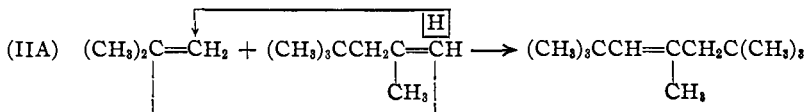
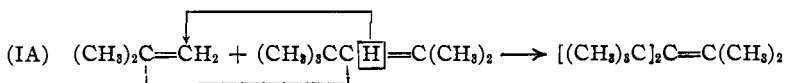


would produce the above olefins without requiring the presence of an alcohol or other derivative.



(IIIA) Same as III

### Conclusions

From the data thus obtained, it appears that the hydrocarbon, known as tri-isobutylene, prepared by the action of sulfuric acid on tertiary butyl alcohol exists in at least three isomeric forms. These olefins are present in an approximate ratio of two moles of pentamethyl-2,2,4,6,6-heptene-3,4 (formula II) and one mole each of trimethyl-1,2,4,4-*tert.*-butyl-3-pentene-2,3 (formula I) and dimethyl-4,4-neopentyl-2-pentene-1,2 (formula III). It also seems apparent that during hydrolysis of the ozonide the hydrocarbon of formula I undergoes a pinacolone rearrangement instead of breaking down into two simple ketones.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

## STUDIES IN THE CHLOROPHYLL SERIES. IV. THE DEGRADATION OF CHLOROPHYLL AND ALLOMERIZED CHLOROPHYLL TO SIMPLE CHLORINS

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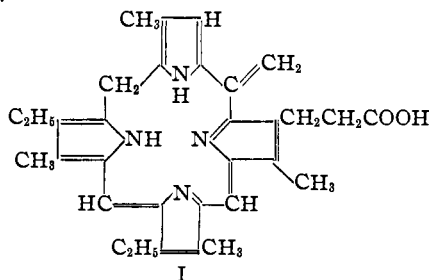
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The thermal decomposition of chlorin *e* was described in the first paper of this series; the chief product of the reaction is pyrochlorin *e*, a monobasic acid whose spectrum resembles that of the parent compound. We have now studied this relatively simple chlorin in more detail and found that it is very closely related to phylloporphyrin. The ester of pyrochlorin *e* appears to be isomeric with the ester of phylloporphyrin, although the pyrochlorin *e* itself contains an additional molecule of water (a similar relationship between the hydrated free acid and the ester is found with chlorin *e*). By catalytic hydrogenation or reduction with hydrogen iodide by Fischer's method<sup>1</sup> and reoxidation, phylloporphyrin is formed in good yields. In the light of Fischer's recent syntheses,<sup>2</sup> phylloporphyrin appears to have one more carbon atom than was formerly supposed. This explains

<sup>1</sup> Fischer and Bäumlner, *Ann.*, **474**, 65 (1929).

<sup>2</sup> Fischer and Helberger, *ibid.*, **480**, 235 (1930).

why this porphyrin ( $C_{32}H_{36}O_2N_4$ ) and pyrochlorin *e* have the same carbon skeleton although only *two* carbon atoms were eliminated from chlorin *e* ( $C_{34}H_{38}O_7N_4$ ) in the pyrolysis. We suggest formula I for pyrochlorin *e*; the transformation to phylloporphyrin would involve the change of a methylene to a methyl group by virtue of a shift of hydrogen. This formulation corresponds to one of the two alternative formulas for phylloporphyrin suggested by Fischer.



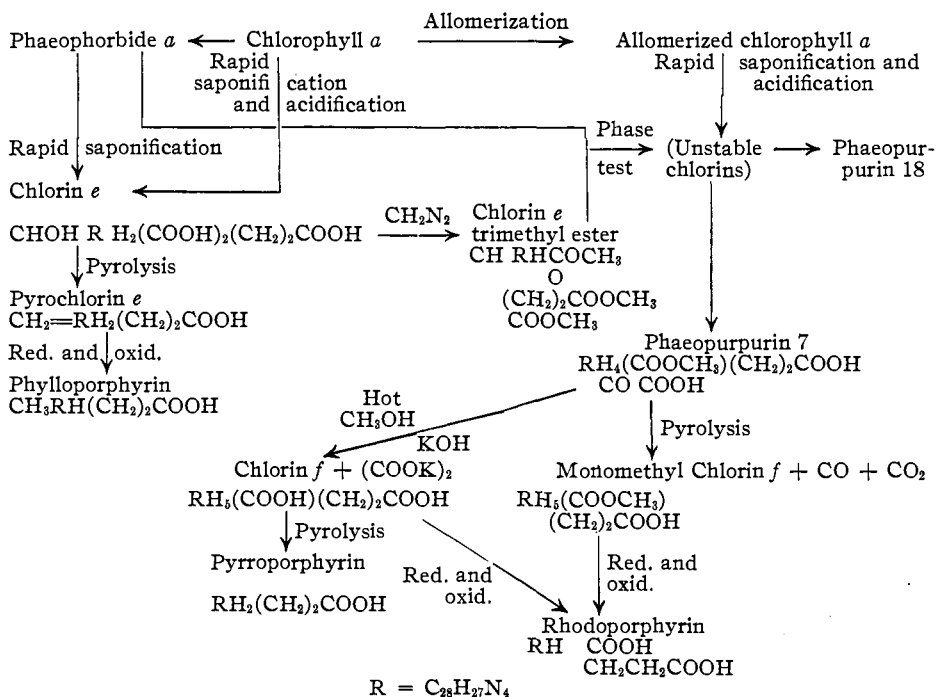
Pyrochlorin *e* may be considered as a normal degradation product of chlorophyll *a* since the relationship between chlorin *e* and the phaeophorbides was shown in the third paper to be closer than was at one time thought. (Both the phaeophorbides and the trimethyl ester of chlorin *e* give the phase test.)

After passing through the phase test the original structure of the chlorophyll molecule is in some way altered. This is apparent both from the failure of the products to give the phase test and from the fact that rhodoporphyrin or pyrroporphyrin and not phylloporphyrin is obtained from them in the reactions which convert chlorins to porphyrins. We have now been able to show that phaeopurpurin 7, a characteristic product of the phase test, is formed by the rapid saponification of allomerized chlorophyll. Thus it is clear that the changes brought about by the phase test and allomerization are essentially the same as Willstätter pointed out. The degradation products of the phaeopurpurins are related to allomerized chlorophyll *a*, just as pyrochlorin *e* is related to unallomerized chlorophyll *a*.

