2. The methods of preparation and reactions of these substances indicate that they are halogen salts of the hypothetical ortho benzene seleninic acid, $C_6H_5Se(OH)_3$, and that this latter compound has basic properties.

SWARTHMORE, PENNSYLVANIA

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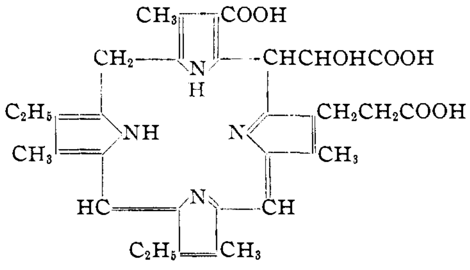
Studies in the Chlorophyll Series. X. The Esters of Chlorin *e*

BY J. B. CONANT AND K. F. ARMSTRONG[1]

The structure of chlorin *e* has been established by a variety of transformations as dihydroisorhodoporphyrin glycolic acid (Formula I).[2]



I

When esterified with diazomethane, hydrochloric acid and methyl alcohol, or by the action of dimethyl sulfate on the potassium salt, a trimethyl ester is formed. The identity of these esters has been investigated by converting them into the copper and zinc complex salts and taking mixed melting points. The results indicated that all the esters are identical. The complex metal salts of the esters of chlorin *e* are very suitable for the characterization of these compounds as they have sharp melting points; however, in our opinion all identification by mixed melting points in the chlorophyll series must be accepted with some reservation. Since this work was completed, a paper by Fischer and Siebel[3] has appeared in which the identity of these same esters was concluded from the mixed melting points of the esters themselves, and from their transformations with hydrogen iodide.

On hydrolysis with alkali, the trimethyl ester regenerates chlorin *e*. The saponification was carried out at room temperature and in an atmosphere of nitrogen to avoid the possibility of oxidation. The sole

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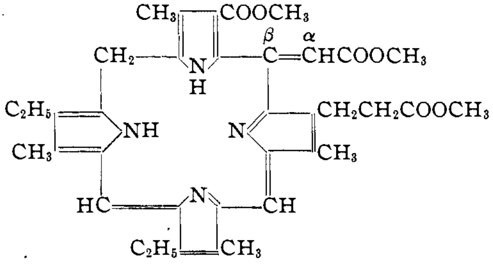
(1) Henry Fund Fellow at Harvard University.

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

(3) Fischer and Siebel, *Ann.*, **499**, 84 (1932).

product was a crystalline chlorin *e* which on oxidation with molybdicyanide gave an almost quantitative yield of carbon dioxide; the other product of the reaction was chlorin *k*. This reaction is characteristic of the grouping —CHOHCOOH as has been shown in a study of chlorin *e* prepared from phaeophorbide.[2] The analysis of this regenerated chlorin *e* corresponds closely to the empirical formula $C_{34}H_{36}N_4O_6$ while the material prepared from phaeophorbide usually has the composition $C_{34}H_{38}N_4O_7$. In spite of this difference in analysis we are convinced of the essential identity of the two materials because of their identical behavior in the rather specific reaction with molybdicyanide. This reaction excludes the possibility that in the esterification of chlorin *e* the secondary alcohol group has been reduced to a CH_2 group as at one time postulated by Fischer[4] in order to account for the analyses of the trimethyl ester and the regenerated chlorin *e*. As will be shown in the course of this paper, the behavior of chlorin *e* and its esters indicates that the characteristic grouping of these compounds is $>$CHCHOHCOOH(R) which in most instances is in equilibrium with $>$C═CHCOOH(R). Whether the hydrated or unhydrated form will be obtained as the isolated material may be expected to depend on slight variations of procedure. There is also the possibility that stereochemical modifications of chlorin *e* exist, both of which in solution are hydrated but one of which can be isolated in the solid state only as the unsaturated compound (dehydrated).

The most probable formula for the trimethyl ester is that of an unsaturated ester (II), a possibility previously suggested in a paper from this Laboratory.[5]



II

In his most recent paper Fischer[3] has also considered the possibility of the trimethyl ester being an unsaturated ester. He has pointed out that the addition of water to such a system might proceed so that the hydroxyl group is attached to the α carbon, instead of to the β carbon (formula II) as would normally be expected. The presence of two pyrrole nuclei might direct the addition in this manner.

