

iodide and picrate, tri-*p*-tolylselenonium chloride and iodide, di-*m*-tolyl selenide, di-*m*-tolyl selenium dichloride, tri-*m*-tolylselenonium iodide and tri-*m*-tolylselenonium chloride-mercuric chloride.

3. Triphenylselenonium hydroxide has been prepared in dilute solution. The solution when concentrated decomposes into benzene (?) and diphenyl selenium oxide.

4. The formation of triphenylselenonium chloride by fusing di-*p*-tolyl selenide with mercury diphenyl and the thermal decomposition of diphenyl-*p*-tolylselenonium iodide into phenyl iodide and phenyl *p*-tolyl selenide indicate the *p*-tolyl group to be more negative than the phenyl.

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[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]

## STUDIES IN THE CHLOROPHYLL SERIES. VIII. THE STRUCTURE OF CHLOROPHYLL *B*

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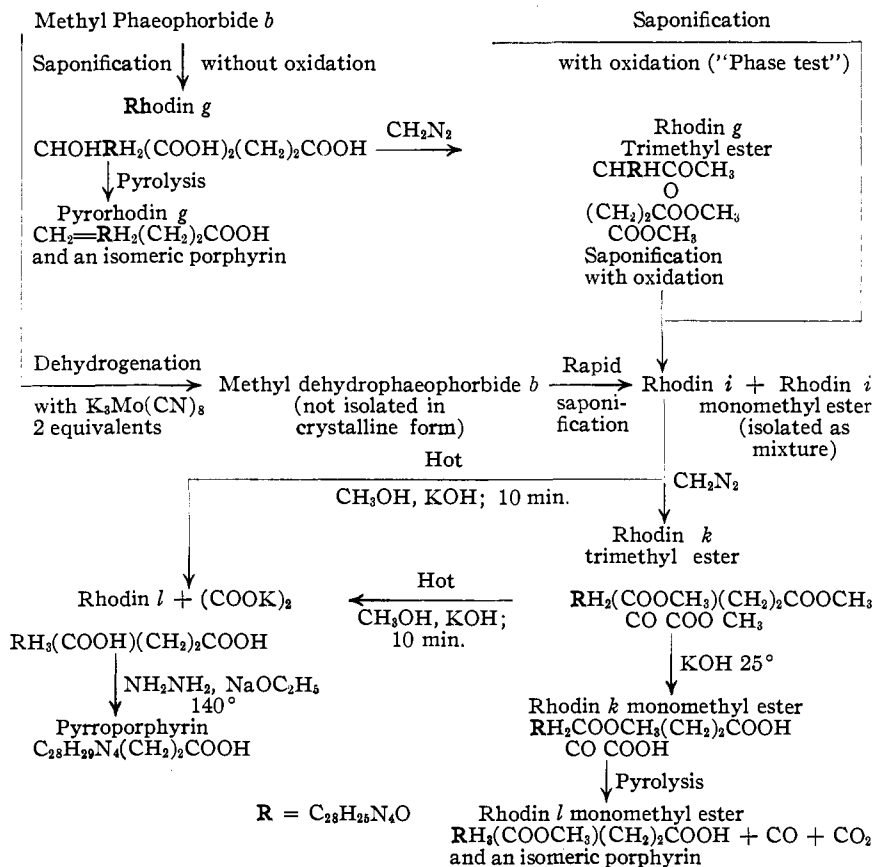
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A study of the transformations of methylphaeophorbide *a* and chlorin *e* has led us to propose structural formulas for these substances and chlorophyll *a*.<sup>1</sup> We have now to report the results of the application of our methods to the *b* series. The analyses of chlorophyll *b* and its transformation products, (phaeophorbide *b*, rhodin *g*) led Willstätter to conclude that the compounds in the *b* series differed from the corresponding *a* compounds by an extra atom of oxygen. More recent analyses by Fischer and co-workers, and in this Laboratory have confirmed this conclusion. Practically no information has been hitherto available about the nature and position of this extra oxygen atom except that the *b* compounds like the *a* compounds can be transformed into phyllo, pyrro and rhodoporphyrins, and therefore this oxygen must be such that it is removed in the drastic autoclave treatment used in preparing the porphyrins.

In all our transformations we have found that the behavior of the *b* compounds is closely analogous to that observed in the *a* series. In every case, however, the reactions are accompanied by more side reactions and the products are more difficult to isolate and purify. Because of the poor yields we have had to be content with evidence which is less satisfactory than in the *a* series. The results are summarized diagrammatically below and are to be compared with those given by the diagrams on pages 361 and 2383 of papers IV and V which deal with the transformations in the *a* series. In this connection it must be recalled that the formulas given in paper IV for

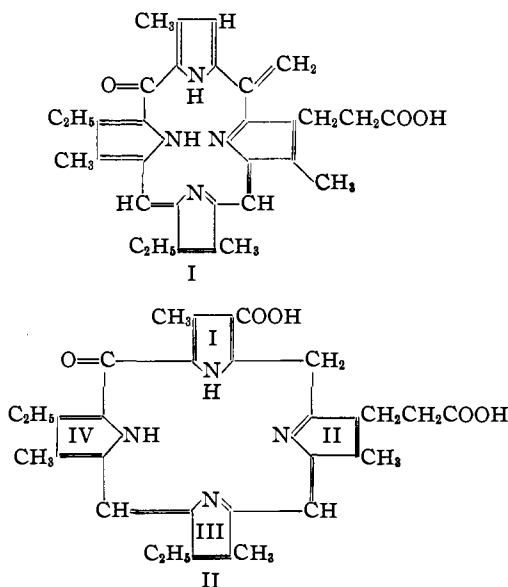
<sup>1</sup> Paper V, THIS JOURNAL, 53, 2382 (1931); see also papers VI and VII, THIS JOURNAL, 53, 3171, 3522 (1931).

the series of allomerized compounds contain two extra hydrogen atoms which we subsequently showed were removed in the phase test or in allomerization.



