bath temperature of 250–270° in an oxygen-free, dry nitrogen stream. The carbon dioxide slowly evolved (none in the first hour) corresponded to 0.35 mole. By comparison, both alpha and beta monomethyl esters lose the bulk of their carbon dioxide in the first hour. The product consists of a mixture of unidentified alkali-soluble porphyrins.

Summary

1. The trimethyl ester of chlorin *e* has been prepared by a number of methods and the identity of the products established through the melting points of their metallic derivatives.

2. On hydrolysis, the trimethyl ester regenerates a chlorin *e* in which the glycolic acid side chain has been shown to be present.

3. Two isomeric monomethyl esters and one dimethyl ester have been prepared and their structures established by their transformations. Two isomeric diethyl methyl esters have been prepared.

4. The bearing of these results on the structure of phaeophorbide *a* has been discussed.

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Studies in the Chlorophyll Series. XI. The Position of the Methoxyl Group

BY JAMES B. CONANT AND EMMA M. DIETZ

In the fifth paper of this series we proposed a formula for chlorophyll *a*,[1] in which a carbomethoxy group was placed in the beta position of one of the pyrrole rings. This seemed necessary as the facts in regard to oxidative hydrolysis (phase test) given in the third and fourth papers[2] showed that a methoxyl group survived this degradation and was present in phaeopurpurin 7. This compound on pyrolysis yielded a monomethyl chlorin *f* and thus was shown to contain a carbomethoxy group attached to one of the pyrrole nuclei. The yield of phaeopurpurin 7 (a monomethyl ester) was always very low, however, and all attempts to increase the yield by changing the conditions of oxidative hydrolysis failed. The chief product was always the unstable chlorin which on standing yields phaeopurpurin 18. In this connection we may mention that we have not been able to observe the conversion of phaeopurpurin 7 to phaeopurpurin 18 on standing in solution as reported by Fischer.[3]

The small yield of phaeopurpurin 7 led us to suspect that it might arise from some impurity in our phaeophorbides, and this has proved to be the case. We have been able to obtain a sample of methyl phaeophorbide *a*

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[1] THIS JOURNAL, **53**, 2382 (1931).

[2] *Ibid.*, **52**, 3013 (1930); **53**, 359 (1931).

[3] Fischer, *Ann.*, **498**, 196 (1932).

which after several recrystallizations yielded no phaeopurpurin 7 on oxidative hydrolysis. It is difficult, however, to remove the precursor of phaeopurpurin 7 from the phaeophorbides. Material prepared from the chlorophyllides yields small amounts of phaeopurpurin 7 unless carefully recrystallized. Crude methyl phaeophorbide *a* prepared from commercial phaeophytin by the usual Willstätter procedure is very rich in this impurity. It can be purified by a partial hydrolysis procedure described in detail in the experimental portion of this paper, which also removes other impurities. The methyl phaeophorbide *a* thus purified, crystallizes beautifully and we believe it to be as pure as the material prepared through the chlorophyllides.

The analyses of the pure methyl phaeophorbides (from phaeophytin or through the chlorophyllides) correspond closely to the formula $C_{36}H_{38}N_4O_5$ instead of $C_{36}H_{40}N_4O_6$ previously assigned. This fact has recently been emphasized by Stoll and by Fischer,[4] and led the latter to modify his formula for chlorophyll *a*. Stoll's formulas for chlorophyll *a* and chlorophyll *b* seem to us to be excluded by a great number of considerations, only one of which will be considered here. According to his formula the hydrolysis of phaeophorbide *a* to chlorin *e* is an oxidative reaction and he states that he is able to prevent chlorin formation by adding a reducing agent to the hydrolyzing mixture.[5] (He has as yet published no experimental details, however.) Mr. J. L. Hartwell[6] in this Laboratory has shown that the formation of chlorin *e* by hydrolysis of the phaeophorbides not only proceeds in a nitrogen atmosphere, but that as powerful a reducing agent as a sulfonated anthrahydroquinone may be present without affecting the result. It seems to us impossible that an oxidative reaction is involved in the formation of chlorin *e* from phaeophorbide *a*.

The parallelism between the behavior of the esters of chlorin *e* and the phaeophorbides has been pointed out in the preceding paper.[7] The analysis of chlorin *e* and its esters corresponds to an oxygen content of O_6 while the reactions call for a formula with O_7; the phaeophorbides of the *a* series behave as if they contained six oxygen atoms but the analyses correspond to a content of five oxygen atoms. We believe that the most probable explanation is that in both cases one is dealing with an unsaturated compound which easily adds water in solution forming an hydroxy compound, the water being lost again on crystallizing and drying. If this is the case, at least *in solution*, the phaeophorbides like the chlorin *e* esters are hydroxy compounds.

