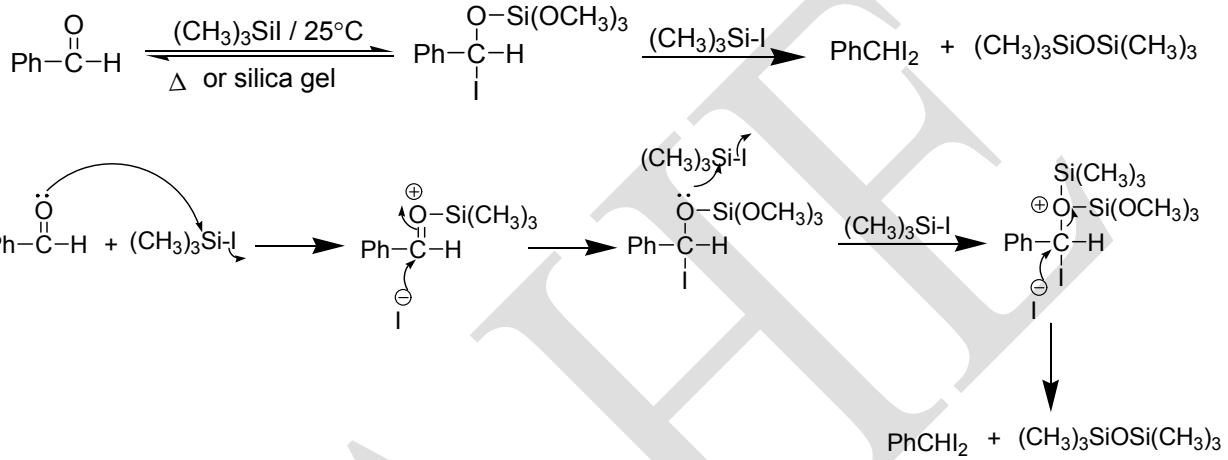
Red phosphorous and iodine or HI converts alcohols to alkyl iodides. TMS-I is also an excellent reagent for this purpose. TMS-I (2 equivalents) react with alcohols at 25°C in methylene chloride, chloroform, or CCl_4 to yield alkyl iodides, HI, and hexamethyldisiloxane. The presence of HI makes this reaction unsuitable for alcohols which possess acid-sensitive functional groups.



Alkoxytrimethylsilanes are cleaved regiospecifically by TMS-I under neutral conditions to yield alkyl iodides and hexamethyldisiloxane. This reaction is general for primary, secondary, and tertiary alkoxytrimethylsilanes.

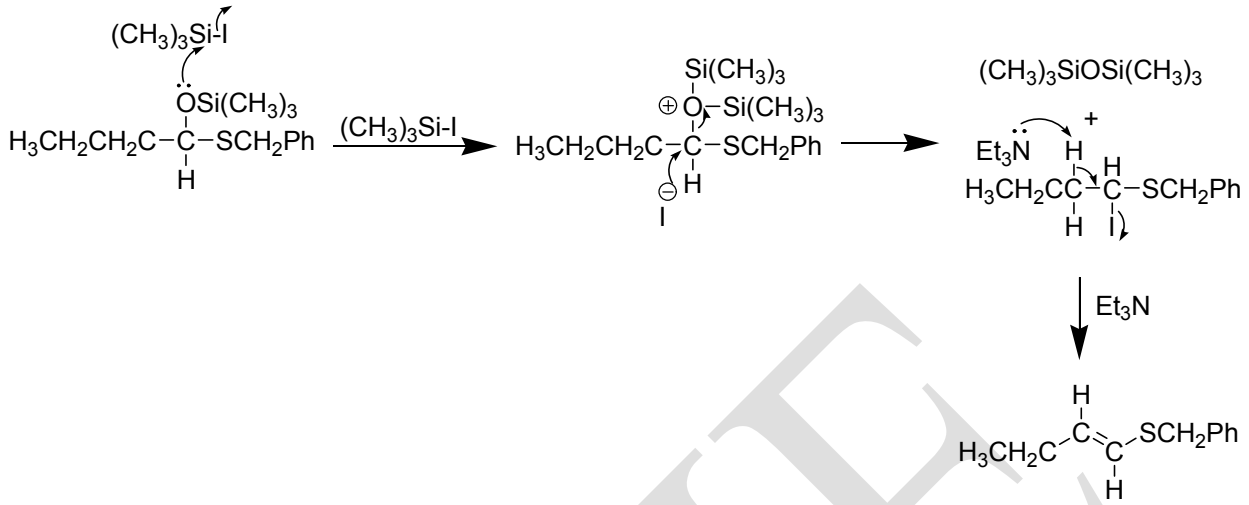


TMS-I and aldehydes react in a 1: 1 ratio to yield iodohydrin trimethylsilyl ethers. Attempts to purify these compounds by distillation or chromatography led to regeneration of the starting aldehyde.



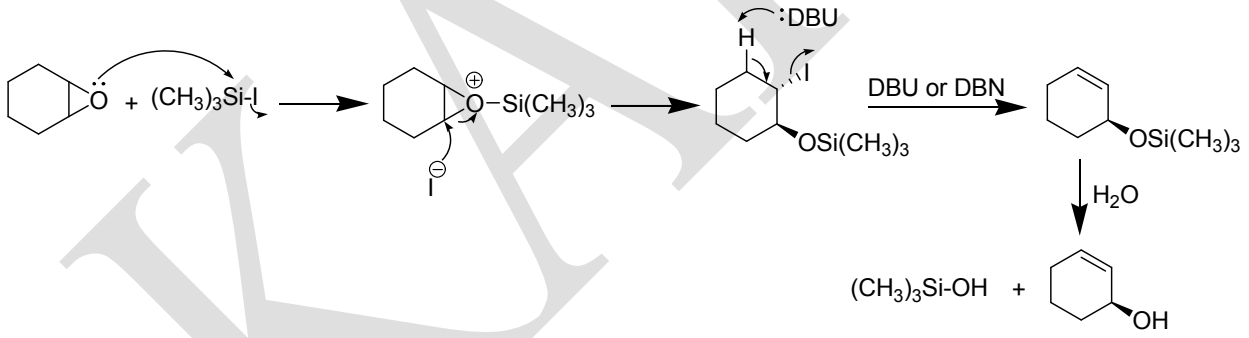
If a 2: 1 ratio is used, the initial aldehyde iodohydrin trimethylsilyl ether is converted to a 1,1-diiodide and hexamethyldisiloxane. This reaction is related to the cleavage of alkoxytrimethylsilanes by TMS-I to yield alkyl iodides. Phenylacetaldehyde is an exception. It undergoes slow reaction with excess TMS-I at O°C to give 2,3,6,7-dibenzo-9-oxabicyclo[3,3,l]nona-2,6-diene.

O-Trimethylsilyl hemithioacetals and ketals react with TMS-I to form α-iodosulfides and hexamethyldisiloxane. α-Iodosulfides were previously virtually unknown. They undergo facile dehydrohalogenation on treatment with triethylamine or with sodium hydroxide under PTC conditions to yield vinyl sulfides.



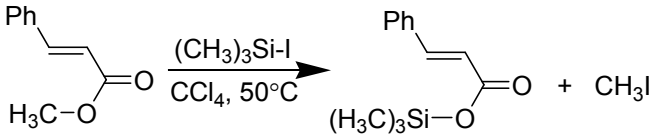
Reaction of Oxiranes with TMS·I - Conversion to Allylic Alcohols

Epoxides react with TMS-I, which was generated by reaction of hexamethyldisilane with iodine, to give 2-iodoalkoxytrimethylsilanes. These can be converted to allylic alcohols by treatment with tertiary amine bases, such as DBU or DBN, followed by hydrolysis.

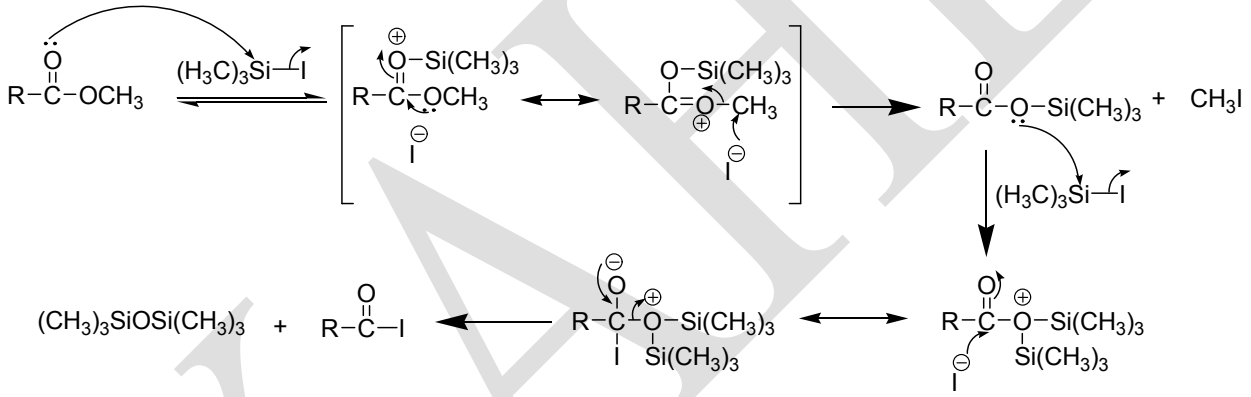


Hydrolysis of Esters

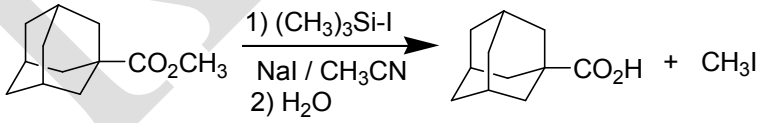
The hydrolysis of alkyl esters has been accomplished in a number of ways. TMS-I rapidly cleaves methyl, ethyl, i-propyl, t-butyl, and benzyl esters in CCl_4 at 50°C to yield the corresponding trimethylsilyl esters and alkyl iodides. The wide generality of ester hydrolysis by TMS-I makes this method a major synthetic advance. Phenyl esters do not react. The reaction tolerates a large number of functional groups such as C-C double bonds.



The following reaction mechanism has been proposed. TMS-I reacts in a rapid reversible reaction with the carbonyl oxygen of the ester to yield trimethylsilyloxy alkoxy-stabilized carbocation/iodide ion pair. Rate limiting nucleophilic attack by iodide on the a-carbon of the alkoxy group yields alkyl iodide and the trimethylsilyl ester. In the presence of excess TMS-I the trimethylsilyl ester is converted to an acyl iodide (IR C=0 1,830-1,800 cm^{-l}) and hexamethyldisiloxane. The fact that TMS-I is usually contaminated by traces of HI acid may account for the hydrolysis of *t*-butyl esters, since nucleophilic attack on a *t*-butyl group is improbable.

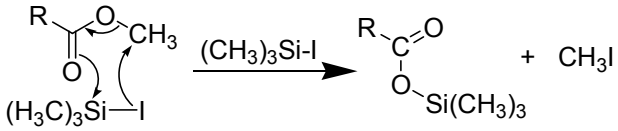


Similar results were obtained for ester hydrolysis with TMS-I generated *in-situ* by the reaction of trimethylphenylsilane or hexamethyldisilane, with iodine or by reaction of TMS-Cl and sodium iodide in acetonitrile.

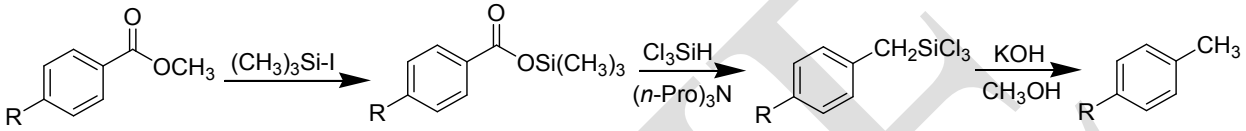


TMS-Br is ineffective in this reaction.

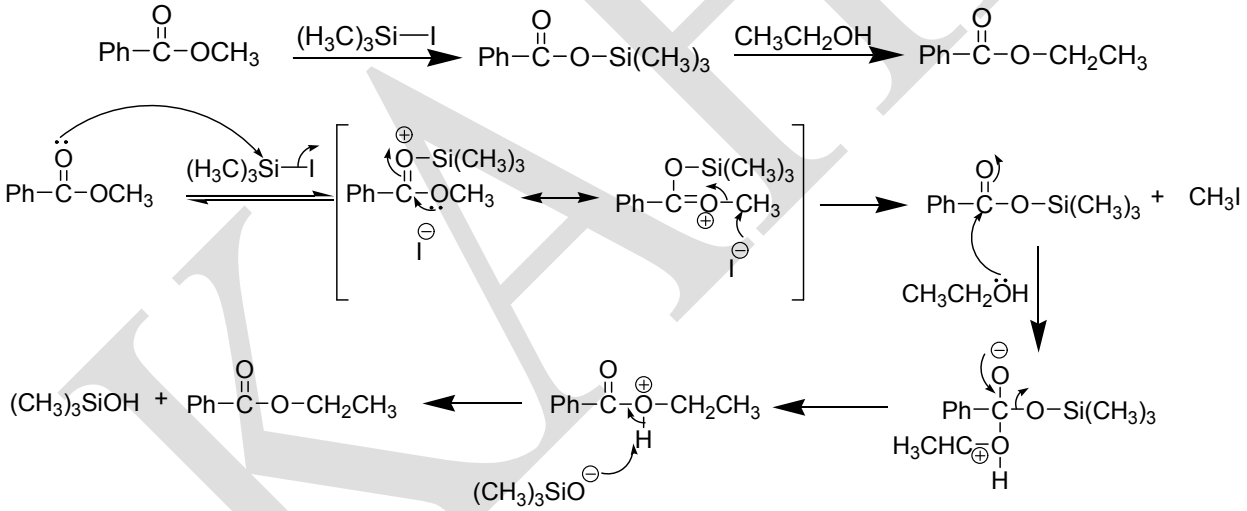
An alternative molecular mechanism which involves a six-membered cyclic transition state has been proposed. The silyl center of TMS-I serves as a hard acid which may coordinate to the carbonyl oxygen of the ester while the soft iodide attacks the a-carbon of the alkoxy group.



Trimethylsilyl esters of aromatic carboxylic acids can be reduced by trichlorosilane and tertiary aliphatic amines to benzyltrichlorosilanes which then can be cleaved by potassium hydroxide in methanol to yield the corresponding methyl aromatics. Alkyl esters of aromatic carboxylic acids, are not reduced under these conditions.

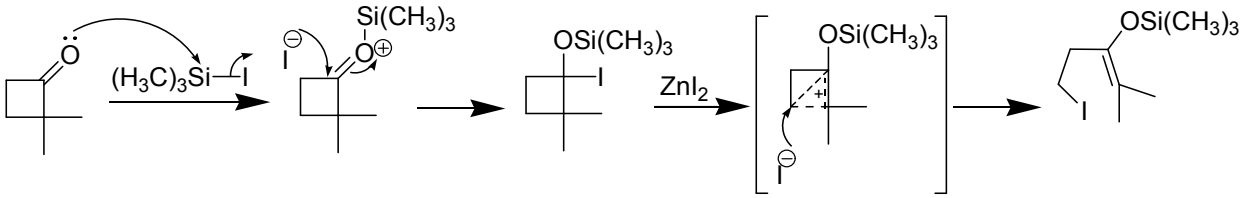


Trimethylsilyl esters react with two equivalents of primary or secondary alcohols to yield the corresponding alkyl esters. This permits transesterification under neutral conditions.

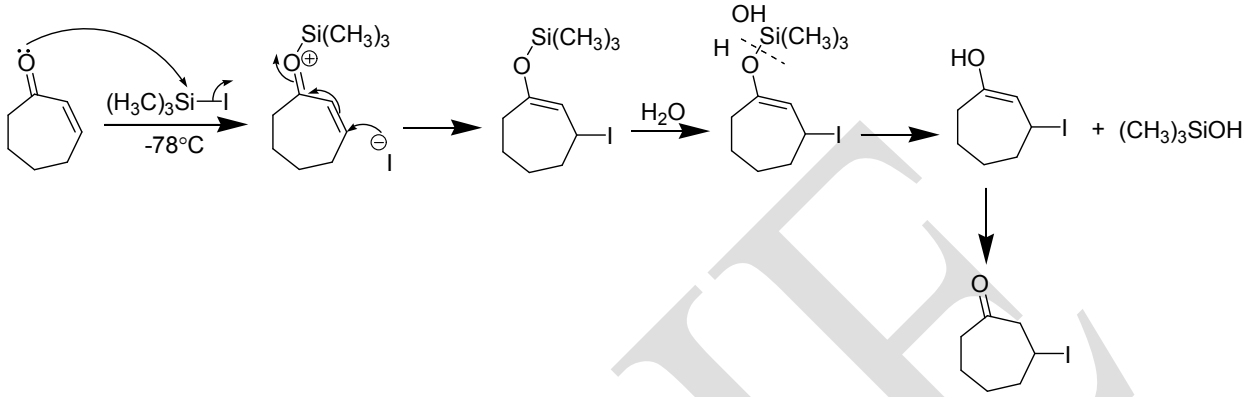


Preparation of β- and γ-Iodo Ketones

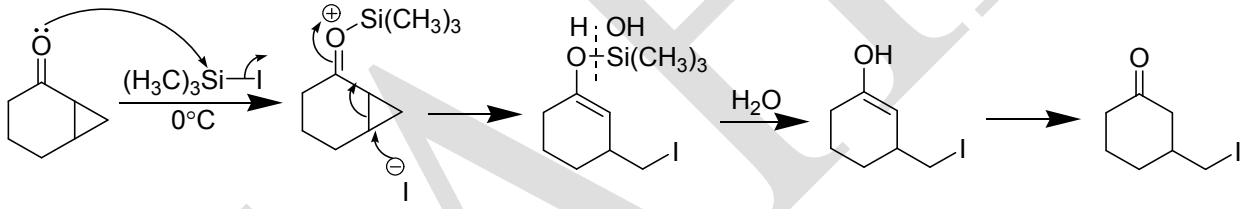
Zinc iodide catalyzed reaction of TMS-I with cyclobutanones yields after hydrolysis ring opened, β-iodoketones. This may occur as outlined below.