The simple rhodins, pyrro-rhodin *g* and rhodin *l* are the analogs of pyrrochlorin *e* and chlorin *f*. They may be regarded as "stripped" rhodins from which certain functional groups have been removed. Their analyses correspond to the formulas of the *a* series minus two hydrogen atoms plus an extra atom of oxygen. It is thus clear that the extra oxygen atom is not concerned with that portion of the molecule which is removed by our transformations, but is present in the nucleus which persists to the stage of the simple rhodins (pyrro-rhodin *g* and rhodin *l*). The fact that these compounds react with semicarbazide to form semicarbazones shows that they contain a carbonyl group. We therefore write formulas I and II for pyrro-rhodin *g* and rhodin *l*, respectively, arbitrarily placing the CO group between pyrrole rings I and IV. This group might be equally well be-

tween rings II and III or perhaps even between IV and III, although this latter would require a peculiar disposition of linkages.



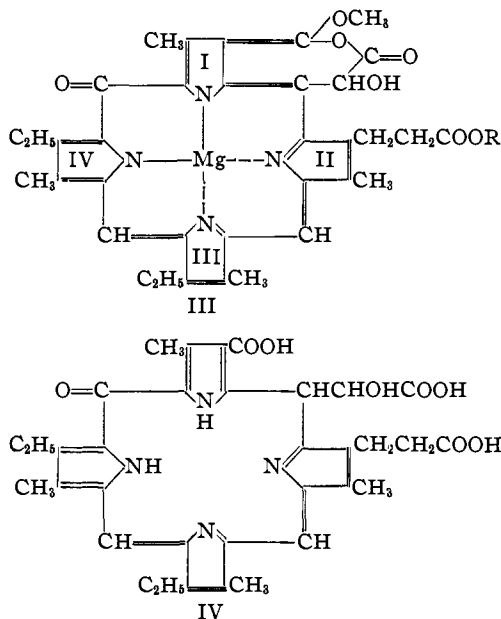
The characteristic difference between the rhodin spectrum and the typical porphyrin spectrum is easily accounted for on the basis of these formulas, along the lines<sup>2</sup> developed in paper VII. Rhodin *l*, like chlorin *f*, has the conjugated system of the porphyrin broken; its absorption bands are therefore not as sharp and narrow as those of a porphyrin.

The structure for chlorophyll *b* that follows from all these considerations is given below (III), R = phytol. The exact position of the carbonyl group is again somewhat arbitrary. The fact that rhodin *g* (formula IV) and methylphaeophorbide *b* (III, R = CH<sub>3</sub>, Mg replaced by two hydrogen atoms), form semicarbazones shows that the carbonyl group is present as such throughout the series. The positions of the phytol and methyl groups rest on the behavior of phaeophorbide *b* and methylphaeophorbide *b* on heating and cannot be considered as definitely established. The phaeophorbides of the *b* series like those of the *a* series lose carbon dioxide at 250° in diphenyl, the reaction occurring at about the same rate.<sup>3</sup> The methoxy group is here lost as in the *a* series. This we discovered by a

<sup>2</sup> THIS JOURNAL, 53, 3522 (1931). It has been called to our attention that the footnote on page 3529 of this paper might be construed as a claim to priority in regard to the structure of phylloporphyrin. No such claim was intended; Fischer and Helberger discussed both the alternative formulas in the paper describing the syntheses [*Ann.*, 480, 235 (1930)].

<sup>3</sup> Paper I, THIS JOURNAL, 51, 3669, Table I (1929).

methoxy determination of methylpyropheophorbide *b*, which was a monomethyl ester instead of the expected dimethyl ester. The pyrolysis product from free phaeophorbide *b* is alkali soluble, thus showing that the free acidic group survives the pyrolysis and is therefore the propionic acid group. Unfortunately the very poor yields and the relatively high expense of obtaining the initial methylphaeophorbide *b* has prevented us as yet from obtaining sufficient quantities of the pyropheophorbides in the *b* series for complete analysis.



The argument for the nature of the functional groups in methyl phaeophorbide *b* and rhodin *g* parallels that applied to the *a* series. Referring to the diagram given above and comparing with the similar summary of our results in the *a* series,<sup>1</sup> it is evident that rhodin *i* corresponds to the unstable chlorins of the *a* series and rhodin *k* monomethyl ester to phaeopurpurin 7. Both the unstable chlorins and rhodin *i* (isolated as a mixture of the free rhodin and its monomethyl ester) are formed by the oxidative hydrolysis brought about under "phase test conditions" in which, as we have shown, oxygen of the air is involved.<sup>4</sup> The spectrum of rhodin *k* differs markedly from that of rhodin *i*, but since there is no striking difference in the color of the solutions, we have not used the name phaeopurpurin in the *b* series. Rhodin *i* is much more stable than the unstable chlorins and is only transformed by methylation with diazomethane into the analog of phaeopurpurin 7 dimethyl ester. Like phaeopurpurin 7, the mono-

<sup>4</sup> THIS JOURNAL, 53, 1615 (1931); also paper V.

methyl ester of rhodin *k* loses carbon monoxide as well as carbon dioxide on heating, yielding a simple rhodin (monomethyl ester of rhodin *l*); this fact together with the formation of oxalic acid on vigorous saponification indicates the presence of the grouping  $\text{COCOOH}$  attached to one of the bridge carbon atoms. In all these transformations, however, the yields are very poor. The presence of the group  $\text{—CHOHCOO—}$  in methylphaeophorbide *b* (and hence in chlorophyll *b*) follows from the fact that potassium molybdicyanide readily dehydrogenates the compound and that the product on saponification yields rhodins *i* and *k* which contain the grouping  $\text{—COCOO—}$ . It should be emphasized that here, as in the *a* series, the evidence for the  $\text{—CHOHCOO—}$  group in the phaeophorbides rests on the dehydrogenation and the formation by subsequent hydrolysis of compounds containing the  $\text{COCOOH}$  group; the complete structure of these compounds (the phaeopurpurins and rhodins *i* and *k*) is not essential to the argument.