The oxidative hydrolysis of pure phaeophorbide *a* in the presence of very dilute alkali resulted in the formation of a monomethyl ester of a chlorin of

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[4] Stoll and Wiedemann, *Naturwissenschaften*, 628, 706, 791 (1932); *Helv. Chim. Acta*, **15**, 1128, 1280 (1932); Fischer, *Ann.*, **499**, 84 (1932).

[5] Stoll, *Helv. Chim. Acta*, **15**, 1284 (1932).

[6] Unpublished results.

[7] This Journal, **55**, 829 (1933).

the "oxidized series." It is stable in solution but on treatment with diazomethane forms dimethyl phaeopurpurin 7 (a triester). A diethyl phaeopurpurin 7 (a diethyl methyl ester) was made by treating the chlorin with diazoethane. On heating this compound with pyridine and sodium carbonate according to Fischer's method,[8] a diethyl ester of isorhodoporphyrin was formed. This shows that the carbomethoxy group of the chlorin was part of the bridge grouping; the formation of a methoxyl-free isorhodoporphyrin on heating the chlorin itself also demonstrated the position of the OCH_3. These transformations thus serve to confirm the position of the methyl group in chlorophyll *a* assigned by Fischer.[9] We propose to designate this new chlorin as the alpha monomethyl ester of chlorin *g*, since it contains the grouping $—COCOOCH_3$ on the bridge. As pointed out in the third paper of this series, the "unstable chlorins" are probably identical with Willstätter's phytochlorin *g*. It is interesting that when the bridge carboxyl group is esterified the chlorin passes into the phaeopurpurin structure only on treatment with diazomethane. On hydrolysis the ester is transformed into the unstable chlorin (chlorin g) which spontaneously passes into phaeopurpurin 18.

The precursor of phaeopurpurin 7 is a substance of considerable interest. It is clearly very similar to the phaeophorbides (or the chlorophyllides as the case may be) as it is difficult to purify these compounds from it. Since phaeopurpurin 7 can be transformed into dimethyl phaeopurpurin 7 by the action of diazomethane, and on pyrolysis yields chlorin *f* monomethyl ester, we are clearly dealing with a compound with the same nucleus and carbon skeleton as in the normal form of phaeophorbide *a*. The position of the methoxy group, however, is different. The phaeopurpurin 7 prepared from crude chlorophyll or phaeophytin appears to have the same structure as the phaeopurpurin 7 formed by hydrolysis of dimethyl phaeopurpurin 7. Both compounds yield monomethyl chlorin *f*, carbon monoxide and carbon dioxide on pyrolysis, and have very similar properties except for a slight difference in color, spectrum and acid number. The position of the methoxyl group in both substances is established by its survival in chlorin *f* monomethyl ester (pyrolysis) and its resistance to hydrolysis by dilute alkali; as will be shown in a later paper, an ester group involving the propionic acid is rapidly hydrolyzed by even dilute alkali. On vigorous hot alkaline hydrolysis, methoxyl-free chlorin *f* is formed. All these facts show that the grouping $—COOCH_3$ is on the pyrrole ring in phaeopurpurin 7.

The precursor of phaeopurpurin 7 occurs in relatively small quantities (1 to 3%) in the crude chlorophyll of a number of green plants which we have so far examined (spinach, Datura, and representatives of filamentous chlorophyceae and marine phaeophyceae) and in commercial phaeophytin. The largest amount (20%) was found in the mother liquors from crystalline

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

(9) Fischer, *ibid.*, **490**, 42 (1932).

ethyl chlorophyllides prepared from Datura. The estimation of its presence is possible by carrying out a vigorous oxidative hydrolysis ("phase test" conditions) of the crude chlorophyll or phaeophytin (preferably free from the carotenoids), allowing the solutions of the unstable chlorins to stand for three to five days and carefully fractionating with acid. The normal chlorophyll *a* component is thus transformed into phaeopurpurin 18 which is easily separated by its high acid number. The oxidized rhodins are also removed only by strong acid. If the oxidative stage of the hydrolysis is not rapid enough, small amounts of chlorin *e* and rhodin *g* may also be formed. The latter is difficult to separate from phaeopurpurin 7 but a procedure described in detail in the experimental section of this paper may be employed.

