Pyrroles and Related Compounds. Part XXIV.[1] Separation and Oxidative Degradation of Chlorophyll Derivatives

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A convenient, large-scale method for separation of the *a* and *b* series metal-free chlorophyll derivatives is described, utilising the Girard ' T ' reagent with subsequent chromatography. Efficient procedures for conversion of phaeophytin-*a* (3) into rhodoporphyrin-XV dimethyl ester (1) and the corresponding 2-vinyl compound (2), involving the classical oxidation to purpurin derivatives, followed by further degradation, are described; n.m.r. data for several important chlorophyll degradation products are reported. Novel oxidative reactions of the isocyclic ring in methyl mesopyrophaeophorbide-*a* (19) are also described.

Our recent studies on syntheses of porphyrin β-keto-esters and their cyclisation to phaeoporphyrins[2,3] and on the protective modification of porphyrin vinyl groups[4] required relatively large amounts of rhodoporphyrin-XV dimethyl ester (1) and the corresponding 2-vinyl compound (2). Though we have obtained these compounds by total synthesis *via* the *b*-oxobilane route,[5] we sought efficient routes to them from the

[1] Part XXIII, J. A. S. Cavaleiro, G. W. Kenner, and K. M. Smith, *J.C.S. Perkin I*, 1973, 2478.

[2] M. T. Cox, T. T. Howarth, A. H. Jackson, and G. W. Kenner, *J. Amer. Chem. Soc.*, 1969, **91**, 1232.

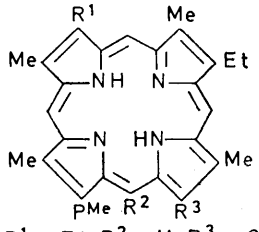
[3] G. W. Kenner, S. W. McCombie, and K. M. Smith, *J.C.S. Chem. Comm.*, 1972, 844.

[4] G. W. Kenner, S. W. McCombie, and K. M. Smith, *J.C.S. Chem. Comm.*, 1972, 1347; *Annalen*, 1973, in the press.

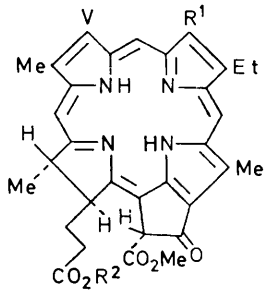
[5] For a general account of this, and other, porphyrin syntheses, see A. H. Jackson and K. M. Smith in ' The Total Synthesis of Natural Products,' vol. 1, ed. J. W. ApSimon, Wiley, New York, 1973, p. 143.

available phaeophytin-*a* (3) and -*b* (4) mixture by means of oxidative degradation of the isocyclic ring.

The *a* and *b* series chlorins can be separated by chromatography on sucrose, but the scale of operation is strictly limited by the necessarily high adsorbent-to-chlorin ratio. A report[6] on the use of the Girard 'T' reagent for some separations in this field prompted us to study this method further. With the phaeophytin mixture, the condensation product (5) formed from (4) was not extracted satisfactorily into water, and even in more favourable cases troublesome emulsions were frequently encountered; thus a chromatographic procedure was developed. After reaction with an excess of reagent 'T' in chloroform–methanol–acetic acid, the solvents were removed and the residue was chromatographed on deactivated, neutral alumina. The condensation product (5) was strongly adsorbed and phaeophytin-*a* could be eluted readily, in high yield. Upon drastic increase of the solvent polarity, compound (5) was eluted, and was converted into methyl phaeophorbide-*b* (6) by treatment with methanol containing small amounts of acetone and sulphuric acid. Methyl phaeophorbide-*a* (7) was obtained by methanolysis



(1) R^1 = Et, R^2 = H, R^3 = CO_2Me
(2) R^1 = V, R^2 = H, R^3 = CO_2Me
(13) R^1 = Et, R^2 = R^3 = H
(14) R^1 = Et, R^2 = R^3 = CO_2Me



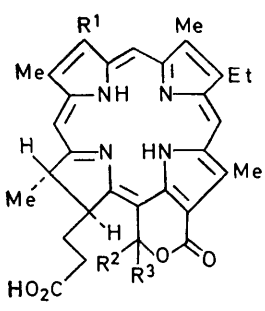
(3) R^1 = Me, R^2 = phytyl
(4) R^1 = CHO, R^2 = phytyl
(5) R^1 = CH:N·NH·CO·CH_2·$\overset{+}{N}Me_3$ Cl^-
R^2 = phytyl
(6) R^1 = CHO, R^2 = Me
(7) R^1 = R^2 = Me

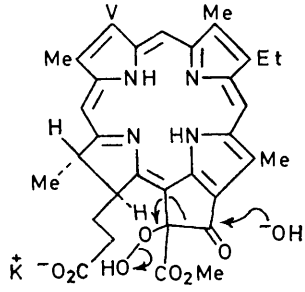
P^{Me} = CH_2·CH_2·CO_2Me; V = CH:CH_2

of the phaeophytin mixture, followed by separation with the Girard reagent, or else by methanolysis of the pure phaeophytin-*a*.

* Porphyrins of the *b* series were not encountered, presumably owing to degradation of the macrocycle during the acid-induced decarboxylation.

