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STUDIES IN THE CHLOROPHYLL SERIES. III. PRODUCTS OF THE PHASE TEST

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RECEIVED MAY 22, 1930

PUBLISHED JULY 3, 1930

One of the most characteristic reactions of the pure chlorophyll compounds is their behavior on treatment in ether solution with strong methyl alcoholic potassium hydroxide. In the "a" series the unallomerized chlorophyllides and the phaeophorbides give a yellow-brown color which soon changes to an intensive green. This appearance of a transitory characteristic color is known as the phase test. On diluting with water the material is found in the aqueous layer. In the "b" series the phase is reddish-brown instead of yellowish-brown. Although this method of saponifying the phaeophorbides is used as a test of the purity of the material, since the "altered" compounds do not give the characteristic yellow or red phase, the products of the reaction have been relatively little studied. Thus in Willstätter's directions for the saponification of the phaeophorbides¹ (phaeophytin) he recommends either treating the solid compound with hot or cold alcoholic potassium hydroxide or allowing a pyridine solution to react with alcoholic potash at the boiling point for half a minute. These procedures yield chlorin *e* (phytochlorin *e*), which has been the chlorin most studied. Another chlorin, phytochlorin *g*, is described in one paper by Willstätter² as being the principal product of the action of alcoholic potash on an ethereal solution of phaeophytin (phase test conditions). The chlorin was described as unstable and its transformation products were not isolated. It seemed to us a matter of interest to pursue this investigation further and we have done so, using the pure members of the "a" series.

Our first experiments were with methyl phaeophorbide *a*, prepared and purified according to the procedure outlined by Willstätter. The compound was dissolved in a small quantity of pyridine (20 cc. for each gram) and then diluted with much ether, so that the resulting solution contained 100 mg. in 150 cc. This solution was shaken vigorously with one-tenth its volume of a 25% solution of potassium hydroxide in methyl alcohol. The yellow color first appeared and gradually changed in the course of three minutes to bright green. In these concentrated solutions and on a large scale the yellow phase is never so bright as when a test with a more dilute solution is carried out on a small scale. At the end of five to ten minutes the mixture was diluted with water, the alkaline aqueous solution separated and the material in it transferred to fresh ether by acidification. The

¹ Willstätter and Stoll, "Investigations on Chlorophyll," translated by Schertz and Merz, Science Printing Co., 1928, p. 263.

² Willstätter and Utzinger, *Ann.*, **382**, 129 (1911).

product was then fractionated by the usual Willstätter–Mieg procedure.

The major portion of the product was a mixture of chlorins in which unstable chlorins with acid numbers between 6 and 12 predominate. These chlorins cannot be isolated in a pure condition since they change more or less rapidly in ether solution, producing two compounds of a purplish brown color which we shall designate as phaeopurpurins. The two phaeopurpurins have acid numbers of 7 and 18, respectively, and crystallize readily; the yields from 1 g. of methyl phaeophorbide were, of phaeopurpurin 18, 350–435 mg., and of phaeopurpurin 7, 100–125 mg. In addition to the unstable chlorins which change into the phaeopurpurins, the phase test at room temperature yields approximately 150 mg. per gram of material of a stable chlorin fraction. This was composed of chlorin *e* (identified by conversion to the trimethyl ester with diazomethane) and a chlorin of acid number 5 containing one methoxyl group. The spectrum and behavior of the latter substance indicated that it might be a monomethyl ester of chlorin *e* but the action of diazomethane yielded an ester with different crystal form from the characteristic trimethyl ester of chlorin *e*.

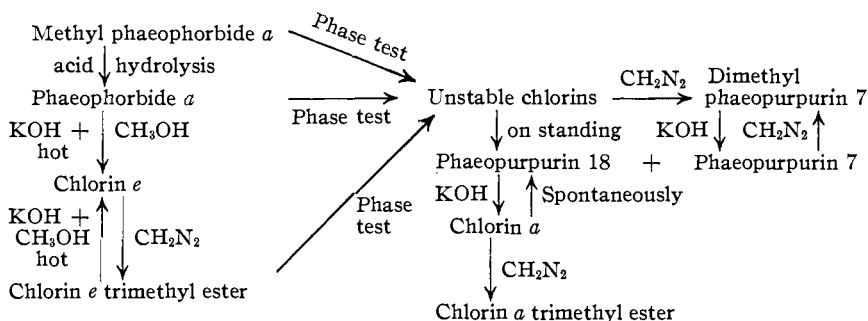
At 23°, the phase test saponification is not complete in less than three minutes; five to ten minutes is a suitable time for preparative purposes. If the action is continued for five hours no phaeopurpurin 7 can be isolated; the products are chlorin *e* and phaeopurpurin 18.

