RECENT PROGRESS IN DETERMINING THE CHEMICAL STRUCTURE OF CHLOROPHYLL

CATHERINE C. STEELE[1]

Received November 30, 1936

I. INTRODUCTION

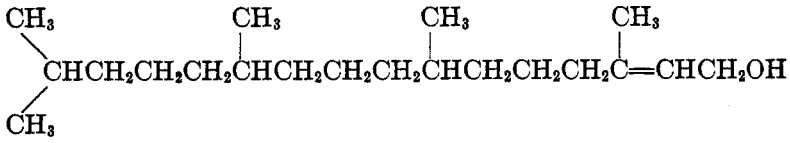
The name "chlorophyll" was first given by Pelletier and Caventou (1817) to the green coloring material in the chloroplasts of plant leaves; it was not, however, until much later that extensive chemical investigations were made on the pigment. In 1864 the English physicist Stokes (81) showed spectroscopically that the chloroplast pigment was a mixture, and Sorby (76) separated it into four pigments, two yellow and two green, by partition between immiscible solvents. This work was ignored until Willstätter (1906) repeated and extended the investigation. In the intervening years many attempts were made to isolate the chloroplast pigments; the isolation of two yellow pigments was repeated by several investigators, but the use of too drastic methods gave very impure chlorophyll extracts, so that, for instance, one worker reported that from a single plant species alone he had obtained some twenty different chlorophylls.

From 1906 to 1914 Willstätter (98) and his collaborators not only succeeded in preparing pure chlorophyll for the first time, but their chemical investigations laid the foundation of our present knowledge of the structures of the green pigments. Willstätter showed that there were two green pigments, chlorophylls *a* and *b*, present in all plant leaves, and that they could be separated by the preferential solubility of chlorophyll *a* in petroleum ether and of chlorophyll *b* in 90 per cent methyl alcohol. The later method devised by Winterstein and Stein (100) of chromatographic adsorption of the pigments on powdered sugar affords an even simpler means of separation. Willstätter found that the ratio of chlorophyll *a* to chlorophyll *b* was remarkably constant in the higher plants, being about three to one. The function of the chlorophylls in the photosynthetic process will not be considered in this paper; reviews, e.g. those of Spoehr (77) and of Stoll (83), and physiological textbooks may be consulted.

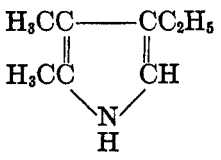
[1] Ph.D. (St. Andrews); former Commonwealth Fund Fellow at the University of Illinois and at Harvard University. Present address: 301 Hartel Street, Philadelphia.

Chlorophyll and its derivatives are very sparingly soluble in organic solvents and are difficult to purify. The chief means of identifying individual compounds is by their unique absorption spectra. Willstätter introduced a general method for the separation and purification of chlorophyll derivatives; they were extracted from ether with hydrochloric acid of different concentrations. This method of acid fractionation depends on the large differences in the distribution ratios of these substances between ether and dilute acid, brought about by small differences in basicity and in solubility. Chlorophyll derivatives are therefore all characterized by an *acid number*, defined as that percentage concentration of hydrochloric acid which extracts two-thirds of the substance from an equal volume of an ether solution.

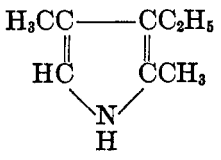
Willstätter determined the correct empirical formula for chlorophyll *a* as $C_{55}H_{72}N_4O_5Mg$ (later substantiated by Fischer (15) and by Stoll (82)), and found that chlorophyll *b* contained an additional oxygen atom, and two hydrogens less than *a*. He proved for the first time the presence of magnesium in the chlorophyll molecule, and showed that in physical properties and indeed to some extent in chemical nature chlorophyll was a wax. On hydrolysis it yields two alcohols, methyl alcohol and phytol, $C_{20}H_{39}OH$. The latter, by the brilliant synthesis of Gottwalt Fischer (14), was shown to have the following structural formula:



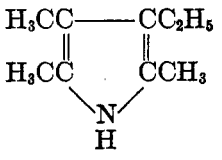
The earliest obtained derivatives of chlorophyll were the pyrroles and the porphyrins. Nencki (72) and later Willstätter (95) reduced chlorophyll to substituted pyrroles, identified by the latter investigator as hemopyrrole, kryptopyrrole, and phyllopyrrole:



Ia
Hemopyrrole

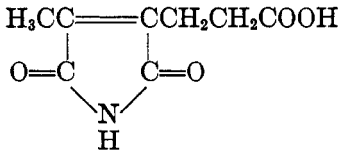


Ib
Kryptopyrrole

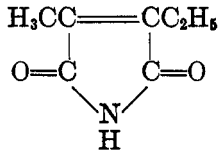


Ic
Phyllopyrrole

Kuster (68) obtained pyrroles by the oxidative degradation of chlorophyll, and isolated hematinic acid and methylethylmaleic imide:



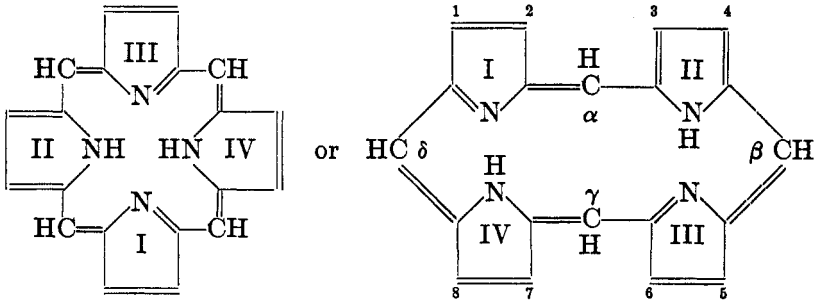
II a
Hematinic acid



II b
Methylethylmaleic imide

Hemoglobin, on reduction and oxidation respectively, gives the same series of pyrroles.

Again, both pigments on drastic alkaline degradation give porphyrins, red, crystalline compounds with characteristic absorption spectra. These porphyrins were originally obtained by Hoppe-Seyler (67) and also by Schunck (75). Indeed, this formation of porphyrins was the first indication of a similarity in chemical structure between the blood pigment and chlorophyll. In the chlorophyll series of porphyrins, Willstätter characterized the dicarboxylic acid rhodoporphyrin, the monocarboxylic acids phylloporphyrin and pyrroporphyrin, and the oxygen-free compound pyrroetioporphyrin. Porphyrins all contain four pyrrole nuclei, and Kuster (69) suggested that these were arranged in a symmetrical fashion according to the following skeletal structure.



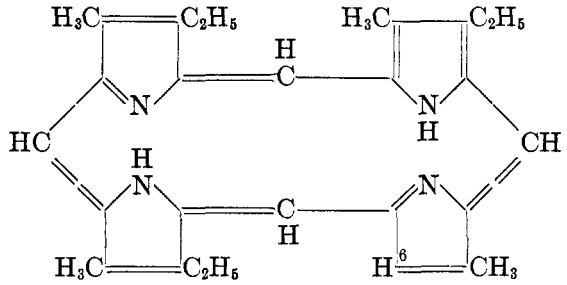
III
The porphin nucleus

This unsubstituted nucleus, termed the *porphin* nucleus, possesses a conjugated system similar to that of benzene, and hence the distribution of the double bonds is admittedly arbitrary. Since the second alternative method of writing the nucleus is that at present in use by Hans Fischer, it will be adopted in this review for the sake of uniformity.

In a series of publications extending over some twenty years, Hans Fischer has proved by synthesis that Kuster's formulation for the porphyrin nucleus was correct.

II. The Structure of the Porphyrins

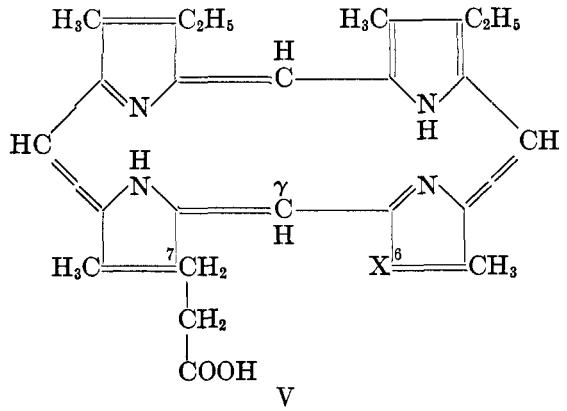
Rhodoporphyrin can be converted into pyrroporphyrin by pyrolysis, with the loss of a molecular proportion of carbon dioxide. Pyrroporphyrin, in turn, can be decomposed with loss of carbon dioxide to give the oxygen-free, alkylated porphyrin,—pyrroetioporphyrin (IV).



IV
Pyrroetioporphyrin

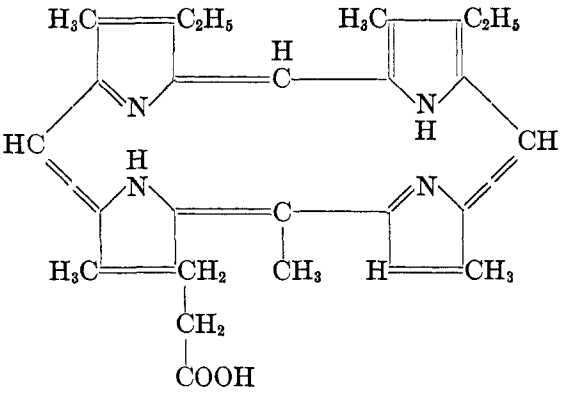
The alternation of methyl and ethyl groups round the nucleus is noteworthy; in the case of the simplest blood porphyrin, mesoetioporphyrin, position 6 is occupied by an ethyl group. That this position was unsubstituted in the chlorophyll porphyrins, phyllo-, pyrro-, and pyrroetioporphyrins, was shown by bromination followed by oxidation. Bromine replaces the hydrogen in the unsubstituted position, and on oxidation, porphin ring fission occurs and bromocitraconimide is obtained.

Fischer developed methods not only for synthesizing substituted pyrroles, but for combining these into dipyrrylmethenes, and further for uniting two such methenes to form porphyrins (19, 36). In this way several series of isomeric porphyrins were obtained, and these included porphyrins identical with rhodoporphyrin (V a), pyrroporphyrin (V b), and phylloporphyrin (V c).



V

a. X = COOH Rhodoporphyrin
b. X = H Pyrroporphyrin

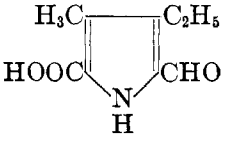


Vc
Phylloporphyrin

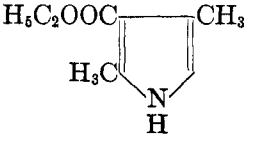
Phylloporphyrin, which contains a methyl group on the γ-bridge carbon atom (see formula III for the system of numbering), can be converted into pyrroporphyrin by the action of sodium ethoxide.

Rhodoporphyrin, as shown above, possesses besides a propionic acid group in position 7 a carboxyl group in the 6-position, which is occupied by the unsubstituted hydrogen atom in pyrro- and phyllo-porphyrins. In systematic nomenclature, rhodoporphyrin is 1,3,5,8-tetramethyl-2,4-diethyl-6-carboxyporphin-7-propionic acid. The length of these systematic names has precluded their use for the various degradation products of chlorophyll in favor of the unsystematic names given by Willstätter, but they are retained by Fischer for the purely synthetic methenes and porphins.

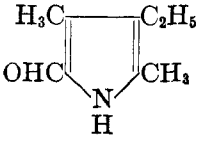
The synthesis of rhodoporphyrin is outlined below to show the general method for porphyrin synthesis. The ethyl ester of the 5-carboxy derivative of kryptopyrrole (I b), treated with three molecules of sulfuryl chloride, gives as one of the products 2-formyl-3-ethyl-4-methylpyrrole-5-carboxylic acid (VI a). This is condensed with the ethyl ester of 3,5-dimethylpyrrole-4-carboxylic acid (VI b) by trituration in the presence of hydrogen bromide and glacial acetic acid. The dipyrrylmethene so obtained is the 4-ethyl ester of 3,5,4′-trimethyl-3′-ethylpyrromethene-4,5′-dicarboxylic acid (VII A). In numbering the positions in the dipyrrylmethenes, the 2 and 2′ positions are always those occupied by the methene bridge.



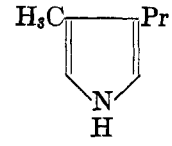
VI a



VI b

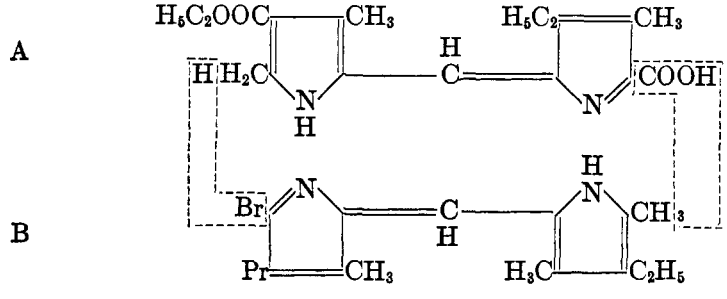


VI c



VI d
(Pr = propionic acid)

Similarly from 5-formylkryptopyrrole (VI c) and the ethyl ester of 4-methylpyrrole-3-propionic acid (VI d) is obtained the dipyrrylmethene, 3,5,3′-trimethyl-4-ethylpyrromethene-4′-propionic acid (VII B; H in place of Br). Compound B is obtained by bromination of the hydrobromide of this dipyrrylmethene with free bromine at 50° to 60°C. It is then condensed with the hydrobromide of A by melting the mixture over a free flame in the presence of succinic acid. Condensation occurs as indicated by the dotted lines in the following formulas, and fractionation of the product leads to the isolation of rhodoporphyrin (Va).



