Chlorophyll-A.

THE FOURTH PEDLER LECTURE, DELIVERED BEFORE THE CHEMICAL SOCIETY AT THE ROYAL INSTITUTION, LONDON, ON FEBRUARY 22ND, 1934.

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As we know from the work of Willstätter and his collaborators (R. Willstätter and A. Stoll, " Untersuchungen über Chlorophyll "; also numerous publications, especially in Liebig's *Annalen der Chemie*), chlorophyll is a wax-like substance of complex structure, consisting of a pyrrole derivative containing magnesium, and the two alcohols phytol and methyl alcohol, which are esterified by two carboxyl groups of the pyrrole component.

The structure of phytol has since been completely elucidated and confirmed by synthesis (F. G. Fischer and Löwenberg, *Annalen*, 1929, **475**, 183) as

$$CH_3\cdot\underset{\underset{CH_3}{|}}{CH}\cdot CH_2\cdot CH_2\cdot CH_2\cdot\underset{\underset{CH_3}{|}}{CH}\cdot CH_2\cdot CH_2\cdot CH_2\cdot\underset{\underset{CH_3}{|}}{CH}\cdot CH_2\cdot CH_2\cdot CH_2\cdot\underset{\underset{CH_3}{|}}{C}\!:\!CH\cdot CH_2\cdot OH$$

The enzyme chlorophyllase, discovered by Willstätter, acts on chlorophyll in alcoholic media, replacing phytol by the corresponding alcohol. In this way the crystalline methyl and ethyl chlorophyllides are obtained. Their formulæ are:

methyl chlorophyllide-a $(C_{32}H_{30}ON_4Mg)(CO\cdot O\cdot CH_3)_2 + \frac{1}{2}H_2O$
ethyl chlorophyllide $(C_{32}H_{30}ON_4Mg)(CO\cdot O\cdot CH_3)(CO\cdot O\cdot C_2H_5)$

Willstätter and his pupils laid the foundations of our knowledge of chlorophyll and its transformation and degradation products in a series of brilliant investigations which I cannot describe in detail now: for particulars, I refer you to the work by Willstätter and Stoll, cited above, in which will be found also the proof that natural chlorophyll is a mixture of two components, a and b. This lecture will deal only with chlorophyll-a.

The presence of water of crystallisation in methyl chlorophyllide has not yet been proved. By the action of acids on chlorophyll, phæophytin is obtained; this contains no magnesium and, like chlorophyll itself, may be separated into two components, a and b. Phæophytin, in its turn, when treated with hydrochloric acid, yields the two phæophorbides, a and b, or, if methyl alcohol is present, the methyl phæophorbides. These can be separated relatively easily, and crystallise exceedingly well:

phæophorbide-a $C_{32}H_{32}ON_4(CO\cdot O\cdot CH_3)(CO\cdot OH)$
methyl phæophorbide-a $C_{32}H_{32}ON_4(CO\cdot O\cdot CH_3)_2$

These formulæ of Willstätter are confirmed by our own experiments (H. Fischer, Moldenhauer, and Süs, *Annalen*, 1931, **486**, 158; compare *ibid.*, 1932, **499**, 108, and p. 250 below).

As the formulæ show, phæophorbide-a is the monomethyl ester of a dicarboxylic acid, and methyl phæophorbide-a is its dimethyl ester.

When either phæophytin or phæophorbide is subjected to hydrolysis with methyl-alcoholic potash for 30 seconds, the products are phytochlorin-e and phytorhodin-g (usually abbreviated to chlorin-e and rhodin-g). Chlorin-e is the " a " component, rhodin-g the " b." Chlorin-e gives a well-crystallised trimethyl ester, $C_{37}H_{42}O_6N_4$, which is most conveniently obtained with diazomethane, according to Treibs and Wiedemann.

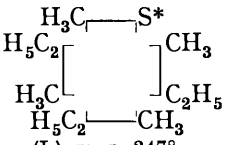
By energetic degradation of chlorophyll and its derivatives, Willstätter and his collaborators obtained numerous phyllins and porphyrins. Among these should be mentioned phylloporphyrin, pyrroporphyrin, and rhodoporphyrin, as well as ætioporphyrin, which is obtained from these by pyrogenetic reactions and was considered to be identical with the ætioporphyrin from hæmin.

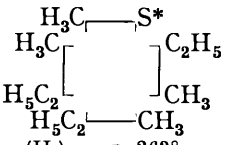
Willstätter and his pupils described numerous isomerides of rhodoporphyrin, which were thoroughly investigated by Treibs and Wiedemann. The result of these investigations was the discovery of a new porphyrin, verdoporphyrin, which is very easily transformed into rhodoporphyrin. An admixture of this verdoporphyrin is the probable explanation of the numerous isomerides of rhodoporphyrin described by Willstätter,

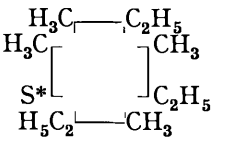
such as erythro-, cyano-, rubi-, and glauco-porphyrins. Later we isolated still another isomeride, ψ-verdoporphyrin. The identity of verdoporphyrin or ψ-verdoporphyrin with any of Willstätter's porphyrins is out of the question, since their basicities are less than those corresponding to the hydrochloric acid and distribution numbers given by Willstätter. A more exact comparison is impossible, because detailed spectroscopic data and the melting points of the esters are not given in his publications. Moreover it seems that the different methods of work in vogue in different laboratories result in the isolation of different isomerides. Conant, for instance, could not isolate Treibs and Wiedemann's verdoporphyrin (*Annalen*, 1929, **471**, 146), but obtained "*iso*rhodoporphyrin," which is identical with our ψ-verdoporphyrin (Conant, Hyde, Moyer, and Dietz, *J. Amer. Chem. Soc.*, 1931, **53**, 370; 1933, **55**, 796; compare H. Fischer and Klebs, *Annalen*, 1931, **490**, 44, 88).

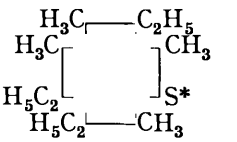
We were, naturally, very interested in determining the constitutions of these porphyrins. In the meantime we had succeeded in developing general methods for synthesising these substances, and we hoped to be able to apply them successfully in the investigation of the chlorophyll porphyrins.