The formation of chlorin *e* in the phase test can be completely suppressed by substituting ethyl alcohol or *n*-propyl alcohol for methyl alcohol in making the alcoholic solution of potassium hydroxide. The same result may also be brought about by carrying out the phase test at -10°. At this temperature the yellow phase persists much longer and the saponification is not complete even in fifteen minutes, but no chlorin *e* is formed. On the other hand, if the saponification of methyl phaeophorbide *a* is brought about by the action of boiling alcoholic potassium hydroxide in pyridine solution (Willstätter's procedure), chlorin *e* is the chief product and no unstable chlorins are formed. Thus by relatively slight changes in the conditions, the saponification of methyl phaeophorbide *a* yields either the unstable chlorins or the stable chlorins (chiefly chlorin *e*). With the same lot of methyl phaeophorbide we have been able to obtain the one type of product or the other; the phase test as ordinarily carried out at room temperature represents a set of conditions in between the two extremes but much nearer the optimum for the production of the unstable products.

We have found that the trimethyl ester of chlorin *e* is surprisingly like methyl phaeophorbide *a* in its behavior toward strong alkali. If one uses a dilute ether solution and methyl alcoholic potassium hydroxide, a yellow phase is distinctly visible; apparently this observation is new. The "phase" is somewhat slower in forming and persists longer than with the phaeophorbides. The product formed is a mixture of unstable chlorins

which are identical in their behavior with those formed in the phase test with the phaeophorbides. Even at room temperature the unstable chlorins are the principal product and no chlorin *e* is formed. Just as in the case of the phaeophorbides, however, if the saponification is brought about rapidly at a higher temperature (using a pyridine solution and boiling alcoholic potash), no unstable chlorins are formed; the product was chlorin *e*, which was identified by the formation of the characteristic trimethyl ester on re-methylation.

The relationships of the phaeopurpurins to each other and the normal chlorophyll derivatives (phaeophorbides, chlorin *e*) is shown in the accompanying diagram.



Phaeopurpurin 7 contains a methoxyl group and at least one free carboxyl group. This is evidently the propionic acid group since it has been shown³ that this is the free group in phaeophorbide *a*, and phaeophorbide *a* yields phaeopurpurin 7 through the phase test. The formation of dimethyl phaeopurpurin 7 by the action of diazomethane probably involves the esterification of one carboxyl and in addition an enol or carboxyl group. Phaeopurpurin 18 contains no methoxyl group, one free carboxyl group, and two masked carboxyl groups. The analyses indicate that there are but five oxygen atoms in the molecule, therefore a lactam and a lactone ring are probably present. One of these rings may be present in the closely related phaeopurpurin 7. The analyses indicate that in the conversion of the normal compounds (the phaeophorbides or the trimethyl ester of chlorin *e*) into phaeopurpurin 7, water has been added and that this water is eliminated in the formation of phaeopurpurin 18. The saponification of phaeopurpurin 18 yields a chlorin which appears to be identical with Willstätter's chlorin *a*. Its trimethyl ester is isomeric with the corresponding ester of chlorin *e*. Chlorin *a* slowly changes to phaeopurpurin 18 on standing; the process may be accelerated by heating a solution in a neutral solvent. Both the phaeopurpurins yield rhodoporphyrin on decomposition with alkali at 150°.

³ Conant and Hyde, THIS JOURNAL, 51, 3668 (1929).

The existence of more than one unstable chlorin in the products from the phase test is indicated by the wide range of acid number and by the spontaneous formation of both phaeopurpurin 7 and phaeopurpurin 18. Probably two chlorins are present which differ only in a methoxyl group; the methoxy compound forms phaeopurpurin 7 spontaneously, the other yields phaeopurpurin 18 with loss of water. The action of diazomethane on the ether solution or dimethyl sulfate on the alkaline solution converts both of the unstable chlorins at once into dimethyl phaeopurpurin 7, as shown by the fact that the yield of this compound is equal to the combined yields of phaeopurpurin 7 and 18 when they are formed spontaneously. It seems probable that the unstable chlorin which yields phaeopurpurin 18 is identical with Willstätter's phytochlorin *g*. The method of formation is similar, the acid number approximately the same, and the instability is characteristic; we also found that our mixture gave an unstable red compound on heating with alcohol, as noted by Willstätter.⁴

We have summarized in Table I the empirical formulas which we consider most probable for the compounds described in this paper, and in the first paper of this series, together with representative analytical data. Each formula has been chosen to fit into a logical scheme and at the same time to give as good agreement as possible between the calculated and found percentages. When one makes due allowance for the difficulties of puri-