It seems to us that there can be no escape from the conclusion that there are two very similar forms of chlorophyll *a*. In the predominant form the carbomethoxy group is located on the bridge; in the other form it is located on the pyrrole ring. We are now engaged in an attempt to separate in a pure state this second form of chlorophyll *a* or its related chlorophyllide or phaeophorbide; we also intend to examine a greater variety of green plants for the relative distribution of the two forms. It appears probable that a similar situation exists in regard to chlorophyll *b*, and we are engaged in a similar line of investigation in regard to the location of the methoxyl group in this compound.

The formula for chlorophyll *a* which we suggested in the previous paper is impossible for the predominant form of chlorophyll *a* because of the position of the methoxyl group; it may represent the second form, however. Fischer's formula containing a carbocyclic ring is, of course, satisfactory as far as the position of the methyl group is concerned for the predominant form, but is not possible for a compound containing the potential carbomethoxy group on the pyrrole ring (as appears to be the case in the second form). We are still inclined to favor a formula for chlorophyll *a* that contains a secondary hydroxyl group attached to the bridge because of the dehydrogenation results considered in our previous papers; the analogy between the chlorin *e* esters and the phaeophorbides also influences us in this choice. We do not feel that the presence of a carbocyclic ring in chlorophyll *a* or the phaeophorbides is proved by the transformation of these substances into phaeoporphyrin a_5 and phylloerythrin which has been carried out by Fischer.[10] The possibility is not excluded that the carbocyclic ring which Fischer has shown to be present in phylloerythrin was closed in the reaction by which the chlorophyll compounds are transformed. Such a ring closure does in fact occur under only slightly more drastic conditions in the preparation of phylloerythrin from the chloroporphyrins.[11]

It would seem extremely probable that the structural formulas of the two

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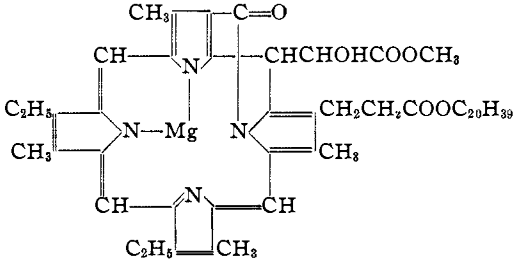
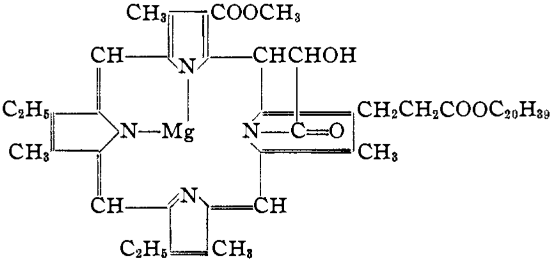
(10) Fischer, *Ann.*, **486**, 107 (1931).

(11) Fischer and Moldenhauer, *ibid.*, **481**, 152 (1930).

forms of chlorophyll *a* are very similar if the compounds differ only slightly in their properties. A formula is therefore desirable which allows the interchange of the methoxyl group between the potential carboxyl groups of the bridge and pyrrole ring without serious structural modification. The lactam formula which we mentioned in the fifth paper[12] as an alternative to our anhydride formula satisfies these requirements. The formula for the predominant form of chlorophyll *a* (a_1) would then be as shown in I, and the other form (a_2)—the precursor of phaeopurpurin 7—would be represented as II.



I Chlorophyll a_1



II Chlorophyll a_2

We are indebted to Miss G. M. Ware and Mrs. S. E. Kamerling for the microanalyses reported in this paper.

Experimental

Purification of Methyl Phaeophorbide *a*.—The methyl phaeophorbide *a* obtained in the usual way from commercial phaeophytin gives unsatisfactory analyses even after several recrystallizations. On hot rapid saponification in nitrogen as much as 15% of chlorin *f* may be obtained along with the chlorin *e*, indicating the presence of an oxidized impurity in the methyl phaeophorbide. After a carefully controlled rapid treatment with dilute alkali, however, the methyl phaeophorbide which remains appears to be as pure as preparations from methyl chlorophyllide *a*. Its analysis shows a content of five oxygen atoms and on hot rapid saponification only a trace of high chlorin results. A typical purification experiment is as follows: three grams of methyl phaeophorbide *a* was dissolved in 150 cc. of pyridine and 750 cc. of acetone in a three-liter three-necked flask, fitted with a gas inlet tube for nitrogen, a mercury-sealed stirrer and a conical glass funnel of 750 cc. capacity. The neck of the funnel was closed by a one-holed rubber stopper at the end of a glass rod. This arrangement permitted the rapid addition

