

[CONTRIBUTION FROM THE CONVERSE MEMORIAL LABORATORY OF HARVARD UNIVERSITY]
**STUDIES IN THE CHLOROPHYLL SERIES. I. THE THERMAL
DECOMPOSITION OF THE MAGNESIUM-FREE COMPOUNDS**

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Chlorophyll *a* is the magnesium derivative of phaeophytin *a*, which contains the groupings $-\text{OC}_{20}\text{H}_{39}$ and $-\text{OCH}_3$ presumably as ester groups. By the replacement of the phytol alcohol radical by methyl, methyl phaeophorbide *a* results, which contains, therefore, two methoxyl groups. The replacement of the phytyl group by hydrogen produces phaeophorbide *a*, a monobasic acid, from which by alkaline hydrolysis the dibasic acid chlorin *e* is formed. In this last reaction a deep-seated change has probably taken place in addition to hydrolysis.¹ A similar set of transformations of chlorophyll *b* yields methyl phaeophorbide *b*, phaeophorbide *b* and rhodin *g*.

We have found that all of these magnesium-free compounds lose carbon dioxide when heated in dilute diphenyl solutions to 180–250°. The process may be followed quantitatively by passing nitrogen through the solution and absorbing the carbon dioxide in the usual way. Crystalline products may be isolated by dissolving the reaction mixture in ether and fractionating with hydrochloric acid according to the Willstätter–Mieg procedure.² The results are summarized in Table I.

It is evident from an inspection of Table I that all the magnesium-free derivatives of both the *a* and the *b* series which we have examined, with the exception of the trimethyl ester of chlorin *e*, lose carbon dioxide under the conditions of our experiment. The phaeophorbides lose one molecule of carbon dioxide whereas chlorin *e* and rhodin *g* lose two molecules. The analyses of the crystalline products which we have isolated in the *a* series correspond to the loss of the number of moles of carbon dioxide noted in our quantitative decompositions. Definite but irregular amounts of water are formed in the reaction, as shown by the increase in weight of the calcium chloride absorption tube, but the blank was so large that no conclusions could be drawn from the results. It is possible that water may be eliminated and then added during the isolation of the products.

It is significant that the trimethyl ester of chlorin *e* does not lose carbon dioxide, whereas methyl phaeophorbide *a* loses one molecule. Since the methyl phaeophorbide contains no free carboxyl group, this carbon dioxide must come from some special grouping of the molecule. The decomposition of an ester group is made unlikely by the fact that chlorin *e* trimethyl ester does not lose carbon dioxide on heating. A lactone seems to be the

¹ Willstätter and Stoll, "Investigations on Chlorophyll," translated by Schertz and Merz, Science Printing Company, 1928, p. 25.

² Willstätter and Mieg, *Ann.*, 350, 1 (1906).

TABLE I
SUMMARY OF DATA OBTAINED IN THE THERMAL DECOMPOSITION OF THE MAGNESIUM-FREE CHLOROPHYLL COMPOUNDS

(1) <i>a</i> Series						
Substance	Moles of CO ₂ per mole	Temp. in oil-bath, °C.	Time of heating, hours	Product	Yield, %	
Methyl phaeophorbide <i>a</i> (m. w. = 614)	0.84	200-240	6.6	Methyl	21	
	0.70	220-250	7.0	pyropheo-	..	
	0.94	250	4.0	phorbide <i>a</i>	21	
Phaeophorbide <i>a</i> (m. w. = 600)	1.2	210-225	4	Pyropheo-	..	
	1.1	220-240	4	phor-	} 23	
	1.1	200-250	1	bide <i>a</i>		
Chlorin <i>e</i> (anhydrous) (m. w. = 630)	0.94	140-150	1	Pyrochlorin <i>e</i>	20	
	1.91	140-170	6	Pyrochlorin <i>e</i>	23	
				Porphyrin	3	
	2.0	250	5	Pyrochlorin <i>e</i>	4	
				Porphyrin	15	
	1.9	250	11		..	
1.4	160-175	2.5		..		
Chlorin <i>e</i> (Lactam hydrate)	1.6	160-185	2.5	Pyrochlorin <i>e</i>	44	
	1.8	240-250	3	Porphyrin	3	
Chlorin <i>e</i> trimethyl ester	0.07 ^a	250	3	Unchanged ester		
(2) <i>b</i> Series (products not yet isolated)						
Methyl phaeophorbide <i>b</i> (m. w. = 638)	0.83	220-230	3			
	0.62	250	0.5			
Phaeophorbide <i>b</i> (m. w. = 624)	0.98	250	1.5			
	1.3	250	4.0			
Rhodin <i>g</i> (m. w. = 640)	1.9	250	8			

^a Within the experimental error (± 0.8 mg.).