VII

In all such syntheses, each of the methenes can react with itself, and therefore other porphyrins are always present. Further, decarboxylation frequently occurs, resulting in more porphyrins, and the isolation of the required porphyrin is often difficult. Therefore the proof of the structure of a given porphyrin rests not alone on the synthesis, but on that coupled with the knowledge of its structure derived from degradation reactions.

Unsubstituted porphin (III) itself has also been synthesized. Fischer (29) condensed in one step four molecules of 2-pyrrolealdehyde by means of boiling formic acid in the presence of alcohol to give porphin. Rothemund (73) prepared porphin by condensing pyrrole with an equimolecular proportion of formaldehyde. When other aldehydes were used, then porphins substituted in the α-, β-, γ-, and δ-positions were obtained.

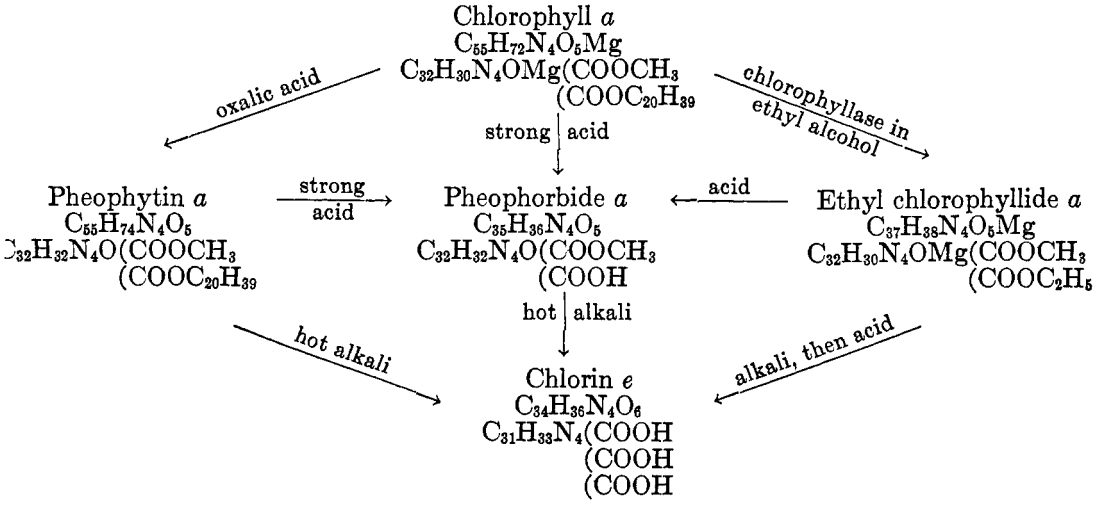
III. Chlorophyll *a*

Investigations of Willstätter

Willstätter prepared a number of derivatives by the action of acid and of alkali on chlorophyll *a*. Chlorophyll, on careful treatment with oxalic acid, loses magnesium, and yields a waxy substance called pheophytin. This has no acid properties, and therefore the magnesium cannot be present as a salt, but must be attached to nitrogen. Hydrolysis of pheophytin with strong acid removes phytol, yielding pheophorbide *a*, a monomethyl ester of a dicarboxylic acid. Esterification with diazomethane gives the dimethyl ester, methyl pheophorbide *a*.

If sections of the green leaves of certain plants (e.g., *Heracleum sphondylium*) are soaked in alcohol, large crystals are developed in the chloroplasts; the substance concerned was called "crystalline chlorophyll." Willstätter showed that these plants contain an enzyme, chlorophyllase, which hydrolyzes the phytyl group and replaces it by the alcohol present. The products are therefore now called ethyl chlorophyllide, if ethyl alcohol is used, methyl chlorophyllide from methyl alcohol, and so forth.

Saponification of pheophorbide *a* or of its ester with hot alkali yields phytochlorin *e* (usually abbreviated to chlorin *e*), which gives with diazomethane a trimethyl ester, according to Treibs and Wiedemann (91). These reactions of chlorophyll are summarized as follows:



VIII

After the brilliant syntheses of the chlorophyll porphyrins by Hans Fischer, the relationship of the structure of chlorophyll to the porphin

nucleus and the nature of the labile groups in chlorophyll required elucidation. The problem was taken up almost simultaneously by Fischer and by J. B. Conant. It seems advisable to separate to some extent the account of the work of these investigators, but in each case historical sequence will not necessarily be observed, for reasons of brevity.

INVESTIGATIONS OF CONANT

The phytyl group

Conant (7, 5) first correctly placed the phytyl group in chlorophyll on the propionic acid side chain. Pheophorbide *a* contains a methoxyl group derived unchanged from chlorophyll *a* (VIII), and a free acid residue resulting from the loss of phytol through hydrolysis. On pyrolysis, pheophorbide *a* is converted into pyropheophorbide *a*, which has lost a methoxyl group, but still retains a carboxylic acid group; the latter acid group must be the propionic acid residue, otherwise it would be eliminated in the pyrolysis. Hence the propionic acid group which survives pyrolysis must be esterified with phytol in the chlorophyll molecule. Fischer later provided an independent proof of this in the hydrogen iodide reactions (*vide infra*).

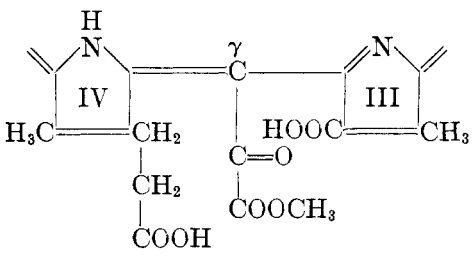
The phase test

Willstätter found that a characteristic reaction of chlorophyll, pheophytin, and pheophorbide in ether solution is the sequence of color changes (green, then yellow, reverting to green, in the *a* series) obtained by the action of cold alcoholic alkali. Conant (11) found that the first product of the "phase test" reaction is an unstable chlorin (apparently identical with Willstätter's (99) phytochlorin *g*), which on being allowed to stand is converted into pheopurpurin 18, a substance with a vivid purple color in ether solution and of "acid number" 18. Immediate esterification of the reaction mixture converts the unstable chlorin into dimethyl pheopurpurin 7. Dimethyl pheopurpurin 7 (a trimethyl ester) on careful hydrolysis loses two methyl groups, forming pheopurpurin 7, a monomethyl ester.

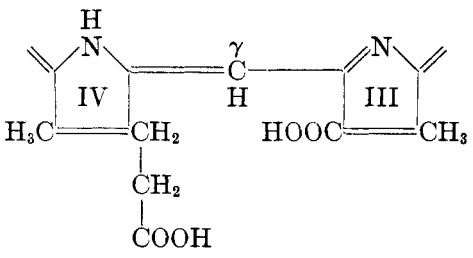
This phase test reaction was shown by Conant (10, 78) to be an oxidative hydrolysis, with atmospheric oxygen acting as hydrogen acceptor. Still milder conditions of oxidative hydrolysis (4) result in a stable chlorin, which is a monomethyl ester. Hydrolysis of this ester yields the unstable chlorin, hence the former is monomethyl chlorin *g* (IX), while diazomethane further esterifies it directly to dimethyl pheopurpurin 7.

Hot saponification of either pheopurpurin 7 or its ester (8, 5) gives rise to a new chlorin (X), chlorin *f* (which Fischer later prepared and called rhodochlorin). Reduction of this dibasic acid with hydrogen iodide in

acetic acid, and subsequent re-oxidation in air, results in the formation of the dibasic acid, rhodoporphyrin (Va). Since the latter contains no side chain on the γ-bridge carbon atom, neither does chlorin *f*. Chlorin *f* therefore contains a carboxyl group in the 6-position, and a propionic acid residue in the 7-position, according to the partial formula (X; see also XXVIII a, and XXIX a and b).



IX
Monomethyl chlorin *g*

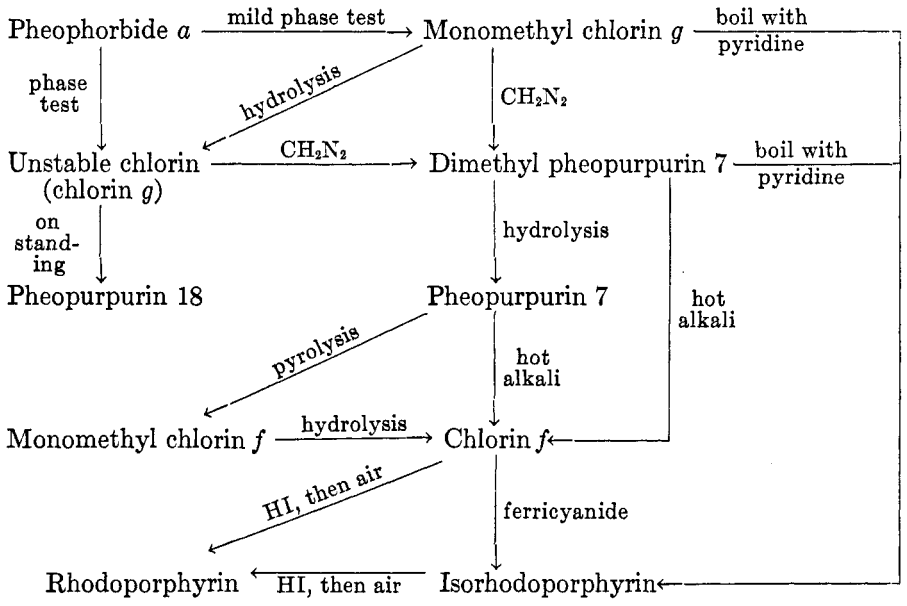


X
Chlorin *f*

Direct dehydrogenation of chlorin *f*, using ferricyanide in an alkaline solution at room temperature (8), gives not rhodoporphyrin but isorhodoporphyrin, which differs from chlorin *f* by containing two hydrogen atoms less. This same porphyrin can also be obtained more directly from pheopurpurin 7; Conant (2) found that pyrolysis of the latter yields small amounts of the porphyrin, while Fischer (66) prepared it by heating dimethyl pheopurpurin 7 in pyridine. He called it pseudoverdoporphyrin, since it also appeared to be isomeric with a verdoporphyrin obtained from the decomposition of chlorophyll by Treibs and Wiedemann (91). This "verdoporphyrin" has now been shown by Fischer (40) to be a mixture of porphyrins. Isorhodoporphyrin can be readily converted into rhodoporphyrin by reduction with hydrogen iodide, followed by atmospheric oxidation. Their relationship is discussed more fully later.

The schematic representation of the phase test products is shown on page 10.

If the isorhodoporphyrin molecule is represented by P, then chlorin *f* is PH_2. Hence according to Conant (2), the fundamental nuclear structure of the pheophorbides and of chlorophyll *a*, which is that of chlorin *f*, is also a dihydroisorhodoporphyrin ring. This result had been foreshadowed by Conant and Kamerling (9), who studied the visible absorption spectra of these compounds at liquid air temperatures. Porphyrins under such conditions show a unique pattern of groups of narrow bands, comparable to the ultra-violet absorption spectrum of benzene, for instance. On the other hand, chlorophyll derivatives such as the pheo-

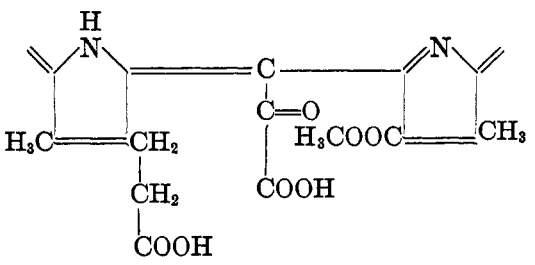


XI

The phase test products

phorbides and the chlorins have spectra with wider bands similar to the spectrum of cyclohexadiene (dihydrobenzene).