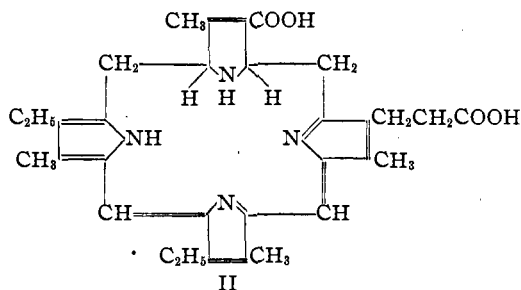
The degradation of both the "normal" and "allomerized" series are outlined in the accompanying diagram. The decomposition of the phaeopurpurins, and the reactions of pyrochlorin *e* are here reported for the first time and the details are given in the experimental portion of this paper. The most satisfactory account of all these changes is indicated by the formulas given in the diagram. The symbol R is taken for  $C_{28}H_{27}N_4$ : thus, rhodoporphyrin is  $RH \begin{matrix} \text{COOH} \\ \text{CH}_2\text{CH}_2\text{COOH} \end{matrix}$ . The analyses of the compounds fit into this scheme as well as could be expected—it is, of course, impossible to be certain of a few hydrogen atoms. (The calculated values given in the experimental portion all correspond to the formulas given in this diagram.)

The following facts seem established by these transformations. (1) Allomerization and the phase test are equivalent and result in the change from a potential phylloporphyrin structure to a potential rhodoporphyrin and pyrroporphyrin structure. (The last part of this statement is implied in Willstätter's diagram if not explicitly expressed.) (2) The  $\text{=C-OCH}_3$  group of methyl phaeophorbide is connected to the  $\beta$ -position of the pyrrole ring which in rhodoporphyrin holds the  $\text{COOH}$  group. This follows from the formation of rhodoporphyrin from monomethyl chlorin *f*, whose methoxyl group can be traced back to that of methyl phaeophorbide *a*. (3) Allomerization (phase test) changes the nature of one carbon atom of the chlorophyll skeleton so that it is easily eliminated together with carbon dioxide. Such elimination occurs in the change from phaeopurpurin 7 to chlorin *f* and monomethyl chlorin *f*. On the other hand, the same carbon atom is retained in the pyrolysis of chlorin *e* (unallomerized series).

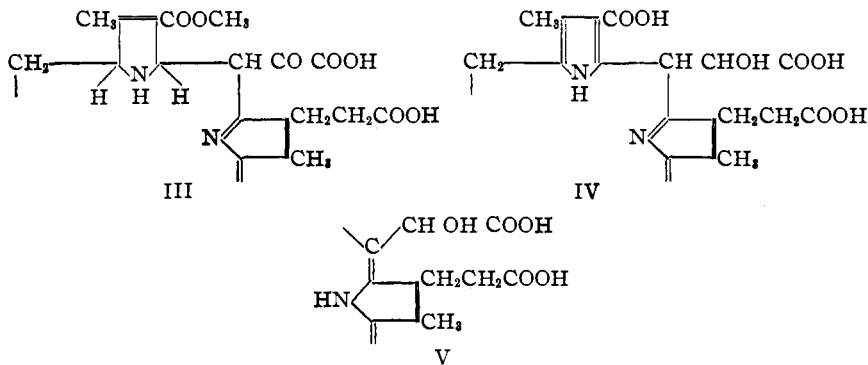
It seems to us that the most satisfactory explanation of the phase test (allomerization) is to be found in the assumption that it is essentially an oxidation reaction involving the "extra" carbon atom. The transfer of hydrogen may be intra-molecular to a pyrrole ring or intermolecular to reduction products not yet isolated. The first explanation has been incorporated in the formulas given in our diagram.



Chlorin *f* is the simple chlorin of the allomerized series; it differs essentially from pyrochlorin *e*, a simple chlorin of the unallomerized series, in that the extra carbon on the bridge has been eliminated. We suggest the following formula (II) for chlorin *f*:



If the foregoing account is correct, the essential group in phaeopurpurin 7 is an  $\alpha$ -keto acid on the bridge; this grouping has been suggested by Fischer as occurring in chlorin *e*. We have strong evidence for its presence (as shown in formula III) in phaeopurpurin 7, in that carbon monoxide is evolved in the pyrolysis and potassium oxalate is split off in the alkaline decomposition to chlorin *f*. For the corresponding compounds in the unallomerized series we might postulate such a grouping as that shown in IV, the change between the two being the internal oxidation and reduction previously referred to.



This grouping could also take the form V (with or without lactone formation from the  $\beta$ -carboxyl); the presence of the linkage  $\text{C}=\text{C}-\text{CHOHCOOH}$  would account for the ease with which chlorin *e* loses carbon dioxide without losing the "extra" carbon atom.

The nomenclature we have adopted warrants a word of explanation. The chlorin obtained from phaeopurpurin 7 by saponification is probably the same compound that Willstätter isolated from hydrolysis of altered

chlorophyll; we have therefore retained this investigator's name for it; it is perhaps identical with the chlorin 10 of Treibs and Wiedemann.<sup>3</sup>

A number of interesting by-products were obtained in connection with the transformations outlined in the diagrams. In addition to pyrochlorin *e* there is formed in the pyrolysis of chlorin *e* a porphyrin which we designated as "pyrochlorin *e* porphyrin" in our first paper. After this was published Fischer and Moldenhauer<sup>4</sup> reported the preparation of a compound which they designated as chloroporphyrin *e*<sub>3</sub>, from chlorin *e* by the action of hydrogen iodide. The absorption spectrum and properties of this compound appeared to be identical with those of our pyrochlorin *e* porphyrin and this was confirmed by preparing some of Fischer's compound by his method and comparing the two spectra in a Hartridge revision spectrocope. However, further study of our pyrochlorin *e* porphyrin convinced us that it was a mixture of phylloporphyrin with another porphyrin. We were able to separate this mixture by a long and tedious fractionation with hydrochloric acid. The chloroporphyrin *e*<sub>3</sub> was also resolved by the same method. The absorption spectrum of the new porphyrin which was thus separated from the admixed phylloporphyrin was distinctly different from phylloporphyrin and from that reported by us for pyrochlorin *e* porphyrin or by Fischer for chloroporphyrin *e*<sub>3</sub>. However, on placing solutions of phylloporphyrin and the new porphyrin in two glass cells and passing a beam of light through both the resulting absorption spectrum was identical with that given for both our porphyrin and chloroporphyrin *e*<sub>3</sub>. We propose to designate the porphyrin thus purified and freed of phylloporphyrin as pyrochloroporphyrin. Its analyses indicate that it is isomeric with phylloporphyrin.