The molecular weights used in calculating the number of moles of carbon dioxide are given; because of the uncertainties of the analyses these values are only approximate.

most probable explanation. As pointed out in our preliminary notice of this work,³ these results point to the presence of some such grouping as an unsaturated lactone bridge which could lose carbon dioxide.

Methylation experiments now in progress should yield further evidence in regard to the presence of a lactone linkage or a hydroxyl group. The fact that the spectra of the parent substances and the pyro compounds are so similar suggests that the pyrrole rings are in the same condition in both compounds.

Our analytical data, as well as the recently published results of Treibs⁴ and of Fischer⁵ indicate that there are five or six oxygen atoms in the

³ Conant and Hyde, *Science*, **70**, 1806 (1929).

⁴ Treibs and Wiedemann, *Ann.*, **471**, 146 (1929).

⁵ Fischer and Bäumlner, *ibid.*, **474**, 65 (1929).

phaeophorbide *a* molecule. The grouping $-\text{COOCH}_3$ is clearly present in methyl phaeophorbide and, if one accepts the conclusion that a lactone group is also present, this leaves the extra methoxyl group without sufficient oxygen to constitute a carbomethoxy group if there are but five oxygens. It should be pointed out, however, that there is no clear evidence that there are two carbomethoxy groups in methyl phaeophorbide *a*. The second methyl group may well be present as the methyl ether of an enol, for example, the enol of a lactam. A similar interpretation is possible with chlorin *e*; indeed, the fact that it is possible to obtain the trimethyl ester by the action of diazomethane on chlorin *e* as shown by Treibs⁴ seems to us strong evidence that the third methyl group in this instance is derived from an enol and not by the opening of a lactone or lactam ring. There seems to be no reason to believe that diazomethane would be effective in opening such groups.

Pyro-phaeophorbide *a* is an acid; this shows that the carbon dioxide from this compound must have resulted from some group other than the free carboxyl and that the acidic group in phaeophorbide *a* is stable at high temperatures. The final decomposition by alkali of all the compounds we have been mentioning leads to porphyrins,^{1,5} which contain the very stable grouping $-\text{CH}_2\text{CH}_2\text{COOH}$; it is quite clear that it is this carboxyl group which is free in phaeophorbide *a* and which is, therefore, attached to the phytol group in phaeophytin and in chlorophyll.

The two products formed by the pyrolysis of chlorin *e* we shall designate by the terms pyrochlorin *e* and pyrochlorin *e*-porphyrin. The former substance is green and has a spectrum somewhat similar to that of chlorin *e* itself, whereas the porphyrin has the general appearance, spectrum and characteristics of this very large class of substances. The two compounds are isomeric. The green pyrochlorin *e* is unstable and on standing for a few months undergoes a partial decomposition. By acid fractionation it is possible to obtain from the decomposed material a substance which is very similar to pyrochlorin *e*-porphyrin, but not identical with it. It is clear that in the pyrolysis of chlorin *e* we have stripped the molecule of all but one carboxyl group and in the green substance have a relatively simple representative of the chlorophyll series, which still has a spectrum similar to the more complex members. A further study of these products, as well as those from the *b* series and the magnesium-containing compounds, is now in progress and should throw more light on the question of the structure of the first transformation products of chlorophyll. It seems extremely probable that the carbon skeleton of the chlorophyll porphyrins as established recently by Fischer⁶ is present in all the chlorophyll series, as he has indicated. The ease of transformation of our green

⁶ Fischer and Treibs, *Ann.*, **466**, 188 (1928); Fischer and Schormüller, *ibid.*, **473**, 211 (1929).

pyrochlorin *e* into a porphyrin is more evidence pointing in this same direction.

In connection with the analytical data given in the experimental part of this paper, it should be mentioned that it is impossible to decide at present between several possible formulas for each compound. The same difficulties have been encountered by other workers in this field as pointed out by Fischer.⁴ We have given the calculated values for several different formulas in each case. Of the more probable formulas for methyl phaeophorbide *a* ($C_{36}H_{38}O_6N_4$, $C_{36}H_{38}O_5N_4$, $C_{35}H_{38}O_6N_4$, $C_{35}H_{38}O_5N_4$) we prefer the formula $C_{36}H_{38}O_6N_4$, since all our results can be best interpreted on this basis. Our analyses and quantitative results of pyrolysis are consistent in each case. At first sight, there appears to be a certain inconsistency between the formulas of the monobasic porphyrins and our pyro-compounds from chlorin *e*. The recent work of Fischer⁶ has established that there are 31 carbon atoms and 2 oxygen atoms in the chlorophyll monobasic porphyrins, yet our substances, which should be isomeric, have at least 32 carbon atoms and 3 oxygen atoms. It would seem that our method of decomposition has left some group in the molecule which is eliminated through the usual alkaline decomposition; it will be a matter of great interest to determine the nature and position of this group.