Like chlorin *e*, rhodin *g* is oxidized very rapidly by potassium molybdicyanide, carbon dioxide being evolved. This reaction shows that rhodin *g* is an  $\alpha$ -hydroxy acid (formula IV). A further discussion of the oxidation products of both chlorin *e* and rhodin *g* will be given in a later paper.

We have not studied the allomerization of chlorophyll *b* as we have not had at hand any pure material. There is every reason to believe, however, that the process is strictly analogous to the phenomenon in the *a* series. Attempts to measure the oxygen absorption during the "phase test saponification" of methylphaeophorbide *b* and of rhodin *g* ester, led to some interesting observations. Oxygen was absorbed rapidly but, unlike the experiment with methylphaeophorbide *a*, the reaction showed no signs of completion after one mole of oxygen was absorbed. This further absorption of oxygen by members of the *b* series in the presence of alkali probably represents a fairly complete decomposition of the molecule by an oxidation process and may account for the low yields which are often met with.

Two very interesting porphyrins were obtained in relatively large quantities in our pyrolysis experiments. One of these, pyrrohodin *g* porphyrin, accompanies pyrrohodin *g* and is isomeric with it, the other (porphyrin 11) is isomeric with rhodin *l* monomethyl ester and is formed in large quantities when rhodin *k* monomethyl ester is pyrolyzed. Both these porphyrins contain an extra atom of oxygen, and since the side-chain group has been removed by pyrolysis, the oxygen must be attached to one of the bridge carbon atoms. Presumably, the oxygen atom is in an hydroxyl group; pyrrohodin *g* porphyrin is to be regarded as oxyphyllporphyrin and porphyrin 11 (from rhodin *k*) as oxyrhodoporphyrin. We intend to investigate further these compounds which appear to represent a new class of porphyrins. It should be noted that according to our formulas a simple rhodin like rhodin *l* and an oxyporphyrin (OH on the bridge) are isomeric

substances; this may explain why porphyrin 11 is obtained in such large quantities in the pyrolysis, since the shift of a single hydrogen atom of the rhodin should suffice to form the porphyrin.

We are greatly indebted to Dr. C. F. Bailey for the preparation of large quantities of methylphaeophorbide *b* used in this work, and to Miss H. F. Hawes and Miss G. M. Ware for carrying out the microanalyses.

### Experimental

**Pyrolysis of Rhodin *g*.**—Pyrorhodin *g*, as well as pyrorhodin *g* porphyrin, was prepared by the pyrolysis of pure rhodin *g* in a manner exactly analogous to that applied in the *a* series for the preparation of pyrochlorin *e* and its porphyrin. Pure rhodin *g* is best prepared by the hot rapid saponification of pure methylphaeophorbide *b*. A typical pyrolysis is as follows: 200 mg. of pure rhodin *g* was thoroughly mixed with 8 g. of talc in a mortar, and this mixture added in small increments over a period of ten to fifteen minutes to 80 g. of boiling diphenyl in a 200-cc. long-necked flask through which a stream of nitrogen was passed. The boiling was continued for one-half hour after the last addition of rhodin-talc mixture. Cold water passing through a lead coil wrapped around the neck of the flask served as an effective reflux condenser for the diphenyl. After cooling somewhat, the contents of the flask were dissolved in 1.5 liters of ether and the solution filtered. The use of talc and a large amount of diphenyl diminished the large proportion of ether-insoluble material which was always formed. The red ether-diphenyl solution was fractionated with hydrochloric acid in the usual way and, as in the *a* series, two principal products were found: pyrorhodin *g* with an acid number of 12.5–13, and pyrorhodin *g* porphyrin with an acid number 3. Two other compounds with acid numbers of about 1 and 8 were also formed, but in such small quantities as to prevent further investigation. No unchanged rhodin *g* was found.

Due to the small amount of material obtained, no attempt was made to isolate pyrorhodin *g* or its porphyrin as the free acids. Instead, the dried ether solutions were methylated directly with diazomethane and these two compounds crystallized from ether as the esters. For the purpose of purification and crystallization, several runs were combined.

**Methyl Ester of Pyrорhodin *g*.**—Pyrорhodin *g* ester crystallized in beautiful dark red rosettes: 1.2 g. of pure rhodin *g* gave 135 mg. of pure pyrорhodin *g* ester, a yield of about 11%. The acid number of the ester is 13.5–14. Its color in acid and ether is the same as that of rhodin *g*. The melting point was 235° (block).

*Anal.* of methyl ester. Calcd. for  $C_{83}H_{36}O_2N_4$ : C, 73.9; H, 6.7; N, 10.1;  $OCH_3$ , 5.8. Found: C, 73.2, 72.9; H, 7.03, 7.08; N, 10.8, 10.9;  $OCH_3$ , 6.3, 6.6.

Spectrum of pyrорhodin *g* ester in ether: I, 661–637; II, 601–589; III, 568–552; IV, 538–512; E.A. 454. Order I, IV, III, II.

In 16% hydrochloric acid: I, 651–623; II, 584–562; III, 539–528; E.A. 465. Order: I, II, III. The spectrum of pyrорhodin *g* is identical with that of its ester in both ether and acid.