In his extensive studies[7] on chlorophylls, Fischer showed that aerial oxidation of the enolate ion of phaeophytin-*a* resulted in cleavage of the C(9)–C(10)





(8) R^1 = V, R^2 = OH, R^3 = CO_2H (9)
(10) R^1 = V, R^2R^3 = O
(12) R^1 = Et, R^2R^3 = O

bond; the phytyl group was also lost, and after acidification the product was the 'unstable chlorin' (8). The key step in this process may involve fragmentation of the 10-hydroperoxide (9), as indicated in the formula. On evaporation of the solution, (8) was converted into purpurin-18 (10), whereas esterification with diazomethane gave purpurin-7 trimethyl ester (11). We decided to utilise these purpurins as sources of rhodoporphyrin derivatives, and our initial experiments were directed towards the 2,4-diethyl compound (1). Catalytic hydrogenation of the phaeophytin mixture, followed by oxidation of the enolate and evaporation of the chlorin solution, gave a crude mixture containing mesopurpurin-18 (12); upon heating in hydrochloric–acetic acid, followed by esterification and oxidation of any chlorins with dichlorodicyanobenzoquinone (DDQ), a porphyrin mixture was obtained which was readily separated by chromatography on alumina. The major component was the desired porphyrin (1), formed in about 15% yield from phaeophytins; this was accompanied * by minor amounts of pyrroporphyrin-XV methyl ester (13) and δ-carboxyrhodoporphyrin-XV trimethyl ester (14). The latter porphyrin had a melting point considerably higher than reported by Fischer,[7] but the structure was confirmed by mass and ^1H n.m.r. spectroscopy, and by comparison with a sample prepared unambiguously from the cyclic anhydride (10) by treatment with base, esterification, hydrogenation of the vinyl substituent, and oxidation to porphyrin with DDQ.

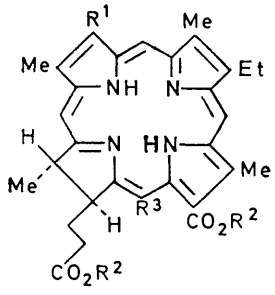
The degradation with acid was not applicable to the preparation of (2), owing to the sensitivity of the vinyl group; hence non-acidic methods for removal of the γ-substituent were investigated. Fischer[7] has reported the loss of the glyoxylic ester residue from (11) under the influence of strong base, producing rhodochlorin (15); the behaviour of (11) in basic solvents

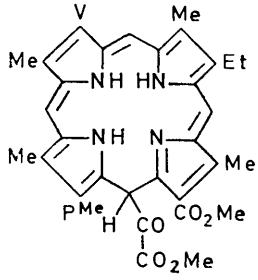
[6] H. R. Wetherell and M. J. Hendrickson, *J. Org. Chem.*, 1959, **24**, 710.

[7] H. Fischer and H. Stern, 'Die Chemie des Pyrrols,' Akademische Verlag, Leipzig, 1940, vol. IIii.

was therefore examined. The desired conversion into (2) was achieved by refluxing (11) for several hours in pyridine, which produced the porphyrin in 55% yield; the yield was raised to 76% by refluxing for a shorter period in 2,4,6-trimethylpyridine (180°). Since phaeophytin-*a* can be converted into (11) in 65—70% yield (essentially by Fischer's procedure, but with chromatographic work-up), the overall conversion into (2) was *ca.* 50%. The analogous decomposition of mesopurpurin-7 trimethyl ester (16) in refluxing collidine produced rhodoporphyrin-XV dimethyl ester (1) in 81% yield.

The mechanism of the purpurin-into-porphyrin conversion is open to conjecture; thermolysis of (11) in collidine containing deuterium oxide gave deuteriated (2) which consisted mainly of a [$^2H_2$]-species, with appreciable amounts of [$^2H_1$]- and [$^2H_3$]-compounds, the deuterium being situated at the *meso*-positions. Presumably, the base-induced loss of the glyoxylic ester residue requires preliminary isomerisation to the phlorin (17) (*cf.* ref. 8); cleavage of the γ-substituent from this





(17)

(11) R^1 = V, R^2 = Me, R^3 = CO·CO_2Me
(15) R^1 = V, R^2 = R^3 = H
(16) R^1 = Et, R^2 = Me, R^3 = CO·CO_2Me

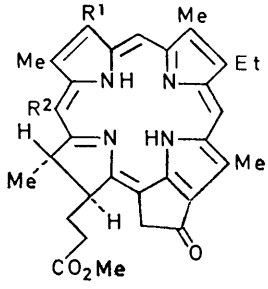
species finds analogy in one of the steps of the Harvard approach to chlorophyll-*a*,[9] and would be followed by protonation and subsequent oxidation of the resulting *meso*-unsubstituted phlorin.

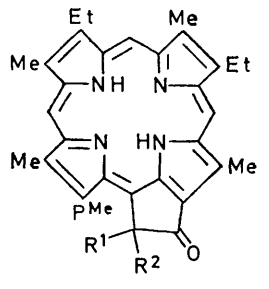
The use of refluxing collidine in place of pyridine was found to have a beneficial effect in the conversion of methyl phaeophorbide-*a* (7) into the corresponding 'pyro' compound (18); heating under reflux during 90 min in collidine gave an essentially quantitative reaction, whereas the usual procedure [7,10] of prolonged reflux in pyridine often gives rise to decomposition products. Methyl phaeophorbide-*b* also underwent the 'pyro' reaction very efficiently under these new conditions.

Oxidation of methyl mesopyrophaeophorbide-*a* (19) was examined, with a view to cleavage of the isocyclic ring in the 'pyro' series, in connection with our biosynthetic studies on chlorophylls. Treatment of (19) with DDQ produced, in addition to the expected phyllo-

[8] R. B. Woodward and V. Škarić, *J. Amer. Chem. Soc.*, 1961, **83**, 4676.
[9] R. B. Woodward, *Angew. Chem.*, 1960, **72**, 651; *Pure Appl. Chem.*, 1961, **2**, 383; *J. Amer. Chem. Soc.*, 1960, **82**, 3800.
[10] H. Wolf, H. Brockmann, jun., I. Richter, C.-D. Mengler, and H. H. Inhoffen, *Annalen*, 1968, **718**, 162.