Experimental Part

Preparation and Analysis of Methyl Phaeophorbide *a*, Phaeophorbide *a*, Chlorin *e* and Chlorin *e* Trimethyl Ester.—Phaeophytin was used for the preparation of these substances. In all cases the methods of preparation used were those described by Willstätter and Stoll.¹ The products were all obtained in a nicely crystalline condition, and agreed closely in acid number, spectrum and crystal form with the descriptions given by the same authors. In most cases, however, the yields obtained were only one-half to two-thirds of those mentioned by Willstätter and Stoll.

In the preparation of chlorin *e* by using the cold hydrolysis over a long period of time, the product seemed to correspond to the form described as the lactam hydrate, while in other cases, using hot hydrolysis with pyridine, the so-called anhydrous form was obtained. Chlorin *e* trimethyl ester was prepared both by using dimethyl sulfate according to Willstätter, and according to Treibs and Wiedemann using diazomethane.⁴ Both products melted on the block at 202–204° and a mixed melting point showed no depression.

For analysis the materials were in most cases redissolved in ether and refracted with hydrochloric acid to insure purity. In all cases the samples were dried over phosphorus pentoxide for a period of from three to five days at a pressure of 10–20 mm. The following table gives the analytical results; these and all the other results reported in this paper were obtained by the Pregl micro methods.

Anal. Methyl phaeophorbide *a*: C, 70.38, 70.90, 71.21, 69.70; H, 6.23, 5.95, 6.06, 6.16; N, 9.38, 9.53, 9.33. Phaeophorbide *a*: C, 67.96, 68.72, 68.29, 68.00; H, 5.94, 6.10, 6.20, 6.53; N, 9.33. Chlorin *e*: C, 67.14; H, 6.49; N, 8.81, 8.96. Chlorin *e* trimethyl ester: C, 69.78, 69.03; H, 7.25, 6.88; N, 8.75.

Preparation of Pyro-compounds.—The method of pyrolysis involved heating the substance in diphenyl solution to the desired temperature for the required length of time.

The apparatus consisted of a side arm U-tube made from tubing thirty millimeters in diameter, and was about 150 mm. in height. This tube contained the diphenyl solution and was heated in an oil-bath. The outlet tube was attached to a 200-mm. length of 3-mm. tubing bent in the form of a U-tube. This served as an air-condenser to assist in preventing diphenyl from being carried along in the gas stream. To this tube was attached another U-tube containing calcium chloride followed by a potash absorption bulb. The whole system was swept out during the experiment by a slow stream of dry nitrogen, which served to agitate the diphenyl solution as well as to transfer any carbon dioxide or water formed to the absorption train. By frequent weighing of the absorption tubes the progress of the decomposition could be followed. The outlet side of the reaction tube was surrounded by a small copper cooling coil to prevent the diphenyl from distilling.

In starting an experiment, 20 g. of diphenyl was heated to boiling for a short time, a slow stream of nitrogen being passed through the apparatus. The absorption tubes were then weighed, replaced and the heating continued for one to three hours to determine any blank which might be involved. The blank on the carbon dioxide tube was negligible while that on the water tube was usually about a milligram per hour. The sample (150–50 mg.) was then introduced quickly into the hot diphenyl and the heating carried out at the desired temperature.

The results of the pyrolysis experiments have been summarized in Table I; some typical results are given in more detail below. Pyrolysis of chlorin *e*: temp., 160–175°; time, 2.5 hrs.; 0.1137 g. yielded 0.0116 of CO₂ and 0.003 g. of H₂O after subtracting a blank of 0.002 g. (0.0008 g. per hr.). Temp. 250°; time 5 hrs.; 0.1252 g. yielded 0.0171 g. of CO₂ and 0.0024 g. of H₂O after subtracting a blank of 0.0060 g. Because of the large blank (due to the volatility of the diphenyl), not much significance can be attached to the increase in weight of the calcium chloride tube. However, some water appears to be formed and in one experiment with chlorin *e* it was condensed in a freezing mixture.