**Methyl Ester of Pyrорhodin *g* Porphyrin.**—This porphyrin was obtained from rhodin *g* as described above. It crystallized in beautiful fine red needles; 1.2 g. of pure rhodin *g* gave 81 mg. of pure pyrорhodin *g* porphyrin ester. Its acid number is 4. In ether it dissolves with a red color, in hydrochloric acid it is blue-green; m. p. 228–230° (block).

*Anal.* of methyl ester. Calcd. for  $C_{83}H_{36}O_2N_4$ : C, 73.9; H, 6.7; N, 10.1;  $OCH_3$ , 5.8. Found: C, 73.6, 74.0; H, 6.42, 6.65; N, 10.49, 10.56;  $OCH_3$ , 6.0, 6.0.

Spectrum of pyrrohodin *g* porphyrin ester in ether: I, 655—642; II, 603—587; III, 565—547; IV, 530—505; E.A. 449. Order: IV, III, II, I.

In 7% hydrochloric acid: I, 630—612 - - 610; II, 581—558; III, shadow at 527; E.A. 449. Order: II, I, III. The spectra of the ester and the free acid are identical.

**Semicarbazone of Pyrrohodin *g* Ester.**—A solution of 70 mg. of pure pyrrohodin *g* ester in 20 cc. of pyridine was treated with 0.3 g. of anhydrous sodium carbonate and 0.3 g. of semicarbazide hydrochloride at room temperature for forty minutes. The color changed immediately from red-violet to brown, the spectrum bands spread apart, and the acid number decreased. Acid fractionation of a small sample after ten minutes showed the absence of the original material and that the product was spectroscopically homogeneous. The main reaction mixture was filtered and added to 2 liters of ether. The solution was washed thoroughly with successive cooled portions of water, 1% hydrochloric acid, and dilute alkali. Black crystalline nodules separated on concentrating the dried ether solution; yield, 55 mg. Warming with strong hydrochloric acid regenerates the pyrrohodin *g* spectrum. The solubility in ether is less than that of the original rhodin ester. The acid number is 7.5; the color in ether is orange-brown with a red fluorescence and in acid is green with a red fluorescence.

The spectrum in ether: I, 673—639; II, - - 608—593 - - ; III, - - 560—543; IV, 531—500 - - . E.A. 460. Order: I or IV, III, II.

The spectrum in 9% hydrochloric acid: I, - - 673—621 - - ; II, 570 - - 531. E.A. 478. Order, I, II.

*Anal.* Calcd. for  $C_{34}H_{39}O_3N_7$ : C, 68.8; H, 6.6; N, 16.5;  $OCH_3$ , 5.2. Found: C, 68.39, 68.4; H, 7.3, 6.8; N, 15.2, 14.79;  $OCH_3$ , 6.53, 6.09.

**Semicarbazone of Methylphaeophorbide *b*.**—This derivative was prepared from methyl phaeophorbide *b* and isolated as described for pyrrohodin *g* ester semicarbazone. The color changed to brown-olive in a few minutes at room temperature and the acid number decreased to 12.5. The original color and spectrum returned on warming with strong hydrochloric acid. The yield was 110 mg. of black micro crystals from 200 mg. of starting material. The color in ether is olive-green with a red fluorescence, in hydrochloric acid blue-green with a slight red fluorescence.

The spectrum in ether: I, 683—648; II, 618—599; III, 565 - - 558 (hazy); IV, 550 - - 542 (hazy); V, 531—500. E.A. 463. Order: I or V, II, IV, III.

The spectrum in 16% hydrochloric acid: I, 686—633; II, - - 616—591 - - ; III, 547 - - 526 - - . E.A. 476. Order: I, III, II.

*Anal.* Calcd. for  $C_{37}H_{41}O_7N_7$ : C, 63.9; H, 5.8; N, 14.1. Found: C, 63.87, 64.42; H, 6.14, 6.25; N, 13.34.

**Semicarbazone of Rhodin *g* Trimethyl Ester.**—This substance was prepared from pure rhodin *g* ester and isolated in the manner described above for the pyrrohodin *g* derivative. The same type of marked color and spectrum change took place immediately and treatment with strong acid again regenerated the spectrum of the original pure rhodin. The yield in this case from 70 mg. was 28 mg. of black powder. The ether solution is brown-olive, with a red fluorescence; the acid solution is green.

The acid number is 6.

The spectrum in ether: I, 679—645; II, - - 614 - - 596; III, 561 - - 542; IV, 530—498. E.A. 460. Order: I or IV, II, III.

The spectrum in 9% hydrochloric acid: I, 690—637; II, 620 - - 604 - - ; III, 573 - - 556 (hazy). E.A. 477. Order: I, III, II.

*Anal.* Calcd. for  $C_{38}H_{43}O_7N_7$ : C, 64.3; H, 6.1; N, 13.8;  $OCH_3$ , 13.1. Found: C, 62.55, 62.89; H, 6.4, 6.4; N, 12.5, 12.6;  $OCH_3$ , 12.4.

**Action of Potassium Molybdicyanide on Rhodin *g*.**—In a preliminary experiment,

rhodin *g* was treated with molybdcyanide in a pyridine-acetone solution under the conditions described for the preparation of chlorin *k* (Paper V, p. 2392). As in the case of chlorin *e*, carbon dioxide was evolved and the barium carbonate collected corresponded to 84% of one mole of gas. The main product was a rhodin (rhodin *n*) which formed a monomethyl ester of acid number 18 on treatment with diazomethane. The color in ether is brown-red, in hydrochloric acid, green, with a red fluorescence in both solvents.

