CHLOROPHYLL[1]

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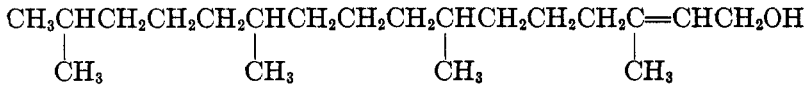
The pigment of green leaves is a complex compound of waxy character, consisting of a magnesium-containing pyrrole component and of the alcohols, phytol, $C_{20}H_{40}O$, and methyl alcohol, which are esterified to two carboxyl groups of chlorophyll, as we know from the investigations of Willstätter and his coworkers (71).

The constitution of phytol has in the meantime been fully elucidated and proved by synthesis to be as follows (29):



Chlorophyllase, discovered by Willstätter, in an alcoholic medium brings about the replacement of phytol by the alcohol employed, and thus the crystalline methyl- or ethyl-chlorophyllides are obtainable, which prove to be mixtures, like chlorophyll itself. The latter consists of two components, *a* and *b*, of which the *b* component is separable only with difficulty and was very recently obtained pure for the first time by Winterstein and Stein (76, 77) by the chromatographic method; and ethylchlorophyllide *b* was obtained by us (50) by a method of partial synthesis. The latter method is also by far the most convenient for obtaining ethylchlorophyllide *a*. The chlorophyllides possess the following composition (73):

Methylchlorophyllide *a*	$[C_{32}H_{30}ON_4Mg](COOCH_3)_2 + 1/2\ H_2O$
Ethylchlorophyllide *a*	$[C_{32}H_{30}ON_4Mg](COOC_2H_5)(COOCH_3)$
Methylchlorophyllide *b*	$[C_{32}H_{28}O_2N_4Mg](COOCH_3)_2 + 1/2H_2O$
Ethylchlorophyllide *b*	$[C_{32}H_{28}O_2N_4Mg](COOC_2H_5)(COOCH_3)$

By treatment with acids, there is obtained from chlorophyll the magnesium-free pheophytin, which may also be separated into the components *a* and *b*. Under the action of hydrochloric acid or of hydrochloric acid and methyl alcohol, pheophytin gives the pheophorbides, methylpheo-

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phorbide $a + b$. Both crystallize extremely well; a is obtainable pure with relative ease, even in large amounts by employing Dr. Neumann's extraction apparatus, while b is more difficult to obtain pure. These compounds have the following formulas (74):

Pheophorbide a	$[C_{32}H_{32}ON_4](COOCH_3)(COOH)$
Methylpheophorbide a	$[C_{32}H_{32}ON_4](COOCH_3)_2$
Pheophorbide b	$[C_{32}H_{30}O_2N_4](COOCH_3)(COOH)$
Methylpheophorbide b	$[C_{32}H_{30}O_2N_4](COOCH_3)_2$

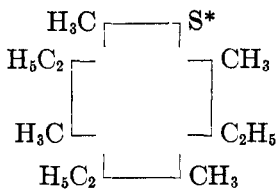
If pheophytin or pheophorbide is subjected to a quick (30 sec.) saponification with methyl alcoholic potassium hydroxide, phytochlorin e and phytorhodin g are produced, more briefly designated as chlorin e and rhodin g. Chlorin e corresponds to the a component, rhodin g to the b component. Chlorin e was observed by Willstätter (75) as occurring in two modifications, as a lactam hydrate of the formula $C_{34}H_{36}O_6N_4$ and as a lactam $C_{34}H_{34}O_5N_4$. In the theoretical introduction to his book on chlorophyll, only the latter formula is given on page 15.

Phytorhodin g (72) possesses the composition $C_{34}H_{32}O_7N_4$. Phytochlorin e was regarded as a tricarboxylic acid with two carboxyl groups free and one bound as a lactam. Phytorhodin g was considered a tetracarboxylic acid (72), with only two or three carboxyl groups free.

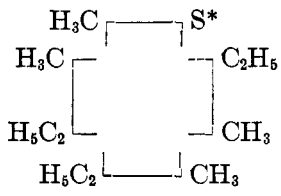
Chlorin e gives a beautifully crystalline trimethyl ester which, according to Treibs and Wiedemann (69), can be prepared with particular ease by means of diazomethane, and possesses the formula $C_{37}H_{42}O_6N_4$. Phytorhodin g also gives with diazomethane a beautifully crystalline trimethyl ester, of the formula $C_{37}H_{40}O_7N_4$.

By energetic degradation of chlorophyll and its derivatives Willstätter and his coworkers obtained numerous phyllins and porphyrins, notable among which are phyllo-, pyrro-, and rhodo-porphyrin, and the etioporphyrin obtainable from them by pyrolysis, which has been held to be identical with the parent substance of hemin.

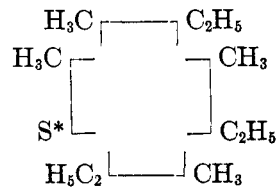
It was naturally a matter of outstanding interest to elucidate the structure of these porphyrins, and since in the meantime we had achieved general methods of porphyrin synthesis, there was thus a prospect of clarifying the constitution of the chlorophyll porphyrins by synthesis. Rhodoporphyrin is a dicarboxylic acid; phyllo- and pyrro-porphyrins are monocarboxylic acids, which were held to be isomers by the earlier workers. It seemed most probable to regard them as tetramethyltriethylmonopropionic acid porphins, of which the theory predicts the following eight isomers, henceforth designated as monocarboxylic acids:



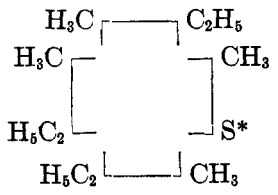
I
m.p. 247°C.



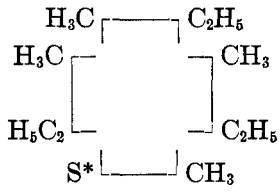
II
m.p. 263°C.



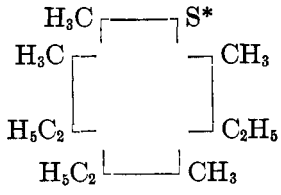
III
m.p. 271°C.



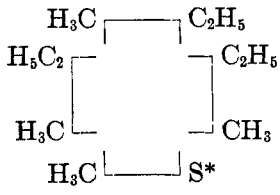
IV
m.p. 208°(220°)C.



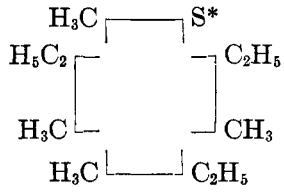
V
m.p. 238°C.



VI
m.p. 246°C.



VII
m.p. 216°C.



VIII
m.p. 214°C.

*S = CH_2CH_2COOH

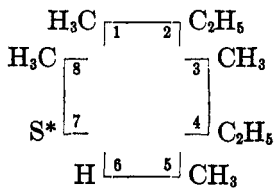
The internal structure of the porphin ring is omitted and each of the four pyrrole nuclei is given by a bracket, as in this review only the positions of the substituents will be interchanged.

These eight isomers were synthesized (13, 56) and all proved to be different from phyllo- and pyrro-porphyrin.

By analytical methods the presence of a free β-methine group in the latter porphyrins could then be demonstrated, which was lacking in rhodoporphyrin, but was still present in etioporphyrin from chlorophyll. The free methine group in the porphyrins named was demonstrated by bromination and oxidation; a bromine atom entered as a substituent into the porphins, and oxidation gave bromocitraconimide. Hence the most probable view concerning constitution was that the porphyrins mentioned were derived from the monocarboxylic acids by removal of an ethyl group. As this might occur in three ways, theory predicts twenty-four isomeric

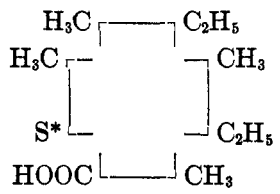
pyrroporphyrins and the same number of rhodoporphyrins, since it was highly probable, through the transition from rhodoporphyrin to pyrroporphyrin with splitting off of carbon dioxide, that in place of the free methine group in pyrroporphyrin a nuclear carboxyl group was present in rhodoporphyrin. The synthesis of certain pyrro- and rhodo-porphyrins completely confirmed this assumption (46, 6, 5, 47).