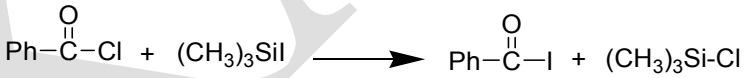
β-Iodoketones undergo facile reaction with various nuc1eophiles, such as cyanide and phenylthiolate ions. β-Iodoketones can also be prepared by Michael addition of TMS-I to α, β-unsaturated ketones.



TMS-I reacts with α, β -cyclopropylketones to yield γ-iodoketones.



TMS-I and TMS-Br undergo halogen exchange with acyl chlorides to yield respectively, acyl iodides and acyl bromides.

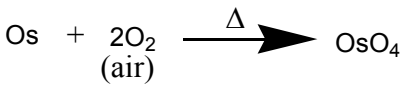


OSMIUM TETROXIDE (OsO_4)

- This reagent is used for **cis-hydroxylation** of carbon-carbon double bond.

Preparation

It is prepared by the oxidation of osmium metal by strong heating in the presence of air. It is also prepared by heating osmium compounds in air.

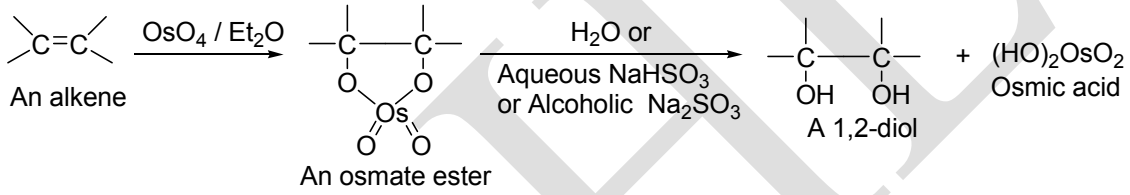


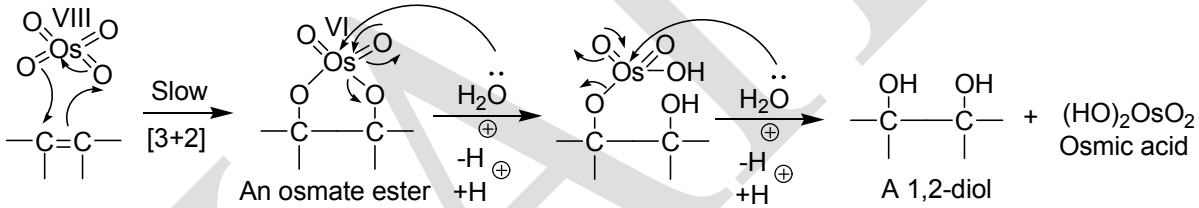
- Reaction with osmium tetroxide is probably the best method for cis-perhydroxylation of alkenes, but the stoichiometric reaction is suitable for **small-scale work with valuable compounds, because of the expense and toxicity of the reagent.**

- However the same result can be obtained more economically by the use of hydrogen peroxide with osmium tetroxide present in catalytic amounts.

Mechanism

The reaction proceeds via a cyclic osmium(VI) complex which on reductive or oxidative hydrolysis yields the corresponding cis-diol.

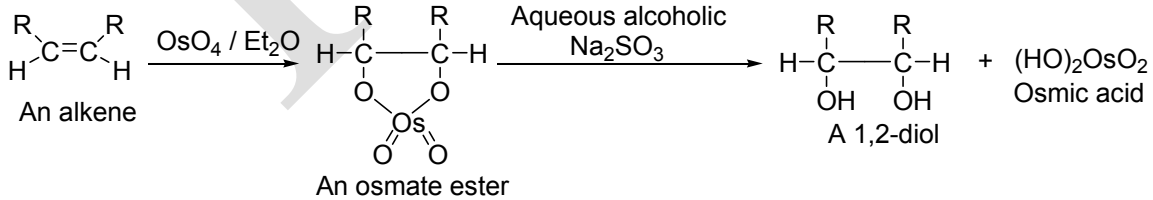


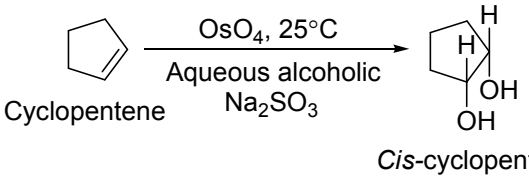


Uses

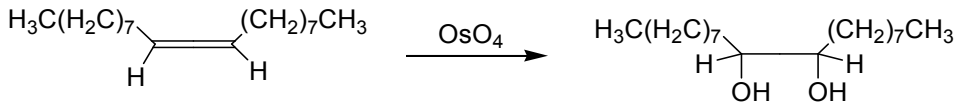
1. Preparation of *cis*-1,2-diols

Osmium tetroxide undergoes *cis*-cycloaddition with olefin from the less hindered side to yield *cis*-1,2-diol. For example,





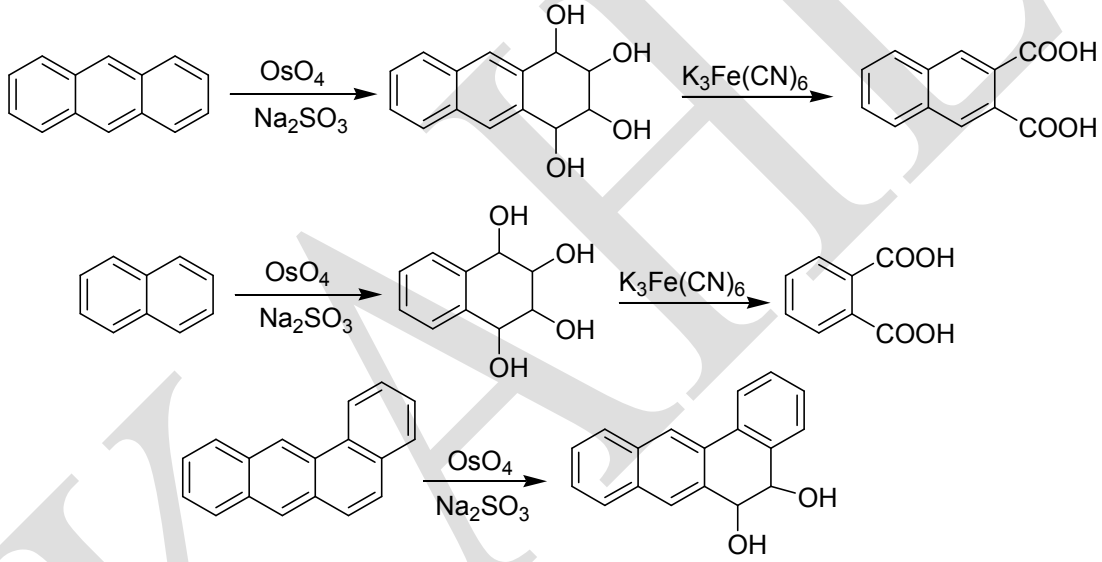
Cis-cyclopentan-1,2-diol



2. Ascertaining structure of polynuclear hydrocarbons

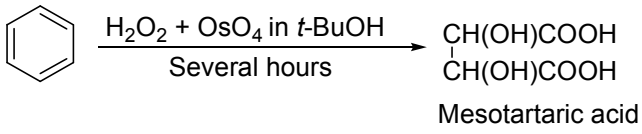
Osmium tetroxide first oxidizes anthracene to tetrol which on further oxidation with potassium ferricyanide yields naphthalene-2,3-dicarboxylic acid. The presence of two carboxylic groups at positions 2and 3 reveal ortho fusion of the third ring in anthracene. This also confirms linear fusion of the three rings.

A similar treatment of OsO_4 with naphthalene confirms ortho fusion of the two rings in naphthalene.

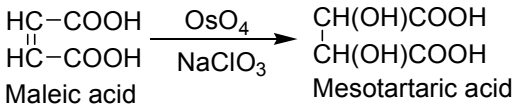


3. As a catalyst

Due to its expensive and toxic nature, osmium tetroxide in small catalytic quantities is used with other oxidizing agents (e.g., H_2O_2) which are able to reoxidise osmium its lower valence states to OsO_4 to continue the further oxidation of the substrate.



A mixture of H_2O_2 and OsO_4 is able to hydroxylate conjugated double bonds.

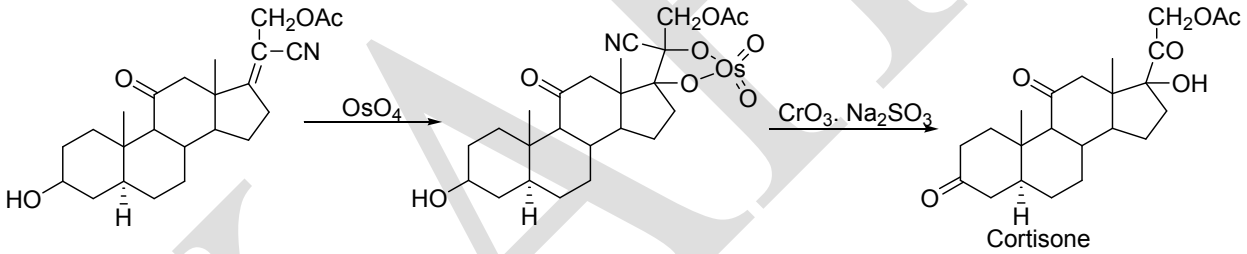


4. Ascertaining structure of unsaturated hydrocarbon

Osmium tetroxide brings about hydroxylation of carbon-carbon double bonds to yield products which are cleaved with specific reagents (e.g., lead tetraacetate or peracids). This method is a good procedure for the degradation of unsaturated compounds. The degraded products can be identified. This gives the presumptive structure of the unsaturated compound.

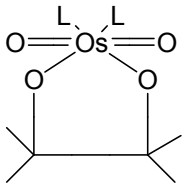
Osmium tetroxide is also used in the synthesis of cortisone.

In synthesis of cortisone by introducing >C=O and –OH group at C-20 and C-17 positions to compounds **I**.



Acceleration by bases

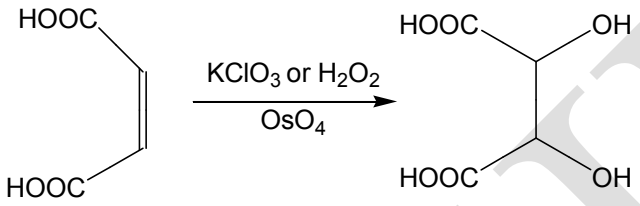
- The reaction is accelerated by tertiary bases usually pyridine and pyridine usually added to the reaction medium.
- Brightly coloured complexes (I) in which osmium is coordinated with two base molecules, separate in almost quantitative yield.
- Using optically active bases lead to generation of optically active diols.

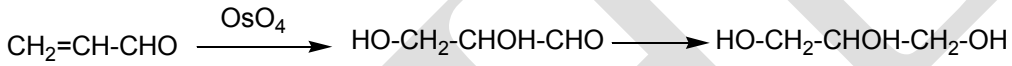


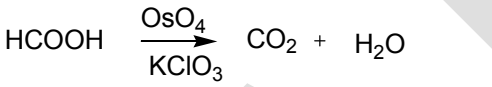
Oxidation with catalytic quantities of OsO_4 in conjunction with other oxidants

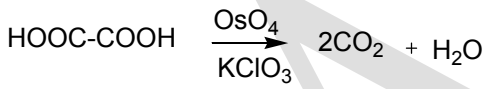
- Osmium tetroxide is frequently used catalytically in conjunction with other oxidants. Formerly, chlorates and hydrogen peroxide were used but the results are better with *t*-butylhydroperoxide and tertiary amine oxides.

Examples





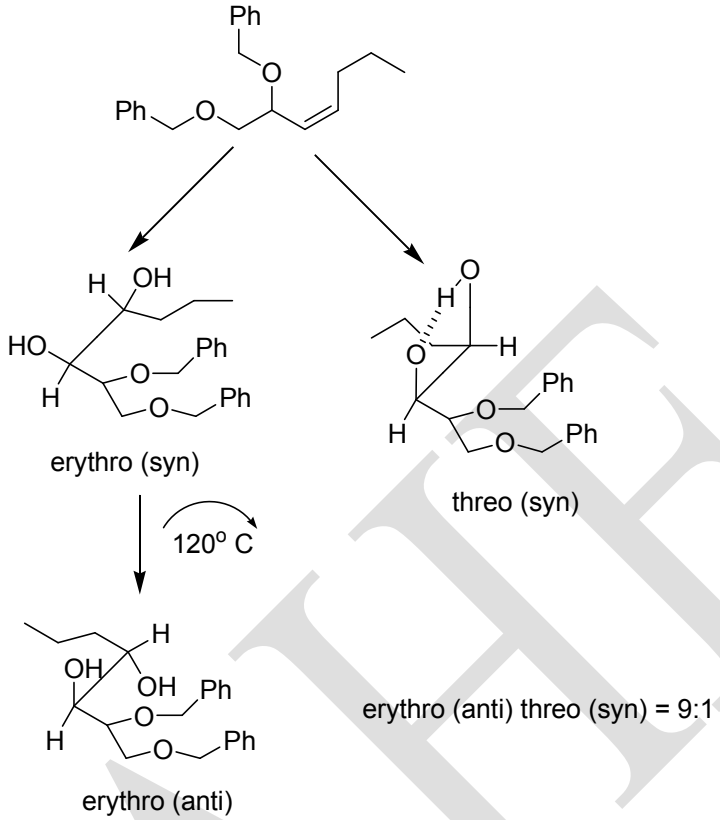




Mechanism

- The reaction proceeds via the oxidative hydrolysis of the initially formed osmate ester resulting in the regeneration of the osmium tetroxide, which continues the reaction so that small amount suffices.
- Osmium tetroxide oxidations in presence of chlorates and hydrogen peroxide suffer from over oxidation of the formed products while t-BuOOH avoids this difficulty.
- t-BuOOH is also effective for the dihydroxylation of di- and tri- substituted alkenes while the chlorates and hydrogen peroxides are fail.

Stereoselectivity in the oxidation of alkenes



Oxidative cleavage of olefins

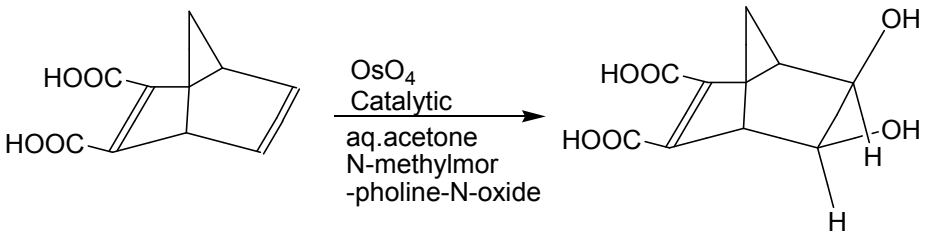
- The reactions of alkenes with periodate and catalytic amounts of osmium tertroxide lead to the formation of two carbonyl compounds (aldehyde and/or ketone).

Advantage

- This method is advantageous of the oxidation with permanganate-periodate method since the aldehydes can be isolated without further oxidation as in case of permanganate.

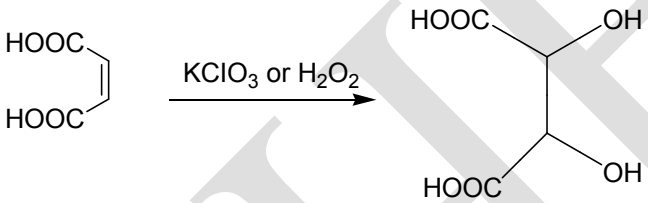
Examples

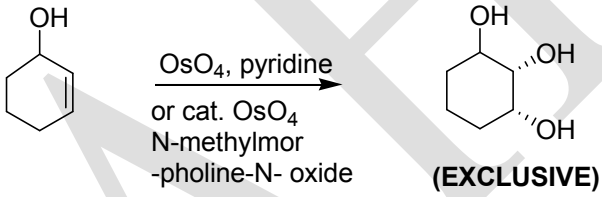
- Because of the large steric requirements of this reagent, reactions with osmium tetroxide usually take place predominantly from the less hindered side of the double bond when there is a choice, as evident from the following example.



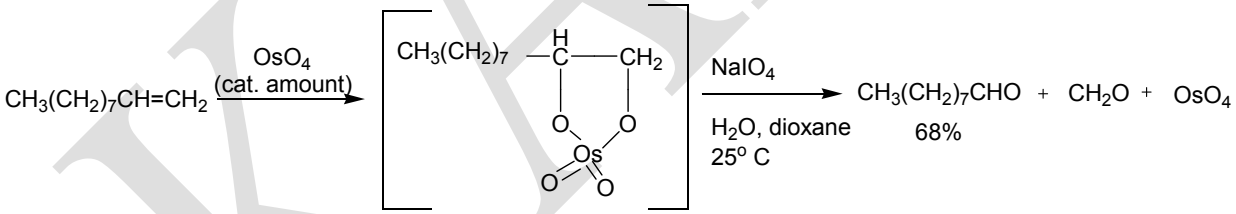
Oxidation of allylic alcohols

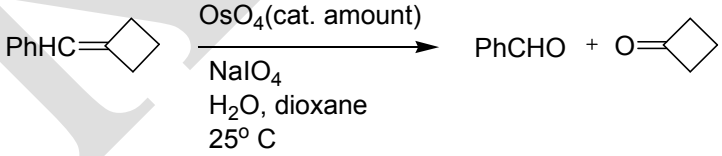
The reaction of allylic alcohols with tetroxide provides 1,2,3-triols in a stereoselectivity manner.