The products were all purified in the same way. The reaction mixture was dissolved in 300 to 500 cc. of ether and fractionally extracted with various concentrations of hydrochloric acid according to the method of Willstätter and Mieg. The main acid fractions were then washed twice with ether to remove any traces of diphenyl and the colored substance retransferred to ether either by dilution of the acid layer or by partial neutralization with ammonia. These ether solutions were then washed with water or with very dilute potassium hydroxide in the case of an alkali insoluble product, dried with sodium sulfate and concentrated to a small volume. Usually concentration to 10 or 15 cc. caused the material to crystallize. For purification these materials were dissolved in ether and refractionated with acid.

The yields given in the table are obviously not true measures of the amounts of the compounds formed in the reactions; the losses during purification of such small samples are very large. In no case was the formation of carbon monoxide detected. In most cases a few milligrams of black ether-insoluble residue was found in the reaction mixture. In one instance, using a 0.5-g. sample of chlorin *e* and adding to the cold diphenyl, 0.3 g. of this black residue was formed. It would thus seem desirable to add the sample to the hot diphenyl.

Chlorin *e* trimethyl ester was the only substance recovered from the reaction mixture unchanged.

Methyl Pyrophaeophorbide *a*.—This substance crystallizes from ether in beautiful long flat needles which tend to grow in radiating clusters. Their color is deep blue with a metallic luster. The crystals redissolve readily though slowly in ether imparting to it an olive-green to brown color, indistinguishable from that of an ether solution of

pure methyl phaeophorbide *a*. It dissolves very readily in pyridine and rather slowly in acetone. The acid number is 15, the acid extract being colored a greenish-blue. The product is not itself extracted from ether by mild alkali but is hydrolyzed slowly in acid solution yielding a material which can be extracted with alkali. This substance is identical in acid number, spectrum and behavior toward alkali with pyropheophorbide *a*. The melting point of methyl pyropheophorbide *a* on the block is 220–225°. The spectrum by visual comparison is identical both in position and relative intensities with that of pure methyl phaeophorbide *a*. Unlike methyl phaeophorbide *a*, the pyro-compound does not give the phase test.

The spectra given below and with the other new compounds were measured in ether solution (concn. 0.1 g. per 5 liters); the thickness of the solution is given in mm. Dotted lines indicate fainter absorption.

100 mm.: 701.2--689.9—646.8; 622.9--619—601.2--597.4; (hazy) 565.6—553.5; 542.7—529.5; 519.2—491.7; 478.6—464.6; E. A. 444.6.

20 mm.: 679.5—656.3; 617.8—603.4; 540.1—531.9; 512.1—504.2--494.7; E. A. 433.

Anal. Calcd. for $C_{33}H_{38}O_4N_4$: C, 72.66; H, 6.57; N, 9.68—for $C_{33}H_{38}O_3N_4$: C, 75.00; H, 6.79; N, 9.65—for $C_{34}H_{38}O_4N_4$: C, 72.1; H, 6.72; N, 9.9—for $C_{34}H_{38}O_3N_4$: C, 74.2; H, 6.9; N, 10.2. Found: C, 74.03, 73.83, 73.93; H, 6.40, 6.04, 6.03; N, 10.14, 9.90.

Pyropheophorbide *a*.—This substance was extracted from ether solution by 13–14% acid to give a bluish-green solution. Its acid number is between 12 and 13. It crystallizes from ether in a micro-crystalline condition, probably needles, with a steel blue luster. The ether solution is colored olive-green to brown, practically identical in color with a solution of pure phaeophorbide *a*, and possesses the same reddish fluorescence. Toward other solvents its behavior is the same as phaeophorbide *a*. With dilute alkali an ether solution gives a yellowish-brown precipitate at first which gradually redissolves in the ether as well as the water layer, imparting a brown color suggestive of the phase test. It appears to give the phase test. On the block it melts between 210 and 220°. Spectroscopically this product is identical with phaeophorbide *a*, which in turn is identical with methylpyropheophorbide *a*.

20 mm.: 682.0--679.7—657.6--655.0; 615.4—604.4; 549.0—531.6; 512.9—491.5; E. A. 433.7.

100 mm.: 699.1--687.3—647.5--644.0; 621.6--619—601.7; 565.5—555.9; 541.0—529.4; 517.2—491.0; 481.3—462.1; E. A. 443.6.

Anal. Calcd. for $C_{34}H_{38}O_4N_4$: C, 72.34; H, 6.38; N, 9.92—for $C_{33}H_{38}O_4N_4$: C, 71.7; H, 6.52; N, 10.13—for $C_{34}H_{38}O_3N_4$: C, 74.4; H, 6.57; N, 10.21—for $C_{33}H_{38}O_3N_4$: C, 73.88; H, 6.71; N, 10.44.

