

Anal. Subs., 0.1015: cc. of 0.1 *N* AgNO₃, 3.68. Calcd. for C₁₄H₁₃OBr: Br, 23.84. Found: Br, 29.03.

Summary

Para-cresol was benzylated by both the Claisen and the aluminum chloride methods and the yields were compared.

Bromination of 2-benzyl-4-methylphenol gave 6-bromo-2-benzyl-4-methylphenol, which was also prepared from 6-bromo-4-methylphenol by the Claisen reaction.

EAST LANSING, MICHIGAN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

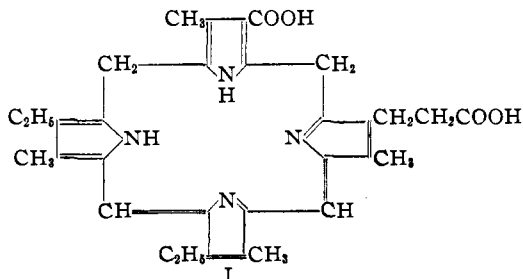
STUDIES IN THE CHLOROPHYLL SERIES. V. THE STRUCTURE OF CHLOROPHYLL A

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The experiments reported in the fourth paper of this series¹ indicated that chlorophyll *a* and the closely related compound phaeophorbide *a* contained the grouping $\text{—CHOH—C}\begin{smallmatrix} \text{O} \\ // \\ \text{O} \end{smallmatrix}$. In allomerization or "phase test saponification," we supposed that this structure was transformed into the α -ketonic acid grouping which was subsequently easily removed as potassium oxalate by boiling the compounds with alkali. The resulting simple chlorin, chlorin *f*, was thus to be regarded as a partially hydrogenated rhodoporphyrin. It was uncertain whether the two hydrogen atoms which were removed from the secondary alcohol group were transferred to some other molecule or intra-molecularly to a pyrrole ring. A further study² has shown that the first alternative is correct and that the dehydrogenating agent is oxygen of the air. The structure of chlorin *f* is therefore that of a dihydrorhodoporphyrin (I); this formula replaces

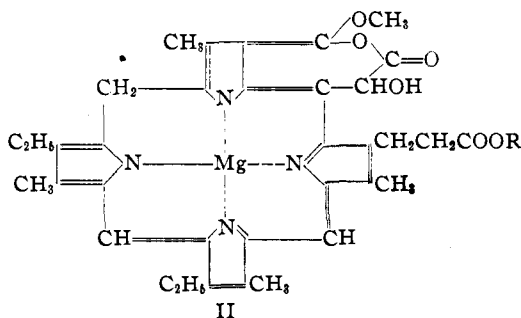


¹ THIS JOURNAL, 53, 359 (1931). For other papers of this series see *ibid.*, 51, 3668 (1929); 52, 1233, 3013 (1930).

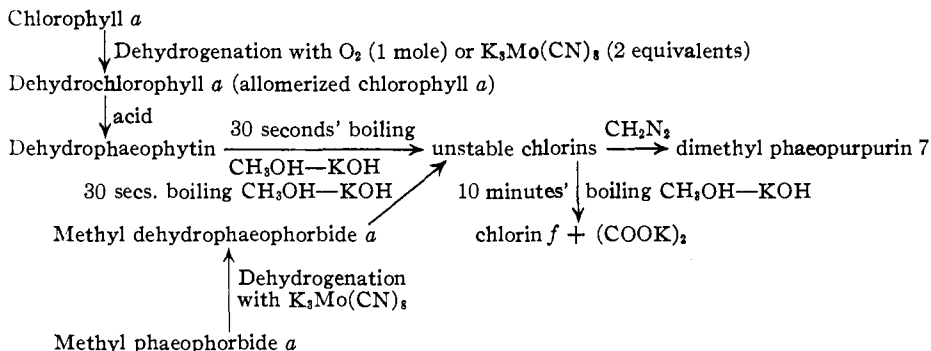
² A brief statement of these results was given in a letter to the editor, THIS JOURNAL, 53, 1615 (1931). The corresponding experiments are recorded in this present paper except for the study of the phase test, which will form a separate paper by Miss C. C. Steele.

the one given in our previous paper. The position of the double linkages is arbitrary and many tautomeric modifications of Formula I may be written. The quantitative dehydrogenation of this simple chlorin is now being studied in order to throw more light on such problems.

We are now in a position to write a structural formula for chlorophyll *a* with some assurance. Such a formula is given below (II) R = phytyl, and the recent evidence is summarized in the accompanying diagram.



The corresponding formulas for methyl phaeophorbide *a* and phaeophorbide *a* are obtained by letting R = CH₃ and H, respectively, and substituting two hydrogen atoms for the magnesium atom.³



We may briefly review the arguments for Formula II for chlorophyll *a* and related compounds. The skeleton structure of chlorin *f* follows from its easy conversion to rhodoporphyrin (paper IV) whose structure has been established by Fischer by synthesis. From the above diagram it is clear that the nucleus of dehydrochlorophyll *a* is chlorin *f* plus the oxalic acid grouping; this agrees in the number of carbon atoms with the analyses of methyl phaeophorbide, etc. (paper III, p. 3016). Two mole-

³ The distinction indicated by the heavy and dotted lines from the magnesium is only useful in showing that two hydrogen atoms are replaced by the metal; there is no reason, of course, to believe that there is a real difference between any of the four coordinate links.