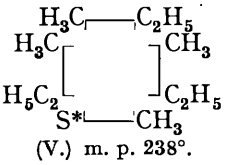
Now rhodoporphyrin is a dicarboxylic acid; phylloporphyrin and pyrroporphyrin are monocarboxylic acids which were held to be isomerides by the earlier investigators. The most probable assumption was that they were tetramethyl-triethyl-porphin-propionic acids, of which theoretically there must be eight isomerides, *viz.*,

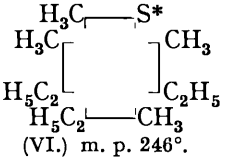

(I.) m. p. 247°.

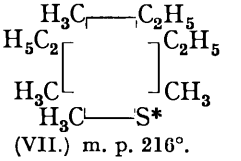

(II.) m. p. 263°.

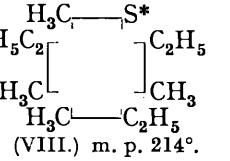

(III.) m. p. 271°.


(IV.) m. p. 208° (220°).


(V.) m. p. 238°.


(VI.) m. p. 246°.


(VII.) m. p. 216°.


(VIII.) m. p. 214°.

* S = $CH_2 \cdot CH_2 \cdot CO \cdot OH$.

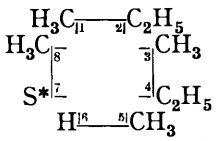
(The formulæ are abbreviated; each square bracket represents one of the four pyrrole rings of the porphyrin molecule; the four bridge CH groups are omitted; the melting points are those of the methyl esters.)

All eight isomerides were synthesised, and all proved to be different from phylloporphyrin and pyrroporphyrin (H. Fischer, Grosselfinger, and Stangler, *Annalen*, 1928, **461**, 221; H. Fischer, Weichmann, and Zeile, *ibid.*, 1929, **475**, 241).

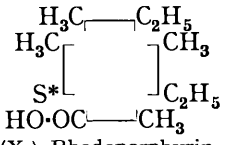
Then we succeeded in proving, analytically, that in both these porphyrins one of the CH groups in the β-position of a pyrrole ring is unsubstituted; this is not the case in rhodoporphyrin, but is so in the ætioporphyrin from chlorophyll. This unsubstituted group was detected by bromination, followed by oxidation. The hydrogen was replaced by bromine and on oxidation bromocitraconimide was formed. According to this, the most probable explanation of the constitution of these two porphyrins was that they corresponded to two of the eight isomeric acids but contained one ethyl group less. Since an ethyl group can be removed from three different positions in each acid, there are twenty-four isomeric acids of the required structure. The same would hold for rhodoporphyrin, since, as it loses carbon dioxide on heating and passes into pyrroporphyrin, it was very probable that the two porphyrins had the same structure, except that where pyrroporphyrin had an unsubstituted CH group, rhodoporphyrin possessed a carboxyl group attached directly to the ring. This view of the structure of rhodoporphyrin was completely confirmed by the synthesis of several pyrro- and rhodo-porphyrins (H. Fischer, Berg, and Schormüller, *Annalen*, 1929, **473**, 211; 1930, **480**, 109, 189; 1930, **482**, 232).

The first step in the proof of the constitution of natural pyrroporphyrin was its conversion into the acid (III) by the introduction of an ethyl group. Since porphyrin III corresponds to ætioporphyrin III in the arrangement of the side chains, this showed that this arrangement is fundamentally the same in both hæmin and chlorophyll; both are derived from ætioporphyrin III.

But this did not settle the structure of pyrroporphyrin, since compounds with an unsubstituted CH group in any of the positions, 2, 4, and 6 would all give porphyrin III on the introduction of an ethyl group. It was then proved by synthesis that position 6 is the one in question. 1 : 3 : 5 : 8-Tetramethyl-2 : 4-diethyl-6-carboxyporphin-7-propionic acid (X) was synthesised and found to be identical with rhodoporphyrin from natural sources; as has been mentioned, this yields pyrroporphyrin on decarboxylation. At the same time 1 : 3 : 5 : 8-tetramethyl-2 : 4-diethylporphin-7-propionic acid (IX) was synthesised, and was found to be identical with natural pyrroporphyrin. By this it was

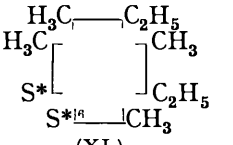


(IX.) Pyrroporphyrin.



(X.) Rhodoporphyrin.

* S = $CH_2 \cdot CH_2 \cdot CO \cdot OH$.



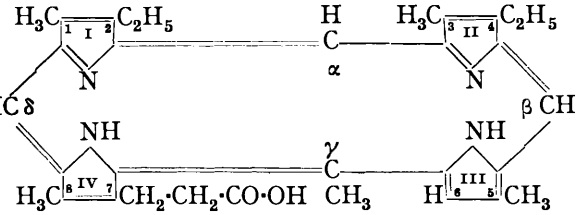
(XI.)

also proved that the unsubstituted CH group in phylloporphyrin is in position 6, since phylloporphyrin also is converted into pyrroporphyrin by the action of sodium ethoxide. The synthesis shows that pyrroporphyrin is closely related to the important hæmin derivative mesoporphyrin (XI).

If the propionic acid group in position 6 of the formula for mesoporphyrin is replaced by hydrogen, one obtains the formula for pyrroporphyrin. In agreement with this we were able to convert pyrroporphyrin (or pyrrohæmin), by the action of chloromethyl ether and hydrobromic acid, into bromomethyl pyrroporphyrin (or hæmin), which, when heated with sodiomalonic ester, gave mesoporphyrin (H. Fischer and Riedl, *Annalen*, 1931, **486**, 178). Thus was obtained—even though indirectly—for the first time a porphyrin which was common to both the chlorophyll and the hæmin series. The ætioporphyrins of chlorophyll have, of course, nothing to do with the ætioporphyrins of hæmin. The former proved to be mixtures of phylloporphyrin and pyrroporphyrin (XII and IX). If one imagines the propionic acid side chains decarboxylated to ethyl groups, one has the formulæ of the corresponding true ætioporphyrins of chlorophyll. This was confirmed by synthesis (H. Fischer, Berg, Helberger, and Schormüller, *Annalen*, 1930, **480**, 109, 235; 1930, **482**, 232). The ætioporphyrin of hæmin, by the way, is not, strictly speaking, decarboxylated mesoporphyrin but decarboxylated protoporphyrin, which we synthesised recently (H. Fischer, Kirstahler, and v. Zychlinski, *Annalen*, 1932, **500**, 1). Recently, too, we succeeded in converting mesoporphyrin (or mesorhodin) through the stage of rhodoporphyrin-γ-carboxylic acid into pyrroporphyrin; the product was mainly 1 : 3 : 5 : 8-tetramethyl-2 : 4-diethylporphin-6-propionic acid (IX) (*Annalen*, 1934, **509**, 19).