In the saponification of pheopurpurin 7 to chlorin *f* (PH_2), oxalic acid is also obtained, hence pheopurpurin 7 (and the isomeric unstable chlorin) is represented by $PH_2C_2O_3$. Again, pyrolysis of pheopurpurin 7 (a monomethyl ester) produces the monomethyl ester of chlorin *f*, accompanied by the evolution of carbon dioxide and carbon monoxide. Hence pheopurpurin 7 contains an α-keto acid (or glyoxalic acid) residue on the γ-bridge carbon atom, and may be represented by the following partial formula (8, 12):



XII

Pheopurpurin 7

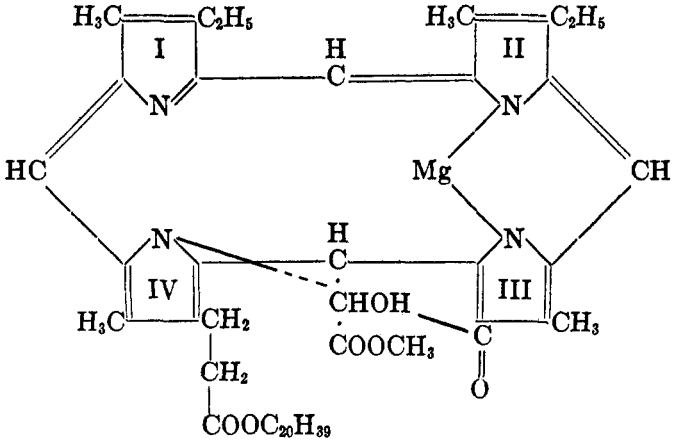
Fischer placed the methyl group in chlorophyll *a* and pheophorbide *a* on the side chain of the γ-bridge carbon atom as a carbomethoxyl residue (*vide infra*). Conant originally placed it on the 6-carboxyl, but later corroborated Fischer's result by independent evidence (4). Monomethyl chlorin *g* (IX) can be esterified with diazoethane to give diethyl pheopurpurin 7 (a diethyl monomethyl ester). If the latter is heated with pyridine the methoxyl is lost, and there results a diethyl ester of isorhodoporphyrin, which has no substituent on the γ-bridge. Hence the methoxyl must be part of the γ-bridge grouping in monomethyl chlorin *g*. If the latter is subjected to pyrolysis, methoxyl-free isorhodoporphyrin is formed, hence again in monomethyl chlorin *g*, and therefore also in chlorophyll and pheophorbide, the methoxyl is in the γ-bridge grouping.

Allomerization

Willstätter described a reaction of chlorophyll which he termed allomerization. When an alcoholic solution of chlorophyll is allowed to stand for some time, the resulting material, "allomerized chlorophyll," is no longer capable of giving the phase test. From the alcoholic solution he prepared phytochlorin *g* (99). Conant (8) showed that "allomerized chlorophyll" on hydrolysis, removal of magnesium by acid, and esterification with diazomethane, gives the same product—dimethyl pheopurpurin 7—as does the phase test. This is in contrast with unallomerized chlorophyll, which under identical treatment yields the trimethyl ester of chlorin *e* (VIII). That allomerization is an oxidative reaction was shown by Conant (10) using a modified Warburg apparatus; two equivalents of oxygen are absorbed per mole of chlorophyll. He further found that chlorophyll and the pheophorbides can be dehydrogenated (5), using two equivalents of potassium molybdicyanide, and again dimethyl pheopurpurin 7 is obtained. Hence the phase test, allomerization, and direct dehydrogenation all lead through dimethyl pheopurpurin 7 and chlorin *f* to isorhodoporphyrin, or to rhodoporphyrin, while rapid saponification, which excludes oxidation, results in chlorin *e* (VIII), from which phylloporphyrin is derived. To explain these results, Conant (4) suggested a structure for chlorophyll (XIII) which has a dihydroporphyrin nucleus, and an esterified lactic acid side chain on the γ-bridge carbon atom. To account for the fact that although crystallized chlorophyll *a* has only five oxygen atoms, it has two ester groups and a potential carboxyl group in the 6-position (which appears in chlorin *e*), two further postulates were made:—*viz.*, that a lactam bridge is present between the potential carboxyl group and the nitrogen atom in pyrrole ring IV, and that the secondary alcoholic grouping in the lactic acid side chain was dehydrated, at least in crystallized chlorophyll. The dehydrogenation results are ex-

plained by the hydration of this unsaturated linkage, followed by oxidation to the α-keto grouping (1):

$$\rangle C{=}CH\cdot COOCH_3 \rightleftarrows \rangle CH\cdot CHOH\cdot COOCH_3 \rightarrow \rangle CH\cdot CO\cdot COOCH_3$$



XIII

Chlorophyll *a* (Conant, 1933)

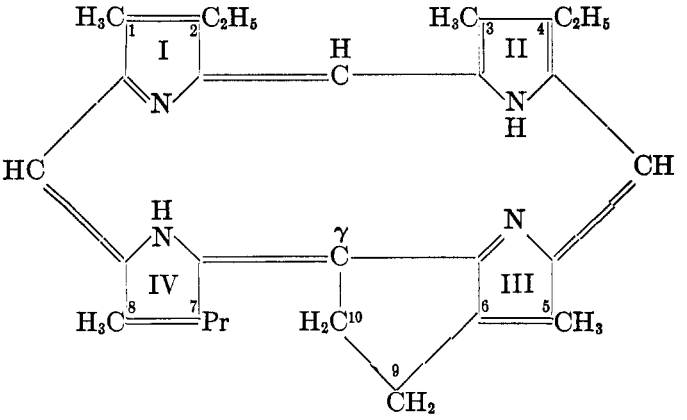
INVESTIGATIONS OF FISCHER

The carbocyclic (or isocyclic) ring

Fischer found a mild reagent for the degradation of chlorophyll in glacial acetic acid solutions of hydrogen iodide at 50°C. By such treatment, many chlorophyll derivatives are reduced to colourless leuco compounds, which re-oxidize in air to porphyrins (47, 18, 65, 48); these, however, unlike the chlorophyll porphyrins previously described, retain the full carbon skeleton of the original compound. In this manner the pheophorbides give rise to pheoporphyrins, while chlorin *e* gives rise to a different series, the chloroporphyrins. Thus pheophorbide *a* with hydrogen iodide yields pheoporphyrin a_5 (65); this contains, like the parent compound, a carbomethoxyl group and a carboxyl group. Further treatment with hydrogen iodide, or the action of hydrogen bromide in acetic acid, eliminates the carbomethoxyl group, giving phylloerythrin, which is spectroscopically a porphyrin. Phylloerythrin may also be obtained directly from pheophorbide *a*, and from pheophytin and the chlorophyllides, by refluxing with 20 per cent hydrochloric acid.

Now phylloerythrin, $C_{33}H_{34}O_3N_4$, was already known as a biological decomposition product of chlorophyll; Löbisch and Fischler (70), finding it in ox bile, called it bilipurpurin, while Marchlewski (71) isolated it from

the feces of herbivora. These biological sources of phylloerythrin convinced Fischer that the hydrogen iodide treatment was mild, and that chlorophyll, the phorbides, and phylloerythrin were closely related in chemical structure. Phylloerythrin contains a reactive carbonyl group which forms an oxime, and which can be reduced by the Wolff–Kishner method (the action of hydrazine and sodium ethoxide in a sealed tube) to form desoxophylloerythrin (48). This compound, a porphyrin with one carboxylic acid group, may also be prepared directly from pheophytin *a* or from pheophorbide *a*, by the action of hydrogen bromide in acetic acid at 180°C. (49). Fischer determined the structure of desoxophylloerythrin by synthesis (51). It is represented by the following formula:



XIV

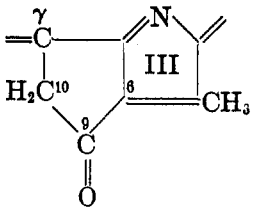
Desoxophylloerythrin

(Pr = —CH_2CH_2COOH)

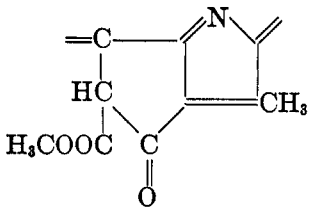
Desoxophylloerythrin can be oxidized with oleum and sulfur to regenerate phylloerythrin. That the carbonyl group in phylloerythrin (XV) is in position 9 and not 10 follows from its alkaline decomposition to rhodoporphyrin, containing a carboxyl group in position 6 (Va).

The structure of pheoporphyrin a_5 (49) is established by its relationship to phylloerythrin and to the chloroporphyrins. Pheoporphyrin a_5 contains a carbomethoxyl group, that is, it always occurs as the monomethyl ester. It contains in addition a carbonyl group, and on hydrolysis yields chloroporphyrin e_6 (XVII), so-called because it is obtainable by the action of hydrogen iodide on chlorin *e* trimethyl ester. Chloroporphyrin e_6 is the monomethyl ester of rhodoporphyrin-γ-acetic acid. Formic acid converts it to γ-methylrhodoporphyrin (chloroporphyrin e_4), and this in turn can be converted to phylloporphyrin (Vc). Pheoporphyrin a_5 (XVI)

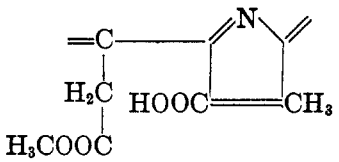
therefore contains the carbocyclic ring of phylloerythrin with a carbomethoxyl group in position 10.



XV
Phylloerythrin

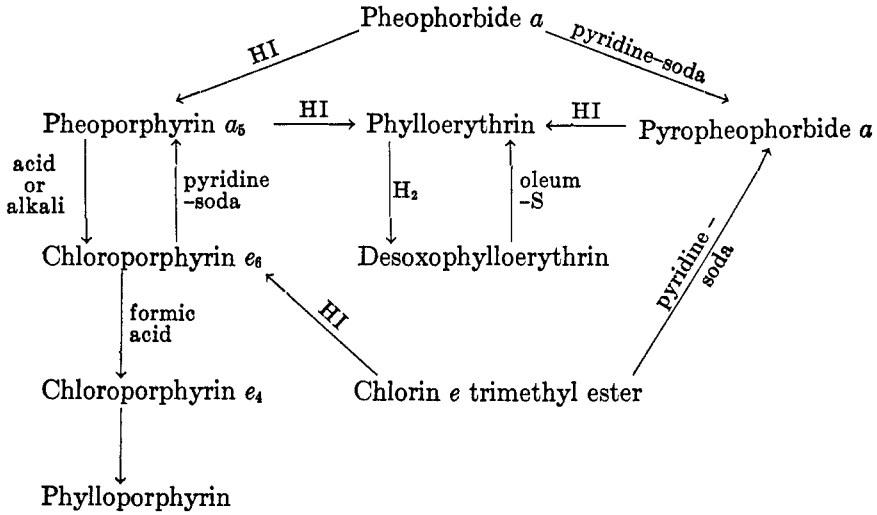


XVI
Pheoporphyrin a_5



XVII
Chloroporphyrin e_6

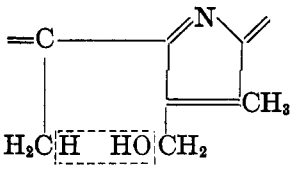
The following table summarizes some of these relationships. For a more complete discussion of the pheo- and chloro-porphyrins, two summaries by Fischer may be consulted (16).



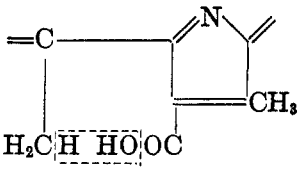
XVIII
The hydrogen iodide reaction

Other proofs of the position of the carbonyl group in position 9 in pheoporphyrin a_5 and in phylloerythrin have been found through partial synthesis of these substances. Desoxophylloerythrin has been obtained by ring closure (59) between positions 6 and γ in 6-hydroxymethylphylloporphyrin (XIX a). The latter is obtained from phylloporphyrin by the action of bromine, to yield 6-bromophylloporphyrin, followed by

treatment with methyl alcoholic alkali, which converts the bromine to the primary alcoholic group (—CH_2OH). Similarly phylloerythrin was obtained by a condensation between positions 6 and γ in chloroporphyrin e_4 (γ-methylrhodoporphyrin) under the influence of sodium ethylate (XIX b) (50).



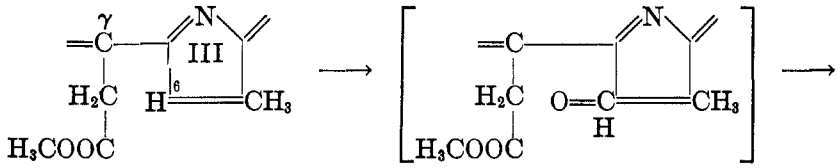
XIX a



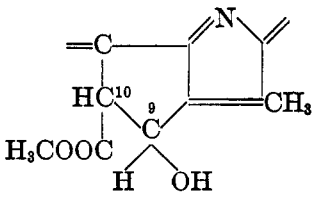
XIX b

This extreme ease of formation of the carbocyclic ring is actually comparable with the methods employed by Fischer in converting the pheophorbides to substances such as phylloerythrin and desoxophylloerythrin, where the existence of the carbocyclic ring has been definitely established by synthesis. Even in view of the biological data cited above, it would appear that the presence of the carbocylic ring in pheophorbide and in chlorophyll has not yet been unequivocally proved.

Again, esterification of chlorin *e* by means of methyl alcohol and hydrochloric acid gives a diester in which the carboxyl group in the 6-position is free. This diester on pyrolysis loses the carboxyl group, and subsequent catalytic reduction to the leuco compound, followed by re-oxidation in air, gives rise to isochloroporphyrin e_4 diester (XX) (41). Here position



XX
Isochloroporphyrin
e_4 ester



9-Hydroxydesoxopheoporphyrin a_5

6 is unsubstituted, and the γ-carbon atom possesses an acetic ester residue. Fischer found means of introducing the formyl group into porphyrins by treating the hemin (complex iron salt) of the porphyrin with dichloromethyl ether in the presence of stannic chloride or bromide (57). In this instance the formyl group enters at position 6, and ring closure (a Claisen condensation) takes place spontaneously, so that the product isolated is 9-hydroxydesoxopheoporphyrin a_5 (42). The latter compound was already known; it is obtainable by catalytic hydrogenation of pheoporphyrin a_5 (34).