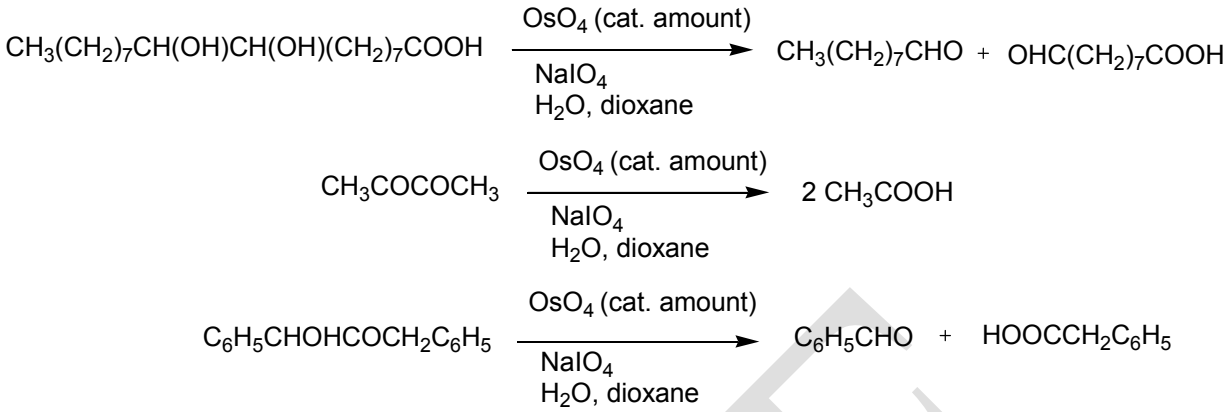




(EXCLUSIVE)







SELENIUM DIOXIDE (SeO_2)

Selenium dioxide is a white crystalline solid which melts at 340°C, may be sublimed at atmospheric pressure.

Preparation

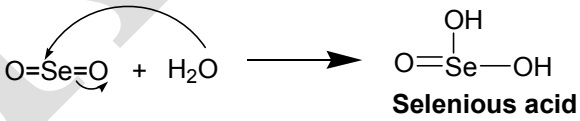
Uses

Used for the oxidation of a variety of organic compounds.

Solvent used: dioxane, acetic acid, acetic anhydride, water and ethanol.

Active species:

In aqueous or alcoholic solutions, selenium dioxide is converted into selenious acid, $(HO)_2SeO$ or to the corresponding dialkylselenite ester.

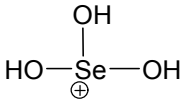


Selenious acid

Methods of addition:

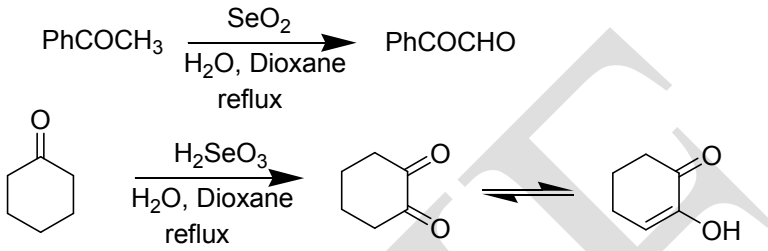
- The reagent may be added to the reaction mixture as selenious acid.
- During oxidation process, the selenium(VI) reactant is reduced to metallic selenium, a red to black insoluble solid.

Catalysis by acid: Protonation of the selenious acid leads to enhanced reactivity.

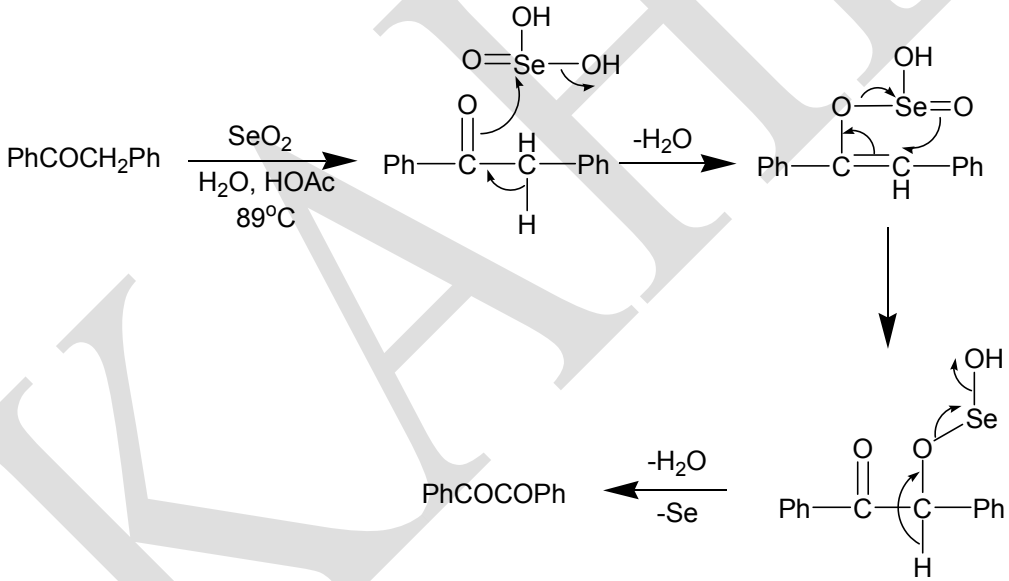


Reactions:

1. Conversion of ketones or aldehydes to 1,2-dicarbonyl compounds

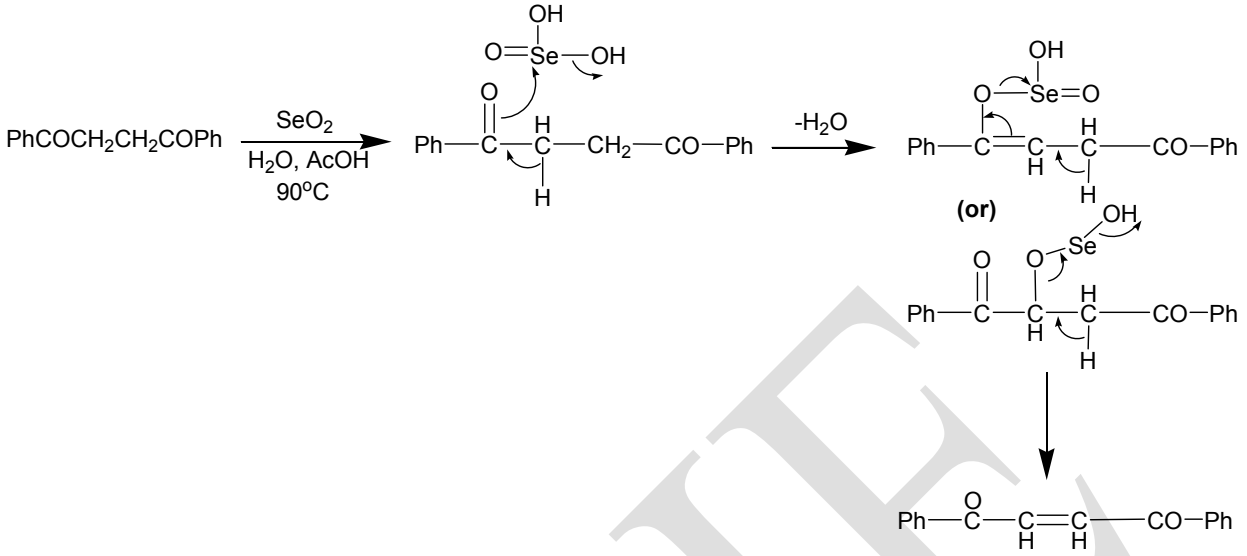


Catalysis by acid:



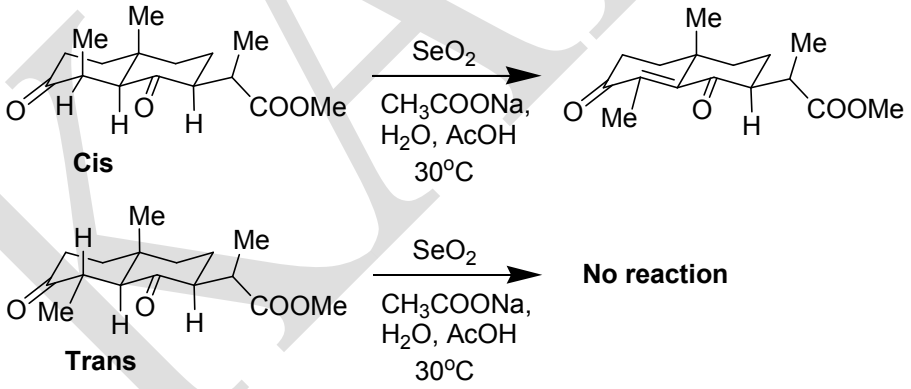
2. Conversion of 1,4-diketones to 2,3-unsaturated-1,4-diketones

A similar intermediate may be involved in the dehydrogenation of 1,4-diketones.

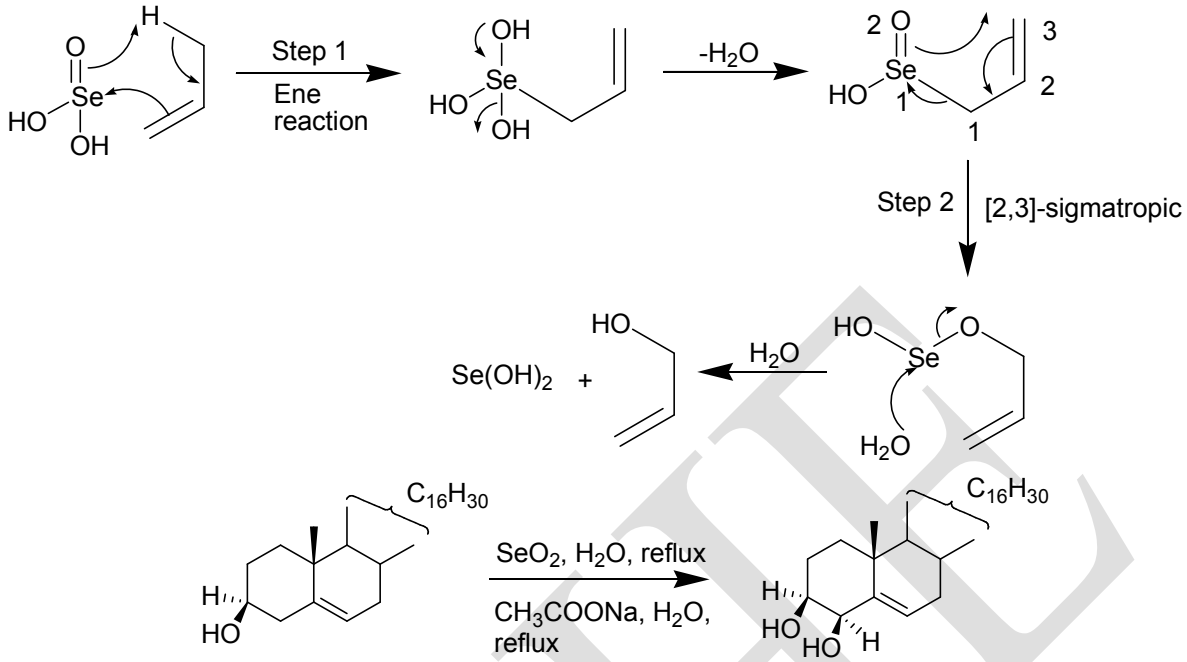


3. Removal of *cis*-hydrogens preferred

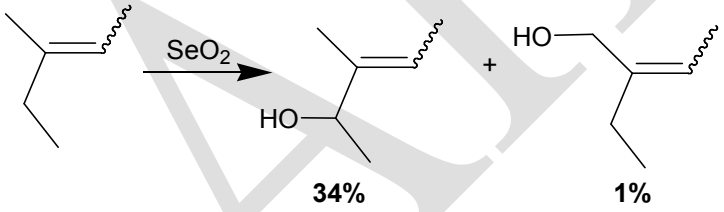
It has been noted with certain polycyclic diketones that this dehydrogenation process is more rapid when the two hydrogen atoms being removed bear a *cis*-relationship to one another than when the hydrogens atoms are *trans* to each other.



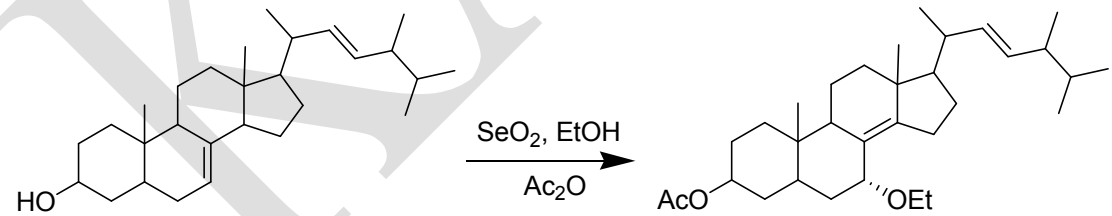
4. Oxidation of olefins to allylic alcohol

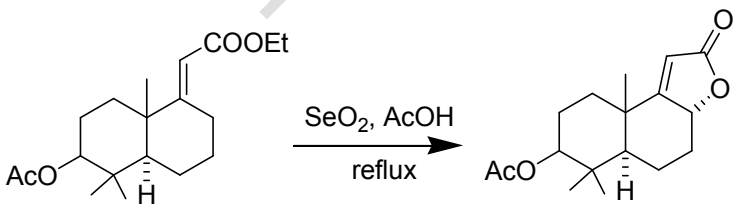


Hydroxylation occurs at the more substituted end of the double bond.



Allylic migration also is found in certain cases.

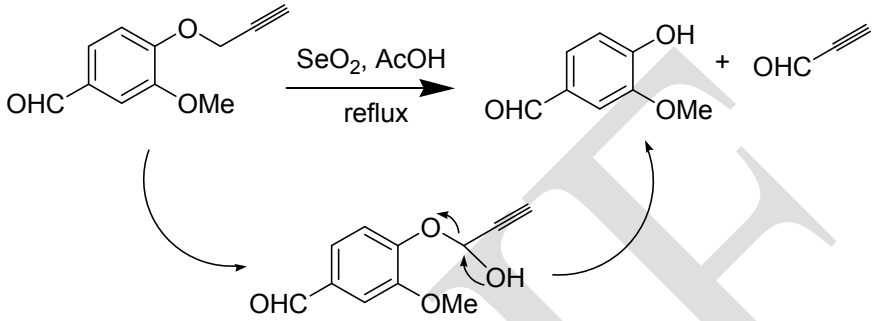




Lactone formation

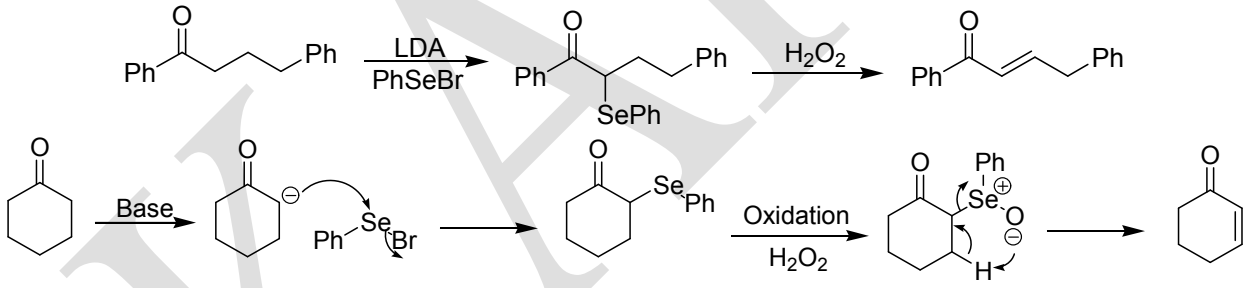
5. Oxidative cleavage

Selenium dioxide attacks activated positions resulting in oxidative cleavage when appropriate leaving groups are present. Aryl propargyl ethers undergo oxidation at the α-alkynyl position to afford a phenolic species and propargyl aldehyde.

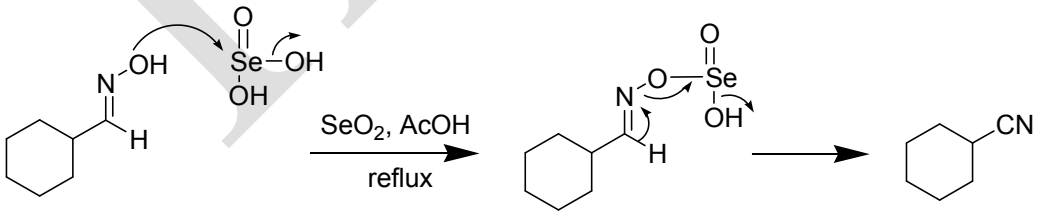


6. Preparation of α,β-unsaturated carbonyl compounds

The reaction involves α selenylation of carbonyl compounds followed by oxidation of selenium with peroxides or peracids and subsequent elimination provides the desired α,β-unsaturated carbonyl compounds



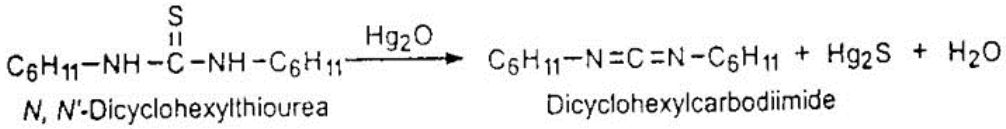
Other transformations:



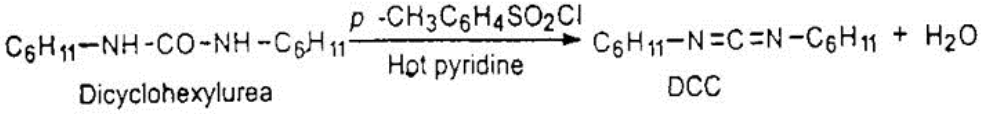
DICYCLOHEXYLCARBODIIMIDE (DCC, C_6H_{11}-N=C=N-C_6H_{11})

Dicyclohexylcarbodiimide may be prepared by any of the methods given below:

(i) By the oxidation of *N,N*-dicycloheylthiourea with mercuric oxide.



(ii) By the dehydration of dicyclohexylurea with *p*-toluenesulphonyl chloride in hot pyridine

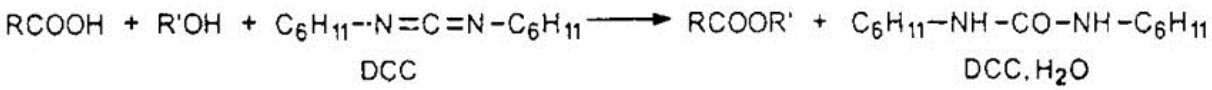


The reagent will be represented by the abbreviation, DCC.