The problem of the constitution of pyrroporphyrin was solved by transforming it into the monocarboxylic acid III by introduction of an ethyl group (2). As this is derived from etioporphyrin III, the fundamental correspondence in the grouping of the side chains in blood and leaf pigment was demonstrated. Both are derivatives of etioporphyrin III. The constitution of pyrroporphyrin, however, was not yet fixed, as the free methine group might be located in the 2-, 4-, or 6-position, all three of which would give the monocarboxylic acid III on introduction of an ethyl group. The 6-position was proved through the synthesis of 1,3,5,8-tetramethyl-2,4-diethylporphin-6-carboxylic acid-7-propionic acid (X) which therefore carries the carboxyl group in the 6-position, and proved identical with the "natural" rhodoporphyrin, whose ready conversion into pyrroporphyrin was analytically demonstrated. At the same time the synthesis of 1,3,5,8-tetramethyl-2,4-diethylporphin-7-propionic acid (IX) was achieved. This proved to be identical with the "natural" pyrroporphyrin. Thus the free 6-position was also demonstrated for phylloporphyrin, for phylloporphyrin can be converted with alcoholate into pyrroporphyrin.



IX

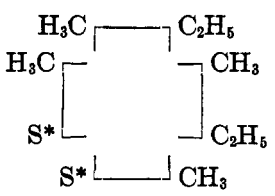
Pyrroporphyrin



X

Rhodoporphyrin

*S = CH_2CH_2COOH



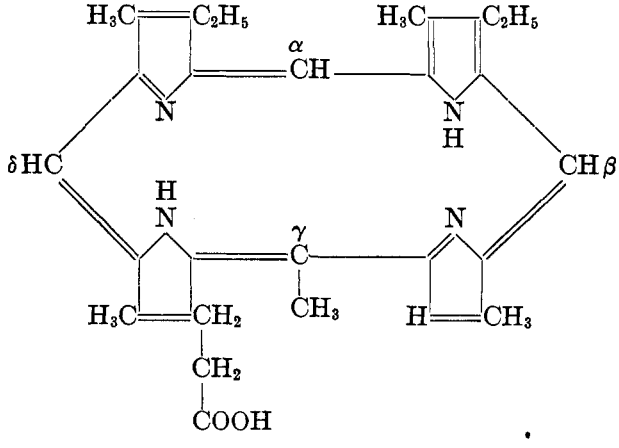
XI

*S = CH_2CH_2COOH

Pyrroporphyrin (IX) is closely related on synthetic grounds to the important blood-pigment porphyrin, mesoporphyrin (XI).

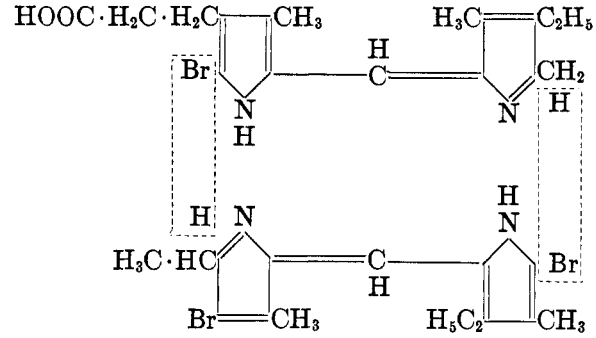
If the propionic acid residue at the 6-position in mesoporphyrin be replaced by a hydrogen atom, the formula of pyrroporphyrin results; hence we could convert pyrroporphyrin or pyrrohemin by treatment with chloromethyl ether and hydrogen bromide into bromomethyl-pyrrohemin or -porphyrin, respectively. This gave mesoporphyrin by conversion with sodium malonic ester (36). *Thus for the first time—although indeed indirectly—a common porphyrin was obtained from blood and leaf pigment;* for indeed the etioporphyrins of chlorophyll have nothing to do with the etioporphyrins of blood pigment. The "etioporphyrin of chlorophyll" proved to be a mixture of pyrro- and phyllo-etioporphyrin, corresponding in constitution to IX or XII. If both propionic acid residues are decarboxylated to ethyl groups, the formulas of the two etioporphyrins result. The proof was obtained by synthesis (6, 18, 47). Strictly speaking, moreover, the etioporphyrin of blood pigment should be considered to be, not the carboxyl-free mesoporphyrin, but the carboxyl-free protoporphyrin, whose synthesis was recently achieved (25). Recently, also, the breakdown of mesoporphyrin or mesorhodin by way of rhodoporphyrin-γ-carboxylic acid to pyrroporphyrin has been accomplished, whereby among other products the "natural" pyrroporphyrin (IX) was obtained (9).

Phylloporphyrin proved to be a homologue of pyrroporphyrin. Spectroscopically it is markedly different from the latter, like proto- and mesoporphyrin. It is particularly sensitive to the action of hydrogen iodide, and it was necessary to consider a vinyl group linking the pyrrole nuclei in the α-position, or the methyl substitution of a methine group (see XII).



XII

Ninety-six isomers were possible, but through the synthesis of pyrroporphyrin the number was limited to four. All four phylloporphyrins were synthesized (18, 48). The scheme of the synthesis of the "natural" phylloporphyrin may be given here:



The complications in these syntheses were theoretically and practically very great. Each of the pyrromethenes employed can react with itself, partial decarboxylation then taking place, so that in every synthesis ten different porphyrins were possible, which in large part were actually formed and for the most part also isolated from the syntheses of all four phylloporphyrins.

γ-Methylpyrroporphyrin (1,3,5,8-tetramethyl-2,4-diethyl-γ-methylporphin-7-propionic acid) proved to be identical with the "natural" phylloporphyrin; the mixed melting point of the esters gave no depression, while with the three isomeric esters marked depressions were found.

Phylloporphyrin was thereby synthesized, though the constitution was not unequivocally established. It was still possible that a vinyl group could bind the pyrrole nuclei 3 and 4. Final proof of constitution was achieved through clarification of structure and synthesis of phylloerythrin, which on breakdown passes over into phyllo-, rhodo-, and pyrro-porphyrins.

For the separation of these porphyrins, as indeed for porphyrins in general, the fractionation method developed by Willstätter and Mieg (70) proved itself of great value. The basicity of the porphyrins, because of the imino groups contained in them, is extraordinarily varied, and by treatment with hydrochloric acid of graded concentrations, especially by several repetitions of the process, a far-reaching separation even of quite complicated porphyrin mixtures can be achieved. This method also proved itself of outstanding value in the separation of the pheophorbides.

Tswett's adsorption analysis is also fruitful in porphyrin chemistry. With its aid A. Treibs (65, 66, 68, 67) isolated the vanadium salts of chlorophyll porphyrins and blood-pigment porphyrins from bitumen and petro-

leum, among others desoxophyllerythroetioporphyrin, whose separation from the accompanying etioporphyrins after its synthesis (23) was obtained only by the aid of adsorption analysis.

Willstätter and his pupils described numerous isomers of rhodoporphyrin, which have been subjected to a more intensive study by Treibs and Wiedemann. The result of this investigation was the discovery of a new porphyrin, verdoporphyrin, which passes over with extraordinary ease into rhodoporphyrin. The presence of verdoporphyrin probably explains the numerous porphyrins described by Willstätter and his pupils, such as erythro-, cyano-, rubi-, and glauco-porphyrin. Later pseudoverdoporphyrin was also isolated by us; this is identical with the "isorhodoporphyrin" of Conant, which the latter had obtained from purpurin (1). Isorhodoporphyrin and pseudoverdoporphyrin (26; see also 24) contain the vinyl group, as was shown through the action of diazoacetic ester and likewise through the reaction with phenyl azide. A vinyl group could thus be unambiguously demonstrated. One of the outstanding properties of pseudoverdoporphyrin is its capacity for taking up rhodoporphyrin. The separation of the two is then difficult, and the spectroscopic behavior of such a mixture is identical with the spectrum of verdoporphyrin (24).