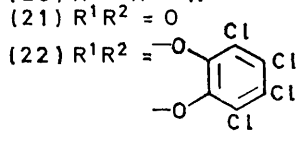
erythrin methyl ester (20), a more polar compound which proved to be the 10-oxo-compound (21). The reactivity of the 10-methylene group was again demonstrated in the reaction of (19) with an excess of *o*-chloranil; the product was characterised by n.m.r. data and elemental analysis as the spiro-acetal (22). Treatment of (21) with selenium dioxide was without effect, and attempted oxidation of the methylene group with dimethyl sulphoxide–hydrogen bromide [11] resulted in





(18) R^1 = V, R^2 = H
(19) R^1 = Et, R^2 = H
(23) R^1 = Et, R^2 = Br

(20) R^1 = R^2 = H
(21) R^1R^2 = O



(22) R^1R^2 =

bromination at the δ-position, a reaction course which finds ample precedent in other studies [8,12] of electrophilic attack on chlorins. The bromo-compound (23) was, however, formed more efficiently by treatment of (19) with pyridinium perbromide.[13]

EXPERIMENTAL

M.p.s were measured on a microscopic hot-stage apparatus. Unless otherwise stated, neutral alumina (Merck; Brockmann grade V) was used in all chromatographic separations. N.m.r. spectra were determined with the Varian HA-100 instrument (deuteriochloroform as solvent with tetramethylsilane as internal standard) and visible absorption spectra were measured with a Unicam SP 800 spectrophotometer (methylene chloride as solvent). Mass spectra were recorded on an A.E.I. MS12 or MS902 instrument (direct insertion probe, source temperature 200—220° at 50 μA and 70 eV).

Methyl Phaeophorbide-a *and* -b *from Phaeophytin*-a *and* -b.—Phaeophytin mixture (5·0 g) was stirred during 15 h in the dark with 5% v/v sulphuric acid in methanol (400 ml). The blue-green solution was diluted with chloroform (400 ml) and the mixture was washed with water (2 × 1 l), dried (Na_2SO_4), and then evaporated to dryness. The residue was crystallised from methylene chloride–methanol, giving the chlorin mixture (2·85 g, 83%) which was normally used without further purification.

Methyl Phaeophorbide-a (7) *and* -b (6) *from the Mixture.*—The foregoing chlorin mixture (2·50 g) was heated under reflux in chloroform (125 ml) with a solution of Girard's reagent 'T' (1·5 g, 2·5 equiv.) in methanol (125 ml) and acetic acid (25 ml) during 1 h. The solvent was evaporated

[11] E. Schipper, M. Cinnamon, L. Rascher, Y. H. Chiang, and W. Oroshnik, *Tetrahedron Letters*, 1968, 6201.
[12] R. Bonnett, I. A. D. Gale, and G. F. Stephenson, *J. Chem. Soc.* (*C*), 1966, 1600.
[13] L. F. Fieser and M. Fieser, 'Reagents for Organic Synthesis,' vol. 1, Wiley, New York, 1967, p. 966.

off *in vacuo* and toluene (*ca.* 100 ml) was added and then evaporated off to remove residual acetic acid. The residue was dissolved/suspended in methylene chloride (*ca.* 50 ml) and chromatographed on alumina (300 g). Elution with methylene chloride gave a deep blue-grey band, and evaporation of the corresponding eluates gave substantially pure methyl phaeophorbide-*a* (1·5 g). Recrystallisation from methylene chloride–methanol gave blue-black plates (1·12 g, 62%), m.p. 224—226° (lit.,[14] 228°), τ (0·2M), 0·89, 1·12, and 1·54 (each 1H, s, 3 *meso*-H), 2·40 (1H, m), and 4·05 (2H, m) ($CH{:}CH_2$), 3·76 (1H, s, 10-H), 5·57 and 5·80 (each 1H, m, 7- and 8-H), 6·10 (3H, s, 10-CO_2Me), 6·41 (3H, s) and 7·6 (4H, m) ($MeO_2C{\cdot}CH_2{\cdot}CH_2$), 6·40 (2H, q) and 8·50 (3H, t) ($CH_2{\cdot}CH_3$), 6·43, 6·80, and 7·18 (each 3H, s, 1-, 3-, and 5-Me), and 8·16 (3H, d, 8-Me).

Further elution of the column with chloroform containing 1% ethanol gave a minor grey band, presumably containing allomerised chlorophylls, which was discarded. The deep yellow-brown *b* series derivative was eluted with chloroform–methanol (20 : 1); the eluates were evaporated to dryness and the residue was stirred overnight with a mixture of methanol (200 ml), acetone (10 ml), and concentrated sulphuric acid (6·5 ml). The solution was diluted with chloroform (400 ml) and washed with water (2 × 750 ml). The organic layer was evaporated and the residue was chromatographed on alumina (150 g) (elution with methylene chloride). Methyl phaeophorbide-*b* was eluted as a deep brownish-grey band, and the residue from the evaporated eluates was recrystallised from methylene chloride–methanol (0·42 g, 67%) to give grey lustrous plates, m.p. 264—267° (lit.,[15] 269°), τ (0·05M), −0·62 (1H, s, CHO), 0·19, 1·04, and 1·55 (each 1H, s, 3 *meso*-H), 2·28 (1H, m) and 3·90 (2H, m) ($CH{:}CH_2$), 3·84 (1H, s, 10-H), 5·61 and 5·85 (each 1H, m, 7 and 8-H), 6·11 (3H, s, (10-CO_2Me), 6·3 (2H, m) and 8·32 (3H, t) ($CH_2{\cdot}CH_3$), 6·42 (3H, s) and 7·6 (4H, m) ($MeO_2C{\cdot}CH_2{\cdot}CH_2$), 6·57 and 6·77 (each 3H, s, 1- and 5-Me), and 8·17 (3H, d, 8-Me).