Uses:

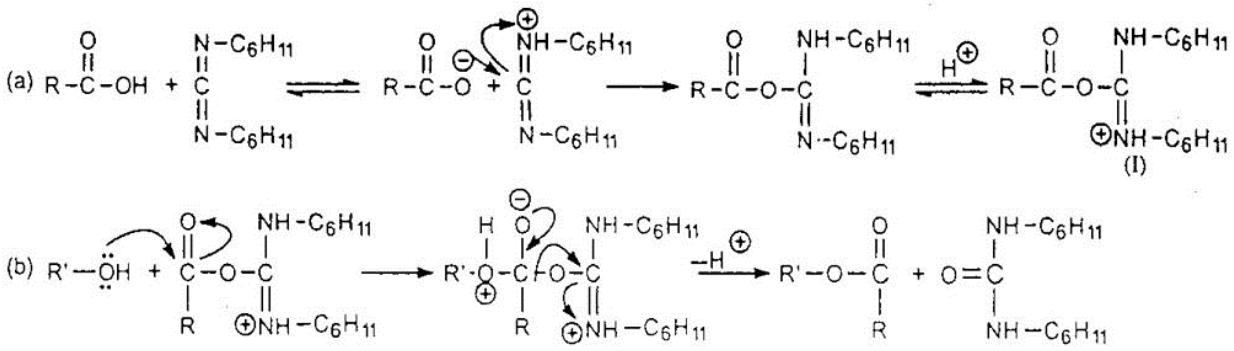
Dicyclohexylcarbodiimide finds application as a dehydrating agent usually under mild conditions. Thus, the reagent has been employed as dehydrating agent in the synthesis of various compounds such as, esters, ethers, anhydrides, amides, lactones, lactams, peptides etc.

1. **Esters:** Esterification of acid with primary or secondary alcohols is promoted by the reagent.



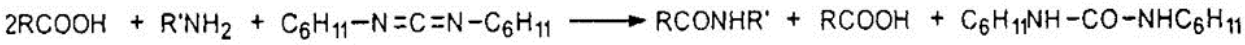
Mechanism:

The reaction is catalyzed by acid. The acid reacts with reagent to form the compound (I) with a good leaving group. Subsequent reaction of the compound (I) with alcohol gives the ester with the expulsion of dicyclohexylurea. The sequential steps of the reaction are given.



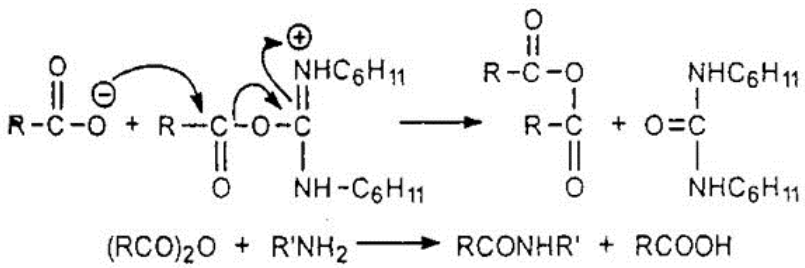
The driving force of the reaction is the formation of a very stable compound, dicyclohexylurea.

2. **Amides**: Amides are not directly obtainable by the treatment of acids with amines. In the presence of this dehydrating reagent however, amides in good yield are obtained at about the room temperature.



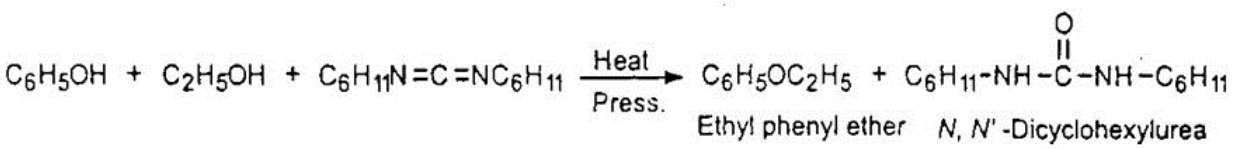
Mechanism- It is suggested that the reagent first converts the acid to its anhydride which with amine gives the amide.

The acid first reacts with the reagent, as in the case of esterification to form (I) which reacts with another molecule of the acid to form the anhydride. The anhydride then reacts with amine to form amide.

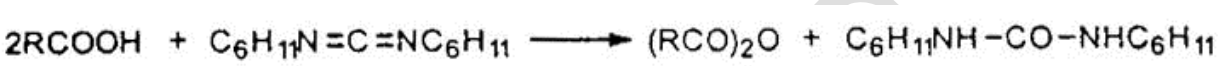


The reaction has been employed to prepare lactams (cyclic amides).

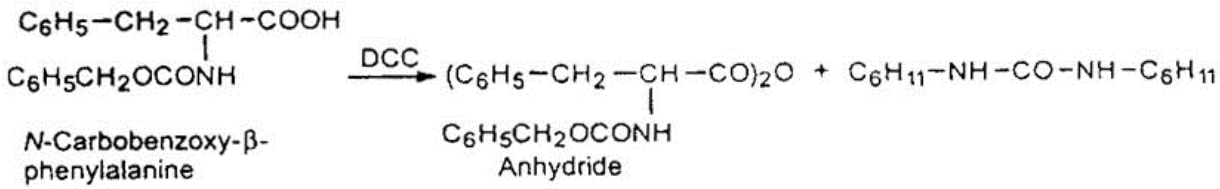
3. **Ethers:** A mixture of phenol, alcohol and the reagent on prolonged heating under pressure gives aryl alkyl ether.



4. **Acid anhydrides:** The reagent gives an excellent yield of acid anhydride from carboxylic acids.

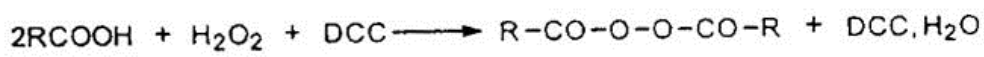


The reaction has been employed to produce symmetrical anhydrides from *N*-substituted amino acids.

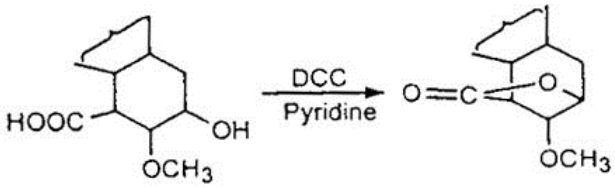


The amino group of the amino acid is substituted by carbobenzoxy group to prevent the amino group from reacting with the carboxyl group. The carbobenzoxy group may be easily removed by hydrogenolysis after the reaction.

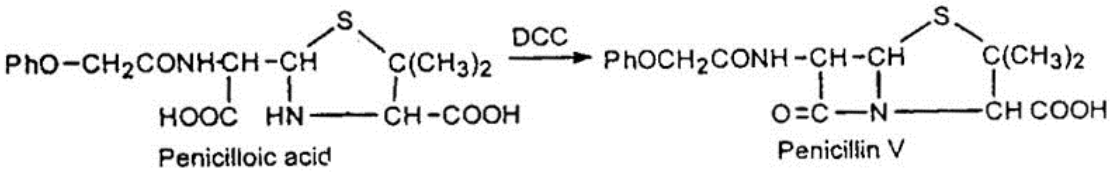
5. **Diacyl peroxide:** Diacyl peroxide can be prepared by the treatment of carboxylic acids with hydrogen peroxide under mild condition in the presence of this reagent.



6. **Lactones:** Dicyclohexylcarbodiimide in pyridine has been found to be a better reagent than acetic anhydride in pyridine for the lactonisation of γ-hydroxy acids. The method has been used during the synthesis of reserpine.

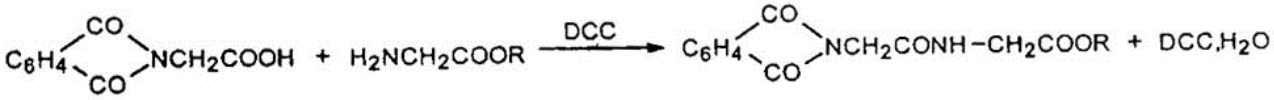


7. **β-Lactams:** Lactams are cyclic amides. β-Lactamas are highly strained rings, sensitive to acids while most reagents for amide linkage involve strongly acid reagents. This caused serious problems in the synthesis of penicillin. However, the difficulty was overcome by using dicyclohexylcarbodiimide for the amide ring.



Diisopropylcarbodiimide has been found to be a better reagent for amide ring formation as it is a very mild reagent.

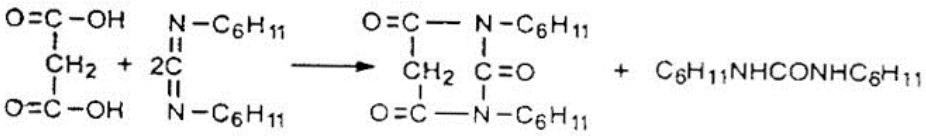
8. **Peptides:** Amino acids in which the amino function has been protected by phthalyl or carbobenzoxy ($C_6H_5CH_2O$-CO-) group condense with amino acid esters in the presence of *N*, *N*'-dicyclohexylcarbodiimide at room temperature to give peptides.



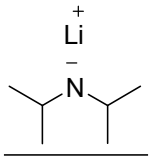
After hydrolysis of the product, the protecting group is removed by treating with hydrazine.

Phthalyl and carbobenzoxy groups are used as protecting groups since they can be easily substituted and easily removed. Carbobenzoxy group may be removed by hydrogenolysis (H_2+ Pt).

9. **Barbituric acid**: Barbituric acid derivatives may be prepared by the treatment of malonic acid with the reagent.



LITHIUM DIISOPROPYAMINE (LDA)



- LDA is one of the strongest bases used to remove less acidic proton.

Preparation

- It can be prepared from the reaction of n-butyl lithium on diisopropyl amine under low temperature.

$$i\text{-}Pr_2NH + C_4H_9Li \longrightarrow (i\text{-}Pr)_2N\text{-}Li$$

Uses

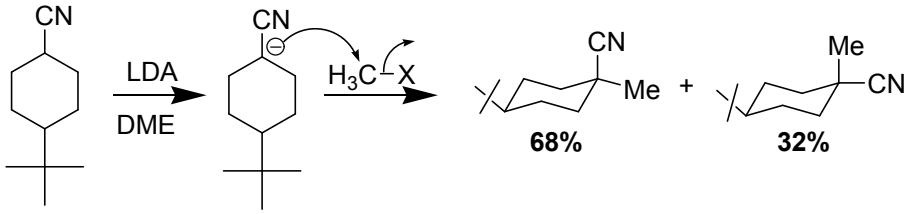
Normally it is used to generation of carbanions.

- Thus alkylation of simple aliphatic nitriles, esters and carboxylate anions can be achieved in the presence of LDA.

- Because LDA is sterically hindered, it reacts with R_1X very slowly favouring alkylation reaction of substrate.
- Further due to steric hindrance, LDA abstracts protons from less hindered position favouring the formation of kinetically controlled abstraction of protons.
- Thus considerable regioslectivity is achieved in the generation of enolates.

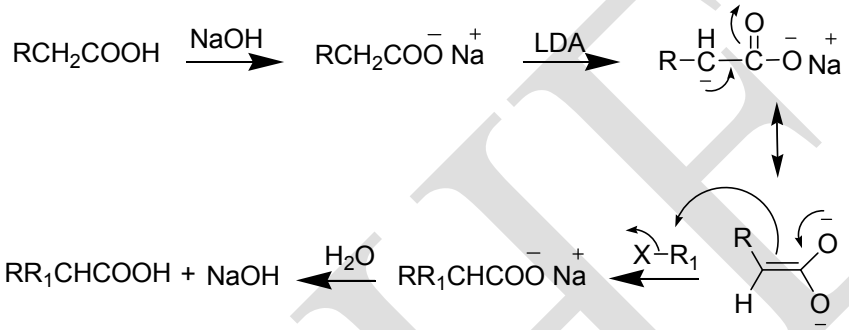
Alkylation of active methylene compound

- LDA in 1,2-dimethoxyethane is usually used for alkylation of active methylene compounds.



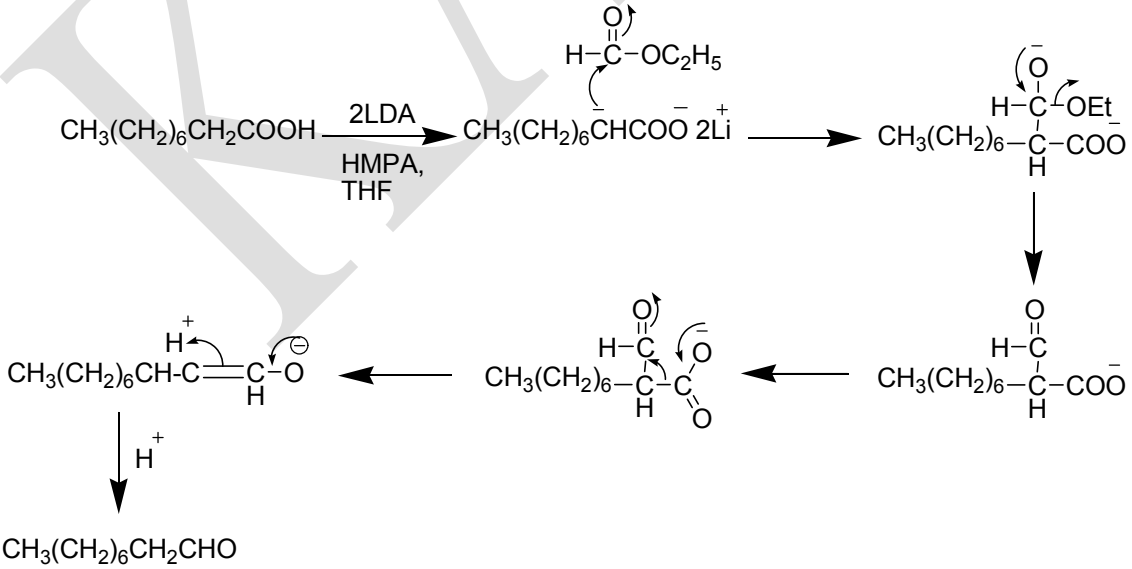
Alkylation of carboxylic acid

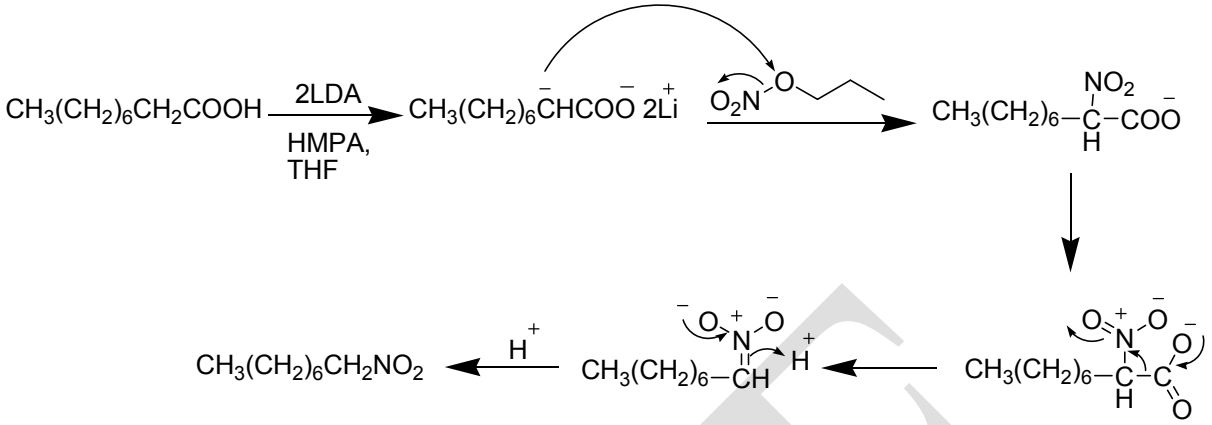
- The strong base LDA is able to abstract the proton of carboxylate anions which is used to generate α-alkylated carboxylic acids.
- The use of Li as counter ion is important because it increases the solubility of the dianionic salt.



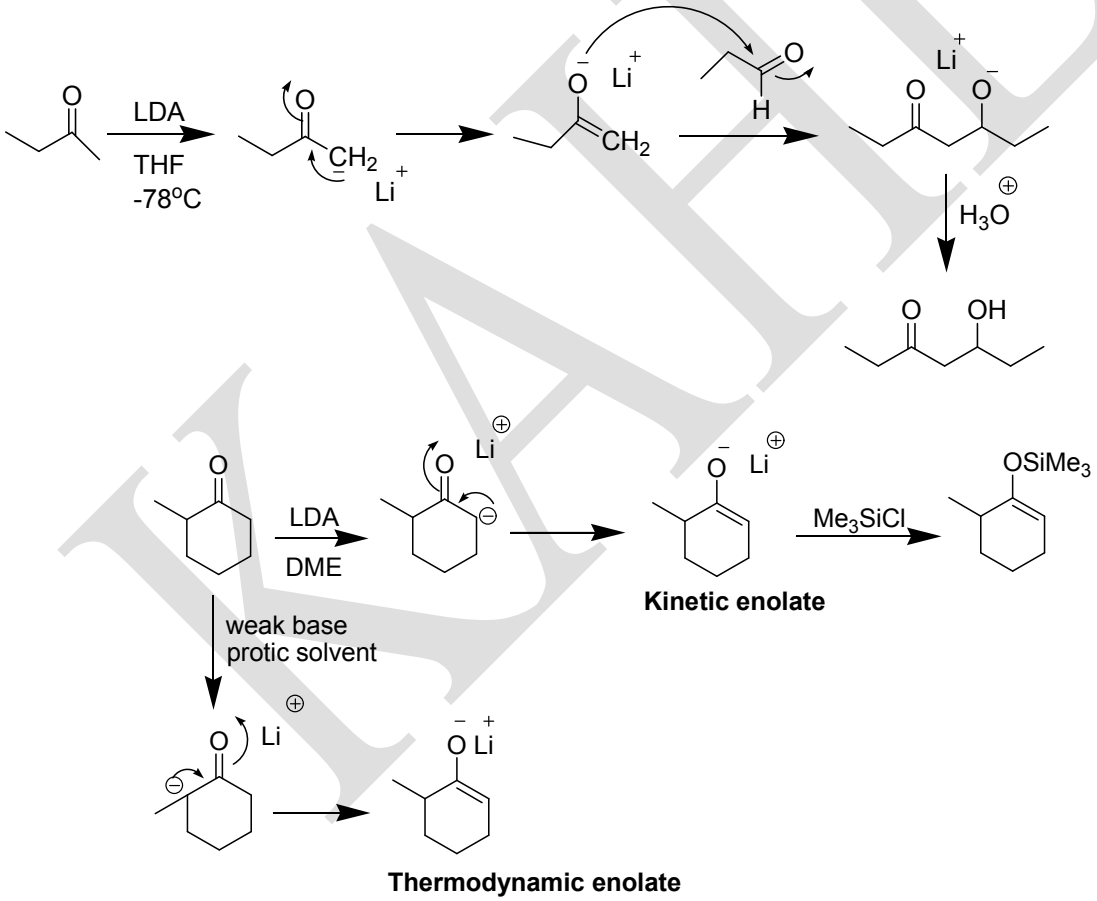
Synthesis of aldehydes and nitroparaffins

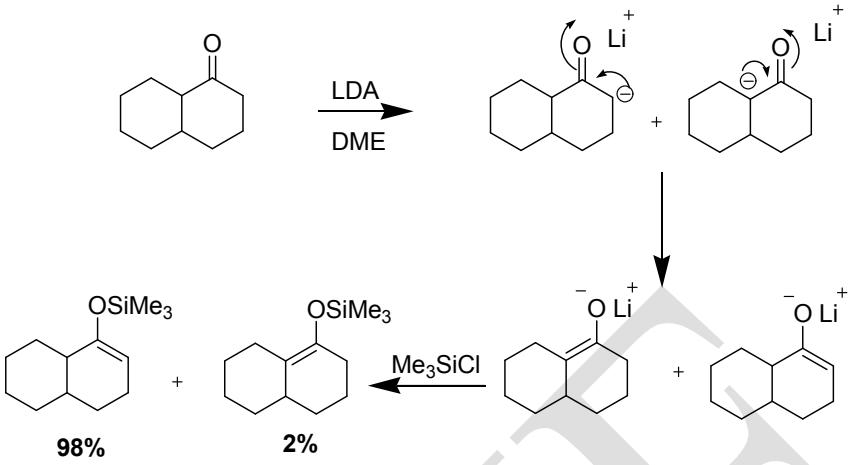
- The dianions of straight chain carboxylic acids can be readily produced by reaction with LDA in the presence of HMPA/HMPT as co-solvent.
- Reaction of dianions with ethyl formate by neutralization with 10% HCl furnishes an aldehyde.



