PORPHYRINS

6.1. HAEMOGLOBIN (HEMOGLOBIN)

Haemoglobin is an iron containing oxygen transport metalloprotein in red blood cells in mammals and almost all other vertebrates. Only few vertebrates, such as eel larvae and some antarctic ice fish have been found to lack haemoglobin. On the other hand, some invertebrates, such as annelid worms, employ haemoglobin in oxygen transport. Haemoglobin binds loosely and reversibly with oxygen in lungs and gills and delivers it to peripheral tissues to maintain viability of cells.

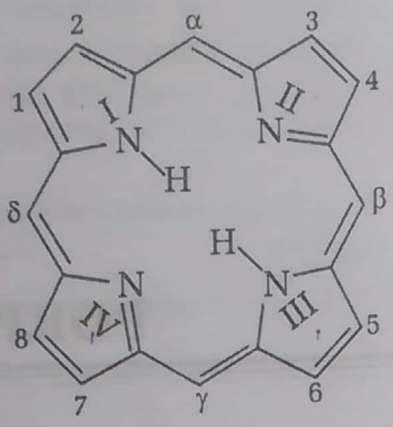
Haemoglobin is made of two parts; a protein part called **globin** (94%) and a non-protein part (prosthetic group) called **haem** (6%). The composition of haemoglobin in various species varies slightly and the variation is only in the globin part of the molecule.

A haemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a prosthetic group, haem. Each individual protein chain arranges in a set of alpha helix structural segments connected together in a myoglobin fold arrangement. This folding pattern contains a pocket that is suitable to tightly bind the haem group.

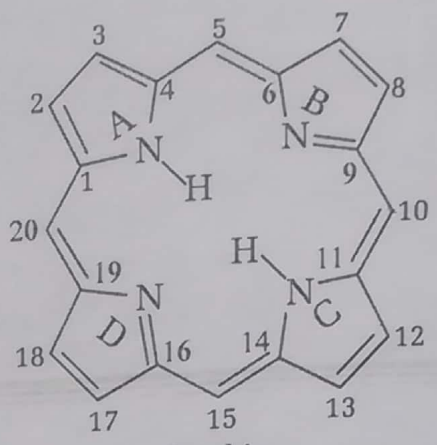
There are two types of protein chains, called α-chain and β-chain, in human haemoglobin and these chains differ in terminal acid group. Two α-chains (141 amino acids each) and two β-chains (146 amino acids each) are present in a molecule of haemoglobin of a normal adult human.

Haem is a prosthetic group that consists of an iron ion contained in the center of a large heterocyclic organic ring called porphyrin (substituted porphin).

Porphin is a chelating agent which can form coordinate bonds to a metal ion through nitrogen. It is a cyclic compound made of four pyrrole rings joined through methine. Thus a porphin molecule has four pyrrole rings and four methine carbon atoms arranged alternatively to make a large heterocyclic ring. Each of the four nitrogen atoms in the center of molecule can coordinate to a metal ion. Carbon atoms forming methine bridges are labelled as either α, β, γ and δ (Fischer numbering) or as 5, 10, 15 and 20 (IUPAC rules). The hydrogen atoms attached to methine bridge carbon atoms are called meso-hydrogens. Pyrrole rings were earlier labelled as I, II, III and IV but are now labelled as A, B, C and D. Porphin has an 18-membered inner aromatic ring which is highly stable because of many contributing resonance structures. Porphin molecule may be a combination of two types of electronic structures.



Porphin
(Fischer Numbering)



Porphin
(IUPAC Numbering)

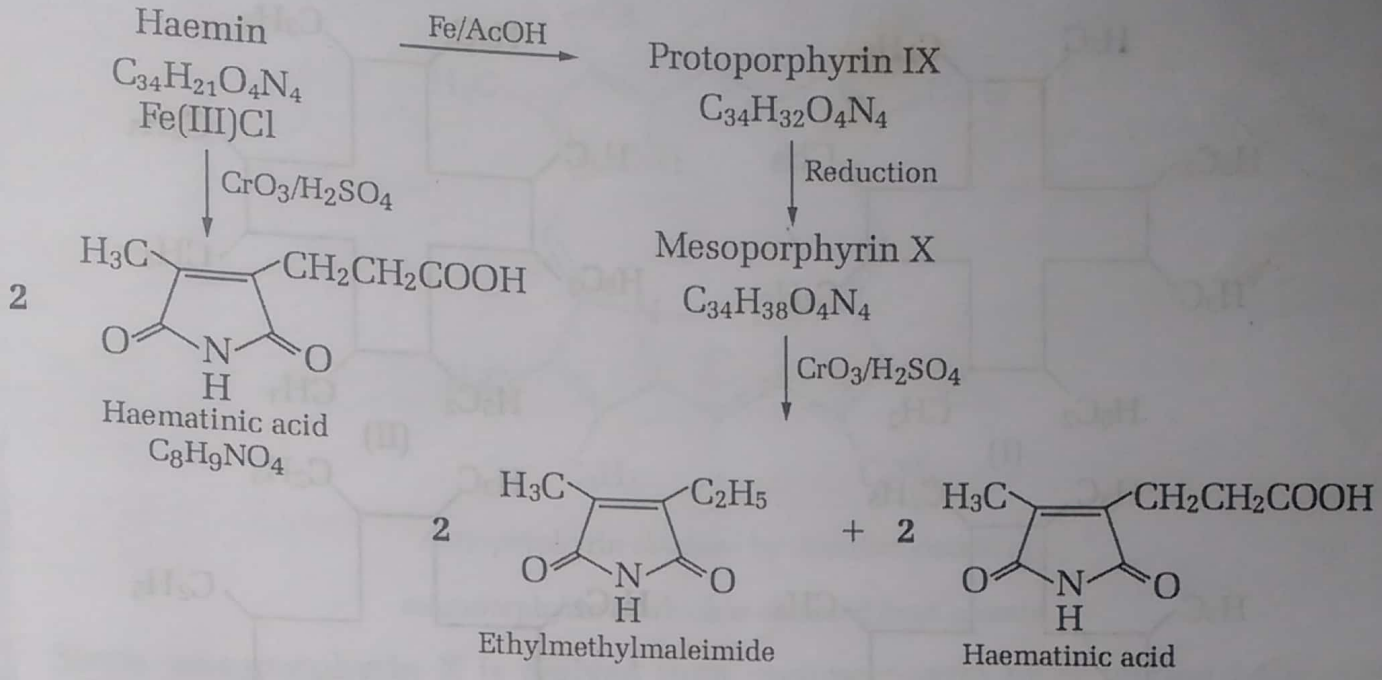
Porphyrins are derivatives of porphins formed by replacement of hydrogen atoms of the pyrrole rings, situated at the outer rim, with some other groups of atoms.

Iron can form six coordinate bonds. In haem, four pyrrole groups of protoporphyrin form a square-planar complex with iron via nitrogen atoms. Two remaining coordination positions of iron are perpendicular to this plane of porphyrin ring. The fifth position is occupied by an imidazole ring of the histidine amino acid residue. It appears that iron is bonded to histidine-87 in the α-chain and to histidine-92 in the β-chain. The sixth valency is probably occupied by water and when haemoglobin combines with one molecule of oxygen to form oxyhaemoglobin, it is the sixth valency, which coordinates with oxygen molecule by replacing water molecule if present.

When the iron atom is in ferrous state, the complex is called protohaem or **haem** and the molecule is neutral. When the iron atom is in ferric state, the molecule carries a unit positive charge and is called **haemin**.

Some common porphyrins are given below.

Porphyrin	Substituents (Fischer Numbering)							
	1	2	3	4	5	6	7	8
Aetioporphyrin I	M	E	M	E	M	E	M	E
Aetioporphyrin II	M	E	E	M	M	E	M	E
Aetioporphyrin III	M	E	M	E	M	E	E	M
Aetioporphyrin IV	E	M	M	E	M	E	E	M
Coproporphyrin I	M	P	M	P	M	P	M	P
Uroporphyrin I	A	P	A	P	A	P	A	P
Protoporphyrin IX	M	V	M	V	M	P	P	M
Deuteroporphyrin IX	M	H	M	H	M	P	P	M,
Haematoporphyrin IX	M	hE	M	hE	M	P	P	M
Mesoporphyrin IX	M	E	M	E	M	P	P	M
Pyrroporphyrin IX	M	E	M	E	M	H	P	M
Rhodoporphyrin XV	M	E	M	E	M	C	P	M

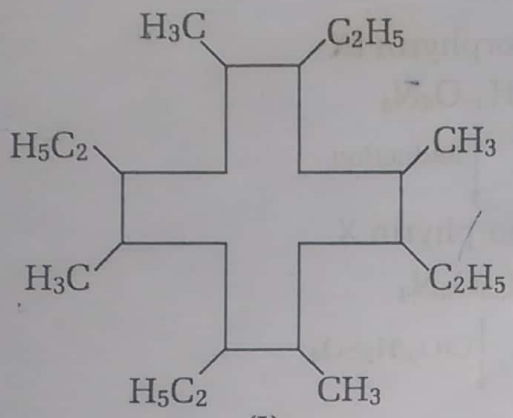


Structures of haematinic acid and ethylmethylmaleimide have been confirmed by their synthesis. Formation of haematinic acid and ethylmethylmaleimide from protoporphyrin suggests that the pyrrole nuclei are linked through the α-position (oxidation at α-carbon). Further, the presence of methyl group at α-position in II, III, IV, VI, VII and VIII suggests that pyrrole nuclei are joined together at α-position by means of one carbon atom only.

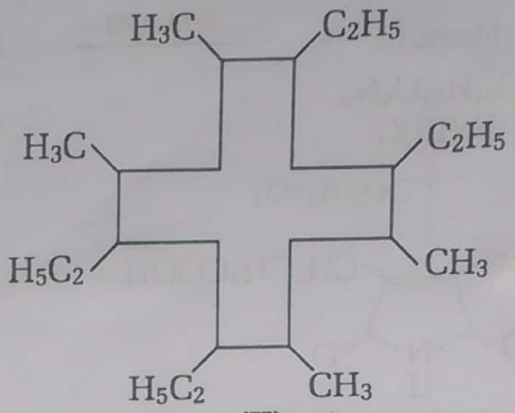
Formation of two molecules of haematinic acid reveals the presence of two propionic acid groups on two pyrrole rings in the β-position. Further the formation of ethylmethylmaleimide as an oxidation product of mesoporphyrin IX suggests the presence of two vinyl groups in the β-position of pyrrole nuclei which on reduction gets converted to ethyl groups. These groups are lost during direct oxidation (due to this reason haemin does not give ethylmethylmaleimide on direct oxidation). The presence of vinylic groups at β-position is also supported by the formation of reductive degradation products (I) to IV from haemin (vinyl group converts into ethyl groups during reduction process).

The above data when pooled together reveals that haemin contains four pyrrole nuclei linked at α-position via one carbon atom. Moreover, four β-positions are substituted by methyl groups, two β′-positions by vinyl groups and two β′-positions by propionic acid groups in protoporphyrin IX.

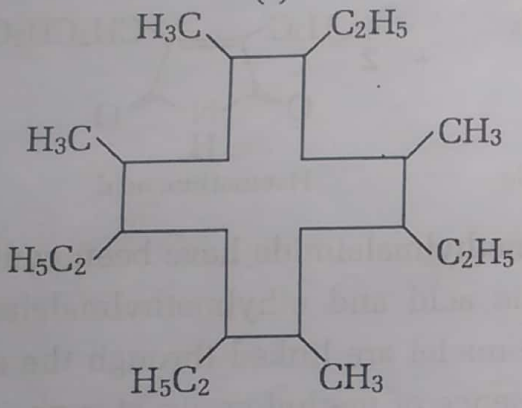
(7) **Position of substituents in four pyrrole nuclei:** Mesoporphyrin on decarboxylation gives aetioporphyrin which was identified as tetraethyltetramethylporphyrin. Aetioporphyrin can exist in four different isomeric forms (positional isomers).



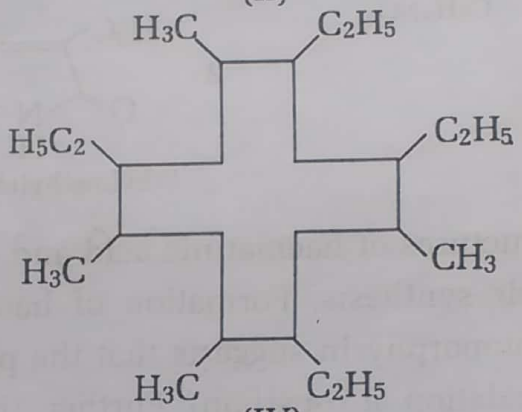
(I)



(II)



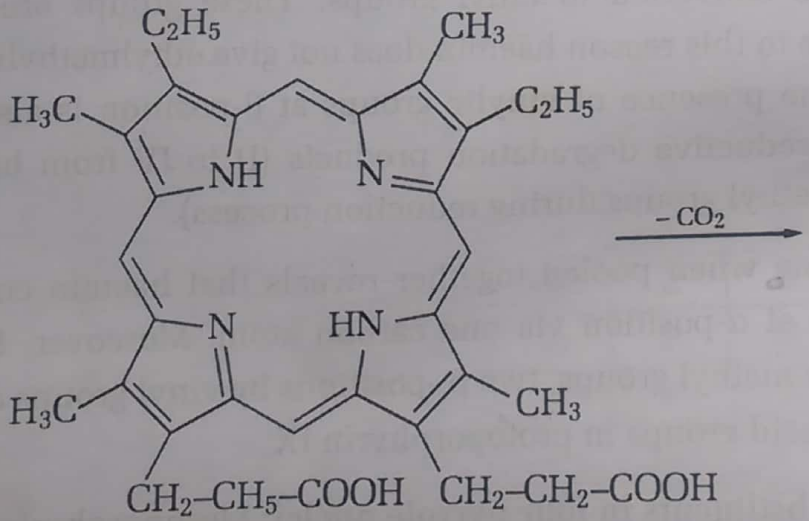
(III)



(IV)

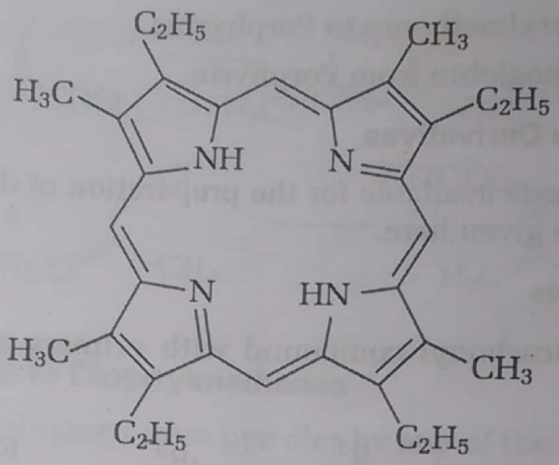
All the four isomers were synthesised but unfortunately they resembled very much in their properties so the aetioporphyrin obtained from haemin could not be identified. The correct substitution pattern was obtained from the study of the precursor of aetioporphyrin *i.e.*, mesoporphyrin. Fifteen different positional isomers of mesoporphyrin having three different substituents (methyl, CH=CH, —CH_2—CH_2—COOH) are possible.