A hydrogen bromide–glacial acetic acid mixture will add to pseudoverdoporphyrin, and through reaction with methyl alcohol a methyl alcohol addition product is obtained, which on heating—exactly like tetramethylhematoporphyrin—again splits off methyl alcohol with regeneration of pseudoverdoporphyrin (27).

Many of these findings were first brought up after the demonstration of a vinyl group in chlorophyll, and thence today the complicated results of the alcoholate degradation, as employed by other authors and by us, have become completely understandable. All the complications which have been exactly studied in hemin are here also at least theoretically possible, and must indeed have actually entered into individual researches. In hemin, which as is well known contains two vinyl groups, saturation of both vinyl groups to form ethyl residues occurs under the action of alcoholate; mesoporphyrin is formed. Besides, one of the two vinyl groups may be split off and one reduced, so that both isomeric hemoporphyrins arise. Besides, chlorin formation occurs from the porphyrin mixture which has arisen. Deuteroporphyrin must certainly be present also. Similar complications are naturally to be expected for chlorophyll breakdown, especially since here in the action of alcoholate widely different techniques in the manner of heating have often been employed. It is also not a matter of indifference whether one starts from phorbides or chlorophyllides. We have not as yet been able to obtain evidence for the formation of further vinylporphyrins except pseudoverdoporphyrin—

which is conveniently designated as vinylrhodoporphyrin—by alkaline degradation (by other methods all have been isolated). The individual fractions were negative to diazoacetic ester. Such vinylporphyrins might most readily be expected in the *b* series, in which, because of the neighboring position of the vinyl group and the formyl residue in the 2- and 3-positions, particular opportunity is afforded for an abnormal course of the reaction.

Parallel to this analytic-synthetic research went the study of the biological breakdown of chlorophyll, because the clarification of the constitution of substances biologically related to hemin had supplied essential starting points for the determination of the constitution and also the way to the synthesis of this substance.

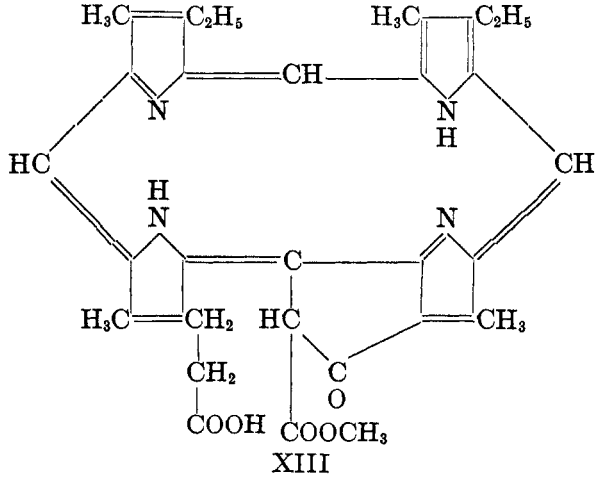
Through the investigations of Löbisch and Fischler (57) and of Marchlewski (58) phylloerythrin was known, which has been obtained from the bile of cattle or the feces of animals. From sheep feces we could obtain probophorbides *a*, *c*, and *d* (19, 21), isomeric with phylloerythrin, as well as pheophorbide *a*, pyropheophorbide *a*, dihydropyropheophorbide *a*, and pyropheophorbide *b*, which with the exception of pheophorbide *a* and the last-named *b* derivative pass over readily into phylloerythrin and are important because, as indeed their name indicates, they are spectroscopically closely related to pheophorbide and thus to the phorbides generally. Hence we must conclude that the pheophorbides as well as phylloerythrin stand in the closest relation to chlorophyll itself. In agreement with this their formation occurs in the Omasus and Abomasus (third and fourth stomachs of ruminants) as Rothemund and Inman (59) showed. Now phylloerythrin is a porphyrin (22), on the grounds of its spectroscopic behavior. Thus for the first time a basis was obtained for the view that the chlorophyll molecule also stood in close relation to the porphyrins; a possibility further supported by synthetic studies through conversion of blood pigment porphyrins into chlorins and rhodins (55, 17, 12). By bacteriological methods (19, 20) chlorophyll derivatives were likewise converted into porphyrins, again a confirmation of the relations stated above. From the conversion of chlorophyll and its derivatives into phyllo-, pyrro-, and rhodo-porphyrins the presence of the porphin nucleus could not be inferred, because in view of the brutal methods of treatment involved (alcoholate breakdown at high temperature in a sealed tube) secondary synthesis might be involved; all the more since these porphyrins are poorer in carbon than the starting material. Willstätter likewise in his book on chlorophyll (72a) had expressed himself as follows: "Also between chlorophyll and aetiophyllin and even between chlorophyll and the first dibasic porphyrins such as cyanoporphyrin or erythroporphyrin lie two steps which essentially transform the molecule, and which cannot be brought into parallel with the reactions of hemin.

"One step is the delactamization and relactamization of the chlorophyll components, through which one ring system is broken and a new one synthesized. The second rearrangement of the molecule, which takes place less obviously, occurs through the action of alkali at high temperatures and leads from the chlorophyllins to the more simply constituted dibasic phyllins, or from phytochlorin and phytorhodin to the corresponding porphyrins."

And somewhat further on: "Therefore it is more probable that the lactam group is of the pyridone type, its carboxyl therefore itself a ring constituent, and that the fourth pyrrole nucleus of cyanoporphyrin first arises through removal of a carbon atom."

It was necessary to search for methods which, while preserving the carbon framework of chlorophyll, should lead to porphyrins, for which synthetic methods stood available. Cautious reduction with hydriodic acid and glacial acetic acid proved fruitful in results here; application of this method to pheophorbide and to chlorin *e* yielded fundamentally different results. The pheophorbides gave pheoporphyrins; chlorin *e* gave chloroporphyrins (3, 4, 31, 32, 33, 34, 10). Spectroscopically, essential differences between the two classes were manifest. In the pheoporphyrins the second and third absorption bands appear compressed, as in the spectroscopic picture of phylloerythrin, while in the chloroporphyrins the spectral phenomena are much more similar to those of the blood pigment porphyrins.

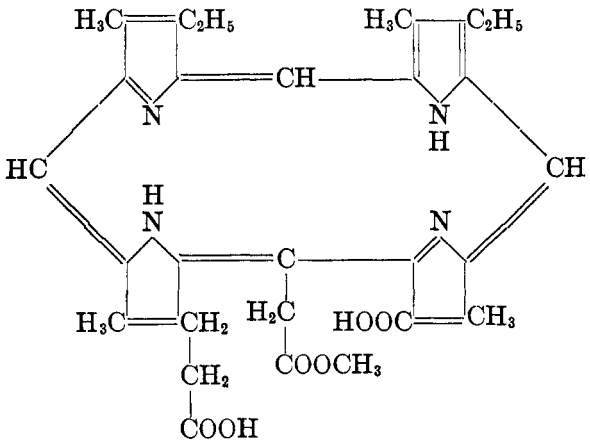
Numerous porphyrins were isolated, and their constitution determined, which cannot be discussed in more detail in a short lecture. The most important among them is pheoporphyrin a_5 (11, 15) (XIII) which is obtainable from the pheophorbides, also from pheophytin and chlorophyll-



XIII

lide, and which always occurs as a monomethyl ester (51, 33, 34); indeed the carbomethoxyl residue in position 10 is only saponifiable with diffi-