If isochloroporphyrin e_4 ester is brominated, one atom of bromine enters the molecule apparently in position 6, for on heating the brominated compound above its melting point, ring closure is effected with the formation of phylloerythrin.

If the formyl group is introduced into phylloporphyrin (Vc) by the method outlined above, the ester of the resulting 6-formylphylloporphyrin gives rise to an interesting series of 9-substituted derivatives of desoxophylloerythrin methyl ester (50), by the same type of ring closure. For instance, 6-formylphylloporphyrin ester condensed with malonic acid gives the 9-acetic acid derivative of desoxophylloerythrin ester, while with methyl alcohol and concentrated hydrochloric acid, the 9-methoxy derivative is obtained.

Conant (7) attempted the pyrolysis of several chlorophyll derivatives in diphenyl, and from the results obtained from methyl pheophorbide *a* established the position of the phytyl group. Fischer (27) found better conditions for pyrolysis in the use of pyridine and sodium carbonate. If pheophorbide is so treated, the carbomethoxyl group is eliminated. The product is pyropheophorbide *a*, containing a carbonyl group in a carbocyclic ring, and spectroscopically a phorbide, but isomeric with phylloerythrin, into which it is convertible by treatment with hydrogen iodide. Fischer isolated from sheep feces (37) a mixture of degradation products of chlorophyll. The name probophorbide was used for some of these, but later (62) phylloerythrin, pyropheophorbide *a*, and its dihydro derivative were isolated from the mixture. It should be noted (XVIII) that pyrolysis of chlorin *e* trimethyl ester also results in pyropheophorbide (58). In this case, synthesis of a carbocyclic ring has taken place, and a similar synthesis occurs in the pyrolysis of chloroporphyrin e_6 to pheoporphyrin a_5.

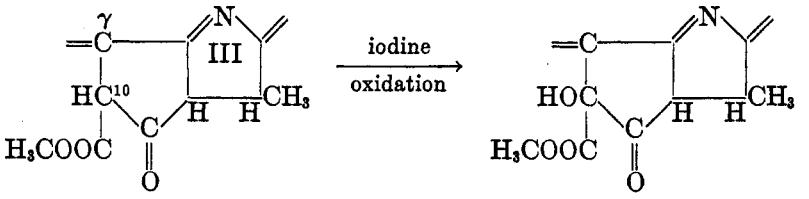
The carbocyclic rings of pyropheophorbide *a* and of phylloerythrin are the same, and according to Fischer pheophorbide *a* and pheoporphyrin a_5 form a similar pair. This latter identity Fischer deduced from the relationships outlined in the preceding paragraphs, and from the following additional facts. The chlorophyllides, methyl pheophorbide, and pheo-

phorbide at ordinary temperatures in an atmosphere of nitrogen form oximes (53), which analyze as substitution, not addition, products and hence show the presence of a carbonyl group. The original compounds can be regenerated from the oximes, and the latter are converted by hydrogen iodide into the oxime of pheoporphyrin a_5. The enolic modification must also be possible, since methyl pheophorbide and pheophorbide form benzoyl substitution products, as was shown by Stoll (87). The β-ketonic acid grouping ($R \cdot CO \cdot CHR' \cdot COOCH_3$) of the carbocyclic ring admits of these modifications, and also explains the reaction of chlorophyll and the pheophorbides to acid and to hot alkali. The latter hydrolyzes the ester groups and opens the carbocyclic ring with the formation of chlorin *e* (XXIII); acid, on the other hand, in addition to hydrolysis of the ester groups, removes carbon dioxide from the carbomethoxyl group, leading to phylloerythrin. Again, Stern and Klebs (79) conducted calorimetric investigations on chlorophyll derivatives, and concluded that the energy values of pheophorbide *a* and of pheoporphyrin a_5 were the same, indicating that they were isomeric. But it is doubtful if a small difference, e.g., of two hydrogen atoms, could be detected by this method.

Fischer's independent proof of the position of the phytyl group in chlorophyll may now be mentioned. When ethyl chlorophyllide (VIII) is treated with hydrogen iodide, the ethyl ester of pheoporphyrin a_5 is formed. This, on heating, is converted into the ethyl ester of phylloerythrin, which has only one acid group, *viz.*, the propionic acid grouping in position 7. Hence the ethyl group in ethyl chlorophyllide, and therefore also the phytyl group in chlorophyll, must be in the propionic acid side chain (66). Stoll's suggestion that since pheophytin is usually prepared with an alcoholic solution an interchange of ester groups may have taken place is not valid for the above argument (84). In any case, Fischer, using pheophytin prepared with acetone in place of alcohol, finds no difference in the results (24).

Many of the chlorophyll porphyrins can be oxidized by iodine, and analysis of the products shows that oxidation has taken place in position 10 (35). For example, pheoporphyrin a_5 (XVI) with iodine in alcohol in the presence of sodium acetate is converted into 10-hydroxypheoporphyrin a_5 (or neopheoporphyrin a_6). If sodium carbonate is substituted for the acetate, the product is 10-ethoxypheoporphyrin a_5 (or pheoporphyrin a_6; XXII d) (53). A series of homologous ethers has been prepared by the use of the corresponding series of alcohols in this oxidation (55). Pheophorbide *a* itself can be oxidized with iodine in glacial acetic acid (53), yielding 10-hydroxypheophorbide *a* (XXI). This Fischer considers as further confirmation of his hypothesis that the carbocyclic ring

in the pheophorbides (and hence in chlorophyll) is identical with that of the derived chlorophyll porphyrins such as phylloerythrin.



XXI

Pheophorbide *a*
(see XXVII a)

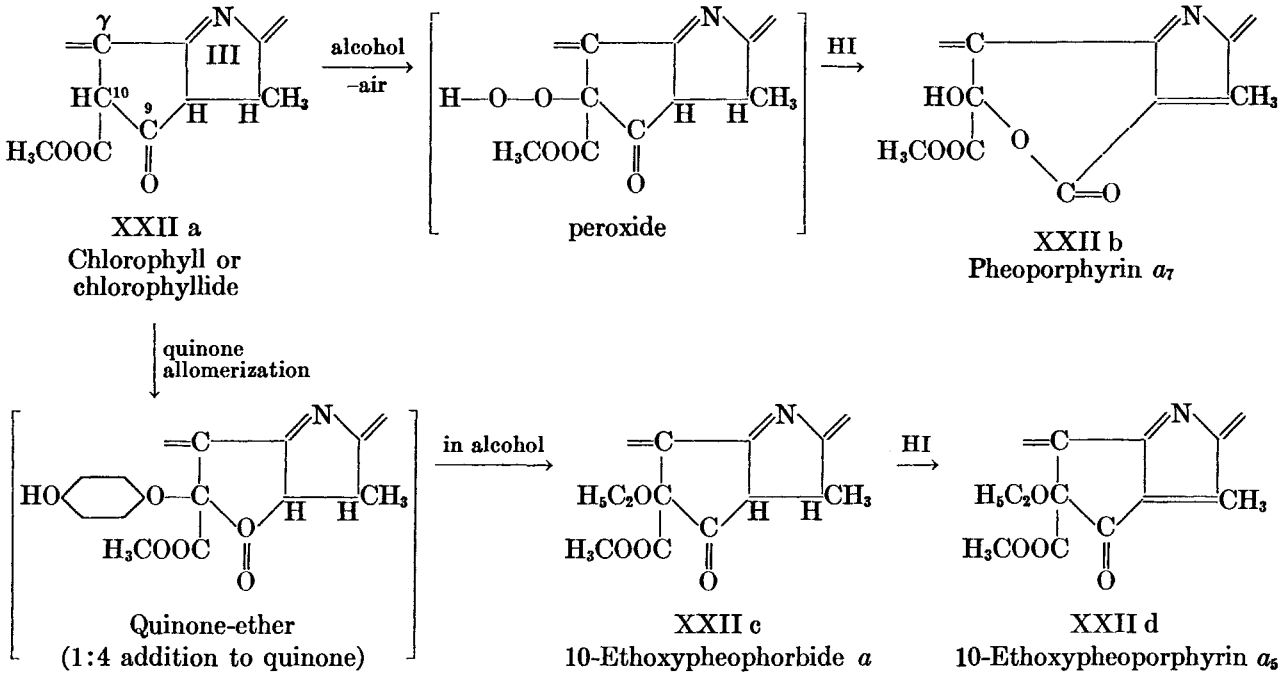
10-Hydroxypheophorbide *a*

Allomerization and the phase test

Fischer found that if allomerized chlorophyll (page 11) is treated with hydrogen iodide, followed by hydrolysis with hydrochloric acid, pheoporphyrin a_7 results (66). Similar treatment of chlorophyll itself yields pheoporphyrin a_5 as does pheophorbide *a* (XVIII). Now pheoporphyrin a_7 is also obtained from chloroporphyrin e_6 by the iodine–glacial acetic acid oxidation described above, and from the latter relationship formula XXII b has been deduced. On esterification this compound forms a triester, with hydrolysis of the lactone ring. Fischer has abandoned the theory that the first step in allomerization is dehydrogenation between positions 10 and γ, and now suggests peroxide formation; no proof for this has been cited so far. But Conant (5) found that allomerized chlorophyll (allomerized with one mole of oxygen) was apparently identical with dehydrochlorophyll, prepared by oxidation of chlorophyll with two equivalents of oxidizing agent; in other words, the reaction appears to be chlorophyll *a* + O_2 → dehydrochlorophyll *a* (or allomerized chlorophyll) + H_2O_2. Fischer's peroxide calls for four equivalents. (See page 19.)

Fischer also carried out allomerization experiments in alcohol, using quinone as hydrogen acceptor in place of atmospheric oxygen (66, 28). Treatment of the resulting allomerized chlorophyll with hydrogen iodide gave 10-ethoxypheoporphyrin a_5. Fischer's latest interpretation of these reactions is given above (63). (The removal of the two hydrogen atoms in nucleus III in the hydrogen iodide reaction will be discussed later.)

With regard to the phase test, Conant insisted that esterification with diazomethane could not accomplish an oxidation of the unstable chlorin, and that the latter must therefore be at the same oxidation level as pheopurpurin 7. Fischer finally agreed with this interpretation. According to Fischer, in the presence of alkali enolization takes place in position 9 with the formation of a double bond between 9 and 10. Then follows



XXII a
Chlorophyll or
chlorophyllide

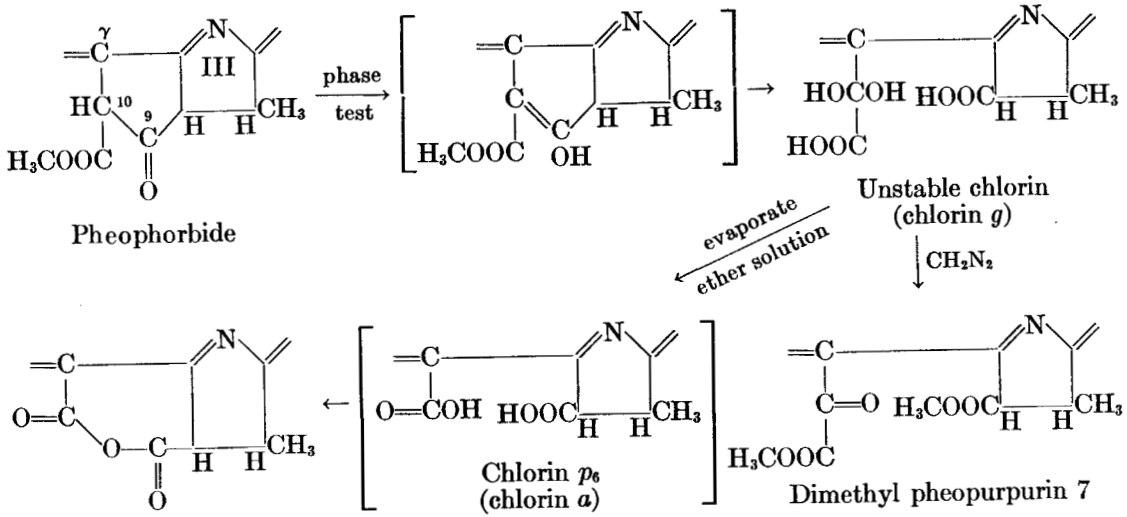
peroxide

XXII b
Pheoporphyrin a_7

Quinone-ether
(1:4 addition to quinone)

XXII c
10-Ethoxypheophorbide *a*

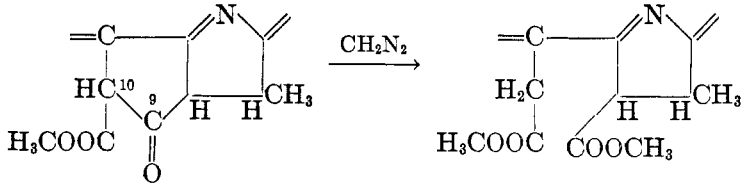
XXII d
10-Ethoxypheoporphyrin a_5



XXIII
The phase test (Fischer)

rupture of this bond by oxidation, accompanied by hydrolysis of the carbomethoxyl group on 10, and the unstable chlorin is, he suggests, a hydrated keto acid (17). (Conant's monomethyl chlorin *g* (IX) might also be a hydrate.) Immediate esterification converts the unstable chlorin to the trimethyl ester, dimethyl pheopurpurin 7. If an ethereal solution of the unstable chlorin is allowed to stand, or is evaporated to dryness, pheopurpurin 18 results (11). Fischer showed that formic acid was split out in this reaction (32), and suggests (17) that chlorin p_6 (*vide infra*) is probably an intermediate product, although it was not isolated in this reaction. (See page 20.)