TABLE I
EMPIRICAL FORMULAS AND ANALYTICAL DATA

Name	Formula	Composition calculated				Analyses found			
		C	H	N	OCH ₃	C	H	N	OCH ₃
Methyl phaeophorbide <i>a</i>	C ₃₅ H ₃₃ O ₈ N ₄	69.5	6.11	9.0	9.98	69.7	6.16	9.38	10.23
		70.38	6.23	9.34	10.58				
Phaeophorbide <i>a</i>	C ₃₅ H ₃₅ O ₈ N ₄	69.08	5.92	9.21	5.1	70.17	6.28	9.36	4.33
						69.11	6.16	9.43	
Methyl pyropheophorbide <i>a</i>	C ₃₄ H ₃₀ O ₈ N ₄	74.45	6.57	10.22	5.66	74.20	6.98	10.1	5.54
						73.9	6.2		5.33
Pyropheophorbide <i>a</i>	C ₃₃ H ₃₄ O ₈ N ₄	74.15	6.36	10.49	0	73.0	6.65	10.1	0
						72.6	6.33		
Phaeopurpurin 7	C ₃₅ H ₃₈ O ₇ N ₄	67.1	6.07	8.95	4.95	67.26	5.92	8.74	5.19
						67.10	5.88	8.91	5.33
Dimethyl phaeopurpurin 7	C ₃₇ H ₄₂ O ₇ N ₄	67.9	6.42	8.56	14.2	68.38	6.51	8.65	14.5
						68.46	6.28	8.53	
Phaeopurpurin 18	C ₃₄ H ₃₂ O ₈ N ₄	70.85	5.55	9.72		70.18	5.87	9.26	0
		70.35	6.21	9.66		70.04	5.64	9.85	
Methyl phaeopurpurin 18	C ₃₅ H ₃₄ O ₈ N ₄	71.2	5.76	9.50	5.26	69.80	5.63	9.18	5.34
						[C ₃₅ H ₄₀ O ₈ N ₄	70.45	6.10	9.62
Chlorin <i>e</i>	C ₃₄ H ₃₆ O ₇ N ₄	66.70	5.88	9.15		66.7	6.4	8.8	
						[C ₃₄ H ₄₀ O ₇ N ₄	67.0	6.6	9.2
Chlorin <i>e</i> trimethyl ester	C ₃₇ H ₄₀ O ₈ N ₄	69.8	6.29	8.81	14.62	69.3	6.5	8.8	14.33
		69.4	6.88	8.75		69.7	6.9	9.0	
Chlorin <i>a</i>	C ₃₄ H ₃₄ O ₈ N ₄	68.7	5.72	9.43		68.2	6.4	9.04	
						[C ₃₄ H ₃₈ O ₈ N ₄	68.25	6.36	9.37
Chlorin <i>a</i> trimethyl ester	C ₃₇ H ₄₀ O ₈ N ₄	69.8	6.29	8.81		69.4	6.9	8.97	14.47
						[C ₃₇ H ₄₄ O ₈ N ₄	69.4	6.88	8.75

⁴ Ref. 1, p. 276.

fication and analysis of these compounds, the agreement is fairly satisfactory; the possibility of the compounds being richer in hydrogen must always be considered. This is particularly so in the case of phaeopurpurin 18 and its ester and chlorin *a* and its ester; the addition of four more hydrogen atoms to the empirical formulas would lead to a much more satisfactory agreement between the calculated and found compositions. This is shown in the table by the empirical formulas enclosed in brackets. Processes of reduction and oxidation may be involved in the transformations for which we have assumed merely saponification and hydrolysis. The formulas for pyropheophorbide *a* and methyl pyropheophorbide *a* have been derived from the parent compounds by assuming loss of carbon dioxide and formaldehyde since recent work in this Laboratory (which will be published shortly) has shown that the methoxyl group is involved in the thermal decomposition of the phaeophorbides.

Although it is too soon to attempt to write satisfactory structural formulas for the compounds involved in the phase test, certain facts are evident from our work. Both the phaeophorbides and the trimethyl ester of chlorin *e* must have in them a structure which is responsible for the formation of the unstable chlorins under the conditions of the phase test. This structure cannot be the lactam linkage which Willstätter postulated, since the results of the pyrolysis of chlorin *e* have shown the absence of a lactam ring in the compound. The opening of a lactone group might be involved but the mere opening and closing of such rings could not alone account for the shift of color to the phaeopurpurins. The functional groups themselves are apparently of no significance in determining the absorption spectrum, since pyrochlorin *e* from which two of these groups have been removed by pyrolysis has the same type of spectrum as the parent substance. Evidence in the same direction is afforded by the resemblance in color of the two phaeopurpurins, which nevertheless differ in the manner in which the potential carboxyl groups are combined. We are inclined to believe that while the changes involved in the normal series (phaeophorbides, chlorin *e*, and pyro-compounds) are due to changes in the functional groups, the transition to the other chlorins and phaeopurpurins is due to a reaction involving the unsaturated system in these molecules which, in contrast to that in the porphyrins, is very reactive.⁵