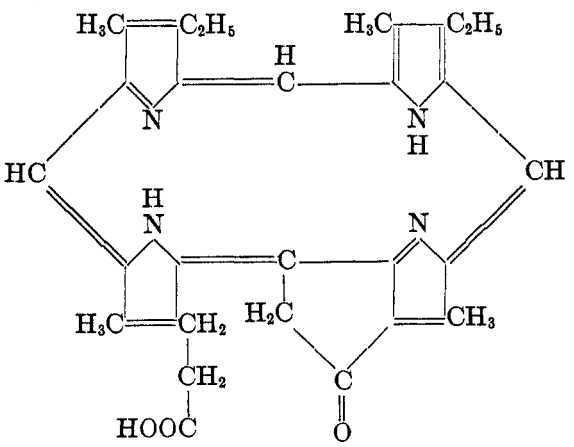
culty. Pheoporphyrin a_5 reacts with ketone reagents and can be split by hydrolysis to chloroporphyrin e_6, which is likewise a monomethyl ester and possesses the following formula (XIV):



XIV

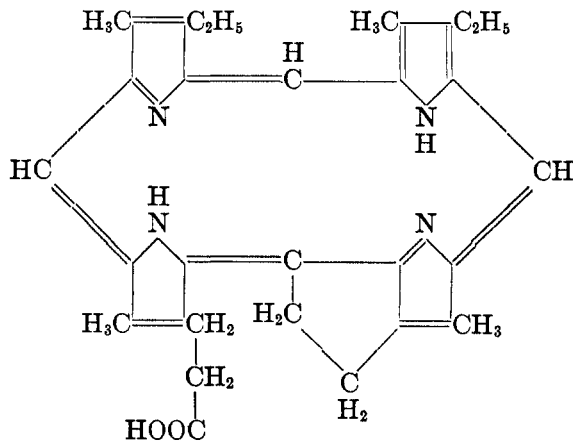
For, on the one hand, it can readily be reconverted into pheoporphyrin a_5; on the other hand, it passes over on treatment with 30 per cent methyl alcoholic potassium hydroxide into chloroporphyrin e_4 (through replacement of the $COOCH_3$ residue by H (XVIII) and rhodoporphyrin (through replacement of the CH_2COOCH_3 residue by H) (X). Through the transformation of chloroporphyrin e_6 into pheoporphyrin a_5, and its reversal, a transition between the chloroporphyrins and the pheoporphyrins was brought about for the first time, and the presence of a carbomethoxylated isocyclic ring in chlorophyll was made probable.

Pheoporphyrin a_5 (XIII) on decarboxylation passes over readily into phylloerythrin (XV):



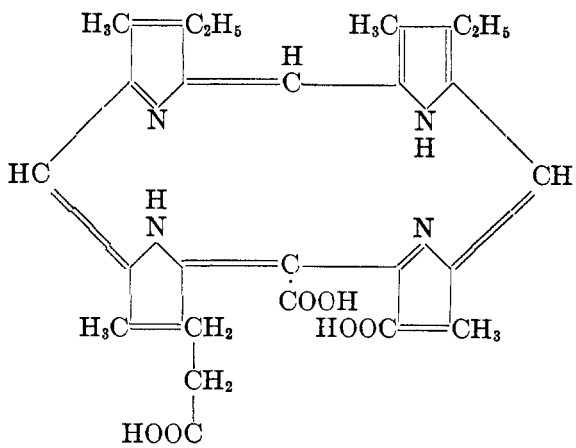
XV

Phylloerythrin may be readily reduced to desoxophylloerythrin (XVI), which contains only two atoms of oxygen, and these are in a carboxyl



XVI

group. The constitution of phylloerythrin was demonstrated as follows: Phylloerythrin does not react with alkalies in an atmosphere of nitrogen, but in the presence of oxygen breakdown occurs to the characteristic chlorophyll porphyrins, phyllo-, pyrro-, rhodo-porphyrin, and rhodo-porphyrin-γ-carboxylic acid (XVII) which was first obtained by this method; this behavior can only be explained by an ethanone bridge between C_6

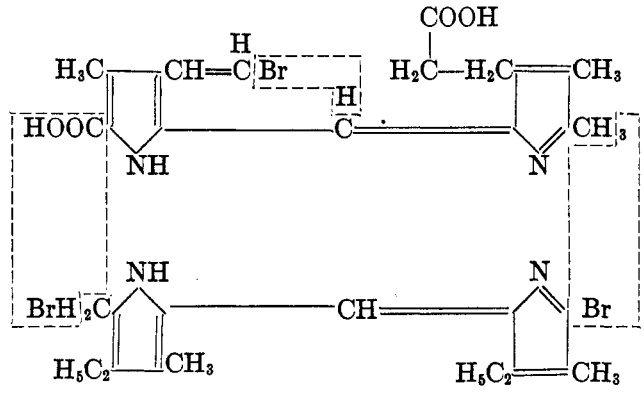


XVII

and the γ-methine group. The keto group could be certainly demonstrated through reaction with ketone reagents.

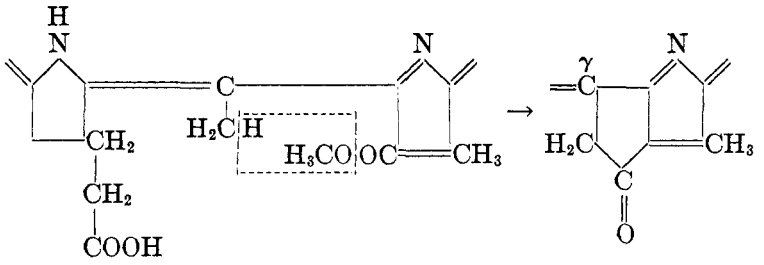
The constitution of desoxophylloerythrin and of phylloerythrin, however, could also be demonstrated by synthesis (37, 39, 40, 16). On the basis of elementary analysis only the formula of a monocarboxylic acid (page 43) or the ring formula (XVI) with two less hydrogen atoms, stood available for desoxophylloerythrin. Fortunately all eight monocarboxylic acids (page 43) had already been synthesized. Spectroscopic observations had already excluded identity (the spectroscopic difference due to the linked isocyclic ring is not in itself large). A new spectral type arises as the C=O group adjoining nucleus III enters (compare formula XV). The spectroscopic influence will be of interest in the iso compound, with CO adjoining the γ-position. The synthesis of an is ophylloerythrin is under way; likewise the mixed melting points gave marked depressions. Only the ring formula, therefore, remained possible.

The synthesis was carried out according to the following scheme, and gave a substance which proved to be identical with the "natural product" in all its properties (38). Desoxophyllerythrin gave, on oxidation with



fuming sulfuric acid containing sulfur, phylloerythrin along with chloroporphyrin e_5. We admit that the position of the CO group at 9 in phylloerythrin is not proved by this synthesis. It might equally well be in the 10-position; but the result of the degradation of phylloerythrin which leads, as stated, to the porphyrins pyrro-, phyllo-, and rhodo-porphyrin, demonstrates unequivocally the position of the carbonyl group.

Recently (35) we have demonstrated in another manner by direct synthesis the constitution of phylloerythrin, employing the action of sodium ethylate on chloroporphyrin e_4, according to the following formulation.