In order to find out the substitution pattern in mesoporphyrin, all the fifteen isomers of mesoporphyrin were synthesised and one of them was found to be identical with that obtained from haemin.



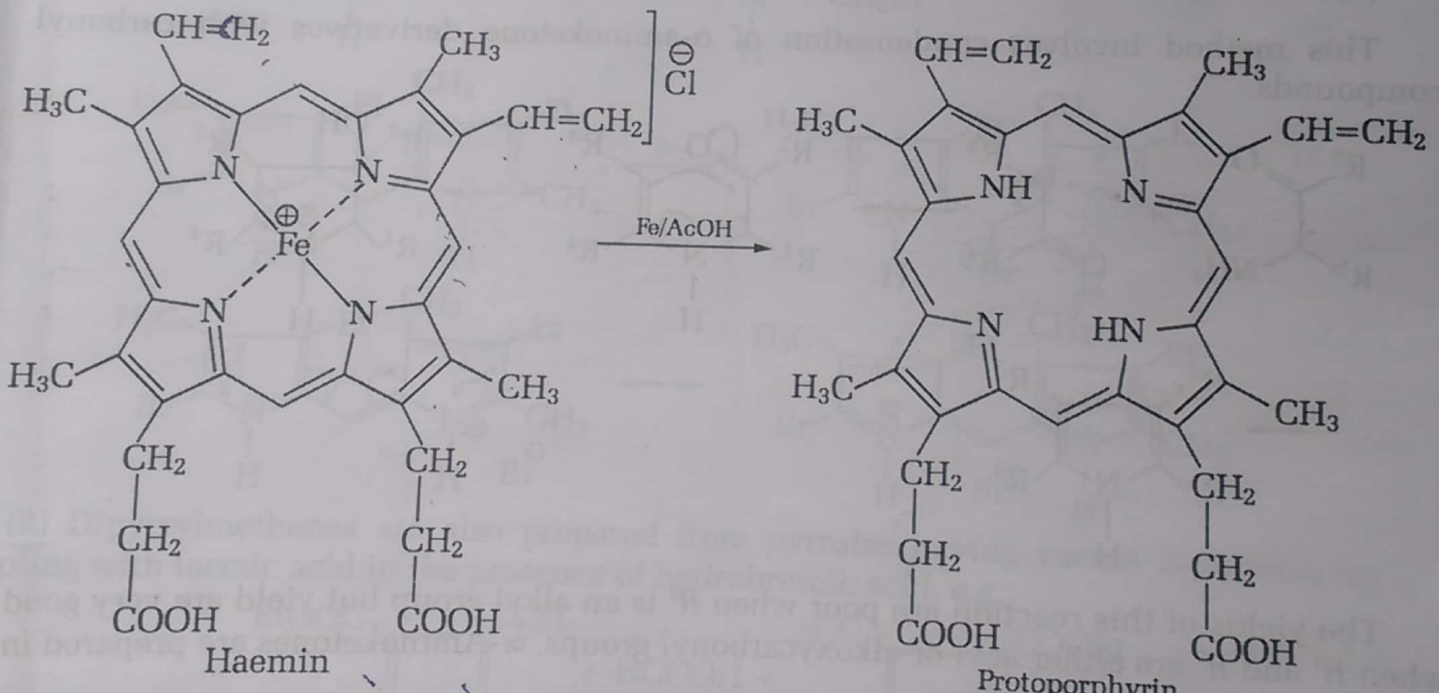
Mesoporphyrin obtained from haemin

The structure of aetioporphyrin was confirmed based on the structure of mesoporphyrin.



Aetioporphyrin obtained by decarboxylation of mesoporphyrin X which is obtained from haemin.

Since mesoporphyrin X is derived from protoporphyrin by reduction ($-CH=CH_2 \rightarrow CH_2-CH_3$) and protoporphyrin is obtained from haemin by removal of iron, the structure of protoporphyrin and haemin may be represented as follows :



Haemin

Protoporphyrin

(8) Finally the structures of haemin and haemoglobin have been confirmed by their synthesis.

6.3. SYNTHESIS OF HAEMOGLOBIN

Synthesis of haemoglobin involves following four stages :

1. Synthesis of Pyrrole derivatives.
2. Conversion of Pyrroles to Dipyrrylmethenes.

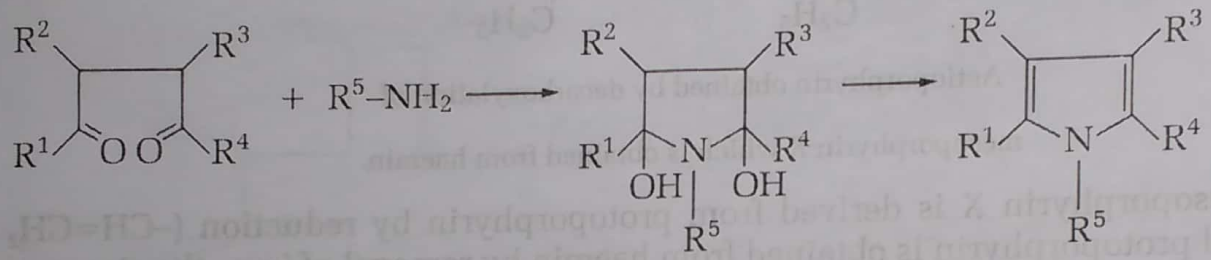
3. Conversion of Dipyrrylmethenes to Porphyrins.
4. Preparation of Haemoglobin from Porphyrin.

6.3.1. Synthesis of Pyrrole Derivatives

There are several methods available for the preparation of desired pyrrole derivatives. Few important methods are given here.

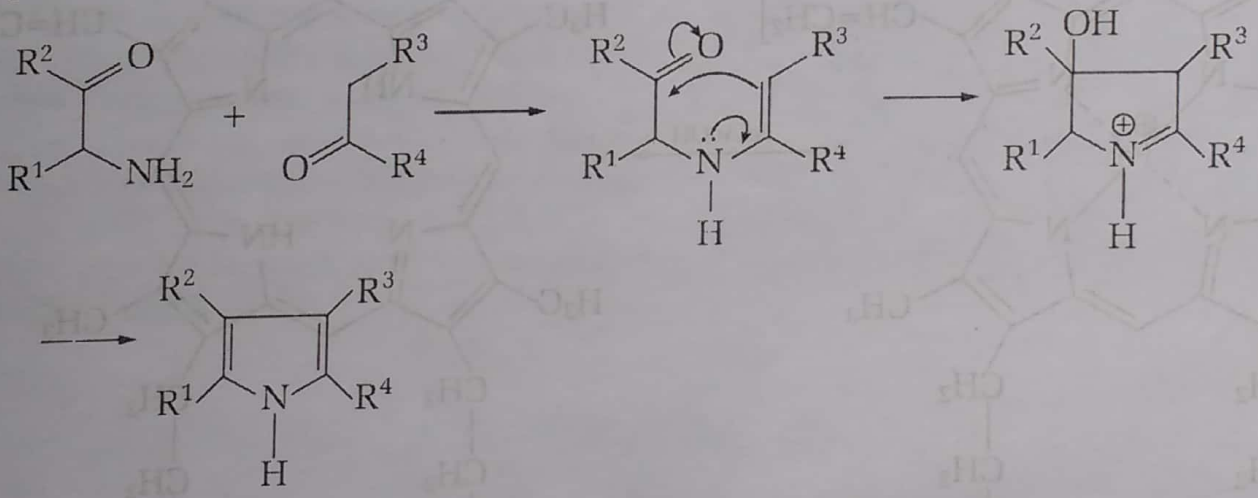
(1) Paal Knorr Synthesis

Treatment of a 1, 4-dicarbonyl compound with primary amine (or ammonia) gives pyrrole.

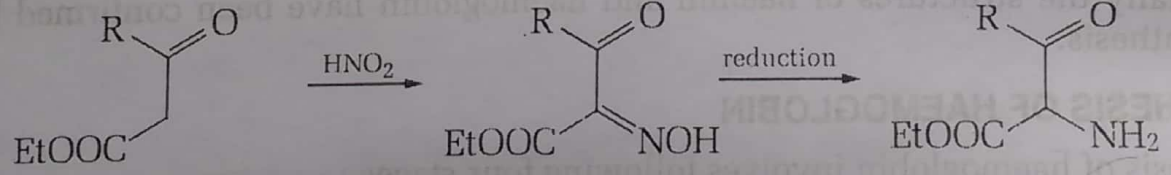


(2) Knorr Pyrrole Synthesis

This method involves condensation of α-aminoketone derivatives with carbonyl compounds.

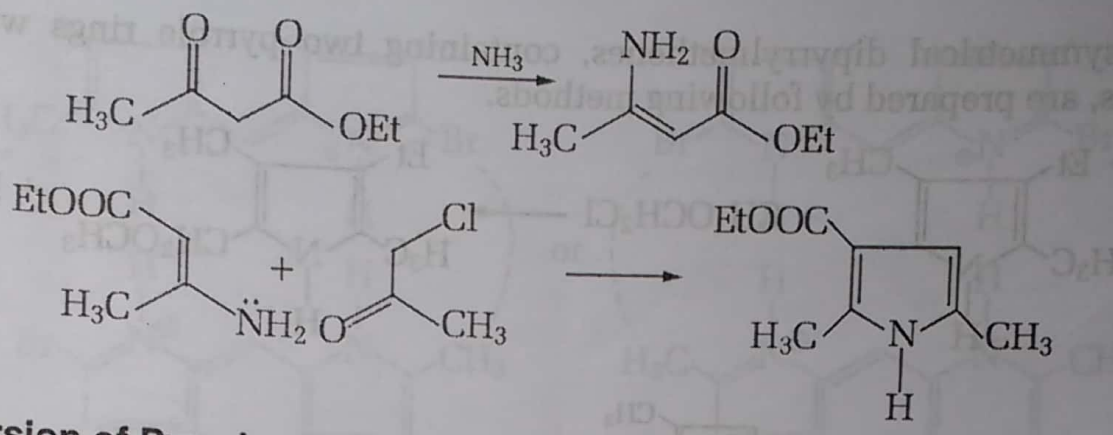


The yields of this reaction are poor when R^3 is an alkyl group but yield are very good when R^1 and R^3 are either acyl or alkoxycarbonyl groups. ∝-Aminoketones are prepared *in situ* in the following manner.



(3) Hantzsch Synthesis

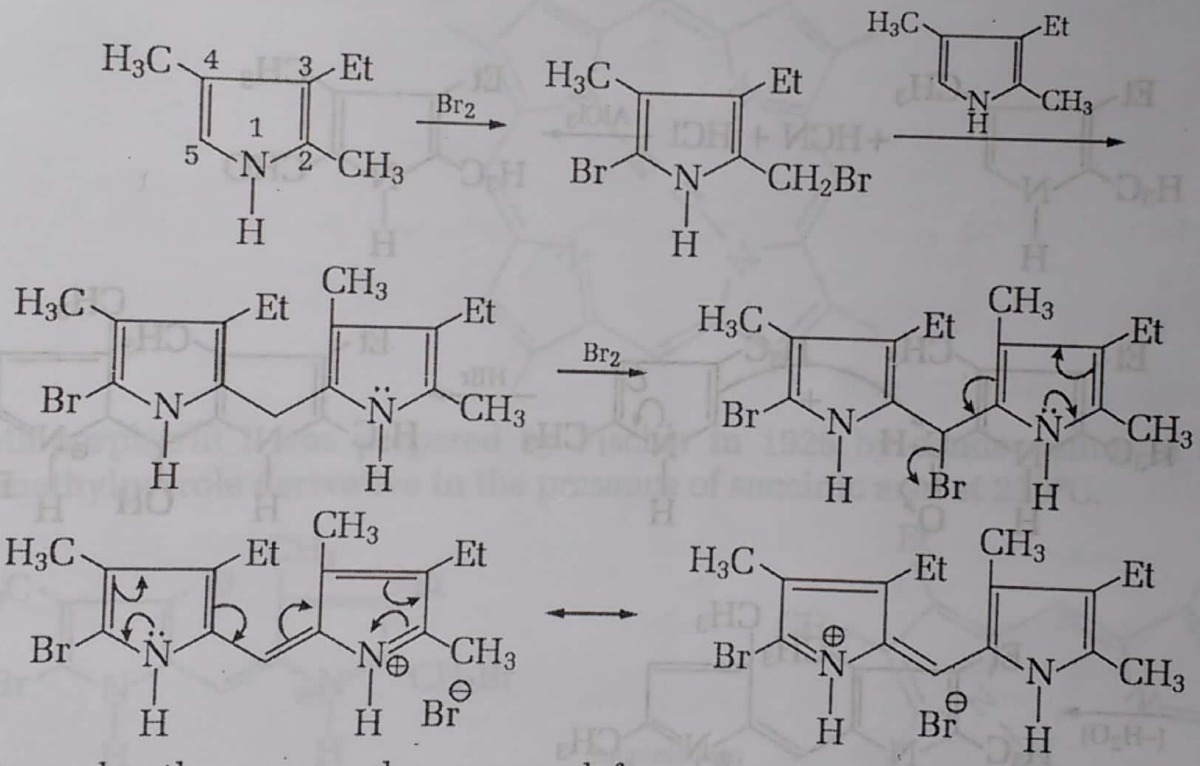
This involves condensation of a chloroacetone derivative, a β-ketoester and a primary amine, for example;