Phaeophytin-a (3) *from the Mixture.*—Phaeophytin mixture (14·5 g) was dissolved in chloroform (400 ml) and heated under reflux during 1 h with a solution of Girard's reagent 'T' (6 g) in methanol (500 ml) and acetic acid (70 ml). The solution was evaporated *in vacuo*, the last traces of acetic acid being removed by addition of toluene and re-evaporation. The residue was dissolved in warm methylene chloride (*ca.* 75 ml) and filtered through glass wool to remove most of the excess of Girard reagent, which was washed with a little methylene chloride, and the combined filtrates were chromatographed (elution with methylene chloride). Evaporation of the eluates gave a black, slightly sticky solid, and recrystallisation from methylene chloride–methanol gave pure phaeophytin-*a* (8·8 g, 89%) as soft, black crystals. The *b* series condensation product was converted into methyl phaeophorbide-*b* as described in the previous preparation, giving 2·5 g (68%) after recrystallisation from methylene chloride–methanol.

Purpurin-7 *Trimethyl Ester* (11).—Phaeophytin-*a* (2·65 g) was dissolved in warm pyridine (40 ml) and the solution was diluted with ether (1·25 l). The solution was stirred with a stream of air passing through it, and a solution of potassium hydroxide (20 g) in n-propanol (70 ml) was added. The bright green mixture (containing precipitated potassium hydroxide) was stirred and aerated for 30 min and then extracted with water (2 × 500 ml). The ethereal solution was discarded; the aqueous extracts were combined, acidified with concentrated sulphuric acid (20 ml) in water (100 ml), and then extracted with methylene chloride (2 × 600 ml). The extracts were washed with water (500 ml) and immediately treated with an excess of ethereal diazomethane. The brownish-purple solution was left at room temperature during 10 min, and then evaporated *in vacuo*. Chromatography of the residue (elution with methylene chloride) gave a single band (purple-brown) of the desired product. The appropriate eluates were evaporated, and the residue was crystallised from methylene chloride–n-hexane, giving purpurin-7 trimethyl ester (1·31 g, 67%) as blue-black plates, m.p. 228—230° (lit.,[16] 234°) (Found: C, 68·4; H, 6·2; N, 8·9. Calc. for $C_{37}H_{40}N_4O_7$: C, 68·1; H, 6·2; N, 8·6%), τ (0·08M), 0·46, 0·81, and 1·53 (each 1H, s, 3 *meso*-H), 2·22 (1H, m) 3·88 (2H, m) ($CH{:}CH_2$), 5·30 and 5·69 (each 1H, m, 7- and 8-H), 5·87, 6·14, 6·48, 6·74, and 6·84 (each 3H, s, 1-, 3-, and 5-Me and 2 OMe), 6·2 and 8·38 (2H, q and 3H, t, $CH_2{\cdot}CH_3$), 6·42 and 7·7 (3H, s and 4H, m, $MeO_2C{\cdot}CH_2{\cdot}CH_2$), and 8·19 (3H, d, 8-Me).

Mesopurpurin-7 *Trimethyl Ester* (16).—This compound was prepared and purified in a manner analogous to the foregoing preparation: phaeophytin-*a* (2·0 g) was hydrogenated in acetone (400 ml) over palladised charcoal, during 1 h. The resulting *meso*-compound was dissolved in pyridine and ether and oxidised exactly as above. Recrystallisation of the chromatographically purified product from methylene chloride–n-hexane gave the mesopurpurin (0·78 g, 52%) as a greenish-black powder, m.p. 211—214° (lit.,[17] 215—216°), τ (0·08M), 0·54, 1·01, and 1·69 (each 1H, s, 3 *meso*-H), 5·41 and 5·82 (each 1H, m, 7- and 8-H), 5·95, 6·22, 6·52, 6·56, 6·90, and 6·96 (each 3H, s, 3 CO_2Me and 1-, 3-, and 5-Me), 6·41, 8·40, and 8·42 (4H, q, 3H, t, and 3H, t, 2 $CH_2{\cdot}CH_3$), 7·95 (4H, m, $CH_2{\cdot}CH_2$), and 8·29 (3H, d, 8-Me).

Purpurin-18 *Methyl Ester.*—This chlorin was prepared by a modification of the method of Fischer:[18] phaeophytin-*a* (0·50 g) was oxidised in ethereal solution, following the method described for the preparation of purpurin-7 methyl ester. The methylene chloride extract containing 'unstable chlorin' was subjected to repeated evaporation and redissolution in methylene chloride–benzene, until no further increase in visible absorption at 695 nm was observed. The product was esterified with ethereal diazomethane, and chromatographed on a short column of alumina. Elution with methylene chloride gave a purple-brown band and recrystallisation of the residue from the appropriate evaporated eluates gave the purpurin as a fine black powder (220 mg, 66%), τ, (0·07M), 0·73, 0·81, and 1·50 (each 1H, s, 3 *meso*-H), 2·16 and 3·85 (1H, m and 2H, m, $CH{:}CH_2$), 4·83 and 5·70 (each 1H, m, 7- and 8-H), 6·4 and 8·48 (2H, q and 3H, t, $CH_2{\cdot}CH_3$), 6·42, 6·74, and 6·98 (1-, 3-, and 5-Me), 6·46 and 7·7 (3H, s and 4H, m, $MeO_2C{\cdot}CH_2{\cdot}CH_2$), and 8·29 (3H, d, 8-Me).