To obtain further evidence in regard to the structure of the trimethyl

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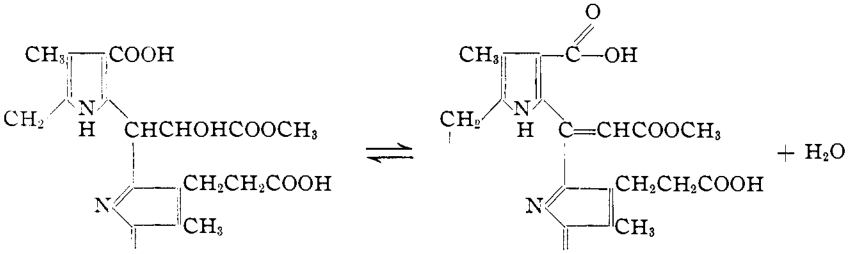
(4) Fischer, Moldenhauer and Süs, *Ann.*, **486**, 126 (1931).

(5) Catherine C. Steel, THIS JOURNAL, **53**, 3174 (1931).

ester of chlorin *e* we have prepared two different monomethyl esters. One of these (the beta ester) is formed by the partial methylation of chlorin *e* with diazomethane; the other (the alpha ester), by the partial hydrolysis of the trimethyl ester. On treatment with diazomethane in excess they are both converted into the trimethyl ester (identity established by mixed melting points of the copper derivatives). On ethylation with diazoethane they yielded two different diethyl methyl esters each having a copper derivative with a characteristic melting point. On further hydrolysis, the alpha ester (obtained by hydrolysis from the trimethyl ester) yields chlorin *e*.

By controlled hydrolysis of the trimethyl ester in cold 12% hydrochloric acid we have obtained a dimethyl ester, which appears to be different from the dimethyl ester obtained by Fischer and Siebel[3] by partial methylation with methyl alcohol and hydrochloric acid.

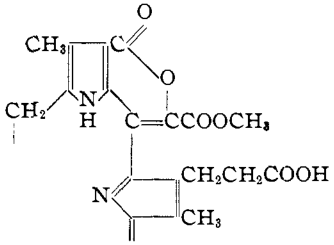
The structure of the alpha ester is established by the following facts. It is oxidized by molybdicyanide but no carbon dioxide is evolved; the product of oxidation (a new chlorin) on treatment with diazomethane yields only a *diester*. On pyrolysis the alpha ester readily loses only one mole of carbon dioxide yielding a monomethyl ester (a chlorin) which on treatment with diazomethane is methylated to a diester. If one considered only these reactions one would write formula III for the essential grouping in the molecule; the analytical data show, however, that a molecule of water has been lost; the anhydro form IV, therefore, suggests itself as the most probable formula.



(Anhydro form)

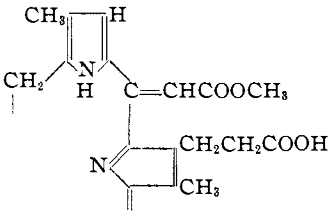
III IV

In solution the two forms would have to be interconvertible in order to explain the oxidation results; the mode of addition of a molecule of water was discussed in a preceding paragraph. Since the oxidation product of the alpha ester forms only a diester it must be analogous to chlorin *k*.[2] The keto group formed by the oxidation enolizes and the grouping $>C{=}C(OH)$-$COOCH_3$ on the bridge then loses water with the carboxyl group in the beta position. Formula V thus formulates the groups in this compound. The pyrolysis product of the alpha ester would have the grouping shown in VI. The analytical data correspond to these formulas.



V

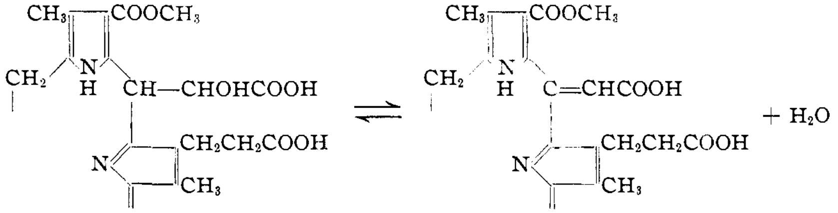
Monomethyl ester of chlorin *k* carboxylic acid
(oxidation product of alpha ester)



