THE SYNTHESIS OF PORPHYRINS DERIVED FROM CHLOROBIUM CHLOROPHYLLS[1,2]

J. L. ARCHIBALD,[3,4] D. M. WALKER,[3,5] K. B. SHAW,[3,6] A. MARKOVAC,[3] AND S. F. MACDONALD

Division of Pure Chemistry, National Research Council, Ottawa, Canada

Received September 23, 1965

ABSTRACT

The proofs of the structures proposed for the *Chlorobium* pheophorbides 650, fractions 1–5, and for the *Chlorobium* pheophorbides 660, fractions 5 and 6, are completed and the degradational evidence, where parallel, confirmed through the synthesis of derived porphyrins.

The photosynthetic bacteria *Chlorobium thiosulfatophilum*, strain L and strain VN, contain *Chlorobium* chlorophyll 650 and *Chlorobium* chlorophyll 660 respectively, the numbers indicating the wavelength of their absorption maxima in the red. These are magnesium complexes of farnesyl (not phytyl) esters of the *Chlorobium* pheophorbides 650 and 660. Like ordinary chlorophyll, both these chlorophylls are mixtures, for their pheophorbides can be resolved into several components, which are termed fractions: *Chlorobium* pheophorbide 650, fractions 1–6, and *Chlorobium* pheophorbide 660, fractions 1–6.

These fractions (pheophorbides) are more closely related to the pyrropheophorbide *a* than to the pheophorbide *a* from chlorophyll *a*, for they lack the 10-carbomethoxy group of the latter. All were shown to be homologues of 2-(α-hydroxyethyl)-2-desvinyl-pyrropheophorbide *a* (I, R^1 = Et, R^2 = Me, R^3 = H) (Reaction Scheme 1) and were formulated (2, 3) as shown in Table I, wherein the structures of the related porphyrins as well as some chlorophyll *a* derivatives are also indicated. It will be noted that some trivial names such as pyrroporphyrin (strictly pyrroporphyrin 15), phylloporphyrin, and desoxo-phylloerythrin are used in two senses. Without qualification, they refer to the prototypes derived from chlorophyll *a*; otherwise, they refer to the homologues of these, derived from the *Chlorobium* chlorophylls.

The analytical and degradational evidence (2, 3) did not completely prove these structures for, in the absence of special features, it could only reveal the pairs of substituents on positions 1–8 but not their order. In both the 650 and 660 series other structures in which the substituents on any of the pyrrole rings 1, 2, or 4 were reversed in order (e.g. 7-methyl-8-propionic acid) were equally compatible with it. The indicated structures were preferred, because they were more obviously related to pheophorbide *a*.

In the 650 series, there was no direct evidence concerning the nature of R^2 in fractions 3, 5, and 6, for citraconimide had not been detected among their oxidation products.

In the 660 series, improved analytical methods revealed the natures of R^2 in all fractions. However, in the absence of adequate models, the nuclear magnetic resonance evidence which placed the R^3 groups on the δ-positions was not conclusive (3, 4). Further, as

[1]*Issued as N.R.C. No. 8760.*

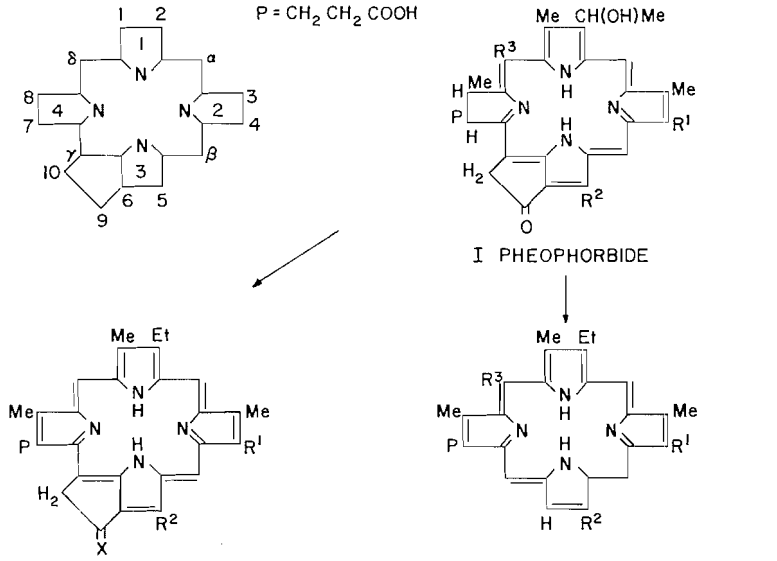
[2]*Part of this work has been reported briefly (1), and the results have been summarized in connection with the analytical work (2, 3).*

[3]*National Research Council Postdoctorate Fellow.*

[4]*Present address: Wyeth Laboratories Incorporated, Philadelphia, Pennsylvania.*

[5]*Present address: Imperial Chemical Industries Ltd., Petrochemical and Polymer Laboratory, Runcorn, Cheshire, England.*

[6]*Present address: Defence Research Board, Shirley's Bay, Ottawa, Canada.*



REACTION SCHEME 1. *Chlorobium* pheophorbides and derived porphyrins.

TABLE I
Pheophorbides and derived porphyrins

	Structure (Reaction Scheme 1)	R^1	R^2	R^3
Derivatives of pheophorbide *a* (from chlorophyll *a*)				
Pyrropheophorbide *a*	I ($CH{=}CH_2$ for CH(OH)Me)	Et	Me	H
Mesopyrropheophorbide *a*	I (Et for CH(OH)Me)	Et	Me	H
Phylloerythrin	II*a*	Et	Me	H
Desoxo-phylloerythrin	II*b*	Et	Me	H
Pyrroporphyrin 15	III*a*	Et	Me	H
γ-Phylloporphyrin 15	III*a* (Me for H on γ)	Et	Me	H
Chlorobium pheophorbide 650				
Fraction 1	I	CH_2CHMe_2	Et	H
Pyrroporphyrin from fraction 1	III*a*	CH_2CHMe_2	Et	H
Fraction 2	I	$CH_2CH_2CH_3$	Et	H
Pyrroporphyrin from fraction 2	III*a*	$CH_2CH_2CH_3$	Et	H
Fraction 3	I	CH_2CHMe_2	Me	H
Pyrroporphyrin from fraction 3	III*a*	CH_2CHMe_2	Me	H
Fraction 4	I	Et	Et	H
Pyrroporphyrin from fraction 4	III*a*	Et	Et	H
Fraction 5	I	$CH_2CH_2CH_3$	Me	H
Desoxo-phylloerythrin from fraction 5	II*b*	$CH_2CH_2CH_3$	Me	H
Fraction 6	I	Et	Me	H
Chlorobium pheophorbide 660				
Fraction 1	I	CH_2CHMe_2	Et	Et
Fraction 2	I	CH_2CHMe_2	Et	Me
Fraction 3	I	$CH_2CH_2CH_3$	Et	Et
Fraction 4	I	$CH_2CH_2CH_3$	Et	Me
Pyrroporphyrin from fractions 3 and 4	III*a* (cf. 650, fraction 2)	$CH_2CH_2CH_3$	Et	H
Fraction 5	I	Et	Et	Me
δ-Phylloporphyrin from fraction 5	III*b*	Et	Et	Me
Fraction 6	I	Et	Me	Me
δ-Phylloporphyrin from fraction 6	III*b*	Et	Me	Me

