M.Sc Semester-III, Paper: CC-11 (Bio-Inorganic Chemistry)

Unit-2 Bio energetics & ATP Cycle [By Dr. Birendra Kumar, Maharaja College]

⇒ Chlorophylls:

Chlorophyll is the green pigment of plants, especially leaves. It is most important group of pigments, embedded in thylakoids of chloroplasts. The natural chlorophyll is a mixture of two different chlorophylls: Chlorophyll-a & Chlorophyll-b (3:1). Biologically, Chlorophyll is very important natural pigment as it is responsible directly or indirectly for the synthesis of all kinds of food (photosynthesis in plants).

Chlorophyll-a: Its empirical formula is $C_{55}H_{72}O_5N_4Mg$. It has a porphyrin ring (tetrapyrrole ring) with isocyclic ring having Mg atom in the centre of porphyrin ring and phytol tail. Porphyrin ring shows alternate double & single bonds. Its dimension is 15×15 Å. It has $-CH_3$ (methyl) at C-3. Phytol tail has hydrophobic tail ($C_{20}H_{39}$) which is esterified with carboxylic group at C-7 of porphyrin ring. It is 20 Å long.

Chlorophyll-b: Its empirical formula is $C_{55}H_{70}O_6N_4Mg$. It has $-CHO$ group at C-3 of porphyrin ring. In rest of matter, it is similar to Chlorophyll-a. Precursors of Chlorophyll synthesis are: Succinyl CoA & glycine and protochlorophyll due to hydrogenation in one of pyrrole rings.

⇒ Structure/Constitution of Chlorophylls: The structure of Chlorophylls was elucidated by the degradation reactions of 'Willstatter' and proved by synthetic work of 'Hans Fischer'. The various points which help in elucidation of structure of Chlorophyll-a may be summarised as follows:

1. On the basis of analytical data, molecular formula of Chlorophyll-a is found to be $C_{55}H_{72}O_5N_4Mg$.
2. On hydrolysis with cold and dilute KOH, it gives phytol ($C_{20}H_{40}O$), methyl alcohol and Chlorophyllide-a indicating thereby that Chlorophyll-a is a diester.

$$C_{32}H_{30}N_4OMg\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix} \xrightarrow[(2\,steps)]{KOH(aq.)} \underset{(Chlorophyllide\text{-}a)}{C_{32}H_{30}N_4OMg\begin{matrix}COOH\\COOH\end{matrix}} + \underset{(Phytol)}{C_{20}H_{40}O} + CH_3OH$$

3. On treatment with dilute mineral acid or ethanolic oxalic acid, it gives magnesium-free compound, phaeophytin-a.

$$C_{32}H_{30}N_4OMg\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix} \xrightarrow{+2HCl(aq.)} \underset{(Phaeophytin\text{-}a)}{C_{32}H_{32}N_4O\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix}} + MgCl_2$$

4. On treatment with mineral acid, phaeophytin-a yields phaeophorbide-a (more easily hydrolysable group, phytyl, is detected).

$$C_{32}H_{32}N_4O\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix} \xrightarrow[(Hydrolysis)]{HCl(aq)} \underset{(Phaeophorbide\text{-}a)}{C_{32}H_{32}N_4O\begin{matrix}COOCH_3\\COOH\end{matrix}} + C_{20}H_{39}OH$$

5. On catalytic reduction, phaeophorbide-a take up two hydrogen atoms indicating the presence of one double bond in phaeophorbide molecule and hence also in chlorophyll molecule. The presence of double bond (vinyl group) is further proved by following reaction:

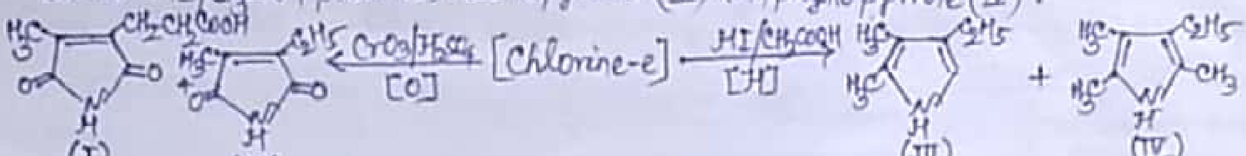
$$-CH{=}CH_2 \xrightarrow{HI} CHI{-}CH_3 \xrightarrow{H_2O} -CH(OH){-}CH_3 \xrightarrow{[O]} -CO{\cdot}CH_3$$