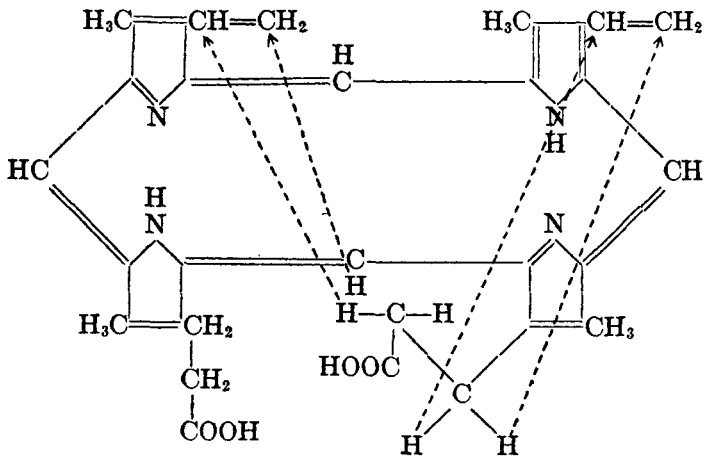


XVIII XV

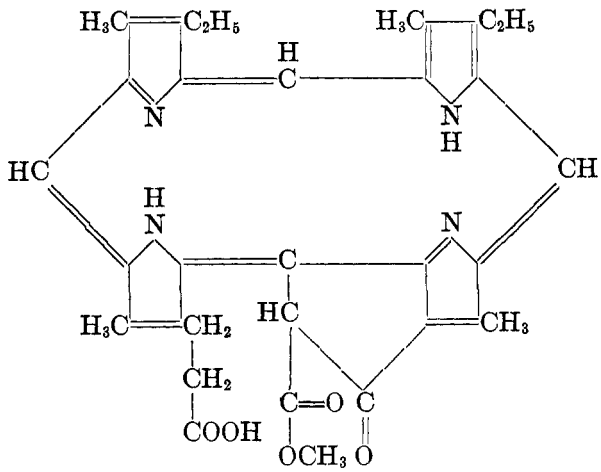
Through the proof of the constitution of phylloerythrin, that of phylloporphyrin as a methyl-substituted pyrroporphyrin was also determined, and the γ-position once more proved.

Let us now summarize briefly the most important results of this section:

Chlorophyll is a pyrrole pigment, esterified with phytol and methyl alcohol. It contains magnesium, bound in complex linkage. Fundamental to it is the porphin system, built into which is an isocyclic ring with a keto group at 9 and a carboxymethyl group at 10; otherwise in respect to the arrangement of the substituents it corresponds to etioporphyrin III and therefore to the hemin of hemoglobin. One may also picture the most important of the chlorophyll porphyrins, pheoporphyrin a_5, as derived from protoporphyrin through β-oxidation of the propionic acid residue in the 6-position to a ketopropionic acid, and condensation, with loss of water, between carbon atom 10 and the γ-methine group of the porphin nucleus, with saturation of both vinyl groups of protoporphyrin to give ethyl residues, as illustrated by the following formulas:



XIX



XX

From a purely formal point of view, one may thus regard pheoporphyrin a_5 as a monoxide of protoporphyrin, and pheoporphyrin b_6 as an oxidation product of pheoporphyrin a_5, in which the methyl group in the 3-position is replaced by the formyl group.

For the porphyrins in general A. Stern and H. Wenderlein (62) have determined the pyrrolenine structure of two opposite nuclei on the basis of the varying influence on light absorption by the carbonyls in the porphin system, according to the position on the nucleus.

Further, the attachment of the phytol group to the propionic acid residue has been demonstrated, and that of the methoxyl to the carboxyl group of the isocyclic ring.

Through degradation of pheophorbide with diphenyl at 180–250°C., Conant has obtained free pyropheophorbide. This method of investigation is open to possible objection because of the high temperature and possible re-esterification of the pheophytin through the alcohol employed. The decarboxylation, however, can be carried through with the same result using boiling pyridine, and pheoporphyrin a_7 (rhodoporphyrin-γ-glyoxalic acid methyl ester) has always been observed only as a monomethyl ester, esterified on the γ-glyoxyl side chain, giving on degradation free rhodoporphyrin (52). Ethyl chlorophyllide also gave the ethyl ester of phylloerythrin (52). Finally pheophytin, obtained with acetone, after preparation and separation of the pheophorbide components *a* and *b*, gave on decarboxylation the free pyro compounds (8).

CONCERNING PHEOPHORBIDE, PYROPHEOPHORBIDE, AND CHLORIN *e*

Pheophorbide, just like chlorophyllide and chlorophyll, is transformed to pheoporphyrin a_5 (XIII) and chlorin *e* into chloroporphyrin e_6 (XIV) by

means of glacial acetic acid–hydrogen iodide mixture. The constitution of the former is confirmed as above and their reciprocal interconversion is possible. Thus it appears that in pheophorbide the same isocyclic ring must exist as in pheoporphyrin a_5, while in the side chain chlorin *e* must correspond to chloroporphyrin e_6. This is confirmed also by the fact that pheophorbide is transformed practically quantitatively by means of diazomethane–methyl alcohol into chlorin *e* trimethyl ester; the same transformation also occurs with the aid of phosgene–alcohol. Pheophorbide, as well as pheoporphyrin a_5, yields a well-crystallized oxime which is convertible by means of hydriodic acid into pheoporphyrin a_5 oxime (43; cf. 28, 49). Accordingly chlorin *e* should be convertible back to pheophorbide. Actually this reaction occurs, but with a yield of only 10 per cent. Decarbomethoxylation occurs so that not pheophorbide but pyropheophorbide results. The latter may also be obtained by boiling pheophorbide with pyridine. Biologically this process occurs in the digestive tract of the ruminants.

The splitting of the carbomethoxyl residue and the low yield of the resynthesis is quite surprising. In remarkable fashion, however, the yield may rise to about 100 per cent if chlorin *e* is esterified in the 6-position with glycol.

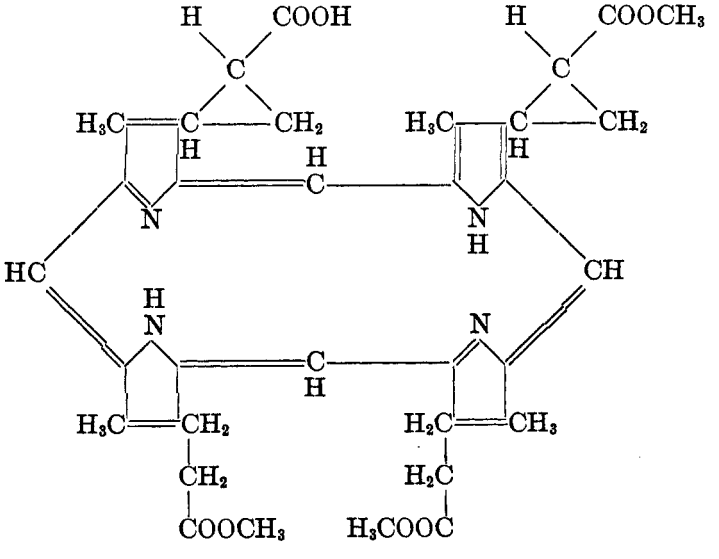
Reduction of pheophorbide as well as unchanged chlorophyllide and chlorophyll by means of hydrogen iodide yields only pure pheoporphyrin a_5 (53). In the digestive tract, where strong reduction processes may often be found, there occurs among other porphyrins phylloerythrin, and thus the porphyrins were early considered to be reduction products of chlorophyll. Synthetically porphyrins were converted into chlorins by reduction methods, and accordingly the porphyrins should be regarded as oxidation products of the phorbides and chlorins. Finally it was possible by means of synthetic methods, this time by oxidation of the porphyrins, to produce substances similar to the chlorins, whereby the latter appeared to be oxidation products of the porphyrins. Clarification of the difficulty was obtained only slowly. Elementary analyses first showed the extraordinary similarity in composition between pheoporphyrin a_5 dimethyl ester and methylpheophorbide as well as between chlorin *e* trimethyl ester and chloroporphyrin e_6 trimethyl ester, so that the possibility of isomerization was brought to the fore.