definite evidence about the nature of the R^3 groups was lacking, the 660 fractions were formulated by assuming the R^3 groups to be the simplest possible: R^3 = Me in all fractions except either 1 and 3 or 2 and 4, wherein R^3 = Et. The molecular weights of fractions 2, 4, 5, and 6, determined since the synthetic work was completed, are consistent only with the former alternative.

In the case of *Chlorobium* pheophorbide 650, fraction 6, the structure was subsequently confirmed and the assumptions validated by converting it into pyrropheophorbide *a* (3), wherein the order of the substituents and the 5-methyl group was known through Fischer's syntheses of related porphyrins. In the present work, the synthesis of the degradation products listed in Table I provides similar confirmation of the structures of the remaining *Chlorobium* pheophorbides 650, fractions 1–5, and of the *Chlorobium* pheophorbides 660, fractions 5, 6, and (in part) 3 and 4. This also supports the assumptions upon which these and the remaining structures were based. All the identities were established through the melting points, the mixed melting points, and the X-ray powder photographs of both the methyl esters and their copper complexes.

In the 650 series, the pyrroporphyrins from fractions 1–4 and the desoxo-phylloerythrin from fraction 5 were identified with synthetic specimens.

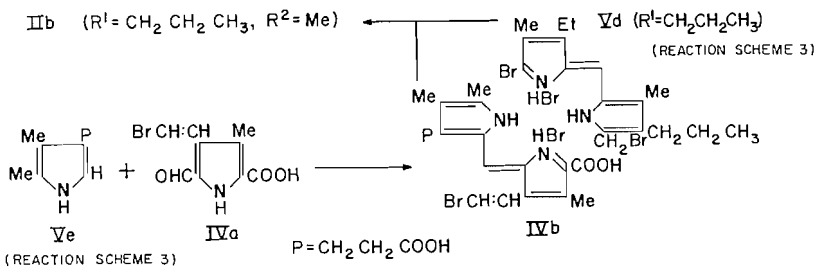
In the 660 series, not enough of fractions 1 and 2 were obtained for the degradations to be attempted. The two phylloporphyrins III*b* (R^1 = $CH_2CH_2CH_3$, R^2 = Et, R^3 = Me) and III*b* (R^1 = $CH_2CH_2CH_3$, R^2 = R^3 = Et) were synthesized for comparison with those from fractions 3 and 4, without prejudice about which should correspond to which. It was initially concluded from the X-ray powder photographs of the esters that the synthetic δ-methyl- and δ-ethyl-porphyrins might correspond with those from fractions 3 and 4 respectively, but the limited amounts of material were used before either of these identities had been established. Further, in view of the polymorphism of the specimens, none of the possible identities can be excluded on this evidence. Other difficulties here were the low yields in the syntheses, the relatively high solubilities, and the relative difficulty with which fractions 3 and 4 are separated. As these two synthetic δ-phylloporphyrins were neither well characterized (see Experimental) nor identified with material of natural origin, they are omitted from Table I. However, both fractions 3 and 4 were also degraded to one pyrroporphyrin which was shown (3) to be identical with the synthetic pyrroporphyrin corresponding to the 650 pheophorbide, fraction 2. This confirmed the structures of fractions 3 and 4 except for the nature and position of R^3. The δ-phylloporphyrins from fractions 5 and 6 were identified with synthetic specimens. That corresponding to fraction 6 (δ-phylloporphyrin 15) had been synthesized by Fischer (5), but none of his specimen remained. When his synthesis was repeated in a considerably modified form, we found that the melting point of its methyl ester – copper complex had been reported incorrectly.

Porphyrins of the 650 Series

The obvious attack was to synthesize pyrroporphyrins for comparison with those obtained by degradation. This degradation seemed to be impracticable for, in contrast to that of pheophorbide *a*, the isocyclic ring of pyrropheophorbide *a* is not easily oxidized by Fischer's methods. In the first instance (fraction 5), this difficulty was avoided by comparing a synthetic desoxo-phylloerythrin with analytical ones. This confirmed the isocyclic ring, but the synthesis of desoxo-phylloerythrins is more laborious than that of pyrroporphyrins and is probably unsuited to 5-ethyl derivatives, for which the pyrrole intermediate providing ring 3 is reportedly unstable (6). Later, methods were found by which

the pheophorbides 650 were degraded to pyrroporphyrins, or the pheophorbides 660 to δ-phylloporphyrins and pyrroporphyrins (3). It was then possible to compare synthetic and analytical pyrroporphyrins, as was done with fractions 1–4.

The synthetic work was done concurrently with the analytical, before the potential of the developing analytical methods was known. A structure tentatively suggested for fraction 2 required it to be degraded to 2- or 4-*n*-propyl-2- or -4-desethyl-desoxophylloerythrin. Because the required pyrromethenes were known, the second of these was synthesized (Reaction Scheme 2) by the method which Fischer had used for desoxophylloerythrin itself (7, 8). All the intermediates were obtained by the reported methods or by modifications thereof (see Experimental).

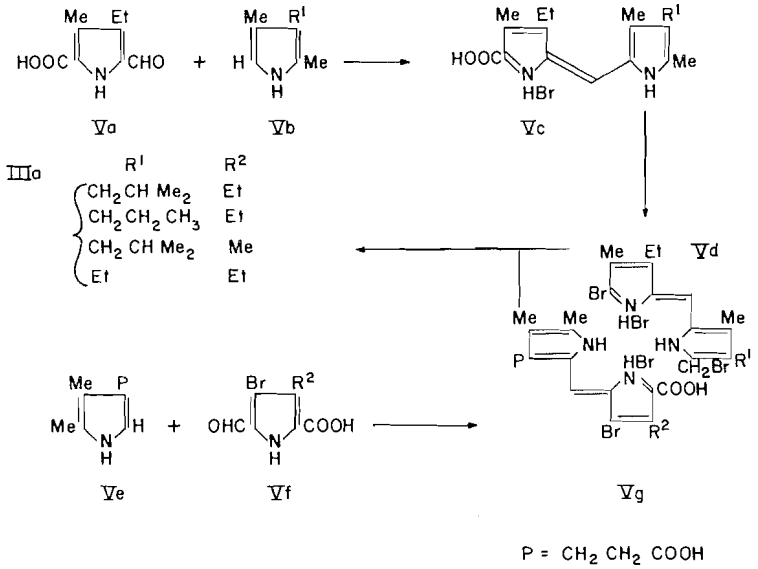


REACTION SCHEME 2. The synthesis of 4-*n*-propyl-4-desethyl-desoxo-phylloerythrin (II*b*, $R^1 = CH_2CH_2CH_3$, R^2 = Me).