VI

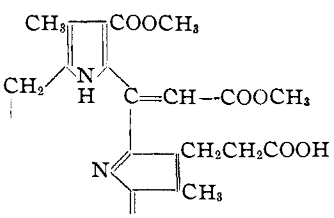
Monomethyl ester of pyrochlorin *e* carboxylic acid
(Pyrolysis product of alpha ester)

The structure of the beta ester is established by the fact that on oxidation with molybdicyanide, carbon dioxide is evolved. Unfortunately, the product which should be an aldehyde could not be isolated as it seemed to form an insoluble complex with the reagent. Experiments designed to obtain this interesting compound by the use of other oxidizing agents are in progress. On pyrolysis the beta ester loses carbon dioxide and yields a mixture of compounds from which an unidentified monomethyl ester of a porphyrin and a monomethyl ester of chloroporphyrin e_4 both of which were alkali soluble, were obtained. The fact that an ester of the latter porphyrin which contains a carboxyl group directly attached to the pyrrole ring was obtained after a drastic pyrolysis shows that the beta carboxyl group must have been esterified. This fact together with the oxidation results conclusively places the methyl group on the carboxyl group directly attached to the pyrrole ring. Like the alpha ester, however, the analyses of the beta ester indicate that the solid is an anhydro compound. In solution we must imagine that there is an interconversion of forms VII and VIII.



VII VIII

The positions of the methoxyl groups in the two isomeric monomethyl esters can be considered as well established; the nature of the anhydro linkage may be regarded as possibly less certain.



IX

The structure of the dimethyl ester obtained by acid hydrolysis is established by these facts: its analysis corresponds to $C_{36}H_{40}O_6N_4$, it is not oxidized by molybdicyanide, and does not yield carbon dioxide on pyrolysis. It differs therefore from the trimethyl ester in that the propionic acid group is free (IX).

We have examined the behavior of the various esters of chlorin *e* in the "phase test." In addition to chlorin *e* triester, the diester and monomethyl ester made by its partial hydrolysis yield on shaking their ether solutions with 25% methyl alcoholic potassium hydroxide, unstable chlorins which on methylation at once yield phaeopurpurin 7, or on standing are converted into phaeopurpurin 18.

Chlorin *e* and beta monomethyl chlorin *e* do not yield phaeopurpurin. According to the ideas suggested in this Laboratory the essential reaction in an oxidative hydrolysis is the conversion of the group $—CHOHCOOCH_3$ (or its anhydro derivative) into $—COCOOCH_3$; this should be possible with the trimethyl, dimethyl and alpha monomethyl esters, but in chlorin *e* and beta monomethyl chlorin *e* the group $—CH(OH)COO—$ would not be readily oxidized by the action of oxygen, or, if oxidized, would yield carbon dioxide.

The parallelism between the behavior of the ester of chlorin *e* which has the group $>C=CHCOOCH_3$ and the phaeophorbides is very striking. In both cases the oxidative hydrolysis under the special phase test conditions is rapid and the products are identical. In the case of the alpha monomethyl ester the analogy is even closer, as the compound is oxidized by potassium molybdicyanide, thus behaving as though it had the grouping $>CHCHOHCOOCH_3$ and yet its analyses correspond to the grouping $>C=CHCOOCH_3$. As has been pointed out elsewhere,[6] the same situation exists in regard to the phaeophorbides; this parallelism seems to us to find a satisfactory explanation if the predominant form of phaeophorbide also contains the grouping $>CHCHOHCOOCH_3$ in equilibrium with its anhydro form $>C=CHCOOCH_3$.

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

Experimental

Chlorin *e* Trimethyl Ester.—Chlorin *e* was esterified with methyl alcohol as follows: 500 mg. of chlorin *e* was dissolved in 100 g. of methyl alcohol saturated with 30 g. of dry hydrogen chloride. After standing for forty hours at room temperature over half the product was alkali-insoluble trimethyl ester; yield, 200 mg. crystallized from ether. The alkali-soluble material was extracted with 6% hydrochloric acid and crystallized from ether; its analysis was: OCH_3, 8.12, 7.86; calcd. for two groups: 9.9.

Melting Points of the Trimethyl Esters of Chlorin *e*.—Samples of the trimethyl ester prepared by esterification with diazomethane, dimethyl sulfate and alkali and methyl alcohol and hydrogen chloride, respectively, were investigated by taking mixed melting points. All melting points recorded in this paper were taken with a block, with the same thermometer, and are uncorrected.