Phylloporphyrin proved to be a homologue of pyrroporphyrin. On the assumption that methyl is substituted for the hydrogen of one of the four " bridge " CH groups (compare formula XII), there are ninety-six possible isomerides; however, the knowledge of the structure of pyrroporphyrin leaves only four possible structures for phylloporphyrin.

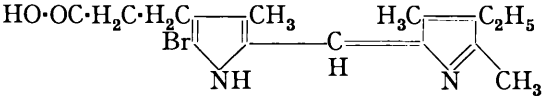
(XII.)

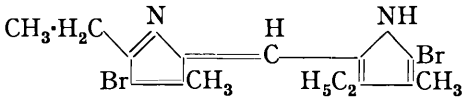


Phylloporphyrin.

All four were synthesised (H. Fischer and Helberger, *Annalen*, 1930, **480**, 235; H. Fischer, Siedel, and Le Thierry d'Ennequin, *ibid.*, 1933, **500**, 137). The scheme of the synthesis of the isomeride which was identical with the product from natural sources is given here (XIII and XIV).

(XIII.)





(XIV.)

There were many theoretical and practical difficulties. Each of the pyrromethenes we used could react with itself; further, partial decarboxylation could take place so that in each synthesis ten different porphyrins could be produced, which, in fact, did for the most part appear, and which, in all four syntheses, were nearly all isolated.

For the separation of these porphyrins (and, in general, for all porphyrin mixtures), the method of fractionation developed by Willstätter and Mieg (*Annalen*, 1906, **350**, 1) proved most successful. The basicities of the porphyrins, due to their imino-groups, vary widely, and hence, by using hydrochloric acid of different degrees of concentration to extract them from a solution in ether, a very complete separation of even complex mixtures can be attained, especially by frequent repetition of the operation. The method is also suitable for separating the phæophorbides.

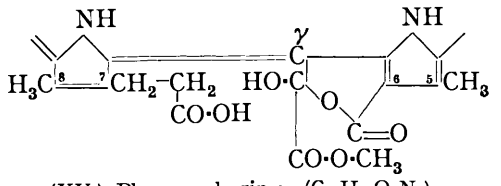
γ-Methyl-pyrroporphyrin (1 : 3 : 5 : 8-tetramethyl-2 : 4-diethyl-γ-methylporphin-7-propionic acid) (XII) proved to be identical with " natural " phylloporphyrin. The mixed melting point of the two esters showed no depression, whereas a distinct depression was obtained with the other three isomeric esters.

While these analytical and synthetical investigations were in progress, the biological degradation of chlorophyll was being studied, since in the study of hæmin we had found that the examination of biologically related substances was of great service in throwing light on the constitution of hæmin itself and in preparing the way for its synthesis.

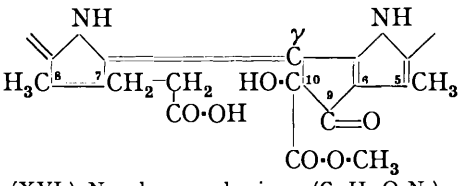
The investigations of Löbisch and Fischler (*Monatsh.*, 1903, **24**, 335) and of Marchlewski (*Z. physiol. Chem.*, 1904—1905, **43**, 464; 1905, **45**, 176) had made known the substance phylloerythrin which they obtained from ox-bile and from the fæces of herbivora. From sheep dung we were able to isolate probophorbides (H. Fischer and Hendschel, *Z. physiol. Chem.*, 1931, **198**, 33; 1933, **222**, 250); these are isomeric with phylloerythrin, into which they are easily converted by heat. They are important because, as the name signifies, they show a close spectroscopic relationship to phæophorbide and the phorbides in general. From this it must be concluded that the phæophorbides and phylloerythrin are closely related to chlorophyll itself. In agreement with this is the fact that, as Rothemund and Inman have shown (*J. Amer. Chem. Soc.*, 1932, **54**, 4702), they are already present in the omasus and abomasus (third and fourth stomach) of ruminants. Now phylloerythrin must be classed among the porphyrins on account of its spectroscopic properties (*Z. physiol. Chem.*, 1925, **143**, 1). This was the first reason for supposing that chlorophyll also was closely connected with the porphyrins; this view was supported, on the synthetical side, by the conversion of hæmin porphyrins into chlorins and rhodins (H. Fischer, Treibs, and Helberger, *Annalen*, 1928, **466**, 243; 1929, **471**, 285; H. Fischer, Helberger, Platz, and Niemer, *ibid.*, 1930, **479**, 27; H. Fischer, Gebhardt, and Rothaas, *ibid.*, 1930, **482**, 1). A further confirmation of the relationship between the two classes of compounds was the conversion of chlorophyll derivatives into porphyrins by bacteriological means (H. Fischer and Hendschel, *loc. cit.*). The presence of the porphyrin ring in chlorophyll and its derivatives could not be inferred from their conversion into phylloporphyrin, pyrroporphyrin, and rhodoporphyrin, for under the conditions of this experi-

ment (action of sodium ethoxide at a high temperature in a sealed tube) secondary syntheses are possible, especially as the products are poorer in carbon than the original material. Hence methods had to be found by which porphyrins could be produced from chlorophyll without destroying its carbon skeleton; for the synthesis of such porphyrins methods were already available. Reduction with hydriodic acid in glacial acetic acid proved successful. When this method was applied to phæophorbide and to chlorin-e, essentially different results were obtained. The former yielded phæoporphyrins, the latter chloroporphyrins (H. Fischer and Bäumler, *Annalen*, 1929, **474**, 65; 1930, **480**, 197; H. Fischer, Moldenhauer, Süs, Hagert, and Filser, *ibid.*, 1930, **478**, 54; 1930, **481**, 132; 1931, **485**, 1; 1931, **486**, 107; 1931, **490**, 1). Even spectroscopic examination showed the essential difference between the two classes of porphyrins. In the absorption spectra of the phæoporphyrins the second and the third band are close together, as in the case of phylloerythrin, while the spectra of the chloroporphyrins resemble those of the hæmin porphyrins much more closely.