6. Phytyl phaeophorbide-a, on hydrolysis with boiling methanolic-KOH provides a tricarboxylic acid chlorin-e (two acidic groups are produced from two ester groups), the formation of third $-COOH$ group indicates the presence of an easily hydrolysable group viz; lactone ring or cyclic ketone.

$$C_{32}H_{32}N_4O\begin{matrix}COOCH_3\\COOC_{20}H_{39}\end{matrix} \longrightarrow C_{32}H_{32}N_4O\begin{matrix}COOH\\COOH\end{matrix} \longrightarrow \underset{(Chlorin\text{-}e)}{C_{31}H_{33}N_4(COOH)_3}$$

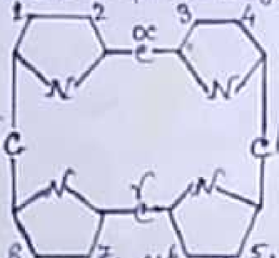
7. On oxidation with chromic acid (CrO_3), Chlorin-e gives well established compounds haematinic acid (I) and ethyl methyl maleimide (II). On the other hand, reduction of chlorine-e

with HI/CH_3COOH produces haemopyrrole (III) and phyllo pyrrole (IV).



(I) (II) (III) (IV)

These reactions indicate the presence of following skeleton for Chlorin-e and also Chlorophyll-a

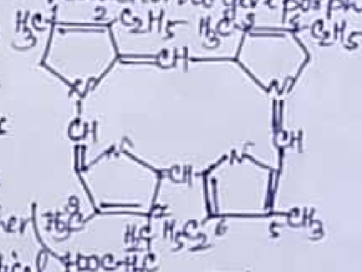


8. Alkaline degradation of Chlorophyll or heating of Chlorin-e with ethanolic KOH yields mainly pyrroporphyrin, rhodoporphyrin and phylloporphyrin. Both phyllo- and rhodo-porphyrins can be converted into pyrroporphyrin; the former by heating with C_2H_5ONa (loss of $-CH_2$) and the latter by heating (loss of CO_2). Moreover, pyrroporphyrin on decarboxylation yields pyrroaetio porphyrin, $C_{30}H_{34}N_4$.

9. Pyrro- & phyllo-porphyrins (but not rhodo-porphyrin) on bromination followed by oxidation with Chromic acid give bromo citraconimide (V) indicating that at least one of the β-positions in one pyrrole ring of these two porphyrins is free.

(V)

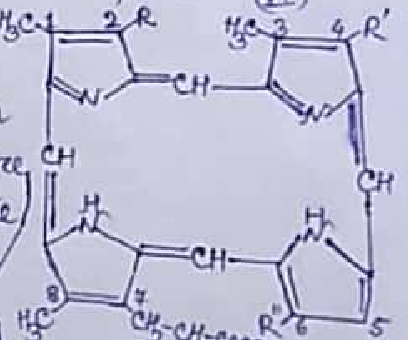
The presence and position of the free hydrogen atom in one pyrrole ring of pyrroporphyrin was confirmed by its acetylation followed with reduction to give porphin-monopropionic acid (VI). Now, we see that by above reactions ethyl group is introduced in place of free hydrogen atom of pyrrole nuclei and since in structure (VI) there are three ethyl groups on positions 2, 4 & 6; following three (VII), (VIII) & (IX) are possible structures for pyrroporphyrin. But the synthetic work of Fischer showed that pyrroporphyrin obtained from Chlorin-e was identical with the (X).



(VI)

10. Rhodoporphyrin is a dicarboxylic, on treatment with CH_3ONa, it evolves CO_2 with the formation of pyrroporphyrin (a monocarboxylic acid) and since it does not possess any free hydrogen atom as it does not form bromocitraconimide, the second $-COOH$ group must be present on position 6 (R'') of the pyrroporphyrin (IX) to give rhodoporphyrin.

11. Phylloporphyrin ($C_{31}H_{35}N_4COOH$) contains a $-CH_2$ more than pyrroporphyrin ($C_{30}H_{33}N_4COOH$) and as its position 6 is found to be free, therefore the additional C-atom ($-CH_3$) must be present on one of the four bridge C-atoms: α, β, γ & δ. But it was seen that the synthetic phylloporphyrin with a methyl group on the γ-C-atom was identical with the phylloporphyrin obtained from Chlorophyll-a.