Conant and Fischer differ in their proposed formulas for chlorin *e* and its triester. Willstätter prepared chlorin *e* by the action of hot alkali on the pheophorbides (VIII), and showed that it formed a triester on esterification. Fischer (32) found that the action of diazomethane in methyl alcohol, or of phosgene and alcohol, was sufficient to convert pheophorbide *a* into chlorin *e* trimethyl ester, and considers that the reaction is a simple methanolysis between carbon atoms 9 and 10.



XXIV
Pheophorbide *a*

Chlorin *e* trimethyl ester

Similar opening of the pheophorbide carbocyclic ring has been achieved by the use of methylamine and of ammonia, the products being the corresponding acid amides of chlorin *e* ester (substituted in the 6-position) (31). The trimethyl ester of chlorin *e* is, according to Fischer (58), isomeric with the triester of chloroporphyrin e_6 (XVII); and from calorimetric data (79) these appear to have equal energy values. Fischer concludes that the side chains have the same state of oxidation, *viz.*, a γ-acetic acid side chain, as shown in the formula above. Conant (1), on the other hand, proposed for chlorin *e* a formula containing a potential lactic acid side chain

$$>CH\cdot CHOH\cdot COOCH_3 \rightleftarrows >C{=}CH\cdot COOCH_3$$

similar to that in his formula for chlorophyll *a* (XIII). This would explain the identical behavior of chlorin *e* triester and pheophorbide toward the phase test, resulting in the unstable chlorin and the pheopurpurins. That chlorin *e* triester, but not chlorin *e* itself, gives the phase test with

formation of the unstable chlorin, is explained by Fischer as activation of the hydrogen atoms on carbon atom 10 by the carbomethoxyl group in the former case (17); but it is difficult to see why this should admit of the addition of two oxygen atoms in the 10-position, as is required by Fischer's formula for the unstable chlorin. Again, Conant's formula would explain the clear-cut quantitative oxidation of chlorin *e* by molybdicyanide (5) (two equivalents of oxidizing agent being used), analogous to the molybdicyanide oxidation of pheophorbide and chlorophyll. With chlorin *e*, carbon dioxide is evolved, and the product isolated is chlorin *k*, containing a lactone ring (2). This quantitative oxidation would indicate that the state of oxidation of the 10-position in chlorin *e* is $>CHOH$, not $>CH_2$; and by analogy, it also throws in doubt the state of oxidation of position 10 in Fischer's formula for pheophorbide *a*. This difficulty is entirely separate from any argument for or against the carbocyclic ring. Fischer himself at one time (66) had a hydroxyl group in place of a hydrogen in position 10 of pheophorbide. The other reactions of chlorin *e* and its esters, e.g., the reactions of the diester with hydrogen iodide, by Fischer and Kellerman (41), are as easily explained on Conant's formulation as on Fischer's.

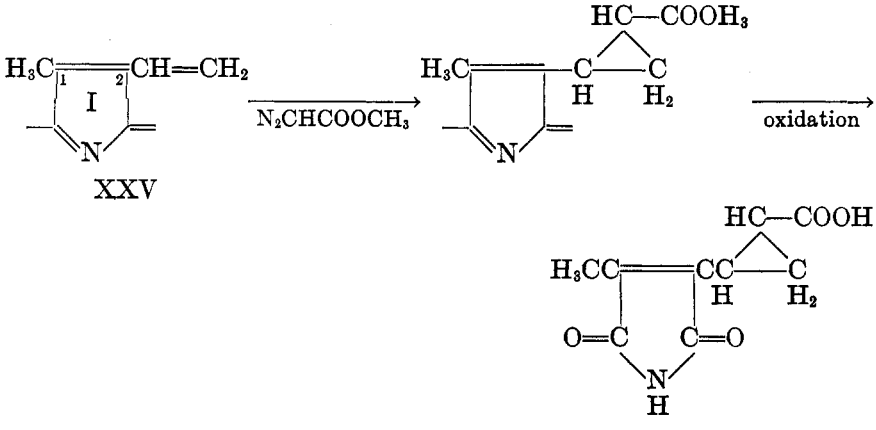
The vinyl group

At the time Conant proposed his formula for chlorophyll *a* he insisted that the chlorophyll nucleus should be considered to have a dihydroporphyrin structure. Fischer, on the other hand, on the basis of isomerization with hydrogen iodide, contended that chlorophyll was an isomerized porphyrin. These two views became reconciled by the later work of Fischer. Three types of reaction established that chlorophyll, the phorbides, chlorins and purpurins—in fact all the non-porphyrin derivatives (but including isorhodoporphyrin) of chlorophyll *a*—contained an unsaturated side chain. In the first place, if pheophorbide is hydrogenated with addition of three molecules of hydrogen, a leuco compound is formed, which in an acid medium is converted into a porphyrin, *viz.*, pheoporphyrin a_5. If however a neutral medium is employed, or the hydrogenation is stopped on the addition of two moles of hydrogen, dihydropheophorbide *a* is obtained (44). Stoll and Wiedemann also obtained this dihydro reaction (85). Dihydro compounds are also obtainable from chlorin *e* and from purpurin 7; spectroscopically they differ very slightly from the parent substances,—an indication that the unsaturated group involved is not part of the nuclear structure.

Secondly, Fischer found that if his hydrogen iodide reaction was carried out in the cold, instead of at temperatures of 50° or 60°C., a new series of compounds, ketoporphyrins in structure, were obtained (52, 53). For instance, chlorophyll *a* and pheophorbide *a* both yield oxopheoporphyrin

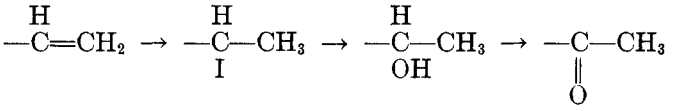
a_5, pyropheophorbide yields oxophylloerythrin, chlorin *e* trimethyl ester yields oxochloroporphyrin e_6, while chlorin *e* yields oxochloroporphyrin e_5. The two former oxo compounds contain two keto groups each. On heating oxophylloerythrin, or on heating oxochloroporphyrin e_5 with sulfuric acid, an oxorhodoporphyrin is formed. Fischer first suggested a methylene group in chlorophyll as giving rise to the oxo reaction (the oxo derivative would then contain a formyl group), then changed it to an ethylidene residue (the oxo derivatives would then possess an acetyl group). That the acetyl residue was the oxo group was shown by the conversion of oxorhodoporphyrin to normal rhodoporphyrin by the Wolff–Kishner reaction ($—COCH_3 \rightarrow —CH_2 \cdot CH_3$). The position of the acetyl residue and therefore also of the parent unsaturated group was established by the fact that oxophylloerythrin heated in a sealed tube with concentrated hydrochloric acid (which replaces the oxo group by hydrogen), followed by esterification, gave a desethylphylloerythrin and a desethylpyrroporphyrin. The latter compound was identical with a synthetic 2-desethylpyrroporphyrin. Also, the desethylphylloerythrin could be reduced to a desethyldesoxophylloerythrin, and the latter was identical with one of the two possible synthetic desethyldesoxophylloerythrins, *viz.*, the 2-desethyl (54). Hence the unsaturated group in the chlorophyll molecule is in the 2-position, and the oxo group must be an acetyl group in order to give rise to a desethyl- and not a desmethylporphyrin.

The third reaction, that of diazoacetic ester, proved conclusively that the unsaturated group was a vinyl residue (46). This too will produce an acetyl group on oxidation. Hemin and one of the blood porphyrins, protoporphyrin, have been shown by synthesis to contain a vinyl group. Diazoacetic ester adds on to this vinyl group with evolution of nitrogen, and on drastic oxidation of the addition compound with chromic acid, one of the products isolated was methyl maleic imide-cyclopropyl carboxylic acid.



The pheophorbides, the chlorins derived from chlorophyll, and the purpurins, but not the porphyrins (except isorhodoporphyrin), add diazoacetic ester in a completely analogous manner. Drastic oxidation of some of these addition products gave the same imide as was obtained from protoporphyrin. The action of hydrogen iodide and of pyrolysis on diazoacetic ester addition products of these chlorophyll derivatives gave diazoacetic ester derivatives of the corresponding compounds. For instance, hydrogen iodide on the derivative of pheophorbide *a* gave the derivative of pheoporphyrin a_5, while pyrolysis of the former gave the diazoacetic ester derivative of pyropheophorbide *a*, identical with that obtained by direct action of diazoacetic ester on pyropheophorbide. In short, the relationships shown on page 14 are also valid for the diazoacetic ester derivatives.

The existence of a vinyl group in position 2 in the molecules of chlorophyll and of its immediate derivatives gives a simple explanation of the oxo reaction. Hydrogen iodide adds on to the vinyl group, hydrolysis occurs with replacement of iodine by the hydroxyl group, and spontaneous dehydrogenation takes place to the acetyl residue:



XXVI
The oxo reaction

Since pheoporphyrin a_5 is isomeric with pheophorbide a_5, the hydrogen iodide isomerization must be in effect a migration of two hydrogen atoms from somewhere in the nucleus to the vinyl group. The porphyrins which result contain an ethyl residue in the 2-position.

Pheopurpurin 7 gives a diazoacetic ester addition product (64) and also undergoes the oxo reaction; it therefore contains the vinyl group. This group is retained in chlorin *f*, obtained by alkaline hydrolysis of purpurin 7 (XII). Isorhodoporphyrin also contains the vinyl group in contrast to the other chlorophyll porphyrins. It gives addition products with bromine and with diazoacetic ester, and the latter compound is identical with that obtained by the action of boiling pyridine on the diazoacetic ester addition product of purpurin 7 (43).

Again, 10-hydroxypheophorbide *a* (XXI) both shows the diazoacetic ester reaction and undergoes the oxo reaction. The product in the latter case is oxorhodoporphyrin (43), shown to be (2-desethyl)-2-acetylrhodoporphyrin (i.e., it contains $—COCH_3$ in place of $—C_2H_5$ in the 2-position), as follows. The action of bromine replaces the acetyl group by bromine, and the bromo compound can then be debrominated by the use of hydra-

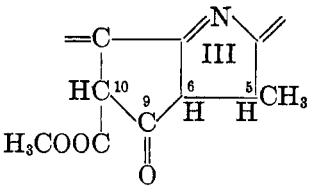
zine hydrate with a catalyst; the resulting 2-desethylrhodoporphyrin had already been synthesized (20). It was also possible to synthesize the acetylrhodoporphyrin; the acetyl group was introduced into 2-desethylrhodoporphyrin by the action of acetic anhydride and stannic bromide on the iron salt (hemin) (43).

The relationship between isorhodoporphyrin and rhodoporphyrin was elucidated by the following reactions. Introduction of the formyl residue (57) into 2-desethylrhodoporphyrin gave a (2-desethyl)-2-formylrhodoporphyrin (43). The action of methylmagnesium iodide on this compound replaces the formyl group with the hydroxyethyl group (—CHOH·CH_3), and the latter was spectroscopically identical with (2-desvinyl)-2-hydroxyethylisorhodoporphyrin, prepared by the action of hydrogen bromide in acetic acid on isorhodoporphyrin. Again, if isorhodoporphyrin is heated with phenazine, the vinyl group is oxidized to a formyl group, and the product is identical with (2-desethyl)-2-formylrhodoporphyrin. Hence isorhodoporphyrin is a 2-vinylrhodoporphyrin, and is not a true isomer of rhodoporphyrin, since it contains two hydrogen atoms fewer. Confirming this, pheopurpurin 7 (39) on being heated with pyridine gives isorhodoporphyrin, while dihydropheopurpurin 7 gives rhodoporphyrin. Rhodochlorin (Conant's chlorin *f*) with hydrogen iodide gives rhodoporphyrin, but the diazoacetic ester addition product of rhodochlorin gives the corresponding addition product of isorhodoporphyrin. The difference between the two porphyrins is therefore in the vinyl group. A further proof of this relationship is afforded by drastic oxidation with chromic acid. In the blood pigment series it was found that an unsaturated side chain such as the vinyl group caused the complete destruction of the nucleus concerned; if, however, an ethyl group were present, methylethylmaleic imide (II b) was obtained. Similarly in the chlorophyll series, Fischer (24) found that the dihydro compounds yielded double the amount of methylethylmaleic imide that was given by the unhydrogenated compounds, and in particular that rhodoporphyrin gave twice as much imide on oxidation as isorhodoporphyrin. The establishing of the relationship between rhodo- and isorhodo-porphyrins was made more difficult by the attempt to fit the verdoporphyrin of Treibs and Wiedemann into the chlorophyll system. These authors (91) discovered that a large number of the chlorophyll porphyrins isolated by Willstätter were not single compounds but mixtures, and they isolated what they considered to be a new single chlorophyll porphyrin, verdoporphyrin (90). Conant (13) was never able to obtain this compound by any method, but isolated only isorhodoporphyrin. Fischer isolated verdoporphyrin by the method of Treibs, and also obtained it from pheoporphyrin a_5 (27). In the former case the product appears to be a mixture of rhodo- and isorhodo-

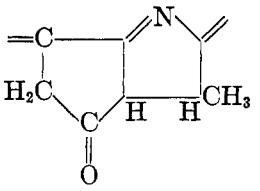
porphyrins, and in the latter a mixture of rhodoporphyrin and chloroporphyrin e_5, according to recent findings of Fischer (40).