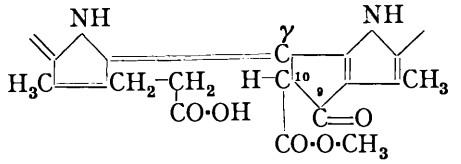
We give a summary of those porphyrins (with partial formulæ) which were most important in arriving at the constitution of chlorophyll, especially in proving the presence of 34 carbon atoms in the chlorophyll molecule (that is, if we omit the ester groups) :



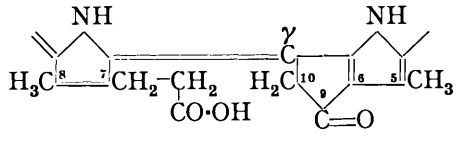
(XV) Phæoporphyrin-a_7 ($C_{34}H_{34}O_7N_4$).



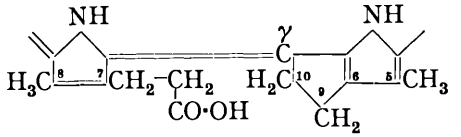
(XVI.) Neophæoporphyrin-a_6 ($C_{34}H_{34}O_6N_4$). [H_5C_2·O instead of HO in position 10 = phæoporphyrin-a_6.]



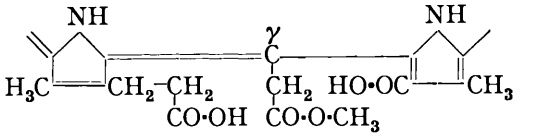
(XVII.) Phæoporphyrin-a_5 ($C_{34}H_{34}O_5N_4$).



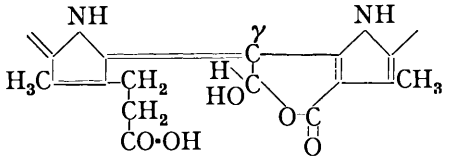
(XVIII.) Phylloerythrin ($C_{33}H_{34}O_3N_4$).



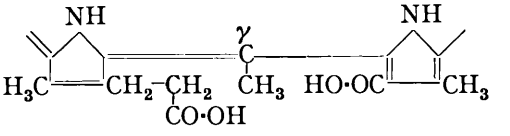
(XIX.) Desoxophyllerythrin ($C_{33}H_{36}O_2N_4$).



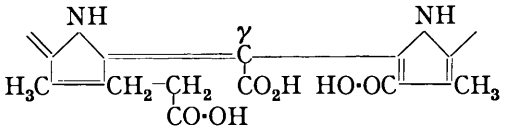
(XX.) Chloroporphyrin-e_6 ($C_{34}H_{36}O_6N_4$).



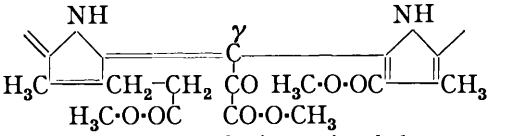
(XXI.) Chloroporphyrin-e_5 ($C_{33}H_{34}O_6N_4$).



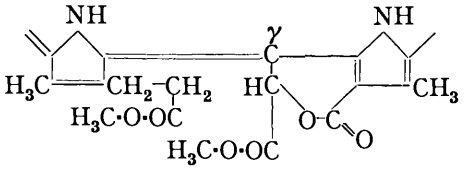
(XXII.) Chloroporphyrin-e_4 ($C_{33}H_{36}O_4N_4$).



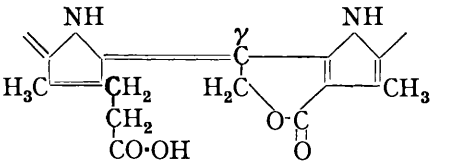
(XXIII.) Rhodoporphyrin-γ-carboxylic acid ($C_{33}H_{34}O_6N_4$).



(XXIV.) Phæoporphyrin-a_7 trimethyl ester (= "allo-a_7") ($C_{37}H_{40}O_7N_4$).



(XXV.) Chloroporphyrin-e_7-lactone dimethyl ester ($C_{34}H_{34}O_6N_4$).



(XXVI.) Hydroxymethylrhodoporphyrinlactone ($C_{33}H_{34}O_4O_4$).

In the empirical formulæ XV—XXVI (except XXIV) the ester groups have been omitted in order to facilitate comparison of the hydrogen content of the different porphyrins.

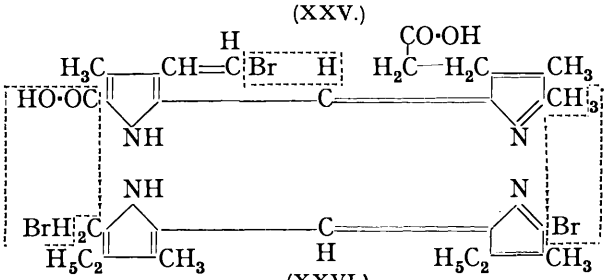
The porphyrins crystallise well and are further characterised by crystalline esters and other derivatives. The esters melt sharply and mixtures of them show a distinct depression of the melting point. In the preparation of porphyrins from phæophorbide or chlorin-e ester, the ester groups are not always completely hydrolysed. The recognition and explanation of this fact were beset with many difficulties. Systematic methoxyl determinations are absolutely necessary as a guide to the purity of the preparations. A further difficulty lies in the ease with which these porphyrins form mixed crystals. For example, if 10% of chloroporphyrin-e_6 is added to phylloerythrin, it cannot be detected with the spectroscope, and the phylloerythrin now crystallises in needles instead of cubes. One is inclined to believe that one is dealing with a new chemical individual, a molecular compound.