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[12] THIS JOURNAL, **53**, 2384 (1931).

of large volumes of alkali or acid as needed. The solution was refluxed in nitrogen for ten minutes to remove oxygen, then cooled to 0° with nitrogen bubbling through. At the same time 750 cc. of 1% potassium hydroxide solution was similarly refluxed for ten minutes and cooled to 0° in nitrogen. Then the stopper carrying the nitrogen inlet tube was loosened and the alkali was rapidly added through the funnel to the violently stirred pyridine–acetone solution. The plunger was replaced in the funnel and after fifteen seconds, 11% hydrochloric acid was added in the same way, precipitating the material in fine amorphous form. This dissolved readily on shaking with five liters of ether and formed a violet-red solution. The alkali-soluble material was removed with 0.02 *N* potassium hydroxide solution. The residue was then extracted with portions of iced 11 to 14% acid until the extracted material had the same spectrum as the main solution, which was finally concentrated without further treatment. The yield in several experiments was 40 to 65%. The alkali-soluble by-products are mainly monomethyl chlorin *e*, and pheopurpurin 18 resulting from hydrolysis of impurities and from oxidative hydrolysis of good methyl phaeophorbide *a* due to unremoved traces of oxygen. The product separated in well-formed black crystals with a blue luster. The spectra in ether and acid are identical with those of methyl phaeophorbide *a* prepared from the chlorophyllides, and the yellow phase color is very marked on shaking the ether solution with methyl alcoholic potassium hydroxide.

Analyses of several samples of methyl and free phaeophorbides are listed below. Those from chlorophyllides were obtained by acid treatment and fractionation of crystalline ethyl or methyl chlorophyllide mixtures of the alpha and beta compounds, prepared from Datura according to the Willstätter procedure. The "regenerated" methyl phaeophorbide *a* was prepared by diazomethane treatment of phaeophorbide *a* from purified methyl phaeophorbide *a*.

Anal. Calcd. for $C_{36}H_{38}O_5N_4$: C, 71.30; H, 6.3; N, 9.24; OCH_3, 10.2.

	C	H	N	OCH_3
Methyl phaeophorbide *a* purified as above	71.15	6.6	9.01	10.61
	71.38	6.9		9.28
Methyl phaeophorbide *a* from methyl chlorophyllides	71.04	6.57	9.28	10.36
	71.27	6.75	9.38	10.68
	71.44	7.03		
Methyl phaeophorbide *a* regenerated	71.44	6.55	9.05	9.3
	71.07	6.55	8.95	10.0
	70.84	6.35		
Methylated phaeophorbide *a* from ethyl chlorophyllides	71.18	6.45	9.56	
	71.12	6.45	9.31	

Calcd. for $C_{35}H_{36}O_5N_4$: C, 70.95; H, 6.1; N, 9.46; OCH_3, 5.3

Calcd. for $C_{35}H_{38}O_6N_4$: C, 67.72; H, 6.25; N, 9.66; OCH_3, 5.35

	C	H	N	OCH_3
Phaeophorbide *a* from purified methyl phaeophorbide *a* dried at 10^{-3} mm. press.	69.78	6.26	9.28	4.7
	69.70	6.63	9.10	5.1
Phaeophorbide *a* from ethyl chlorophyllides	70.3	6.35	9.50	

Rate of Hydrolysis of Methyl Phaeophorbide *a*.—Preliminary experiments were carried out on the hydrolysis of methyl phaeophorbide *a* at 0° in an aqueous butyl carbitol–pyridine mixture 0.04 *N* in potassium hydroxide and in a nitrogen atmosphere. The results indicate that there is a rapid conversion to monomethyl chlorin *e* with loss of one methoxyl group and apparently without the intermediate formation of phaeophorbide *a*. The rate of formation of monomethyl chlorin *e* was as follows: 40% in five minutes, 55% in ten minutes, and 80% in forty-five minutes. The concentration of phaeophorbide *a* was never greater than 5%. The second methoxyl group is lost with much greater difficulty. After thirty-six hours under the same conditions only one-fourth of the product was free chlorin *e*. Phaeophorbide *a* in aqueous pyridine at 0° in nitrogen and with an alkali concentration of 0.45 mol., also changes rapidly to monomethyl chlorin *e*. The analysis of the product corresponds to a quarter of a methoxyl group even after twenty-four hours.