Mesopurpurin-18 *Methyl Ester.*—Phaeophytin mixture (4·0 g) in pyridine (40 ml) and acetone (300 ml) was hydrogenated at room temperature during 1 h over 10% palladised charcoal (1 g). The catalyst was removed by filtration through Celite and the filtrate was evaporated to leave only the pyridine. This solution was diluted with ether (1·5 l) and then treated with a solution of potassium

[14] Ref. 7, p. 64.
[15] Ref. 7, p. 247.
[16] Ref. 7, p. 111.
[17] Ref. 7, p. 112.
[18] Ref. 7, p. 118.

hydroxide (20 g) in n-propanol (70 ml). The resulting solution was shaken vigorously during 5 min, and a mixture of 30% hydrogen peroxide solution (10 ml) and water (15 ml) was added; shaking was continued for a further 10 min. The mixture was extracted with water (600 and then 300 ml) and the ethereal layer was discarded. The aqueous extracts were combined and acidified with a mixture of concentrated sulphuric acid (20 ml) and ice (*ca.* 200 g) and then extracted with chloroform (3 × 500 ml). The combined extracts were washed with water (500 ml), dried ($MgSO_4$), and evaporated *in vacuo*. The residue was dissolved in chloroform (100 ml) and benzene (200 ml) and the solution was again evaporated. This process was repeated until no further increase in visible absorption at 690 nm was observed. The final product was dissolved in chloroform (200 ml), cooled to 0°, and then treated with an excess of ethereal diazomethane. After 5 min at 0° the solution was evaporated and the residue was chromatographed (elution with methylene chloride). A minor green band, presumably containing mesochlorin-e_6 ester, was discarded, and the slower-running purple-grey anhydride was collected. The eluates were evaporated and the residue was recrystallised from methylene chloride–n-hexane, to give mesopurpurin-18 methyl ester (390 mg, 19%) as shiny grey plates, τ, (0·08M), 0·88, 0·90, and 1·49 (each 1H, s, 3 *meso*-H), 4·90 and 5·67 (each 1H, m, 7- and 8-H), 6·33 and 6·63 (each 2H, q) and 8·33 and 8·51 (each 3H, t) (2 CH_2·CH_3), 6·44 and 7·6 (3H, s and 4H, m, MeO_2-C·CH_2·CH_2), 6·69, 6·82, and 6·96 (each 3H, s 1-, 3-, and 5-Me), and 8·29 (3H, d, 8-Me).

2,4-*Diethyl*-6-*methoxycarbonyl*-7-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (' *Rhodoporphyrin*-XV *Dimethyl Ester* ') (1).—(a) *From mesopurpurin*-7 *trimethyl ester* (16). Mesopurpurin-7 trimethyl ester (325 mg) was heated during 2 h at 200° in 2,4,6-collidine (40 ml). The cooled solution was evaporated and the residue was crystallised from methylene chloride–methanol, giving the required porphyrin (228 mg, 81%) as purple needles, m.p. 266—268°, identical (mixed m.p., t.l.c., and mass and n.m.r. spectra) with an authentic sample prepared by total synthesis (m.p. 267—269°).

(b) *From mesopurpurin*-18 *methyl ester*. Mesopurpurin-18 methyl ester (100 mg) was heated at 100° during 2 h with a mixture of acetic acid (20 ml) and concentrated hydrochloric acid (15 ml) containing water (15 ml). The resulting brown solution was diluted with water (250 ml) and extracted with methylene chloride–pyridine (10 : 1). The extract was washed with water, dried ($MgSO_4$), filtered, and then treated with a large excess of ethereal diazomethane at 0° for 10 min. The solution was then evaporated and the residue was chromatographed on alumina (grade III) (elution with methylene chloride). The purple band, consisting of a porphyrin–chlorin mixture was collected and treated with DDQ (20 mg); the solution was concentrated, and then chromatographed as before. A single porphyrin was eluted with methylene chloride; the solution was evaporated and the residue was crystallised from methylene chloride–methanol, to give purple needles (61 mg, 65%), m.p. 266—268, identical with the material prepared in (a).

(c) *From phaeophytins, with concurrent production of pyrroporphyrin*-XV *methyl ester* (13) *and γ-carboxyrhodoporphyrin*-XV *trimethyl ester* (14). Phaeophytin mixture (5·0 g) was hydrogenated and oxidised as described for mesopurpurin-18 methyl ester. The crude purpurin obtained on evaporation of the chloroform extract was dissolved in acetic acid (100 ml), water (70 ml), and concentrated hydrochloric acid (70 ml) and then the solution was refluxed gently during 2 h. The mixture was diluted with iced water (*ca.* 1 l), neutralised with concentrated ammonia solution (*d* 0·88; 70 ml) and then extracted with 10 : 1 chloroform–pyridine (3 × 400 ml). The extracts were washed with water (1 l), dried ($MgSO_4$), filtered, and esterified with ethereal diazomethane (from 7 g of ' diazald '). The solution was then evaporated, and the residue was chromatagraphed on alumina (grade III) (elution with methylene chloride). The porphyrin–chlorin mixture was oxidised by addition of small portions (*ca.* 20 mg) of DDQ until the chlorin visible absorption band at 650 nm was at a minimum. The product was then rechromatographed on alumina (grade III) [elution with benzene–methylene chloride (1 : 1)]. The most mobile porphyrin (red-brown band) was collected and crystallised from methylene chloride–methanol, giving pyrroporphyrin-XV methyl ester (35 mg) as purple plates, m.p. 235—237° (lit.,[19] 240—242°), τ (0·05M) 0·02, 0·04, and 0·07 (1H, s, 1H, s, and 2H, s, 4 *meso*-H), 0·99 (1H, s, 6-H), 5·64, 6·83, and 6·34 (2H, t, 2H, t, and 3H, s, CH_2·CH_2·CO_2Me), 6·02 and 6·06 (each 2H, q) and 8·18 and 8·20 (2H, t and 3H, t) (2 CH_2·CH_3), 6·34, 6·48, and 6·52 (3H, s, 3H, s, and 6H, s, 1-, 3-, 5-, and 8-Me).