Optical activity and the dihydroporphin nucleus

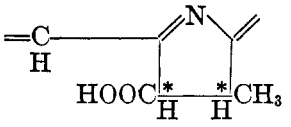
Stoll and Wiedemann (88), using monochromatic light, found that chlorophyll, pheophytin, and pheophorbide were optically active. Fischer and Stern (63) used white light, whereby higher concentrations of substance could be employed, and found that not only chlorophyll and the phorbides, but also the chlorins and purpurins were optically active. Pyropheophorbide, prepared either from pheophorbide or by ring closure of chlorin *e* (XVIII), showed the same specific rotatory power. Now the carbocyclic ring of pheophorbide contains an asymmetric carbon atom in position 10, but in pyropheophorbide this asymmetry is no longer present. Hence there must be at least one other asymmetric center. Fischer therefore suggested that the two surplus hydrogen atoms in the chlorophyll nucleus which convert the vinyl group to ethyl in the hydrogen iodide isomerization to the porphyrin nucleus be placed one on the γ-carbon atom, and one on a nitrogen atom in one of the pyrrole rings. The γ-carbon atom would then be asymmetric; pyropheophorbide could be optically active, but conversion to phylloerythrin would destroy this asymmetry. Phylloerythrin (formula XV) was in fact found to be optically inactive. The decisive argument against asymmetry in the γ-position was that rhodochlorin (Conant's chlorin *f*; formula X), which has no substituents in the γ-position, is still optically active. Hence there must be at least one asymmetric center somewhere else than in positions 10 and γ in the chlorophyll molecule. Fischer therefore then placed the two surplus hydrogen atoms of the dihydroporphin nucleus of chlorophyll, the phorbides, and the chlorins and purpurins, in positions 5 and 6 of nucleus III, thus forming a dihydropyrrole nucleus (50).



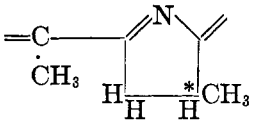
XXVII a
Pheophorbide



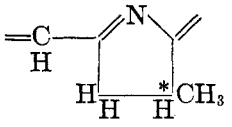
XXVII b
Pyropheophorbide



XXVIII a
Rhodochlorin (Fischer)

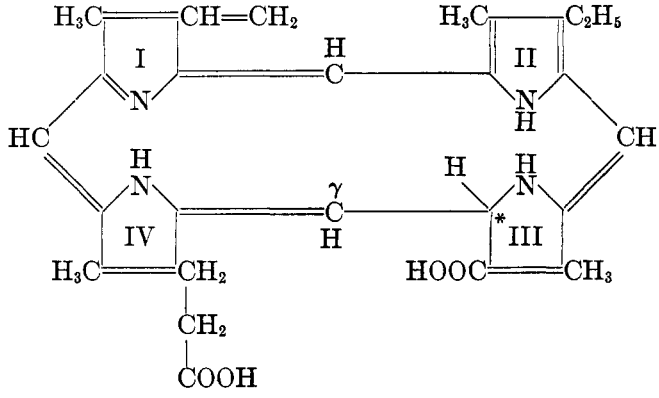


XXVIII b
Phyllochlorin



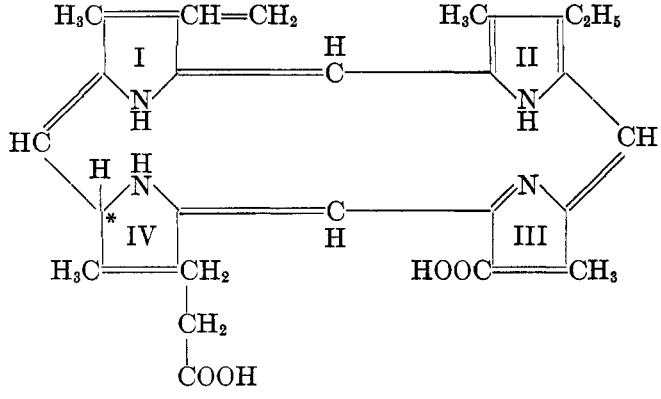
XXVIII c
Pyrrochlorin

Conant (3) had previously made a similar suggestion on the basis of titration results on the basicity of chlorophyll derivatives (*vide infra*). These results indicated that the phorbides and the chlorins contained a nucleus of basicity comparable to the amide R—NH—COR, and this nucleus was either III or IV, since only these two carried acidic groups. The following formulas indicate Conant's suggestions for the possible structure of chlorin *f* (the vinyl group proved by Fischer has been incorporated). Both formulas contain one asymmetric carbon atom, marked by asterisks.



XXIX a

or



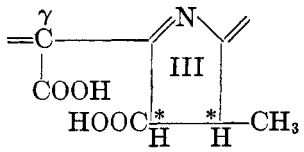
XXIX b

Chlorin *f* (Conant) = rhodochlorin

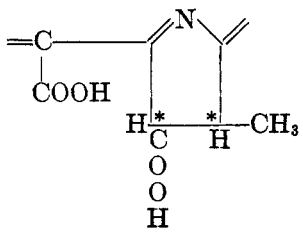
Fischer found that another simple chlorin, phyllochlorin (Conant's pyrochlorin *e*, obtained by pyrolysis of chlorin *e* (8)) is also optically active

(41). It differs from rhodochlorin in that it contains a methyl group on the γ-carbon atom, and no carboxyl group in the 6-position (*cf.* the difference between rhodo- and phyllo-porphyrins). Pyrrochlorin, with no substituents in either the γ- or the 6-position, has been prepared by the decarboxylation of rhodochlorin (39); it also is optically active. Fischer's formulas for these three chlorins are given above (XXVIII); the asymmetric centers are indicated by asterisks. The formulas for phyllo- and pyrro-chlorins derivable from either of Conant's formulas for chlorin *f* would also retain the asymmetric center. Hence the optical activity of these three chlorins does not determine the position of the two "extra hydrogens."

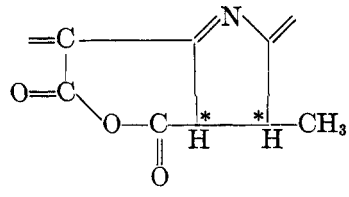
In Fischer's formula for rhodochlorin there are two asymmetric carbon atoms, therefore theoretically two racemic and four optically active modifications are possible. Fischer claims to have found this type of isomerism in compounds closely related to rhodochlorin (40). Conant (11) showed that pheopurpurin 18 (an anhydride; figure XXIII) on alkaline hydrolysis yielded the tribasic acid, chlorin *a*, which formed a trimethyl ester on treatment with diazomethane. Fischer (32) called the new substance chlorin p_6, and established its structure as that of a rhodoporphyrin-γ-carboxylic acid, except that it contains the vinyl group and the two extra nuclear hydrogen atoms. It is a dextrorotatory substance. Fischer also obtained a compound apparently isomeric with chlorin p_6 by the action of dilute sodium hydroxide in the presence of air on pheophorbide *a*. The new compound, pseudochlorin p_6, differs from chlorin p_6 spectroscopically, and in optical activity; it is levorotatory. They are not, however, optical antipodes. Similarly the dihydro derivatives (40) and the diazoacetic ester addition products of the two chlorins differ in spectrum and in optical activity. The chlorins also differ in chemical activity. Chlorin p_6 and its dihydro compound revert spontaneously to pheopurpurin 18 and its dihydro compound, respectively, while pseudochlorin p_6 only gives pheopurpurin 18 on heating, and the dihydro compound is completely stable. Fischer's explanation is that the carboxyl groups in the normal series are adjacent to each other, and thus anhydride formation is possible (formulas XXX).



XXX a
Chlorin p_6

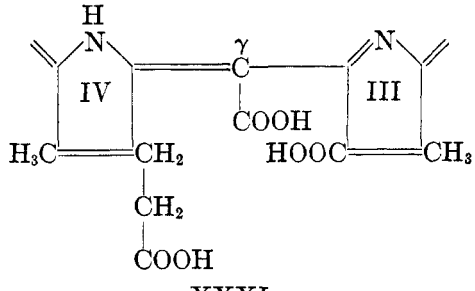


XXX b
Pseudochlorin p_6

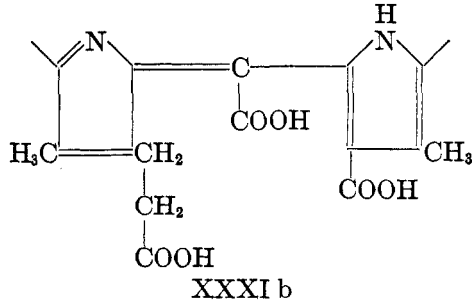


XXX c
Pheopurpurin 18

Further, chlorin p_6 with hydrogen iodide gives rhodoporphyrin-γ-carboxylic acid, which with concentrated sulfuric acid forms a green anhydride. Pseudochlorin p_6 with hydrogen iodide gives a rhodoporphyrin-γ-carboxylic acid which differs in mixed melting point and spectrum, and is decomposed by sulfuric acid. A similar result is obtained when the diazoacetic ester addition products of the two chlorins are used. Since there cannot be optical isomerism dependent on position 6 in rhodoporphyrin-γ-carboxylic acid, Fischer asumes that the form which gives no anhydride must have undergone isomerization in the hydrogen iodide treatment, with rearrangement of hydrogen atoms in all the pyrrole nuclei (XXXI). So far, no investigator has ever shown the existence of two such isomers of one compound in the chlorophyll series, especially in the porphyrins (which have been exhaustively investigated for such isomers).



XXXI a
Rhodoporphyrin-γ-carboxylic acid from chlorin p_6; forms anhydride



XXXI b
Rhodoporphyrin-γ-carboxylic acid from pseudochlorin p_6; forms no anhydride

If the two chlorins had given the same rhodoporphyrin-γ-carboxylic acid, the evidence for an asymmetric center at position 6 would have been conclusive. But the postulation of this new rhodoporphyrin acid throws doubt on the whole question of the relation between chlorin p_6 and pseudochlorin p_6.[2]

The exact structure of the chlorophyll molecule

Adopting the method of Tschugaeff and Zerewitinoff (92), in which active hydrogen atoms are measured by their reaction with methylmagnesium iodide, and later the modified method of Schmitz-Dumont and Hamon (74), Fischer in a series of papers estimated the active hydrogen atoms in chlorophyll derivatives. In the simple esterified porphyrins, e.g., rhodoporphyrin dimethyl ester, two active hydrogens were found; these appeared to be the hydrogens in two imino groups, corresponding to formula Va. Methyl pheophorbide *a* and pheoporphyrin a_5 dimethyl ester contain a third active hydrogen (30), and this is the labile hydrogen in position 10, which in the former case is responsible for enol formation in the phase test. The presence of the carbomethoxyl group in position 10 appears to influence the lability of the hydrogen atom, for pyropheophorbide *a* (XXVII b) shows only two active hydrogen atoms, and does not give the phase test reaction. But a hydroxyl group in position 10 of pheophorbide *a* would also give these results. Isorhodoporphyrin (2-vinylrhodoporphyrin) shows anomalous results, having apparently only one active hydrogen atom.