The spectrum in ether: I, 669—634; II, - - -606 - - -587 - - -; III, 564—548; IV, 538—502. E.A. 457. Order: I, IV, II, III.

The spectrum in 20% hydrochloric acid: I, 665—622; II, 594 - - -575; III, - - -542 - - -525 - - -. E.A. 473. Order: I, II, III.

*Anal.* Calcd. for  $C_{34}H_{36}O_5N_4$ :  $OCH_3$ , 5.3. Found:  $OCH_3$ , 5.2, 5.48.

Rhodin *n* and its ester are being prepared in quantity and will be reported more fully in a later paper.

**Dehydrogenation of Methylphaeophorbide *b* with Molybdcyanide.**—A preliminary experiment was carried out under conditions previously used in the *a* series (paper V, p. 2389). A solution of 0.33 g. of potassium molybdcyanide in 20 cc. of water and 2 cc. of glacial acetic acid was oxidized with 0.1 *M* potassium permanganate solution. To this was added 100 cc. of acetone and 14 cc. of pyridine, and the mixture filtered. A sample of 100 mg. of methylphaeophorbide *b* was then added and the whole allowed to stand for fifteen hours. In a parallel experiment, 100 mg. of methylphaeophorbide was dissolved in the same solvent mixture *without* the oxidizing agent and allowed to stand for the same length of time. Both products were transferred to ether and washed with 0.1 *N* alkali. The two ether solutions containing the alkali-insoluble material were evaporated to dryness and analyzed for relative oxidation by saponification with boiling 25% methyl alcoholic potassium hydroxide. Methylation with diazomethane followed by acid fractionation showed the proportion of oxidized products, rhodin *k* and rhodin *l* esters, to be approximately 80% in the oxidation experiment and 30% in the control. The product of lower acid number was rhodin *g* ester formed from unchanged methylphaeophorbide *b*.

**Phase Test Dehydrogenation of Methylphaeophorbide *b*.**—In a manner analogous to that observed in the *a* series (Paper III) cold alkali treatment of methylphaeophorbide *b* in the presence of oxygen does not convert it to the normal saponification product, rhodin *g*, but effects an oxidation as well as a saponification to an unstable rhodin *i*. This, on methylation with diazomethane, changes to the trimethyl ester of rhodin *k*, a stable compound. This nomenclature is borrowed from Willstätter,<sup>6</sup> who reports two stable rhodins, *i* and *k*, formed through the action of cold alkali on methylphaeophorbide *b*, or from allomerized chlorophyll *b*.

The preparation of rhodin *k* ester from methylphaeophorbide *b* is as follows: 500 mg. of pure methylphaeophorbide *b* was dissolved in 10 cc. of pyridine and diluted with one liter of ether. To this solution was added 100 cc. of 25% potassium hydroxide in *n*-propyl alcohol containing 5 cc. of 30% hydrogen peroxide, and the mixture was shaken for ten minutes. Dilution with water changed the brilliant green color to red-brown and the completely alkali-soluble product was transferred by acidification to 3 liters of ether. On treatment with diazomethane, the bright red ether solution became brown-red while the spectrum changed from that of the unstable rhodin *i* to that of the trimethyl ester of rhodin *k*. After washing with 12% acid, the solution was extracted exhaustively with 15–17% acid. Careful refractionation and a spectroscopic comparison

<sup>6</sup> Willstätter and Stoll, "Investigations on Chlorophyll," translated by Schertz and Merz, Science Printing Co., 1928, p. 278.



of first and last fractions failed to show the presence of a second rhodin as reported by Willstätter. The solution was washed with 0.1 *N* potassium hydroxide, dried and concentrated. The yield of shiry black platelets was 150 mg.

In another preparation, fractionation was carried out without methylation and the main product, rhodin *i*, was contained in a 14–16% extract. Refractionation showed that only one rhodin (with perhaps its monomethyl ester) was present. Its spectrum in ether: I, 677—643; II, 608-- -595; III, -- -564—548; IV, 534—498; E.A., 460. Order: I, IV, III, II.

Spectrum in 17% hydrochloric acid: I, -- -699—629- - -; II, 583 (weak center); III, 547-- -525 (very weak); E.A. 476. Order: I, III, II. The spectrum did not change while the ether solution stood for three days at room temperature. On treatment with an ethereal solution of diazomethane, the two central bands grew less intense and the wide red band broadened, forming the spectrum of rhodin *k* ester.

*Anal.* Found: C, 65.40, 65.67; H, 5.47, 5.69; OCH<sub>3</sub>, 1.43, 1.35.

**Trimethyl Ester of Rhodin *k*.**—This was recrystallized for analysis from a mixture of acetone and methyl alcohol. The acid number is 16 and the color in acid is green without fluorescence. The ether solution is rose-brown in color. The solubility in ether approximates that of rhodin *g* ester. It is very soluble in acetone, less soluble in methyl alcohol. Comparative hydrolysis with alkali showed it to be rendered alkali-soluble much more readily than rhodin *g* or rhodin *l* esters and thus analogous to dimethylphaeopurpurin 7 in the *a* series. The substance softened at 245° and melted on the block at 250–252°. The spectrum in ether of a 50-mm. layer of solution (1 mg. in 30 cc. of ether): I, 683—642; II, 614- - -599 (very faint); III, 566- - -552; IV, -- -533.5—509- - -; E.A., 458. Order: I, IV, III, II.

The spectrum in 20% acid: I, 697—624- - -580; II, 545- - -526 (very faint); E.A., 479. Order: I, II.

*Anal.*<sup>6</sup> Calcd. for C<sub>37</sub>H<sub>40</sub>O<sub>8</sub>N<sub>4</sub>: C, 66.5; H, 6.0; N, 8.36; OCH<sub>3</sub>, 13.9. Found: C, 66.50, 66.80; H, 6.15, 6.30; N, 8.57, 8.57; OCH<sub>3</sub>, 14.1, 14.5.