Experimental Part

Saponification under Phase Test Conditions of Methyl Phaeophorbide *a*.—One gram of methyl phaeophorbide *a* was dissolved in 20 cc. of pyridine, the solution diluted with 1500 cc. of ether, and 150 cc. of 25% potassium hydroxide in methyl alcohol added; the mixture was shaken vigorously for eight minutes. The yellow-green color of the phase appeared at the beginning and gradually changed to bright green. One liter of water was then added and the color at once changed to brown, all of the acidic material

⁵ Conant and Hyde, *THIS JOURNAL*, **52**, 1233 (1930).

going into the aqueous layer, leaving the upper ether layer almost colorless. The alkaline water layer was acidified with dilute hydrochloric acid in the presence of fresh ether until all of the product was transferred to the ether. The ether solution, which was olive green in color, gave the following spectrum: 50 mm.: I, 706.9—646.1; II, 619.6—605.1 (hazy); III, 562; IV, 547.8—536.6—524; V, 513.6—483; E. A., 443. Order: I, V, IV, II, III.

The ether solution was then extracted with 6% hydrochloric acid until the blue color of the extracts began to diminish. The material in the acid extracts was at once retransferred to 800 cc. of ether. Spectrum of the 6% fraction in ether: I, 700—692.2—649.2; II, 617.1—604.1 (hazy); III, 536.2—528.4 (hazy at each side); IV, 511.4—486.7; E. A., 436.4. Order: I, IV, III, II. The residual ether solution then showed the following spectrum: I, 707.7—648.2; II, 618.9—606.2 (more prominent than in 6 per cent. spectrum); III, 544.8—533.8—524.1; IV, 512.2—484.8; E. A., 444.1; Order: I, IV, III, II. The solution was then further extracted with 9, 10, 11 and 12% acids successively. The extraction was stopped when the 12% extracts became only slightly colored. The acid solutions were of a deep green color. The ether solution from the combined extracts (1500 cc.) was olive green with a brownish tinge. Spectrum: I, 709.0—648.3; II, 620.6—605.7 (hazy); III, 546.8—534.5—524.3; IV, 512.6—484.4; E. A., 443.6; Order, I, IV, III, II. A small amount of material of a reddish-brown color was not extracted by even the 12% acid. The ether spectrum of it was: I, 717.6—652.3—628.2; II, 552.7—532.6; III, 514.0—497.4; IV, 482.5—471.2 (faint and hazy); E. A., 449.0; Order: I, II, III, IV. No attempt was made to isolate a pure substance from this product.

The two ether solutions from the 6% and 9–12% extractions were washed with water and allowed to stand over a small amount of anhydrous sodium sulfate for one week. At the end of this time the solution from the 6% extract was colored a greenish-brown and the other solution was a rich purplish-brown; each of these was then carefully fractionated according to the Willstätter-Mieg procedure.

The ether solution from the 6% extract was found to contain chlorin *e*, a monomethyl chlorin, phaeopurpurin 7, and a small quantity of phaeopurpurin 18. Chlorin *e* was removed with 3% acid, the monomethyl chlorin with 5% acid, phaeopurpurin 7 with 7% acid, while the phaeopurpurin 18 remained in the ether after extraction with 12% acid.

The other ether solution contained chiefly phaeopurpurin 18, together with some phaeopurpurin 7, which was removed with 7% acid. Extraction with stronger acids removed an intermediate fraction and a solution of pure phaeopurpurin 18 remained.

The yields from a number of experiments were 350–425 mg. of phaeopurpurin 18, and 100–125 mg. of phaeopurpurin 7. The total chlorin fraction was approximately 150 mg., and consisted of about an equal mixture of chlorin *e* and the monomethyl chlorin.

The same products were obtained by the saponification of phaeophorbide *a*. One run starting with 1 g. of phaeophorbide *a* and employing the procedure described above, except that phase-test alkali treatment was limited to three minutes, yielded the mixture of the two chlorins, 442 mg. of phaeopurpurin 18 and 72 mg. of phaeopurpurin 7.

The change in ether solution from the unstable chlorins first obtained to the brownish phaeopurpurin is very slow. The major part of the transformation takes place in two days, but at least a week is necessary to reach a state approaching completion. The ether solution may stand for two weeks or longer without decreasing the yields of phaeopurpurins. As described under the discussion of dimethyl phaeopurpurin 7, the same transformation may be brought about instantly by methylation with diazomethane.