In the conversion of the simple chlorins to porphyrins by means of reduction and oxidation, certain chlorins were formed which resembled in spectrum Fischer's synthetic chlorins. Thus, by the hydrogen iodide method we obtained from pyrochlorin *e*, a chlorin very similar to Fischer and Treib's phyllochlorin<sup>8</sup> which these investigators prepared from phylloporphyrin. A small but definite shift in one of the lines in the absorption spectrum and a difference of 15° in the melting point of the esters distinguishes our phyllochlorin from that of Fischer and Treibs. Some of the same phyllochlorin was obtained by catalytic hydrogenation and reoxidation of pyrochlorin *e*; this method was the most advantageous for the conversion to phylloporphyrin. The same phenomenon was met with in the allomerized series. In the conversion of chlorin *f* to rhodoporphyrin by means of hydrogen iodide, a chlorin was obtained in small amounts whose type of spectrum resembled the "synthetic" chlorins rather more than the parent chlorin or chlorin *e*. If the chlorin spectrum is characteristic of a partially

<sup>3</sup> Treibs and Wiedemann, *Ann.*, **471**, 146 (1929).

<sup>4</sup> Fischer and Moldenhauer, *ibid.*, **478**, 54 (1930).

hydrogenated porphyrin (or its equivalent, see formula I for pyrochlorin *e*), it is evident that many isomeric chlorins may be expected. From the pyrolysis of phaeopurpurin 7 we have also obtained two porphyrins in small amounts which appear to be new. The analysis of one of these (designated as porphyrin 8 in the experimental portion) indicates that it is isomeric with rhodoporphyrin monomethyl ester. The nature of this isomerism as well as that of pyrochloroporphyrin with phylloporphyrin (see above) remains to be explained.

The preparation of relatively large quantities of phaeopurpurin 7 and its dimethyl ester has been simplified by starting directly with phaeophytin *a* and *b* and putting this material through the phase test using as the reagent at room temperature a propyl alcoholic solution of potassium hydroxide. The unstable chlorins were treated with diazomethane, yielding dimethyl phaeopurpurin 7. From this by alkaline hydrolysis phaeopurpurin 7 may be obtained. As noted in the previous paper, it is particularly interesting that one methoxyl group is so much more resistant to alkaline hydrolysis than the other two. It was possible to prepare dimethyl phaeopurpurin 7 in the pure state without first separating the *a* and *b* series by virtue of the fact that in the *b* series the products of the phase test are rhodins *i* and *k*, which Willstätter showed to be relatively stable; they are not transformed by treating with diazomethane. Starting with pure methyl phaeophorbide *b* we found that there was no substance formed with acid number corresponding to dimethyl phaeopurpurin 7. In this new procedure from phaeophytin *a* and *b* all the *b* series is lost, but, in spite of this, because of the great saving in labor, this is very much the best method of preparing the compound.

### Experimental

**Pyrochlorin *e*.**—The preparation of this compound has been improved by using boiling diphenyl and a shorter time of heating. A typical procedure is as follows: a 100-g. portion of diphenyl was heated to boiling over a free flame and 1 g. of chlorin *e* was then quickly added and the boiling continued for five minutes with vigorous shaking. On adding the chlorin there was a short, but vigorous, evolution of gas. The hot reaction mixture was then poured cautiously into a liter of ether in a two-liter flask. The reddish-brown ether solution thus obtained was extracted repeatedly (about 10 times) with 200-cc. portions of 1% hydrochloric acid until the color was very light, each fraction being washed twice to remove diphenyl. These extracts were retransferred to about 2 liters of ether. The ether solution of the 1% acid extract was re-extracted with 0.5% hydrochloric acid. This retreatment leaves the impurity with the red line at 650  $m\mu$  in the ether. The "pyrochlorin *e* porphyrin" was transferred to 2 liters of ether from which it crystallized in prismatic needles as usual on evaporating the solution to 100 cc. In this way from two 1-gram runs 250 mg. of porphyrin was obtained, which represents a 15% yield. The combined mother liquors from the two runs containing the pyrochlorin *e* were then worked up in the usual way and yielded 0.615 g. of pyrochlorin *e*, which is a 36% yield.

*Anal.* Calcd. for  $C_{32}H_{38}O_2N_4$  ( $C_{32}H_{36}O_2N_4 \cdot H_2O$ ): C, 73.0; H, 7.22; N, 10.65. Found: C, 74.1, 74.3, 74.5; H, 7.0, 7.4, 7.5; N, 10.3, 10.4.

The spectrum in ether has been published (paper I). The spectrum in 8% hydrochloric acid was as follows: I, 672.7---669.2—628.1---625.0; II, 613.5—592.4; III, 563.4—541.8---525.2; E. A. 442.2. Order of intensity: I, III, II.

**Methyl Ester of Pyrochlorin *e*.**—The pyrochlorin *e* was methylated in ether solution with diazomethane. The ester crystallizes only after concentrating the ether solution to a very small volume and diluting with ethyl alcohol. It comes out in beautiful, almost perfectly equilateral, triangles of bright luster; it melted sharply at 184°. The spectrum was identical line for line in a revision spectroscope with the spectrum of the parent substance.

**Spectrum of Pyrochlorin *e* Ester in Ether.**—Concn. 0.2 g./5 liters at 65 mm. depth: I, 677.1—637.1---627.4; II, 614.5---610.3—595.8---594.2; III, 595.5---548.2 (hazy); IV, 534.3---532.5—525.5; V, 517.9---516.2—480.7; E. A. 441.8. Order: I, V, II, IV, III.

In 10% Hydrochloric Acid.—I, 674.8---672.6—626.4; II, 614.2—595.6---590.0; III, 563.9---550—543.5—525.9; E. A. 444.1. Order: I, III, II.

*Anal.* Calcd. for  $C_{33}H_{38}O_2N_4$ : C, 75.85; H, 7.28; N, 10.73;  $OCH_3$ , 5.94. Found: C, 75.5, 75.7; H, 7.2, 7.8; N, 10.6, 10.7;  $OCH_3$ , 5.7, 6.2.

The statement in our previous paper that pyrochlorin *e* was unstable has not been verified. Carefully purified material, even after long standing, has been found to be unchanged. A solution of pyrochlorin *e* ester in glacial acetic acid was boiled for twenty-four hours without apparent change. No other product could be found. Decomposition with potassium hydroxide (alcoholic) and pyridine at 220° for five hours yielded phylloporphyrin, identified by mixed melting point of the esters. The copper, zinc and iron salts were made by heating pyrochlorin *e* ester with the corresponding acetates in glacial acetic acid. The only one of these which could be obtained in nicely crystalline condition was the copper salt.