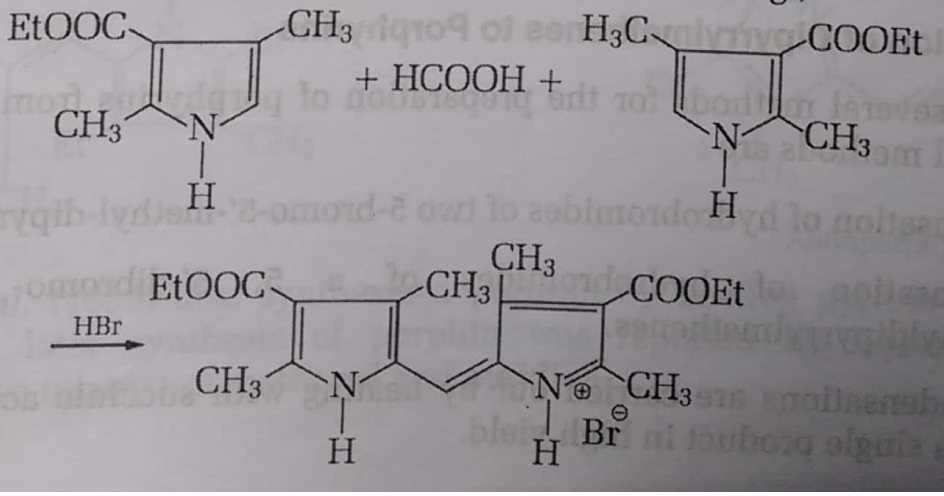
6.3.2. Conversion of Pyrroles to Dipyrrylmethenes

Dipyrrylmethene may be obtained from pyrroles by any of the following methods :

(1) 2-Methylpyrrole derivative having unsubstituted 5-position are converted to dipyrrylmethene by bromination, for example :

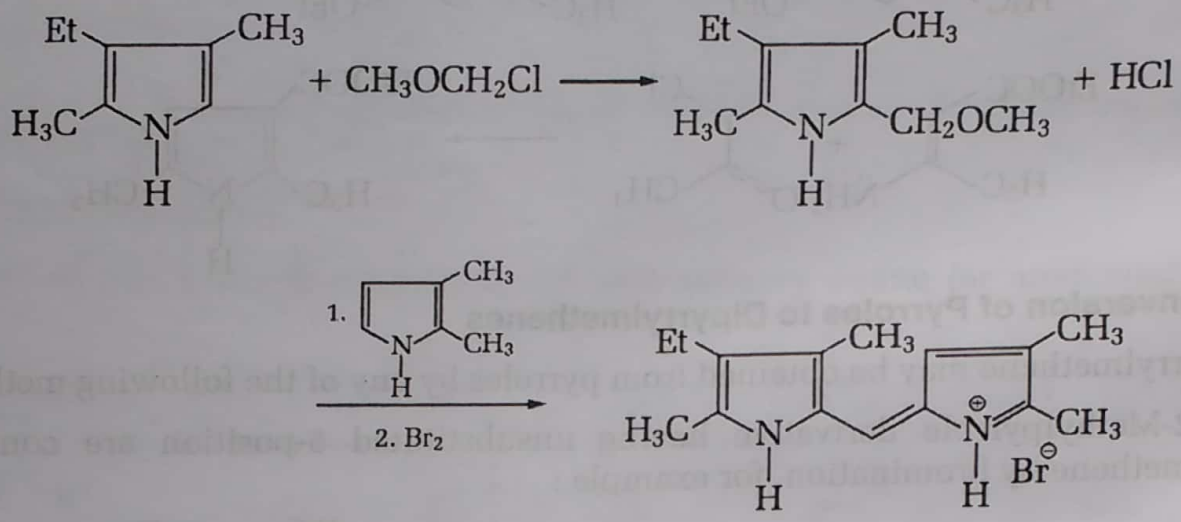


(2) Dipyrrylmethenes are also prepared from pyrroles having vacant 5-position by coupling with formic acid in the presence of hydrobromic acid, e.g.,

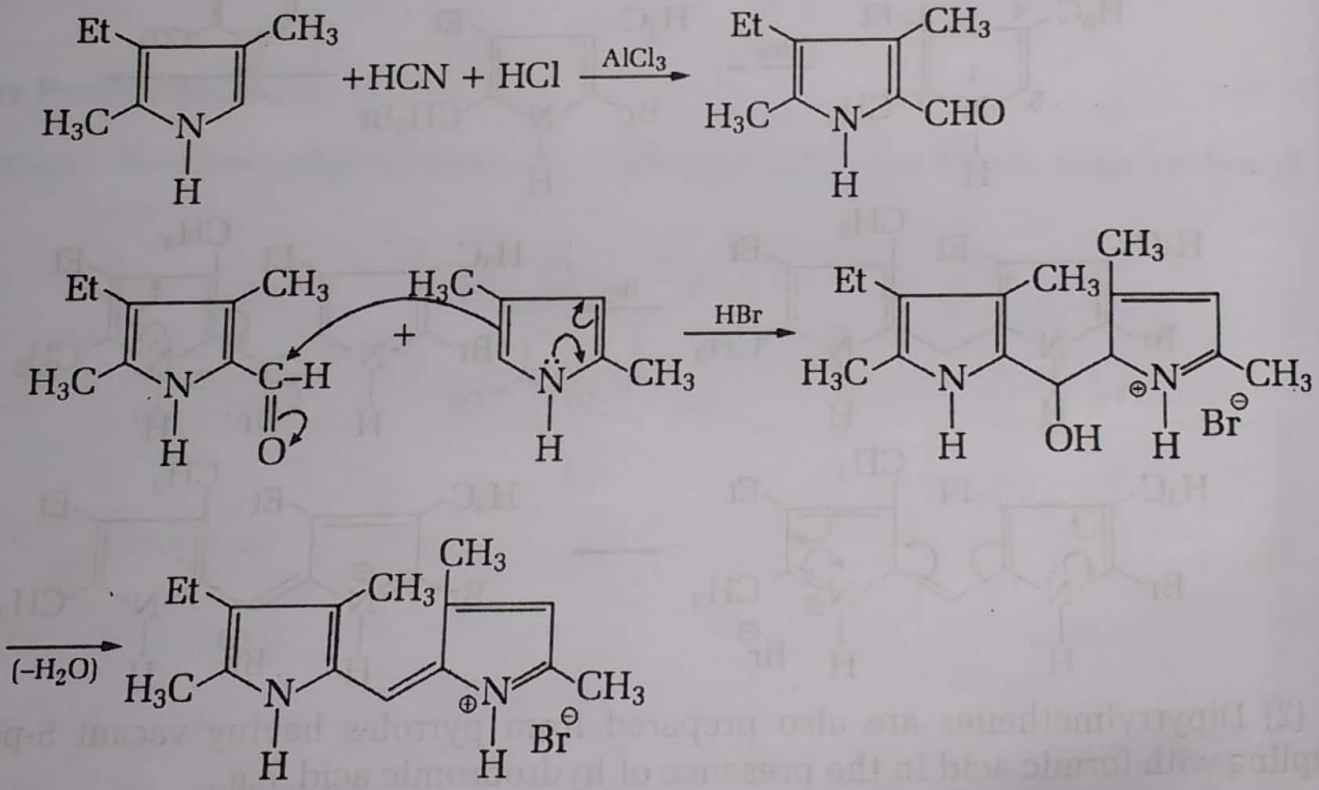


(3) Unsymmetrical dipyrrylmethenes, containing two pyrrole rings with differe substituents, are prepared by following methods.

(a)



(b)



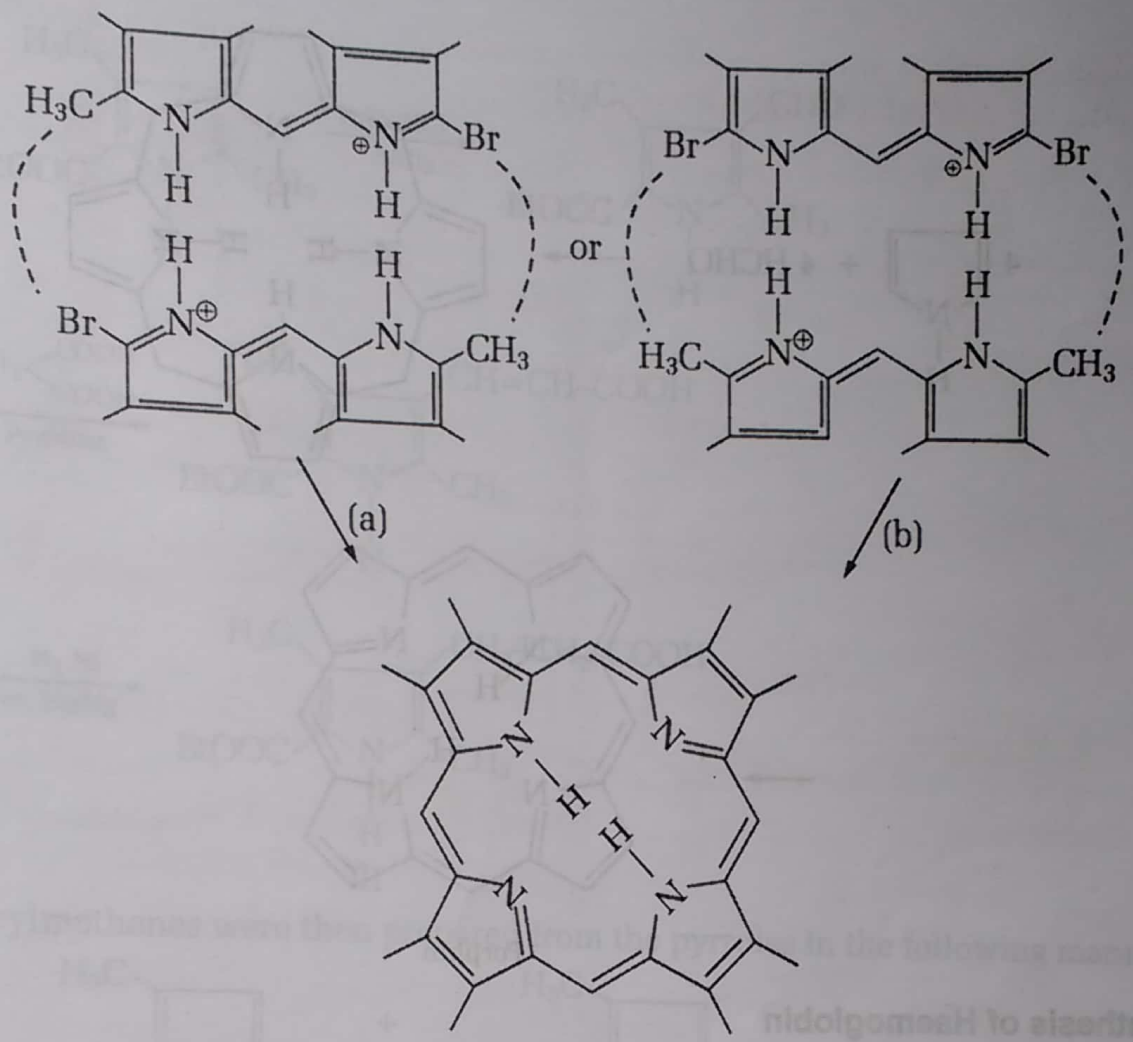
6.3.3. Conversion of Dipyrrylmethenes to Porphyrins

There are several methods for the preparation of porphyrins from dipyrrylmethene but most useful methods are :

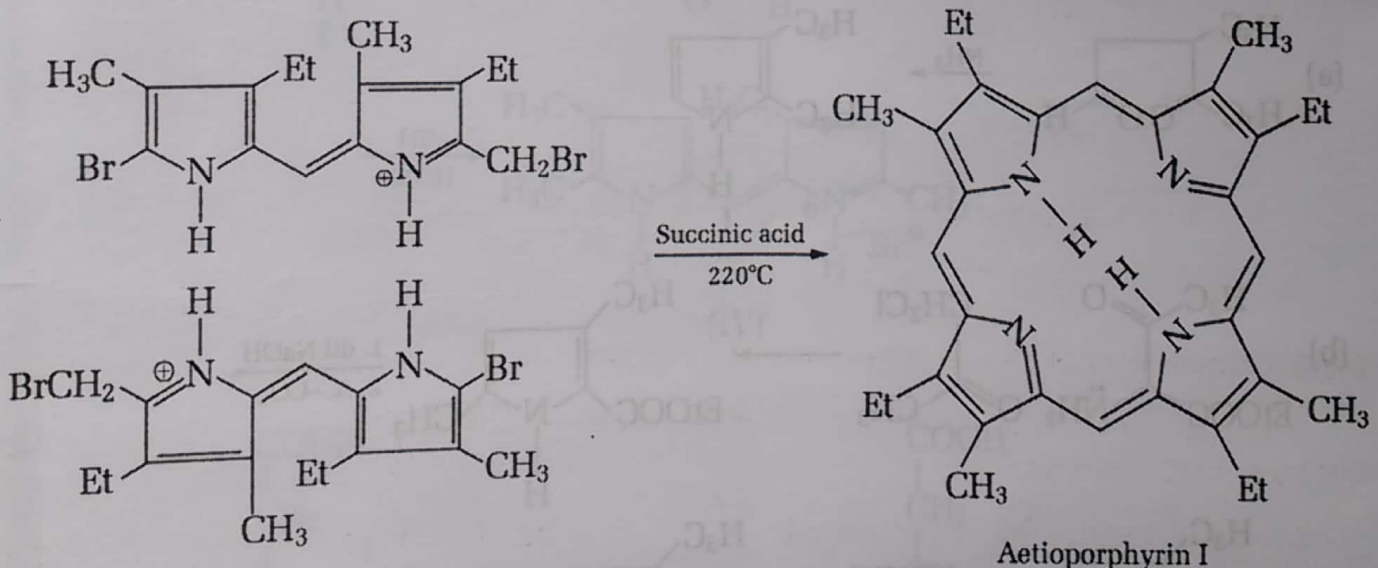
(a) Condensation of hydrobromides of two 5-bromo-5′-methyl-dipyrrylmethenes and

(b) Condensation of hydrobromides of a 5, 5′-dibromo- and a 5, 5 dimethyldipyrrylmethenes.