The synthetic 4-*n*-propyl-4-desethyl-desoxo-phylloerythrin (II*b*, $R^1 = CH_2CH_2CH_3$, R^2 = Me) differed from that obtained when fraction 2 was degraded. Later analytical work showed that, in both fractions 2 and 5, R^1 was indeed *n*-propyl and was on ring 2 rather than on ring 1. However, it also showed that R^2 was ethyl in fraction 2, and thus was probably methyl in fraction 5. Fraction 5 was then degraded to a desoxo-phylloerythrin which was shown to be identical with the synthetic one.

Some work on the above degradations should be mentioned. In model experiments, the degradation of pyrropheophorbide *a* with hydrogen iodide in acetic acid to phylloerythrin, and of the latter to desoxo-phylloerythrin by the Wolf–Kishner method (9), was repeated. This gave an initially low melting desoxo-phylloerythrin ester (m.p. ca. 255°) which required extensive purification. The same method was used to degrade fraction 2. Further model experiments showed that the Wolf–Kishner reduction of mesopyrropheophorbide *a* gave a mixture of desoxo-phylloerythrin and desoxo-mesopyrropheophorbide *a* (10), and the latter gave more desoxo-phylloerythrin when heated with dilute acid. Here a high-melting product (ester m.p. 262–264°) resulted directly, so this method was used to degrade fraction 5.

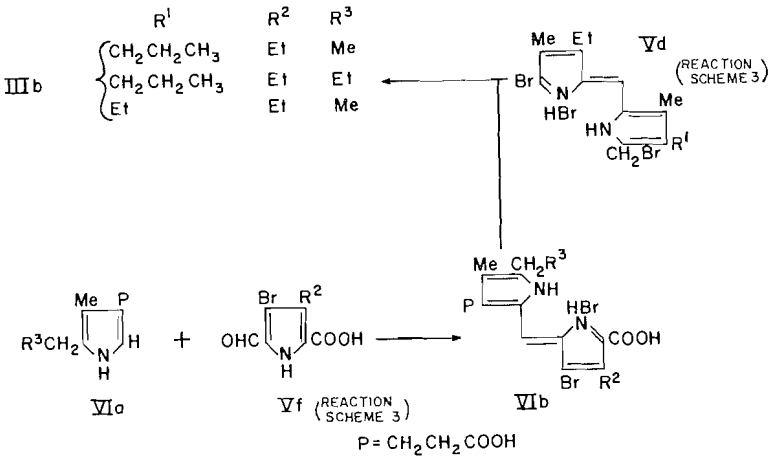
The four pyrroporphyrins were synthesized by the scheme used by Fischer for one synthesis of pyrroporphyrin 15 (11) (Reaction Scheme 3). Unlike two possible alternatives, it utilized some pyrromethenes which could also be used in the synthesis of δ-phylloporphyrins (see below); also, one methene (V*d*, $R^1 = CH_2CH_2CH_3$) had already been prepared. Further, and unlike the fourth alternative, this scheme used the more easily available pyrroles, derivatives of 2,4- rather than 2,3-dimethyl-pyrrole, to construct rings 2 and 3 on which R^1 and R^2 varied. All the pyrroles and pyrromethenes in Reaction Scheme 3 were obtained essentially by the reported methods, or by standard ones (see Experimental). However, there had been no satisfactory preparation of V*b* (R^1 = isobutyl), and the following are new: V*c* (R^1 = isobutyl), V*d* (R^1 = isobutyl), V*f* (R^2 = Et), and V*g* (R^2 = Et).



REACTION SCHEME 3. The synthesis of the pyrroporphyrins (V*d* (R^1 = Et)) is obtained directly by brominating 2,4-dimethyl-3-ethyl-pyrrole.

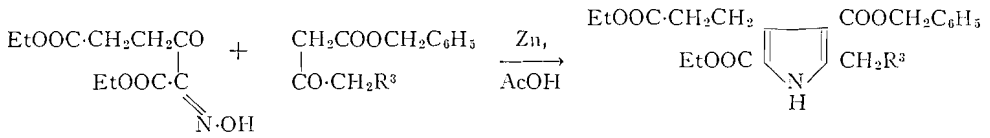
Porphyrins of the 660 Series

The syntheses of the δ-phylloporphyrins was undertaken by the method indicated in Reaction Scheme 4, for reasons mentioned in connection with the analogous synthesis of pyrroporphyrins. The 5-ethyl-5-desmethyl-δ-phylloporphyrin corresponding to fraction 5 was thus obtained. The δ-phylloporphyrins presumably corresponding to fractions 3 and 4, III*a* (R^1 = $CH_2CH_2CH_3$, R^2 = Et, R^3 = Me) and III*a* (R^1 = $CH_2CH_2CH_3$, R^2 = R^3 = Et), were also obtained, but, as mentioned above, were inadequately characterized. The data here (see Experimental) gave some misleading indications of identities, whereas the usual problem has been that of polymorphism obscuring identities (cf. 650, fraction 4, and 660, fraction 6); in both cases the criteria are near the limits of their usefulness. By using V*d* (R^1 = Et) and VI*b* (R^2 = R^3 = Me), this method failed to give δ-phylloporphyrin itself (III*a*, R^1 = Et, R^2 = R^3 = Me), which corresponds to fraction 6; the spectrum of the product suggested that it was the corresponding pyrroporphyrin.



REACTION SCHEME 4. The synthesis of homologues of δ-phylloporphyrin.

The syntheses of the pyrroles VI*a* (R^3 = Me and R^3 = Et) were analogous to a synthesis of VI*a* (R^3 = H) (12):



followed by $COOCH_2C_6H_5 \rightarrow COOH \rightarrow H \rightarrow CHO \rightarrow CH_3$, and then hydrolysis and decarboxylation. Here, as in analogous cases, the acids produced by hydrogenating the benzyl esters are most smoothly decarboxylated by heating the crude acids containing Raney nickel, a small amount first being decarboxylated to wet the walls of the flask with the product. We have since found that homologues such as the above are more readily obtained through the reaction of α-bromomethyl-pyrroles with Grignard reagents (13).