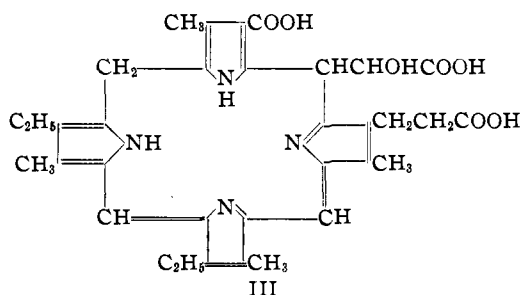
cules of potassium molybdcyanide (a powerful oxidizing agent) are involved in the dehydrogenation of chlorophyll *a* (or methyl phaeophorbide *a*). This fact, together with the formation of potassium oxalate on alkaline decomposition of the dehydro compounds, shows the presence of an esterified or otherwise masked α -hydroxy acid group in the original molecule. The methoxyl group of chlorophyll *a* is attached to the β -position of a pyrrole ring because the transformations described in the fourth paper yielded a monomethyl ester of chlorin *f* which, unlike chlorin *f* itself (a dibasic acid), does not readily lose carbon dioxide on heating. The well-known relationship of chlorophyll *a* to phaeophorbide *a* shows that the phytol group is attached to the acid group which is free in the latter compound. This is the propionic acid residue since on pyrolysis of phaeophorbide *a* this acid group is not eliminated as carbon dioxide (paper I).

The position of the $-\text{CHOH}-\text{C}\begin{smallmatrix} \text{O} \\ // \\ \text{OH} \end{smallmatrix}$ group and the masking of its acidic hydrogen remain to be considered. The latter necessity arises from the fact that phaeophytin *a* and methyl phaeophorbide *a* are only di-esters although they contain three potential carboxyl groups. The analyses of methyl phaeophorbide *a* indicate six oxygen atoms; the masking of one carboxyl group as a lactam (but not as a lactone) or as an anhydride (as shown in Formula II) is consistent with these analyses. The second alternative seems preferable to us because on pyrolysis phaeophorbide *a* loses carbon dioxide and methyl alcohol, the methoxyl group being destroyed (see experimental portion of this paper). This points to a close interconnection of the masked carboxyl group and the carboxyl group in the β -position of the pyrrole ring, the propionic acid group being free. We therefore write Formula II, placing the CH_2 link arbitrarily, there being at present no direct evidence in regard to this portion of the molecule.⁴

The analyses of chlorin *e* correspond to Formula III, as do its transformations into pyrochlorin *e* described in the last paper. It is rapidly oxidized by potassium molybdcyanide (two equivalents are involved)

⁴ Since this paper was sent to the editor, an article by Fischer, Moldenhauer and Süs [*Ann.*, 486, 107 (1931)] has appeared on the same subject. These authors write a structural formula for chlorophyll *a* (largely based on their investigation of phylloerythrin) which is very different from the one proposed in the present paper. The chief differences are as follows: they consider the chlorophyll nucleus to be an oxidized porphyrin ring instead of a partially reduced porphyrin as we have postulated; they write a five-membered carbon ring joining one pyrrole ring to the bridge, a tertiary hydroxyl (instead of a secondary in our formula) and the phytol and methyl groups in different positions. Without going into a long discussion of this new paper, we can only say that we do not believe that the formula for chlorophyll *a* there proposed is capable of explaining the dehydrogenation reactions and certain other transformations described in this and our previous papers.

and like all α -hydroxy acids loses carbon dioxide during the oxidation.⁵ The resulting chlorin (designated hereafter as chlorin *k*) gives a monomethyl ester on treatment with diazomethane. Presumably the aldehydic group formed by the oxidation has closed a lactone ring with a carboxyl group. The further transformations of chlorin *k* will be reported in detail in a later paper. The trimethyl ester of chlorin *e* (formed by the action of diazomethane) unlike the parent compound is not oxidized by potassium molybdicyanide. This fact together with the analyses, which show a loss of water during the esterification, indicate a lactone formation between a carboxyl and the hydroxyl of the $-\text{CHOHCOOCH}_3$ group; enolization would account for the ability to form a trimethoxyl derivative.



The unstable chlorins appear to be a tribasic acid and its monomethyl ester corresponding to the removal of the two hydrogen atoms of the CHOH group of chlorin *e*. (They are chlorin *f* + $-\text{C} \begin{smallmatrix} \text{O} \\ // \\ \text{COOH} \end{smallmatrix}$.) The nature of their transformation into the phaeopurpurins has not yet been established with certainty and a discussion of the structure of the latter will be postponed. Although they are important as a method of characterizing the dehydro series, the details of their structure are not involved in the evidence which leads to Formula II for chlorophyll *a*. As would be expected, the phaeopurpurins and methyl dehydrophaeophorbide are not oxidized by potassium molybdicyanide. It may be mentioned that methyl phaeophorbide *b* is more rapidly oxidized than the corresponding *a* compound. A study of the *b* series from the point of view presented in this paper is now in progress.