These condensations are carried out by heating with succinic acid at 220°C. Thes methods give a single product in high yield.

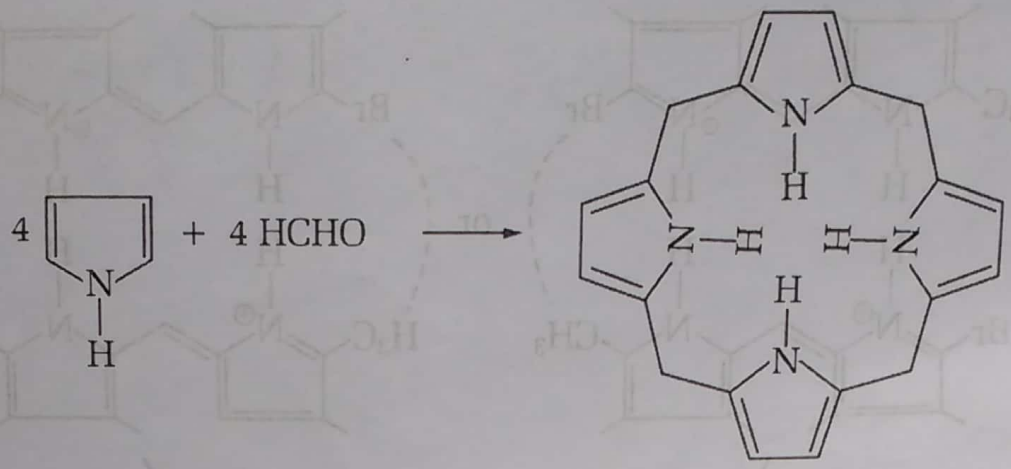


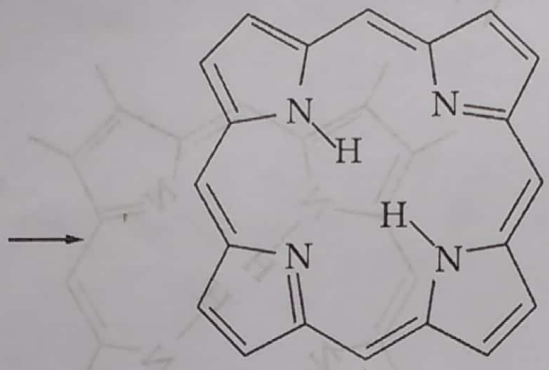
Aetioporphyrin I was prepared by Fischer in 1926 by condensation of 5-bromo-5′-bromomethylpyrrole derivative in the presence of succinic acid at 220°C.



Aetioporphyrin I

Fischer *et. al.* (1939) also synthesized porphin by heating pyrrole-2-aldehyde with formic acid. A later synthesis of porphin was reported by heating pyrrole with formaldehyde in a mixture of methanol and pyridine.



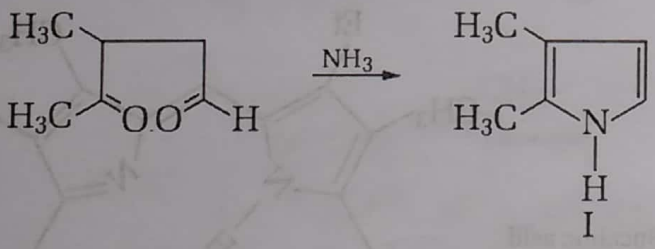


Porphin

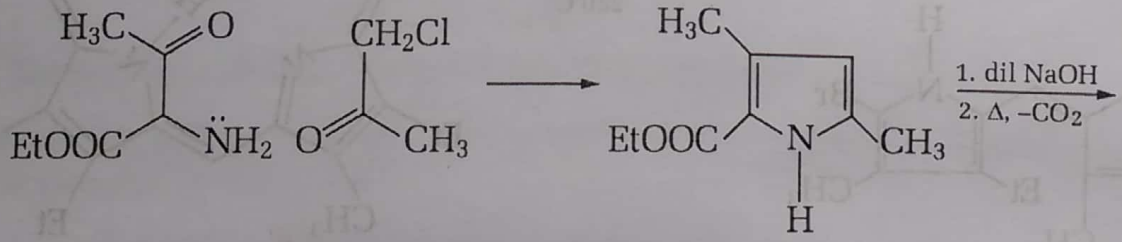
6.3.4. Synthesis of Haemoglobin

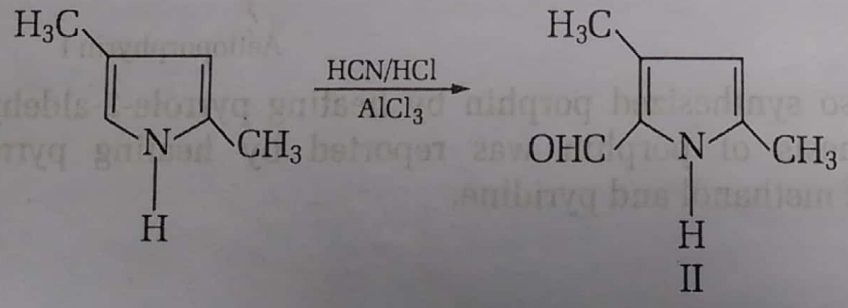
Pyrroles containing methyl groups and propionic acid residue in desired position could be obtained in the following manner by methods described earlier.

(a)

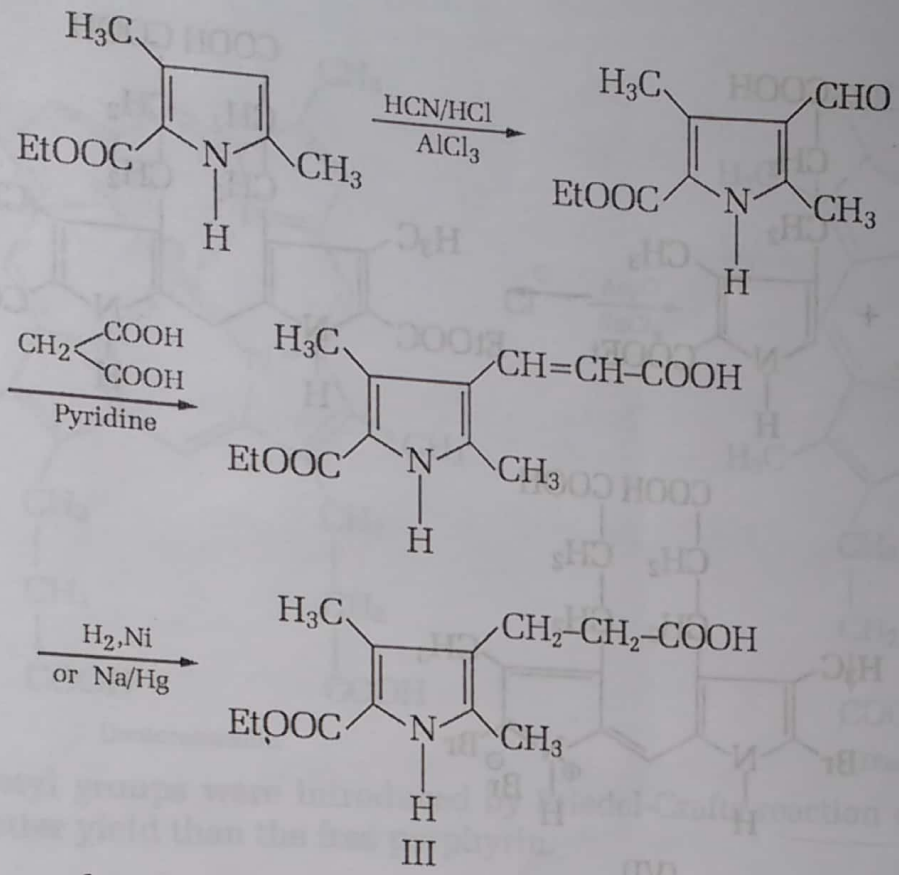


(b)



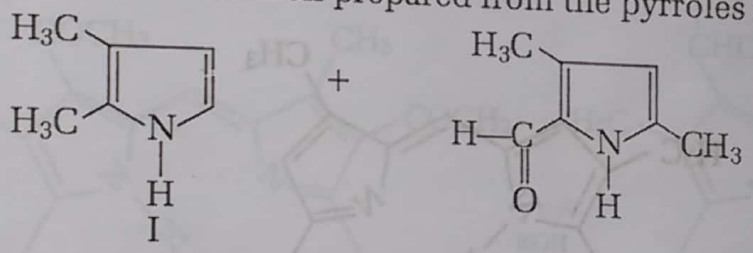


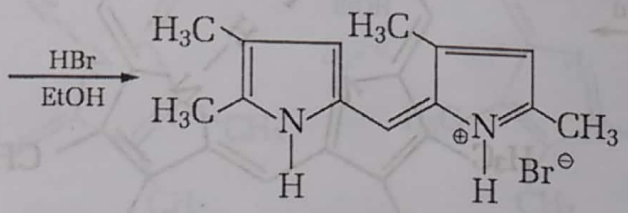
(c)



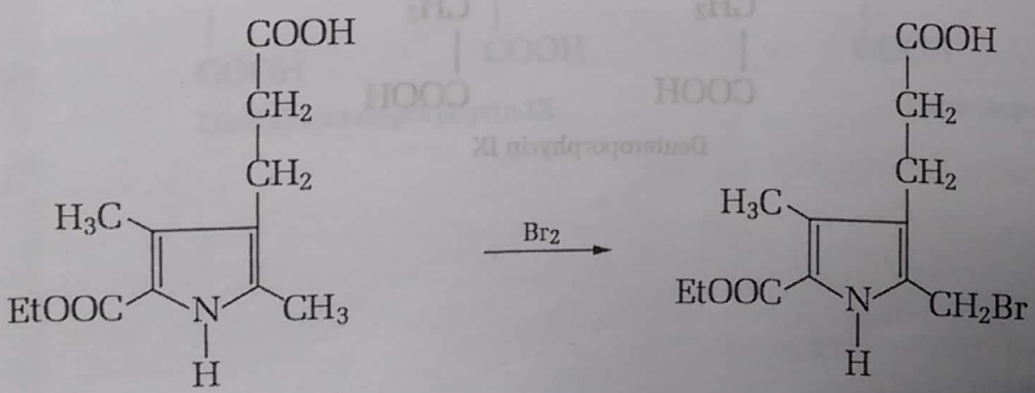
III

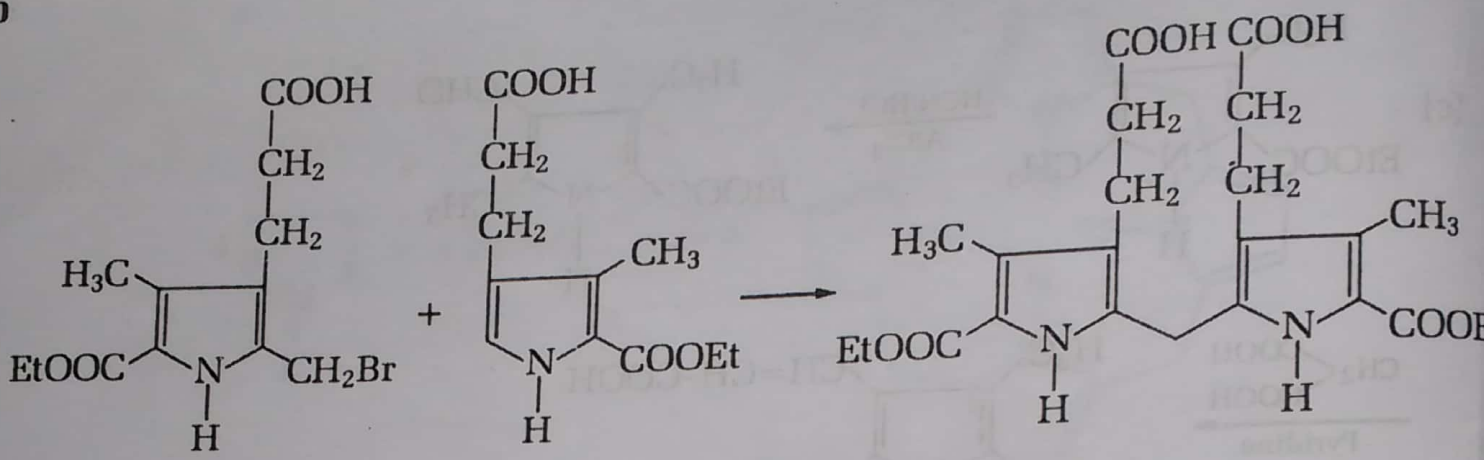
Dipyrrylmethenes were then prepared from the pyrroles in the following manner.