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(6) THIS JOURNAL, **55**, 839 (1933).

Ester, mode of preparation	M. p., °C.
(1) Diazomethane	209–210
(2) Dimethyl sulfate	209–210
(3) Methyl alcohol	213.5–214.5

Mixed melting points: (1) + (2), 209–210°; (1) + (3), 211.5–213.5°; (2) + (3), 210–211.5°.

A sample of the dimethyl sulfate ester, very slowly crystallized, melted at 213.5–214.5°. The diazomethane ester m. p. 209–210° did not have its melting point raised on recrystallizing from chloroform–methyl alcohol, nor lowered again on subsequent crystallization from ether. The melting point of the trimethyl ester is dependent on the rate of heating. Thus, samples placed on the block at temperatures up to 185° melt at the normal temperature; placed on the block at above 185° they melt at once, resolidify, and melt again at the normal temperature. A sample placed on the block at 205° melts but does not resolidify. It appears that a form melting at about 187° is transformed into the higher melting modification on melting, or slowly, on heating below its melting point.

Copper Derivative of Chlorin *e* Trimethyl Ester.—The method of Treibs and Wiedemann was followed.[7] To 100 mg. of ester in 3 cc. of chloroform, a solution of 30 mg. of copper acetate in 3 cc. of methyl alcohol was added, and the solution evaporated to dryness. The product was three times crystallized from acetone–petroleum ether (58–70°), forming slender dark green needles.

Spectrum in ether (1 mg. in 30 cc.; 5-cm. tube): I, 660.9–615.5; II, 591.8–581.5; III, 505.4–495.6. E.A. 434.2. Order, I, III, II.

Anal. Calcd. for $C_{37}H_{40}N_4O_6Cu$: C, 63.44; H, 5.76; N, 8.00; Cu, 9.08; OCH_3, 13.30. Found: C, 62.33, 62.12; H, 5.90, 5.70; N, 7.91, 8.10; Cu, 9.56, 8.84; OCH_3, 14.44, 14.64.

The ether solution is blue green and does not fluoresce. The complex is extremely slowly decomposed by concentrated hydrochloric acid when in ether solution. Dissolved in equal parts of acetic acid and hydrochloric acid, it regenerates a chlorin in which the main red band is displaced further to the blue region than in chlorin *e*.

The copper derivatives of the trimethyl ester prepared in the three different ways were made separately and compared. Their spectra in ether were identical.

Esters, mode of preparation	M. p., copper derivatives, °C.
(1) Diazomethane	217–219
(2) Dimethyl sulfate	218–220
(3) Methyl alcohol	218–220

Mixed melting points: (1) + (2), 217–219°; (2) + (3), 218–220°; (1) + (3), 218–220°.

Zinc Derivative of Chlorin *e* Trimethyl Ester.—This was prepared exactly as the copper derivative, using zinc acetate: dark green needles; ether solution blue with strong red fluorescence.

Spectrum in ether (1 mg. in 30 cc.; 5-cm. tube): I, 658.5–622.2; II, 605.9–585.7; III, 525.6–513.4; E.A. 440.5. Order, I, II, III.

Anal. Calcd. for $C_{37}H_{40}N_4O_6Zn$: C, 63.26; H, 5.74; N, 7.98; Zn, 9.31; OCH_3, 13.26. Found: C, 63.18, 63.24; H, 6.16, 5.84; N, 7.63, 7.84; Zn, 9.06, 8.87; OCH_3, 14.19, 14.04.

The chlorin regenerated on shaking the ether solution for a few minutes with 15% hydrochloric acid is identical in spectrum, color, and acid number with chlorin *e* trimethyl ester.

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[7] Treibs and Wiedemann, *Ann.*, **471**, 171 (1929).

The zinc derivatives of the three differently prepared chlorin *e* trimethyl esters have the same spectrum.

Esters, mode of preparation	M. p. Zn derivative, °C.
(1) Diazomethane	237–238
(2) Dimethyl sulfate	243–245
(3) Methyl alcohol	236–237

Mixed melting points: (1) and (2), 239–243°; (2) and (3), 236–243°; (1) and (3), 236–237°. Repeated recrystallization did not raise the melting points of the diazomethane and methyl alcohol esters' zinc derivatives to that of the dimethyl sulfate ester. It may be observed that our unsaturated formula for the triester gives a possibility for geometrical isomerism.

