formed. ω-Polybromoprene, a granular, insoluble, rubber-like mass, is produced under conditions that lead to very slow polymerization.

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Studies in the Chlorophyll Series. IX. Transformations Establishing the Nature of the Nucleus

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In previous papers of this series, the view has been put forward that the fundamental nucleus of chlorophyll *a* and the related phaeophorbides and chlorins is a reduced porphyrin ring. Such a formulation was largely based on spectroscopic data obtained at low temperatures.[1] We have now been able to obtain very convincing evidence of the correctness of this hypothesis by showing that a typical chlorin (chlorin *f*) can be oxidized to a porphyrin.

The dehydrogenation of chlorin *f* can be brought about either by the action of potassium ferricyanide on an alkaline solution at room temperature (the reaction is very rapid) or by the action of air on a hot alkaline solution of the complex metal salts of the chlorin. The latter method of converting chlorins to porphyrins has been used by Fischer in reoxidizing the "synthetic chlorins" prepared by reduction of the porphyrins.[2] It is clear that chlorin *f* is entirely analogous and is a reduced porphyrin. Since in chlorin *f* the side chain attached to the bridge has been removed, the failure of chlorin *e* to be so readily oxidized to a porphyrin can be attributed to the presence of the other functional groups. The number of hydrogen atoms present in chlorin *f* in excess of a porphyrin can be taken quite definitely as two, since one or three is excluded by valency considerations and four seems excluded by the analyses of chlorin *f* and its ester. The found and calculated percentages of hydrogen in the dimethyl ester and the free chlorin are as follows (paper IV): ester, found 7.05, 6.9, calcd. dihydro 7.04, tetrahydro 7.37; free chlorin, found 6.3, 6.6, calcd. dihydro 6.67, tetrahydro 7.02.

Chlorin *f* is a dibasic acid containing no group on a bridge atom, since it is readily converted to rhodoporphyrin by reduction with hydrogen iodide and reoxidation. However, the porphyrin which is formed from it by direct dehydrogenation is not rhodoporphyrin but an isomeric porphyrin which we shall designate as isorhodoporphyrin. It is identical with the

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[1] Conant and Kamerling, THIS JOURNAL, **53**, 3522 (1931). Since the publication of this paper, the measurements of Kistiakowsky and Arnold there referred to have been published [*ibid.*, **54**, 1713 (1932)]. A comparison of the ultraviolet absorption spectra at low temperature of benzene and cyclohexadiene (Fig. 2, K. and A.) with the absorption spectra of aetioporphyrin and chlorin *e* (Figs. 1 and 3, C. and K.) also at low temperature shows in a very striking way the resemblance of the porphyrin-chlorin relationship to that of benzene *vs.* cyclohexadiene.

[2] Fischer and Helberger, *Ann.*, **471**, 285 (1929).

porphyrin described in paper IV which was obtained as by-product in the pyrolysis of phaeopurpurin 7 and whose isomerism with rhodoporphyrin we commented on at that time. Subsequently the same substance was isolated by Fischer[3] by boiling dimethylphaeopurpurin 7 with pyridine; one would be inclined to assume that it was identical with verdoporphyrin except that Fischer explicitly states that it is not and calls it pseudoverdoporphyrin. We have never been able to prepare verdoporphyrin in this Laboratory and therefore could not make a direct comparison.