The calorimetric investigations of A. Stern and G. Klebs were then of great importance. These authors showed that the energy contents of methylpheophorbide and pheoporphyrin a_5 dimethyl ester were equal. Likewise the energy contents of chlorin e_6 and chloroporphyrin e_6 trimethyl ester were equal. The formula, therefore, of pheoporphyrin a_5 must be essentially the same as that of pheophorbide, and the formula of chloroporphyrin e_6 essentially the same as chlorin *e*. We thus enter into the dis-

cussion of the phorbide or chlorin state of chlorophyll, in which extraordinary difficulties appeared, since in the side chains chlorophyll and its derivatives were generally regarded as saturated. Consequently isomerism among the porphyrins, the phorbides and the chlorins could not be a correct explanation and contradictions appeared on every side. The possibility of methylene groups as bridges between the pyrrole nuclei or of pyrroline formation (17, 12) was brought into discussion. The latter explanation, which was primarily applied to the synthetic chlorins, was difficult to apply to the "natural" products, since according to the state of knowledge at that time, a higher hydrogen content must occur in the latter molecules than in the porphyrins. Actually, however, isomerism did exist.

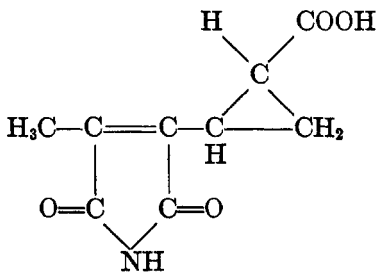
THE DISCOVERY OF A VINYL RESIDUE IN THE PHORBIDES, CHLORINS, PURPURINS, AND PORPHYRINS OF CHLOROPHYLL

If one allows colorless hydrogen iodide to act upon phorbides or chlorins, acetylporphyrins are obtained (42). The acetyl group occupies the 2-position, a fact which was verified synthetically (44). A still more positive indication of the presence of the vinyl group is shown by the reaction with diazoacetic ester (30). Protoporphyrin contains two vinyl groups and reacts with two molecules of diazoacetic ester to yield 1,3,5,8-tetramethyl-2,4-dicyclopropylcarboxylic acid ester-porphin-6,7-dipropionic acid:



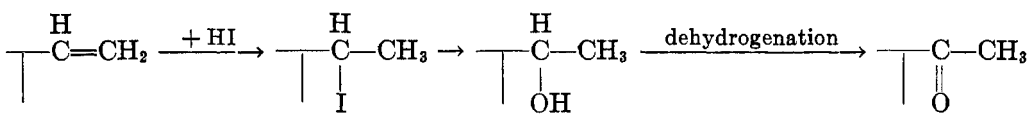
XXI

The constitution of this substance was proved, not only by the elementary analysis, but by oxidation. Besides hematinic acid, methylmaleic imide-cyclopropyl-carboxylic acid was obtained, with the following formula:



XXII

In the same fashion, the phorbides, chlorins, and purpurins reacted with diazoacetic ester. For example, pheophorbide after the reaction and subsequent oxidation yields methylmaleic imide-cyclopropyl-carboxylic acid as well as methylethylmaleic imide and hematinic acid. Therefore the vinyl group must exist in pheophorbide and at the same time it is shown that the diazoacetic ester reaction is generally applicable to the detection of vinyl radicals in the porphyrins as well. Thus the vinyl group may be detected in pseudoverdoporphyrin, in phyllochlorin, and in rhodochlorin. The appearance of the acetyl radical in the oxoporphyrins may be explained by the presence of the vinyl group, according to the following scheme:



I II III IV

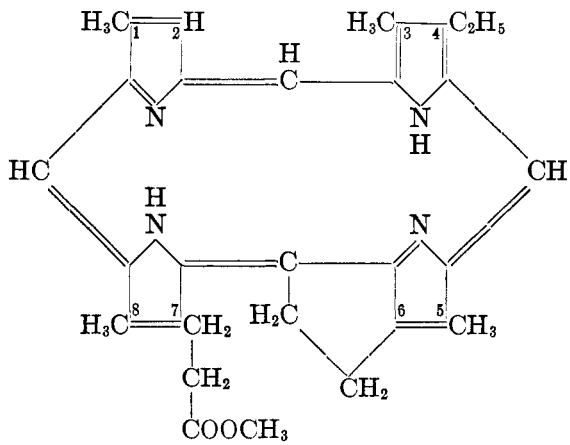
At first hydrogen iodide adds to the vinyl group, then the iodine is exchanged for hydroxyl, and finally spontaneous dehydrogenation leads to the acetyl radical. The reaction is completely analogous to the hematoporphyrin reaction of hemin, except that state III represents the end point of the latter.

Hydrogen bromide as well as methyl alcohol (26) combine with the vinyl groups of chlorophyll derivatives as they do with those of hemin. By means of catalytic reduction or glacial acetic acid–hydrogen iodide reduction, hemin is converted into mesohemin, containing four more hydrogen

atoms. This reaction similarly converts bilirubin to mesobilirubin. Exactly in this fashion the meso derivatives of chlorophyll may be obtained,[2] and one may prepare mesopheophorbide, mesochlorin, etc., in this way. The mesoderivatives are spectroscopically still phorbides or chlorins, but contain two hydrogen atoms more than the corresponding porphyrins. Consequently, during the conversion into the porphyrin a dehydrogenation must take place.

Proof of the 2-position of the vinyl group

It was of especial interest to determine the position of the vinyl group in the molecule. This was accomplished after treatment of oxophylloerythrin with concentrated hydrochloric acid in the pressure bomb (14). Two porphyrins resulted, a phylloerythrin with a free methine group and, by means of a secondary degradation, a pyrroporphyrin which contained a second free methine group. The latter was synthesized (7). No depression of the mixed melting point was observed. A further proof was started by the degradation of oxophylloerythrin with hydrogen bromide–glacial acetic acid. There resulted a reduction of the keto group of the isocyclic ring, together with the splitting off of the acetyl group. The product was a desoxophylloerythrin with a free methine group. Inasmuch as at that time it was not decided whether a formyl or an acetyl group was present in the oxoporphyrins, the four theoretically possible desoxophylloerythrins with a free methine group in either position 1, 2, 3, or 4 were synthesized (44). The desoxophylloerythrin ester of the following formula



XXIII

[2] The addition of two hydrogen atoms was first correctly indicated by Stoll and Wiedemann (Helv. Chim. Acta **16**, 191 (1932)), but no experimental details were given. The authors assumed addition to a double bond in the nucleus.

gave no depression in mixed melting point with the analytical preparation, whereas the other three showed differences even spectroscopically.

With this determination, definite proof of the presence of an acetyl residue in the oxoporphyrins was obtained and thereby that of the vinyl group in the original material. Likewise the position of the substituents in "natural" hemin was confirmed.

A third proof was yielded by the displacement of the ethyl residue in oxorhodoporphyrin by means of bromine. The bromine was removed by catalytic reduction and there resulted a rhodoporphyrin with a free methine group. That it was a 2-desethylrhodoporphyrin was proved by means of a mixed melting point with the synthetic material (7). The acetyl group was re-introduced into the 2-desethylrhodoporphyrin and oxorhodoporphyrin re-obtained. Thus through synthesis the constitution of oxorhodoporphyrin and the oxo compounds was confirmed (26).

In this manner the detection of the acetyl group in the oxoporphyrins was confirmed at the same time. Earlier we had assumed a formyl residue; however, of course, only an ethyl group can be located in the 2-position.

For the determination of the constitution of chlorophyll and its derivatives, the demonstration of a vinyl group was fundamental, for then for the first time the easy isomerization of the green substances to the red substances was understandable. There was required merely a rearrangement of hydrogen to the vinyl group in order to obtain the saturated porphyrin system, for the porphyrins obtained from chlorophyll did not contain the vinyl group at all. Also the biological origin of phylloerythrin becomes understandable. In addition, the higher energy content, corresponding to two hydrogen atoms, of the meso compounds and of the diazoacetic ester addition products becomes clear (60).