(VII) to (X)
(Pyrroporphyrins)

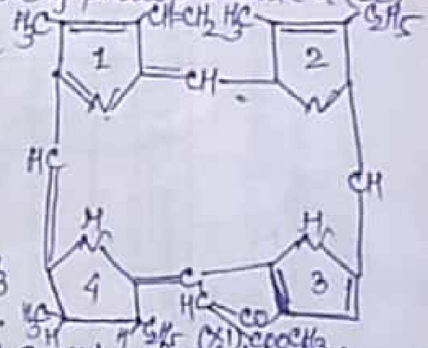
(VII) R=H, R' & R'' = C_2H_5
(VIII) R & R'' = C_2H_5 & R' = H
(IX) R & R' = C_2H_5 & R'' = H
(X) Rhodoporphyrin, R & R' = C_2H_5, and R'' = COOH.

12. Phaeophorbide-a (obtained from Chlorophyll by elimination of Mg and phytyl group) on mild reduction with HI/CH_3COOH at 50-60°C followed by atmospheric oxidation gives an isomeric saturated substance phaeoporphyrin-a. The latter compound contains a -COOH and a $-COCH_3$ group, and behaves as a β-keto ester.

13. As the chlorin-e contains three -COOH groups whereas it degraded product (rhodoporphyrin) contains two -COOH groups, the third -COOH group of Chlorin-e will be on the γ-carbon atom of phylloerythrin.

14. On catalytic dehydrogenation, phaeophorbide-a, gives the dihydro derivative having the ketonic group of the former intact. This suggests the presence of a readily reducible double bond in it. Oxidation experiments on phaeophorbide-a and its dihydro derivative showed the presence of a vinyl group at 2-position in the former.

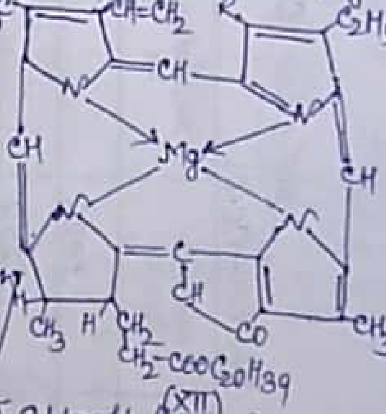
15. Oxidation of phaeophorbide-a gives citronimide, ethyl methyl haematinimide. The former two products arise from pyrrole nuclei having double bonds whereas the last from pyrrole nucleus having no double bond but having propionic acidic group, i.e; from ring 4 of phaeophorbide-a. So now we know the complete structure of phaeophorbide-a (XI) and to elucidate the structure of Chlorophyll we must introduce one phytyl group and one Mg-atom in it.



(XI)

16. Position of phytyl group: Phaeophorbide-a contains a -COOH group derived from $-COOC_{20}H_{39}$ and a $-COOCH_3$ group; pyrolysis of it results in the loss of $-COOCH_3$ but not of -COOH, hence $-COOCH_3$ must be attached directly to porphyrin ring and the -COOH gr. which survived decarboxylation must be present as $-CH_2CH_2-COOH$ to which phytyl group is attached.

17. The magnesium atom is not linked through O-atom since the oxygen-free compound aetiophyllin ($C_{31}H_{34}N_4Mg$) still contains Mg-atom. Magnesium atom is linked to N-atoms because Mg-free compounds, porphyrins, are more basic than Chlorophyll (N-Mg→NH). Hence, Chlorophyll may be represented as (XII).