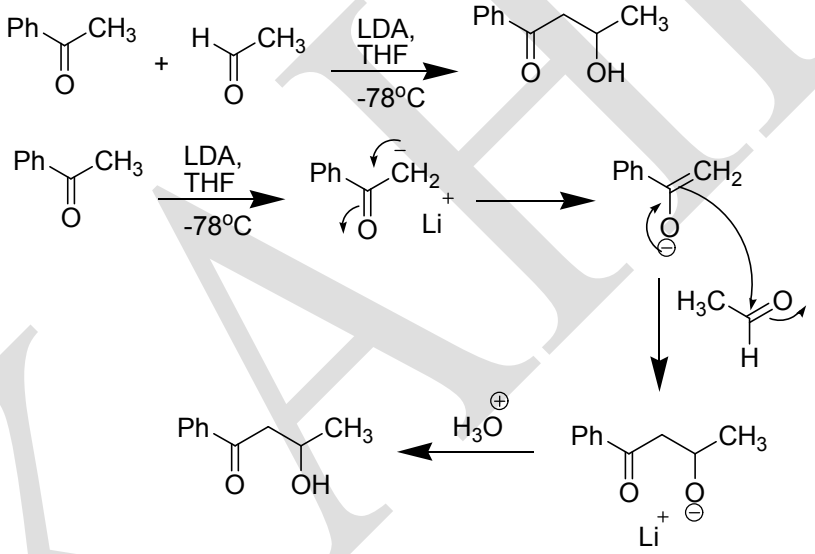


Regioselectivity in case of unsymmetrical ketones

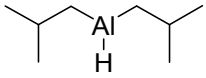




Direct aldol condensation



DI-ISOBUTYLALUMINIUM HYDRIDE (DIBAL-H)



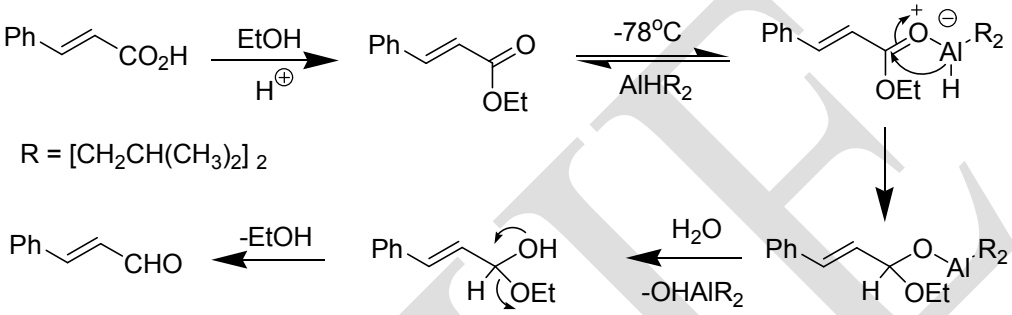
- It is a versatile reducing agent.
- At ordinary temperature esters and ketones are reduced to alcohols, nitriles gives amine and epoxides are cleaved to alcohols.

Uses

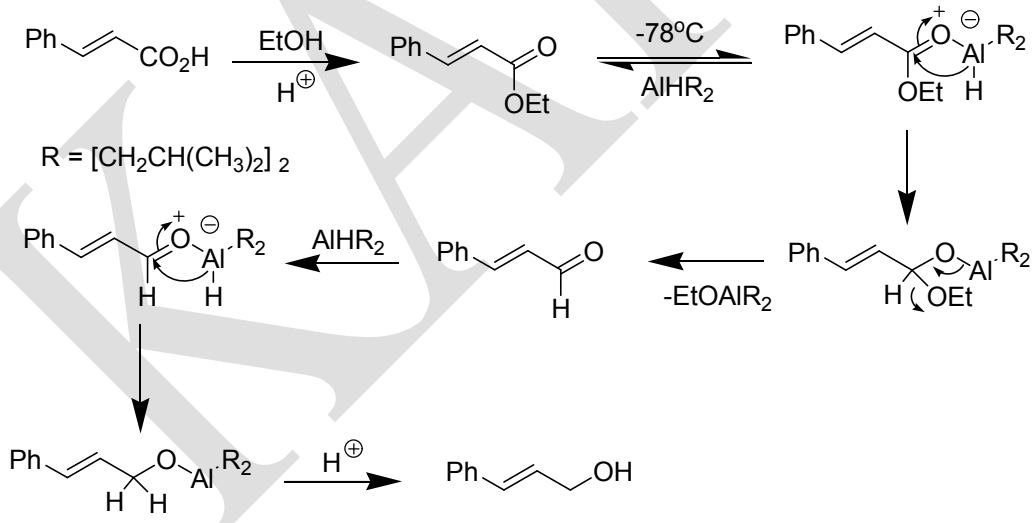
- The greatest use probably in the preparation of aldehydes.

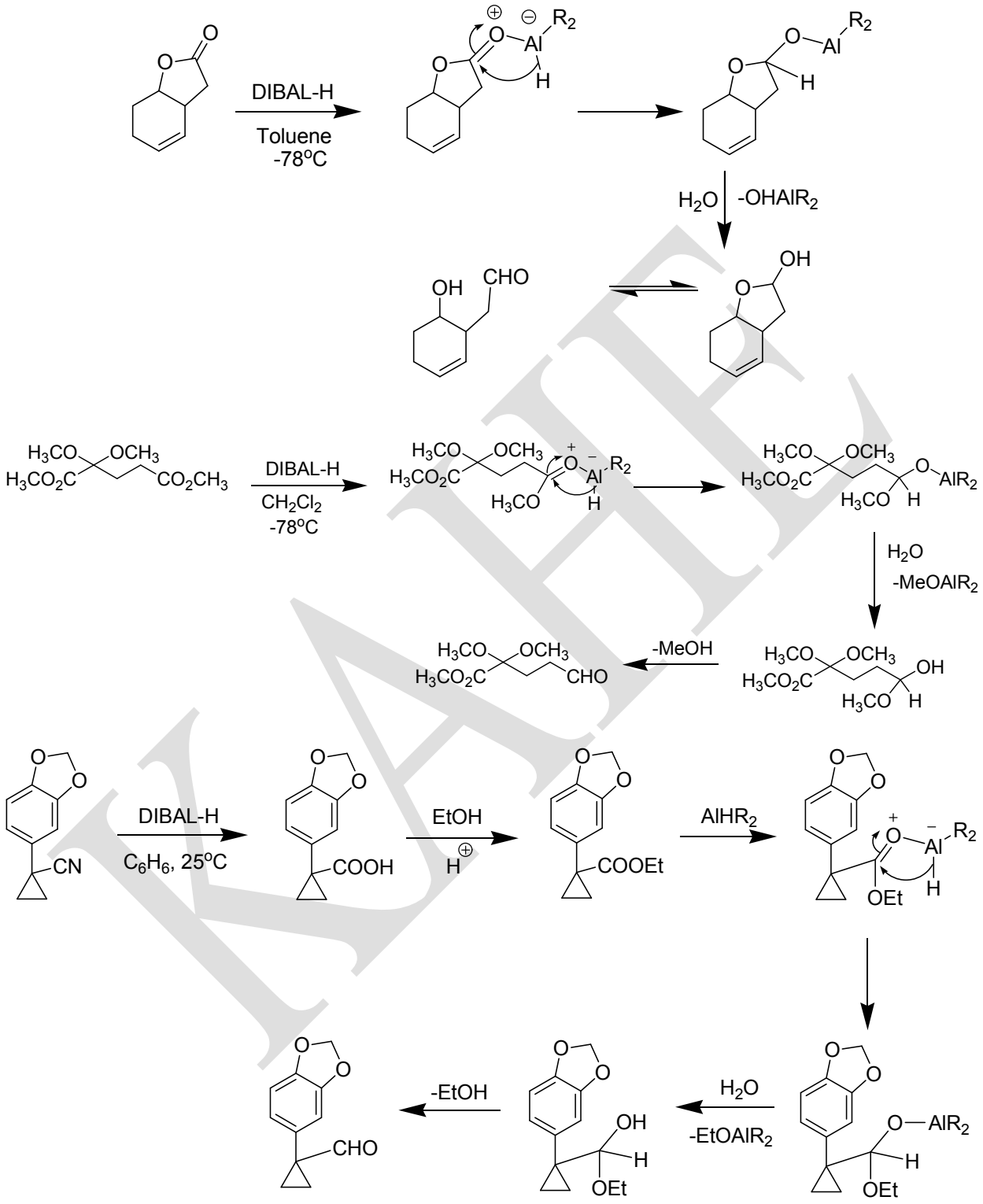
- It is also a useful reagent for the reduction of disubstitued alkynes to *cis* alkenes and for selective 1,2-reduction of α,β-unsaturated carbonyl compounds to allylic alcohols.

- At room temperatures DIBAL-H reduces ester to the primary alcohol. However -78°C the intermediate alkoxide is stable enough to accumulate and can be trapped by adding water, which also destroys the residual reducing agent:

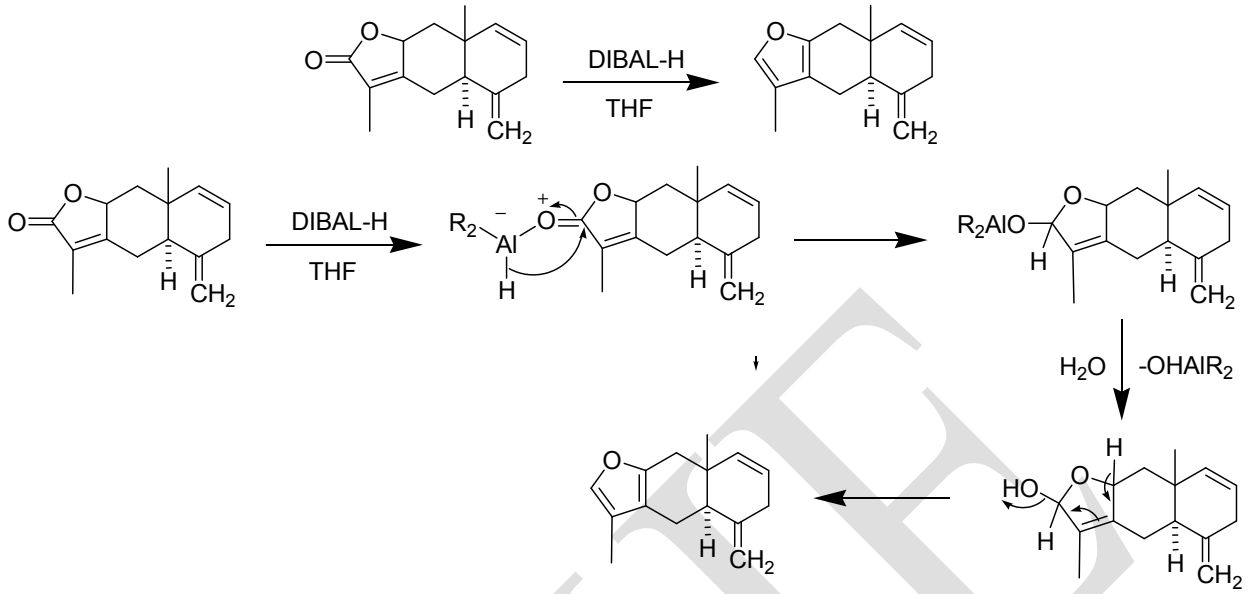


- The cinnamic acid is converted to the ester which is reduced at room temperatures with DIBAL-H to the primary alcohol.





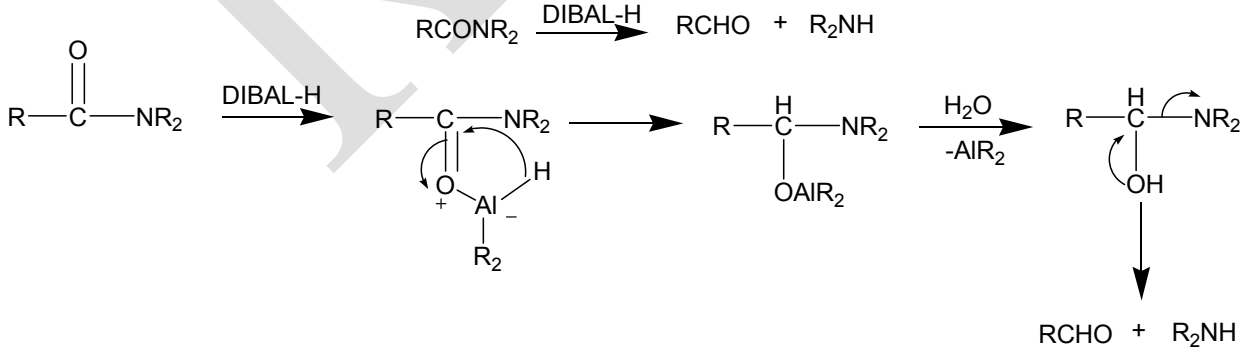
- Reduction of unsaturated γ-lactone to furan derivative has been effected. This reaction proceeds via reduction to lactol and elimination of water.



Diastereoselctive reduction

- In the reduction of carbonyl carbon adjacent to chiral centre bulky DIBAL-H preferentially chooses the less hindered face of the carbonyl group.
- DIBAL-H reduces only the carbonyl group of α,β-unsaturated ketone and aldehyde.

- DIBAL-H reduced *N*,*N*-disubstitued amide into aldehyde in good yield, $LiAlH_4$ also brings about the reduction but sometimes further oxidation to primary alcohol also occurs.



MERCURIC ACETATE, [$Hg(OAc)_2$]

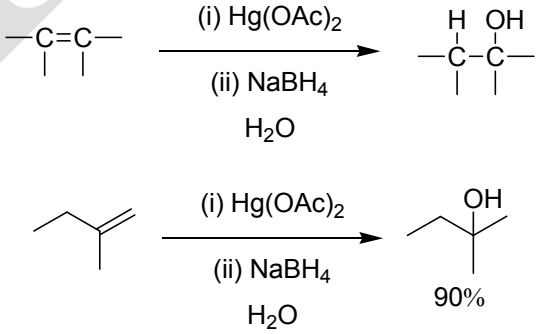
Oxymercuration-demercuration

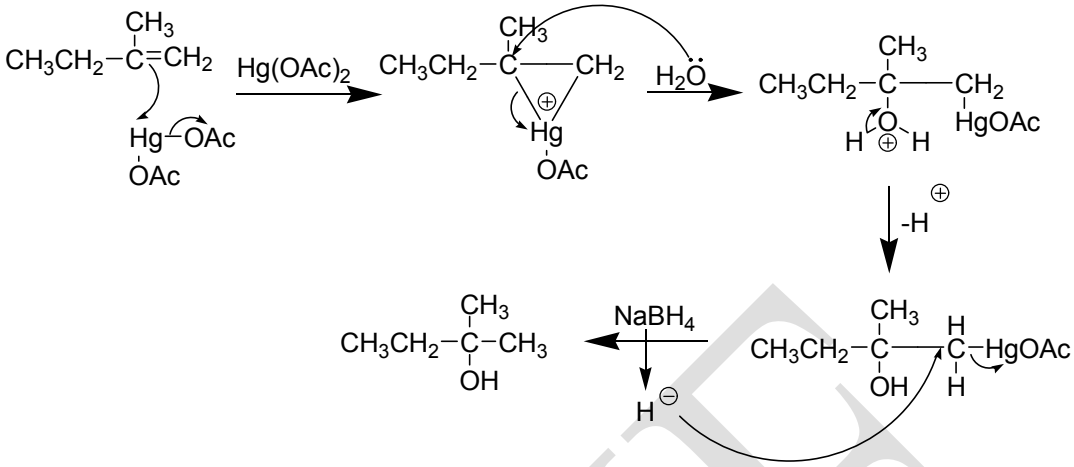
- Alkenes react with mercuric acetate in the presence of water to give hydroxymercurial compounds, which on yield alcohols.

- The first stage oxymercuration involves addition of OH and HgOAc to C=C bond. Then in demercuration, the HgOAc is replaced by H. This reaction sequence amounts to hydration of the alkene, but is much more widely applicable than direct hydration.