The most important of these porphyrins is phæoporphyrin-a_5 (XVII), which can be obtained from the phæophorbides as well as from phæophytin and chlorophyllide; it always occurs as the monomethyl ester (H. Fischer and Süs, *Annalen*, 1930, **482**, 225; 1931, **485**, 1; 1931, **486**, 107). The carbomethoxy-group in position 10 is therefore hydrolysed with difficulty. Phæoporphyrin-a_5 reacts with ketonic reagents and undergoes hydrolytic fission, yielding chloroporphyrin-e_6, which also is a monomethyl ester. It has the structure (XX), since on the one hand it is easily reconverted into phæoporphyrin-a_5, and on the other hand, 30% methyl-alcoholic potash converts it into chloroporphyrin-e_4 (XXII) and rhodoporphyrin. The conversion of chloroporphyrin-e_6 back into phæoporphyrin-a_5 was the first example of a transition from the group of chloroporphyrins to the phæoporphyrins.

Phæoporphyrin-a_5 (XVII) is easily converted by decarboxylation into phylloerythrin (XVIII). The latter may be reduced to desoxophyllerythrin (XIX), which contains only 2 atoms of oxygen, and these in a carboxyl group. The constitution of phylloerythrin was proved as follows: In an atmosphere of nitrogen it is unaffected by alkalis; in the presence of oxygen, decomposition sets in and the characteristic chlorophyll porphyrins, phylloporphyrin, pyrroporphyrin, and rhodoporphyrin, are produced, as well as rhodoporphyrin-γ-carboxylic acid, which was first obtained in this way. This behaviour can only be explained by the presence of an ethanone bridge between the positions 6 and γ; the presence of a carbonyl group was proved by reaction with the usual ketonic reagents.

The structure of desoxophyllerythrin and phylloerythrin was also proved by synthesis (H. Fischer and Riedmair, *Annalen*, 1931, **490**, 91; 1932, **497**, 181; 1932, **499**, 288; H. Fischer, Heckmaier, and Riedmair, *ibid.*, 1932, **494**, 86). Two formulæ for desoxophyll-

(XXV.)



(XXVI.)

erythrin presented themselves for consideration—(XIX) or one of the porphinpropionic acids (I—VIII), which have two more hydrogen atoms. These eight isomerides were all synthesised, and none was identical with desoxophyllerythrin. The difference in absorption spectra could only be due to an isocyclic ring in the molecule. (XIX) was then synthesised according to the following scheme: the product was identical with the desoxophyllerythrin from natural sources. On oxidation with oleum and sulphur, desoxophyllerythrin was converted into phylloerythrin and some chlorophoryrin-e_5 (XXI). We admit that the synthesis does not prove that the carbonyl group in phylloerythrin is in position 9—it could just as well be in position 10; however, the conversion of phylloerythrin into phylloporphyrin, pyrroporphyrin, and rhodoporphyrin, which has already been mentioned, is a clear proof of the position of the carbonyl group.

The constitutional formula of chloroporphyrin-e_6 is further supported by the action of formic acid on the porphyrin, which produces chloroporphyrin-e_4 (XXII) in good yield. Chloroporphyrin-e_4 in its turn can be converted into rhodoporphyrin-γ-carboxylic acid (XXIII) and phylloporphyrin as well as into chloroporphyrin-e_5. The transformation into chloroporphyrin-e_5 takes place very easily under the mildest conditions, when oxygen is present; and conversely chloroporphyrin-e_5 is very easily converted into chloroporphyrin-e_4. The ease with which the methyl group of chloroporphyrin-e_4 is oxidised is remarkable; it is caused by the carboxyl group in position 6. The substituent in this position can activate the substituent in the γ-position, just as is observed in the case of *o*-substituents in the benzene ring.

The majority of the chlorophyll porphyrins are oxidised by iodine in glacial acetic acid in the presence of sodium acetate. Under these conditions chloroporphyrin-e_6 (XX) is very easily converted into chloroporphyrin-e_7-lactone dimethyl ester (XXV). Oxidation has therefore taken place at position 10. The lactone ester (XXV) may be decarboxylated to hydroxymethyl rhodoporphyrin lactone (XXVI), which can then be oxidised in the same way to chloroporphyrin-e_5 (XXI). If the lactone ester (XXV) itself is oxidised, it yields phæoporphyrin-a_7 (XV). In the reverse direction chloroporphyrin-e_5 (XXI) may be reduced to hydroxymethyl rhodoporphyrin-lactone (XXVI), and phæoporphyrin-a_7 (XV) to chloroporphyrin-e_7-lactone. Finally, phæoporphyrin-a_5 (XVII) may be oxidised by the iodine method (in *alcohol*) to neophæoporphyrin-a_6 (XVI). If sodium carbonate is used instead of sodium acetate in this case, the product is phæoporphyrin-a_6.

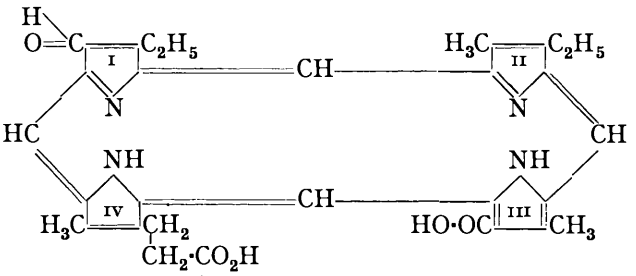
So there can be no doubt as to the relationships between the porphyrins in the table, and their structure must be regarded as determined. One transformation only has not yet been successfully carried out—the hydrolysis of neophæoporphyrin-a_6 (XVI) to chloroporphyrin-e_7-lactone dimethyl ester (XXV); we always obtained instead phæoporphyrin-a_7. Whether this is due to an unavoidable oxidation or not, has not yet been determined.