Methyl dehydrophaeophorbide *a* is a crystalline compound which can be purified and studied in the usual way; it differs only slightly in its visible absorption spectrum from the unoxidized compound. However, in the near ultraviolet there is considerable difference (Fig. 1). In studying chlorophyll *a* itself we have been forced to use spectroscopic methods and chemical transformations since the amount of material at

⁵ It may be noted that phaeophorbide *a* (a free acid) on oxidation does not lose carbon dioxide but behaves exactly like its methyl ester. This is additional proof that the phytol group is not on the bridge carboxyl.

our disposal was small and the compounds containing the phytyl group crystallize with difficulty. The absorption spectra in the near ultraviolet of the compounds are given in Fig. 2. It will be seen that the dehydro-

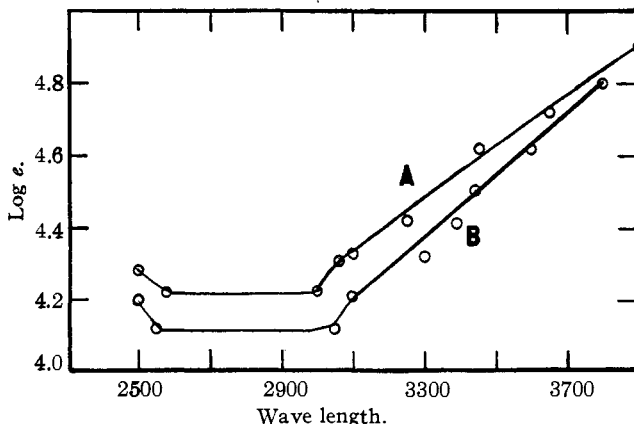


Fig. 1.—Log extinction coefficient-wave length curves for: A, methyl phaeophorbide *a*; B, methyl dehydrophaeophorbide *a*.

chlorophylls prepared with oxygen (allomerization) or with molybdcyanide are identical (curve B) and differ from chlorophyll *a* (curve A) as methyl dehydrophaeophorbide differs from methyl phaeophorbide. However, there is a difference in the absorption spectrum of dehydro-

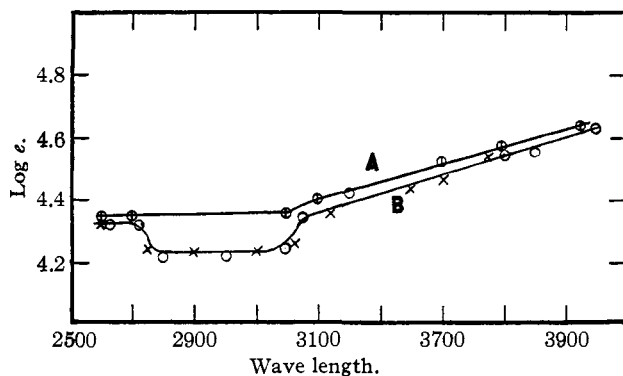


Fig. 2.—Log extinction coefficient-wave length curves for: A, chlorophyll *a*; B, $\times\times\times$, allomerized chlorophyll *a*; $\circ\circ\circ$, dehydrochlorophyll *a*.

chlorophyll *a* in the visible according to whether oxygen or molybdcyanide is used in the preparation. This difference amounts to some 40 Å. in the position of the band at 662 $m\mu$ and the same amount of shift at 530 $m\mu$ is found in the corresponding dehydrophaeophytins. The com-

pound prepared with oxygen is the one which resembles dehydrophaeophorbide in regard to the position of this one band which represents a shift from phaeophorbide *a* itself. Since the chemical transformations of the two dehydrochlorophylls are the same, and they are both formed by the removal of two hydrogen atoms, any difference between them must be slight. We cannot be certain at present whether we are dealing with a subtle isomeric shift or the presence of an impurity in the material made with molybdicyanide.

The possible significance of the easily oxidized group $-\text{CHOHC}=\overset{\text{O}}{\text{C}}$ in chlorophyll from the point of view of photosynthesis has been discussed in a brief note⁶ and will be considered in more detail in a later paper of this series.

We are indebted to Miss Helen F. Hawes for carrying out the micro-analyses reported in this paper.