Copper Derivative of Chlorin *e* Triethyl Ester.—This was prepared by adding copper acetate in boiling methanol to the ester in chloroform. The triethyl ester was prepared by the use of diazoethane and agreed in its properties with those reported by Fischer and Siebel.[3] After two minutes of boiling, methanol and excess copper acetate were washed out with water, the chloroform solution dried and evaporated to dryness and the copper derivative crystallized from ether–petroleum ether. This procedure was used in all triethyl and mixed ethyl–methyl esters to avoid ester interchange in crystallizing from methanol; m. p. 154–155°. Spectrum in ether (1 mg. in 30 cc.; 5-cm. tube): I, 655.4–617.4; II, 594.0–582.2; III, 506.6–494.4. E. A. 429.7. Order: I, III, II.

Anal. Calcd. for $C_{40}H_{46}N_4O_6Cu$: C, 64.70; H, 6.25; N, 7.55; Cu, 8.57; OC_2H_5, 18.22. Found: C, 64.49; H, 6.53; N, 8.95; Cu, 8.49; OCH_3, 18.72.

Regeneration of Chlorin *e* from the Trimethyl Ester.—700 mg. of crystalline trimethyl ester in 20 cc. pyridine was hydrolyzed with 7.5 g. potassium hydroxide dissolved in 100 cc. methyl alcohol and 5 cc. of water on standing overnight in a nitrogen atmosphere at room temperature. Almost the whole product was of acid number 3, and agreed in color, spectrum and acid number with chlorin *e*; yield 350 mg. of crystalline material (from ether). Dried for analysis *in vacuo* at 60°. Calcd. for $C_{34}H_{36}O_4N_6$: C, 68.42; H, 6.08; N, 9.39. Found: C, 69.21, 69.08; H, 6.37, 6.17; N, 9.39, 9.51; OCH_3, 0.0.

One hundred mg. of this chlorin was oxidized in a filtered oxidizing mixture made up as follows: 3 g. of potassium molybdocyanide, 2 cc. of acetic acid, 7 cc. of water, oxidized with permanganate and made up to 100 cc. with 10 cc. of pyridine and a molar solution of pyridine in acetone. An 80% yield of carbon dioxide (determined as barium carbonate) was obtained, together with chlorin *k*, identified by color and spectrum in ether and acid, and acid number. A duplicate experiment, conducted on the material which had been dried for analysis, gave 53% of carbon dioxide yield together with chlorin *k* and a small amount of unidentified porphyrin (acid number eight).

Experiments on the regeneration of chlorin *e* from its trimethyl ester at higher temperatures were unsatisfactory; 400 mg. of ester in 2 cc. of pyridine was given a hot quick saponification for four minutes with 30 cc. of boiling 25% potassium hydroxide in methyl alcohol in a nitrogen atmosphere. Fractionation of the product gave 160 mg. of crystalline chlorin extracted by 4% acid; found: OCH_3, 0.7%. Oxidation with molybdicyanide gave a yield of carbon dioxide of only 40%, even after correcting for the presence of a methoxy compound which would not yield carbon dioxide. Another experiment in which the solution was boiled for five minutes with 25% methyl alcoholic potash and for five more minutes after addition of an equal volume of water gave 175 mg. of chlorin; found: OCH_3, 0.95%. The corrected carbon dioxide yield was 54%. Since chlorin *e* and some of its derivatives give almost quantitative yields of carbon dioxide in oxidation with molybdicyanide, it is clear that in the above experiments, in addition to the methoxy derivative, and the chlorin which yields carbon dioxide and chlorin *k*, there is produced another chlorin which does not yield carbon dioxide on oxidation.

Alpha Monomethyl Ester of Chlorin *e*.—1.5 g. of chlorin *e* trimethyl ester dissolved in 10 cc. of pyridine was hydrolyzed with 300 cc. of 12% potassium hydroxide in methyl alcohol in a flask freed of oxygen and refilled with nitrogen. After standing for ten minutes at room temperature, the product was transferred to 2.5 liters of ether, washed free of pyridine and methyl alcohol, and acid fractionated. There was only a trace of unhydrolyzed trimethyl ester. The bulk of the material was a chlorin of acid number 5, identical in color and spectrum with chlorin *e;* yield of crystalline material from acetone–petroleum ether, 45%. There remained in the ether, alkali-soluble chlorin extractable with 7 or 8% hydrochloric acid.