The copper compound dissolved in ether with a deep blue-green color from which it was not extracted by strong acid and showed the following spectrum: I, 648.7---646.0—609.8---606.9; II, 589.1---576.2; III, 550.5---532.3; IV, 613.8—493.7; E. A. 434.5. Order: I, IV, II, III.

*Anal.* Calcd. for  $C_{33}H_{36}O_2N_4Cu$ : C, 67.9; H, 6.16; N, 9.6;  $OCH_3$ , 5.3; Cu, 1.09. Found: C, 66.6, 68.3; H, 6.4, 6.7; N, 9.3;  $OCH_3$ , 4.7; Cu, 1.01, 1.05.

The iron salt gave a deep green ether solution, from which it was not extracted with concentrated hydrochloric acid. Its ether solution behaved in a peculiar way in that when shaken with dilute ammonia the color became brown and the spectrum was altered considerably. On shaking with acid the original color and spectrum returned. Dilute potassium hydroxide does not appear to effect the same change. The initial spectrum in ether was: I, 671.4—646.1---644.1; II, 619.2---617.1—600.3---596.1; III, 564---545.8 (very faint); IV, 533.1—526.2; V, 513.3---509.0—499.6---497.2—487.5 (double max.); E. A. 444.9. Order: I, V, II, IV, III.

The spectrum after treatment with ammonia was as follows: I, 690.7---673.4—646.6---644.6; II, 532.4---526.8; III, 513.6---508.4—500.0—495—488.5; E. A. 452. Order: I, III, II.

The color of the zinc complex in ether was pure blue. It was readily attacked by 10 or 15% hydrochloric acid, which extracted it with a pure green color. Its spectrum in ether was: I, 672.0---669.3—641.8; II, 634.2---633.3—622.3---620.0; III, 603.6---591.0; IV, 557.8---544.5; V, 534.1—525.1; VI, 517.3---512.9—487.6 (hazy); E. A. 446.6. Order: I, II, VI, III, V, IV.

**Catalytic Hydrogenation of Pyrochlorin *e*.**—Pyrochlorin *e* was reduced in glacial acetic acid in the usual way; 5.5 moles of hydrogen per mole of compound was absorbed when the reaction mixture had become colorless, which required about an hour and a half. On exposure to air without removal of the catalyst the solution became brown

and on working up the product a porphyrin was obtained which was identical in spectrum in ether and acid solution with phylloporphyrin with the exception of a weak line at 652-645. In addition to this porphyrin a small amount of a chlorin was obtained which could be extracted with 7.5% hydrochloric acid; its spectrum was similar but not identical with Treibs and Wiedemann's phyllochlorin. This same phyllochlorin was obtained in better yields by the reduction using hydrogen iodide.

**Reduction of Pyrochlorin *e* with Hydrogen Iodide.**—Since the ester of pyrochlorin *e* was more readily available in a pure condition than the free acid, it was used for preparative purposes; in smaller runs with the free pyrochlorin *e* the results were identical. A 250-mg. sample of the pyrochlorin *e* ester was dissolved in 30 cc. of glacial acetic acid and 5 cc. of 45% hydriodic acid added; the color of the solution turned from a deep indigo-blue to a pure green. The flask containing the reaction mixture was then placed on the steam cone and heated for eight to ten minutes with frequent shaking. During this time the green color of the solution became lighter and the solution took on a yellowish-brown shade. The reaction mixture was immediately poured into 500-600 cc. of ether contained in a separatory funnel. On adding about 200 cc. of water and shaking vigorously the material went back in solution, and on standing for about an hour the ether solution became intensely green with a strong red fluorescence. Partially neutralizing the acid with ammonia immediately after transferring to ether seemed to accelerate the change to green. The extraction was then carried out in the usual manner after washing the ether solution several times with water to remove the acetic acid. Extraction with 1% hydrochloric acid removed phylloporphyrin along with a large proportion of another porphyrin with a line near 650. Extractions with 4 and 5% acid removed only small amounts of the green compound, which came out readily with 6 and 7% acid. These latter extracts contained the bulk of the coloring matter present and were colored pale blue. This main fraction, after transferring to ether, was esterified with diazomethane and concentrated for crystallization, a double volume of low-boiling petroleum ether being added. On standing overnight the material crystallized in round clusters of triangular plates with a deep blue luster and having a melting point of 148-150° on the block. In some preparations clusters of long hair-like needles appeared, but three recrystallizations from petroleum ether failed to raise the melting point above 150°. The yield in this case was 140 mg. or 56% of the theoretical if the molecular weight of the material is considered to be the same as that of pyrochlorin *e* ester. A sample was tested for homogeneity by carefully fractionating and comparing the spectra of various fractions with the mother liquor. In every case the spectra compared were identical. In catalytic hydrogenation this chlorin required 3.3 moles of hydrogen per mole of compound for its conversion to the corresponding leuco-compound. On reoxidation phylloporphyrin was formed, as indicated by the comparison of its spectrum with known phylloporphyrin. There was, however, a weak line near 650 due to some impurity which is usually found in impure phylloporphyrin. In fact, this line appears in the reduction and reoxidation of pure phylloporphyrin.

The spectrum of the new phyllochlorin was as follows, in ether: I, 662.1--660.4--632.1--620.4--615.1; II, 606.6--599.5--594.1--591.6--586.8; III, 552.4--549.7--544.3--540.5; IV, 525.3--519.5; V, 512.7--508--479.4; VI, --455.8--; E. A., 432.4. Order: I, V, II, IV, III, VI.

**In 7% Hydrochloric Acid.**—I, 656.4--653.3--624.6--621.1; II, 606.6--588; III, 556--546.2; IV, 536--521.8; E. A., 436.0. Order: I, II, IV, III.

*Anal.* (monomethyl ester). Calcd. for  $C_{33}H_{40}O_2N_4$ : C, 75.6; H, 7.6; N, 10.7;  $OCH_3$ , 5.9. Found: C, 75.44, 75.42; H, 7.75, 7.54; N, 11.06, 11.03;  $OCH_3$ , 6.77, 6.55.

The fraction of material containing phylloporphyrin from the hydrogen iodide reduction was esterified with diazomethane, after drying its ethereal solution, and re-



fractionated with 0.5% hydrochloric acid. The ester thus obtained crystallized in the way characteristic of phylloporphyrin ester and melted at 230° on the block. A mixture with some known phylloporphyrin ester of melting point 235° melted at 230–233°. Comparison of its spectrum in ether and acid solution with known phylloporphyrin in the revision spectroscope showed the identity of the two spectra throughout.