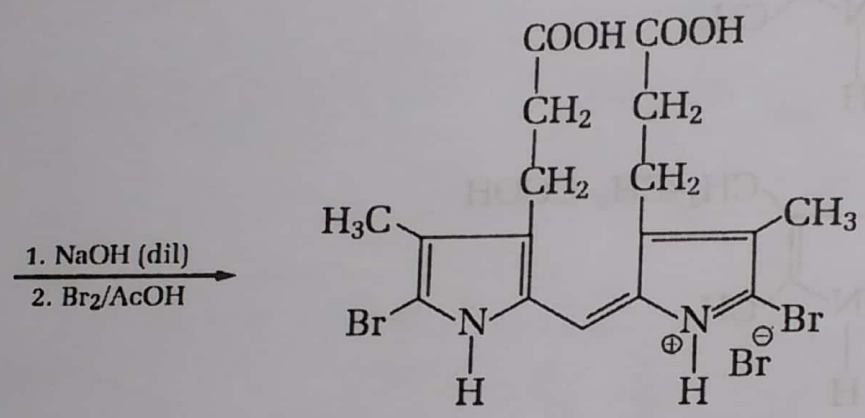




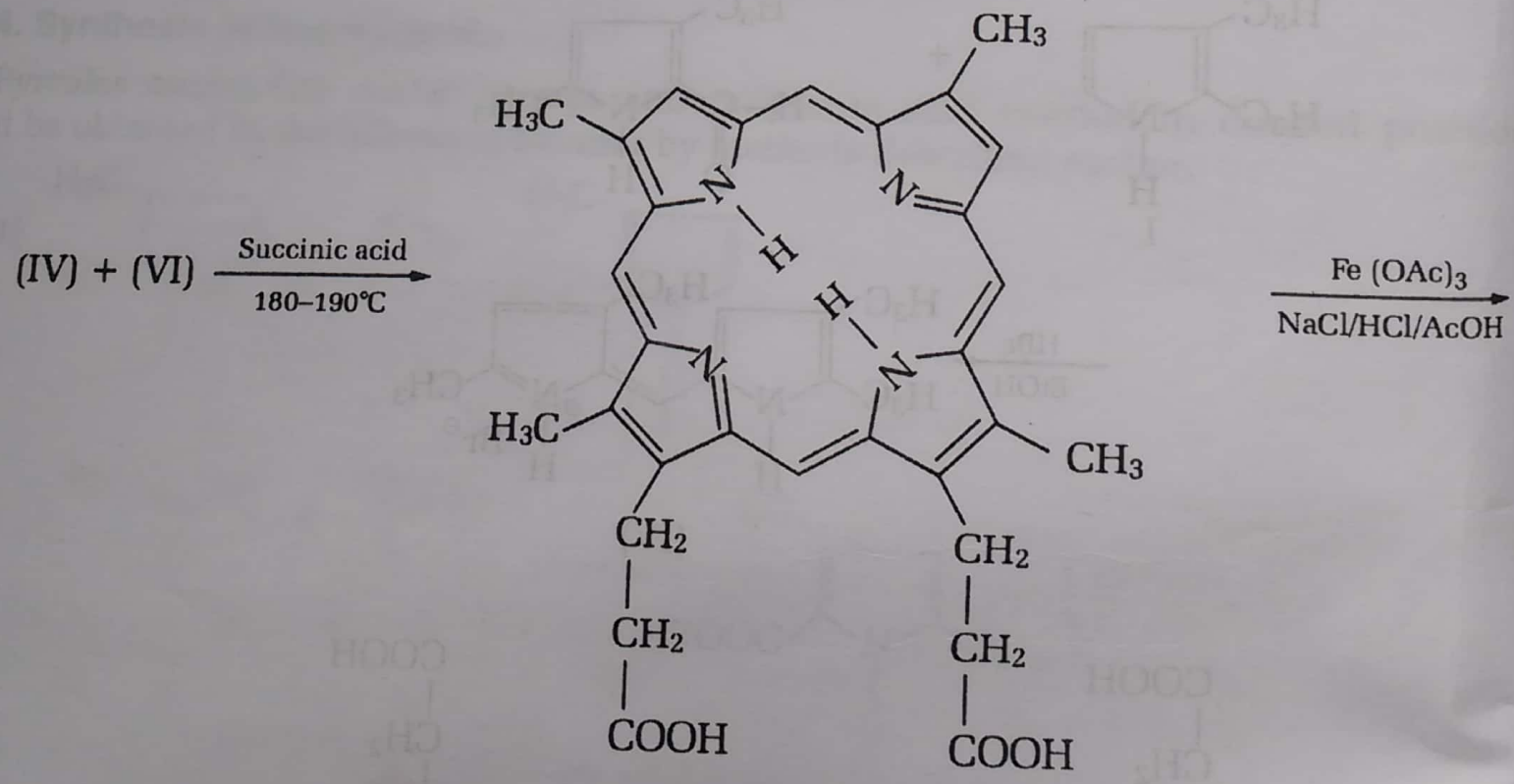
(IV)



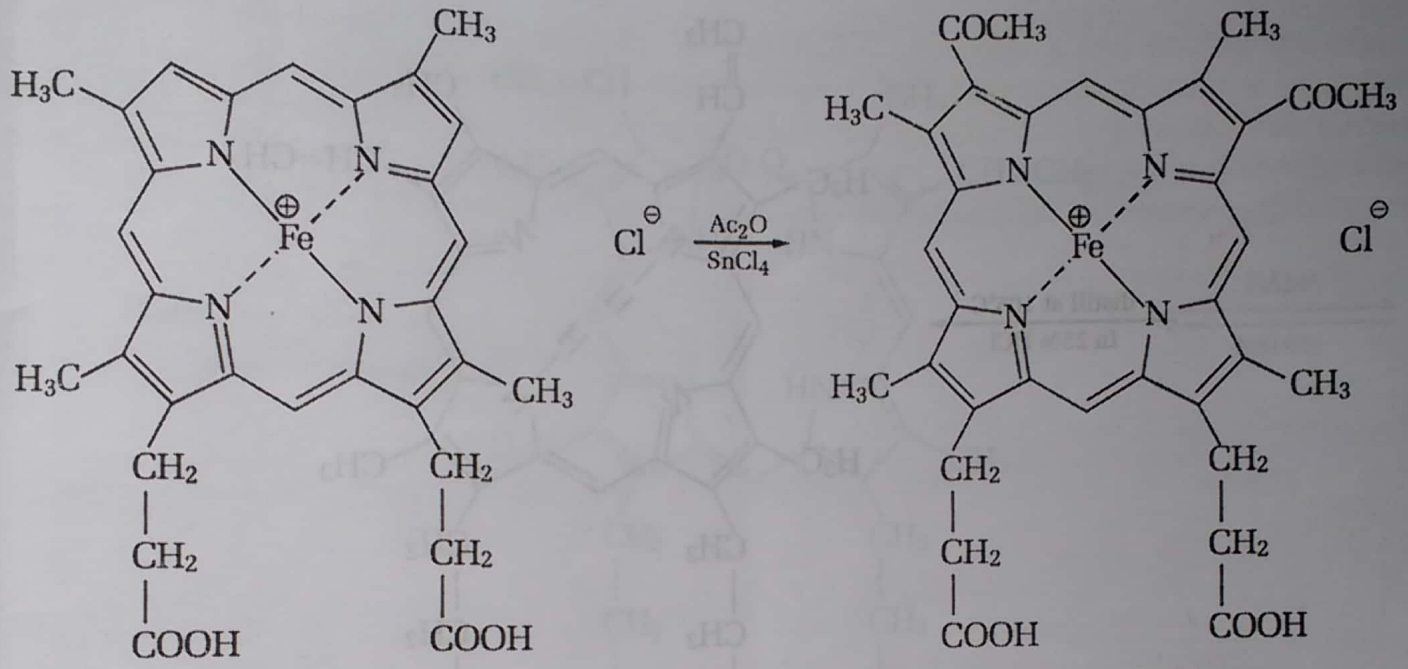




(VI)