(XII)
[Chlorophyll-a, $R = -CH_3$
Chlorophyll-b, $R = CHO$]

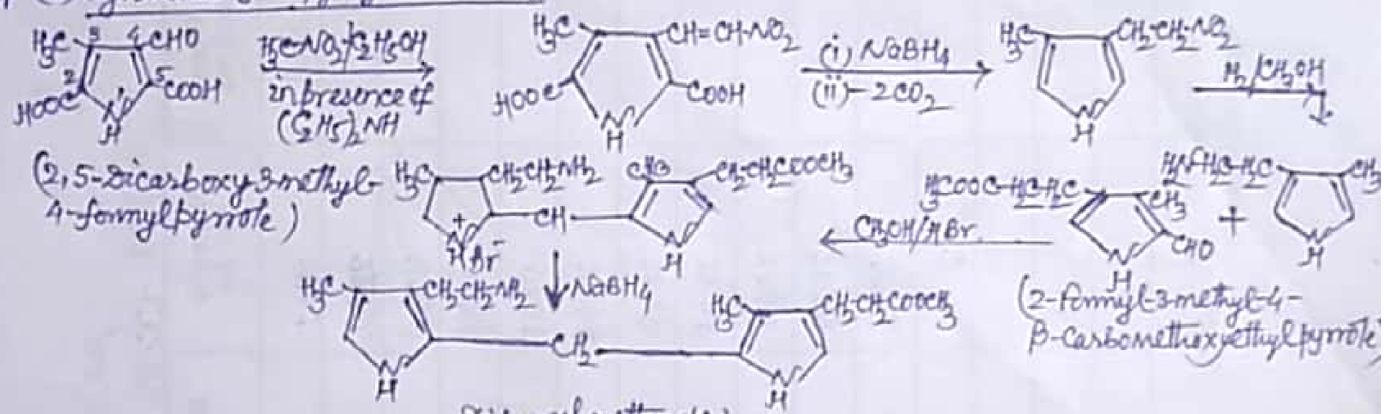
Structure of Chlorophyll-b:

The molecular formula for chlorophyll-b is found to be $C_{55}H_{70}N_4O_6Mg$, it contains one oxygen atom more and two hydrogen atoms less than the Chlorophyll-a ($C_{55}H_{72}N_4O_5Mg$). This difference is due to the presence of -CHO group on position 3 in place of methyl group. The structure is elucidated in the same manner as that for Chlorophyll-a (XII). The presence of an aldehydic group was indicated by formation of dioxime from methyl phaeophorbide-b, its position was fixed by the conversion of trimethyl ester rhodin-e into a compound, dimethyl deoxophylloerythrin (Known structure).

Synthesis of Chlorophyll:

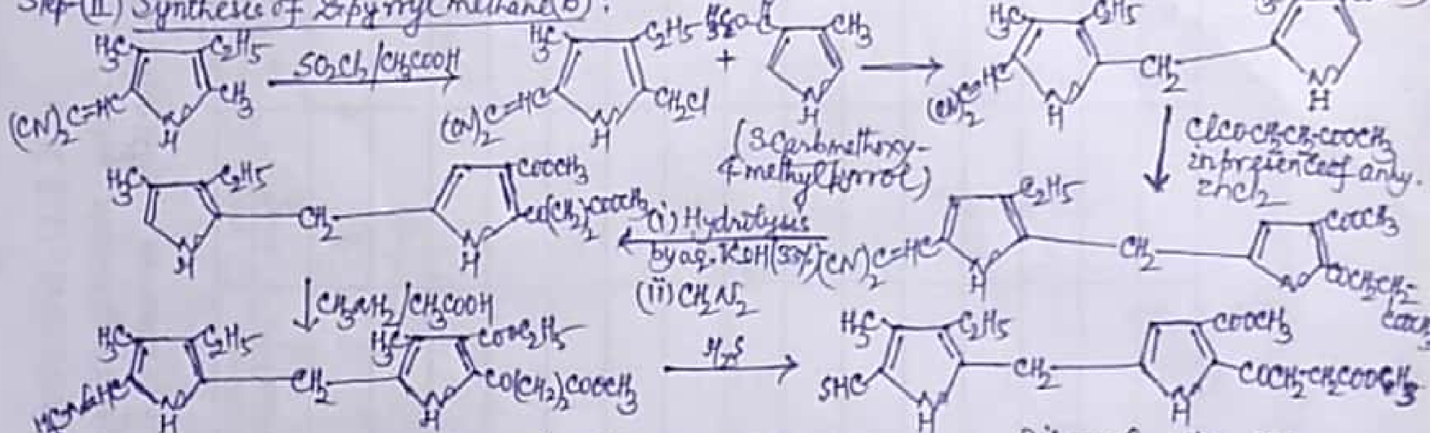
The total synthesis of Chlorophyll-a was given by Woodward et al (1960). It involves three steps:

Step-(I) Synthesis of Dipyrrylmethane(A):