Anal. (dried at 60° *in vacuo*). Calcd. for $C_{35}H_{40}O_7N_4$: C, 66.84; H, 6.42; N, 8.91; OCH_3, 4.94. $C_{35}H_{38}O_6N_4$: C, 68.81; H, 6.28; N, 9.18; OCH_3, 5.08. Found: C, 68.40, 68.66; H, 6.24, 6.51; N, 8.57, 8.56; OCH_3, 4.91, 5.35.

Five mg. was shaken in 10 cc. of ether with 1 cc. of 25% potassium hydroxide in propyl alcohol. Transferred to fresh ether after ten minutes, the product on fractionation was shown to consist of unchanged chlorin *e* alpha monomethyl ester and phaeopurpurin 18. The presence of unchanged material is explained by the separation of an insoluble potassium salt.

Conversion of Alpha Monomethyl Chlorin *e* to Trimethyl Ester of Chlorin *e*.—Sixty mg. of mono ester was introduced in 1 cc. of pyridine into 500 cc. of ether and methylated with diazomethane. The ether solution was successively washed with acid, alkali and water, dried and evaporated to dryness. The residue was dissolved in 5 cc. of chloroform and treated with 20 mg. of copper acetate in 15 cc. of boiling methyl alcohol. On boiling off the chloroform the copper derivative crystallized in green needles. Twice recrystallized from chloroform–methanol it melted at 217–219° (block uncorr.) and showed no depression with the copper derivative of the trimethyl ester made directly from chlorin *e* (m. p. 217–219°).

Oxidation of Alpha Monomethyl Chlorin *e*.—Electrometric titration of the oxidation with potassium molybdicyanide in acetone–pyridine showed that 79% of two equivalents of reagent were used in thirty-five minutes, and 102% in five hours; 60 mg. was oxidized in an apparatus designed to collect any carbon dioxide evolved, in a stream of nitrogen in a filtered oxidizing mixture made up as follows: 0.240 g. of molybdocyanide in 10 cc. of water, with 1 cc. of glacial acetic acid, oxidized with permanganate and then made up with 8 cc. of pyridine, and acetone to 90 cc. No carbon dioxide was evolved, whereas in a control experiment 58 mg. of chlorin *e* gave a 74% yield of carbon dioxide as barium carbonate. The product of the oxidation was a chlorin.

Monomethyl Ester of Chlorin *k* Carboxylic Acid

Chlorin from Oxidation of Alpha Monomethyl Chlorin *e*.—For preparative purposes 300 mg. was oxidized with a filtered mixture made as follows: 0.9 g. of potassium molybdocyanide, 20 cc. of water, 5 cc. of acetic acid oxidized with permanganate, and made up to 250 cc. with molar pyridine in acetone. After four hours the product was transferred to 2 liters of ether and washed free from acetone and pyridine; 7–12% acid extracted a small quantity of a mixture of chlorins which was discarded. The main product was a chlorin, extracted with 14 and 16% hydrochloric acid; yield 50% of the chlorin, crystallized from acetone–petroleum ether; OCH_3 calcd. for $C_{35}H_{36}O_6N_4$: 5.11. Found: 5.23, 5.34. The substance has a typical chlorin color in ether; the acid solution is blue with red fluorescence. A sample was methylated with diazomethane, and the product crystallized from acetone–petroleum ether, m. p. 260–262°. Acid number of ester, 16. Spectrum in ether (1 mg. in 30 cc. 5-cm. tube): I, 684.9–650.4; II, 616.7–603; III, 534.4–525.9; IV, 519.1–487.6. E.A. 432.2. Order: I, IV, III, II. Spectrum in acid: I, 674.8–635.8; II, 606.4–593.1; III, 570.0–557.4; IV, 532.9–517.5. E. A. 443.0. Order: I, IV, III, II.

Anal. Calcd. for $C_{36}H_{38}N_4O_6$: C, 69.40; H, 6.16; N, 9.00; OCH_3, 9.97. Found: C, 69.23, 69.53; H, 6.91, 7.00; N, 8.29; OCH_3, 9.62, 9.18.