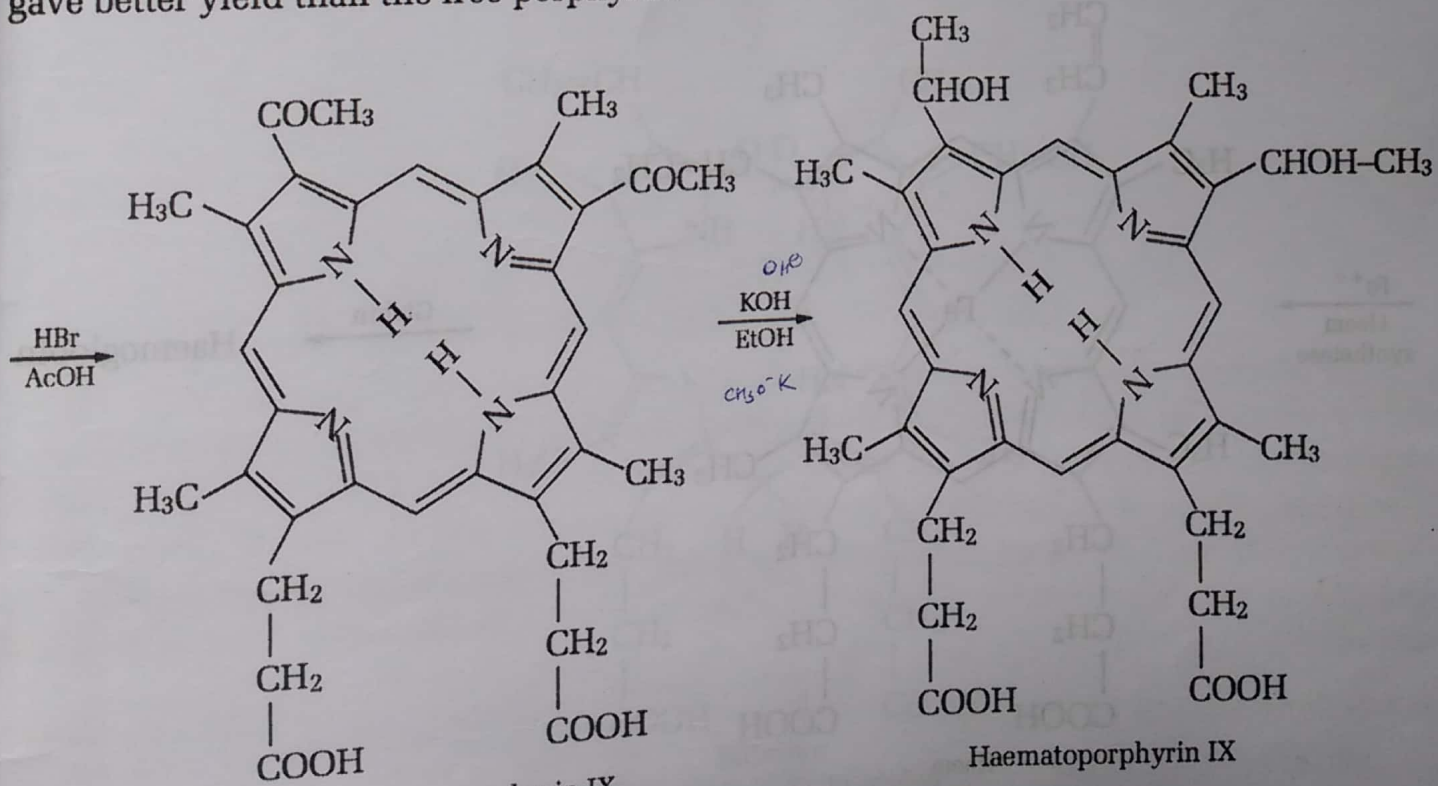


Deuteroporphyrin IX

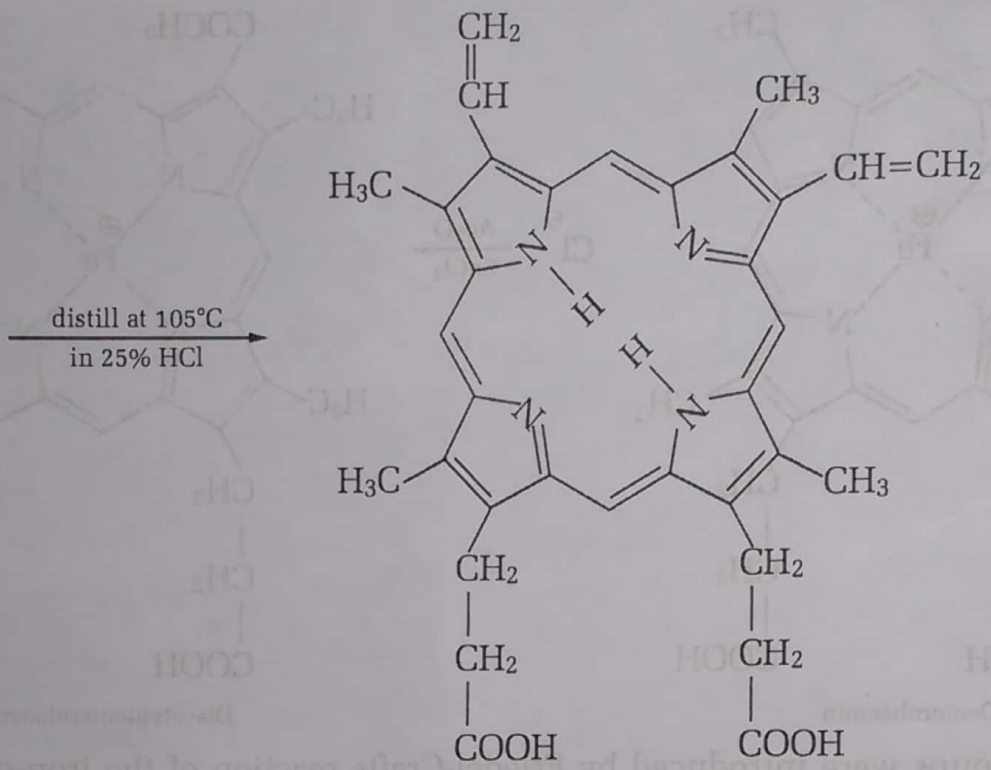


Deuterohaemin — Diacetyldeuterohaemin

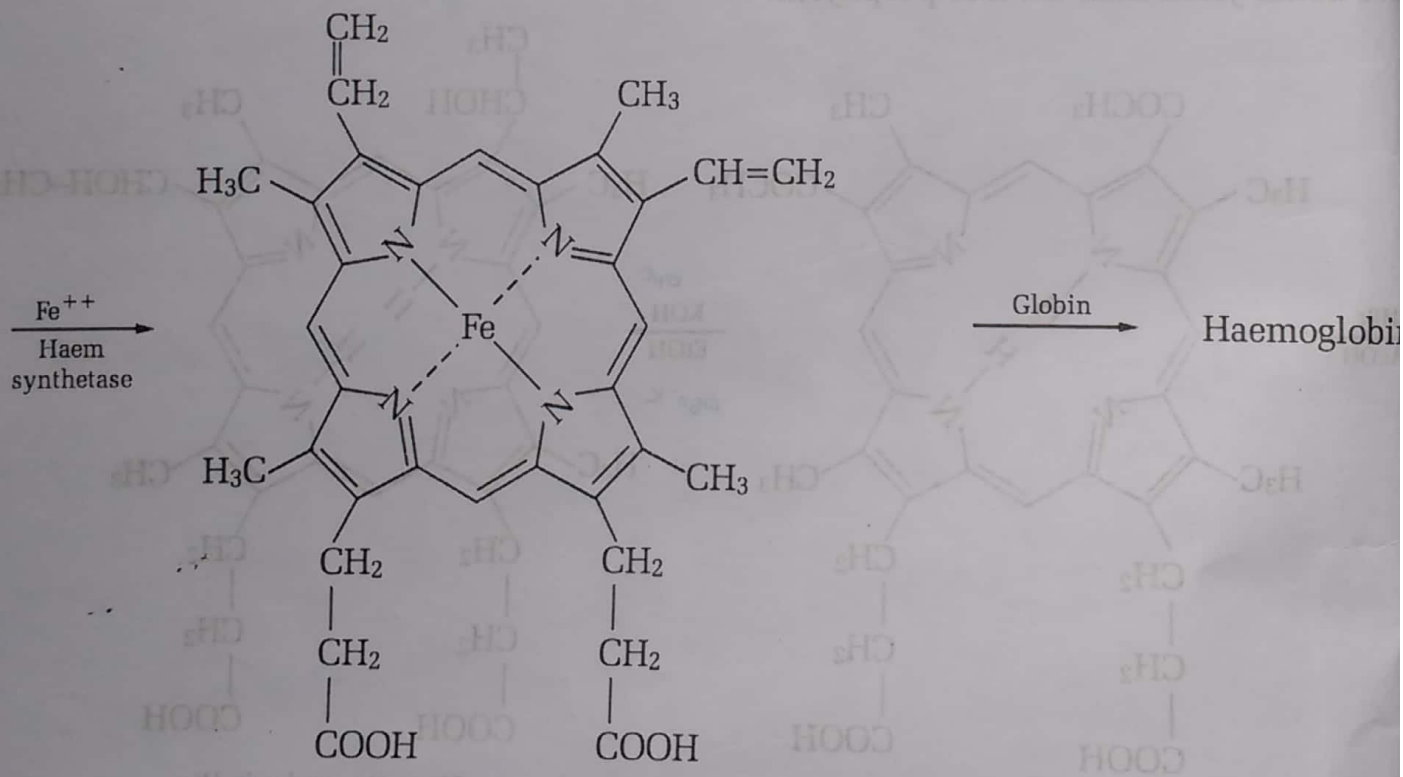
Acetyl groups were introduced by Friedel-Crafts reaction of the iron-complex which gave better yield than the free porphyrin.



Dicetyldeuteroporphyrin IX — Haematoporphyrin IX



Protoporphyrin IX

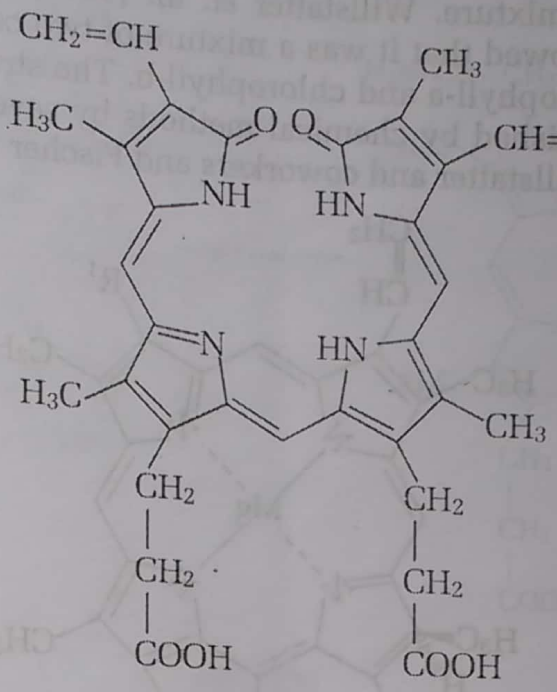


Haem

6.4. Metabolism of Haemoglobin in Mammals

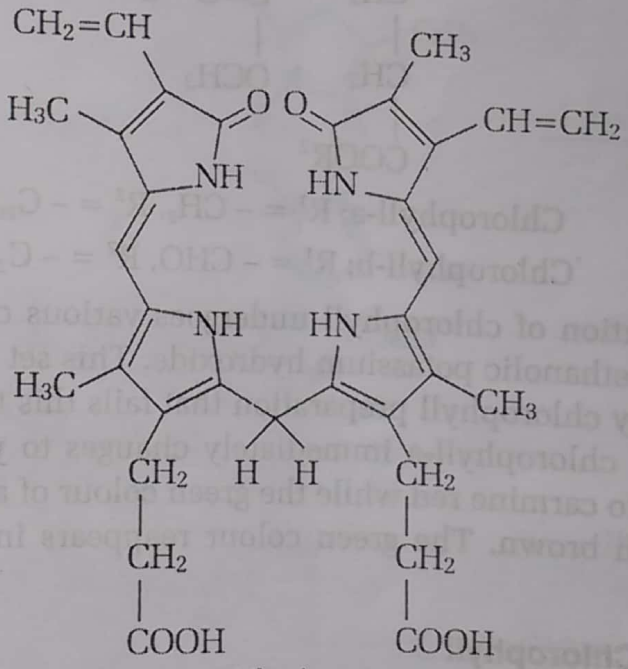
Haem undergoes oxidation in the presence of ascorbate to give biliverdin by the los of Fe atom. Biliverdin is immediately converted by enzymatic reduction to bilirubi which is a yellow pigment. The excessive heam breakdown results in excessive bilirubi formation. Excess bilirubin accumulates in the skin and tissues imparting yellow colou This condition is known as jaundice.

Haem $\xrightarrow[\text{Fe}]{O_2 \quad CO}$ Oxidative cleavage



$\underset{\text{NADPH}}{\overset{\text{NADP}}{\rightleftharpoons}}$

Biliverdin



Bilirubin

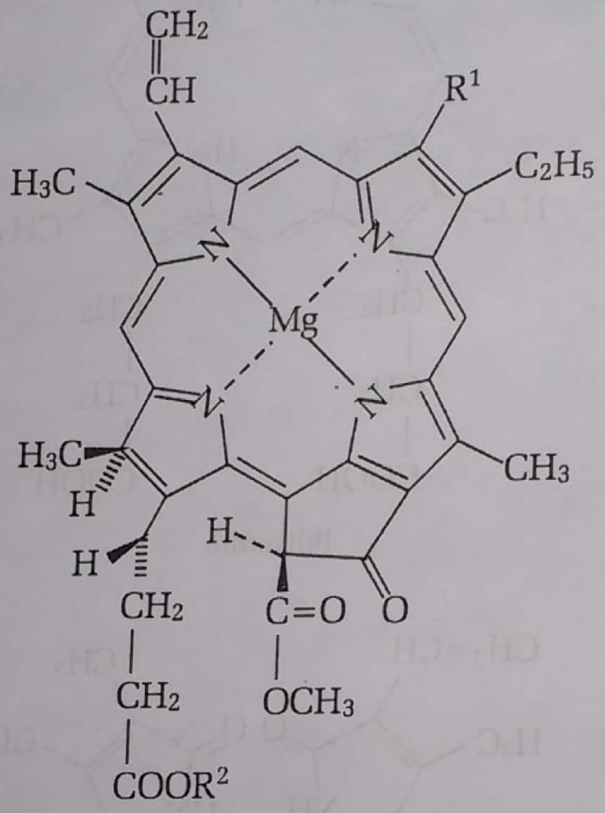
Metabolism of haemoglobin

6.5. Chlorophyll

Chlorophyll is the green colouring material found in leaves and green stems. It absorbs light which is used by plants to synthesize carbohydrates, proteins and fats and the process is known as photosynthesis.

The term 'chlorophyll' was coined by Pelletier and Caventou (1818) for the green pigment found in plant leaves. It was shown by Stokes (1864) that chlorophyll was not a

single compound but a mixture. Willstatter *et. al.* (1912) obtained chlorophyll from variety of sources and showed that it was a mixture of two compounds. He isolated ther and named these as chlorophyll-a and chlorophyll-b. The structures of chlorophyll-a an chlorophyll-b were established by chemical methods by several groups of workers, mos prominent of these are Willstatter and coworkers and Fischer and coworkers.



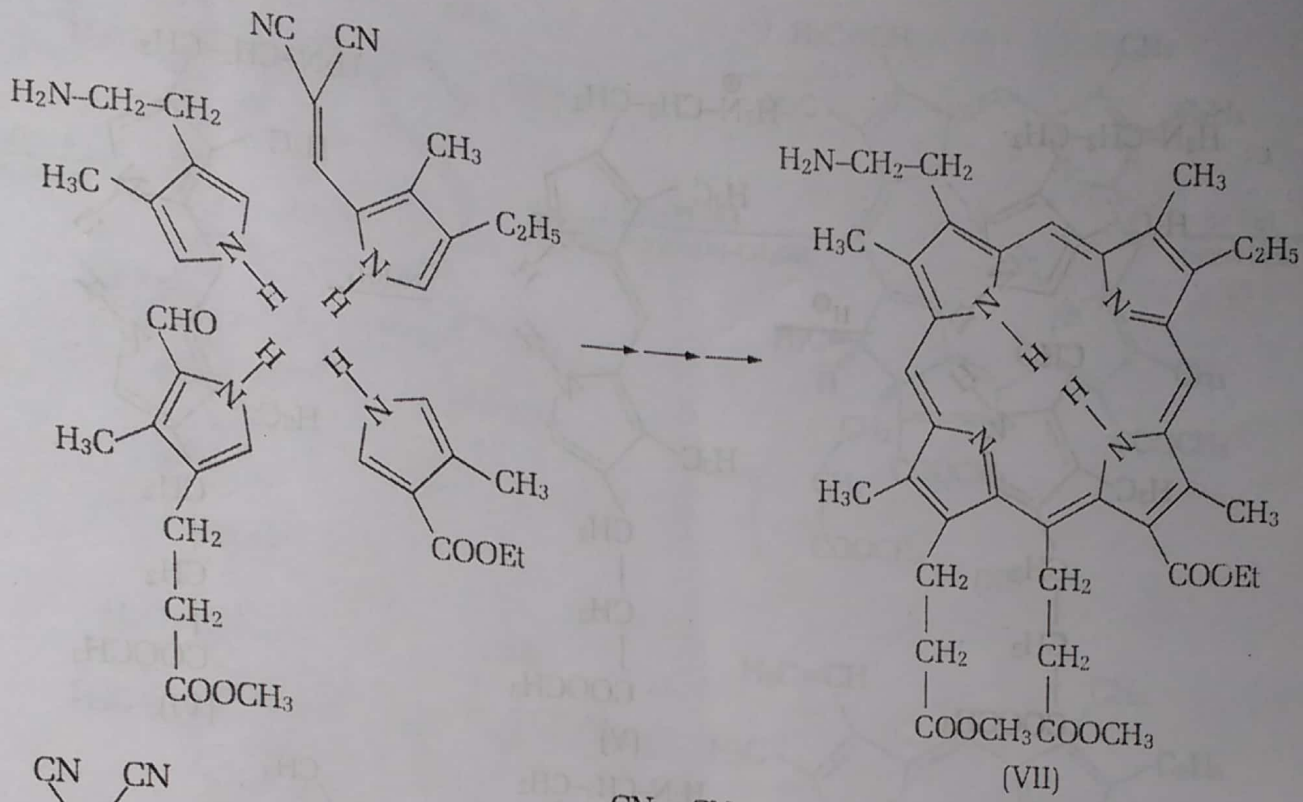
Chlorophyll-a; $R^1 = -CH_3$, $R^2 = -C_{20}H_{39}$