Experimental

Quantitative Dehydrogenation Experiments with Potassium Molybdicyanide.—The molybdicyanide reagent was freshly prepared before each set of experiments as follows:⁷ 5 cc. of 0.06 *M* $\text{K}_4\text{Mo}(\text{CN})_8$ and 0.7 cc. of glacial acetic acid were mixed and approximately 0.1 *N* potassium permanganate added drop by drop until present in very slight excess; the mixture was then made up to a total volume of 10 cc. In each experiment approximately 10^{-6} mole of compound (accurately weighed) was dissolved in 5 cc. of acetone containing enough anhydrous pyridine to be molar with respect to this base. One cc. of the molybdicyanide reagent was then added, the time noted and the mixture made up to 10 cc. with more molar pyridine in acetone. Definite samples (1 cc. or 0.7 cc.) were withdrawn from the reaction mixture from time to time, diluted with 5-fold aqueous acetate buffer solution and the molybdicyanide content determined by electrometric titration with 0.001 *M* $\text{K}_4\text{Fe}(\text{CN})_6$ using a micro-buret and a very small titration vessel. The end-point is very sharp even at these high dilutions and the accuracy is determined by the error in sampling (about $\pm 5\%$). With each set of experiments a "control experiment" was carried out, by introducing 1 cc. of the same reagent into the pyridine-acetone solution, making the volume exactly 10 cc. and withdrawing a sample at the same time as the samples were withdrawn from the various reaction mixtures. There was very little change in the control titration even after twenty-four hours but it was deemed best to correct for this change by taking the extent of reduction of the molybdicyanide as being the difference between the "control titration" and the titration of the reaction mixture. A typical experiment was as follows: 4.97 mg. of methyl phaeophorbide *a* plus 1 cc. of molybdicyanide reagent (prepared as described above, approximately 0.03 *M*) in molar pyridine in acetone with a total volume of 10 cc.; the control was exactly like the experiment but the methyl phaeophorbide *a* was omitted. The titrations of 0.7-cc. samples with 0.001 *M* $\text{K}_4\text{Fe}(\text{CN})_6$ were as follows: 20 min. control 1.65 cc., reaction 1.42, diff. 0.23; 90 min. control 1.65 cc., reaction 1.30, diff. 0.35; 390 min. control 1.65 cc., reaction 0.75, diff. 0.90; 1400 min. control 1.49 cc., reaction 0.50, diff. 1.04; since the 4.97 mg. of methyl phaeophorbide *a* is 8×10^{-6}

⁶ Conant, Dietz and Kamerling, *Science*, **73**, 1888 (1931).

⁷ For the use of molybdicyanide in organic reactions, see Conant and Pratt, *This Journal*, **48**, 3229 (1926).

mole, two equivalents of oxidizing agent is 16×10^{-6} mole for the whole sample or 1.12 cc. for a 0.7-cc. sample. The extent of the reaction is thus 93% based on two equivalents after 1400 min., with an increase of only 13% in the last 1000 min.; the limit is clearly two equivalents per mole within the experimental error. To save space the other quantitative experiments may be summarized by giving the extent of the reaction based on two equivalents at each time: *chlorophyll a*: 12 min. 67%; 60 min. 87%; 400 min. 110%; *chlorin e*: 30 min., 90%; 90 min., 96%; 390 min., 95%; 600 min., 99%; *methyl phaeophorbide b*: 30 min., 35%; 90 min., 66%; 390 min., 102%; 1400 min., 125%; *dimethyl phaeopurpurin 7*: none in 390 minutes; *trimethyl ester chlorin e*: none in 1400 minutes. In all these experiments at least three moles of reagent per mole of substance was employed. The rates are roughly reproducible if all the conditions are kept rigidly constant. The potassium molybdocyanide crystallizes on the sides of the container during the reaction since it is much less soluble than the molybdcyanide in acetone. This separation of the reduced reagent probably helps to keep the reaction proceeding at a definite pace as the oxidation potential of the solution is thus kept high throughout the experiment.

The Dehydrogenation of Chlorophyll a

(a) With Molybdcyanide.—Twenty-five milligrams of pure chlorophyll *a* were dehydrogenated as follows: 5 cc. of approximately 0.03 *M* $K_3Mo(CN)_8$ (prepared freshly as described above) was diluted with a molar pyridine solution in acetone to exactly 50 cc. Five cc. of this mixture was kept as the control and the 25 mg. of chlorophyll *a* added to the remainder (45 cc.); 0.7-cc. samples of the mixture and the control were titrated for oxidizing power with 0.001 *M* $K_4Fe(CN)_6$ as described above; after 400 minutes the titration corresponded to slightly more than 100%, compared with about 90% at the end of one hour. The mixture was then poured into water, extracted with ether and the ether layer repeatedly washed with water to remove pyridine and inorganic salts. The ether layer was finally dried over sodium sulfate and evaporated to dryness *in vacuo* leaving the dehydrochlorophyll as an amorphous wax. A solution of this in alcohol showed the following spectrum in the visible: principal absorption 678.5–653.0; weak absorption centers 615, 568, 531; E.A. 444. The ultraviolet extinction coefficient curve is given as B (circles) in Fig. 2. On removal of the magnesium by shaking an ethereal solution of this dehydrochlorophyll with dilute hydrochloric acid, a solution of dehydrophaeophytin was obtained which showed the following spectrum in the visible: I, 681.7–653.1; II, 615–604; III, 538.9–529.5; IV, 509.0–490.0; E.A., 439; Order I, IV, III, II. The hot rapid saponification of this dehydrophaeophytin by the usual Willstätter procedure (30 sec. with 35% methyl alcoholic potassium hydroxide) yielded no chlorin *e* but gave the unstable chlorins which were identified by conversion to dimethyl phaeopurpurin 7 with diazomethane (ether and acid spectrum identification). In another experiment, 7 mg. of pure chlorophyll *a* was dehydrogenated in the same manner as just described and the magnesium removed with acid. The resulting dehydrophaeophytin (as an amorphous wax) was treated with boiling methyl alcoholic potassium hydroxide (35%) and boiled for three minutes under a reflux. The reaction mixture was worked up in the usual way, being first methylated with diazomethane and then carefully acid fractionated. No chlorin *e* ester was obtained; the major product was identified spectroscopically in acid and ether as chlorin *f* ester. This was judged to be about 50% of the total; the other product was a porphyrin of acid number lower than 10. (The acid number of dimethyl chlorin *f* is 13.)