The benzyl propionyl acetate and benzyl *n*-butyryl acetate required in the above pyrrole syntheses were obtained, as was benzyl acetoacetate (14), by heating the ethyl esters with benzyl alcohol. The ethyl esters were obtained by boiling ethyl propionyl malonate and ethyl *n*-butyryl malonate with water (cf. refs. 15, 16). This may be the most generally useful method for preparing ethyl acyl acetates. The yields by a standardized procedure are competitive, consistent, and insensitive to the size of the run. Acyl malonic esters (17, 18) have also been split less reliably (19) by heating them with sulfonic acids (18), less conveniently by cold concentrated sulfuric acid (16) (see also Experimental), in low yield by steam distillation (20), and (together with undesirable by-products) by heating them with acid and a catalyst (21). When the product is not readily separated from the by-product (diethyl malonate) by distillation, it can be purified as its bisulfite complex or, if it does not form one, as its magnesium derivative (cf. ref. 22). Related methods employing ethyl acyl acetoacetates as intermediates give ethyl acetoacetate as the by-product, the separation of which, other than by distillation, requires more specialized methods. Although ethyl propionyl acetate is conveniently obtained from ethyl cyanoacetate and ethyl magnesium iodide, this method is less general and requires 2 moles of the Grignard reagent.

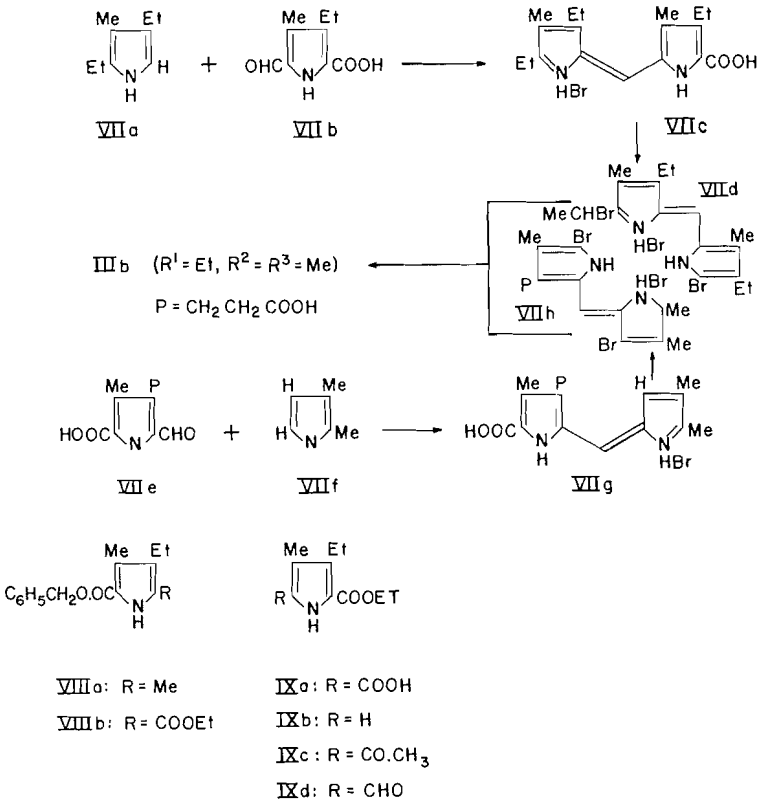
As δ-phylloporphyrin (III*a*, R^1 = Et, R^2 = R^3 = Me) was not obtained as above, we repeated Fischer's synthesis (5) (Reaction Scheme 5). Accordingly, the pyrroles VII*e* and VII*f*, obtained by more recent methods (see Experimental), were converted into VII*g* and hence into VII*h*. The preparation of VII*d* was modified, VII*b* being used instead of 3-methyl-4-ethyl-pyrrole, for it is doubtful that the latter always condenses under the methyl group (23). The two known pyrroles VII*a* and VII*b* were obtained by a method which was particularly convenient, because both were required: VIII*a* was converted through VIII*b*, IX*a*, and IX*b* into IX*c* and IX*d*; the latter gave VII*a* and VII*b* respectively. In effect, this sequence converts a pyrrole into its isomer with the β-substituents reversed, or into higher homologues thereof. Another alternative though unsatisfactory synthesis of VII*a* was found in the modified Knorr synthesis (cf. ref. 24) of its α-carbethoxy derivative from isonitroso-acetoacetic ester and 4-methyl-3,5-heptanedione.

EXPERIMENTAL

Pyrroles

*2-Formyl-3-(ω-bromovinyl)-4-methyl-5-carboxy-pyrrole (IV*a*) (25)*

The reported yield and melting point of the intermediate, 2,4-dimethyl-3-(ω-bromovinyl)-5-carbethoxy-pyrrole, were only realized when the crude product (from ether) was recrystallized by extraction (thimble) successively with benzene (5 parts), with hexane (10 parts), and then with benzene.



REACTION SCHEME 5. The synthesis of δ-phylloporphyrin 15.

Only when the corresponding ester was hydrolyzed by heating it for 2 h under reflux (mantle) was the expected yield obtained.

2-Formyl-3-ethyl-4-methyl-5-carboxy-pyrrole (Va) (26)

2,4-Dimethyl-3-ethyl-5-carbethoxy-pyrrole (27) was oxidized in varying yield to the 2-aldehyde (28) (purified to m.p. ⩾ 90° from aqueous ethanol by high vacuum distillation or by extraction with pentane), and the latter was hydrolyzed (26), the product (m.p. ⩾ 200°) being precipitated by acetic acid. A specimen, m.p. 210° (lit. (26) m.p. 199°), was analyzed.

Anal. Calcd. for $C_9H_{13}O_3N$: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.13; H, 6.88; N, 7.55.

2,4-Dimethyl-3-n-propyl-pyrrole (Vb, $R^1 = CH_2CH_2CH_3$) (29, p. 60)

The method developed for the 3-ethyl homologue (30) gave a 65% yield from 2,4-dimethyl-3-propionyl-5-carbethoxy-pyrrole (29, p. 200).

2,4-Dimethyl-3-isobutyl-5-carbethoxy-pyrrole

The corresponding 3-isobutyryl-pyrrole (31) (colorless prisms, 55%, m.p. 111–113° (lit. (31) m.p. 108°)) was isolated by extraction with ether, distillation (bath temperature 160° at 0.1 mm), and crystallization from aqueous ethanol. This was hydrogenated for 6 h in 2.5 parts of ethanol over Raney nickel (1 900 p.s.i., 160°), more nickel added, and the hydrogenation repeated. The product was filtered off and dissolved in ether. Ether was removed from the filtered solution and the residue crystallized from aqueous ethanol as colorless needles (52%), m.p. 116–117°.