As we have already explained, when chlorophyll, chlorophyllide, phæophytin, phæophorbide, or methyl phæophorbide is subjected to short hydrolysis with alcoholic potash, the products are chlorin-e and rhodin-g, or a precursor of the latter; into the last question we need not enter. Chlorin-e forms with diazomethane, or with hydrochloric acid and alcohol, beautifully crystalline di- and tri-methyl esters. The trimethyl ester is isomeric with that of chloroporphyrin-e_6 (H. Fischer and Siebel, *Annalen*, 1932, **494**, 73), as is shown by the agreement in the analyses as well as in the calorimetric measurements of Dr. Stern (Stern and Klebs, *Annalen*, 1933, **505**, 295). Similarly methyl phæophorbide is isomeric with phæoporphyrin-a_5 dimethyl ester, and, in general, phorbides, chlorins and porphyrins which have the same empirical formulæ have also the same energy values. Chlorophyll itself must therefore have a modified porphyrin ring as its basal structure. This view is supported by the possibility of converting chlorin-e trimethyl ester into pyrophæophorbide, the product of the decarboxylation of phæophorbide. This transformation corresponds to the transition from chloroporphyrin-e_6 into phæoporphyrin-a_5, but with the difference that here the carbomethoxy-group in position 10 remains intact, while in the former case it is split off. This must be due to a constitutional peculiarity of phæophorbide, since in phæoporphyrin-a_5 itself the carbomethoxy-group in position 10 is relatively stable. When treated with pyridine and sodium carbonate under conditions in which methyl phæophorbide

is completely and smoothly decarboxylated, phæoporphyrin-a_5 dimethyl ester is stable, that is, only traces of phylloerythrin ester are formed. (Of course in an acid medium decarboxylation to phylloerythrin ester takes place relatively easily.) The isomerism between phorbides, chlorins, and porphyrins is further illustrated by their behaviour on catalytic hydrogenation. In each case three molecules of hydrogen are taken up and the leuco-stage is reached; on reoxidation porphyrins are formed. Under these conditions chlorophyll itself and methyl phæophorbide yield only phæoporphyrin-a_5, which must therefore have the same basic structure as chlorophyll.

Indications of the nature of the isomeric modifications in the porphin nucleus were obtained by a new method of reduction with hydriodic acid, applied to chlorophyll, the phorbides, the purpurins, and the chlorins (H. Fischer and Riedmair, *Annalen*, 1933, **505**, 87; 1934, **508**, 224). If this is carried out in the cold, new keto-porphyrins are obtained—the product from chlorophyll and methyl phæophorbide-a is oxophæoporphyrin-a_5 [formerly called *iso*phæoporphyrin-a_6 (*loc. cit.*)]. The presence of a second keto-group was clearly proved, and it was further shown that this "oxo-reaction" is a general reaction of phorbides and chlorins. Pyrophæophorbide yields oxophylloerythrin, chlorin-e trimethyl ester gives oxochloroporphyrin-e_6.

The investigation of 10-oxyphæophorbide-a, which is obtained from phæophorbide-a by oxidation with iodine in glacial acetic acid according to the method we have already mentioned, was of special importance. On reduction with hydriodic acid in the usual way (at 65°) it yields neophæoporphyrin-a_6, in confirmation of the constitution we assumed for it. Reduction in the cold leads to oxoneophæoporphyrin-a_6 and oxorhodoporphyrin. The occurrence of the latter is explained as due to hydrolytic fission of the oxoneophæoporphyrin-a_6 and oxidation to oxophæoporphyrin-a_7, the γ-side chain of which is labile, in analogy with phæoporphyrin-a_7; and as phæoporphyrin-a_7 is easily transformed into rhodoporphyrin, here oxorhodoporphyrin is formed. This was obtained well crystallised and its structure was explained as :

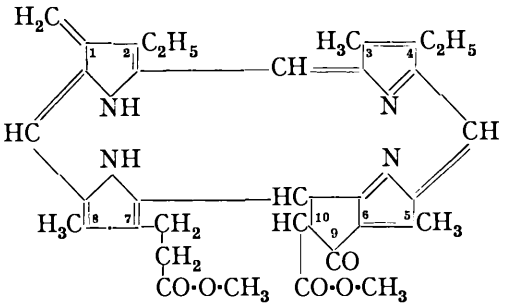


(XXIX.) *Oxo(4-formyl)rhodoporphyrin* ($C_{32}H_{32}O_5N_4$).

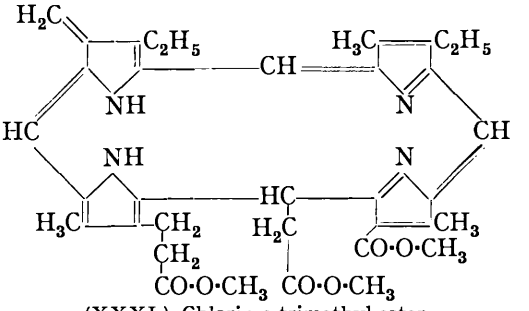
That is to say, we are most probably dealing with a porphyrin containing an aldehyde group joined directly to the ring; the majority of the reactions of oxorhodoporphyrin are in agreement with this view. It was possible to convert it into rhodoporphyrin by the Wolff–Kishner method (action of sodium ethoxide and hydrazine under pressure). Reduction with sodium ethoxide gave an alcohol, from which water could not be split off even by heating in a high vacuum. Hence the presence of an acetyl group (instead of a formyl group) in oxorhodoporphyrin is impossible; for experience has shown that in the hæmin porphyrins, the secondary alcohols, which are obtained by the reduction of acetyl groups, lose water easily and yield the vinyl derivatives. On the other hand, concentrated sulphuric acid causes a shift of the spectrum towards the red. However, for the present we must consider the presence of a formyl group more likely.

Since porphyrins and their leuco-compounds do not undergo the oxo-reaction, there must be in those compounds which do undergo the reaction, namely, chlorophyll, the phorbides, chlorins, and purpurins, some unsaturated side chain which is responsible for it. If the oxo-compounds contain an acetyl group, we must consider an ethylidene side chain as its precursor; if, on the other hand, the oxo-group is a formyl group (as is more likely) the side chain giving rise to it is probably a methylene group. In accordance with this

view are the following formulæ for methyl phæophorbide-a and chlorine-e trimethyl ester, which are, of course, closely related (H. Fischer and Siebel, *Annalen*, 1932, **449**, 84):



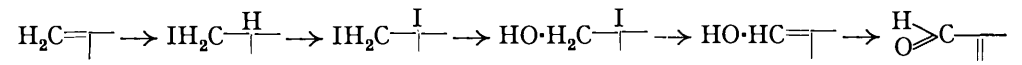
(XXX.) Methyl phæophorbide-a ($C_{36}H_{38}O_5N_4$).



(XXXI.) Chlorin-e trimethyl ester ($C_{37}H_{42}O_6N_4$).

These relationships are readily explained by the given formulæ. The fact that chlorin-e triester is at once decarboxylated under the influence of pyridine and sodium carbonate, yielding pyrophæophorbide-a, while chloroporphyrin-e_6 is converted into phæoporphyrin-a_5, is explained by the unsaturated side chain and by the arrangement of the substituents at the γ-carbon atom, which factors must also account for the phorbide and chlorin spectra.