The major porphyrin, eluted in pure methylene chloride, was the required rhodoporphyrin-XV dimethyl ester; recrystallisation from methylene chloride–methanol gave red needles (390 mg, 15%) identical with the material described in (a) and (b).

Further elution with methylene chloride gave a third porphyrin which was purple on the column. Evaporation of the eluates and crystallisation from methylene chloride–methanol gave γ-carboxyrhodoporphyrin-XV trimethyl ester (55 mg) as flat, purple needles, m.p. 260—262° (lit.,[20] 234°), τ (0·08M) 0·28, 0·37, and 0·62 (each 1H, s, 4 *meso*-H), 5·56 and 5·69 (each 3H, s, 2 CO_2Me), 6·0, 6·95, and 6·33 (2H, m, 2H, t, and 3H, s, CH_2·CH_2·CO_2Me), 6·0 and 8·40 (4H, m, and 6H, t, 2 CH_2·CH_3), and 6·44, 6·60, 6·77, and 6·85 (each 3H, s, 1-, 3-, 5-, and 8-Me), *m/e* 624(100%) and 565(43). This material was identical with an authentic sample prepared from chlorin-p_6 trimethyl ester as described in the experiments below.

Chlorin-p_6 *Trimethyl Ester*.—This compound was prepared from purpurin-18 methyl ester (180 mg) by hydrolysis and treatment with diazomethane, as described by Fischer.[21] The chlorin was purified by chromatography on alumina (elution with methylene chloride) and crystallised from methylene chloride–n-hexane to give deep-green, shiny leaflets (142 mg, 77%), τ (0·08M), 0·35, 0·61, and 1·38 (each 1H, s, 3 *meso*-H), 2·17 and 3·90 (1H, m and 2H, m, CH:CH_2), 4·86 and 5·56 (each 1H, m, 7- and 8-H), 5·85 and 5·89 (each 3H, s, 2 CO_2Me), 6·40 and 8·37 (2H, q and 3H, t, CH_2·CH_3), 6·43 and 7·8 (3H, s and 4H, m, MeO_2-C·CH_2·CH_2), 6·53, 6·69, and 6·88 (each 3H, s, 1-, 3-, and 5-Me), and 8·19 (3H, d, 8-Me).

2,4-*Diethyl*-6,γ-*bis*(*methoxycarbonyl*)-7-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (14).—Chlorin-p_6 trimethyl ester (62 mg) was dissolved in acetone (50 ml) and hydrogenated during 50 min over 10% palladised

[19] H. Fischer and H. Orth, ' Die Chemie des Pyrrols,' Akademische Verlag, Leipzig, 1937, vol. IIi, p. 343.

[20] Ref. 19, p. 541.

[21] Ref. 7, p. 122.

charcoal (100 mg). The resulting red solution was filtered through Celite, and the filtrate was warmed at 50° with DDQ (47 mg, 2·2 equiv.). The colour of the solution immediately became blue-green, then changed to purple-red during 10 min. After a further 10 min, the mixture was evaporated and the residue was chromatographed on alumina (grade III). Elution with methylene chloride gave a single porphyrin, which crystallised from methylene chloride–methanol to give the required porphyrin (14) (28 mg, 47%), m.p. 258—259·5°, not depressed upon admixture with the material (m.p. 260—262°) from degradation of phaeophytin.

4-Ethyl-6-methoxycarbonyl-7-(2-methoxycarbonylethyl)-1,3,5,8-tetramethyl-2-vinylporphin (' *2-Vinylrhodoporphyrin*-XV *Dimethyl Ester* ') (2).—(a) *From purified purpurin-7 trimethyl ester.* Purpurin-7 trimethyl ester (320 mg) was heated in an oil-bath at 190—200° in 2,4,6-collidine (40 ml) during 2 h. After cooling, the solvent was evaporated off at 0·1 mmHg and the residue was recrystallised from methylene chloride–methanol, giving the required porphyrin (207 mg, 76%) as purple needles, m.p. 271—272·5°, not depressed upon admixture with a synthetic sample of m.p. 272—274°.

(b) *From phaeophytin*-a *or methyl phaeophorbide*-a. These two chlorins were transformed into 2-vinylrhodoporphyrin-XV dimethyl ester in 42—48% yield by oxidation to purpurin-7 trimethyl ester, chromatography, and then pyrolysis and crystallisation as described in (a).

(c) *Deuteriation experiment.* Distilled 2,4,6-collidine was dried by passage through a column of alumina (grade I). Recrystallised purpurin-7 trimethyl ester (100 mg) and deuterium oxide (0·3 ml) were dissolved in the purified collidine (25 ml) and the solution was refluxed during 2 h and then evaporated *in vacuo*. The residue was chromatographed on alumina (grade III) (elution with methylene chloride) and the product was recrystallised from methylene chloride–methanol (twice). The deuteriated porphyrin (46 mg, 53%) was obtained as needles, m.p. 273—274°. The mass spectral parent ion cluster was compared with that of the undeuteriated material, and the following deuteriation pattern was calculated: $^2H_0$ 5%; $^2H_1$ 20%; $^2H_2$ 45%; $^2H_3$ 25%; $^2H_4$ 5%.