The substitution of ethyl alcohol for methyl alcohol in the phase test results in a

more rapid reaction and the formation of only the unstable chlorins, as the following experiment shows.

To a solution of 200 mg. of methyl phaeophorbide *a* in 300 cc. of ether (by use of pyridine) in a separatory funnel, was added 20 cc. of a 20% solution of potassium hydroxide in absolute ethyl alcohol. The yellow color of the phase appeared, but quickly turned green. The saponification proceeded with greater rapidity than when methyl alcoholic potash was used. A solid precipitated from solution. After shaking for four minutes, water was added. All the material went into the aqueous layer. The saponification products transferred to ether with a brownish-green color. Methylation with diazomethane produced the instantaneous color change to the phaeopurpurin color. After fractionation in the usual way, dimethyl phaeopurpurin 7 was obtained as the chief product; no chlorin *e* ester was found, the other product being weakly basic. In another experiment *n*-propyl alcohol was substituted for the methyl alcohol, the procedure being identical. Dimethyl phaeopurpurin 7 was obtained in excellent yields, no chlorin *e* ester being formed. The identity of the dimethyl phaeopurpurin 7 was checked by analysis.

A saponification at -10° was carried out as follows. A solution of 200 mg. of methyl phaeophorbide *a* in pyridine was transferred to 300 cc. of ether in a 500-cc. three-necked flask equipped with a mechanical stirrer and thermometer. The flask was surrounded by an ice-salt mixture and the ether solution cooled to -12° . Thirty cc. of a cold 25% methyl alcoholic potash solution was added and the mixture stirred mechanically for sixteen minutes. The temperature was kept at -10 to -12° . The mixture was then transferred to a separatory funnel and shaken with water. A small amount of unreacted methyl phaeophorbide *a* remained in the ether layer, but most of the material went into the aqueous solution. The alkali-soluble material was transferred to ether. The color of the ether was decidedly brown, and a treatment with 4% acid showed that no strongly basic chlorins were present. The ether solution was washed, dried and treated with diazomethane. The color became purplish-brown on methylation. The chief product was dimethyl phaeopurpurin 7. The side products were weakly basic materials of mixed green-brown color, not extracted by 10% acid. No chlorin *e* trimethyl ester was found. The yield of crystalline dimethyl phaeopurpurin 7 was 72 mg.

Phase Test Saponification of Chlorin *e* Trimethyl Ester.—A solution of 200 mg. of chlorin *e* trimethyl ester in 300 cc. of ether (the ester being first dissolved in pyridine) was shaken for fifteen minutes with 30 cc. of 25% methyl alcoholic potash. At the end of this time all the material was alkali soluble. Water was added and the product transferred to fresh ether. The color of the ethereal solution was a deep olive green with a brownish tinge; extraction with 4% acid showed the complete absence of any strongly basic chlorins. The ether solution was washed with water and dried with sodium sulfate. It was then shaken with an ethereal diazomethane solution until a test showed that all the material was alkali insoluble. Upon the addition of the diazomethane, the color of the solution changed at once to the rich purplish-brown color of the phaeopurpurins. On working up the product there was no trace of chlorin *e* ester; the chief product was dimethyl phaeopurpurin 7; the yield was 104 mg. It is interesting to note that chlorin *e* itself in an identical experiment yielded only the usual trimethyl ester and no phaeopurpurins.

Hot Saponification of Phaeophorbide *a*.—A solution of 600 mg. of phaeophorbide *a* in 2 cc. of pyridine was warmed to 80° in a silver crucible, and to this was added a boiling solution of 16 g. of pure potassium hydroxide in 25 cc. of methyl alcohol, while stirring with a silver spatula. The mixture was boiled for thirty seconds, cooled and washed with water in a separatory funnel. The reaction products in 1500 cc. of ether were

completely methylated by diazomethane, and the esters were fractionally extracted with acid. The largest amount of material came out with 7 and 8% acid, and later proved to be chlorin *e* trimethyl ester. A very small amount of dimethyl phaeopurpurin 7 was found in the 10% extract. The remainder of the material was weakly basic chlorin esters which were extracted with 12–16% acid. The yield of chlorin *e* trimethyl ester (prismatic needles) was 320 mg.

Hot Saponification of Chlorin *e* Trimethyl Ester.—In a silver crucible, 138 mg. of the ester was dissolved in 0.4 cc. of pyridine. The crucible was warmed on a hot plate, and to it was added a boiling solution of 3.5 g. of potassium hydroxide in 6 cc. of methyl alcohol. The mixture was boiled for thirty seconds, cooled and washed into a separatory funnel with water. The saponification product was transferred to ether and the ether solution washed and dried. The olive-green chlorin solution was then completely methylated by means of diazomethane in the usual way. There was no change of color. No dimethyl phaeopurpurin 7 was found, but a small amount of weakly basic chlorin material remained in the ether mother liquor. The chlorin ester extracted by 8% acid, upon concentration of the ether solution, gave 74 mg. of chlorin *e* trimethyl ester as long prismatic needles, identical with the starting compound.