The oxo-reaction very probably occurs as follows:

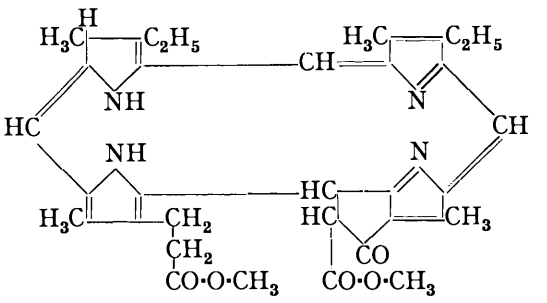


First, hydrogen iodide is added on at the double bond of the methylene group, just as in the formation of the dihydro-compounds. Then the tertiary hydrogen is replaced by iodine, the other iodine atom is replaced by hydroxyl, hydrogen iodide is split off, and the hydroxymethylene group undergoes rearrangement to form an aldehyde group, while at the same time rearrangement takes place in the ring with formation of the dihydroporphin structure. Finally oxidation to porphyrin takes place, probably through the stage of a leuco-compound. The whole reaction takes several hours for completion, and it is probable that primarily a hydrogenated intermediate compound is formed, recognisable by the fading of the colour, and this in a secondary reaction gives rise to the oxo-compound.

At first sight this complete reaction mechanism seems surprising. But the facility with which chloroporphyrin-e_4 (XXII) is oxidised by iodine, acetic acid, and sodium acetate to chloroporphyrin-e_5 (XXI) is beyond doubt; as is also the oxidation which takes place under these conditions of the carbon attached to the ring in position 10. The difference between this and the oxo-reaction is obviously due to the concentrated hydriodic acid present in the latter case, which causes the iodine to attack the molecule in a different way.

If we assume the above structure for methyl phæophorbide, the fact that chlorophyll and its nearest derivatives readily take up two atoms of hydrogen (H. Fischer and Lakatos, *Annalen*, 1933, **506**, 123; cf. also Stoll and Widemann, *Naturwiss.*, 1932, 791) is explained at once. The dihydro-compounds are formed by saturation of the methylene group, so that methyl dihydrophæophorbide-a must have the following formula:

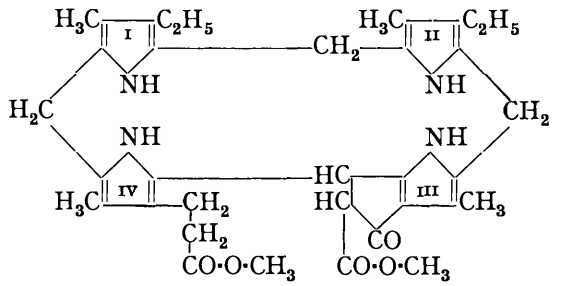
(XXXII.)

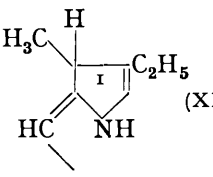


There is no essential difference in the conjugated system between this structure and that of methyl phæophorbide-a; it explains the slight difference in the spectrum and the possibility of ring opening to form dihydrochlorin-e trimethyl ester, as well as the production of the other chlorophyll porphyrins. These are formed from chlorin-e and its dihydro-derivative simply by heating with glycol; in the latter case two hydrogen atoms are lost.

It is a remarkable fact that, in the case of the dihydro-compounds, the oxo-reaction is complete only after two days, and the leuco-compounds, whether of the dihydro-compounds, the phæophorbides, or the porphyrins, do not react at all. The most probable explanation of this is that in the leuco-compounds the pyrroline structure of pyrrole ring I is no longer present. The leuco-compound of phæophorbide-a, its dihydro-compound, and of phæoporphyrin-a_5, has formula (XXXIII). From this a transformation into (XXXII) and (XXX) is no longer possible. On hydrogenation of the phæophorbides and the dihydrophæophorbides, the unstable leuco-compound (XXXIV) is first formed and this then undergoes intramolecular rearrangement to the structure (XXXIII). The ex-

(XXXIII.)

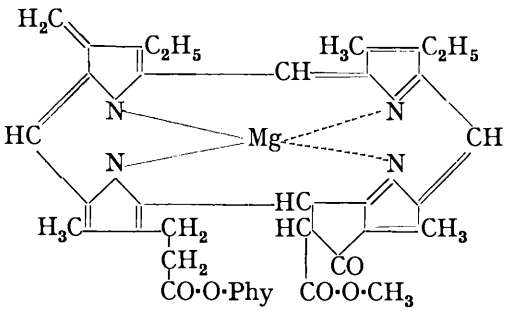




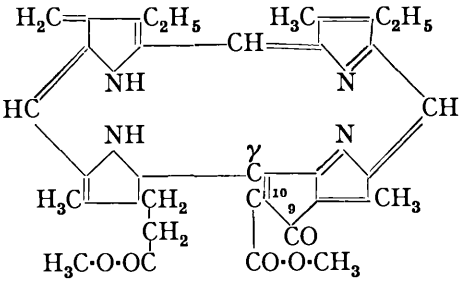
(XXXIV.)

planation of the fact that the dihydrophorbides and dihydrochlorins do undergo the oxo-reaction—though more slowly—is that, in part, the leuco-compound (XXXIII) is formed; as a result, iodine is set free, which then oxidises the dihydro-compound (XXXII) to the state represented by (XXX). The reaction now proceeds as on page 253, that is, the oxo-compound is formed.

Chlorophyll itself must have the structure (XXXV), which explains all its reactions. In the reaction with diazomethane in methyl alcohol, chlorin-e trimethyl ester is formed, which involves a simple methanolysis between carbon atoms 9 and 10 (compare XXX and XXXI).



(XXXV.) Chlorophyll-a ($C_{55}H_{72}O_5N_4Mg$).



(XXXVI.) ($C_{36}H_{36}O_5N_4$).

Two very important reactions of intact chlorophyll are allomerisation (Willstätter) and the phase test (Molisch). By allomerisation is meant the change undergone by chlorophyllide when its alcoholic solution is evaporated to dryness, whereby it loses its power of crystallisation. The allomerised material is sharply differentiated from the original material by its failure to give the phase test. This reaction is shown by intact chlorophyll and by fresh leaves; on the addition of methyl-alcoholic potash an intense brown colour is formed which soon changes to green. Allomerised chlorophyll gives the green phase at once.