Chlorophyll-b; $R^1 = -CHO$, $R^2 = -C_{20}H_{39}$

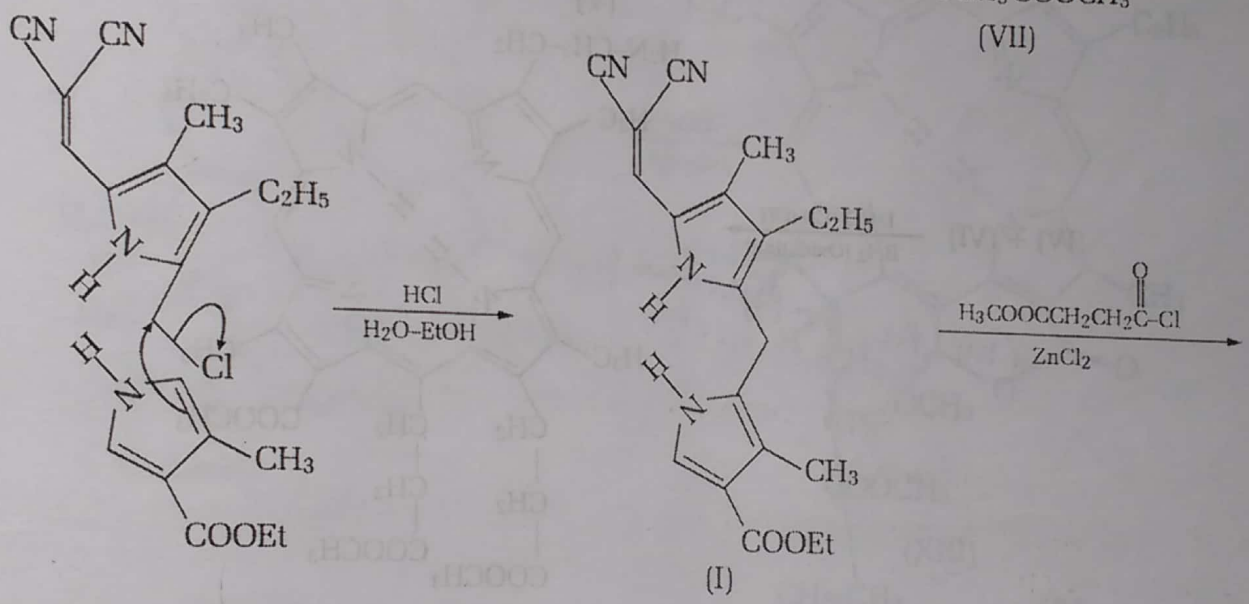
An ethereal solution of chlorophyll undergoes various colour changes when shake with a solution of methanolic potassium hydroxide. This set of colour reactions is know as phase test and any chlorophyll preparation that fails this test is said to be allomerized The green colour of chlorophyll-a immediately changes to yellow, that of chlorophyll-b changes from green to carmine red while the green colour of a mixture of two chlorophyll changes to yellowish brown. The green colour reappears in the lower layer after a few moments.

6.5.1. Synthesis of Chlorophyll

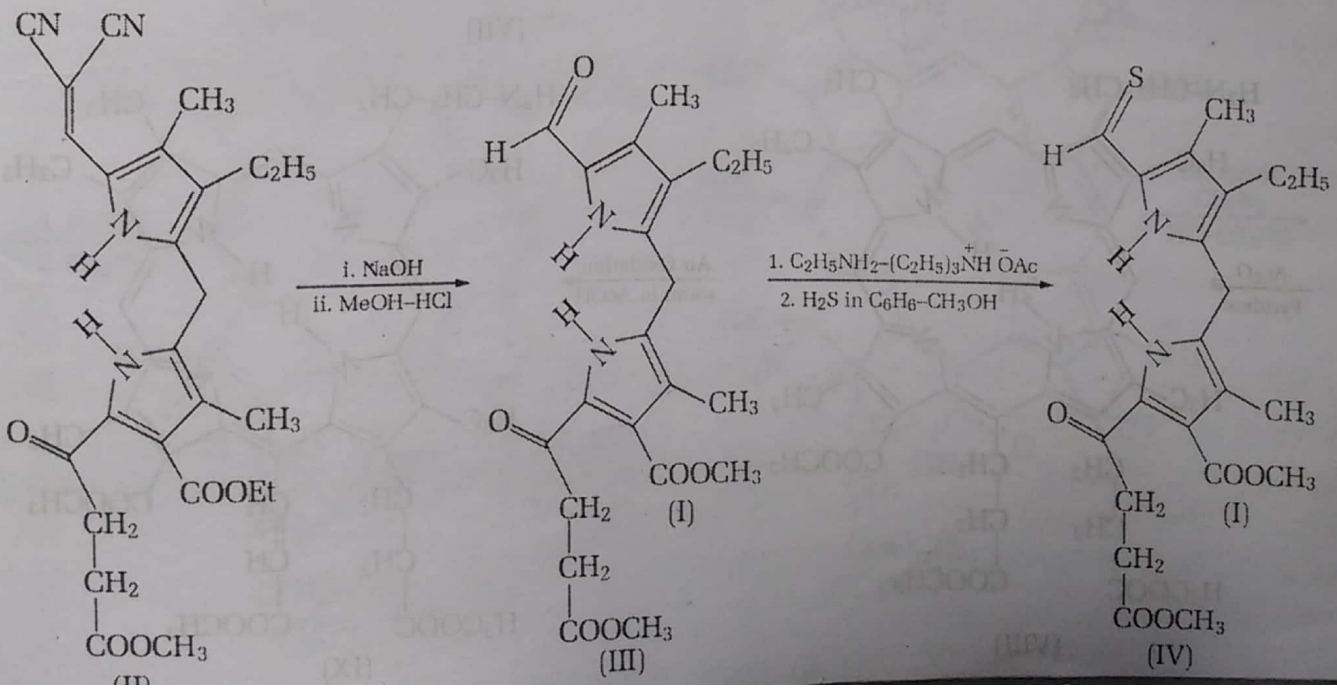
Woodward and coworkers accomplished the total synthesis of chlorophyll in 1960 The Key intermediate chosen for the synthesis was porphyrin (VII), which has desired substituents at the desired positions. The only exception was vinyl group, which was generated at a later stage from aminoethyl group. Porphyrin (VII) was then converted to chlorin (chlorin-e trimethyl ester) (XVI), which had already been converted to chlorophyll-a prior to this synthesis. Porphyrin (VII) was prepared by coupling of four pyrrole derivatives.



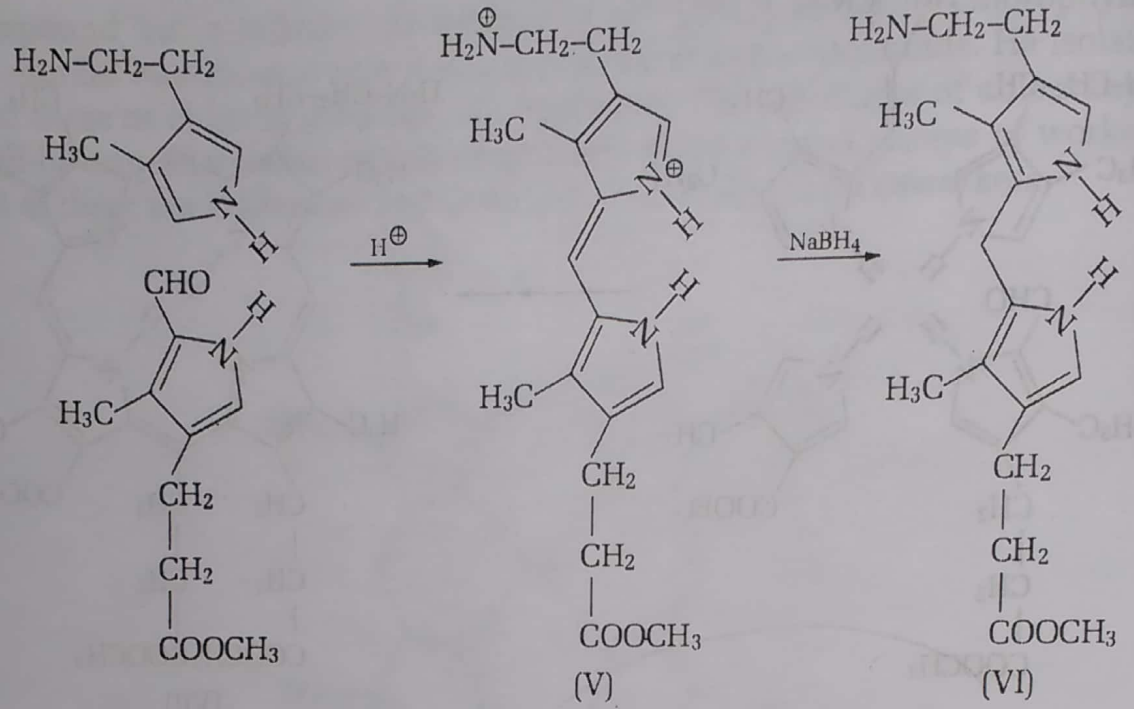
(VII)

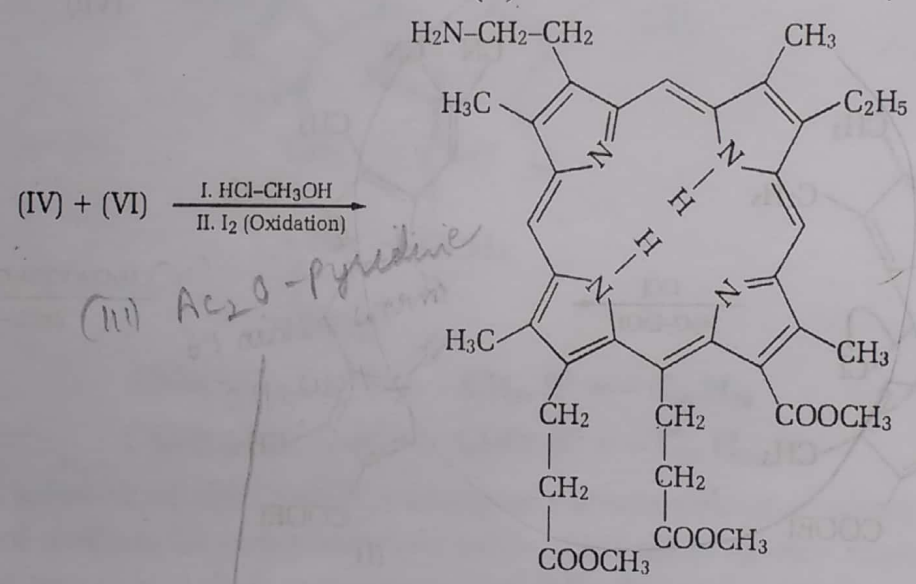


(I)

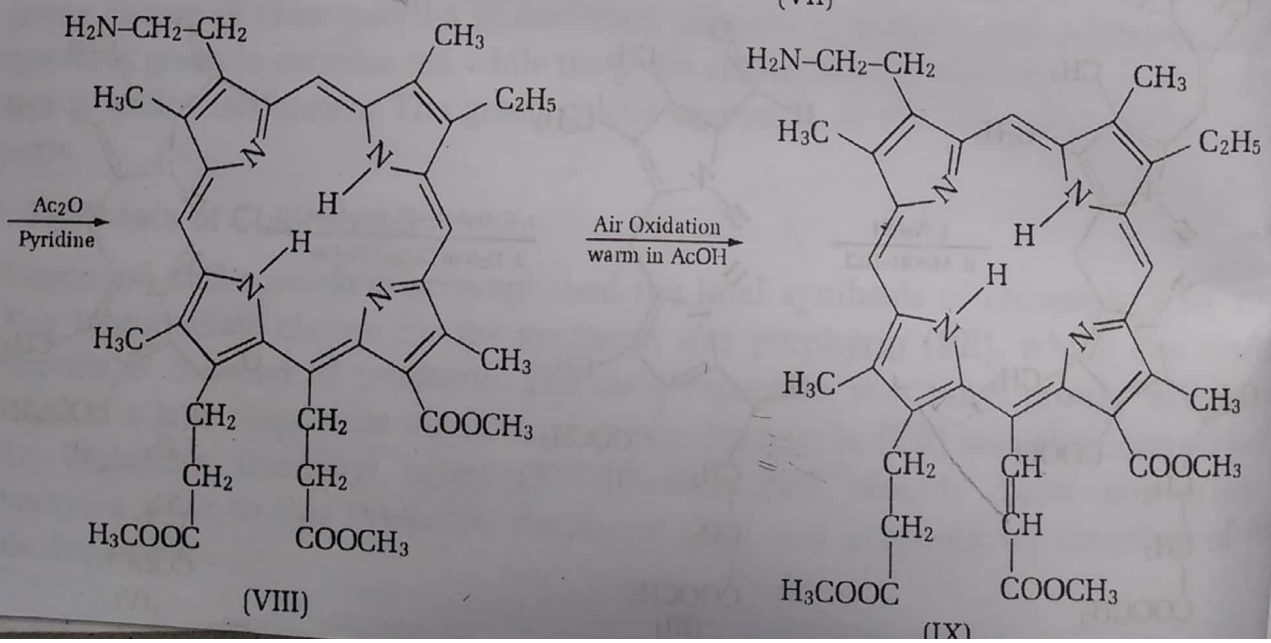


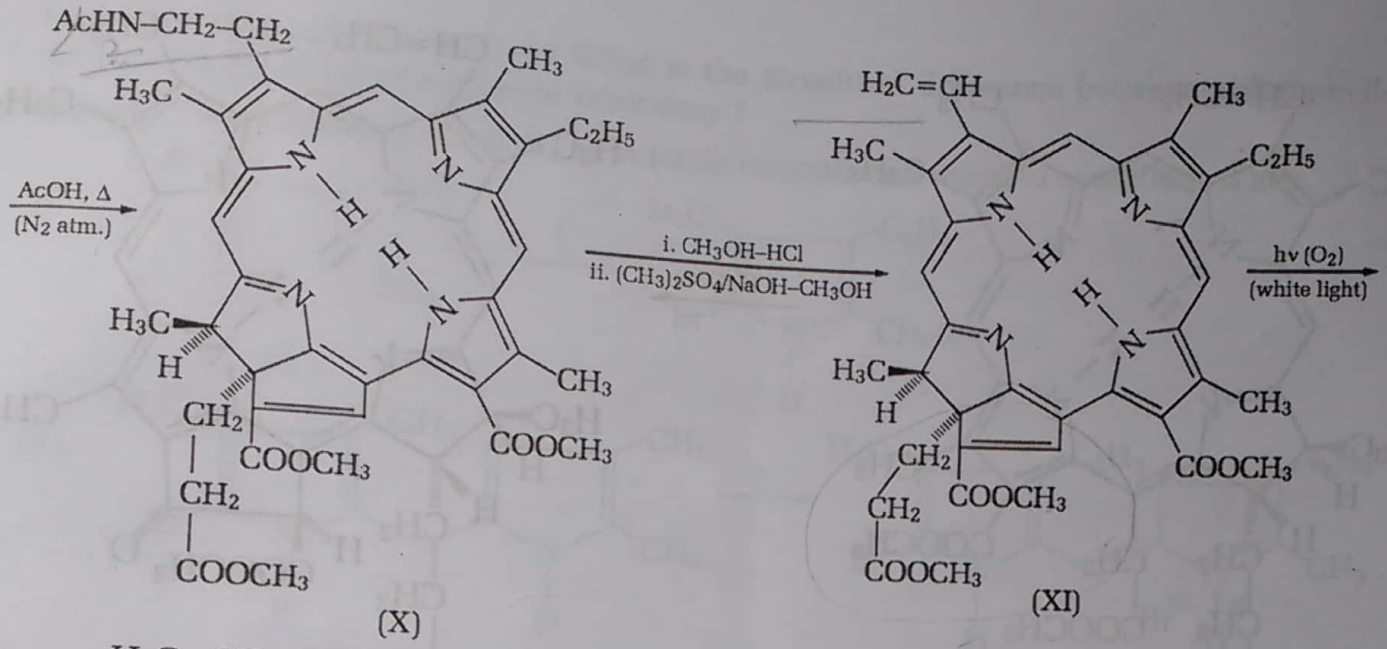
(II) (III) (IV)