**Monomethyl Ester of Rhodin *k*.**—This substance was prepared from the trimethyl ester by shaking an ether solution for five minutes with one-tenth its volume of 25% potassium hydroxide in methyl alcohol. Remethylation with diazomethane produced the trimethyl ester in the normal way. The acid number is 14 and the colors and spectra in acid and ether are the same as those of the trimethyl ester. The melting point was 206° (block).

*Anal.* Calcd. for C<sub>35</sub>H<sub>38</sub>O<sub>8</sub>N<sub>4</sub>: C, 65.6; H, 5.6; N, 8.72; OCH<sub>3</sub>, 4.83. Found: C, 62.42, 62.32; H, 5.83, 5.68; N, 8.49, 8.86; OCH<sub>3</sub>, 6.0, 5.6; ash, 0.2, 0.06.

**Pyrolysis of Monomethyl Rhodin *k*.**—As in the case of phaeopurpurin 7 in the *a* series, monomethyl rhodin *k* loses carbon monoxide and one mole of carbon dioxide very readily on heating. A sample of 0.1351 g. pyrolyzed in diphenyl at 240–260° for thirty minutes and swept with pure nitrogen lost 88% of a mol of carbon dioxide. Further heating for one hour failed to change appreciably the weight of gas collected in the potash absorption bulb. A second sample of 0.1273 g. lost 96% of a mol of carbon dioxide in one hour of heating. The evolution of carbon monoxide was demonstrated in both cases by passing the gas through dilute hemoglobin solution and noting the characteristic bands of CO-hemoglobin.

For preparative purposes the method was varied somewhat in an attempt to avoid too drastic decomposition. To a solution of 100 g. of boiling diphenyl saturated with

<sup>6</sup> All samples were dried *in vacuo* at 66° and usually required from twenty-four to thirty-six hours to reach constant weight.

nitrogen was slowly added 1 g. of rhodin *k* which had been previously thoroughly ground up with 5 g. of talc. The solution was boiled for ten minutes, cooled somewhat, poured into 2 liters of ether and filtered. The colored material was taken into 0.1 *N* potassium hydroxide, washed twice with ether to remove diphenyl and transferred to 8 liters of fresh ether. A large proportion of scum was formed. The 12–14% extract contained a porphyrin and after washing the solution with 16% acid, the rhodin was the only remaining product and was extracted readily with 18% acid. The yield from 1 g. was 90 mg. of porphyrin and 55 mg. of the monomethyl ester of rhodin *l*.

**Monomethyl Ester of Rhodin *l*.**—From ether solution this ester formed shiny black crystalline nodules. It is somewhat more soluble in ether than its isomer, the porphyrin; its acid number is 16.5. The color in ether is violet-red, in hydrochloric acid it is green-blue and in both solvents there is a red fluorescence. It melted at 187° (block). The spectrum in ether: I, 667–635; II, 602–590; III, 564–547; IV, 534–502 (hazy edge); E.A., 455. Order: I, IV, III, II.

The spectrum in 20% hydrochloric acid: I, 670- -665–615- -607; II, 592- -557 (very faint); III, 542- -526 (very faint); E.A., 469. Order: I, (II or III).

*Anal.* Calcd. for  $C_{33}H_{36}O_5N_4$ : C, 69.7; H, 6.4; N, 9.80;  $OCH_3$ , 5.4. Found: C, 69.04, 68.13; H, 6.22, 6.18; N, 10.30, 10.02;  $OCH_3$ , 5.82, 5.77.

**A Porphyrin from the Pyrolysis of Monomethyl Rhodin *k* (Porphyrin 11 Monomethyl Ester).**—This substance crystallized from ether in the form of a dark red microcrystalline powder. The acid number is 11.5, the hydrochloric acid solution is blue-green and the ether solution is red. Both have a red fluorescence. It is rather insoluble in ether, slightly soluble in methyl alcohol and quite soluble in chloroform.

The spectrum in ether: I, 667- -656 (very faint); II, 649–639; III, 596–583; IV, 556–546; V, 526–502; E.A., 454. Order: V, IV, II, III, I. The spectrum in 16% hydrochloric acid: I, 624–604; II, 593- -582–549; III, weak center at 524. E.A. 463. Order: I, II, III.

*Anal.* Calcd. for  $C_{33}H_{36}O_5N_4$ : C, 69.7; H, 6.4; N, 9.80;  $OCH_3$ , 5.4. Found: C, 69.65, 69.08; H, 6.23, 6.13; N, 9.48, 9.23;  $OCH_3$ , 5.80, 5.53.

**Dimethyl Ester of Porphyrin 11.**—This substance was prepared by the action of diazomethane on an ethereal solution of the monomethyl ester. It crystallized from ether in the form of a dark red crystalline powder. The acid number is 13. The colors and spectra of the acid and ether solutions are the same as those of the monomethyl ester. The substance melted at 185–187° (block).

*Anal.* Calcd. for  $C_{34}H_{38}O_5N_4$ : C, 70.1; H, 6.5; N, 9.56;  $OCH_3$ , 10.6. Found: C, 69.54, 69.70; H, 5.79, 6.26; N, 9.83, 9.63;  $OCH_3$ , 10.61, 9.18.