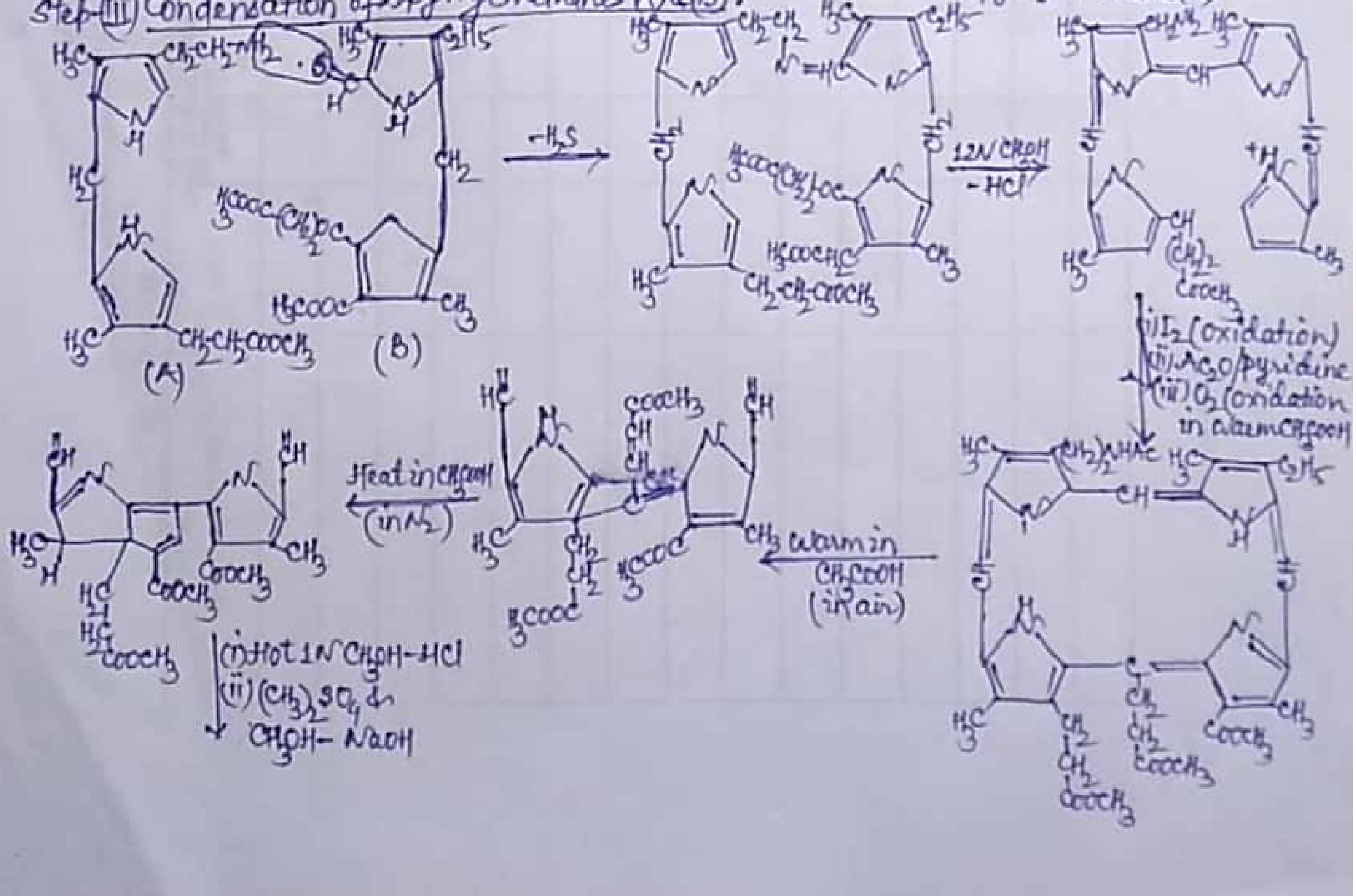
Dipyrrylmethane(A)