Isorhodoporphyrin can be converted into rhodoporphyrin by reduction with hydrogen iodide and reoxidation of the resulting leuco compound with air. It can also be transformed into rhodoporphyrin by the action of strong alkali at 185–190°. The cause of the isomerism between isorhodoporphyrin and rhodoporphyrin is a matter of great interest and is clearly of the utmost importance to the chemistry of chlorophyll *a* since the fundamental nucleus of this compound is a dihydroisorhodoporphyrin. It seems probable that the isomerism may be due to a difference in the position of the two hydrogen atoms attached to the nitrogen atoms of the pyrrole nuclei in the porphyrin. If such isomers are capable of independent existence, a number are possible for an unsymmetrical porphyrin like rhodoporphyrin. However, one would expect that such isomers would yield the same complex metallic salt with a given metal—zinc for example. Isorhodoporphyrin and rhodoporphyrin, however, both form individual metallic complex salts with zinc from which each is regenerated without any evidence of interconversion. If the two zinc salts of these porphyrins are to be formulated as complex salts with all four nitrogen atoms involved, they are electronic isomers, which seems unlikely. Similar unexplained porphyrin isomers have appeared elsewhere in the degradations of chlorophyll *a* and in each case reduction with hydrogen iodide and reoxidation causes a conversion to the porphyrin of known structure. Pyrochlorin *e* porphyrin (pyrochloroporphyrin) for example[4] is isomeric with phylloporphyrin and by the decarboxylation of isorhodoporphyrin we have obtained small quantities of what appears to be an isopyrroporphyrin. We hope that experiments which are in progress in this Laboratory on the alkylation of porphyrins may throw light on this peculiar isomerism.

The establishment of the fact that chlorin *f* is a dihydroporphyrin can be used to settle the question of the state of oxidation of phaeophorbide *a*. If we let the entire porphyrin molecule be represented by the symbol P, then chlorin *f* is PH_2 and the unstable chlorin (or the isomeric phaeopurpurin) from which it is formed by loss of carbon monoxide and carbon dioxide must be $PH_2C_2O_3$. These compounds contain two hydrogen atoms less than the phaeophorbides or the tribasic acid, chlorin *e*, which is,

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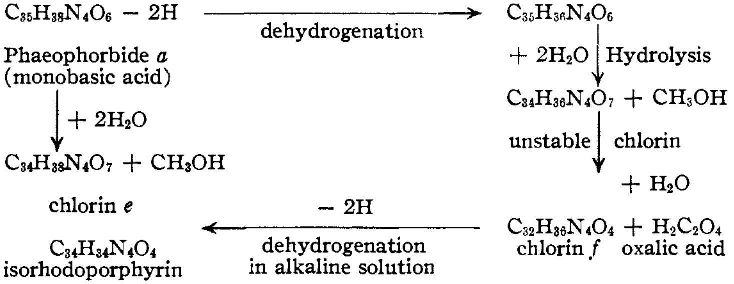
(3) Fischer, *Ann.*, **490**, 87 (1931).

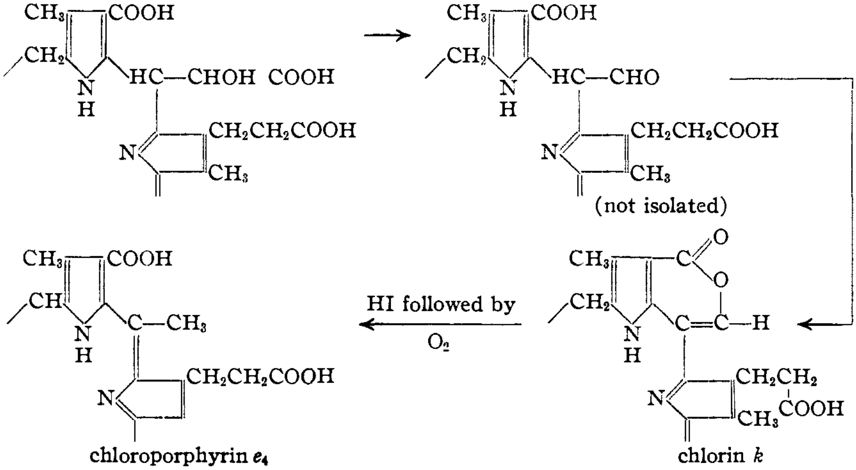
(4) Conant and Hyde, THIS JOURNAL, **51**, 3674 (1929); **53**, 367 (1931).

therefore, $PH_4C_2O_3$ = $C_{34}H_{38}N_4O_7$ which corresponds to our previous formula (see I below). Free phaeophorbide corresponds in composition to a monomethyl chlorin *e minus* water, and is, therefore, $C_{35}H_{38}N_4O_6$.[4a] Its transformation to isorhodoporphyrin is given by the following reactions in each of which there can be no doubt of the stoichiometric relations of factors and products.