**Hot Saponification of Monomethyl Rhodin *k*.**—A boiling solution of 250 cc. of 25% methyl alcoholic potassium hydroxide was saturated with nitrogen and a mixture of 1.05 g. of rhodin *k* trimethyl ester and 10 g. of talc was slowly added. The whole was boiled vigorously under reflux for ten minutes. The intense green mixture was diluted, filtered, and the completely alkali-soluble product transferred by acidification to 4 liters of ether. There was considerable scum. The aqueous layer was concentrated *in vacuo*, and the oxalic acid from the reaction precipitated as usual as the calcium salt, redissolved in dilute sulfuric acid and titrated with standard potassium permanganate solution. A 15% yield of oxalic acid was thus indicated. The dark red ether solution was carefully fractionated with hydrochloric acid. The main product was contained in 16–18% extracts and was identical in spectrum with monomethyl rhodin *l*, previously described and prepared by the pyrolysis of rhodin *k*, and with rhodin *l* prepared directly from methylphaeophorbide *b* as described below. There are probably at least two rhodins formed in the reaction and contained in the 8–10 and 13–14% fractions but these were

not present in sufficient quantities to be investigated further. The 16–18% fraction was refractionated, methylated and concentrated. The yield of small black crystals of rhodin *l* dimethyl ester was 100 mg.

**Rhodin *l*.**—This substance was recrystallized for analysis from acetone–methyl alcohol. The color in ether is violet-red with a red fluorescence, in hydrochloric acid it is green-blue with a red fluorescence. The acid number is 15. The spectrum in ether: I, 666–636; II, 602–591; III, 564–547; IV, 534–501 - - -. E.A., 453. Order: I, IV, III, II.

The spectrum in 20% hydrochloric acid: I, - - 660–607 - - ; II, 592–573 (very faint); III, 539–522 (very faint). E.A., 469. Order: I, II, III.

*Anal.* Calcd. for  $C_{32}H_{34}O_6N_4$ : C, 69.3; H, 6.1; N, 10.1;  $OCH_3$ , 0. Found (prepared directly from methylphaeophorbide *b* as described below): C, 69.04, 69.47; H, 6.10, 6.20; N, 9.57;  $OCH_3$ , 0.

**Rhodin *l* Dimethyl Ester.**—A sample of this substance prepared by saponification of methylphaeophorbide *b* was recrystallized from acetone and analyzed to prove its identity with the ester of the pyrolysis product of rhodin *k*. Considerable difficulty was experienced in obtaining good analyses on samples crystallized from ether. This may have been due to traces of contaminating rhodin, but was more likely due to solvent of crystallization, since values improved in some cases on long drying *in vacuo* and in others on recrystallization from acetone.

The colors and spectra in ether and hydrochloric acid are the same as those of the unmethylated compound. The acid number is 17.

*Anal.* Calcd. for  $C_{34}H_{38}O_6N_4$ : C, 70.1; H, 6.5; N, 9.6;  $OCH_3$ , 10.6. Found (prepared directly from methylphaeophorbide *b*): C, 69.48, 68.50; H, 6.40, 6.79; N, 8.74, 8.63;  $OCH_3$ , 10.38, 9.16. (Prepared from monomethyl rhodin *k*): C, 67.90, 67.93; H, 6.31, 6.08.

**Semicarbazone of Rhodin *l* Dimethyl Ester.**—A solution of 50 mg. of pure rhodin *l* ester in 15 cc. of pyridine was heated with 0.2 g. of anhydrous sodium carbonate and 0.2 g. of semicarbazide hydrochloride at 100° for 20 minutes. The mixture was filtered and poured into 2 liters of ether. The olive-green solution was washed with ice water and with 4% hydrochloric acid, then extracted three times with iced 13% acid and rapidly transferred to ether. After washing with 0.05 *N* alkali and concentrating the ether solution, 18 mg. of a black crystalline powder separated out. The acid number is 10.5, and the color in acid is bright green with a slight red fluorescence. The ether solution shows a strong, red fluorescence. Warming with strong hydrochloric acid changes the color and spectrum to those of the original rhodin. The spectrum in ether: I, 694–644; II, 615 - - 598 - - ; III, 562 - - 541 (very faint); IV, 529–496. E.A. 465. Order: I or IV, II, III.

The spectrum in 14% hydrochloric acid: I (edge unsharp), - - 676–624 - - ; II, 606 - - 582 (very faint); III, 544–526. E.A. 574. Order I, III, II.

*Anal.* Calcd. for  $C_{38}H_{41}O_6N_7$ : C, 65.2; H, 6.35; N, 15.15. Found: C, 63.01; H, 6.54; N, 13.71, 13.67.

**Hot Oxidative Saponification of Methylphaeophorbide *b*.**—For preparative purposes rhodin *l* is best obtained by cold, then hot, alkali treatment of methylphaeophorbide *b* without isolating an intermediate. To 4 g. of pure methyl phaephorbide *b* dissolved in 40 cc. of pyridine was added 500 cc. of 25% methyl alcoholic potassium hydroxide which had been stirred with an oxygen stream for an hour and to which had just been added 3 cc. of superoxol. A few more cc. of superoxol was added, the mixture was cooled slightly to keep it at room temperature, and the oxygen stream continued for a further half hour. Nitrogen was then passed in for thirty minutes and finally the

mixture was quickly heated to boiling and boiled for eight minutes. The brown phase did not disappear until the solution was heated. After rapid cooling and dilution with 2 liters of water, the product was transferred to 10 liters of ether. There was considerable scum formation. The aqueous layer was concentrated and the calcium oxalate, determined as usual by permanganate titration, was 150 and 250 mg. (17 and 28 per cent. yield) in two preparations. The red-brown ether solution was carefully fractionated with hydrochloric acid and each extract was transferred to a fresh portion of ether and examined spectroscopically. The main product was contained in the 16-18% extract and was spectroscopically identical with monomethyl rhodin *l* obtained by pyrolysis of rhodin *k*, and with the main product of hot saponification of rhodin *k*. There appear to be at least two rhodins formed in much smaller quantity and contained in the 9-11 and 14% fractions, respectively.