**Pyrochloroporphyrin.**—The porphyrin mixture obtained in the pyrolysis of chlorin *e* as previously described amounts to about 150 mg. per gram of chlorin *e*. This material from 2 g. of chlorin *e* was dissolved in about three and one-half liters of ether with the aid of 10% acid. Fractionation of the resulting solution was begun with 0.3% acid. Each fraction of acid was washed twice with 300-cc. portions of ether before retransferring the colored matter to ether. With ten or fifteen extractions approximately one-third of the total material was removed, which appeared to be fairly pure phylloporphyrin as judged by a comparison of the spectrum with known phylloporphyrin. By continuing the extraction and gradually increasing the strength of acid to 0.4%, approximately one-quarter of the remaining coloring matter was removed. Comparison of its spectrum with the first fraction showed it to be a mixture. This second fraction was discarded. The wash ether was now replaced by fresh ether and the extractions continued using 0.5 and 0.6% acid. In this way approximately another third of the colored material was removed without finding any further change in spectrum. The spectrum of this material was easily distinguished from that of phylloporphyrin in the revision spectroscope. The mother liquor, though still quite colored, was discarded. The first and last fractions were then esterified and the resulting ether solutions of the esters subjected to fractionation. It was necessary in this case to use slightly more concentrated acid, since the esters were slightly higher in acid number than the free porphyrin acids. Thus the phylloporphyrin ester was extracted with 0.4 and 0.5% acid, while 0.6 or 0.8% acid was necessary for the convenient extraction of the pyrochloroporphyrin ester. This latter porphyrin solution was likewise washed first with 0.4 and 0.5% acid to remove further possible amounts of phylloporphyrin ester. In each case when about half of the coloring matter had been extracted, the mother liquors were discarded. The ethereal solutions of the extracted porphyrin esters were concentrated to a small volume for crystallization. The phylloporphyrin ester crystallized in the usual red prismatic needles, while the pyrochloroporphyrin ester appeared as long hair-like needles. Approximately 80 mg. of the former and 45 mg. of the latter were obtained. The yield is no criterion of the relative proportions of the two compounds present in the original mixture, since a large amount of the material was discarded. The mixture was judged to consist of approximately equal amounts of the two compounds. The material described in our first paper as "pyrochlorin *e* porphyrin" was undoubtedly more than 50% pyrochloroporphyrin, since this compound is much less soluble than phylloporphyrin, so that by recrystallization a considerable proportion of phylloporphyrin would be left in the mother liquor. The melting point of the phylloporphyrin ester was 235–236°, that of the pyrochloroporphyrin ester 226–237°. The former compound mixed with phylloporphyrin ester melted at 236–237°, while the latter also mixed with phylloporphyrin melted at 228–230°.

Spectrum of pyrochloroporphyrin ester in ether: I, 641.7–631.8; II, ---606.6---III, 593.7---581.3---574.7---573.2; IV, 550.9---549.4---534.6---532.6; V, 522.8---521.1---487.8; E. A., 445.2. Order: V, IV, III, I, II.

The line previously recorded at 664–658 was obviously due to an impurity which has been removed in this fractionation. Spectrum in 1% acid: I, 609.0---599.0; II, 585.5---570.5---548.8---545.7; III, 526.2---514.2; E. A., 437.5. Order: II, I, III.

*Anal.* of methyl ester. Calcd. for  $C_{55}H_{78}O_2N_4$ : C, 75.85; H, 7.28; N, 10.73;  $OCH_3$ , 5.94. Found: C, 75.99; H, 7.45; N, 10.6;  $OCH_3$ , 6.3, 5.9.

*Anal.* of free acid. Calcd. for  $C_{32}H_{36}O_2N_4$ : C, 75.6; H, 7.09; N, 11.0. Found: C, 75.3, 75.0; H, 7.0, 7.2; N, 10.4, 10.2.

The spectrum of the phylloporphyrin ester fraction was identical in every detail with the spectrum of a known sample, compared line for line in the revision spectroscope.

A sample of the material designated as chloroporphyrin  $e_3$  by Fischer and Moldenhauer<sup>4</sup> was prepared in the manner described by them. This sample was subjected to careful acid fractionation in the same way as the "pyrochlorin  $e$  porphyrin" mixture. The results were identical. The phylloporphyrin ester after its refractionation amounted to 45 mg. The pyrochloroporphyrin ester amounted to 43 mg. The crystal form in each case was the same as that of the corresponding fractions in the pyrolysis experiment. The spectra of the corresponding fractions in ether and acid were likewise identical in every detail as shown by direct comparison. The phylloporphyrin ester melted at 225–228°, and its mixture with a known sample melted at 230–234°. The melting point of the pyrochloroporphyrin ester was 226–230°, and when mixed with a sample of pyrochloroporphyrin ester prepared as above, gave no depression.

**Reduction of Pyrochloroporphyrin.**—A sample of pyrochloroporphyrin ester was dissolved in a few cc. of glacial acetic acid. A few drops of 45% hydriodic acid was added and the mixture was then heated on the steam cone for ten minutes. On transferring to ether and working up in the usual way, a major portion of the colored matter was extracted with 0.5% hydrochloric acid. This material was identical in spectrum both in acid and ether by direct comparison with known phylloporphyrin.

The spectrum as measured in ether was as follows: I, 633.0—628.5—615.0 (hazy shadow); II, 606.6—601.8; III, 592.3—589.8—578.5—573.1; IV, 543.3—540.7—531.3—529.3; V, 519.6—516.8—486.2; E. A. 438.8. Order: V, III, IV, I, II.

Another small sample of material was reduced catalytically to the leuco compound. The reduction may have been carried too far since the reoxidation took place very slowly. On retransferring to ether considerable scum was formed. However, a small amount of material could be extracted with 0.5% acid which checked in spectrum with known phylloporphyrin, though the ether solution was slightly brown and obviously impure.