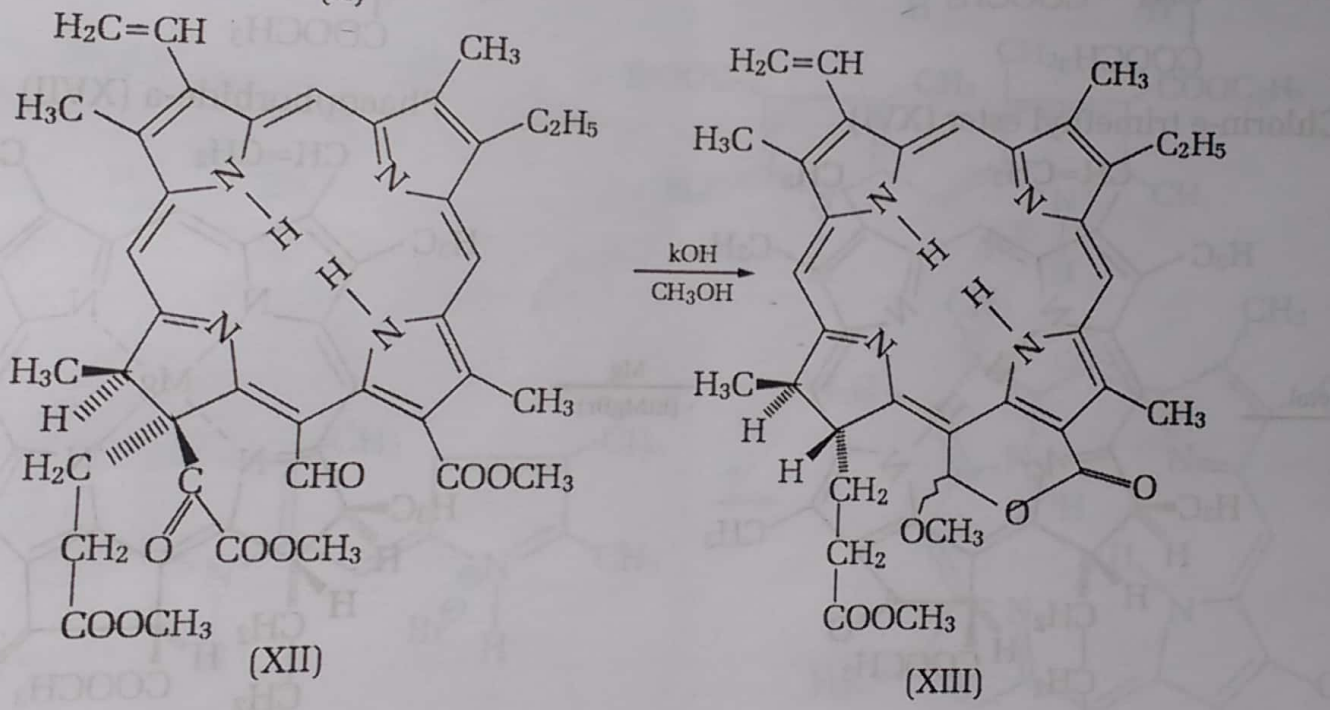


(III) Ac_2O–Pyridine

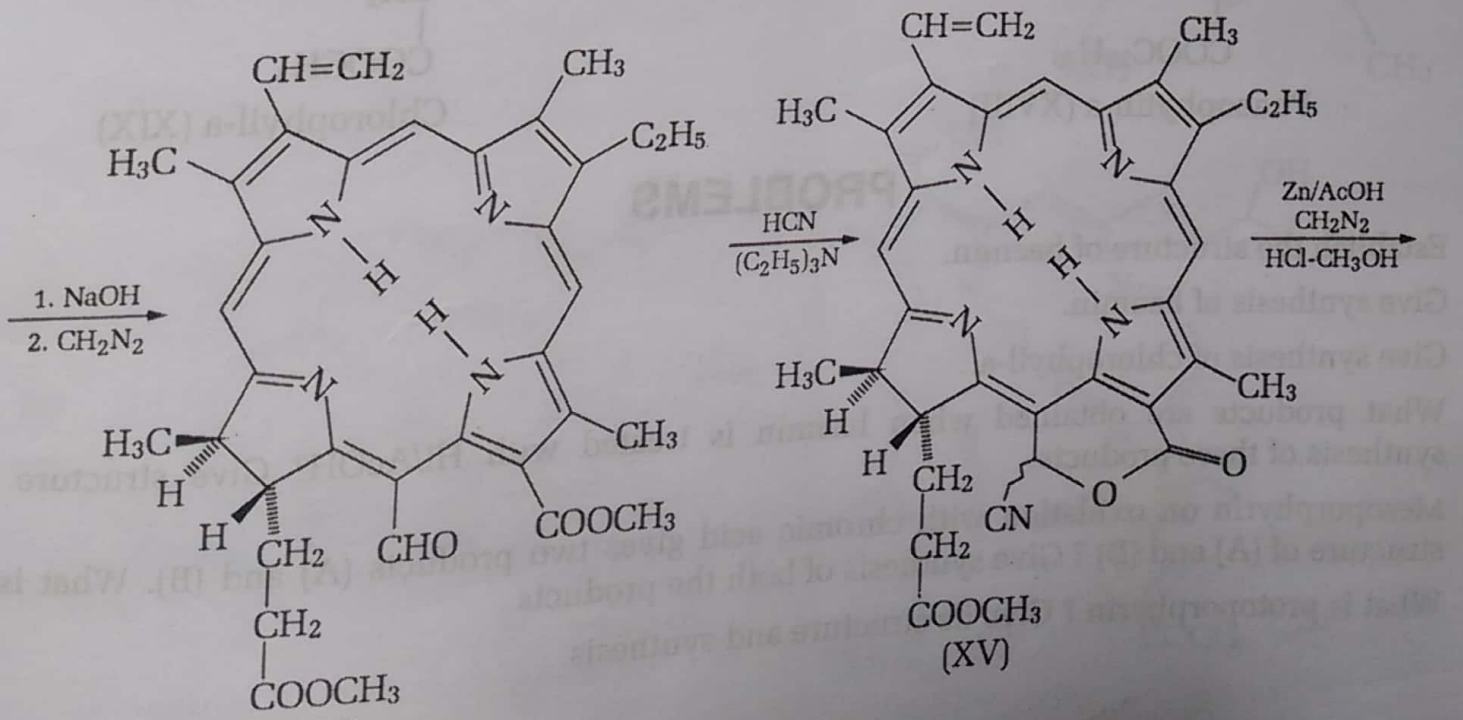




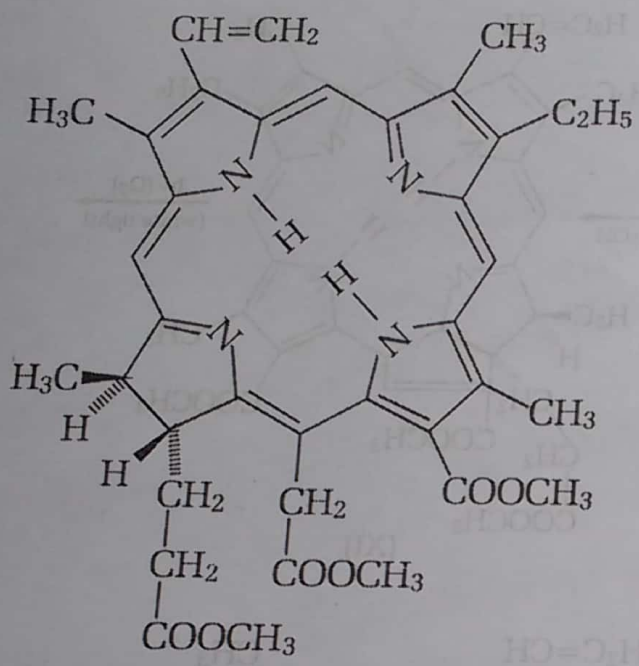
(X) (XI)



(XII) (XIII)

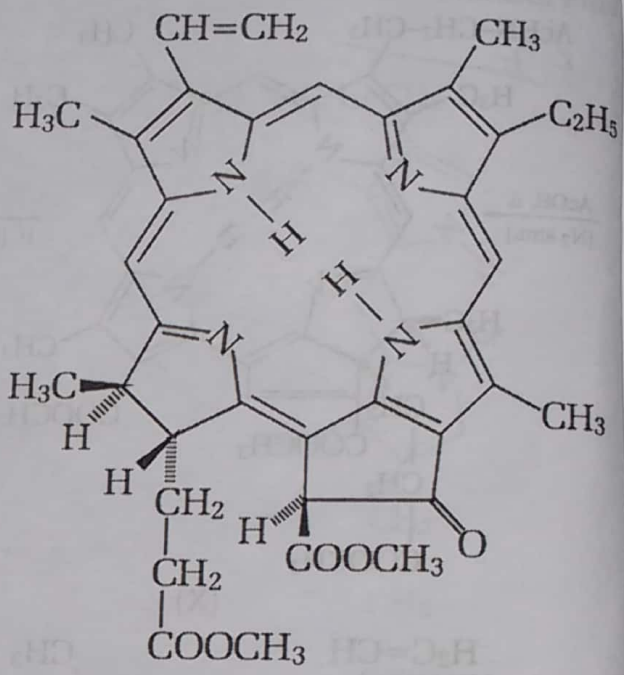


(XIV) (XV)



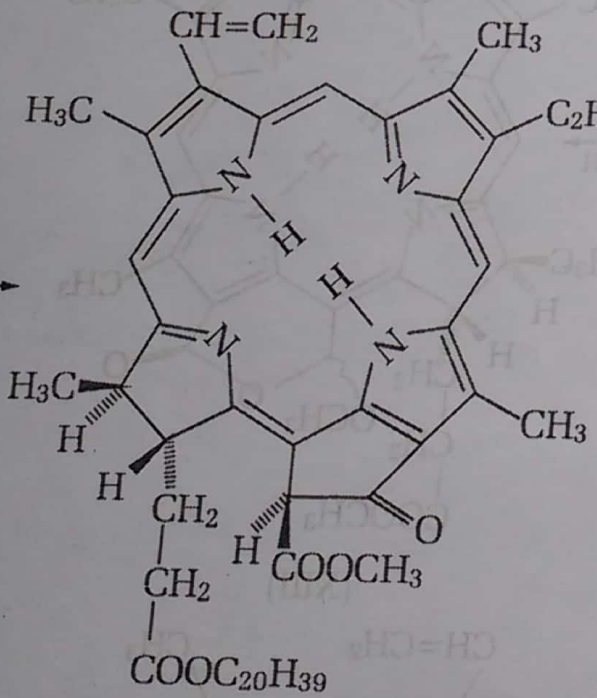
Chlorin-e trimethyl ester (XVI)

CH_3ONa



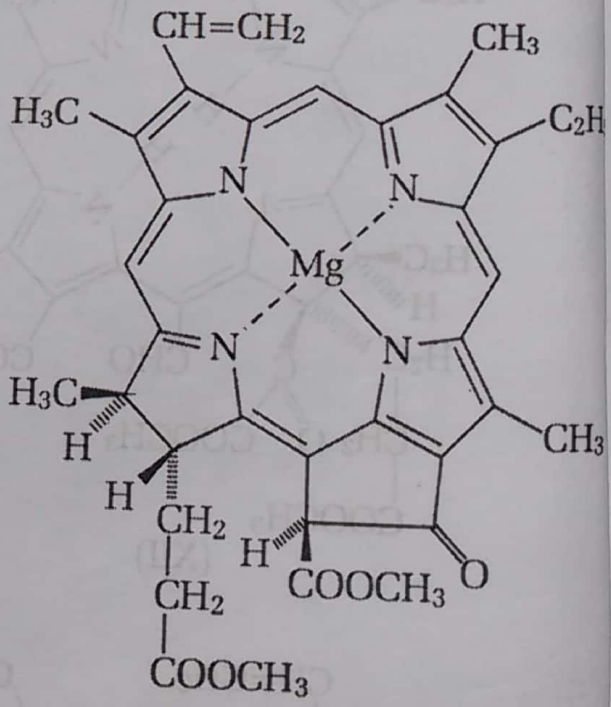
Phaeophorbide-a (XVII)

Phytol



Phaeophytin-a (XVIII)

Mg
(EtMgBr)



Chlorophyll-a (XIX)