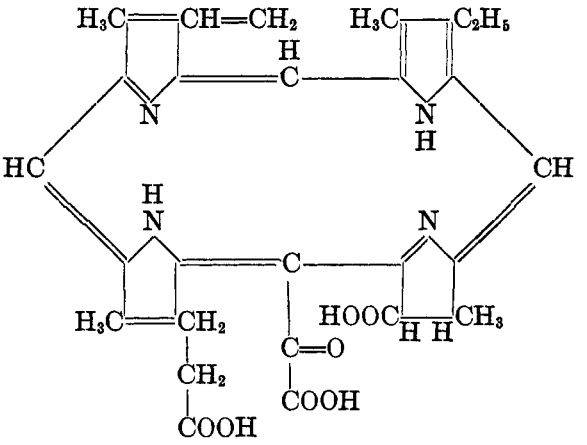
An excess of two hydrogen atoms over the saturated porphyrins was thus known to be present in the green meso compounds, while the pheophorbides were isomeric with the corresponding porphyrins but, exactly like the meso compounds, it was necessary that they contain two hydrogen atoms more in the nuclear system (those atoms removed from the ethyl residue). The question then arose as to the position of these hydrogen atoms. This was answered through the detection of the optical activity of rhodochlorin, of phyllochlorin, of pyrrochlorin, and of their meso compounds.

ANALYSIS OF THE OPTICAL ACTIVITY OF CHLOROPHYLL

Pheoporphyrin a_5 and pheophorbide possess a center of asymmetry in carbon atom 10 and therefore chlorophyll can be optically active (54). The optical activity was first pointed out by Stoll and Wiedemann (64), who observed a small rotation—close to or within the limits of error—which quickly vanished. We could confirm the optical activity of chloro-

phyll and numerous derivatives, but not the rapid inactivation. Even many hours of boiling with pyridine led to no inactivation. Transformation into the leuco compound followed by re-oxidation did not always lead to loss of optical activity. Chlorophyll and its derivatives are levorotatory, with the exception of those substances which are prepared by the aid of propyl alcohol and potassium hydroxide, a reagent which was introduced into chlorophyll chemistry by Conant and leads to the purpurins. These are all dextrorotatory. If one, however, conducts the purpurin reaction with the aid of sodium hydroxide in the presence of air, then levorotatory pseudochlorin p_6 is the result.

This is the place to consider briefly the purpurins which, as mentioned, were discovered by Conant.[3] Propyl alcoholic potassium hydroxide acts readily in the cold on chlorophyll, pheophorbide, and chlorins with cleavage of the isocyclic ring. There result unstable chlorins which are transformed into purpurins by the action of diazomethane. Conant found two purpurins, namely, purpurin 7 and purpurin 18. The latter contains one carbon atom less than purpurin 7. Purpurin 7 has the following formula:

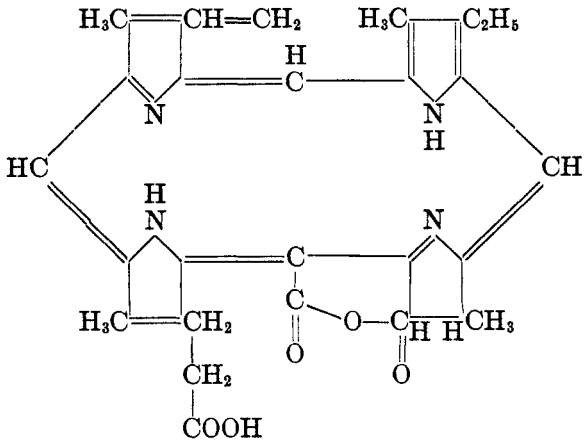


XXIV

That the vinyl group is present was proved by the reaction with diazoacetic ester, the oxo reaction, and by means of the degradation to rhodochlorin, whereby the glyoxalic acid residue is split off. That rhodochlorin contains the vinyl group follows from its transformation into vinylrhodoporphyrin.

[3] According to L. Marchlewski (Biochem. Z. **277**, 17 (1935)) and Conant, purpurin 18 was first observed by the former and described as anhydro-β-phyllotaonin.

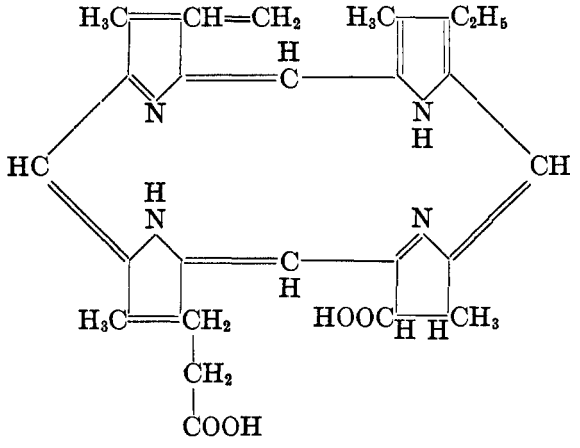
Purpurin 18 has the following formula:



XXV

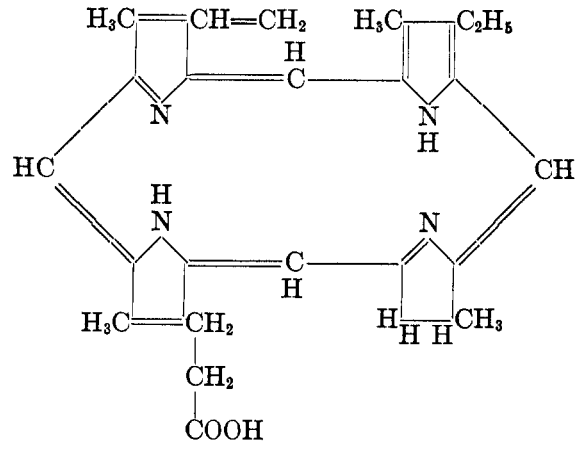
The vinyl group of this compound is identified in the same fashion as was that of purpurin 7.

As the purpurin structures indicate, an extensive oxidation of the isocyclic ring is involved here, very similar to that which can occur by treatment of chlorin *e* with hydrogen iodide in the presence of excess iodine. We cannot enter further into the details of this process here. The purpurins are also optically active. They may be transformed into optically active chlorins, among which the following may be mentioned:

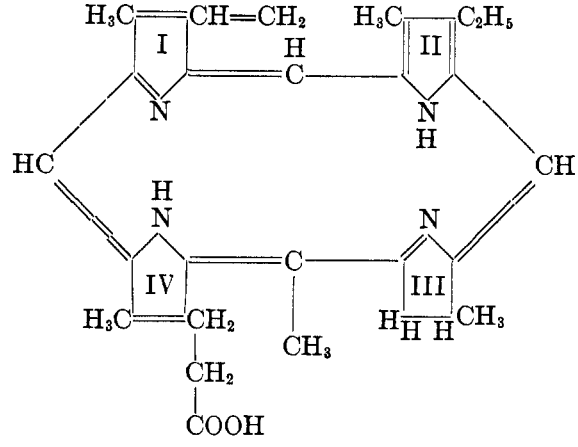


XXVI

Rhodochlorin



XXVII
Pyrrochlorin

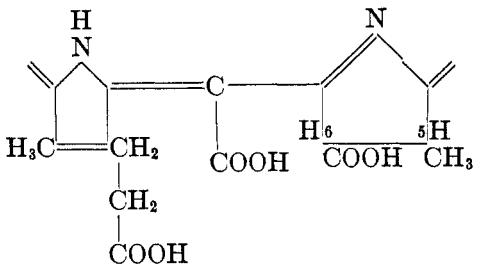


XXVIII
Phyllochlorin

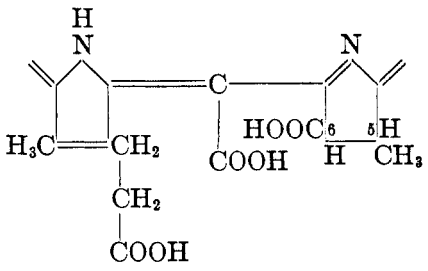
These three substances are also optically active and may isomerize into the corresponding optically inactive porphyrins. For chlorophyll, the chlorins, and the purpurins, accordingly, formulas with methylene groups cannot be considered, since with these optical activity could not be present. There remains only the assumption of a pyrrolenine structure for one ring, and most probably ring III comes into question. In the isocyclic ring double bonds could not occur, inasmuch as in such a system powerful strains would be set up. For this reason the other rings of the system are