**Preparation of Dimethyl Phaeopurpurin 7 from Crude Phaeophytin.**—A solution of 18 g. of crude phaeophytin (*a* and *b*) in 60 cc. of pyridine was added to 4 liters of ether and shaken for ten minutes with 400 cc. of a mixture of 25% potassium hydroxide in *n*-propyl alcohol. The solution turned deep green upon the addition of the alkali. Three liters of water was added to the saponification mixture and shaken; the alkaline layer was removed and the product transferred to 10 liters of ether by acidification. The ether solution, after washing with water and drying with sodium sulfate for ten minutes, was treated with an excess of diazomethane in ether solution. When a test showed that the product was alkali insoluble, the ether solution was extracted from eight to ten times with 1500-cc. portions of 11% acid. The acid solution used should be cold and all operations should be performed rapidly in order to prevent hydrolysis of the ester. Each acid extract was washed with 1500 cc. of ether in a 4-liter separatory funnel before transferring to ether. The material in the acid extracts was transferred to 8 liters of ether in all. This ether solution was washed with a 0.1 *N* solution of potassium hydroxide to remove some acidic material resulting from hydrolysis. Upon concentrating the ether solution, the dimethyl phaeopurpurin 7 crystallized in glistening hexagonal plates; the yield was 2–2.5 g.

**Preparation of Phaeopurpurin 7.**—A solution of 2.6 g. of dimethyl phaeopurpurin 7 in pyridine was added to 3 liters of ether and shaken for five minutes with 300 cc. of 25% *n*-propyl alcoholic potash. Water was added and the aqueous layer was acidified with shaking in the presence of 6 liters of fresh ether. The phaeopurpurin 7 was then extracted by 8% acid. The acid extracts were washed with ether through an inter-

mediate separatory funnel and then the product was transferred to ether, 8 liters for all the extracts. The yield of phaeopurpurin 7 was 1.66 g.

A second procedure was adopted for preparing phaeopurpurin 7 which saved time but resulted in a slightly less pure product. The phase-test saponification of phaeophytin was carried out as described under the preparation of dimethyl phaeopurpurin 7. After methylation, instead of extracting the ester with 11% acid, the dry 10-liter ether solution (from 18 g. of phaeophytin) was shaken for five minutes with 500 cc. of 25% *n*-propyl alcoholic potash. Water was added and then the product was transferred back to the same ether solution by acidification. The resulting ether solution was then extracted with 8% acid until the color of extracts was a weak green. The acid extracts were washed with an equal volume of ether and transferred to 8 liters of fresh ether. The yield was 1.33 g. of phaeopurpurin 7.

Phaeopurpurin 18 and dimethyl phaeopurpurin 7 were relatively resistant to pyrolysis in diphenyl, as might be expected. Thus for the former it required five and one-half hours at 250° for the loss of 0.6 mole of carbon dioxide and for the latter four hours at the same temperature for 0.7 of a mole. The pyrolysis product was a complex mixture of porphyrins and chlorins which has not yet been separated into sufficient quantities of pure materials to warrant description.

**Pyrolysis of Phaeopurpurin 7.**—This substance lost one mole of carbon dioxide with comparative ease. A sample of 0.1630 g. was heated in diphenyl at the boiling point for ten minutes, the oil-bath was removed and the system swept with pure nitrogen for twenty minutes longer. The potash absorption bulb gained 11 mg., which corresponds to 96% of one mole of carbon dioxide lost by the sample. A sample of 0.1520 g. of phaeopurpurin 7, heated in diphenyl at 160–170° for forty-five minutes, lost 9 mg. of carbon dioxide, or 84% of one mole. An hour longer of heating at the same temperature did not change the weight of carbon dioxide. In addition to carbon dioxide, carbon monoxide was evolved. This was established by passing the gas through hemoglobin solution and noting the characteristic bands of CO-hemoglobin. The products resulting from the pyrolysis of phaeopurpurin 7 consisted of a mixture of porphyrins and one chlorin. The chlorin, which had an acid number of 11, and which we shall designate as monomethyl chlorin *f* was easily separated from the porphyrin mixture, since all the porphyrins were readily extracted by 5–9% acid. By laborious and careful fractionation, two porphyrins were isolated from the porphyrin mixture. One porphyrin had an acid number of 8, and the other 6. Besides the porphyrins 6 and 8 there are small amounts of some other porphyrins with lower acid numbers.

For preparative purposes, the pyrolysis was carried out on a larger scale. To a solution of 60 g. of boiling diphenyl was added 500 mg. of phaeopurpurin 7 and the solution heated at the boiling point for five minutes. After cooling, the diphenyl was poured into 500 cc. of ether and dilute alkali was added to extract the pyrolysis product. The alkaline solution was washed twice with fresh ether to remove all but traces of the diphenyl, and the pyrolysis product was then transferred to 2 liters of ether by acidification of the alkaline solution. The ether solution was extracted with 3 and 4% acids and then with 6–9% acids; the 9% extracts were washed with ether to remove traces of the chlorin. The chlorin was then extracted by means of 11–13% acids. The 6–9% acid porphyrin portion was separated by careful acid fractionation. The yields of the porphyrins were small. From 1 g. of phaeopurpurin 7 was obtained 70 mg. of the pyroporphyrin 8 and 30 mg. of the pyroporphyrin 6. The proportions of the two porphyrins and accompanying impurities may have varied with the method of pyrolysis and source of the phaeopurpurin 7, but this could not be checked exactly. Monomethyl chlorin *f*, which could be easily isolated and purified, was formed in 15–16% yields under all conditions; the yield from 1 g. of phaeopurpurin 7 was 150–165 mg.

**Monomethyl Ester of Chlorin *f*.**—This substance crystallizes from ether in eight-sided plates which approach an oval in contour. The acid number is 11, and the hydrochloric acid solution is pure blue. The ether solution is clear green without an olive tinge. The solubility in ether is about the same as that of chlorin *e*. The ether spectrum varies only slightly from that of chlorin *e* (1 mg. in 30 cc. ether): I, 684—647.5—637—631; II, 615—603; III, 563—553; IV, 531—524; V, 508—483.5; E. A. 433. Order: I, V, IV, II, III.

The spectrum in 13% acid: I, 681—594; II, 577—568; III, 534—532—521—520; IV (very faint), 495—487; E. A. 447. Order: I, III, II, IV.

*Anal.* Calcd. for  $C_{33}H_{40}O_4N_4$ : C, 71.2; H, 7.2; N, 10.07;  $OCH_3$ , 5.6. Found: C, 71.4, 71.6; H, 6.6, 6.8; N, 10.0, 10.08;  $OCH_3$ , 6.1, 5.8.

**Dimethyl Ester of Chlorin *f*.**—This substance, prepared by the action of diazomethane on monomethyl chlorin *f*, or by the action of dimethyl sulfate on the alkaline solution, crystallizes from ether and methyl alcohol in rough aggregates of needles or sharp-pointed plates. The acid number lies between 13 and 14, and the acid solution is blue. The color and spectrum of the ether solution is the same as that of the monomethyl ester. The melting point is 182° (block). The hydrolysis of the ester (phase test conditions) yielded an alkali-soluble chlorin apparently identical with chlorin *f*; methylation with dimethyl sulfate regenerated the dimethyl ester.