The hydrolysis of phaeophorbide *a* proceeds very rapidly in the presence of oxygen, forming monomethyl chlorin *g* which is then transformed more slowly with loss of methoxyl content to phaeopurpurin 18. The rate of formation of "unstable chlorins" including the monomethyl ester of chlorin *g* in a phaeophorbide *a* solution 0.02 molar in alkali was as follows: 40% in two minutes, 80% in ten minutes.

Monomethyl Ester of Chlorin *g*.—This substance was prepared by mild oxidative hydrolysis of phaeophorbide *a* obtained from its methyl ester purified as above, or from methyl chlorophyllide *a*. A solution of 450 mg. of phaeophorbide *a* in 125 cc. of pyridine and 250 cc. of water was cooled to 0° in an ice-bath and stirred by a stream of oxygen for thirty minutes. Then 75 cc. of 0.06 *N* aqueous potassium hydroxide was added, making the total alkalinity 0.01 *N*, and the gas stream was continued for thirty minutes. The product was transferred to three liters of ether and allowed to stand overnight to enable any unstable chlorins present to change over to phaeopurpurin 18. Extraction with 6 to 8% acid removed some low chlorin, 12 to 14% acid removed the main product, and the residue contained small amounts of unchanged phaeophorbide and of phaeopurpurin 18 resulting from demethylation of the desired chlorin. A convenient test for the homogeneity of the last extracts of monomethyl chlorin *g* is treatment with diazomethane which changes the spectrum completely and instantly to that of phaeopurpurin 7. The yield of chlorin was 30 to 60% in several experiments.

A sample was recrystallized for analysis from acetone–ligroin, forming small black crystals. The color in ether is bright green with a red fluorescence; in acid, green. The acid number is 12.

The spectrum in ether (a solution of one milligram in 30 cc. measured in a 55-mm. tube): I, 687—646; II, ---620—600---; III, ---566---554--- (very hazy); IV, 533—520; V, 509—481. E. A.: 438. Order: I, IV, V, II, III.

The spectrum in 16% hydrochloric acid: I, 589—569; II, ---541---521--- (very faint). E. A.: 456. Order: I, II.

Anal. Calcd. for $C_{35}H_{38}O_7N_4$: C, 67.08; H, 6.1; N, 8.95; OCH_3, 4.95. Found: C, 66.66, 66.90; H, 5.9, 6.0; N, 8.95, 9.19; OCH_3, 4.6, 4.4.

When monomethyl chlorin *g* is shaken in ether solution with 25% potassium hydroxide in propyl alcohol, an unstable chlorin is obtained which changes to phaeopurpurin 18 slowly on standing, or immediately on warming.

Pyrolysis of Monomethyl Chlorin g.—Pyrolysis in diphenyl results in the removal of the two bridge carbon atoms and methyl group forming *free* isorhodoporphyrin as the main product. A solution of 300 mg. of the chlorin in 50 g. of diphenyl was boiled in nitrogen for five minutes. The product in 6 liters of ether had a brown-red color and was worked up in the usual manner. The main product, a porphyrin, was extracted with 8 to 10% acid, leaving traces of chlorin in the mother liquor. The spectra in acid and ether were identical with those of isorhodoporphyrin; yield, 50 mg. of crystalline isorhodoporphyrin.

Anal. Calcd. for $C_{33}H_{38}N_4O_4$ (monomethyl ester): OCH_3, 5.60. Found: OCH_3, 0.61, 0.84.

Transformation to Dimethyl Phaeopurpurin 7.—Monomethyl chlorin *g* unlike the ordinary "unstable chlorins" is unchanged after standing for a few weeks in ether solution, but on treatment with diazomethane, dimethyl phaeopurpurin 7 (a triester) forms immediately. This product was identified by spectrum in acid and ether, by acid number and by analysis. It was thus shown to be identical with "synthetic" dimethyl phaeopurpurin 7.

The spectrum in ether: I, 712—651; II, ---638—613--- (very weak); III, 554---549—529---; IV, 516---512—489---. E. A. 443. Order: I, IV, III, II.

Anal. Calcd. for $C_{37}H_{42}O_7N_4$: C, 67.9; H, 6.4; N, 8.56; OCH_3, 14.2. Found: C, 67.75; H, 6.53; OCH_3, 14.0, 13.7.