(b) With Oxygen (Allomerization).—The oxygen absorption during allomerization was demonstrated by using a modified Warburg apparatus.⁸ A number of experiments

⁸ Hyde and Scherp, *THIS JOURNAL*, **52**, 3359 (1930).

were carried out, of which the following is typical. Pure chlorophyll *a* (9.03 mg., 0.01 millimole) was dissolved in 10 cc. of absolute ethyl alcohol (prepared by distillation from magnesium ethylate) and the solution immediately placed in one of the bottles of the absorption apparatus; the other bottle contained the corresponding amount of pure solvent. The apparatus was placed in a water-bath at 25° and shaken continuously; pressure readings were taken from time to time and the number of moles of gas absorbed calculated from a previously determined factor (1 cm. of manometer liquid = 0.0017 millimole of gas). The course of the reaction is indicated by the following figures, which give the millimoles of gas absorbed at different times: 1 hr., 0.0017; 3.3 hrs., 0.0037; 7.5 hrs., 0.0053; 31 hrs., 0.0088; 46 hrs., 0.0099. The reaction clearly proceeds at a progressively diminishing rate as illustrated by the fact that the rate in 10⁻⁶ mole per hr. is 1.7 for the first hour, 0.4 for the 3.3-7.5-hr. period and 0.075 for the period between the last readings. The limit is clearly one mole of oxygen per mole of chlorophyll, corresponding to the reaction chlorophyll *a* + O₂ → dehydrochlorophyll *a* + H₂O₂.

As Willstätter emphasized, the rate of the process of allomerization is very sensitive to impurities. Since we now know that allomerization is a kind of autoxidation, this sensitivity to positive and negative catalysts is not surprising. We are planning a thorough study of this interesting problem. For the present, we may note that among the variables are the water content and the alkalinity. By using an amount of barium hydroxide corresponding to a 0.01 *M* solution, the rate of oxygen absorption was greatly accelerated. As would be expected dehydrochlorophyll prepared by the use of potassium molybdicyanide did not absorb any oxygen even after twenty hours of shaking.

The absorption spectrum in the visible of dehydrochlorophyll *a* prepared by the use of oxygen was as follows: principal absorption, 672.8-641; weak absorption centers, 608, 568, 528; E. A., 442. The corresponding dehydrophaeophytin spectrum was as follows: (identical with methyl dehydrophaeophorbide): I, 683.7-653.5; II, 6.16-604.5; III, 535.6-526.5; IV, 508.6-488.9; E. A. 436; Order: I, IV, III, II.

The ultraviolet absorption curve is given as B (crosses) in Fig. 2. The transformation of dehydrochlorophyll prepared with oxygen (*i. e.*, allomerized chlorophyll) into the unstable chlorins was described in the fourth paper.

Preparation of Methyl Dehydrophaeophorbide a.—A typical preparation was as follows. A solution of 2 g. of potassium molybdicyanide in a mixture of 120 cc. of water and 12 cc. of glacial acetic acid was oxidized with 0.1 *M* potassium permanganate solution, and the excess of oxidizing agent destroyed with a few milligrams of the reduced salt. The resulting yellow solution was mixed with 600 cc. of acetone and 80 cc. of dry pyridine, and the amorphous brown precipitate which formed was filtered out. Acetone was added to bring the total volume to 900 cc. and 25 cc. was removed at this point as a control for subsequent titration. Six hundred milligrams of methyl phaeophorbide *a* was then added and the solution shaken for several minutes. Titration of samples showed the reduction of 50% of two equivalents of oxidizing agent after one hour and 20% more than two equivalents after nineteen hours.

The solution was then filtered and one-third of the solvent was removed with a water pump at room temperature. The remaining solution was added to 3 liters of ether and the resulting olive-green ether solution was washed thoroughly with a total of 3 liters of water. The washings were a dark olive-brown in color and were thick with scum. This was filtered off and dissolved in a chloroform-acetone mixture, poured into ether and fractionated separately. Usually, the aqueous washings contained considerable material in colloidal form, which settled out only after standing several hours, preferably in the presence of sodium chloride or hydrochloric acid. Before acid fractionation, the alkali-soluble material was removed with 0.05 *M* potassium hydroxide. The fractionation was carried out with 500-cc. extracts of cooled acid, each being washed with two

500-cc. portions of ether. Five extracts of 14% hydrochloric acid removed some phaeopurpurin 7 and olive-brown material, which on standing for several days changed somewhat further, but not completely, into phaeopurpurin 7. The remaining solution was extracted exhaustively with eight 500-cc. portions of 17% acid, then with one of 18% acid.