Methyl Pyrophaeophorbide-a (18).—Methyl phaeophorbide-*a* (100 mg) was heated under reflux during 90 min in collidine (25 ml) in an oil-bath at 190—200° under a slow stream of nitrogen. The solution was evaporated at 0·1 mmHg and the residue was crystallised from methylene chloride–methanol, to give the required pyro-compound (82 mg, 91%) as blue-black needles, τ (0·08M), 0·74, 0·82, and 1·52 (each 1H, s, 3 *meso*-H), 2·15 and 3·90 (1H, m and 2H, m, $CH{:}CH_2$), 4·91 (2H, q, 10-CH_2), 5·60 and 5·78 (each 1H, m, 7- and 8-H), 6·30 and 8·41 (2H, q and 3H, t, $CH_2{\cdot}CH_3$), 6·42 and 7·7 (3H, s and 4H, m, $MeO_2C{\cdot}CH_2{\cdot}CH_2$), 6·49, 6·70, and 6·94 (each 3H, s, 1-, 3-, and 5-Me), and 8·22 (3H, d, 8-Me).

Methyl Pyrophaeophorbide-b.—Methyl phaeophorbide-*b* (100 mg) was heated under reflux in collidine (25 ml) as in the previous experiment. After evaporation, the product was purified by chromatography on alumina, and crystallised from methylene chloride–n-hexane (78 mg, 83%) as fine, black crystals, τ (0·1M), −0·65 (1H, s, CHO), 0·30, 1·39, and 1·64 (each 1H, s, 3 *meso*-H), 2·30 and 3·87 (1H, m and 2H, m, $CH{:}CH_2$), 4·99 (2H, q, 10-CH_2), 5·60 and 5·83 (each 1H, m, 7- and 8-H), 6·34 and 8·65 (2H, q and 3H, t, $CH_2{\cdot}CH_3$), 6·36 and 7·5 (3H, s and 4H, m, $MeO_2C{\cdot}CH_2{\cdot}CH_2$), 6·74 and 6·76 (each 3H, s, 1- and 5-Me), and 8·15 (3H, d, 8-Me).

Methyl Mesopyrophaeophorbide-a (19).—Methyl phaeophorbide-*a* (1·02 g) was dissolved in pyridine (10 ml) and acetone (400 ml) and hydrogenated at room temperature over 10% palladised charcoal (0·75 g) during 40 min. The deep-blue solution was filtered through Celite and the filtrate was evaporated *in vacuo*. The residue was heated under reflux in an atmosphere of nitrogen in anhydrous pyridine (75 ml) during 15 h. The solvent was removed *in vacuo* and the residue was chromatographed on alumina (grade III) (elution with methylene chloride). The product was eluted as a deep blue-black band, and was recrystallised from methylene chloride–n-hexane to give blue-black needles (751 mg, 78%), m.p. 239—242° (lit.,[22] 242—244°).

Methyl δ-Bromomesopyrophaeophorbide-a (23).—(*a*) Methyl mesopyrophaeophorbide-*a* (100 mg) was dissolved in a mixture of dimethyl sulphoxide (20 ml) and 48% hydrogen bromide in acetic acid (1·0 ml), and the solution was heated at 90—100° during 5 min (the colour turned from blue to green). The mixture was diluted with ether (250 ml) and then washed with water (3 × 250 ml) and then saturated brine (250 ml). The brownish-purple solution was dried ($MgSO_4$) and evaporated. Chromatography of the residue on alumina (elution with methylene chloride) gave a single purple-brown band, leaving polar decomposition products on the column. The required *bromo-compound* (61 mg, 54%) was obtained as black needles, m.p. 142—144° (from methylene chloride) (Found: C, 64·95; H, 5·8; N, 8·7. $C_{34}H_{37}BrN_4O_3$ requires C, 64·8; H, 5·8; N, 8·9%), τ (0·1M), 0·64 and 0·72 (each 1H, s, 2 *meso*-H), 4·86 (2H, q, 10-CH_2), 5·41 and 5·85 (each 1H, m, 7- and 8-H), 6·25, 8·38, and 8·42 (4H, q, 3H, t, and 3H, t, 2 $CH_2{\cdot}CH_3$), 6·45 and 7·70 (3H, s and 4H, m, $MeO_2C{\cdot}CH_2{\cdot}CH_2$), 6·47, 6·55, and 6·84 (each 3H, s, 1-, 3-, and 5-Me), and 8·32 (3H, d, 8-Me), $\lambda_{max.}$ 413·5 (ε 102,000), 516(7200), 547·5(12,500), 613(5400), and 672 nm (38,400); $\lambda_{max.}$ [CH_2Cl_2–$CF_3{\cdot}CO_2H$ (trace)] 422 (ε 132,000), 540(4400), 562·5(6300), 612(6600), and 664 nm (31,100).

(*b*) Methyl mesopyrophaeophorbide-*a* (55 mg) was stirred for 20 min in methylene chloride (15 ml) and pyridine (0·1 ml) with pyridinium perbromide (38 mg, 1·2 equiv.). The solution was evaporated, and the residue was chromatographed and crystallised as in (*a*), giving the bromo-compound (38 mg, 62%), m.p. 142—144°, identical with the material synthesised above.