The nucleus of the phaeophorbides and chlorophyll a is thus a dihydroporphyrin ring. Chlorin *e* is a dihydroisorhodoporphyrin with the lactic acid residue on one of the bridge carbon atoms; in our opinion it is still an open question as to the nature of the ring which is present in the phaeophorbides (and the chlorophyllides) and which opens on hydrolysis to yield chlorin *e*. We shall deal further with this problem in later papers.

The most satisfactory evidence for the presence of the lactic acid residue in chlorin *e* is its oxidation by molybdicyanide, yielding carbon dioxide.[5]



[4a] Since this paper was submitted for publication we have become convinced that the analyses of the purest samples of phaeophorbide *a* correspond to $C_{35}H_{36}N_4O_5$ as given by Willstätter. Stoll [*Die Naturwissenschaften*, 628 (1932)] and Fischer [*Ann.*, **499**, 84 (1932)] have arrived at the same conclusion. The implications of this fact are discussed in two papers which are being submitted for publication.

[5] Conant, Dietz, Bailey and Kamerling, *ibid.*, **53**, 2392 (1931).

The resulting chlorin, chlorin *k*, is a monobasic acid yielding a mono ester whose analysis corresponds to the formula indicated below. On reduction with hydrogen iodide and reoxidation with air it is converted into a dibasic porphyrin which was prepared some time ago by Fischer and named by him chloroporphyrin e_4.

Its structure has been established by Fischer as a methylrhodoporphyrin. A similar transformation in the *b* series is the oxidation of rhodin *k* to rhodin *n*; it would be represented by the same series of reactions except that the group CO replaces CH_2 in the porphyrin (shown to the left in each formula). Rhodin *n* forms a monosemicarbazone, chlorin *k* forms no semicarbazone; this shows that in each case the aldehyde group formed by the oxidation of the hydroxy acid has been masked by lactone formation. The transformation of rhodin *n* to a porphyrin has not yet been studied.

Experimental Part

Isorhodoporphyrin from Chlorin *f*.—A solution of 150 mg. of chlorin *f* (0.28 millimole) in 75 cc. of 0.1 *N* potassium hydroxide was mixed with 30 cc. of 0.03 *M* potassium ferricyanide (0.9 millimole). After two minutes the solution was acidified and the organic material transferred to ether. A great deal of insoluble "scum" was formed which carried some porphyrin. The scum was therefore dissolved in pyridine and the mixture diluted with ether to recover more porphyrin. Acid fractionation of the ethereal solution showed that isorhodoporphyrin is the sole soluble product; it was removed with 6–9% hydrochloric acid and retransferred to ether in the usual manner. The yield of crystalline porphyrin was 25–35 milligrams. Experiments in which the amount of ferricyanide or the time of reaction was varied did not increase the yield. The use of less than three equivalents of reagent left some chlorin unattacked.

A solution of 100 mg. of chlorin *f* in 100 cc. of 10% sodium hydroxide was treated with about 300 mg. of a mixture of magnesium oxide and zinc oxide and boiled for three hours in a current of oxygen. The product worked up in the usual manner after decomposing the complex salts with strong acid yielded 11 mg. of crystalline isorhodoporphyrin; there was no other colored ether-soluble product. The acid number of the porphyrin is 6. Color in acid: violet, with a red fluorescence. Spectrum in ether: (1 mg. in 1 cc. of pyridine and 29 cc. of ether, observed through a thickness of 50 mm.) I 645.1—640.8; II, 594.1---586.0—583.7---578.8—575.0; III, 558.6—539.5---536.9; IV, 519.8---517.1—502.9---498.2. E. A. 447.3. Order: III, IV, II, I. Spectrum in 9% hydrochloric acid: I, 664.5—654.4; II, 622.4—598.0; III, 585.8---575.5—540.9; IV, 528.5—520.4. E. A. 447.0. Order: II, III, I, IV.

Anal. Calcd. for $C_{32}H_{34}O_4N_4$: C, 71.38; H, 6.32; N, 10.41. Found: C, 70.96, 71.17; H, 6.24, 6.51; N, 10.0, 9.8; OCH_3, 0.