The actual arrangement of the double bonds in the chlorophyll molecule appears to be no longer entirely arbitrary. Fischer, Stern, and coworkers examined spectroscopically most of the chlorophyll derivatives, and established certain general laws (80). If a porphyrin contains a carbonyl group in a nucleus, the spectrum is closely related to that of rhodoporphyrin. If the porphyrin contains two carbonyl groups, then two types of spectra are possible. The "rhodo" type appears if one carbonyl is on a nucleus and the second carbonyl is in the γ-position, or

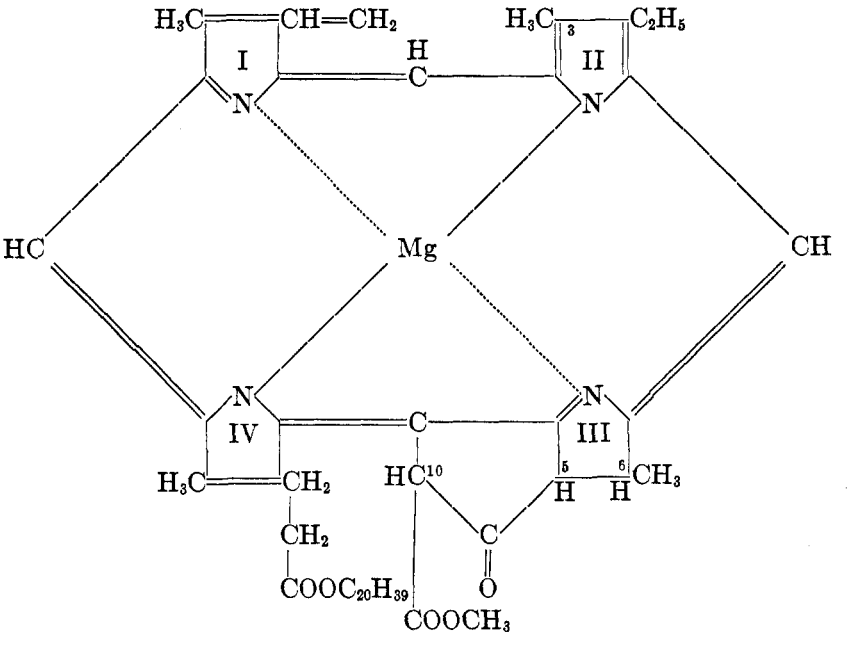
[2] The latter is formed in a reaction which is very closely related to the phase test. This preparation differs from the preparation of monomethylchlorin *g* chiefly in temperature (50°C. compared with 0°C.) and in time (ten hours compared with from 30 minutes to one hour). This suggests that the γ-carbon atom in pseudochlorin p_6 may carry a potential glyoxalic acid side chain; such a compound (an isomer of the unstable chlorins or of monomethylchlorin *g*) could give pheopurpurin 18 on being heated, and with hydrogen iodide might give a rhodoporphyrin-γ-glyoxalic acid, which would naturally form no anhydride with sulfuric acid, but would be decomposed. Against this argument are the analyses, and the fact that pseudochlorin p_6 does not give dimethylpheopurpurin 7 on esterification.

between the γ- and 6-positions in a carbocyclic ring in conjugation with the whole porphin nucleus; or again, if the two carbonyl groups are situated in alternate pyrrole nuclei, e.g., I and III, or II and IV. On the other hand, if the two carbonyl groups are in adjacent nuclei, or not in conjugation with the porphin system, then the "rhodo" type spectrum is absent. Applying this generalization, it can be shown that nucleus IV has a pyrrole structure and nucleus III a pyrrolenine structure, and that the double bond at the γ-carbon atom is attached to IV and not to III. Briefly, chloroporphyrin e_6 dimethyl ester is a γ-formylrhodoporphyrin dimethyl ester, but it does not show a "rhodo" type spectrum; similarly the trimethyl ester of rhodoporphyrin-γ-carboxylic acid has a spectrum different from the "rhodo" type, hence the double bond of the carbon atom cannot be attached to nucleus III. The position of the double bond between the γ-carbon atom and nucleus IV was suggested earlier by Fischer (64), as he found that models constructed with the double bond to nucleus III showed a much larger amount of strain. How far these results apply to the chlorophyll molecule itself is still an open question. Conant and Kamerling's spectroscopic data (9) at low temperatures indicate that in chlorophyll and its non-porphyrin derivatives there is a break in the conjugated system of the porphyrins. Conant's formula for chlorin *f* (XXIX) takes this into account; Fischer's formulas (*cf.* XXVIII a and XXXII) do not.

Conant (3) attempted to establish the types of nuclei present in the chlorophyll molecule by potentiometric titrations in glacial acetic acid with perchloric acid. Comparing chlorophyll derivatives with simple nitrogenous compounds containing the pyrrole, isopyrrole (or maleic imide), and pyrrolenine (tertiary nitrogen as in pyridine) structures, he found that porphyrins showed two strongly basic groups comparable to two pyrrolenine nuclei (*cf.* nuclei I and III in formula III). The chlorins, on the other hand, had only one strongly basic group (nucleus I in formula XXIX a or nucleus III in formula XXIX b), and one group intermediate in basicity between a pyrrolenine and a pyrrole structure (nucleus III or IV, respectively). To accomodate these findings, Conant suggested the hydrogenated pyrrolenine nucleus shown (formula XXIX). In general, therefore, Conant's results agree with those of Stern, and in particular corroborate the fact that in the chlorins, and therefore in chlorophyll, it is a pyrrolenine nucleus of the porphyrins which carries the two hydrogen atoms to form the dihydroporphin nucleus. On the other hand, the basicity of this hydrogenated nucleus is comparable to R—NH—COR, and is less basic than Fischer's nucleus III would be.

The following formula for chlorophyll *a* is the most recent of those sug-

gested by Fischer, on the basis of the results summarized in the previous pages:

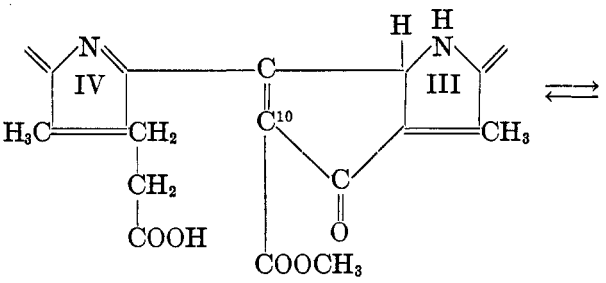


XXXII
Chlorophyll *a* (Fischer, 1936)

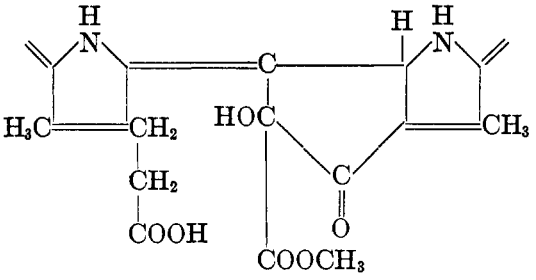
Since a paper by Dr. Fischer summarizing his views follows this review, it will perhaps not seem too critical of his brilliant work if the objections to this present formula—many of them have already been indicated—are summarized:

(a) The position of the hydrogen atoms in positions 5 and 6 needs further proof than the apparent isomerism between chlorin p_6 and pseudochlorin p_6.

(b) The state of oxidation of carbon atom 10 in chlorin *e* and therefore also in pheophorbide *a* appears open to question. In the former case, the experimental data indicate a >CHOH grouping in position 10. How such a grouping could be interpolated in the pheophorbide *a* formula and still explain all the results is more difficult. An unsaturated carbocyclic ring may be the answer; for instance, the following formula for pheophorbide derived from formula XXIX a for chlorin *f* and incorporating the carbocyclic ring is one possibility.



XXXIII a
Pheophorbide



XXXIII b
(1,4-addition of H_2O)

An interesting point in favor of some such unsaturation at position 10 is Fischer's finding that pheoporphyrin a_5 is optically inactive (64), although it contains an asymmetric center in position 10. Either the carbocyclic ring is affected by the hydrogen iodide treatment, and is therefore *not* the same in pheophorbide and pheoporphyrin a_5, or else a racemic mixture of the two forms, whose optical activity depends on this center of asymmetry, is present in pheophorbide. The latter supposition is hardly tenable.

Removal of the two "extra" hydrogen atoms from nucleus III in formula XXXIII a above, might lead to 1,4-addition in position 10 and on the nitrogen of this nucleus. Fischer (45) found that the two hydrogen atoms could be removed by oxidation with silver acetate, and the product was not 2-vinylpheophorphyrin a_5, but 10-acetoxy-2-vinylpheophorphyrin a_5. This fits the above formula equally as well as being explicable by Fischer's theory of activation of the hydrogen on carbon atom 10 by the carbomethoxyl group. The reaction was, however, quantitative after five minutes heating, according to the equation,

$$C_{35}H_{36}N_4O_5 + 4CH_3COOAg = C_{37}H_{36}N_4O_7 + 4Ag + 3CH_3COOH$$

This corroborates Fischer's formula as far as it goes, but the experimental details indicate that oxidation, or at least decomposition, continued on longer treatment with excess reagent.

(c) The extreme ease of formation of the carbocyclic ring, shown by Fischer to occur in various syntheses, throws doubt on the proofs of the presence of this ring in pheophorbide and in chlorophyll. The methods of degradation of these compounds to substances which have been shown by synthesis to contain this ring are at least as drastic as the methods of ring closure. Complete proof of the carbocyclic ring could probably only be afforded by the synthesis of pheophorbide.

IV. Chlorophyll *b*

Chlorophyll *b* presented a more difficult problem than chlorophyll *a*, chiefly because the yields were very low, and the products much more difficult to purify. It appeared, however, that chlorophyll *b* differed from *a* in that it contained an additional oxygen atom. Willstätter (98) established this by analysis, and various suggestions as to the position of this oxygen atom were advanced. Conant (6), by pyrolysis, phase test reaction, and saponification, obtained from methyl pheophorbide *b* a series of compounds entirely parallel to those in the *a* series (XI). These rhodins, corresponding to the chlorins in the *a* series, still retained the extra oxygen atom, and formed semicarbazones, indicating the presence of a carbonyl group. That this group was in some other part of the molecule than the reactive grouping responsible for the phase test was indicated by the ability of rhodin *l*, the simple rhodin corresponding to chlorin *f* of the *a* series, and which contained no substituent on the γ-bridge carbon atom, to form a semicarbazone. To account for the marked difference in absorption spectrum between the *a* and *b* series, Conant placed this carbonyl group in the nucleus. He replaced a methylene bridge grouping by a $>C{=}O$ bridge in one of positions α, β, or δ, but not in γ, because of the properties of rhodin *l*. Warburg (93, 94) also obtained carbonyl derivatives in the *b* series.

Fischer (38), using the hydrogen iodide reaction, showed that pheophorbide *b* and rhodin *g* (analogous to chlorin *e*) gave rise to porphyrins, viz., the pheoporphyrin *b* series and the rhodoporphyrin *g* series, corresponding to the pheoporphyrin *a* and chloroporphyrin *e* compounds of the *a* series (XVIII). Thus he established that pheophorbide *b* contained the carbocyclic ring of the *a* series. He showed that pheophorbide *b* could form an acetal, and also an oxynitrile derivative, while the carbonyl group in the carbocyclic ring is incapable of such reactions. Further, he found that rhodin *g*, in which the carbocyclic ring is no longer present, can still form an oxime with hydroxylamine, indicating that the new carbonyl

group was in some other part of the molecule (25, 38). Since oxidation results on *b* compounds at that time gave no hematinic acid (II a), which is derived from nucleus IV, Fischer originally placed this carbonyl group in the β-position of the propionic acid side chain (26). Meanwhile Stoll and Wiedemann placed the new carbonyl group in the side chain on the γ-bridge as an α-keto acid (86), and later isolated a dioxime of pheophorbide *b* (89).

Fischer's later work definitely placed the carbonyl group in nucleus II of chlorophyll *b*. The action of hydrogen iodide on the trimethyl ester of rhodin *g* gives the trimethyl ester of rhodoporphyrin g_7 (corresponding to chloroporphyrin e_6, formula XVII). This compound forms a monooxime, indicating that it still contains the carbonyl group of the *b* series. It gives the oxo reaction, with the formation of rhodoporphyrin g_8, which can no longer form an oxime, and which has acquired an additional carboxyl group, indicating that the parent compound had a formyl group (21). Hydrogen bromide or hydrogen chloride at high temperatures gave rise to 3-desmethylphylloporphyrin and 3-desmethylpyrroporphyrin, hence the formyl group in the *b* series is in the 3-position, replacing a methyl group of the *a* series (21, 84). Final and definite proof was given by the synthesis of 3-desmethyldesoxophylloerythrin (*cf.* formula XIV). This compound is obtainable from the ester of rhodoporphyrin g_8 by reduction, whereby the 3-carboxyl group is reduced to a hydrogen atom, and ring closure is effected between the γ- and 6-positions. Alternately, pyrolysis of rhodoporphyrin g_8 in pyridine effects ring closure with formation of pheoporphyrin b_7 (*cf.* e_6 to a_5; in the *b* series there is an additional carboxyl group originating from the formyl group). If the oxime (on the carbocyclic carbonyl group) of b_7 is heated with hydrogen bromide, it loses two carboxyl groups, and 3-desmethyldesoxophylloerythrin (*cf.* formula XIV) again results (23). This compound was synthesized (54), and proved the position of a formyl group in the 3-position of chlorophyll *b*.

Chlorophyll *b* and the rhodins contain the vinyl group in the 2-position; diazoacetic ester gives addition products, and that the reaction does not affect the formyl group is proved by the ability of the oximes to form diazoacetic ester addition products (22). The poor yields obtained in the *b* series are due to the presence of the two unsaturated groupings. Fischer showed that if these groups are prevented from reacting, side reactions are eliminated, and the yields parallel and even better those in the *a* series. For instance, pyrolysis of rhodin *g* trimethyl ester gives pyropheophorbide *b* in 10 per cent yield; pyrolysis of the oxime gives a yield of 30 per cent oxime; while pyrolysis of the diazoacetic ester addition product of the oxime gives a 60 per cent yield of the corresponding derivative of pyropheophorbide *b* (17).

It is possible to convert the *b* into the *a* series by converting the formyl group into the methyl group; this was accomplished by catalytic reduction in formic acid of pheoporphyrin b_6, the products being pheoporphyrin a_5 and its reduction product, 9-hydroxydesoxopheoporphyrin a_5 (33). Fischer's formula for chlorophyll *b* is therefore identical with that of *a* (XXXII), except for the replacing of the methyl group in the 3-position by a formyl group.