Conversion of Diethyl Methyl Ester to Diethyl Isorhodoporphyrin.—The carefully fractionated ether solution of a preparation of monomethyl chlorin *g* was treated with diazoethane whereby diethyl phaeopurpurin 7 (phaeopurpurin 7 is a monomethyl ester) was formed. The isolated crystalline product (430 mg.) was dissolved in 75 cc. of pyridine, 1.5 g. of anhydrous sodium carbonate was added and the solution was refluxed for three hours. On transferring to 10 liters of ether the solution had a good porphyrin color, the main product being diethyl isorhodoporphyrin with traces of a low porphyrin and of a high chlorin. The yield was 135 mg. After recrystallization from chloroform-methyl alcohol, the melting point was 240–241°. The recrystallized diethyl ester prepared by the action of diazoethane on the ferricyanide oxidation product of chlorin *f* melted at 236–238°.[13] The mixed melting point lay between these two values.

Anal. Calcd. for $C_{36}H_{44}O_4N_4$ (diethyl ester): C, 71.52; H, 7.6; N, 9.7; OC_2H_5, 15.3. Found: C, 71.90, 71.71; H, 6.91, 6.48; N, 9.30; OC_2H_5, 15.4, 14.0.

A further identification of the product as a diethyl ester was obtained by using a solution of trimethylamine as absorbent in the Zeisel determination.[14] Authentic samples of methyl ethyl and of diethyl isorhodoporphyrin were analyzed at the same time to serve as controls on the method.

Preparation of Phaeopurpurin 7 from Various Plant Sources

(a) **From Commercial Phaeophytin.**—Oxidative hydrolysis under phase test conditions of phaeophytin results in the formation of unstable chlorins which change over in the course of several days to phaeopurpurin 18 and 1 to 2% of phaeopurpurin 7. The evidence indicates that the major form of chlorophyll *a* has a carbomethoxy group on a carbon atom attached to the bridge position as in alpha monomethyl chlorin *g*, which probably precedes phaeopurpurin 18 in the above-mentioned hydrolysis of phaeophytin. The preparation of phaeopurpurin 7 in this way was carried out as follows. Eighteen grams of phaeophytin was dissolved in 60 cc. of pyridine, the solution was diluted with 4 liters of ether and then shaken for eight minutes with a solution of 400 cc. of 20% potassium hydroxide in propyl alcohol which had been previously saturated with oxygen. The product was transferred to ten liters of ether and a large amount of ether-insoluble scum was formed. After standing for five days the solution was fractionated. Some strongly basic chlorin was removed by 4 to 7% hydrochloric acid and the phaeopurpurin 7 was then extracted with 8 to 13% acid. The yield after refractionation was 230 mg. of very small black crystals. Phaeopurpurin 18 was isolated from the residue in 15% yield.

Anal. Calcd. for $C_{35}H_{38}O_7N_4$: OCH_3, 4.95. Found: OCH_3, 3.97, 4.14.

(b) **From Spinach Chlorophyll.**—An oxidative hydrolysis was carried out under similar conditions on chlorophyll isolated from spinach according to the Willstätter procedure. A solution of 25 g. of chlorophyll in 70 cc. of pyridine was added to 4.5 liters of ether and the whole shaken for eight minutes with 450 cc. of 20% potassium hydroxide in propyl alcohol. (The alkali was previously saturated with oxygen.) The green mixture was diluted and the product transferred to 12 liters of ether. After four days the solution was fractionated. Extraction with 6% acid removed some low

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(13) THIS JOURNAL, **55**, 795 (1933).

(14) Pregl, "Microanalysis," second English edition, p. 190.

chlorin. Extraction with 8 to 13% acid removed a mixture of phaeopurpurin 7 and rhodin *g* which it was impossible to resolve by further fractionation. A separation was easily accomplished, however, by making use of the rapid reaction of the rhodin *g* with semicarbazide hydrochloride to form a derivative of low acid number. This could then be readily removed from the unchanged phaeopurpurin 7 by acid fractionation. The presence of small amounts of carotenoid pigments appears to increase the percentage of non-oxidative hydrolysis which produces chlorin *e* and rhodin *g*, and in general seems to decrease the destruction of the rhodins by alkali.

The separation of phaeopurpurin and rhodin was actually carried out as follows. The mixture was taken to dryness and dissolved in 15 cc. of pyridine; 0.7 g. of anhydrous sodium carbonate and 0.7 g. of semicarbazide hydrochloride were added and the mixture was allowed to stand at room temperature for ten minutes. The product was transferred to five liters of ether. Extraction with 5 to 7% acid readily removed the semicarbazone of the rhodin, leaving phaeopurpurin 7 and a little high chlorin as the only other products. The yield of phaeopurpurin 7 after fractionation was 150 mg.