Monomethyl Ester of Pyrochlorin *e* Carboxylic Acid

Pyrolysis Product Alpha Monomethyl Chlorin *e*.—120 mg. was heated in 20 g. of diphenyl in an oil-bath at 215° for three and a half hours in a stream of nitrogen freed from oxygen by passing over red-hot copper wire, and dried with sulfuric acid and phosphorus pentoxide. The carbon dioxide evolved was absorbed in a potash bulb; yield of carbon dioxide 6 mg.; theory for 1 mole 8.65 mg.; 70% yield. The product was dissolved in 15% acid and washed with ether to remove diphenyl and then transferred to 1 liter of fresh ether; 4 and 5% acid extracted a little porphyrin; 6 and 7% acid removed traces of porphyrin and some chlorin. The main product was a chlorin, extracted with 10 and 11% acid; yield 50 mg., crystallized from acetone–petroleum ether, 42%.

Anal. Calcd. for $C_{34}H_{38}O_4N_4$: C, 72.04; H, 6.76; N, 9.88; OCH_3, 5.47. Found: C, 72.20, 71.44; H, 7.15, 6.72; N, 9.76, 9.78; OCH_3, 5.81. Esterified with diazomethane it gave a diester; OCH_3: 10.02, 10.24, m. p. 206–208° (block uncorr.). Spectrum (1 mg. in 30 cc. ether, 5-cm. tube). Ether solution typical chlorin color. I, 682.4–647.6; II, 613.8–602.3; III, 531.6–525.0; IV, 507.6–485.3. E. A. 433.0. Order: I, IV, III, II.

Spectrum in acid. Acid solution greenish blue with red fluorescence. I, 677.2–631.8; II, 611.2–598.4; III, 532.6–521.8; E.A. 446.7. Order: I, III, II.

Diethyl Alpha Monomethyl Ester of Chlorin *e*.—The alpha monomethyl ester was treated with diazoethane; the product was fractionated and extracted with 11% acid, transferred to ether and crystallized; m. p. 152°.

Anal. Calcd. for $C_{39}H_{46}O_6N_4$: C, 70.22; H, 6.96; N, 8.40. Found: C, 70.15; H, 6.78; N, 8.09.

The copper derivative prepared in the usual manner and crystallized from ether–petroleum ether melted at 166.5–167° (block uncorr.).

Beta Monomethyl Ester of Chlorin *e*.—0.614 g. of dry chlorin *e* (0.001 mole) dissolved in 2 cc. of dry pyridine (distilled over potassium hydroxide) and 50 cc. of dry dioxane (freshly distilled from sodium) was treated with a dry ether solution containing 0.0012 mole of diazomethane (standardized with benzoic acid), in a three-necked flask fitted with mercury-sealed stirrer, a calcium chloride tube, and a tap funnel delivering below the surface of the liquid. The diazomethane was added during twenty minutes with stirring, and after standing overnight the mixture was transferred to 2.5 liters of ether and washed free of dioxane. Three extracts with 3% acid and one of 4% sufficed to remove unmethylated chlorin *e*. The main product was isolated with 5% acid.

Anal. 3% fraction 2.80% OCH_3; 4% fraction 4.89% OCH_3; 5% fraction 5.07% OCH_3.

The yield of 5% fraction, crystallized from acetone–petroleum ether, 250 mg., was 40%. The acid number is 5, the color in ether and acid and the spectrum are identical with that of chlorin *e*.

Anal. Calcd. for $C_{35}H_{40}O_7N_4$: C, 66.84; H, 6.42; N, 8.91; OCH_3, 4.94. $C_{35}H_{38}O_6 \cdot N_4$: C, 68.81; H, 6.28; N, 9.18; OCH_3, 5.08. Found: C, 69.00, 68.72; H, 6.67, 6.32; N, 8.48, 8.83; OCH_3, 5.11, 5.05.

No conversion to phaeopurpurin under "phase test" conditions could be obtained.

Unextracted with 5% acid in the products of the partial methylation of chlorin *e*, is an alkali-soluble chlorin of acid number 7. Its yield can be increased by employing 2 moles of diazomethane. The analysis indicates it to be impure dimethyl ester. Calcd. OCH_3, 9.9. Found: 8.3, 8.7.