In the formation of pyropheophorbide *a* there was obtained as a by-product a small amount of black micro-crystalline material. It was extracted from the mother liquor by 9% acid giving a green solution, and on retransferring to ether gave a dark purple-red color which was unusually intense considering the amount of material present. The quantity was too small for careful investigation, but the spectrum is given below.

20 mm.: 670.0—661.6; 588.4—579.5; 566—557.2—546.2; 525—497.4; E. A. 441.7.

100 mm.: 675—653.0; 604--591.9—580.3--574.8; 568.1—557.1--543; 527.3—494.3; E. A. 452.7.

Pyrochlorin *e*.—Pyrochlorin *e* is extracted from an ether solution by 8% acid, giving a deep greenish-blue solution. Its acid number is between seven and eight. It is so soluble in ether that the small quantities available could not be crystallized from that solvent. It was, however, obtained in a micro-crystalline condition by removing most of the ether, adding a small volume of ethyl alcohol and diluting gradually (over

a period of a day) with water to 50%. Prepared in this way it appears as a dark blue micro-crystalline powder. The color of its ether solution is identical with that of chlorin *e*. However, when the solution is shaken with dilute potassium hydroxide, the behavior is similar to that of an ether solution of pyropheophorbide *a*. A brown precipitate appears which only partially redissolves and colors both the ether and water layers yellowish-brown, a color suggesting the phase test. Its melting point is between 165–170°.

The spectrum is the same as that of chlorin *e* in relative intensities, but the corresponding lines are shifted slightly to shorter wave lengths, most noticeably in the red.

20 mm.: 670—649.3; 609—596.0; 532.3—525.5; 509.6—487.3; E. A. 430.8.

100 mm.: 679.6—641.1; 611.9—596.6; 560.9—550.2; 534.1—526.1; 516.5—483.4; E. A. 442.9.

Anal. Calcd. for $C_{32}H_{36}O_3N_4$: C, 73.60; H, 7.06; N, 10.40—for $C_{32}H_{36}O_3N_4$: C, 73.28; H, 6.87; N, 10.68. Found: C, 73.70, 74.14, 73.79, 73.52; H, 6.86, 6.96, 5.87, 6.04; N, 10.41, 10.26, 10.35.

Pyrochlorin *e* seems to be unstable; a carefully purified sample of the solid, after standing for three months, on refractionation with acid yielded a small quantity of material which was extracted with 1% acid. This material was red in ether solution and crystallized in beautiful ruby-red prismatic needles. The color of the ether solution and acid number suggest identity with pyrochlorin *e* porphyrin (isomeric with pyrochlorin *e*). The spectrum, however, seems slightly different.

50 mm.: 666.1—659.3—655.5—647; 594.1—582—574.8; 551.1—533.9; 522.3—487.2; E. A. 452.8.

Pyrochlorin *e* Porphyrin.—This substance was formed as a by-product in rather small quantities along with pyrochlorin *e*. It is extracted from the mother liquor easily with 1% acid and the acid number is between one-half and one. The acid solution is purple and gives a red ether solution on retransferring. The concentrated ether solution gives long red prismatic needles with a purplish-blue iridescence. The solubility was similar to that of phylloporphyrin. By shaking the ether solution with dilute potassium hydroxide, a rather insoluble potassium salt is formed.

20 mm.: 589.1—580.2—573.4; 548.1—533.4; 516—491.0; E. A. 433.3.

100 mm.: 665.6—657.5—651.0; 634.7—628.8; 593.4—580.9—573.5; 547.3—532.2; 519.3—487.0; E. A. 446.3.

Anal. Calcd. for $C_{32}H_{36}O_3N_4$: C, 73.60; H, 7.06; N, 10.40—for $C_{32}H_{36}O_3N_4$: C, 73.28; H, 6.87; N, 10.68. Found: C, 74.03, 74.23, 74.02; H, 6.31, 6.81, 6.73; N, 10.37, 10.67, 10.73.

Summary

1. Phaeophorbide *a*, methyl phaeophorbide *a* and chlorin *e* lose carbon dioxide on heating in dilute diphenyl solution to 180–250°. The amount of carbon dioxide evolved corresponds to the loss of one molecule with the phaeophorbides and two in the case of chlorin *e*. The analyses of the crystalline products confirm this conclusion. Two isomeric products are obtained from chlorin *e*, one a green substance the other a porphyrin.

2. The experiments with the phaeophorbides indicate that a lactone or similar grouping is the source of the carbon dioxide and that the phytol in phaeophytin is attached to the $-\text{CH}_2\text{CH}_2\text{COOH}$ group.