- The two-stage oxymercuration-demercuration is fast and convenient, takes place under mild conditions, and gives excellent yields often over 90%.

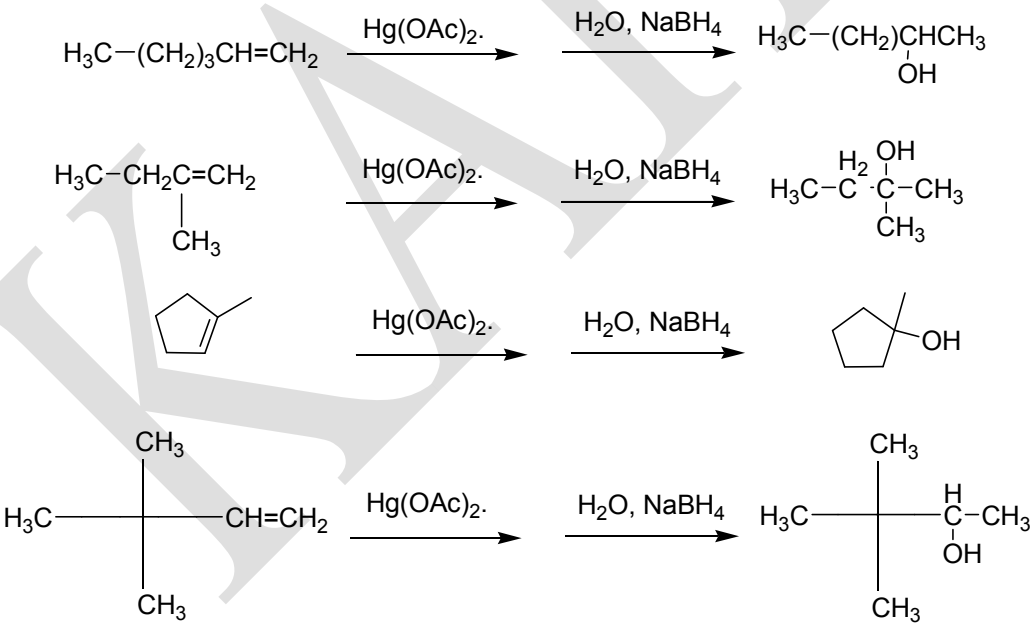
- The alkene is added at room temperature to an aqueous solution of mercuric acetate diluted with the solvent THF.
- Reaction is generally complete within minutes.
- The organomercurial compound is not isolated but is simply reduced in situ $NaBH_4$. (The mercury is recovered as a ball of mercury).

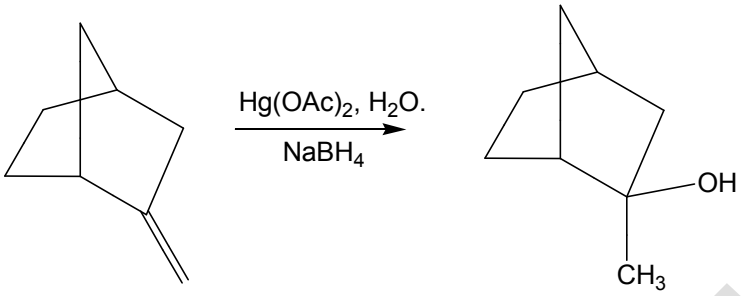




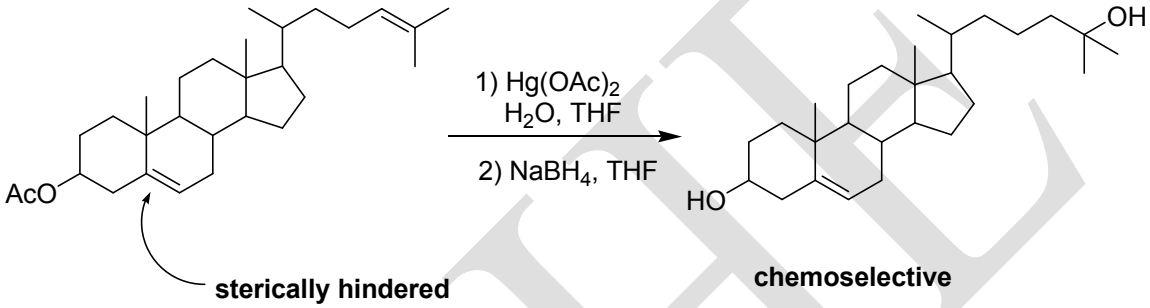
Regioselectivity

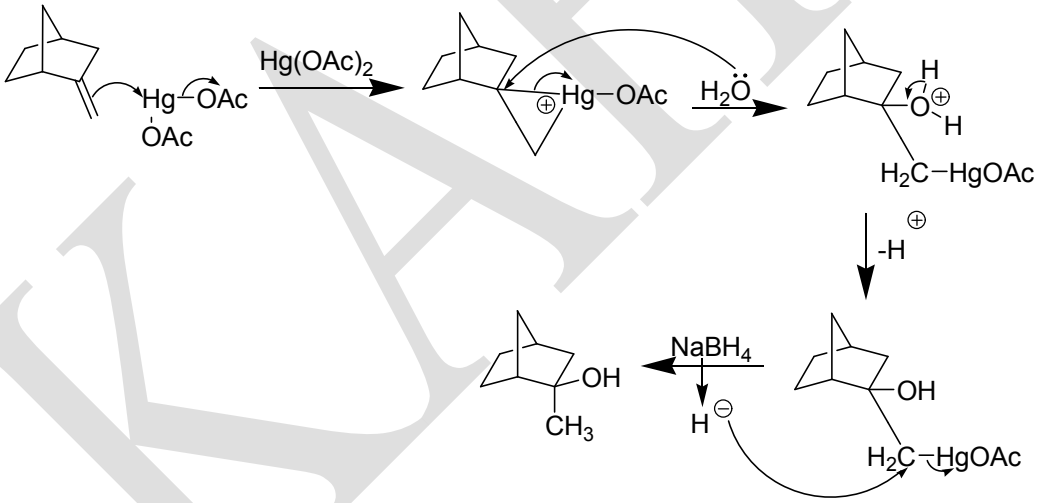
- Oxymercuration-demercuration is highly regioselectivity and gives alcohols corresponding to markonikov addition of water to the C=C bond. For e.g.





(Regio and stereoselectivity)





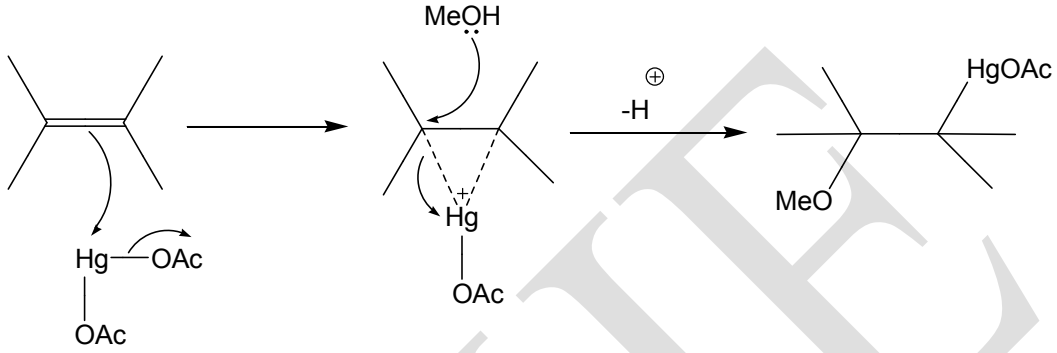
Mechanism

- Oxymercuration-demercuration involves electrophilic addition to the carbon-carbon double bond, with the mercuric ion acting as the electrophilic.
- The absence of rearrangement argues against an open carbocation as intermediate.
- Instead, a cyclic mercurinium ion analogous to the bromonium and chloronium ions is proposed.
- Olah reported spectroscopic evidence for the preparation of stable solutions of such

mercurinium ions and have since then been observed in the phase.

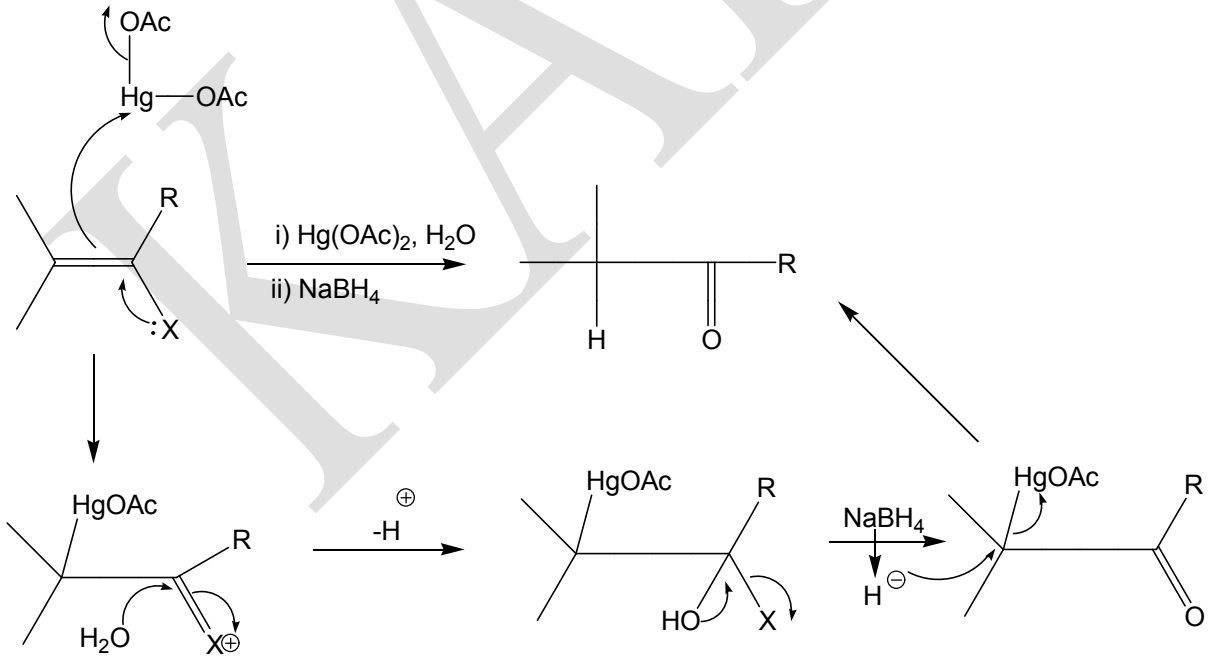
- The mercurinium ion is attacked by the nucleophilic solvent, in the present case water, to yield the addition product.

Methoxymercuration



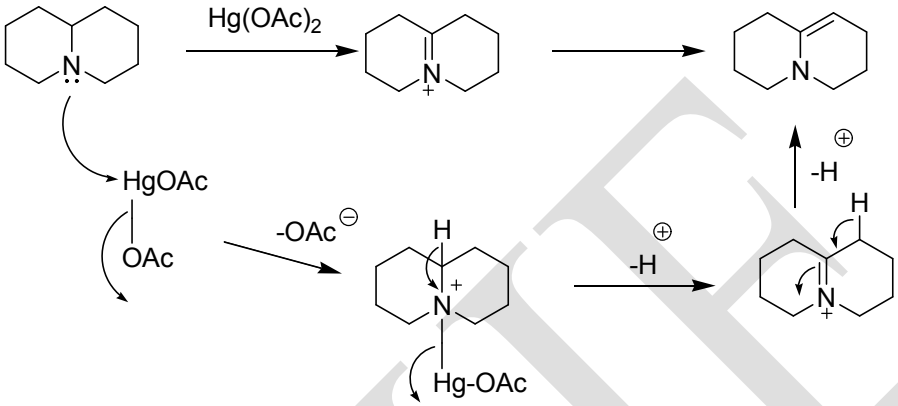
Hydrolysis of vinyl chlorides

Vinyl halides are hydrolysed to ketones at room temperature with mercuric trifluroacetate or with mercuric acetate in either trifluroacetic acid or acetic acid containing BF_3 etherate.

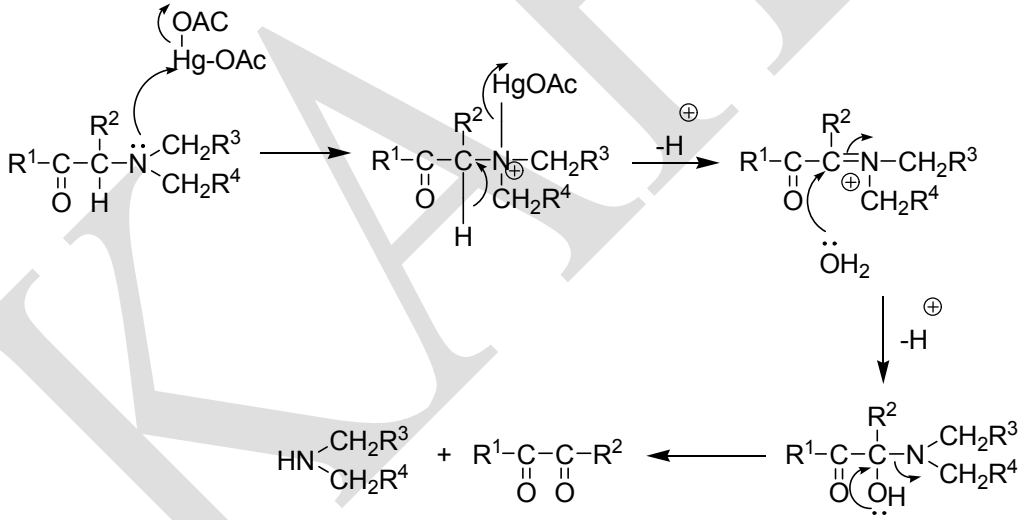


Dehydrogenation of tertiary amines affording enamines

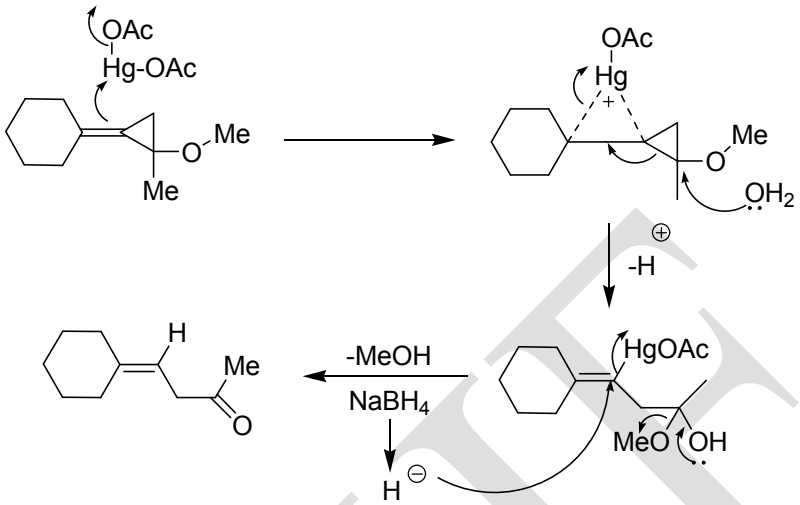
Dehydrogenation of tertiary amines gives enamines when treated with mercuric acetate. In this case the initial product is an iminium ion, which loses a proton to give the enamine.



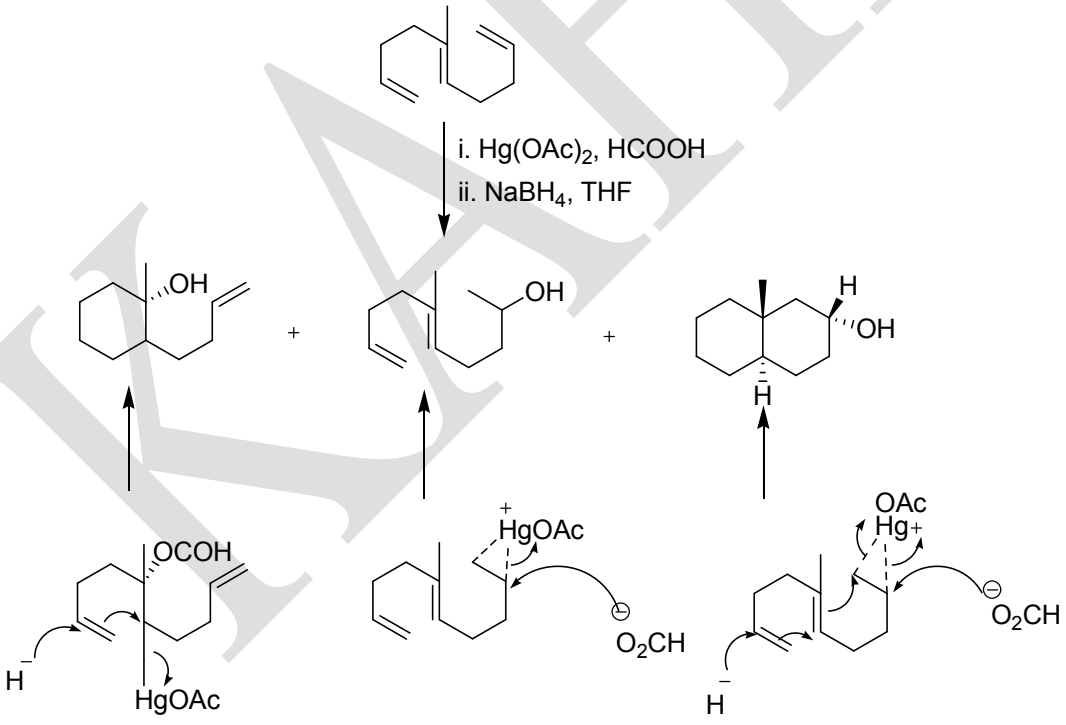
Preparation of 1,2-dicarbonyl compounds



Preparation of β,γ-unsaturated carbonyl compounds



Cyclisation of dienes



Text Book:

1. Smith, M. B. (2015). *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure* (VII Edition). New Jersey: John Wiley & Sons, Inc., Hoboken.

Reference Books:

1. Sanyal, S. N. (2014). *Reactions, Rearrangements and Reagents* (IV Edition). New Delhi: Bharathi Bhawan (Publishers and Distributors).