Anal. Calcd. for $C_{13}H_{21}NO_2$: C, 69.92; H, 9.48; N, 6.27. Found: C, 70.03; H, 9.34; N, 6.31.

2,4-Dimethyl-3-isobutyl-pyrrole (Vb, $R^1 = CH_2CHMe_2$) (31)

The above 5-carbethoxy derivative (5 g) was heated under reflux for 3 h with 3 g of sodium hydroxide, 10 ml of water, and enough ethanol to maintain solution. It was taken to dryness *in vacuo*, the residue extracted with 150 ml of water, and the insoluble ester recycled. Acidifying the aqueous extracts with 10% hydrochloric acid to pH 6 precipitated the α-carboxy-pyrrole (92%), which decomposed at 92°. This was distilled *in vacuo* at 110° to give the product (87%) as a colorless oil.

Anal. Calcd. for $C_{10}H_{17}N$: C, 79.40; H, 11.34; N, 9.27. Found: C, 79.74; H, 11.12; N, 8.98.

The one-step method from 2,4-dimethyl-3-isobutyryl-5-carbethoxy-pyrrole was unsatisfactory by either the original (31) or the modified (30) Wolf–Kishner procedures.

2,3-Dimethyl-pyrrole-4-propionic Acid (Hemopyrrole-carboxylic Acid, Ve)

This (12) was always recrystallized just before use as yellow needles, m.p. 132–133°, by extraction with 50 parts of hexane (thimble) over 12 h while protected from light.

2-Formyl-3-bromo-4-methyl-5-carboxy-pyrrole (Vf, R^2 = Me)

2,4-Dimethyl-3-bromo-5-carbethoxy-pyrrole (29, p. 92) was chlorinated in chloroform (not ether (cf. ref. 11)), the chloroform removed at 20°, and the residue converted into the 2-aldehyde which was obtained as colorless needles (65–80%). This was hydrolyzed (11) under nitrogen and the product recrystallized as microneedles (60%) from aqueous acetone.

Ethyl Propionyl Acetate (with D. K. Dougall)[7]

Ethyl propionyl malonate was prepared (18) on a 5 *M* scale in a 5 l flask, the ether layer containing the crude product being further washed with dilute $NaHCO_3$ and then water before stripping. The combined undistilled residues from two such runs were gently boiled under a 12 inch Vigreux column with 2 l of water for 9 h, with vigorous stirring (cf. ref. 16). The combined ester layers in the flask and distillate were extracted with 10 × 350 ml of aqueous $NaHSO_3$ (4 lb). The extract was washed with pentane, and then stirred in an ice bath while the solution was being neutralized at 25° with 35% NaOH, ice being added as necessary. The mixture was extracted with benzene; the extract was washed with aqueous $NaHCO_3$ and then with water, dried (Na_2SO_4), and filtered; the benzene was removed. Distillation gave 543 g (38% overall), b.p. 89.5–91° at 20 mm, n_D^{25} 1.4213, copper complex m.p. 142–143° (lit. (32) m.p. 144–145°). Gas–liquid chromatography showed about 4% of a second component.

Anal. Calcd. for $C_7H_{12}O_3$: C, 58.31; H, 8.39. Found: C, 59.61; H, 8.41.

In preliminary experiments on a 1.25 *M* scale, the above method and the sulfuric acid method (cf. ref. 16) both gave 28% yields. When the purification with bisulfite was omitted, a 51% yield was reported by the above method (33). Using gas–liquid chromatography, we found a commercial specimen to be 50% pure.

Benzyl Propionyl Acetate

Ethyl propionyl acetate (432 g) and benzyl alcohol (324 g) were heated with a few boiling stones in a 1 l flask in a mantle under a 12 inch Vigreux column until thermometers in the liquid and at the top of the column registered 176° and 91° respectively. The rest was distilled to 83° (0.05 mm). The distillate was recycled by heating to 183° and then distilling to 83° (0.05 mm), this second distillate being recycled again. The three residues were combined and distilled to give the product (575 g, 93%), b.p. 80–85° or higher at 0.5 mm depending on the rate of distillation, n_D^{25} 1.5050.

Anal. Calcd. for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.23; H, 6.42.

2-Methyl-3-carbobenzoxy-4-ethyl-5-carbethoxy-pyrrole

This was prepared by the Knorr synthesis according to our usual procedure (compare the corresponding 4-(2′-carbethoxyethyl)-pyrrole (23)), ethyl propionyl acetate (see above) being nitrosated with amyl nitrite and the product being condensed with benzyl acetoacetate. The crude pyrrole was washed with 50% ethanol and crystallized from ethanol, m.p. 126–127° (51%). For analysis it was recrystallized to m.p. 127–129°.

Anal. Calcd. for $C_{18}H_{21}O_4N$: C, 68.55; H, 6.71. Found: C, 68.10; H, 6.67.

2-Methyl-3-bromo-4-ethyl-5-carbethoxy-pyrrole (29, p. 93)

The corresponding 3-carbobenzoxy-pyrrole (above) was reduced in 3 parts of ethanol over Raney nickel (1 000 p.s.i., 100°) to the 3-carboxylic acid (29, p. 257) (90%). This was decarboxylated at 260° and the resulting 3-free pyrrole (29, p. 240) was distilled at 15 mm and then twice crystallized from aqueous ethanol (69%). A solution of this (19 g) in 60 ml of carbon tetrachloride was stirred and protected from moisture while 5.8 ml of bromine was dropped in. Stirring was continued for ½ h, and the solution was concentrated *in vacuo* to one-half volume and then refrigerated overnight. The crystals were washed with ether and recrystallized from aqueous ethanol as needles (63%), m.p. 127–129° (lit. (29, p. 93) m.p. 124°).

2-Formyl-3-bromo-4-ethyl-5-carbethoxy-pyrrole (34)

Sulfuryl chloride (10.3 ml) was added over 20 min to a stirred solution of the corresponding 2-methyl pyrrole (above, 16.3 g) in 250 ml of chloroform (ethanol free). Next day the solvent was removed *in vacuo*. The residue was twice crystallized from 70% ethanol at 0° to give nearly colorless needles (68%), m.p. 96–97° (lit. (34) m.p. 93–95°).

Anal. Calcd. for $C_{10}H_{12}NO_3Br$: C, 43.81; H, 4.41; N, 5.11; Br, 29.16. Found: C, 43.99; H, 4.28; N, 5.06; Br, 28.69.