Oxidation of Beta-Monomethyl Ester of Chlorin *e*.—A 5-mg. sample used up 101% of 2 equivalents of potassium molybdicyanide in acetone–pyridine solution in sixty minutes; 62 mg. was oxidized with a filtered oxidizing mixture containing 0.4 g. of molybdocyanide, 2 cc. of glacial acetic acid, and 12 cc. of water, oxidized with permanganate and made up with 8 cc. of pyridine and acetone to 90 cc. The carbon dioxide evolved was swept out with nitrogen and weighed as barium carbonate: yield of barium carbonate in four hours, 13.4 mg.; calcd. for 1 mole 19.5 mg. or 69% yield. In a duplicate experiment 125 mg. gave 36.7 mg. of barium carbonate; calcd., 36.8; 100% yield.

On pouring into ether preparatory to working up the product, the whole material separated as an insoluble scum, which dissolved in 1% hydrochloric acid to give a blue, strongly fluorescing solution, which again gave the scum on neutralization. The scum was also soluble in alkalies. It appeared that the oxidation product had formed an insoluble complex with the oxidizing agent. The complex was not broken by hydrogen sulfide in acid, alkaline or ammoniacal solution, nor by continuous ether extraction of the acid solution. Other experiments designed to break down the complex without demethylating the chlorin proved unsuccessful.

Pyrolysis of Beta-Monomethyl Ester of Chlorin *e*.—120 mg. of ester was heated in 20 g. of diphenyl in a bath maintained at 245–265° in an oxygen-free, dry nitrogen stream for three hours. Carbon dioxide equivalent to 0.86 mole was evolved. The product was dissolved in acid, washed free of diphenyl with ether, transferred to fresh ether and the mixture of alkali soluble porphyrins obtained, acid fractionated. The products were a porphyrin extracted with 2% acid, identified by its color in ether and acid, and by its spectrum as chloroporphyrin e_4 (monoester).

Anal. OCH_3, calcd., 5.48. Found: 6.09.

Another porphyrin was extracted with 7% acid, but was not identified; OCH_3 found, 5.09.

Conversion of Beta-Monomethyl Ester to Normal Chlorin *e* Trimethyl Ester.—A sample was methylated with excess diazomethane, fractionated and transferred to fresh ether and converted into the copper derivative as described above. It melted at 217–219° (block uncorr.) and the mixed melting point with the copper derivatives of chlorin *e* trimethyl ester made directly from chlorin *e*, and the trimethyl ester made from alpha monomethyl chlorin *e* showed no depression.

Diethyl Beta-Monomethyl Ester of Chlorin *e*.—A sample of the beta ester treated with diazomethane gave on fractionation a triester, crystallized from ether, m. p. 155–157°. The copper derivative prepared in the usual way, crystallized from ether–petroleum ether, m. p. 187–189°.

Chlorin *e* Dimethyl Ester, by the Acid Hydrolysis of the Trimethyl Ester.—Suitable conditions were found by following the hydrolysis of the trimethyl ester in 12% hydrochloric acid, determining the percentage of alkali solubility and the methoxyl content of the alkali soluble material with time. At room temperature the dimethyl ester first formed is later slowly hydrolyzed to give a monomethyl ester. For preparative purposes, 750 mg. of triester was dissolved in 250 cc. of 12% hydrochloric acid, and stood at room temperature for three hours. On working up the product, about 100 mg. of triester remained unhydrolyzed. The alkali-soluble material, crystallized from ether, gave 310 mg. of excellent crystals, of no definite melting point, sintering on the block above 230°. The spectrum and color is identical with that of chlorin *e*.

Anal. Calcd. for $C_{36}H_{40}O_6N_4$: C, 69.19; H, 6.45; N, 8.97; OCH_3, 9.93. Found: C, 69.40, 69.61; H, 6.52, 5.90; N, 9.07, 8.83; OCH_3, 9.72, 9.31.

The dimethyl ester is not oxidized by molybdicyanide; 5 mg. of dimethyl ester in 15 cc. of ether, shaken with 1.5 cc. of 25% potassium hydroxide in methyl alcohol, gave unstable chlorins which on methylation at once gave dimethyl phaeopurpurin 7, or on standing, phaeopurpurin 18.

Pyrolysis of Dimethyl Ester.—100 mg. of material was heated in diphenyl with a 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

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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).