Isorhodoporphyrin Dimethyl Ester.—This ester was prepared by the action of diazomethane on isorhodoporphyrin. The spectra in acid and ether were identical with those of the free porphyrin. Melting point (block) 264° (softens 261°); mixed melting point with ester from dimethylphaeopurpurin 7 by Fischer's procedure 260–261°; melting point of Fischer's porphyrin 260°; acid number, 9.

Anal. Calcd. for $C_{34}H_{38}O_4N_4$: C, 72.08; H, 6.71; N, 9.89; OCH_3, 10.95. Found: C, 72.08, 72.09; H, 6.92, 7.20; N, 9.70, 9.68; OCH_3, 10.34, 10.15.

The monomethyl ester of isorhodoporphyrin was described in paper IV, p. 370 (including analysis).

Transformations of Isorhodoporphyrin.—A solution of 25 mg. of isorhodoporphyrin in boiling anthracene was heated for three minutes. On fractionation there was obtained pyrroporphyrin (identified by acid number and by comparison of acid and ether spectra with a known sample), and a porphyrin extracted by 4 and 6% acid. This was neither pyrroporphyrin nor unchanged isorhodoporphyrin. It will be investigated later.

A solution of 10 mg. of isorhodoporphyrin in 10 cc. of acetic acid was reduced on a steam-bath for two and a half minutes with 2 cc. of hydrogen iodide (45%), and worked up in the usual way. About one-quarter of the material had been transformed into rhodoporphyrin. There was also considerable unchanged isorhodoporphyrin, which on another reduction gave a further quantity of rhodoporphyrin.

A solution of 15 mg. of isorhodoporphyrin dimethyl ester in 1 cc. of pyridine was mixed with 50 mg. of mixed zinc and magnesium oxides and 5 cc. of 25% methyl alcoholic potash. The whole was heated in a bomb tube filled with nitrogen for seven hours at 185–190°. On treatment with acid to destroy the metallic complexes and acid fractionation in the usual way, rhodoporphyrin and pyrroporphyrin were obtained, representing about one-third of the material, the rest being unchanged isorhodoporphyrin. The identification of rhodoporphyrin and pyrroporphyrin was by acid number and spectrum. Similar experiments at 120–140° for five hours gave only a trace of conversion.

Chloroporphyrin e_4 from Chlorin *k*.—The yield of chlorin *k* from chlorin *e* was increased from that reported in paper IV to 37% when 2 g. of chlorin *e* was oxidized at once. A sample of chlorin *k* (150 mg.) was dissolved in 125 cc. of glacial acetic acid; 15 cc. of hydriodic acid (sp. gr. 1.45) was added and the mixture heated on the steam-bath for two and a half hours. The reaction mixture transferred to ether was washed with dilute ammonia until the acid had been removed and was allowed to stand for about two hours in contact with a slightly ammoniacal aqueous solution (to complete the reoxidation of the leuco compound). The material was then acid-fractionated in the usual manner. Chloroporphyrin e_4 was extracted with 1% acid; from 500 mg. of chlorin *k*, 63 mg. of crystalline material was obtained. It was identified by conversion to the dimethyl ester with diazomethane and a comparison of this with a sample prepared by Fischer's procedure. Analysis, spectrum, acid number and melting point were identical (250–252°). A mixed melting point showed no depression.

In addition to chloroporphyrin e_4, there was obtained another porphyrin or mixture of porphyrins which was extracted with 4% acid. About 36 mg. of this material was obtained from 500 mg. of chlorin *k*. On treatment with diazomethane in ether solution it became alkali insoluble. The methoxyl determination indicated only about a group and a half. Color in acid: blue-green. Spectrum in ether of mixed porphyrins: (chloroporphyrin e_4) I, 635.5—631.4; II, 590.0---579.9—575.6; III, 557.4—545.0---541.1; IV, 523.4---518.8—502.9---500.0. E.A. 439.5. Order: III, IV, II, I. Spectrum in 7% hydrochloric acid: I, 626.4---621.6—615.1---605.9—600.5; II, 582.8---578.4—550.0---545.6. E.A. 442.7. Order: II, I.