Properties of the Unstable Chlorins.—These compounds cannot be isolated in a solid state, as their solutions become brown on evaporation. The spectrum of the mixture as obtained by extraction with 9–12% acid and retransferring to ether was: I, 709.0—648.3; II, 620.6—605.7 (hazy); III, 546.8 - -534.5—524.3; IV, 512.6—484.4; E. A., 443.6; order I, IV, III, II. The action of diazomethane on this mixture and its spontaneous conversion to the phaeopurpurins is described below under these compounds. The ethereal solution on evaporating and boiling with alcohol gave a very unstable light red color similar to that described by Willstätter as characteristic of phytochlorin *g*. This red compound quickly changes to a mixture of the phaeopurpurins in which phaeopurpurin 18 predominates.

Monomethyl Chlorin from Phase Test Saponification.—The spectrum of this substance is identical with that of chlorin *e* trimethyl ester except for an extra line in the red. Spectrum (1 mg. in 30 cc.): I, 705—694; II, 683—646; III, 615—602; IV, 562 - -555; V, 545 - -534—526; VI, 512—485; E. A., 439. Order: II, VI, V, I, III, IV. The acid number is 5, and the acid solution is blue. The ether solution is more green than that of chlorin *e*.

Anal. Calcd. for $C_{35}H_{42}O_6N_4$: C, 68.4; H, 6.84; N, 9.12; OCH_3 , 5.05. Found: C, 68.14, 68.46, 68.64; H, 6.70, 6.61, 6.73; N, 9.26; OCH_3 , 5.04, 5.07.

Phaeopurpurin 7.—This substance, purified by careful acid fractionation, is brown in ether solution, with a purplish tint. A thin layer of solution is more purple than brown. It crystallizes from ether in bluish-black spherical clusters of fine needles. It is very soluble in pyridine and acetone, almost insoluble in cold ether, insoluble in cold methyl and ethyl alcohols, but goes into solution upon heating, and is insoluble in petroleum ether. The acid number is 7, and the acid extract has a deep green color. The compound does not melt sharply, but fuses on the block at 200–205°. The spectrum was taken of an ether solution of a concentration of approximately 2 mg. in 50 cc. through a thickness of 50 mm. I, 714.0—648.6; II, 632.2—613.0 (faint and hazy); III, 549.6—526.8 (edges not sharp); IV, 514.1—487.4 (edges hazy); E. A., 443.4. Order: I, IV, III, II.

Anal. Calcd. for $C_{35}H_{38}O_7N_4$: C, 67.1; H, 6.07; N, 8.95; OCH_3 , 4.95. Found: C, 67.10, 67.26, 67.57, 67.65; H, 5.88, 5.92, 6.54, 5.89; N, 8.74, 8.91; OCH_3 , 5.19, 5.33, 5.45. (In these and all other analyses reported in this paper, the Pregl micro methods were employed; the samples were dried in high vacuum over phosphorus pentoxide for at least twelve hours at 61°.)

Dimethyl Phaeopurpurin 7.—An excess of diazomethane in ether was added to a solution of 80 mg. of phaeopurpurin 7 in 200 cc. ether. After two or three minutes the substance was completely methylated, as shown by shaking a portion of the solution in a test-tube with dilute alkali. The ether solution was fractionated and the product isolated in the usual manner; the yield was 55 mg. A more convenient method of preparation is to treat the mixture of unstable chlorins with diazomethane. Surprisingly enough, the color changes at once from olive green to the deep purplish-brown characteristic of the phaeopurpurins. The product is isolated in the usual way, care being taken in the acid fractionation to remove the trimethyl ester of a chlorin *e* with an acid number of 8. From 1 g. of methyl phaeophorbide *a*, 455–495 mg. of crystalline dimethyl phaeopurpurin 7 may be thus obtained; 150 mg. of the trimethyl ester of a chlorin was obtained as a by-product. A third method of preparation is by alkylation with dimethyl sulfate. Two hundred mg. of methyl phaeophorbide *a* was saponified under phase-test conditions and the product transferred to 200 cc. of water in a separatory funnel. Then dimethyl sulfate, which had previously been washed with dilute alkali, was added a few drops at a time with continuous shaking. Each portion of dimethyl sulfate was used up before more was added. At the neutral point the methylated product began to precipitate from the solution and the solution became almost colorless. Small portions of dilute alkali were added to destroy any dimethyl sulfate that remained. The aqueous layer containing the precipitate was shaken with ether, the ester passing into the ether solution; it was separated from any unmethylated material by washing with 0.1 *N* alkali. The fractionation of this solution with 8 and 10% acids produced a small amount of chlorin ester and dimethyl phaeopurpurin 7.