*Anal.* Calcd. for  $C_{34}H_{40}O_4N_4$ : C, 71.8; H, 7.04; N, 9.86;  $OCH_3$ , 10.9. Found: C, 71.54, 72.0; H, 7.05, 6.9; N, 9.66, 9.88;  $OCH_3$ , 10.6.

**The Porphyrins from Pyrolysis of Phaeopurpurin 7.**—The porphyrin of acid number 8 is bluish-green in acid solution with a slight red fluorescence. It crystallizes from ether solution in stout diamond-shaped prisms. The spectrum in an ether-pyridine solution (1 mg. in 20 cc. of ether and 10 cc. of pyridine): I, 645—639; II, 595—580—571; III, 562—538; IV, 522—504; E. A., 444. Order: III, II, IV, I.

The spectrum in 9% acid: I, 621—601; II, 585—574—543; III, 527—516; E. A., 444. Order: II, I, III.

*Anal.* Calcd. for  $C_{33}H_{36}N_4O_4$ : C, 71.7; H, 6.5; N, 10.15;  $OCH_3$ , 5.6. Found: C, 71.6, 71.8; H, 6.4, 6.5; N, 9.7;  $OCH_3$ , 6.3.

The porphyrin 6 crystallizes from ether in pointed plates with oval sides. The solution in acid is purplish-blue. The spectrum in ether solution: I, 675—660, faint shadow; II, 638—632.5; III, 590—576—571; IV, 552—539; V, 515—502—491; E. A., 436. Order: IV, V, III, II, I.

The spectrum in 6% hydrochloric acid: I, 608—600; II, 581—565—550—540; E. A., 435. Order: II, I.

The substance contains one methoxyl group; found, 6.52, 6.71%  $OCH_3$ .

**Hot Saponification of Phaeopurpurin 7.**—To a boiling solution of 30 g. of potassium hydroxide in 90 g. of *n*-propyl alcohol was added 400 mg. of phaeopurpurin 7 and the solution was refluxed for five minutes. The color of the solution was dark green with a deep red fluorescence. Water and ether were added and the mixture shaken. The aqueous alkaline layer was drawn off and the material transferred to 2 liters of fresh ether by acidification. The resulting ether solution was of a purplish-red color. The saponification product was a mixture of porphyrins and chlorin *f*. Acids of 4–8% extracted the porphyrins, leaving the ether colored green. The chlorin was extracted by 10–11% acids and transferred to fresh ether. Careful fractionation of the porphyrin extract with 5 and 6% acids removed the porphyrin of lower acid number. The material of higher acid number was in lesser amount and was not isolated. As the porphyrin of acid number 5 became more pure, more and more ether had to be used to keep it from crystallizing from solution. Finally, the pure porphyrin was contained

in 4 liters of ether, from which was obtained 30 mg. of substance upon concentrating the ether solution. The yield of chlorin *f* was 111 mg. The same products were obtained when 35% potassium hydroxide in methyl alcohol was employed at the boiling point for thirty seconds.

Another saponification of phaeopurpurin 7 was carried out with the same quantity of material and procedure as described above. The saponification product, transferred to 3 liters of ether, was then methylated with diazomethane and the esters of the chlorin and porphyrin were fractionated from the ether solution. The porphyrin fraction was removed by 9% acid and the chlorin ester by 13-16% acid. The porphyrin ester corresponding to the insoluble porphyrin 5 was obtained by careful fractionation with 8% acid and washing the acid extracts with ether. Much material was lost as the ester during this acid fractionation—the acidic material was removed by washing the final ether solution with 0.1 *N* alkali. The yield of chlorin *f* ester was 146 mg.; of porphyrin 5 ester, 10 mg.

Potassium oxalate as a product of the reaction was demonstrated in another experiment; 400 mg. (0.6 of a millimole) of dimethyl phaeopurpurin 7 was hydrolyzed by boiling with 100 cc. of 25% methyl alcoholic potassium hydroxide for ten minutes. The reaction mixture was worked up in the usual way, the acidified aqueous layer being repeatedly extracted with ether and finally filtered through charcoal to remove all traces of the complex organic products. The aqueous solution was concentrated, made faintly alkaline with ammonia and calcium chloride added to the hot solution. The precipitate was washed with dilute acetic acid and collected on a microfilter; it weighed 39 mg. On treatment with dilute hydrochloric acid 30 mg. (0.23 millimole) of calcium oxalate dissolved. The calcium oxalate was reprecipitated from the hydrochloric acid solution, filtered off, dissolved in sulfuric acid and titrated with standard permanganate. The total oxalate thus determined was 20 mg. of calcium oxalate (0.16 millimole). Considering the losses encountered in the manipulations, the quantity of oxalate found corresponds to the yield of chlorin *f* and the accompanying porphyrin (about 40%). In a "control experiment" chlorin *e* was substituted for phaeopurpurin 7 and the same procedure employed; no calcium oxalate was obtained.

**Chlorin *f*.**—This substance is very insoluble in ether, from which it crystallizes in microscopic prismatic needles barely visible at a magnification of 60 diameters. The acid number is 10 and the color in acid solution is deep blue. The solution in ether is green without the olive tint characteristic of chlorin *e*. The spectrum in ether: I, 685—645—631; II, 617—603; III, 561—554; IV, 531—524; V, 510—481; E. A., 436. Order: I, V, IV, II, III. The spectrum in 12% hydrochloric acid: I, 680—629—611—597; II, 577—568; III, 535—521; E. A. 439. Order: I, III, II.

**Anal.** Calcd. for  $C_{32}H_{38}O_6N_4$ : C, 70.9; H, 7.05; N, 10.34. Found: C, 71.7, 71.4; H, 6.3, 6.6; N, 10.0, 10.3;  $OCH_3$ , 0.

**A Porphyrin from the Saponification of Phaeopurpurin 7.**—This porphyrin was obtained in very small quantities and is characterized by its insolubility in ether and the solubility in dilute alkali. It dissolves readily in 0.1 *N* potassium hydroxide with a pink solution. The acid number lies between 5 and 6; the color of the acid solution is light blue with a purplish-red fluorescence. It contains no methoxyl group. It appears to be the dibasic acid corresponding to porphyrin 6 from the pyrolysis experiments. The spectrum in ether solution: I, 637—632; II, 593—578—573; III, 557—553—542—537; IV, 518—511—503—492; E. A., 436. Order: III, IV, II, I. Spectrum in 0.1 *N* potassium hydroxide: I, 664—650; II, 574—556; III, 549—537; IV, 513—496; E. A., 436. Order: I, IV, III, II. Spectrum in 6% acid: I, 616.5—598; II, 585—573—541; III, 524—511 (very faint); E. A., 440. Order: II, I, III.