generally laid down in terms of the formulas previously employed. That rings I and II are to be formulated differently followed from the spectroscopic findings of the four synthetic β-free desoxophylloerythrins (45). A pyrrolenine structure for rings I and III in the porphyrin nucleus was then found by A. Stern and H. Wenderlein (61), and because of this it is highly probable that chlorophyll itself must have an analogous arrangement, for the spectroscopic appearance of chlorophyll derivatives is fundamentally very similar to that of porphyrins. That ring III possesses the pyrrolenine structure is proved in the following way: As already mentioned, chlorin p_6, which is dextrorotatory, may be obtained from pheophorbide *a*. Degradation by sodium hydroxide and air produces pseudochlorin p_6. The latter is distinguished from chlorin p_6 by means of the melting point of the esters, spectroscopically, and by the opposite sign of rotation, namely, levorotation. The two substances are, however, not optical antipodes.

In similar fashion, mesopheophorbide may be transformed into the analogous meso derivatives; likewise the diazoacetic ester addition products. Both series, however, are isomeric with each other, as the elementary analyses show. The explanation for this behavior is given by considering the isomerism in the sense of the following formulas:



XXIX
Pseudochlorin p_6



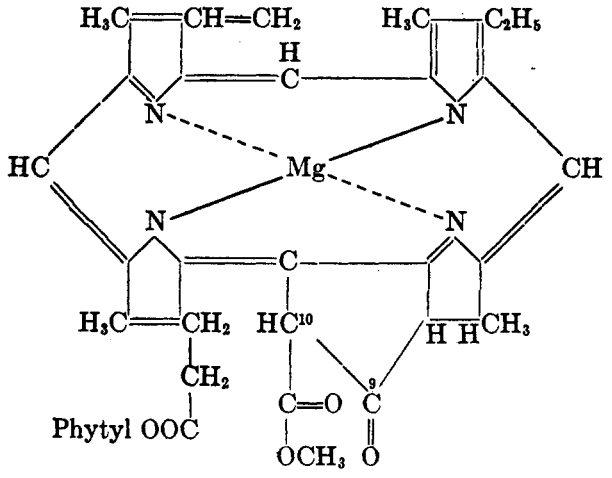
XXX
Chlorin p_6

That the molecules are not optical antipodes may be seen from models without difficulty, for the asymmetric center on carbon atom 5 does not participate in the process.

Since, as already mentioned, the analogous substances, this time with opposite rotations, were prepared from the diazoacetic ester and meso derivatives of the pheophorbides, we regard the formulation of ring III as a pyrrolenine ring as certain, inasmuch as only thus could optical antipodes be formed. That the carboxyl groups on carbon atom 6 are differently arranged in space follows also from the fact that in the chlorin p_6 series the anhydride formation to purpurin 18 is possible, whereas in the pseudo

series this does not take place. A steric difference must therefore exist. The spectrographic investigation of A. Stern and H. Wenderlein of the various substances under consideration confirms the same idea (63).

Consequently, for chlorophyll *a* itself only the following formulation comes into question:

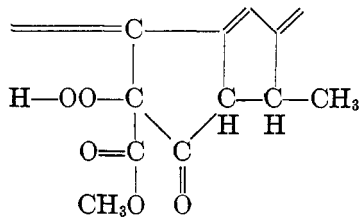


XXXI

This formula is capable of explaining all the transformations, and any other choice in the location of the double and single bonds is now excluded. This structural formula has on the tertiary carbon atom 10 a hydrogen atom which must be characterized by a special lability, since a carbonyl group and a carbomethoxyl group are located next to it. This explains two important properties of chlorophyll,—allomerization and the phase test.

By allomerization is understood the phenomenon that alcoholic solutions of chlorophyll or chlorophyllides, evaporated to dryness, lose their ability to crystallize and the phase test becomes negative, i.e., the green phase immediately appears. We owe to Conant the explanation of this process—namely, that one mole of oxygen is used up per mole of chlorophyll—and we were able to show that oxidation occurred on C_{10}. The allomerization process could be imitated in an alcoholic medium with quinone, which forms hydroquinone, and after hydrogen iodide reduction 10-ethoxy- or 10-methoxy-pheoporphyrin a_5 could be isolated. The process takes place in the following fashion: Chlorophyll adds to quinone in the 1,6-positions to form the hydroquinone ether (Wieland), which then decomposes by alcoholysis. 10-Ethoxypheoporphyrin a_5 as well as 10-ethoxypheophorbide could be isolated. In the presence of alcohol and

air—as in the classic allomerization—the 10-peroxide derivative of the following formulation must arise:



This was, however, not isolated as such, but the hydrogen iodide reduction yielded pheoporphyrin a_7, whereby the oxidation on carbon atom 10 may be said to be proved. It is interesting as well as important to point out that the activation of the hydrogen atom on carbon atom 10 is intensified by the coördinately bound magnesium.

In the case of pheophorbide the process could not be imitated with iodine–alcohol, in contrast to chlorophyllide *a* and pheoporphyrin a_5, which easily undergo the transformation. In pheoporphyrin a_5 the 10-hydrogen atom is therefore more labile than in pheophorbide, while this exhibits no increased reactivity in its phyllin; it is especially insensitive toward atmospheric oxygen.

In regard to the phase test, this is introduced by the formation of the yellow potassium salt arising from the wandering of the hydrogen atom from carbon atom 10 to the carbonyl group in 9. Through this process a double bond is formed between 9 and 10. The ring becomes more labile, as the construction in the model illustrates very clearly, and the tendency to ring cleavage is great. It thus becomes plain why hydrolysis under the influence of methyl alcoholic potassium hydroxide proceeds extraordinarily easily with such enormous reaction velocity that within thirty seconds it is quantitatively complete and chlorin *e* is formed. Pure chlorin *e*, free from unstable chlorins and purpurins, is the criterion for undamaged chlorophyll. Methanolysis with the aid of diazomethane is still easier to carry out; in the presence of uninjured chlorophyll only chlorin *e* trimethyl ester is produced by this reaction (41). In agreement with this the 10-oxy derivatives of pheophorbide give no phase test, inasmuch as the hydrogen atom needed for the formation of the enol group on C_9 is missing. For the same reason, allomerized chlorophyll is phase test negative.

It is impossible to go farther into the question of chlorophyll *b*. It may be mentioned that this is constituted in complete analogy to chlorophyll *a*, except that in place of the methyl group in ring II a formyl residue is present.

Purely formally, hemin and chlorophyll are closely related in respect to the fundamental ring system of each, as follows from the explanation on page 53. The most important differences are the complexly bound metal, in the former case iron, in the latter magnesium, in the former free carboxyl groups, in the latter a double esterification,—once with phytol and once with methyl alcohol.

In historical development we regard hemin as the older dyestuff. How it became transformed into chlorophyll is not clear. Perhaps there resulted at first a methylation on the γ-carbon atom; from our synthetic studies we found that derivatives of phylloporphyrins with unsaturated side chains in the 6-position close to form isocyclic rings even under the mildest conditions.

Further progress in knowledge in this field is to be expected on the synthetic side and also on the analytic through studying the chlorophyll of lower plants. Since the sulfur bacteria possess a modified chlorophyll in which in place of the vinyl group an acetyl radical is present, the possibility exists that further differently constituted types of chlorophyll appear in nature.

These investigations have been performed during the past seven years in the Organic-Chemical Institute of the Technische Hochschule in Munich.

I owe many thanks to my numerous collaborators, who are named in publications appearing in Liebig's *Annalen*.

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