Dimethyl phaeopurpurin 7 crystallizes from ether solution in glistening hexagonal plates of a purplish-blue color. The ether solution is purplish-brown and the acid solution is green. The acid number is 10. It is somewhat more soluble in solvents than phaeopurpurin 7, especially in ether. On a block it melts at 233–235°. Spectrum in ether (1 mg. in 25 cc.) 50 mm. tube: I, 712.1–650.8; II, 634.6 - - 618.0 (very faint); III, 554.5 - - 549.0—532.1 - - 526.3; IV, 514.9 - - 510.5—491.7 - - 485.3; E. A., 442.2. Order: I, IV, III, II.

Anal. Calcd. for $C_{37}H_{42}O_7N_4$: C, 67.9; H, 6.42; N, 8.56; OCH_3 , 14.2. Found: C, 68.38, 68.46, 68.21; H, 6.51, 6.28, 6.44; N, 8.73, 8.65, 8.53; OCH_3 , 14.45, 14.16.

Phaeopurpurin 18.—This substance, purified by recrystallization from ether, has a rich brown color in ether solution that shows a purplish tint in thin layers or dilute solutions. It crystallizes from ether in hair-like needles which form matted clusters and dark, irregular aggregates. The crystals are yellow-brown by transmitted light and dark blue by reflected light. The compound is difficultly soluble in ether and methyl and ethyl alcohol, insoluble in benzene and petroleum ether, easily soluble in pyridine, moderately soluble in acetone. The acid number is 18, and the acid solution is greenish-blue in color. It has no definite melting point but slowly sinters at 250–280° on the block. Spectrum: (1 mg. in 50 cc. of ether containing pyridine) I, 716.0—676.5 - - 645.7—628.7; II, 549.9—544.2 - - 540.5—533.8; III, 511.1—498.0 (shadow); IV, 484.4 - - 470.7 (shadow, faint); E. A., 436.8. Order: I, II, III, IV.

Anal. Calcd. for $C_{33}H_{36}O_6N_4$: C, 70.35; H, 6.21; N, 9.66. Found: C, 70.04, 70.18; H, 5.87, 5.64; N, 9.26, 9.85, 9.71; OCH_3 , none.

Methyl phaeopurpurin 18 was prepared by the methylation of phaeopurpurin 18 with dimiazethane in acetone solution. It crystallizes from ether in prismatic needles or long plates with pointed ends. The crystals are extremely insoluble in ether, but are fairly soluble in acetone and very soluble in pyridine. The color of the ester in solutions is similar to that of the free acid. The acid number is 19. It melts at 275–278° (block). Spectrum (1 mg. in 50 cc. ether): I, 717.1—675.1 - - 645.9—628.5; II, 550—544.2 - -

540.0—533.7; III, 511.1—498.1; IV, 482.1—471.1 (shadow); E. A., 438.5. Order: I, II, III, IV.

Anal. Calcd. for $C_{35}H_{36}O_2N_4$: C, 71.0; H, 6.08; N, 9.46; OCH_3 , 5.24. Found: C, 69.80, 70.15, 70.45; H, 5.63, 6.05, 6.10; N, 9.18; OCH_3 , 5.34.

Chlorin *a*.—A solution of 300 mg. of phaeopurpurin 18 in 800 cc. of ether was shaken for ten minutes with 45 cc. of 25% methyl alcoholic potassium hydroxide; water was added and the product fractionated in the usual way. The 6–7% acid fraction was carefully refractionated to remove some material of lower acid number. Chlorin *a* crystallized from ether in clusters of small needles. The ether solution is slightly more greenish than that of chlorin *e*. The acid number is 6 and the acid solution is blue-green. The spectrum in ether (1 mg. in 30 cc.): I, 691.3—650.0; II, 620 - -606.2; III, 534—526.6; IV, 508.5—485.2; E. A., 432.5. Order: I, IV, III, II.

Anal. Calcd. for $C_{34}H_{36}O_2N_4$: C, 68.25; H, 6.36; N, 9.37. Found: C, 68.31, 68.20; H, 6.4, 6.9; N, 9.22, 9.04.

Chlorin *a* is the chief product, also, from the hot quick saponification of phaeopurpurin 18; there is also a small amount of a chlorin of acid number 3.5.