Methyl δ-Bromomesophaeophorbide-a.—(*a*) The method described in (*a*) above was followed, with methyl mesophaeophorbide-*a* (145 mg). The product was purified in ether, and chromatographed on alumina (elution with methylene chloride). The purple-brown eluates were evaporated and the residue was recrystallised from methylene chloride–n-hexane to give the *bromo-compound* (75 mg, 44%) as blue-black prisms, m.p. 167—169° (Found: C, 62·7; H, 5·7; N, 7·9. $C_{36}H_{39}BrN_4O_5$ requires: C, 62·85; H, 5·7; N, 8·15%), τ (0·1M), 0·54 and 0·70 (each 1H, s, 2 *meso*-H), 3·81 (1H, s, 10-H), 5·18 and 5·86 (each 1H, m, 7- and 8-H), 6·11 (3H, s, 10-CO_2Me), 6·36 and 7·70 (3H, s and 4H, m, $MeO_2C{\cdot}CH_2{\cdot}CH_2$), 6·45, 6·52, and 6·85 (each 3H, s, 1-, 3-, and 5-Me), and 8·2—8·4 (9H, m, 2 $CH_2{\cdot}CH_3$ and 8-Me), $\lambda_{max.}$ 413 (ε 107,000), 482(3000), 514·5(8100), 546(13,700), 613(6300), and 672·5 nm (40,900); $\lambda_{max.}$ [CH_2Cl_2–$CF_3{\cdot}CO_2H$ (trace)] 419 (ε 116,000),

[22] Ref. 7, p. 75.

538(4600), 578·5(7500), 611·5(7500), and 665·5 nm (37,400).

(*b*) The method described in the foregoing preparation (*b*) was used, with methyl mesophaeophorbide-*a* (50 mg) and pyridinium perbromide (35 mg), and gave the bromo-compound (39·5 mg, 62%), identical with the sample prepared in (*a*).

Phylloerythrin Methyl Ester (20) *and* 10-*Oxophyllo-erythrin Methyl Ester* (21).—Methyl mesopyrophaeophorbide-*a* (100 mg) and DDQ (70 mg) were refluxed during 1 h in an anhydrous mixture of acetone (20 ml) and benzene (20 ml). The solution was evaporated *in vacuo* and the residue was chromatographed on alumina (elution with methylene chloride). The eluates from the purple-grey band were evaporated and the residue was recrystallised from methylene chloride–methanol to give phylloerythrin methyl ester (35 mg, 35%) as purple prisms, m.p. 263—265° (lit.,[23] 266°). Further elution of the column with methylene chloride containing 2% acetone gave a deep grey-green band, and recrystallisation of the residue from the evaporated eluates from methylene chloride–methanol yielded 10-*oxophylloerythrin methyl ester* (31 mg, 29%) as greenish-black prisms, m.p. 235—240° (decomp.) (Found: C, 72·6; H, 5·95; N, 10·0. $C_{34}H_{34}N_4O_4$ requires C, 72·6; H, 6·1; N, 10·0%), τ (0·1M), 0·30, 1·05, and 1·11 (each 1H, s, 3-*meso*-H), 6·1, 7·40, and 6·41 (2H, m, 2H, t, and 3H, s, $CH_2{\cdot}CH_2{\cdot}CO_2Me$), 6·1, 8·13, and 8·26 (4H, m, 3H, t, and 3H, t, 2 $CH_2{\cdot}CH_3$), and 6·48, 6·67, 6·72, and 7·03 (each 3H, s, 1-, 3-, 5-, and 8-Me), $\lambda_{max.}$ 416 (ε 111,000), 519sh(4200), 562(8100), 585(10,000), and 637 nm (2600), $\lambda_{max.}$ [CH_2Cl_2–$CF_3{\cdot}CO_2H$ (trace)] 418 (ε 191,000), 564(10,000), 599(4700), and 613sh nm (4400), *m/e* 562 (parent ion).

10,10-(*Tetrachloro-o-phenylenedioxy*)*phylloerythrin Methyl Ester* (22).—A mixture of methyl mesopyrophaeophorbide-*a* (50 mg), tetrachloro-*o*-benzoquinone (50 mg) and dry acetone (25 ml) was heated under reflux during 3 h, and then evaporated *in vacuo*. The residue was chromatographed on alumina (grade III) (elution with methylene chloride). The reddish-green solution was evaporated, and the residue crystallised twice from methylene chloride–methanol, yielding the *spiro-compound* (30 mg, 38%) as small red needles, m.p. >300° (Found: C, 60·3; H, 4·6; N, 6·95. $C_{40}H_{36}Cl_4N_4O_5$ requires C, 60·4; H, 4·6; N, 7·05%), τ ($CF_3{\cdot}CO_2H$) −1·28 and −1·12 (1H, s and 2H, s, 3 *meso*-H), 5·7, 6·72, and 6·20 (2H, m, 2H, t, and 3H, s, $CH_2{\cdot}CH_2{\cdot}CO_2Me$), 5·7, 8·11, and 8·19 (4H, m, 3H, t, and 3H, t, 2 $CH_2{\cdot}CH_3$), and 5·91, 6·13, 6·20, and 6·23 (each 3H, s, 1-, 3-, 5-, and 8-Me), $\lambda_{max.}$ 419 (ε 209,000), 531(8300), 577(21,400), and 626 nm (2400), $\lambda_{max.}$ [CH_2Cl_2–$CF_3{\cdot}CO_2H$ (trace)] 415·5 (237,000), 553(15,000), and 591 nm (13,900), $\lambda_{max.}$ [CH_2Cl_2–$CF_3{\cdot}CO_2H$ (1 : 1)] 414(328,000), 549(9900), 565·5(11,900), 594(5100), and 617 nm (7500).

We thank Professor H. H. Inhoffen (Braunschweig, Germany) for a generous gift of phaeophytin mixture.

[3/1079 *Received*, 25*th May*, 1973]

[23] Ref. 7, p. 192.