The 16-17% fraction was estimated to contain 155 mg. by comparison with a standard solution in a Zeiss comparison spectroscope. After washing with alkali and concentrating the solution, 95 mg. crystallized. The homogeneity of the product was shown by its spectrum and by hot rapid saponification which yielded only the unstable chlorins, which were identified by conversion into dimethyl phaeopurpurin 7 with diazomethane.

Approximately 150 mg. of scum was obtained per gram of methyl phaeophorbide *a* used. This could not be avoided even by taking the solution to dryness in a vacuum desiccator and adding a pyridine solution of the residue to ether. The first 14% extracts were rose-brown in color and showed a marked phaeopurpurin 7 spectrum though they were alkali-insoluble. The material rendered alkali-soluble by acid hydrolysis during fractionation was converted by diazomethane to equal quantities of dimethyl phaeopurpurin 7 and methyl dehydrophaeophorbide *a*.

The purity of the various crops of crystals of methyl dehydrophaeophorbide *a* is best tested by examining the products of hot rapid saponification. As described by Willstätter, unchanged methyl phaeophorbide *a* goes mainly to chlorin *e* under these circumstances although some oxidation by the air seems unavoidable. However, dehydrophaeophorbide *a*, which is in the oxidized series, forms no chlorin *e* but goes to unstable chlorins, which on methylation with diazomethane yield dimethyl phaeopurpurin 7. The detection of chlorin lines in the spectrum of the 8% fraction of methylated saponification products probably indicates from 10-25% of methyl phaeophorbide *a* in the starting material. The absence of chlorin lines in the spectrum of the 8% fraction probably indicates that the fraction of unoxidized methyl phaeophorbide *a* is below 10%. The experimental conditions are as follows, and aim to exclude air oxidation of the sensitive methyl phaeophorbide *a*. Five milligrams of solid is warmed in a silver crucible with 0.1 cc. of dry pyridine, 7 cc. of boiling 35% potassium hydroxide in methyl alcohol is added and the boiling continued over a free flame for thirty seconds. The intensely green alkaline solution is washed into a separatory funnel and the product transferred by acidification to 75 cc. of ether. The aqueous layer is shaken with fresh ether to remove more completely the strongly basic chlorins. The combined ether solutions are washed, dried with sodium sulfate and treated with an ethereal solution of diazomethane.

An instantaneous change in color and spectrum occurs if unstable chlorins are present. Acid fractionation is carried out as usual. With unoxidized material, 8% hydrochloric acid extracts most of the product, namely, chlorin *e* ester. In the oxidized series, the main product, dimethyl phaeopurpurin 7, is removed by 11% acid, although there is always some chlorin *f* ester of slightly higher acid number, which results through further saponification of the unstable chlorins.

Methyl Dehydrophaeophorbide *a*.—This substance was recrystallized for analysis from acetone-methyl alcohol. Black iridescent plates with irregular corners formed slowly. In thin layers they appear red-brown by transmitted light. The acid number is 16.5 and the color in acid solution is the same green-blue as that of methyl phaeophorbide *a*. The ether solution, however, is a much brighter green than that of the unoxidized compound and the solubility is greater. A "mixed solubility" test proved more satisfactory than mixed melting points to show the difference between the two. This is carried out by preparing in small test-tubes thoroughly saturated solutions of each of the two substances in question and of their mixture. If the two samples are identical, the three solutions should show the same shade and intensity of color. If different substances are actually present, the solution containing the mixture will be as

concentrated as the sum of the other two, and its color will be a blend of theirs. Such a comparison indicated methyl dehydrophaeophorbide *a* and the unoxidized compound to be distinct individuals. When a dilute ether solution of methyl dehydrophaeophorbide *a* is treated with concentrated methyl alcoholic potassium hydroxide (phase test), only an uncertain, fleeting, light yellow precedes the green color; under the same conditions methyl phaeophorbide *a* gives a strong yellow persisting for several seconds. The material sinters at 260–265°.

The spectrum in ether differs from that of methyl phaeophorbide *a* by a shift of about 40 Å. in the position of the narrow green band at 530: (1 mg. in 30 cc. of ether) I, 690.5–650; II, 618–603; III, 565–556; IV, 536–524.5; V, 512.4–485; E. A., 444. Order: I, V, IV, II, III. The spectrum in 19% acid: I, 690–642; II, 615–603; III, 584.5–569; IV, 538–523; E. A., 454. Order: I, III, IV, II.

Anal. Calcd. for $C_{38}H_{38}O_6N_4$: C, 69.7; H, 5.8; N, 9.0; OCH_3 , 10.0. Found: C, 68.65, 68.48, 68.30; H, 6.28, 6.28, 6.34; N, 8.88, 8.62; OCH_3 , 8.65, 8.99; ash, 0.34, 0.40.