2-Formyl-3-bromo-4-ethyl-5-carboxy-pyrrole (Vf, R^2 = Et)

The corresponding ester (above, 11.74 g) was heated in the steam bath under nitrogen for 3 h with 36 ml of 5% NaOH. The cooled solution was diluted to 300 ml, filtered, acidified with HCl, and refrigerated. The nearly colorless precipitate (96%) was recrystallized from acetone as needles which decomposed above 230°.

Anal. Calcd. for $C_8H_8O_3NBr$: C, 39.05; H, 3.29; N, 5.69; Br, 32.48. Found: C, 39.07; H, 3.40; N, 5.71; Br, 32.11.

2-Ethyl-3-carbobenzoxy-5-carbethoxy-pyrrole-4-propionic Acid Ethyl Ester

This was prepared from ethyl β-keto-adipate (16), amyl nitrite, and benzyl propionyl acetate (above) on a

[7]*National Research Council Postdoctorate Fellow, 1956–1957.*

1.5 *M* scale exactly as was its 2-methyl homologue (23). It formed colorless prisms (58%) when it was crystallized from ethanol, m.p. 92–93°.

Anal. Calcd. for $C_{22}H_{27}O_6N$: C, 65.82; H, 6.78; N, 3.49. Found: C, 66.03; H, 6.91; N, 3.10.

2-Ethyl-3-carboxy-4-(2-carbethoxyethyl)-5-carbethoxy-pyrrole

The corresponding 3-carbobenzoxy-pyrrole (above) was hydrogenated in ethanol over Raney nickel for 6 h at 125° and 1 500 p.s.i. Ethanol was removed and the residue extracted with ice-cold 0.1 *N* sodium carbonate, from which the product was precipitated with acetic acid. When it was crystallized from 50% ethanol it formed colorless prisms, m.p. 186–188.5°.

Anal. Calcd. for $C_{15}H_{21}O_6N$: C, 57.86; H, 6.80; N, 4.50. Found: C, 57.81; H, 6.91; N, 4.84.

2-Ethyl-5-carbethoxy-pyrrole-4-propionic Acid Ethyl Ester

The corresponding 3-carbobenzoxy-pyrrole (above, 50 g) was hydrogenated as above and the crude acid was slowly distilled at 15 mm from a 250 ml flask heated in a mantle with the neck wrapped in heating tape. The distillate was redistilled from a Vigreux flask to give an oil (78%), b.p. 150° at 0.5 mm, solidifying to colorless prisms, m.p. 40–41°, unstable to air and light.

Anal. Calcd. for $C_{14}H_{21}O_4N$: C, 62.90; H, 7.92; N, 5.24. Found: C, 63.17; H, 7.89; N, 5.13.

2-Ethyl-3-formyl-5-carbethoxy-pyrrole-4-propionic Acid Ethyl Ester

The corresponding 3-free pyrrole (above, 17.5 g) was stirred with dimethylformamide (35 ml, distilled over P_2O_5), while 8.8 ml of phosphorus oxychloride was dropped in. The mixture was heated on the steam bath for 40 min, cooled, and poured into 300 ml of water containing 52 g of sodium acetate. The oily layer solidified when the solution was allowed to cool. It was recrystallized by extraction with 1 600 ml of hexane (thimble) to give colorless needles (79%), m.p. 85–86°.

Anal. Calcd. for $C_{15}H_{21}O_5N$: C, 61.00; H, 7.17; N, 4.74. Found: C, 60.79; H, 6.80; N, 5.08.

2-Ethyl-3-methyl-5-carbethoxy-pyrrole-4-propionic Acid Ethyl Ester

The corresponding 3-formyl pyrrole (above) was reduced in ethanol over Raney nickel at 180° and 1 300 p.s.i. for 4 h. After the catalyst had been separated and the ethanol removed, the residue was crystallized from aqueous ethanol to give colorless needles (82%), m.p. 80.5–81.5°. For analysis, it was recrystallized to m.p. 81–82°.

Anal. Calcd. for $C_{15}H_{23}O_4N$: C, 64.03; H, 8.29; N, 4.98. Found: C, 64.21; H, 8.19; N, 5.18.

2-Ethyl-3-methyl-pyrrole-4-propionic Acid (VIa, R^3 = Me)

The corresponding 5-carbethoxy ester (above, 21.4 g) was heated under reflux for 3 h on the steam bath with 100 ml of water, 30 ml of ethanol, and 22 g of sodium hydroxide. Ethanol was boiled off, more water added, and the solution saturated with sulfur dioxide under a layer of ether and extracted twice with ether. The ether layer was washed with water and dried (Na_2SO_4), and the ether was removed. The residual solid was heated on the steam bath for 5 min with 15 ml of water. The whole was quickly dried (oil pump) and the residue crystallized from 1 200 ml of hexane. The colorless needles (86%) that were initially formed rapidly turned pink, m.p. 85–88°, sensitive to air and light.

Anal. Calcd. for $C_{10}H_{15}O_2N$: C, 66.27; H, 8.34; N, 7.73. Found: C, 66.08; H, 7.95; N, 7.91.

Ethyl n-Butyryl Acetate

Crude ethyl *n*-butyryl malonate was prepared in two 4.1 *M* runs in the same manner as ethyl propionyl malonate was (above), and boiled with water in the same way for 6.5 h. Combining the ester layers in the flask and distillate gave 1 270 ml of crude product. Magnesia mixture (850 ml) (from 330 g of $MgCl_2 \cdot 6H_2O$, 420 g of NH_4Cl, 1 500 ml of water, and 450 ml of ammonium hydroxide), 800 ml of water, and 400 ml of ice were stirred vigorously at 10°, 110 ml of the crude ester was added, and stirring was continued for 20 min. The filtered precipitate was twice slurried (Waring Blendor) and filtered with 60 ml of magnesia mixture which had been diluted with 500 ml of ice water, and then dried to 0.02 mm. The magnesium complex from 310 ml of crude ester (194 g, m.p. 152.5–154° (lit. (22) m.p. 156–157°)) was shaken with 600 ml of ether, 110 ml of hydrochloric acid, and 800 ml of ice water. The ether layer was washed with 10% HCl and both acid layers washed in turn with ether. The combined ether layers were washed with water, aqueous disodium phosphate, and water, and then dried (Na_2SO_4) and the ether removed. The residue was distilled at 14 mm to give 14 g, b.p. 86–91°, $n_D^{16.5}$ 1.4265; 122 g, b.p. 91–92.5°, $n_D^{16.5}$ 1.4275; and 18 g, b.p. 92.5–93°, $n_D^{16.5}$ 1.4275; total 49% overall.