Rhodin *n*.—The oxidation of rhodin *g* to yield rhodin *n* was carried out in exactly the same manner as the oxidation of chlorin *e*.

An oxidizing solution was prepared by dissolving 1.66 g. of potassium molybdocyanide in 100 cc. of water and 10 cc. of glacial acetic acid, oxidizing with potassium permanganate, pouring into a mixture of 500 cc. of acetone and 67 cc. of pyridine, making up to 750 cc. with water, and filtering; 500 mg. of rhodin *g* was added, and after allowing five hours for the reaction, the mixture was poured into 6 liters of ether, washed, and fractionated. Extraction with 12–16% acids removed impure material, which was discarded, while rhodin *n* was extracted by 18 and 20% acid. The material was refractionated to remove small amounts of impurities: yield, 89 mg.; acid number, 17. Spectra reported in paper VIII, p. 4443.

Anal. Calcd. for $C_{33}H_{32}O_5N_4$: C, 70.21; H, 5.67; N, 9.93. Calcd. for $C_{33}H_{34}O_6N_4$:

C, 68.04; H, 5.84; N, 9.62. Found: C, 67.60, 68.44, 68.09; H, 5.50, 6.58, 6.09; N, 9.90, 9.80; OCH_3, 0.58, 1.0.

Rhodin *n* Monomethyl Ester.—This compound was prepared by the action of diazomethane on rhodin *n*; spectra the same as rhodin *n*. The melting point is indefinite; it sinters at 140–150°.

Anal. Calcd. for $C_{04}H_{34}O_5N_4$: C, 70.59; H, 5.88; N, 9.69; OCH_3, 5.36. Found: C, 69.04, 68.36, 68.85; H, 6.68, 6.11, 6.28; N, 8.77, 8.94; OCH_3, 5.20, 5.94.

Semicarbazone of Rhodin *n* Monomethyl Ester.—Sixty mg. of the ester and 250 mg. each of sodium carbonate and semicarbazide hydrochloride were mixed in 20 cc. of pyridine, and allowed to stand at room temperature for forty-five minutes. After two minutes the wine-red color changed to olive-green and the spectrum bands spread apart. The mixture was put into 2 liters of ether and washed well with cold water and cold 1% hydrochloric acid. The ether solution was greenish-yellow. The semicarbazone was rather insoluble and tended to form a scum; this was recovered and added to the main lot; yield, 25 mg.; acid number, 11; acid color, green. Spectrum in ether: I, 681.1—647.1; II, 613.0—601.6; III, 530.1---526.0—498.4. E. A. 451.2. Order: I, III, II. Spectrum in 14% hydrochloric acid: I, 673.9---667.9—643.9---634.9; II, 543.2—529.2. E. A. 459.5. Order: I, II.

Anal. Calcd. for $C_{35}H_{37}O_5N_7$: C, 66.14; H, 5.82; N, 15.43; OCH_3, 4.88. Found: C, 65.13, 65.75; H, 6.33, 6.17; N, 13.79, 13.86; OCH_3, 5.00, 4.66.

Warming with 18% hydrochloric acid on the steam-bath for less than a minute was enough to regenerate rhodin *n*.

Attempt to Prepare a Semicarbazone of Chlorin *k* Ester.—One hundred mg. of chlorin *k* ester and 0.4 g. each of sodium carbonate and semicarbazide hydrochloride were added to 40 cc. of pyridine, and the mixture was refluxed for an hour. The mixture was poured into 2 liters of ether and washed well with cold 2% hydrochloric acid and cold water. There was no color or spectrum change. Analysis of the product proved that it was unchanged chlorin *k*.

Summary

1. A simple chlorin (chlorin *f*) obtained by degradation of chlorophyll *a* can be dehydrogenated to a porphyrin having the same carbon skeleton. This fact affords further evidence that the fundamental nucleus of the chlorophyll *a* molecule is a dihydroporphyrin ring.

2. Chlorin *k*, obtained from chlorin *e* by oxidative degradation, has been transformed into chloroporphyrin e_4. Rhodin *g* has been oxidized to rhodin *n* which contains a carbonyl group as do the other members of the *b* series.

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