The 16-18% extracts containing rhodin *l* were carefully refractionated and esterified with diazomethane. The yield of small black crystals was 200 mg. The 9-11% fraction was also refractionated and methylated with diazomethane without change in spectrum. On concentration of the ether solution 55 mg. of small shiny black crystals separated.

**The Rhodin from the Saponification of Methylphaeophorbide *b*.**—The rhodin of acid number 11 and its methyl ester of acid number 13.5 are rose-brown in ether and green in acid and show a red fluorescence in both solvents. The spectrum in ether: I, 668—638; II, 605--581--; III, --560—545; IV, 533--529—504--. E.A. 452. Order: I, IV, III, II.

The spectrum in 16% hydrochloric acid: I, --654--635--; II, 621—606; III, 577—555; IV, weak center at 526. E.A. 464. Order: III, II, I, IV.

**Wolf-Kishner Reduction of Rhodin *l* and its isomeric Porphyrin.**—Fifteen-milligram samples each of rhodin *l* monomethyl ester, and of its isomeric porphyrin were heated in a sealed tube with 2 cc. of a mixture prepared by dissolving 1 g. of sodium in 13 cc. of absolute ethyl alcohol and adding 1 cc. of anhydrous hydrazine hydrate. After heating for five hours at 140°, the products were diluted and transferred to ether. In each case the dark red ether solution contained a small amount of pyrroporphyrin, identified spectroscopically, and about three times as much of a porphyrin of acid number about 4. This porphyrin had the spectrum of rhodoporphyrin with an extra band in the red which was not removed by refractionation. It seems probable that impure rhodoporphyrin was at hand but this we do not regard as certain.

**Methylpyrophaeophorbide *b*.**—This substance was prepared from methylphaeophorbide *b*. The best results seemed to be with samples of about 0.3 g. heated in 40 g. of diphenyl at about 240° for two and one-half hours. The insoluble residue under these conditions amounted to about 17% of the weight of the starting material, and the yield of crude methylpyrophaeophorbide *b*, 20% or a little better. Considerable scum was also formed during subsequent extractions.

The pyrolysis mixture was dissolved in about 900 cc. of ether, filtered, and then fractionated with 14, 18 and 23% hydrochloric acids; 25% acid completely decolorized the mother liquor. The 14% fraction was green in acid and on transferring to ether gave a red solution. The amount of this material was small and it appeared to be a mixture. No attempt was made to isolate it. The 18% extraction removed all of the methylpyrophaeophorbide *a* which came from the methylphaeophorbide *a* present as an impurity. The 23% fraction contained most of the desired pyro compound.

The methylphaeophorbides and methylpyrophaeophorbides hydrolyze with great ease and, in spite of rapid manipulation, it is impossible to work with 23% acid without hydrolyzing a considerable portion of the methyl compounds. This hydrolyzed material may be removed by extraction with 0.01 molar potassium hydroxide, or the whole

ether solution of retransferred product may be treated with diazomethane, thus re-methylating the hydrolyzed product. The alkali-soluble material, on retransferring to ether by acidification, may also be methylated with diazomethane. In order to obtain pure material, a very careful refractionation with acid was undertaken. The loss of material was great, 200 milligrams of product yielding only 11 and 14 milligrams of crystalline material which were the end fractions with 18 and 19% acid. Only enough substance for methoxyl determinations was available. The methylpyropheophorbide *b* crystallizes in black microcrystals from ether. Its acid number is 21-22. The color of the solution and its spectrum are identical with those of methylphaeophorbide *b*.

*Anal.* Calcd. for  $C_{34}H_{36}O_4N_4$ : N, 9.9;  $OCH_3$ , 5.5. Found: N (on crude material), 9.53, 9.37, 9.48;  $OCH_3$  (19% fraction), 5.66; (18% fraction), 4.22.

**Oxygen Absorption during Phase Test.**—The oxygen absorption during the phase test was measured in the Warburg apparatus at a temperature of 30°. <sup>7</sup> The solid ester was placed in the side arm of the conical vessel out of contact with the 25% methyl alcoholic potassium hydroxide. After mixing, the oxygen absorptions of methylphaeophorbide *b* and of rhodin *g* esters in this medium were as follows, expressed as millimoles of gas absorbed at the given time: *0.01 millimole of rhodin g ester*, 20 min., 0.0039, 30 min., 0.0057, 60 min., 0.0104, 100 min., 0.0143; *0.01 millimole of methylphaeophorbide b*, 10 min., 0.0032, 25 min., 0.0058, 40 min., 0.0086, 55 min., 0.0104, 70 min., 0.0119, 100 min., 0.0133. The latter reaction proceeds at a progressively diminishing rate as shown by the following values in  $10^{-5}$  millimoles per minute for the rate of gas absorption during each interval: 3.2, 2.3, 2.1, 1.8, 1.7, 1.3.

### Summary

The methods developed in this Laboratory for the elucidation of the structure of chlorophyll *a* have been applied to a study of chlorophyll *b*. The transformations in the *b* series closely parallel those in the *a* series and two simple rhodins have been obtained from which certain functional groups have been removed. The formation of semicarbazones shows that a carbonyl group is present in the modified porphyrin ring in these simple rhodins and also throughout the *b* series. Our explanation of allomerization and the "phase test" reaction applies to the *b* as well as the *a* series. A variety of evidence thus leads to the conclusion that chlorophyll *b* is an oxo derivative of chlorophyll *a* in which a carbonyl group connects two of the pyrrole rings in a modified porphyrin nucleus.

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<sup>7</sup> Paper VI, THIS JOURNAL, 53, 3171 (1931).