The phaeopurpurin 7 obtained from both of the above sources is not quite identical in physical properties with the compound prepared by hydrolysis of the dimethyl ester and whose chemical transformations were described in the fourth paper. However, it gives the same pyrolysis and saponification products.[15] It is browner in ether solution, but has the same green color in acid; the acid number is somewhat higher (10) and less sharp. A sample in ether solution was unchanged after two months. A sample of solid material after a year had changed completely to phaeopurpurin 18 and unidentifiable compounds of lower acid number. The spectrum in ether: I, 710—649; II, 545—524.5; III, 515---509—484.5. E. A. 442. Order: I, III, II.

Anal. Calcd. for $C_{35}H_{38}O_7N_4$: C, 67.1; H, 6.1; N, 8.95; OCH_3, 4.95. Found: C, 67.64, 67.46; H, 6.17, 6.39; OCH_3, 4.2, 3.9.

A dimethyl ester of identical spectrum is formed on methylation with diazomethane. The acid number was 13.

Pyrolysis of Phaeopurpurin 7 from Phaeophytin.—Duplicate pyrolyses were carried out in diphenyl in nitrogen, the solution being boiled for ten minutes, then removed from the bath and swept for a further twenty minutes. Under these conditions 0.9 mol of carbon dioxide was evolved and carbon monoxide was detected in both cases. The product from 240 mg. was resolved by acid fractionation into a porphyrin fraction (8 to 9% acid) with the spectrum of isorhodoporphyrin, and a chlorin fraction with the spectrum of chlorin *f*. The yields were 17 mg. and 25 mg., respectively, and the analyses check the formulas for the monomethyl compounds in both cases. This behavior is identical with that of "synthetic" phaeopurpurin 7 and further supports the existence of a β-carbomethoxyl group in both compounds.

Anal. Calcd. for $C_{33}H_{38}N_4O_4$: C, 71.5; H, 6.9; N, 10.11; OCH_3, 5.6. Found for the chlorin: C, 71.44, 71.67; H, 7.0, 6.8; OCH_3, 5.14.

Anal. Calcd. for $C_{33}H_{36}N_4O_4$: C, 71.73; H, 6.5; N, 10.15; OCH_3, 5.62. Found for the porphyrin: C, 71.50, 71.16; H, 7.2, 7.0; N, 9.99; OCH_3, 5.25.

Hydrolysis of Phaeopurpurin 7 from Spinach Chlorophyll.—It has been shown above that the propionic ester group in methyl phaeophorbide *a* is readily hydrolyzed even in 0.04 potassium hydroxide solution at 0°. Unpublished experiments of Mr. J. L. Hartwell in this Laboratory have shown that other derivatives which contain this grouping lose the ester group with similar ease, while β-carbomethoxy ester groups are held for a much longer time. It seemed advisable therefore to carry out a mild hydrolysis under the standardized conditions. The survival of the methoxyl group (OCH_3: 5.0, 4.85) after five hours in 0.03 *N* potassium hydroxide is considered as further evi-

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[15] THIS JOURNAL, **53**, 359 (1931).

dence that it is the β-carboxyl rather than the propionic acid group in phaeopurpurin 7 which is esterified.

A small sample of phaeopurpurin 7 from spinach chlorophyll was heated for five minutes with 25% potassium hydroxide in methyl alcohol in nitrogen. Like "synthetic" phaeopurpurin 7, it readily lost the bridge grouping forming chlorin *f* and a smaller amount of isorhodoporphyrin. Both products were identified by spectra and acid number.

Estimation of the Precursor of Phaeopurpurin 7.—The indicated evidence for the existence of a chlorophyll a_2 with the carbomethoxy group on the beta position of a pyrrole ring makes it important to find a convenient means of its estimation in chlorophylls of various sources, with the hope of isolating it in pure form from some favorable source. No direct method of estimation has been found thus far, due apparently to the very similar chemical and physical properties of chlorophylls a_1 and a_2. The method used involves the estimation in solution of the relative proportions of phaeopurpurins 7 and 18 formed on oxidative hydrolysis under standard conditions. The chlorophyll should be free of carotenoids which otherwise interfere with the oxidative hydrolysis. One hundred milligrams of substance is dissolved in 5 cc. of pyridine and diluted with 150 cc. of ether. The solution is shaken with one-tenth its volume of 20% potassium hydroxide in propyl alcohol for eight minutes. An equal volume of ether is added and the product is transferred to it. After three to four days it is extracted with 6% acid to remove any chlorin *e*, then with 100 cc. extracts of 8, 10 and 12% acid until phaeopurpurin 18 begins to be extracted. The residue, when freed of carotenoids makes up the phaeopurpurin 18 fraction. The spectrum shows only very faint rhodin lines. The phaeopurpurin 7 fractions are combined and the relative proportions of both phaeopurpurins are determined by comparison with standard solutions in a comparison spectroscope.