**Action of Hydriodic Acid on Dimethyl Ester Chlorin *f*.**—A solution of 20 mg.

of chlorin *f* dimethyl ester in 30 cc. of glacial acetic acid was heated on a steam cone for ten minutes with 2 cc. of 45% hydriodic acid. The brownish-red reaction mixture was poured into ether and the acetic acid was washed out with water and ammonia. Finally, the ether solution was extracted with dilute alkali, which removed most of the material, and the product was transferred to fresh ether by acidification. Fractionation with 6% acid removed a porphyrin, leaving a green solution. The porphyrin on further purification proved to be identical with rhodoporphyrin in acid number, color and spectrum in acid and ether solution. The green solution was washed with 9% acid and then the chlorin removed with 12% acid. The chlorin had a spectrum somewhat similar to those of the "synthetic chlorins" reported by Fischer: I, 680—638 (two maxima); II, 627.5—621; III, 608—593; IV, 563—545; V, 531—525—510.5; VI, 508—478.5; E. A. 438. Order: I, VI, III, V, II, IV.

**Pyrolysis of Chlorin *f*.**—A 0.1029-g. sample of chlorin *f* was heated in diphenyl at 240–260° for twenty minutes; 1.7 mg. of carbon dioxide was lost. After heating for two and one-half hours longer at the boiling point of diphenyl, the loss of carbon dioxide in all was 4.6 mg. This corresponds to over 50% of one mole of carbon dioxide. The result may not be accurate due to the small weight of sample. The pyrolysis product was a mixture of porphyrins from which was obtained by fractionation with 1.5–2% acid, a considerable amount of pyrroporphyrin. The remainder of the material was of higher acid number. The pyrroporphyrin was identical in acid number, color and spectra in acid and ether solutions with a known sample of pyrroporphyrin. A mixed melting point of the esters showed no depression.

**Alkali Decomposition of Monomethyl Chlorin *f*.**—A mixture of 65 mg. of monomethyl chlorin *f*, 6 cc. of 25% methyl alcoholic potassium hydroxide, 3 cc. of pyridine and 50 mg. of magnesium oxide was heated at 150–156° for five hours in a silver-lined autoclave.<sup>5</sup> The decomposition product was shaken with 20% hydrochloric acid and transferred to a liter of ether. The red ether solution, without purification, checked in color and spectrum with an authentic sample of rhodoporphyrin. Fractionation showed the presence of only one substance which were removed by 4–5% acid. The color and spectrum in ether and acid solution were identical with those of rhodoporphyrin. The fractionated material was converted to the methyl ester by the use of diazomethane. The ester crystallized from ether in rectangular plates, characteristic of rhodoporphyrin dimethyl ester. The melting point was 255°, and a mixed melting point with a known sample of dimethyl rhodoporphyrin showed no depression.

**Alkali Decomposition of the Porphyrins from the Pyrolysis of Phaeopurpurin 7.**—A 20-mg. sample of porphyrin 6 and a 10-mg. sample of porphyrin 8 were heated in separate silver crucibles with proportionate amounts of alkali, pyridine and magnesium oxide according to the procedure of Treibs and Wiedemann, to 150–160° for five hours. The phyllins were decomposed with 20% acid and the products transferred to ether. In each case rhodoporphyrin was the sole product. The material was identical in color and spectrum in ether and acid solution, and in acid number, with authentic rhodoporphyrin.

**Saponification of Allomerized Chlorophyll.**—Crude chlorophyll<sup>6</sup> (from spinach) was separated into the *a* and *b* components by Willstätter's procedure. The pure chlorophyll *a* gave the "phase test" and on rapid saponification yielded chlorin *e* with only a trace of material with higher acid number. Samples of this pure chlorophyll *a* were allomerized by standing in absolute alcohol solution for twenty-four to forty-eight hours; the allomerization was demonstrated by the failure of the resultant material to give the phase color. A solution of 150 mg. of allomerized chlorophyll in alcohol was diluted

<sup>5</sup> Treibs and Wiedemann, *Ann.*, **471**, 174 (1929).

<sup>6</sup> We are indebted to Dr. H. A. Spoehr for supplying the crude chlorophyll.

with water and ether and the material thus transferred to dry ether in the usual way. The ether solution was evaporated to dryness in a silver container and the residue treated with 15 cc. of boiling alcoholic potassium hydroxide, (35%) and the boiling continued for thirty seconds. On diluting with water and acidification with concentrated hydrochloric acid, the color change showed the removal of magnesium. On working up the product in the usual way, including the treatment of the unstable chlorins with diazomethane, dimethyl phaeopurpurin 7 was the main product. It was identified by its color, acid number, spectrum in ether, in 12% hydrochloric acid and, after hydrolysis, in 0.1 *N* alkali (the spectra were compared with a known sample in a revision spectroscope); as a by-product a porphyrin ester with acid number about 8 was obtained. In another experiment allomerized phaeophytin *a* was prepared by treating allomerized chlorophyll *a* with concentrated hydrochloric acid. The rapid hot saponification of the allomerized phaeophytin *a*, followed by the diazomethane treatment in the usual way, yielded dimethylphaeopurpurin 7 and only a trace of higher chlorins; the porphyrin appeared to be absent.

### Summary

1. It has been shown that the saponification of allomerized chlorophyll *a* yields phaeopurpurin 7, a characteristic product of the phase test; allomerization and the phase test appear to be equivalent processes.

2. Phaeopurpurin 7 (a monomethyl ester) on pyrolysis loses carbon dioxide and carbon monoxide, forming the monomethyl ester of a simple chlorin which appears to be a hydrogenated rhodoporphyrin. The corresponding dibasic acid is formed by the action of hot alcoholic potassium hydroxide on phaeopurpurin 7, oxalic acid being split off. These facts show that phaeopurpurin 7 is an  $\alpha$ -keto acid.

3. Pyrochlorin *e* is a simple chlorin of the unallomerized series; it still contains the carbon atom which is eliminated as carbon monoxide in the allomerized compound. It appears to be isomeric with phylloporphyrin. All these facts point to an oxidation-reduction process as being involved in the phase test and allomerization.

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