Hot Quick Saponification of Methyl Dehydrophaeophorbide *a*.—The identification of phaeopurpurin 7 and its decomposition product, chlorin *f*, and the complete absence of chlorin *e* among the products of this reaction offer strong chemical evidence for the homogeneity of the methyl dehydrophaeophorbide *a* employed and for its relation to the oxidized series. To 165 mg. of methyl dehydrophaeophorbide *a* warmed with one cc. of pyridine in a silver crucible, was added 20 cc. of boiling 35% potassium hydroxide in methyl alcohol. The boiling was continued over a free flame for thirty seconds and the intense green solution was diluted with water and transferred to two liters of ether. The olive-green ether solution was dried and methylated with diazomethane. It was acid-fractionated into an 8–11% fraction containing mainly dimethyl phaeopurpurin 7 and a 12–14% fraction which was mainly dimethyl chlorin *f*. Careful refractionation and concentration of the resulting solutions gave 30 mg. and 55 mg., respectively, of well-formed crystals of each of the two products. These were identified spectroscopically in acid and ether solutions and by mixed melting points with known samples.

In another experiment with 100 mg. of methyl dehydrophaeophorbide *a* the alkaline solution was boiled for ten minutes under reflux in order to achieve a more complete conversion to chlorin *f* and potassium oxalate. The yields were 45 mg. of chlorin *f* and 7 mg. of calcium oxalate isolated in the usual way. One hundred milligrams of methyl phaeophorbide *a* under similar treatment yielded 55 mg. of chlorin *e*, 5 mg. of chlorin *f*, and 1.7 mg. of calcium oxalate. The last two are degradation products of the oxidized series and their presence here must be due to unavoidable oxidation even during the rapid hot saponification or else to the presence of a small fraction of methyl dehydrophaeophorbide in the original methyl phaeophorbide *a*. A phase test saponification of methyl dehydrophaeophorbide *a* follows a parallel course to that of methyl phaeophorbide *a*, with the formation of the same unstable chlorins as intermediates and dimethyl phaeopurpurin 7 as the product of methylation. The yield of crystalline dimethyl phaeopurpurin 7 was 65 mg. from 137 mg. of methyl dehydrophaeophorbide *a*. The identity of the product was tested by acid and ether spectra and by a mixed melting point with an authentic sample.

Preparation of Chlorin *f*.—A more efficient and less laborious method of preparing this compound from methyl phaeophorbide *a* follows from our present knowledge of the chemical steps involved. By bubbling oxygen through a solution of methyl phaeophorbide *a* in concentrated methyl alcoholic potassium hydroxide at 0°, oxidation was accomplished before appreciable hydrolysis to chlorin *e* could take place. The same alkaline solution was then rapidly heated to boiling and refluxed for ten minutes in order to effect the decomposition to potassium oxalate and chlorin *f*. As a precaution against the usual oxidation of chlorin *f* to a porphyrin in the hot saponification, nitrogen was

bubbled through the solution both before and during the heating. The details are as follows. To a solution of 2 g. of methyl phaeophorbide *a* in 10 cc. of pyridine was added 250 cc. of 25% potassium hydroxide in methyl alcohol which had previously been cooled to 0° and saturated with oxygen for fifteen minutes. The intense green solution was kept at 0° and stirred by an oxygen stream for thirty minutes. Nitrogen was then passed through for twenty-five minutes after which the solution was quickly brought to the boiling point with a free flame and boiled vigorously under reflux for ten minutes in the nitrogen atmosphere. The product was cooled rapidly and diluted with one liter of water. The green alkaline solution was acidified in the presence of 4.5 liters of ether and transferred (there was some formation of scum). The olive-green ether solution was extracted as described in paper IV, the only two products being a porphyrin with an acid number of 5-6 and chlorin *f*, which is removed by 11% acid. No chlorin *e* could be detected when the temperature was kept low. The yield of crystalline product (isolated as the dimethyl ester after treatment with diazomethane) was 970 mg.

Conversion of the Unstable Chlorins to Chlorin *f* and Potassium Oxalate.—The unstable chlorins cannot be isolated from an ether solution but are stable in concentrated alkali. They are readily prepared from methyl phaeophorbide *a* in methyl alcoholic potassium hydroxide as was described in the preparation of chlorin *f*. An experiment was performed to demonstrate that potassium oxalate was the other product of the reaction which forms chlorin *f*. The calcium oxalate was precipitated and titrated with standard permanganate solution as previously described (paper IV). The yield from 400 mg. of methyl phaeophorbide *a* was 150 mg. of chlorin *f* and 37 mg. of calcium oxalate.