2. Tewari, N. (2011). *Advanced Organic Reaction Mechanism* (III Edition). Kolkata: Books and Allied (P) Ltd.

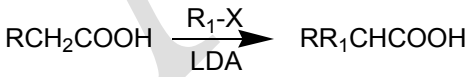
POSSIBLE QUESTIONS

PART- A – Multiple Choice Questions

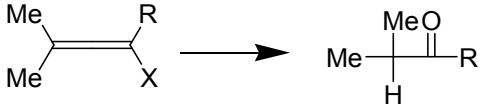
(Each Question Carry One Mark) (Online Examinations)

PART-B (Each Question Carry Two Marks)

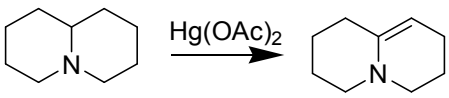
1. Write the preparation of DDQ?
2. Suggest a mechanism for following reaction.



3. Write the preparation and uses of Selenium dioxide?
4. Explain the synthesis and uses of DCC.
5. What are the reagents used to effect the following conversions? Explain the mechanism.



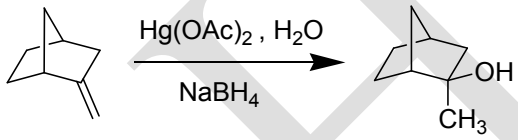
6. Provide a plausible mechanism of the following reaction?



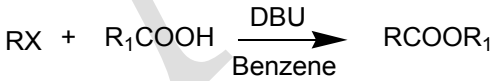
PART-C (Each Question Carry Six Marks)

1. Illustrate with examples the uses of the following reagents in synthetic organic chemistry
   (i) DDQ (ii) Osmium tetraoxide.
2. Explain the preparation synthetic applications of DMSO.
3. (i) Explain the mechanism of the following reactions. Indicate the selectivity's involved, if any.
   (a)

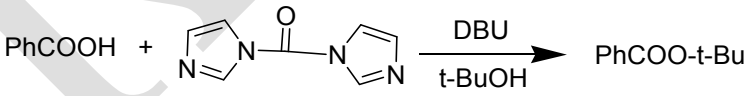
   (b)



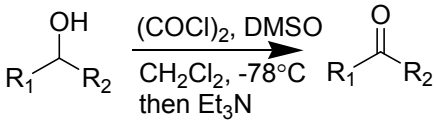
   (ii) Illustrate the synthetic applications of DCC.
4. Write short notes on the following reagents in organic synthesis.
   (i) LDA (ii) DBU
5. (i) Write the mechanism for the following conversions.
   (a)



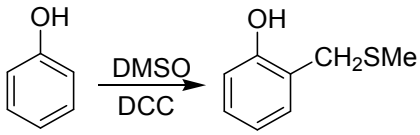
   (b)



   (ii) Discuss the mechanism of the following reactions:
   (a)

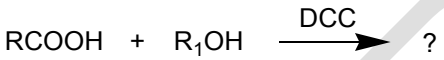


(b)

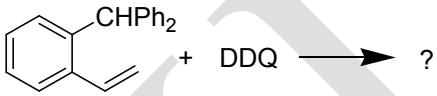


6. Complete the following reactions. Provide the mechanisms.

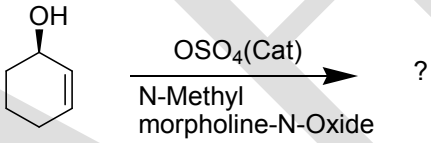
(i)



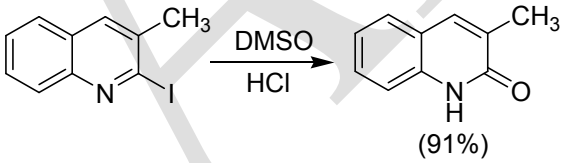
(ii)



(iii)



(iv)

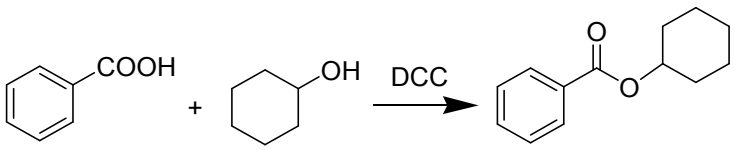


7. (i) Write the product of the following reaction and explain the mechanism.

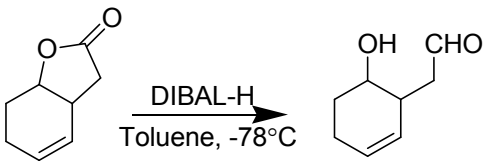
(ii) Illustrate the synthetic application of DBU.

8. Provide a plausible mechanism of the following reactions.

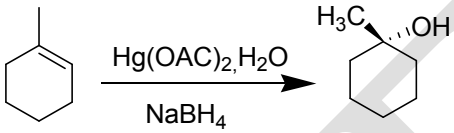
(i)



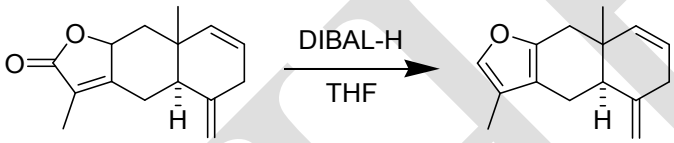
(ii)



(iii)



(iv)

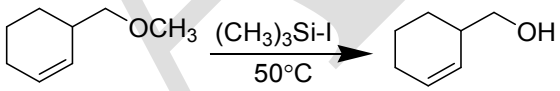


9. (i) Explain how DDQ is prepared from hydroquinone. What are the important synthetic uses of this reagent?

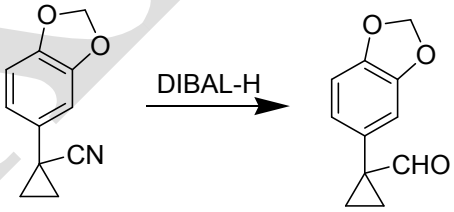
(ii) Illustrate the synthetic application of lithium diisopropylamine.

10. Suggest a reasonable mechanism for the following transformations.

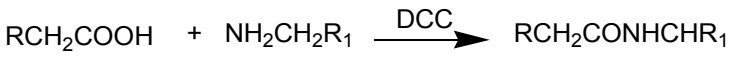
(i)



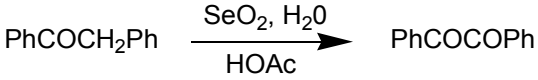
(ii)



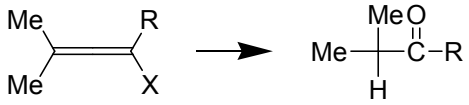
(iii)



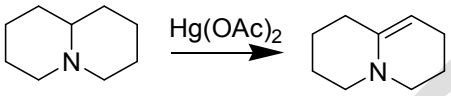
(iv)



11. (i) What is the reagent used to effect the following conversion? Explain the mechanism.



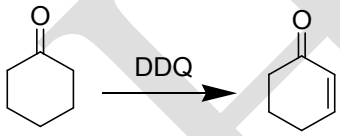
(ii) Provide a plausible mechanism of the following reaction?



(iii) Mention some uses of trimethylsilyl iodide.

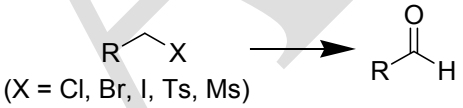
PART-D (Each Question Carry Ten Marks)

1. (a) Write the mechanism of the following conversion.

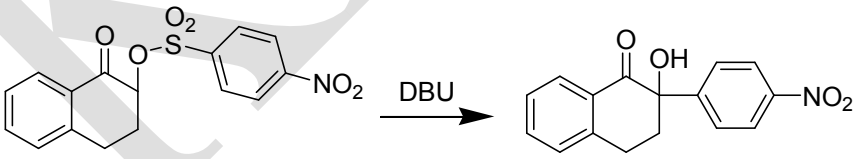


(b) Suggest suitable reagents for effecting the following conversion. Provide mechanism.

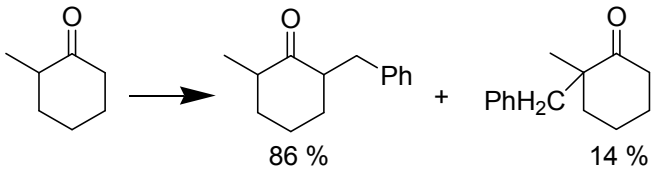
(c) What are the reagents used to effect the following conversion? Explain the mechanism.



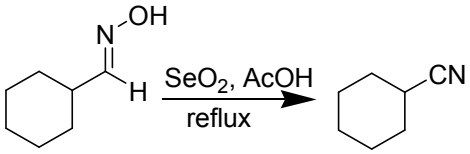
(d) Provide a plausible mechanism for the following reaction?



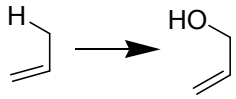
2. (i) Suggest suitable reagents for effecting the following conversions?



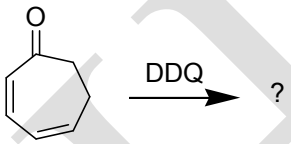
(ii) Suggest a mechanism for the following reaction.



(iii) Suggest suitable reagents for effecting the following conversion. Provide the mechanism.



(iv) Write the product of the following reaction and explain the mechanism.



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Pollachi Main Road, Eachanari Post, Coimbatore–641 021.

DEPARTMENT OF CHEMISTRY

UNIT-V

REAGENTS IN ORGANIC SYNTHESIS

PART-A–Multiple Choice Questions

(Each Question Carry One Mark) (Online Examinations)

1. The DDQ is prepared from

a) hydroquinone b) naphthoquinone c) anthraquinone d) benzoquinone

2. DDQ is mainly used for

a) oxidation **b) dehydrogenation** c) hydrogenation d) reduction

3. In benzene DDQ is red in colour due to

a) f-f transition b) LMCT **c) charge transfer** d) d-d transition

4. The DDQ mechanism the reactive intermediate is

a) carbanion b) free radical c) carbene **d) carbocation**

5. In DDQ the carbocation intermediate have been

a) trapped b) isolated c) detected d) separated

6. 2,3,5,6-tetrachloro-parabenzoquinone is known as

a) DDQ **b) chloranil** c) DBU d) DCC

7. Tetralin can be converted into naphthalene by using

a) DCC b) chloranil c) B_2H_6 **d) DDQ**

8. Choose the statement which is false for OsO_4

a) It converts alkenes into vicinal diols b) the reaction takes place via formation of osmate ester as intermediates **c) the diols formed will be trans**

d) Os (VIII) is converted into Os (VI)

9. The restrictions for use of DDQ is

a) oxidizes alcohols b) oxidizes ketone c) oxidizes aldehyde **d) oxidizes amines**

10. DBU is an organic

a) base b) acid c) amine d) salt

11. DBU is prepared from

a) nylon-6 and acrylonitrile **b) caprolactem and acrylonitrile**

c) nylon-66 and acrylonitrile d) caprolactem and acrolene

12. Chloranil also known as

a) tetrachloro-1,4-benzoquinone b) DCC c) DDQ d) AZQ

13. DBU is used for base-mediated

a) single bond migration b) allylic migration

c) triple bond migration **d) double bond migration**

14. Which reagent is used for the conversion of acid into ester?

a) DBU b) DCC c) DIBAL-H d) DDQ

15. Which reagent is used for the conversion of alcohol into ether?

a) DCC **b) DBU** c) DIBAL-H d) DDQ

16. Which reagent is used for rearrangement reaction?

a) DCC **b) DBU** c) DIBAL-H d) DDQ

17. A reagent used for cis-hydroxylation of double bond is

a) baker's yeast b) sodamide **c) OsO_4** d) NBS

18. 2,3-dichloro-5,6-dicyano-p-benzoquinone is simply abbreviated as

a) DCC b) DBU c) DIBAL-H **d) DDQ**

19. Which one of the following selectively oxidizes reactive methylene group into carbonyl group without affecting the carbonyl group originally present?

a) SeO_2-H_2O b) LTA c) OsO_4 d) periodic acid

20. DBU used as a base in

a) Aldol condensation **b) Michael addition** c) Wittig reaction d) Perkin reaction

21. The addition of an alkene to OsO_4 in ether gives

a) trans diol b) anti diols **c) cis diols** d) germinal diols

22. The addition of dienophile to a diene is known as

a) Pschorr synthesis b) Prevost Reaction

c) Oppenauer oxidation **d) Diels-Alder reaction**

23. DDQ is used for the selective oxidation of

a) allylic alcohols b) vinylic alcohol

c) tertiary alcohols d) benzylic alcohols

24. Which one of the following reagent is used for synthesis of allylic alcohol?

a) O_3 b) periodic acid **c) SeO_2-H_2O** d) perbezoic acid

25. Which one of the following reagent is suitable to convert ethyl cinnamate into cinnamyl alcohol at room temperature?

a) DCC **b) DIBAL-H** c) H_2/Pd d) Zn/AcOH

26. Which one of the following reagent is used for the oxidation of secondary alcohol to carbonyl compounds?

a) DMSO b) DCC c) Br_2-AcOH d) NBS

27. Which one of the following reagent is used for the oxidation reactions?

a) DDQ **b) DMSO** c) DCC d) DBU

28. Which one of the following reagent is used as a solvent as well as oxidant?

a) DDQ b) DCC c) DBU **d) DMSO**

29. The formation of aldehydes by treatment of primary alkyl halides with dimethylsulfoxide and a hydrogen acceptor. Thus it is often known as the

a) Moffatt Oxidation b) Goldman Oxidation

c) Swern Oxidation **d) Kornblum oxidation**

30. The oxidation of primary and secondary alcohols to carbonyl compounds (aldehydes and ketones) from dimethyl sulfoxide (DMSO) in combination with oxalyl chloride and triethylamine under anhydrous conditions, it is generally known as the

a) Moffatt Oxidation b) Goldman Oxidation

c) Swern Oxidation d) Kornblum oxidation

31. The oxidation of alcohols to ketones under dimethyl sulfoxide (DMSO) and dicyclohexylcarbodiimide (DCC) in the presence of phosphoric acid. This method was initially known as the

a) Moffatt Oxidation b) Goldman Oxidation

c) Swern Oxidation d) Kornblum oxidation

32. Which one of the following reagent is used for removal of the protecting group?

a) DCC b) DBU c) diborane **d) trimethylsilyl iodide**

33. The selenium reacts with oxygen gives

a) selenium dioxide b) selenious acid c) selenium tetraoxide d) selenium trioxide

34. The selenium dioxide is used for

a) reduction reaction **b) oxidation reaction** c) oxidation-reduction reaction d) redox reaction

35. Selenium dioxide reacts with water gives

a) metallic selenium b) selenium tetraoxide **c) selenious acid** d) selenium trioxide

36. Which one solvent is used for selenium dioxide oxidation reaction?

a) chloroform b) hexane c) ethyl acetate **d) water**

37. The conversion of cyclohexanone into 1,2-diketo cyclohexane is effected by

a) SeO_2 b) DBU c) B_2H_6 d) DDQ

38. The oxidation of olefins into allylic alcohol by using

a) DBU **b) SeO_2** c) B_2H_6 d) DDQ

39. Which reagent is used for the conversion of cyclohexanecaraldehyde oxime into cyanocyclohexane

a) DBU b) B_2H_6 **c) SeO_2** d) DDQ

40. The dehydration of dicyclohexylurea with p-toluenesulphonyl chloride in hot pyridine gives

a) DBU b) B_2H_6 c) DDQ **d) DCC**

41. DCC is used as a

a) dehydrating agent b) hydrating agent c) oxidizing agent d) reducing agent

42. DCC gives an excellent yield of acid anhydride from

a) acid chlorides **b) carboxylic acids** c) phenol d) alcohols

43. A mixture of phenol, ethanol and DCC on prolonged heating under reduced pressure gives

a) ester b) carboxylic acids **c) ethyl phenyl ether** d) acid anhydride

44. Diacyl peroxide can be prepared by the treatment of carboxylic acids with hydrogen peroxide under mild condition in the presence of

a) DBU b) B_2H_6 c) DDQ **d) DCC**

45. LDA is one of the

a) strongest base b) strongest acid c) weakest acid d) weakest base

46. LDA is used to remove

a) more acidic proton **b) less acidic proton**

c) sterically hindered proton d) neutral proton

47. Why LDA abstracts less acidic proton due to

a) inductive effect b) hyperconjugation **c) steric hindrance** d) resonance effect

48. LDA can be prepared from the reaction of n-butyl lithium on

a) isopropyl amine b) propyl amine c) diisobutyl amine **d) diisopropyl amine**

49. DIBAL-H is a versatile

a) dehydrating agent b) hydrating agent c) oxidizing agent **d) reducing agent**

50. At room temperature DIBAL-H reduces ester to the

a) primary alcohol b) acid c) secondary alcohol d) aldehyde

51. The cinnamic acid is converted to the ester which is reduced at room temperature with DIBAL-H to the

a) cinnamaldehyde b) cinnamoyl chloride **c) cinnamyl alcohol** d) benzaldehyde

52. Which reagent is used for preparation of aldehydes?

a) DBU b) B_2H_6 c) DCC **d) DIBAL-H**

53. DIBAL-H is used as a selective 1,2-reduction of α, β-unsaturated carbonyl compounds to

a) allylic alcohols b) alcohols c) homo allylic alcohols d) vinyl alcohols

54. Reduction of unsaturated γ-lactone to furan derivative has been effected by

a) DBU **b) DIBAL-H** c) DCC d) B_2H_6

55. DIBAL-H reduces only the carbonyl group of α, β-unsaturated ketone is an example for

a) regioselective reduction b) stereoselective reduction

c) chemoselective reduction d) regiospecific reduction

56. DIBAL-H reduced N,N-di-substituted amide into

a) amide b) acid c) alcohol **d) aldehyde**

57. Alkenes react with mercuric acetate in the presence of sodium borohydride and water to give

a) amide b) acid **c) alcohol** d) aldehyde

58. Vinyl halides are hydrolysed to ketones at room temperature with

a) DBU b) DIBAL-H c) DCC **d) $Hg(OAc)_2$**

59. Dehydrogenation of tertiary amines gives enamines when treated with

a) mercuric acetate b) DIBAL-H c) DCC d) DBU

60. Which one reagent is used for the preparation of stilbene?

a) DBU b) DIBAL-H **c) DDQ** d) $Hg(OAc)_2$

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KARPAGAM ACADEMY OF HIGHER EDUCATION