Conant found that, when the mixture of chlorophyllides-a and -b underwent allomerisation in alcohol, 1 mol. of oxygen was consumed. Up to the present this observation has not been repeated with other solvents. But there is really a change in the chlorophyll, as we have shown with the separated components. Further, (1) no acetaldehyde can be detected in the alcohol; (2) when chlorophyll, which has been allomerised with 1 mol. of oxygen, is reduced with hydriodic acid and hydrolysed with hydrochloric acid, it yields phæoporphyrin-a_7 (XV); intact chlorophyll is converted by the same treatment into phæoporphyrin-a_5 (XVII).

The allomerisation of chlorophyll with *p*-benzoquinone gives more insight into the problem. In an atmosphere of nitrogen, 1 mol. of the quinone is reduced to quinol, and the allomerised chlorophyll which is formed is found to have added on a molecule of alcohol, for, on reduction with hydriodic acid, phæoporphyrin-a_6 (XVI; $O{\cdot}C_2H_5$ instead of OH) is produced.

The explanation of the formation of phæoporphyrin-a_7 and phæoporphyrin-a_6 is, in my opinion, only possible if we assume that, on allomerisation, a double bond is formed between the γ-carbon atom and carbon atom 10. In order to obtain the formula for allomerised phæophorbide we must write (XXX) with two atoms of hydrogen less, thus getting (XXXVI).

Apart from this, the formula is analogous to (XXX), and 1 mol. of water or alcohol can easily be added, in the 1 : 4-position, to the conjugated system between pyrrole ring III and the isocyclic ring. In the formation of phæoporphyrin-a_7, this arises in a secondary reaction during the hydrolysis with hydrochloric acid, during which a hitherto unexplained and certainly unavoidable oxidation takes place. The formation of phæoporphyrin-a_6, on the other hand, is due to the stabilisation caused by the ether group. This assumption is supported by the analogous reaction of natural phæophorbide, which is converted by hydriodic acid in the presence of glacial acetic acid and air into neophæoporphyrin-a_6, and by the "iodine method" into 10-hydroxyphæophorbide. Here again 1 : 4-addition of water takes place. Oxidised chlorophyll or phæophorbide is therefore poorer by two atoms of hydrogen than these porphyrins, since they are formed in a secondary reaction by the addition of water or alcohol.

We therefore explain the phase test as an enolisation of the carbonyl group in position 9, the hydrogen from C_{10} wandering to form the hydroxyl group. The brown phase is due to the change in structure and to the formation of an alkali salt; the brown changes to green owing to oxidation. Since allomerised chlorophyll no longer possesses the hydrogen atom which wanders, it cannot show the brown phase. Various oxidations take place in the strongly alkaline medium, which lead finally to the formation of rhodoporphyrin-γ-carboxylic acid, or its green anhydride. Propyl-alcoholic potash on the other hand produces purpurins, through the intermediate stage of an unstable chlorin, as Conant showed.

The purpurins probably resemble the phorbides in structure, since they also undergo the oxo-reaction. With alkalis they immediately form green salts. 10-Ethoxyethyl-phæophorbide does not show the phase test; the green colour appears at once, since no enolisation can take place.

In chlorophyllide, methyl phæophorbide, and phæophorbide there is a keto-group. This view is confirmed by their behaviour with diazomethane in methyl alcohol—they yield chlorin-e trimethyl ester, while the allomerised compounds form only unstable chlorins. As the allomerised compounds have the original nuclear structure, these chlorins must correspond to formula (XXXVI). Lately we succeeded in proving directly the presence of this keto-group in natural phæophorbide, methyl phæophorbide and in chlorophyllide-a. In an atmosphere of nitrogen at the ordinary temperature they form oximes. The oximes can be reconverted into the parent substances, which still show the phase test and on methanolysis yield chlorin-e trimethyl ester. Cautious reduction of the oximes with hydriodic acid yields the oxime of phæoporphyrin-a_5.

In conclusion I will mention briefly the relationship between chlorophyll and hæmin, or hæmoglobin. Chlorophyll is a wax; hæmoglobin, in my opinion, is a molecular compound of globin and hæm. Chlorophyll, as an ester, contains not only phytol but methyl

alcohol; its nucleus is an isomeric modification of the porphin ring; the side chains, however, correspond, not to those of hæmin, but to an isomeric form of mesoporphyrin. The two vinyl groups of hæmin are hydrogenated to ethyl groups; the propionic acid group in position 6 has become a β-ketopropionic acid, which has united with the γ-carbon atom and undergone oxidation to form the isocyclic ring characteristic of chlorophyll; while at the same time a hydrogen atom has wandered from a methyl group in position 1 or 3 to the γ-carbon atom. Finally, magnesium has replaced co-ordinately bound iron.

The last-mentioned wandering of a hydrogen atom has not taken place in mesochlorin, since this does not undergo the oxo-reaction. The synthetic chlorins, therefore, represent a still unknown modification of the porphin system; the oxo-structure, on the other hand, seems to occur in nature. Gaffron has shown that the pigment of the sulphur bacteria (*Beggiatoa thiocystis*) is sensitive to light and oxygen and is non-fluorescent. After a hydrochloric acid extract of the bacteria (*B. thiocystis*) has been precipitated with lead carbonate, a green pigment, which has a fine spectrum, may be extracted with pyridine. Mr. Gaffron sent us some of his material, which, on fractionation, yielded porphyrins spectroscopically identical with oxophæoporphyrin-a_5 and oxophylloerythrin.

When the pure culture is extracted with pyridine, and the extract warmed for a short time with formic acid, the same result is obtained on spectroscopic examination. Reduction for three minutes with hydriodic acid in glacial acetic acid, which converts chlorophyllide into phæoporphyrin-a_5, here yields oxorhodoporphyrin.

Noack (*Naturwiss.*, 1933, 835) recently isolated a chlorophyll derivative from *Beggiatoa purpurea* which, according to the analyses, has a higher oxygen content than usual. He considers it likely that this is a derivative of chlorophyll-b, but I do not think this probable; it is more likely that the oxo-reaction * takes place in the metabolism of the bacteria.