The following percentages of phaeopurpurin 7 referred to the total phaeopurpurin concentration (7 and 18) were obtained from various sources and estimated according to the above procedure: chlorophyll from spinach 2%; commercial phaeophytin 8%; crystalline ethyl chlorophyllides (second crops from mother liquors) from Datura 10 to 23%; crude chlorophyll from marine fucus 1%; crude chlorophyll from a filamentous chlorophycea about 1%; crude methyl phaeophorbide *a* from phaeophytin by Willstätter's procedure 18%; methyl phaeophorbide *a* after purification by partial hydrolysis (see above) 4%; the same after two recrystallizations from acetone–ligroin 0%; free phaeophorbide *a* from chlorophyllides from Datura 6%.

Summary

1. Methyl phaeophorbide *a* may be prepared in a pure condition from methyl chlorophyllide *a*, or from phaeophytin if the first crude product is subjected to a fractional hydrolysis. The composition of pure methyl phaeophorbide *a* is $C_{36}H_{38}N_4O_5$.

2. After several recrystallizations, pure methyl phaeophorbide *a* yields no phaeopurpurin 7 on oxidative hydrolysis with strong alkali. With dilute alkali under oxidative conditions a monomethyl ester of chlorin *g* (the unstable chlorin) is formed. The carbomethoxy group of this compound has been shown to be part of the bridge grouping. This confirms the position of the methyl group in chlorophyll *a* assigned in Fischer's formula.

3. The precursor of phaeopurpurin 7 seems to be a second form of chlorophyll *a*; the carbomethoxy group in this compound is attached to the pyrrole ring. Evidence has been obtained indicating the existence of this second form in crude chlorophyll from a number of sources. A lactam

formula for chlorophyll *a* seems to offer the best explanation at present of the transformations of the predominant form and to allow for the existence of a second similar form with the methoxyl group in a different position.

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Notes

The Intramolecular Rearrangement of Phenyl Ethers with the Aid of Aluminum Chloride

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The literature contains reports concerning the rearrangement of alkyl phenyl ethers to substituted phenols through the agencies of heat, zinc chloride, hydrogen chloride, sulfuric acid, sulfuric and glacial acetic acids, boron fluoride, etc.

Due to the fact that rearrangement of *m*-cresyl isopropyl ether leads to the formation of the commercially important thymol (and its isomers), this transformation has received careful study.[1] Niederl and Natelson first effected the reaction with a sulfuric–glacial acetic acid solution; the same products were obtained by Sowa, Nieuwland and Hinton using boron fluoride as the rearranging agent. The same products obtained by these workers are also obtained when aluminum chloride is used in the role of the agent favoring the intramolecular rearrangement. This agent simplifies the experimental procedure. It is believed that this type of rearrangement holds promise for further study into the interesting field of ether rearrangement, and a more comprehensive study is contemplated.

Procedure.—One mole of aluminum chloride was slowly added (twenty minutes) to one mole of *m*-cresyl isopropyl ether contained in a flask surrounded by a cooling bath. Heat was evolved, and the liquid became discolored. The mixture was allowed to stand for twenty-four hours and then hydrolyzed with ice water. The organic liquid which then separated was water washed and distilled. Practically all the liquid came over between 228 and 224°; it has been shown in the previous rearrangements that such a fraction consists of thymol and the para substituted isomer, *p*-isopropyl-*m*-methylphenol. The product was alkali soluble and ferric chloride colored its alcohol solution; n_D^{22} 1.5274. The yield of redistilled product was 65%. There was no unrearranged ether and no *m*-cresol.

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(1) Niederl and Natelson, THIS JOURNAL, **53**, 1928 (1931); **54**, 1063 (1932); Niederl, Natelson and Smith, Indianapolis Meeting of the American Chemical Society, 1931; Sowa, Nieuwland and Hinton, THIS JOURNAL, **54**, 2019 (1932).