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COIMBATORE-641 021

(For the candidates admitted from 2018 & onwards)

PG DEGREE EXAMINATION, AUGUST 2019

THIRD SEMESTER

INTERNAL TEST-I

CHEMISTRY

ORGANIC CHEMISTRY-III (Natural Products)

Time: 2 hours **Maximum: 50 marks**
Date: 28-08-2019 **Subject code: 18CHP301**

PART- A (20 x 1=20 Marks)
Answer All the Questions

1. Catalytic hydrogenation of zingiberene yields

a) dihydozingiberene b) hexahydrozingiberne

c) trihydrozingiberene d) tetrahydrozingiberene

2. Ozonolysis of zingiberene gives

a) laevulic acid b) acetic acid c) bromoform d) ketodicarboxylic acid

3. Santonin on prolonged heating with barium hydroxide gives

a) santonous acid b) santinic acid c) santoninic acid d) santonic acid

4. Eudalene behaved as an

a) aliphatic compound b) aromatic compound

c) heterocyclic compound d) unsaturated compound

5. Catalytic hydrogenation of abietic acid gives

a) dihydroabietic acid b) tetrahydroabietic acid c) retene d) biphenyl

6. Reaction of santonamine with nitrous acid gives

a) santonic acid b) santoninic acid c) hyposantonin d) santinic acid

7. Catalytic hydrogenation of caryophyllene gives

a) caryophyllenic acid b) norcaryophyllenic acid

c) tetrahydrocaryophyllene d) dihydrocaryophyllene

8. The conversion of abietinol to methylabietin is an example for

a) Wanger-Meerwein rearrangement b) Fries rearrangement

c) Benzilic acid rearrangement d) Wolff rearrangement

9. Steroids have the

a) 1,2–cyclopentenophenanthrene ring b) 1,3-cyclopentenophenanthrene ring

c) phenanthrene ring d) anthracene ring

10. The reaction which produces red colour while chloroform solution of cholesterol reacting with sulphuric acid

a) Liebermann-Burchard reaction b) Liebermann reaction

c) dye test d) Salkowski reaction

11. The infrared spectrum of ergosterol showed a band at 970 cm^{-1} indicates

a) hydroxyl b) trans-alkene group c) cis-alkene group d) nitro group

12. Progesterone forms oxime with

a) semicarbazone b) hydroxylamine c) phenylhydrazine d) hydrazine

13. Testosterone is prepared from

a) cholesterol b) ergosterol c) progesterone d) oestrone

14. Which one is α,β-unsaturated ketone?

a) cholesterol b) oestrone c) equilenin d) progesterone

15. The catalytic hydrogenation of ergocalciferol yields

a) dihydroergocalciferol b) trihydroergocalciferol

c) tetrahydroergocalciferol d) octahydroergocalciferol

16. Cholic acid contains

a) 1 hydroxyl group b) 2 hydroxyl group

c) 3 hydroxyl groups d) 4 hydroxyl groups

17. The naturally plant compounds having a basic character and containing at least one nitrogen in a heterocyclic ring is known as

a) alkaloids b) steroids c) terpeniods d) proteins

18. The most of the alkaloids are

a) optically active b) meso c) racemic mixture d) optically inactive

19. The example for commercially synthesized alkaloid is

a) morphine b) quinine c) papaverine d) atropine

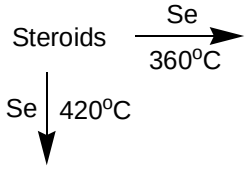
20. Tropinic acid on Hofmann exhaustive methylation followed by reduction yields

a) tropidine b) tropilidine c) pimelic acid d) tropinone

PART- B (3 x 2= 6 Marks)
Answer All the Questions

21. Explain how ultraviolet spectroscopy is useful in terpenoid chemistry?

22. Write the structure of the expected product in the following reaction.



23. What is Herzig-Meyer method?

PART- C (3 x 8= 24 Marks)
Answer All the Questions

24. (a) Elucidate the structure and synthesis of Zingiberene.

(Or)

(b) Explain the general methods of determining structure of Terpenoids.

25. (a) Explain the structural elucidation and synthesis of Santonin.

(Or)

(b) Explain the structural elucidation and synthesis of Oestrone.

26. (a) Explain the positions of the hydroxyl group and double bond in Cholesterol.

(Or)

(b) (i) Explain the structural elucidation of Tropic acid.

(ii) Write note on Robinson's synthesis of Tropinone.

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COIMBATORE-641 021

(For the candidates admitted from 2018 & onwards)

PG DEGREE EXAMINATION, OCTOBER 2019

THIRD SEMESTER

INTERNAL TEST-II

CHEMISTRY

ORGANIC CHEMISTRY-III (Natural Products)

Time: 2 hours **Maximum: 50 marks**
Date: 14-10-2019 **Subject code: 18CHP301**

PART- A (20 x 1=20 Marks)
Answer All the Questions

1. Oxidation of quinine with chromic acid produces

a) 6-methoxy quinoline b) meroquinene c) quininic acid d) quininone

2. Methylmorphenol when demethylated with HCl yields

a) morphol b) morphenol c) trihydroxymorphine d) β-mehtylmorphimethine

3. The Von Braun's method the tertiary cyclic amines is converted into

a) N-cyano derivative b) N-methyl derivative

c) N-nitroso derivative d) N-ethyl derivative

4. The number of double bonds present in morphine is

a) 1 b) 3 c) 2 d) 4

5. Among the following which protein is soluble in water?

a) albumins b) globulins c) prolamins d) glutelins

6. Which one element present in nucleoprotein

a) phosphorus b) calcium c) magnesium d) sulphur

7. Proteins are

a) acidic b) basic c) amphoteric d) neutral

8. Denaturation of protein molecule which result the changes in

a) folding b) configuration c) hydrogen bonding d) conformation

9. Biuret reaction is responsible for

a) carbohydrates b) proteins c) alkaloids d) steroids

10. Benzyloxycarbonyl chloride is used as a

a) side-chain protecting group b) carboxyl protecting group

c) amino protecting group d) activation of carboxyl group

11. When the cofactor has been removed the protein that remains is known as

a) coenzymes b) holoenzyme c) metalloenzyme d) apoenzyme

12. The disease caused by the deficiency of insulin is

a) beri-beri b) diabetes c) cancer d) rickets

13. In DDQ the carbocation intermediate have been

a) trapped b) isolated c) detected d) separated

14. DBU is an organic

a) base b) acid c) amine d) salt

15. Which reagent is used for the conversion of alcohol into ether?

a) DCC b) DBU c) DIBAL-H d) DDQ

16. Which one of the following selectively oxidizes reactive methylene group into carbonyl group without affecting the carbonyl group originally present?

a) SeO_2-H_2O b) LTA c) OsO_4 d) periodic acid

17. The addition of an alkene to OsO_4 in ether gives

a) trans diol b) anti diols c) cis diols d) germinal diols

18. Which one of the following reagent is used for the oxidation of secondary alcohol to carbonyl compounds?

a) DMSO b) DCC c) Br_2-AcOH d) NBS

19. Which one of the following reagent is used for removal of the protecting group?

a) DCC b) DBU c) diborane d) trimethylsilyl iodide

20. Dehydrogenation of tertiary amines gives enamines when treated with

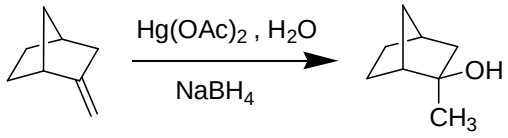
a) mercuric acetate b) DIBAL-H c) DCC d) DBU

PART- B (3 x 2= 6 Marks)
Answer All the Questions

21. Write the conversion of 3, 4-dimethoxy-2-nitro-benzaldehyde into dimethylmorphol

22. Define metalloproteins with suitable examples?

23. Provide a plausible mechanism of the following reaction?



PART- C (3 x 8= 24 Marks)
Answer All the Questions

24. (a) Explain the Gate's synthesis of Morphine.

(Or)

(b) Explain the structural elucidation and synthesis of Quinine.

25. (a) Discuss the biological significance of DNA and RNA.

(Or)

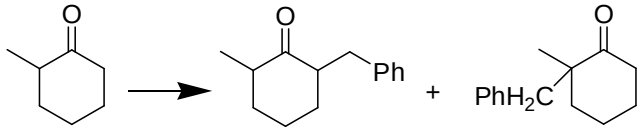
(b) Explain the synthesis of peptide.

26. (a) Illustrate with examples the uses of the following reagents in synthetic organic chemistry

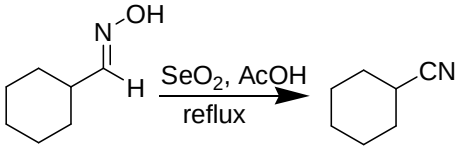
(i) DDQ (ii) DCC.

(Or)

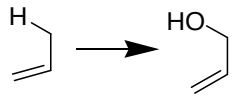
(b) (i) Suggest suitable reagents for effecting the following conversions?



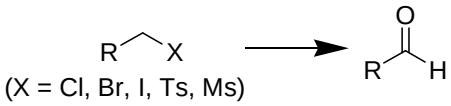
(ii) Suggest a mechanism for the following reaction.



(iii) Suggest suitable reagents for effecting the following conversion. Provide the mechanism.



(iv) What are the reagents used to effect the following conversion? Explain the mechanism.



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[17CHP301]

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(For the candidates admitted from 2017 onwards)

M.Sc., DEGREE EXAMINATION, NOVEMBER 2018

Third Semester

CHEMISTRY

ORGANIC CHEMISTRY – III : (NATURAL PRODUCTS)

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

(Part - B & C 2 ½ Hours)

PART B (5 x 6 = 30 Marks)
Answer ALL the Questions

21. a. How are the positions of carboxylic group and angular methyl group established in abietic acid?

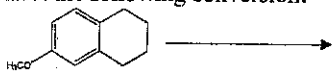
Or

b. Outline the steps involved in the biosynthesis of monoterpenoids.

22. a. How are the positions of double bonds and hydroxyl group established in ergosterol?

Or

b. Effect the following conversion:



Oestrone

23. a. Write the biosynthesis of quinine.

Or

b. Establish the structure of tropine.

24. a. Describe the characteristics of proteins.

Or

b. Explain the biological importance of nucleic acids.

25. a. Describe the use of DMSO and SeO_2 in the synthesis of various organic compounds.

Or

b. Explain the synthesis application of OsO_4 and DDQ.

PART C (1 x 10 = 10 Marks)
(Compulsory)

26. Elucidate the structure of morphine. Confirm the structure by a suitable synthesis.

Reg. No.....

[16CHP301]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2016 onwards)

M.Sc., DEGREE EXAMINATION, NOVEMBER 2017

Third Semester

CHEMISTRY

ORGANIC CHEMISTRY-III
(NATURAL PRODUCTS)

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

(Part - B & C 2 ½ Hours)

PART B (5 x 6 = 30 Marks)
Answer ALL the Questions

21. a. How are essential oils isolated from plant materials by steam distillation method? Indicate how the terpenoids are separated from essential oils.

Or

b. How are position of the carboxylic acid group and position of angular methyl group in abietic acid established?

22. a. Explain the biosynthesis of cholesterol.

Or

b. Describe the constitution of vitamin D.

23. a. How is morphine synthesized from 2,6-dihydroxy naphthalene?

Or

b. Elucidate the structure of tropine.

24. a. Describe the synthesis of insulin.

Or

b. Explain the biological importance of RNA and DNA.

25. a. Bring out the synthetic applications of SeO_2 and DMSO.

Or

b. Illustrate the synthetic applications of LDT and DDQ.

PART C (1 x 10 = 10 Marks)
(Compulsory)

26. Elucidate the structure of equilenin. Confirm the structure by a suitable synthesis

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Reg. No.....

[15CHP301]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education
(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2015 onwards)

M.Sc., DEGREE EXAMINATION, NOVEMBER 2016

Third Semester

CHEMISTRY

ORGANIC CHEMISTRY III
(Natural Products)

Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

(Part - B & C 2 ½ Hours)

PART B (5 x 6 = 30 Marks)
Answer ALL the Questions

21. a) How are the positions of angular methyl group and two double bonds established in abietic acid?

Or

b) With suitable examples, explain the different steps involved in the biosynthesis of mono terpenoids.

22. a) How is Oestrone synthesized?

Or

b) Elucidating the structure of ergocalciferol.

23. a) Give a detailed account on biosynthesis of quinoline alkaloids.

Or

b) Explain the isolations and properties of alkaloids.

24. a) Explain the Bergmann's method of synthesis of polypeptides. How is it modified?

Or

b) Discuss the biological role of RNA and DNA.

25. a) Explain the synthetic applications of OsO_4 and DDQ.

Or

b) Discuss the synthetic applications of DIBAL – H and Diborane.

PART C (1 x 10 = 10 Marks)
(Compulsory)

26. Elucidate the structure of morphine. Confirm its structure by a suitable synthesis.

Reg. No.....

[14CHP301]

KARPAGAM UNIVERSITY

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(Established Under Section 3 of UGC Act 1956)
COIMBATORE – 641 021
(For the candidates admitted from 2014 onwards)

M.Sc., DEGREE EXAMINATION, NOVEMBER 2015

Third Semester

CHEMISTRY

ORGANIC CHEMISTRY III
(Natural Products)

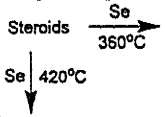
Time: 3 hours Maximum : 60 marks

PART – A (20 x 1 = 20 Marks) (30 Minutes)
(Question Nos. 1 to 20 Online Examinations)

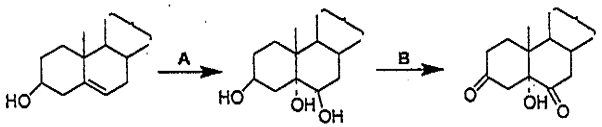
PART B (5 x 8 = 40 Marks) (2 ½ Hours)
Answer ALL the Questions

21. a. (i) Explain the biosynthesis of monoterpenoids.
(ii) Explain the conversion of Santonin into santonic acid.
Or
b. (i) Explain the isolation of monoterpenoids and sesquiterpenoids.
(ii) Elucidate the structure of Zingiberene.

22. a. (i) Write the conversion of Ergosterol into Progesterone.
(ii) Outline the conversion of cholesterol into 5β-cholanic acid.
Or
b. (i) Write the structure of the expected product in the following reaction.



(ii) Complete the following sequence of reactions giving missing reagents **A** and **B**.



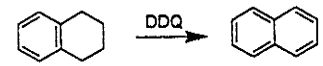
(iii) Write the conversion of Pregnanediol to Progesterone?

23. a. (i) Explain the stereochemistry of Morphine.
(ii) Explain the synthesis of Quinine.
Or
b. (i) Explain the conversion of Thebaine into Thebenine.
(ii) Explain the synthesis of Tropine.

24. a. (i) Explain the classification of proteins.
(ii) Explain how the following act as amino protecting groups in the synthesis of peptide (a) Benzyloxy carbonyl group (b) phthaloyl group
Or
b. Explain the synthesis of peptide.

25. a. (i) Explain the mechanism of the following reactions. Indicate the selectivity's involved, if any.

(1)



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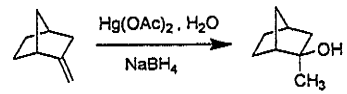

(ii) Illustrate the synthetic applications of DCC.
Or
b. Write short notes on the following reagents in organic synthesis.
(i) LDA (ii) DBU

Reg. No.....

[13CHP301]

KARPAGAM UNIVERSITY

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Third Semester

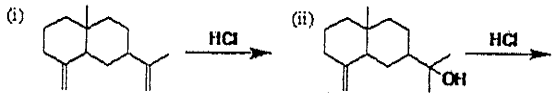
CHEMISTRY

ORGANIC CHEMISTRY – III (Natural Products)

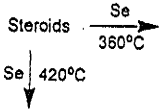
Time: 3 hours Maximum : 60 marks

PART – A (10 x 2 = 20 Marks)
Answer any TEN Questions

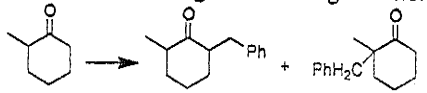
1. Write the structures of three isomers of Eudesmol.
2. Write the structure of the expected product in each of the following reactions.



3. Explain how ultraviolet spectroscopy is useful in terpenoid chemistry?
4. Write the structure of the expected product in the following reaction.



5. Explain the synthesis of Diel's hydrocarbon?
6. Explain what is Salkowski reaction?
7. How the presence of hydroxyl group is confirmed in the alkaloids?
8. How will you confirm the carboxyl group in alkaloids?
9. What is Zeisel method?
10. Define denaturation of proteins?
11. What is meant by annealing?
12. Explain Biuret reaction to test proteins?
13. Write the preparation of DDQ?
14. Suggest suitable reagents for effecting the following conversions?



15. Mention some uses of Ozonolysis.

PART B (5 X 8= 40 Marks)
Answer ALL the Questions

16. (a) (i) Explain the nature of side chain and position of double bond in Ergosterol.
(ii) How will you convert Cholesterol into Progesterone?
Or
(b) Explain the positions of the hydroxyl group and double bond in Cholesterol.

17. (a) (i) Explain the Synthesis of Morphine.
(ii) How will you establish the following?
(a) Position of $-OCH_3$ in Quinine. (b) Position of phenolic –OH group in Morphine.
Or
(b) Explain the structural elucidation and synthesis of Quinine.

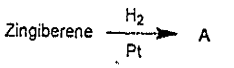
18. (a) Discuss the biological significance of DNA and RNA.
Or
(b) (i) Explain the mechanism of an enzyme action.
(ii) Explain the solid-phase peptide synthesis.

19. (a) Illustrate with examples the uses of the following reagents in synthetic organic chemistry
(i) DDQ (ii) Osmium tetraoxide.
Or
(b) Explain the synthetic uses of the following reagents (i) Diborane (ii) OsO_4 .

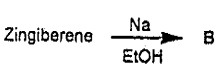
20. **Compulsory : -**

(i) How are Terpenes classified?
(ii) Identify the products **A, B, C, D & E** in the following transformations.

(a)



(b)



(c)