Chlorin *k*.—A typical preparation of this compound by the oxidation of chlorin *e* with molybdicyanide was as follows: 500 mg. of chlorin *e* was added to a filtered oxidizing mixture which was made from 1.66 g. of potassium molybdicyanide in 100 cc. of 10% acetic acid oxidized with a slight excess of permanganate and 500 cc. of molar pyridine in acetone, the total volume being brought to exactly 750 cc. by the addition of water. A sample was removed as a control before the addition of chlorin *e*. After five hours at room temperature the reaction mixture was worked up in the usual way with water and ether; titration had previously shown that about 85% of two equivalents of oxidizing agent had been reduced. A "colorimetric comparison" in a Zeiss comparison spectroscope with chlorin *f* as an assumed standard showed little or no destruction of the organic colored material. The ether solution after repeated washing with water was fractionated with 9 and 14% hydrochloric acid; the lower fraction, which was small, was discarded. The 14% portion was carefully refractionated; the 13 and 14% portions were combined, transferred to ether and the solution concentrated and a small amount of petroleum ether added. The product crystallized with a steel-blue luster. The yield was 109 mg. (24%). The acid number is between 11 and 12; the color in ether is olive-green and in acid, blue. The spectrum in ether: I, 685-644.5; II, 615-613.5-601; III, 561-553; IV, 535.3-522.5; V, 510.5-483; E. A., 437. Order: I, V, IV, II, III. The spectrum in 13% hydrochloric acid: I, 682-630; II, 611-602; III, 582-571; IV, 535-522; E. A. 447. Order: I, IV, III, II.

Anal. Calcd. for $C_{33}H_{34}O_4N_4$: C, 72.0; H, 6.2; N, 10.18. Calcd. for $C_{33}H_{36}O_4N_4$: C, 69.7; H, 6.3; N, 9.86. Found: C, 71.6, 71.4; H, 6.64, 6.55; N, 10.5, 9.7, 10.2.

Chlorin *k* Monomethyl Ester.—Chlorin *k* was esterified in ether solution with diazomethane. The ester was crystallized from ether-petroleum ether. The melting point was 145-147° with some decomposition. The spectrum in ether and acid was identical with that of free chlorin *k*. The acid number was 13.

Anal. Calcd. for $C_{34}H_{36}O_4N_4$: C, 72.3; H, 6.4; N, 9.93; OCH_3 , 5.5. Found: C, 71.4, 71.6; H, 6.5, 6.8; N, 9.66, 9.46; OCH_3 , 4.5, 5.0, 6.2.

Evolution of Carbon Dioxide from Chlorin *e* on Oxidation.—An experiment exactly

similar to that described under the preparation of chlorin *k* was carried out in a flask connected with an inlet tube for purified nitrogen and an outlet tube passing through an empty bottle set in a freezing mixture and then into barium hydroxide solution. Nitrogen was passed through the apparatus with the oxidizing mixture in place for three hours before adding the chlorin *e*; there was no trace of barium carbonate visible. After adding the chlorin *e* the gas stream was continued and in seven minutes time a cloudiness was observed in the barium hydroxide solution. After five hours, the heavy precipitate of barium carbonate which had formed rapidly during the first hour, was filtered, dried at 110° and weighed. It weighed 90 mg. (56% of the theoretical amount of one mole of carbon dioxide per mole of chlorin *e*).

Further Experiments on the Pyrolysis of Methyl Phaeophorbide a.—In our first report of the preparation of pyropheophorbide *a* and methyl pyropheophorbide *a* (paper I), the fact that a methoxyl group was involved in the decomposition was overlooked due to the failure to carry out a methoxyl determination on the pyro compounds. The complete analysis of the two pyro compounds reported in Table I of the third paper of this series (p. 3016) showed that not only was carbon dioxide eliminated but methyl alcohol or formaldehyde was split out of the molecule. To test for the presence of these substances the gases evolved in the pyrolysis of 300 mg. of methyl phaeophorbide *a* in boiling diphenyl were passed into 5 cc. of distilled water, a current of nitrogen being used to sweep through the products. The aqueous solution gave a definite aldehyde test with decolorized fuchsin solution (Schiff's reagent) and with Tollens' reagent. The presence of methyl alcohol was demonstrated by the usual procedure of repeatedly introducing a heated bright copper wire and subsequently applying the aldehyde test, which is greatly increased by this treatment. The possibility that the methoxyl group of phaeophorbide *a* might have been eliminated as methane during the pyrolysis was investigated by using carbon dioxide to sweep the volatile products into a buret filled with potassium hydroxide solution. Half a millimole of methyl phaeophorbide *a* yielded only 1.75 cc. of gas which was shown by combustion analysis to be 63% carbon monoxide, 13% inert (probably nitrogen from air) and 23% methane, corresponding to a total of 0.4 cc. = 0.02 of millimole (in a duplicate experiment the methane was found to be 0.5 cc.). The actual yield of crystalline pyro compound was five times this amount. The possibility of a shift of the methoxyl group from oxygen to nitrogen during pyrolysis was also considered and the pyro compounds were tested for N-methyl groups by Pregl's quantitative method; no such groups were found, however.

Summary

1. The process of allomerization has been shown to be a process of dehydrogenation by atmospheric oxygen.
2. Chlorophyll *a* and methyl phaeophorbide *a* may be dehydrogenated by potassium molybdicyanide, two equivalents of reagent being involved. The dehydro compounds on rapid saponification yield no chlorin *e* but only the unstable chlorins which on longer boiling with alkali yield chlorin *f* and potassium oxalate.
3. Chlorin *e* is oxidized by potassium molybdicyanide with loss of carbon dioxide.
4. A structural formula for chlorophyll *a* has been proposed based on the experiments reported in this and previous papers of this series.