All three fractions were suitable for conversion into the benzyl ester (see below). The middle fraction (copper complex m.p. 126–126.5° (lit. (35) m.p. 125–126°)) appeared on gas chromatography to be homogeneous and indistinguishable from the product which was obtained from ethyl *n*-butyryl acetoacetate (29, p. 405), but both gave a carbon analysis which was 2.5% high.

Benzyl n-Butyryl Acetate

As in the preparation of benzyl propionyl acetate (above), 116.3 g of the above ethyl *n*-butyryl acetate and 93 ml of benzyl alcohol were heated to 195° and distilled to 95° (0.01 mm) through three cycles. Distillation of the combined residues gave 145 g (89%), b.p. (0.02 mm) 89–93° or higher depending on the rate of distillation, n_D^{15} 1.5040.

Anal. Calcd. for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 71.05; H, 7.41.

2-n-Propyl-3-carbobenzoxy-5-carbethoxy-pyrrole-4-propionic Acid Ethyl Ester

This was obtained from ethyl β-keto-adipate (16), amyl nitrite, and the above benzyl *n*-butyryl acetate in the same manner as its 2-methyl homologue was (23), except that more acetic acid was used to maintain solution and prevent foaming. It formed colorless prisms (42%) when it was crystallized from aqueous ethanol, m.p. 84.5–85.5°.

Anal. Calcd. for $C_{23}H_{29}O_6N$: C, 66.49; H, 7.04; N, 3.37. Found: C, 66.36; H, 6.96; N, 3.31.

2-n-Propyl-3-methyl-5-carbethoxy-pyrrole-4-propionic Acid Ethyl Ester

The corresponding 3-carbobenzoxy derivative (above), in the same manner as its 2-ethyl homologue above, was converted successively into the 3-carboxy pyrrole (colorless plates from ethanol, m.p. 178–180°, or changing at 160° to needles, m.p. 184–185°), the 3-free pyrrole (prisms from pentane at −10°, m.p. 35.5–36.5°), the 3-formyl pyrrole (needles from hexane, m.p. 87.5–88.5°), and the 3-methyl pyrrole (ca. 20% overall). The latter formed colorless needles when it was crystallized from aqueous ethanol, m.p. 77–79°.

Anal. Calcd. for $C_{16}H_{25}O_4N$: C, 65.06; H, 8.53; N, 4.74. Found: C, 65.33; H, 8.50; N, 4.78.

2-n-Propyl-3-methyl-pyrrole-4-propionic Acid (VIa, R^3 = Et)

This was obtained, in the same manner as its 2-ethyl homologue (above), from the corresponding 5-carbethoxy ester (above). It separated from hexane when the solution was allowed to stand under nitrogen, finally at 10°, as long colorless needles (68%), m.p. 80.5–81.5°.

Anal. Calcd. for $C_{11}H_{17}O_2N$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.43; H, 8.58; N, 7.42.

4-Methyl-3,5-diethyl-2-carbethoxy-pyrrole

Ethyl acetoacetate (48 g) in 200 ml of acetic acid was nitrosated at 8° with 28 g of sodium nitrite in 50 ml of water, and excess nitrite was destroyed the next day with ammonium sulfamate. This solution, zinc dust (150 g), and 4-methyl-3,5-heptanedione (36) (51 g) were added in 5 portions over 10 min to a stirred solution of ammonium acetate (40 g) in acetic acid (100 ml) maintained at 60–70°. More zinc dust was added at this temperature over 1 h. The mixture was then kept for 1 h at 105° and then decanted into ice water. The product was separated from 2,4-dimethyl-3,5-dicarbethoxy-pyrrole by extraction with pentane, distillation to 90° (1×10^{-4} mm), and crystallization from pentane at −20°. It formed large colorless prisms (7 g, 9%), m.p. 62–63°.

Anal. Calcd. for $C_{12}H_{19}NO_2$: C, 68.86; H, 9.15. Found: C, 68.77; H, 9.07.

References to this in the literature (5; 29, p. 57) refer to 4-formyl-3,5-diethyl-2-carbethoxy-pyrrole.

3-Methyl-2,4-diethyl-pyrrole (VIIa)

(*a*) 4-Methyl-3-ethyl-2-carbethoxy-5-acetyl-pyrrole (IX*c*, see below, 13 g) was heated for 16 h at 160° in an autoclave with 12 ml of hydrazine and 7 g of sodium dissolved in 220 ml of absolute ethanol. Ethanol was then distilled off through a column and 30 ml of water added to the residue, which was then extracted with ether. The extract was dried (potassium carbonate) and the ether distilled off through a column. A drop of hydrazine was added to the residue, which was distilled through a small column (95–98°, 12 mm) to give 7.5 g (94%) of a pale oil, picrate m.p. 110° (lit. (5) m.p. 110°). It is stable in an evacuated sealed tube protected from light.

Anal. Calcd. for $C_9H_{14}N$: C, 78.77; H, 11.02; N, 10.21. Found: C, 78.47; H, 10.76; N, 10.15.

(*b*) 4-Methyl-2,4-diethyl-2-carbethoxy-pyrrole (see above, 1 part) was heated for 5 h at 170° in a Teflon-lined metal tube with 8 parts of ethanolic potassium hydroxide (10% w/v). Ether was then added and the product (82%) isolated as above, picrate m.p. and mixed m.p. with the above picrate 109–110°.

Anal. Found: C, 78.64; H, 11.22; N, 10.01.

4-Methyl-3-ethyl-2-carboxy-pyrrole-5-aldehyde (VIIb)

The corresponding ester (IX*d*, see below, 9.5 g) was boiled for 5 min with 20 ml of 10% sodium hydroxide. The clear solution was cooled, filtered with Darco, and acidified with hydrochloric acid. The crystalline precipitate was separated and recrystallized from 60% ethanol, m.p. 176° (7.5 g, 91%). For analysis it was recrystallized three times from 70% ethanol as long needles, m.p. 180–181° (lit. (37) m.p. 174°).

Anal. Calcd. for $C_9H_{11}O_3N$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.38; H, 5.98; N, 7.67.

2-Formyl-4-methyl-5-carboxy-pyrrole-3-propionic Acid Diethyl Ester

(*a*) A solution of 10.7 g of 2,4-dimethyl-5-carboxy-pyrrole-3-propionic acid diethyl ester (24) in 50 ml of chloroform (dried over calcium chloride) was stirred in an ice bath and protected from moisture while 7.2 ml of sulfuryl chloride was added over 1 h. Next day the chloroform was removed *in vacuo* and the residue was boiled for 5 min with 15 g of sodium acetate in 100 ml of water. The insoluble oil was washed by decantation and dissolved in ether. The washed (aqueous sodium carbonate and then water) and dried ether solution was evaporated and the residue distilled in high vacuum. The product (8 g, 71%) solidified when the solution was allowed to cool, m.p. 62–67°. For analysis it was recrystallized from pentane as pale yellow prismatic needles, m.p. 64.5–65.5° (lit. (38) m.p. 62–66°).