A sample of pure crystalline chlorin *a* after drying at 61° for twelve hours and standing for two weeks at room temperature was found to contain a small amount of phaeopurpurin 18. The transformation to phaeopurpurin 18 is hastened by heating as shown by an experiment in which a freshly purified sample was heated in diphenyl to 140° for thirty minutes; after transferring to ether the solution was purplish-red and a considerable amount of phaeopurpurin 18 was obtained by acid fractionation.

Trimethyl Ester of Chlorin *a*.—Methylation with diazomethane of the 6–7% fraction from the saponification of phaeopurpurin 18 (hot or cold) yielded the trimethyl ester of chlorin *a*. In one experiment 155 mg. was obtained from 300 mg. of phaeopurpurin 18.

This substance crystallizes from ether in hexagonal plates with a blue luster. The acid number is 10. The color of the ether and acid solutions is the same as those of the free acid. An ethereal solution does not show a "phase" with methyl alcoholic potash, but turns a deep blue-green color at once. The spectrum is the same as that of chlorin *a*. The melting point on the block was 239–240°.

Anal. Calcd. for $C_{37}H_{44}O_6N_4$: C, 69.4; H, 6.88; N, 8.75; OCH_3 , 14.52. Found: C, 69.4, 69.54; H, 6.9, 7.13; N, 8.97, 9.13; OCH_3 , 14.47, 14.63.

Alkali Decomposition of the Phaeopurpurins.—Thirty-milligram samples of both phaeopurpurin 7 and 18 were decomposed with 3 cc. of methyl alcoholic potassium hydroxide, 1.5 cc. of pyridine and 25 mg. of magnesium oxide at 150–160° for five hours in a silver-lined autoclave.⁶ The resulting mixtures were shaken with 20% acid to decompose the magnesium complexes and transferred to ether. Fractionation showed that in each case rhodoporphyrin was almost the sole product. Only traces of material with acid numbers lower or higher than four were found. The 4% extracts gave solutions whose spectra in ether and acid solution were identical with those of a sample of rhodoporphyrin.

Summary

The saponification of methyl phaeophorbide *a*, phaeophorbide *a* and the trimethyl ester of chlorin *e* has been studied. Under phase test conditions at room temperature unstable chlorins are formed in large amounts; from these chlorins a new type of compound, designated as the phaeopur-

⁶ Treibs and Wiedemann, *Ann.*, **471**, 174 (1929).

purins, results by a spontaneous change. By the use of ethyl alcohol in place of methyl alcohol, or by lowering the temperature, the unstable chlorins became the sole products: quick saponification at an elevated temperature produces chlorin *e* and no unstable chlorins.

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NOTES

Attempted Use of Activated Silica Gel in the Esterification of Salicylic Acid and β -Naphthol.¹—Korolev,² using an especially prepared silica gel suspended in the liquids, obtained 75–80% yields of methyl salicylate, and a 50% yield of methyl naphthyl ether.

Although the use of silica gel in the liquid phase seems to offer a simple method of preparing esters, the preparation of the silica gel as described by Korolev is far from a convenient process. It seemed worth while, therefore, to study the esterification of salicylic acid and β -naphthol using silica gel which had been activated by passing dry air over the gel at a temperature of 150°, according to the commercial process as devised by Patrick (U. S. Patent 1,297,724) and which has been adapted to small-scale production by Holmes.³

Since silica gel activated in this way is known to be a good absorber of water, the presence of the gel should favor production of ester, not only by its catalytic powers, but also by removing the water formed.

The general method used was to place two samples of the mixture to be esterified in flasks fitted with reflux condensers and to heat them in a water-bath. Silica gel was added to one flask and, after refluxing for at least eight hours, the acid was then determined by titration with a standard base.

It was found that a number of different samples of silica gel activated by the method of Patrick do *not* catalyze the esterification of salicylic acid or β -naphthol with methyl alcohol.

CONTRIBUTION FROM THE
SCHOOL OF CHEMISTRY
UNIVERSITY OF MINNESOTA
MINNEAPOLIS, MINNESOTA
RECEIVED JANUARY 13, 1930
PUBLISHED JULY 3, 1930

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Estimation of Organic Halogen.—In their paper "A General Method for the Determination of Halogens in Organic Compounds" [THIS JOURNAL, 52, 1195 (1930)] the authors, J. J. Thompson and U. O. Oakdale, make no mention in their references to a similar method

¹ The experimental work was done by R. Chelberg, teacher of chemistry at Tracy, Minnesota.

² Korolev, *J. Chem. Ind. (Moscow)*, 4, 547 (1927); *C. A.*, 22, 944 (1928).

³ Holmes, "Laboratory Manual of Colloid Chemistry," 1922, p. 76.