V. Attempts at the Synthesis of Chlorophyll

For a total synthesis of chlorophyll, the starting material would naturally be phylloerythrin, which has been prepared synthetically. The first step would then be the introduction of the carbomethoxyl residue into the isocyclic ring to give pheoporphyrin a_5. Fischer could not achieve this, although he found it possible to introduce the ethoxyl residue by the iodine oxidation method, with the formation of 10-ethoxyphylloerythrin (53). Higher homologues were also prepared (55). However a partial synthesis of pheoporphyrin a_5 was arrived at by ring closure of the 6-formyl derivative of isochloroporphyrin e_4 ester (XX), which already contains the carbomethoxyl residue, to give 9-hydroxydesoxopheoporphyrin a_5 (42). Oxidation of the latter with chromic acid gives pheoporphyrin a_5 itself.

Willstätter (9b) introduced the phytyl group into pheophorbide biologically by reversing the action of the chlorophyllase enzyme. By the use of phosgene, whereby an acid chloride appears to be formed as an intermediate product, Fischer (56) found it possible to esterify pheophorbide *a* with various alcohols of high molecular weight; these included phytol, geraniol, menthol, cetyl alcohol, and others. Thus a synthesis of pheophytin from pheophorbide was achieved, and the synthetic product was in all its properties identical with natural pheophytin.

Willstätter (97) achieved a synthesis of chlorophyll from pheophytin by introducing magnesium through the medium of the Grignard reagent. Fischer (60) found that in this reaction the phytyl group is partially saponified. However he succeeded in introducing magnesium by the Grignard reaction into methyl pheophorbide *a*, with the formation of chlorophyllide *a*, whose identity was established by the hydrogen iodide and allomerization reactions, giving rise to pheoporphyrin a_5 ester and its 10-ethoxy derivative, respectively. Fischer (31) later found better conditions for the Grignard reaction, and introduced magnesium into the pheophytin molecule, giving chlorophyll *a* itself, and into pheoporphyrin a_5, giving pheoporphyrin a_5-phyllin.

Similarly in the *b* series, ethyl chlorophyllide *b* was synthesized from ethyl pheophorbide *b* by the use of the Grignard reagent (61).

The question of color in chlorophyll and its derivatives is of interest. Early striking examples were the acid and anhydride pairs; rhodoporphyrin-γ-carboxylic acid (XXXI a) is red and its anhydride is green, while pheopurpurin 18 (an anhydride) (XXX c) is purplish-red, and the corresponding acid, chlorin *a* (Conant) or chlorin p_6 (Fischer, XXX a), is green. In many cases the difference is merely that of two nuclear hydrogen atoms, as in the simple chlorins, rhodo- (Conant's chlorin *f*), phyllo-, and pyrro-chlorins (XXVIII a, b, and c), compared with the corresponding porphyrins, all of which are red. The difference between the chlorins and the rhodins (red) is in the formyl group. Fischer considers that any oxygen-containing side chain attached to the nucleus will effect this color change, and cites the pheopurpurins, whose colour is analogous to that of the rhodins, and which possess keto groups attached to the γ-carbon atom.

Fischer proposes modifying the nomenclature of the types of chlorophyll derivatives (39); he suggests that they be classified into phorbins, chlorins, and porphins. Phorbins possess the dihydroporphin nucleus and the carbocyclic ring. When this ring is opened, chlorins result; this group therefore includes the rhodins and the purpurins. The prefix "meso" before the phorbins and chlorins indicates an ethyl in place of a vinyl group; i.e., the meso compounds are the former dihydro compounds.

REFERENCES

(1) CONANT AND ARMSTRONG: J. Am. Chem. Soc. **55**, 829 (1933).
(2) CONANT AND BAILEY: J. Am. Chem. Soc. **55**, 795 (1933).
(3) CONANT, CHOW, AND DIETZ: J. Am. Chem. Soc. **56**, 2185 (1934).
(4) CONANT AND DIETZ: J. Am. Chem. Soc. **55**, 839 (1933).
(5) CONANT, DIETZ, BAILEY, AND KAMERLING: J. Am. Chem. Soc. **53**, 2382 (1931).
(6) CONANT, DIETZ, AND WERNER: J. Am. Chem. Soc. **53**, 4436 (1931).
(7) CONANT AND HYDE: J. Am. Chem. Soc. **51**, 3668 (1929).
(8) CONANT, HYDE, MOYER, AND DIETZ: J. Am. Chem. Soc. **53**, 359 (1931).
(9) CONANT AND KAMERLING: J. Am. Chem. Soc. **53**, 3522 (1931).
(10) CONANT, KAMERLING, AND STEELE: J. Am. Chem. Soc. **53**, 1615 (1931).
(11) CONANT AND MOYER: J. Am. Chem. Soc. **52**, 3013 (1930).
(12) DIETZ AND ROSS: J. Am. Chem. Soc. **56**, 159 (1934).
(13) DIETZ AND WERNER: J. Am. Chem. Soc. **56**, 2180 (1934).
(14) FISCHER, GOTTWALT: Ann. **475**, 183 (1929).
(15) FISCHER: Ann. **499**, 84 (1932).
(16) FISCHER: Ann. **502**, 175 (1933); Pedler Lecture, J. Chem. Soc. **1934**, 245.
(17) FISCHER AND BAUER: Ann. **523**, 235 (1936).
(18) FISCHER AND BAUMLER: Ann. **480**, 197 (1930).
(19) FISCHER, BERG, AND SCHORMÜLLER: Ann. **480**, 109 (1930).
(20) FISCHER AND BÖCKH: Ann. **516**, 177 (1935).
(21) FISCHER AND BREITNER: Ann. **510**, 183 (1934).
(22) FISCHER AND BREITNER: Ann. **511**, 183 (1934).
(23) FISCHER AND BREITNER: Ann. **516**, 61 (1935).

(24) FISCHER AND BREITNER: Ann. **522**, 151 (1936).
(25) FISCHER, BREITNER, HENDSCHEL, AND NÜSSLER: Ann. **503**, 1 (1933).
(26) FISCHER, BROICH, BREITNER, AND NÜSSLER: Ann. **498**, 228 (1932).
(27) FISCHER, FILSER, HAGERT, AND MOLDENHAUER: Ann. **490**, 1 (1931).
(28) FISCHER, FILSER, AND PLÖTZ: Ann. **495**, 1 (1932).
(29) FISCHER AND GLEIM: Ann. **521**, 157 (1935).
(30) FISCHER AND GOEBEL: Ann. **522**, 168 (1936).
(31) FISCHER AND GOEBEL: Ann. **524**, 269 (1936).
(32) FISCHER, GOTTSCHALDT, KLEBS, AND PLÖTZ: Ann. **498**, 194 (1932).
(33) FISCHER AND GRASSL: Ann. **517**, 1 (1935).
(34) FISCHER AND HASENKAMP: Ann. **515**, 148 (1935).
(35) FISCHER, HECKMAIER, AND HAGERT: Ann. **505**, 209 (1933).
(36) FISCHER AND HELBERGER: Ann. **480**, 235 (1930).
(37) FISCHER AND HENDSCHEL: Z. physiol. Chem. **198**, 33 (1931); see also FISCHER and HENDSCHEL: Z. physiol. Chem. **222**, 250 (1933).
(38) FISCHER, HENDSCHEL, AND NÜSSLER: Ann. **506**, 83 (1933).
(39) FISCHER, HERRLE, AND KELLERMANN: Ann. **524**, 222 (1936).
(40) FISCHER AND KAHR: Ann. **524**, 251 (1936).
(41) FISCHER AND KELLERMANN: Ann. **519**, 209 (1935).
(42) FISCHER AND KELLERMANN: Ann. **524**, 25 (1936).
(43) FISCHER AND KRAUSS: Ann. **521**, 261 (1936).
(44) FISCHER AND LAKATOS: Ann. **506**, 123 (1933).
(45) FISCHER AND LAUTSCH: Ann. **525**, 259 (1936).
(46) FISCHER AND MEDICK: Ann. **517**, 245 (1935).
(47) FISCHER, MERKA, AND PLÖTZ: Ann. **478**, 283 (1930).
(48) FISCHER, MOLDENHAUER, AND SÜS: Ann. **485**, 1 (1931).
(49) FISCHER, MOLDENHAUER, AND SÜS: Ann. **486**, 107 (1931).
(50) FISCHER, MÜLLER, AND LESCHHORN: Ann. **523**, 164 (1936).
(51) FISCHER AND RIEDMAIR: Ann. **490**, 91 (1931).
(52) FISCHER AND RIEDMAIR: Ann. **505**, 87 (1933).
(53) FISCHER, RIEDMAIR, AND HASENKAMP: Ann. **508**, 224 (1934).
(54) FISCHER AND ROSE: Ann. **519**, 1 (1935).
(55) FISCHER AND SCHERER: Ann. **519**, 234 (1935).
(56) FISCHER AND SCHMIDT: Ann. **519**, 244 (1935).
(57) FISCHER AND SCHWARZ: Ann. **512**, 239 (1934).
(58) FISCHER AND SIEBEL: Ann. **494**, 73 (1932).
(59) FISCHER, SPEITMANN, AND METH: Ann. **508**, 154 (1934).
(60) FISCHER AND SPIELBERGER: Ann. **510**, 156 (1934).
(61) FISCHER AND SPIELBERGER: Ann. **515**, 130 (1935).
(62) FISCHER AND STADLER: Z. physiol. Chem. **239**, 167 (1936).
(63) FISCHER AND STERN: Ann. **519**, 58 (1935).
(64) FISCHER AND STERN: Ann. **520**, 88 (1935).
(65) FISCHER AND SÜS: Ann. **482**, 225 (1930).
(66) FISCHER, SÜS, AND KLEBS: Ann. **490**, 38 (1931).
(67) HOPPE-SEYLER: Z. physiol. Chem. **3**, 339 (1879); **4**, 193 (1880).
(68) KUSTER: Z. physiol. Chem. **82**, 463 (1912).
(69) KUSTER: Ber. deut. pharm. Ges. **12**, 513 (1912).
(70) LÖBISCH AND FISCHLER: Monatsh. **24**, 335 (1903).
(71) MARCHLEWSKI: Z. physiol. Chem. **43**, 464 (1904); **45**, 466 (1905).
(72) NENCKI: Ber. **34**, 997 (1901).

(73) ROTHEMUND: J. Am. Chem. Soc. **58**, 625 (1936).
(74) SCHMITZ-DUMONT AND HAMON: J. prakt. Chem. **139**, 162 (1934).
(75) SCHUNCK: Proc. Roy. Soc. London **50**, 302 (1891); **57**, 314 (1895); Ann. **284**, 81 (1894).
(76) SORBY: Proc. Roy. Soc. London **21**, 442 (1873).
(77) SPOEHR: Annual Review of Biochemistry, Vol. 2, p. 453. Stanford University Press, Stanford (1933).
(78) STEELE: J. Am. Chem. Soc. **53**, 3171 (1931).
(79) STERN AND KLEBS: Ann. **505**, 295 (1933).
(80) STERN AND WENDERLEIN: Z. physik. Chem. **175A**, 405 (1936).
(81) STOKES: Proc. Roy. Soc. London **13**, 144 (1864).
(82) STOLL: Naturwissenschaften **20**, 628 (1932).
(83) STOLL: Naturwissenschaften **24**, 53 (1936).
(84) STOLL: Helv. Phys. Acta **9**, 19 (1936).
(85) STOLL AND WIEDEMANN: Naturwissenschaften **20**, 791 (1932).
(86) STOLL AND WIEDEMANN: Helv. Chim. Acta **15**, 1280 (1932).
(87) STOLL AND WIEDEMANN: Helv. Chim. Acta **16**, 739 (1933); **17**, 163 (1934).
(88) STOLL AND WIEDEMANN: Helv. Chim. Acta **16**, 307 (1933).
(89) STOLL AND WIEDEMANN: Helv. Chim. Acta **17**, 456 (1934).
(90) TREIBS AND HERRLEIN: Ann. **506**, 1 (1933).
(91) TREIBS AND WIEDEMANN: Ann. **471**, 146 (1929).
(92) TSCHUGAEFF AND ZEREWITINOFF: Ber. **40**, 2025 (1907).
(93) WARBURG AND CHRISTIAN: Biochem. Z. **235**, 240 (1931).
(94) WARBURG AND NEGELEIN: Biochem. Z. **244**, 9 (1932).
(95) WILLSTÄTTER: Ann. **373**, 227 (1910); **385**, 188 (1911).
(96) WILLSTÄTTER AND BENG: Ann. **358**, 205 (1907).
(97) WILLSTÄTTER AND FORSEN: Ann. **396**, 180 (1913).
(98) WILLSTÄTTER AND STOLL: Untersuchungen über Chlorophyll, Berlin, 1913; translated by Scherz and Merz, Science Publishing Co., 1928. The original papers are to be found in the Annalen from 1906 to 1914.
(99) WILLSTÄTTER AND UTZINGER: Ann. **382**, 129 (1911).
(100) WINTERSTEIN AND STEIN: Handbuch der Pflanzen Analyse, IV, II, 1403 (1933); also Z. physiol. Chem. **220**, 263 (1933); **230**, 139 (1934).