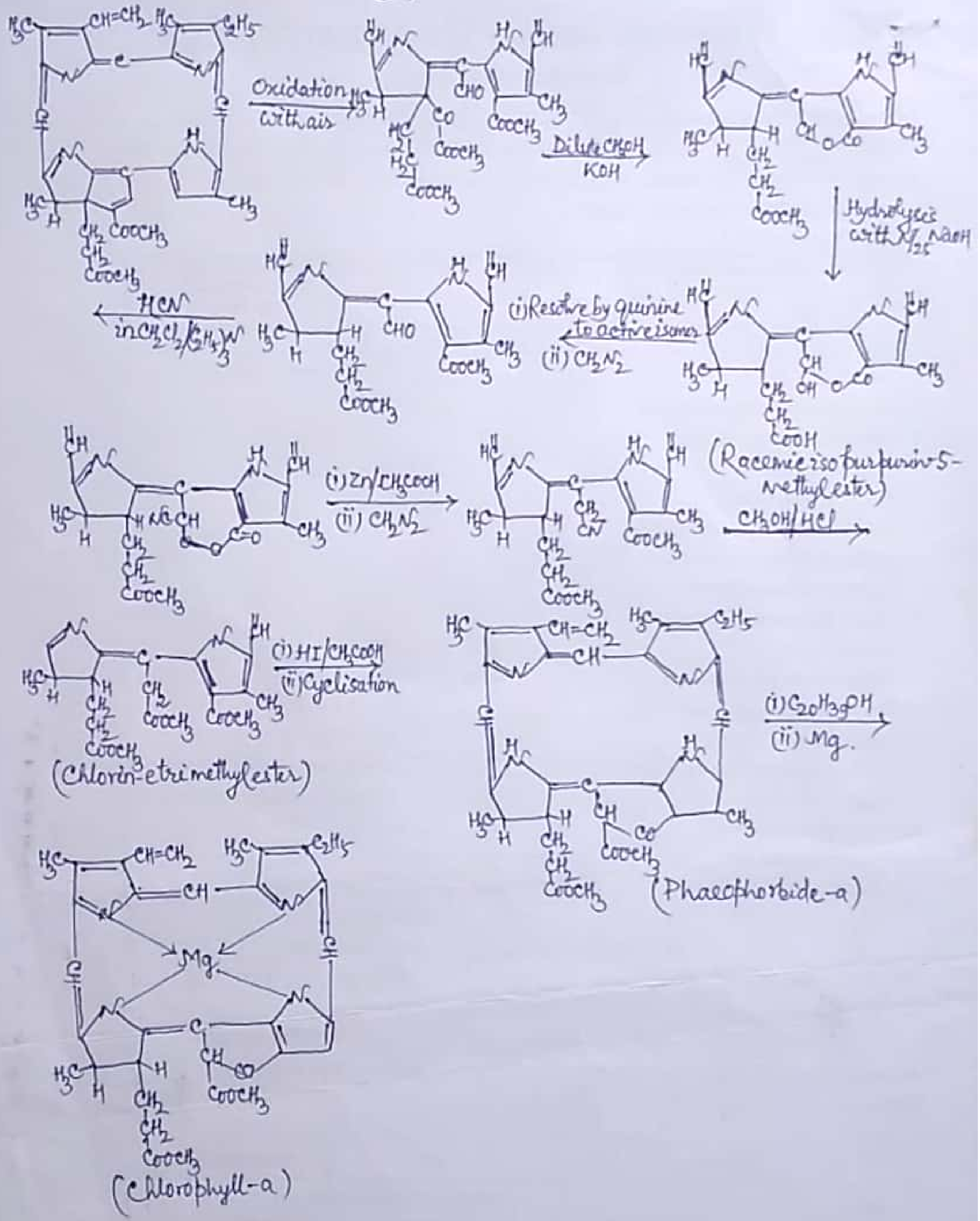
Step-(II) Synthesis of Dipyrrylmethane(B):



Dipyrrylmethane(B)

Step-(III) Condensation of Dipyrrylmethanes (A) & (B):




Oxidation with air
Dilute CH3OH KOH
Hydrolysis with N/25 NaOH
(Racemic isopurpurin-5-methyl ester)
(i) Resolve by quinine to active isomer
(ii) CH2N2
HCN in CH2Cl2/(C2H5)3N
(i) Zn/CH3COOH
(ii) CH2N2
CH3OH/HCl
(i) HI/CH3COOH
(ii) Cyclisation
(Chlorin-e trimethyl ester)
(i) C20H39OH
(ii) Mg.
(Phaeophorbide-a)
(Chlorophyll-a)