Anal. Calcd. for $C_{14}H_{19}NO_5$: C, 59.77; H, 6.81. Found: C, 59.95; H, 6.84.

(*b*) The corresponding half ester (free propionic acid (39)) was esterified at 20° in ethanolic hydrogen chloride and crystallized from pentane to give prismatic needles, m.p. and mixed m.p. with the above diethyl ester 64.5–65.5°.

2-Formyl-4-methyl-5-carboxy-pyrrole-3-propionic Acid (VIIe)

Either the above diethyl ester or the monoethyl ester (39) was hydrolyzed in ethanolic potassium hydroxide, crystallized from formic acid as colorless needles (39), and dried (63°, 1×10^{-4} mm).

Anal. Calcd. for $C_{10}H_{11}NO_5 \cdot HCOOH$: C, 48.71; H, 4.83; N, 5.16; equiv. wt. 90.4. Found in the product from the diethyl ester: C, 48.45; H, 4.73; N, 5.16; equiv. wt. 80.6. Found in the product from the monoethyl ester: equiv. wt. 79.4.

After recrystallization from water, the former melted at 220–235° (decomp.) (lit. (38) m.p. 232–234° (decomp.)) with intervening phase changes.

2,3-Dimethyl-pyrrole (VIIf)

2-Methyl-3-carbethoxy-pyrrole (40) was reduced by lithium aluminium hydride (41).

4-Methyl-3-ethyl-5-carbobenzoxy-pyrrole-2-carboxylic Acid (cf. VIIIb)

Sulfuryl chloride (6.6 g) was dropped into a stirred solution of 2,4-dimethyl-3-ethyl-5-carbobenzoxy-pyrrole (42) (VIII*a*, 3.6 g) in 50 ml of absolute ether at 10–15°. Next day the ether was removed *in vacuo*, hot aqueous sodium acetate (7 g in 30 ml) added to the residue, and the mixture boiled for 5 min. After the solution was allowed to cool, the insoluble material was dissolved in 10% sodium hydroxide, and the solution was washed with ether and then acidified with hydrochloric acid. The precipitate was separated, washed with water, and crystallized from ethanol (thimble) to give 2.5 g (62%), m.p. 166°. For analysis it was recrystallized from ethanol as colorless prisms, m.p. 175°.

Anal. Calcd. for $C_{16}H_{17}O_4N$: C, 66.88; H, 5.96; N, 4.88. Found: C, 67.02; H, 5.95; N, 5.02.

4-Methyl-3-ethyl-2-carbethoxy-5-carbobenzoxy-pyrrole (VIIIb)

As in the preparation of the corresponding carboxylic acid (above), 26.7 g of 2,4-dimethyl-3-ethyl-5-carbobenzoxy-pyrrole in 250 ml of ether was treated with 40.8 g of sulfuryl chloride. Ethanol (150 ml) was added to the residue that was left after the ether had been evaporated. Water (200 ml) was added to the clear solution, and the mixture extracted with ether. The extract was washed with 5% sodium hydroxide, with saturated sodium bisulfite, and with water, and then dried (sodium sulfate) and the ether evaporated. Crystallizing the residue from *n*-hexane gave 28 g (92%), m.p. 51°. For analysis it was recrystallized from *n*-hexane as colorless needles, m.p. 53°.

Anal. Calcd. for $C_{18}H_{21}O_4N_2$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.39; H, 6.66; N, 4.65.

4-Methyl-3-ethyl-2-carbethoxy-pyrrole-5-carboxylic Acid (IXa)

The corresponding benzyl ester (VIII*b*, see above, 15.75 g) in 250 ml of ethanol was hydrogenated at atmospheric pressure over 500 mg of palladium black (uptake 1 100 ml, calcd. 1 180 ml). The mixture was heated to dissolve the precipitate, filtered, and cooled. The colorless product (10.2 g, 91%) separated, m.p. 230°. For analysis it was recrystallized from ethanol as colorless elongated prisms, m.p. 233° (lit. (37) m.p. 218°).

Anal. Calcd. for $C_{11}H_{15}O_4N$: C, 58.65; H, 6.71; N, 6.22. Found: C, 58.74; H, 6.68; N, 6.43.

4-Methyl-3-ethyl-2-carbethoxy-pyrrole (IXb)

The corresponding acid (IX*a*, see above) was decarboxylated (37) to give the distilled product (53%), m.p. 69–70°. For analysis it was recrystallized from *n*-hexane as colorless plates, m.p. 75° (lit. (37) m.p. 76°).

Anal. Calcd. for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73. Found: C, 66.09; H, 8.25; N, 7.90.

4-Methyl-3-ethyl-2-carbethoxy-5-acetyl-pyrrole (IXc)

Anhydrous aluminium chloride (21 g) was added, in portions, to a cooled and stirred solution of the corresponding α-free pyrrole (see above, 14 g) in 150 ml of carbon disulfide and 10 ml of acetyl chloride. The mixture was heated under reflux for 15 min, cooled, and poured into ice water. The product was extracted with ether; the extract was washed with 5% sodium hydroxide and then with water, and dried (sodium sulfate); the solvents were removed *in vacuo*. The residue was crystallized from *n*-hexane (thimble) to give 13 g (75%) of colorless needles, m.p. 107°.

Anal. Calcd. for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.30; H, 7.51; N, 6.04.

4-Methyl-3-ethyl-2-carbethoxy-pyrrole-5-aldehyde (IXd)

The corresponding α-free pyrrole (IX*b*, see above, 10 g) was converted into the aldimine hydrochloride (37). When this was dissolved in 50 ml of ice water (much less than suggested (37), the aldehyde being appreciably soluble), the aldehyde crystallized (9.5 g, 82%), m.p. 55°. For analysis it was crystallized from 60% ethanol, m.p. 65–66° (lit. (37) m.p. 68°).

Anal. Calcd. for $C_{11}H_{15}O_3N$: C, 63.14; H, 7.23; N, 6.69. Found: C, 63.33; H, 7.39; N, 6.88.

Pyrromethenes

4,4',5'-Trimethyl-3-(ω-bromovinyl)-5-carboxy-pyrromethene-3'-propionic Acid Hydrobromide (IVb) (8)

After washing with ethyl acetate until the washings were very pale, and then with ether, the product (57%) decomposed at about 105°.

5-Bromo-4,3'-dimethyl-5'-bromomethyl-3,4'-diethyl-pyrromethene Hydrobromide (Vd, R^1 = Et) (43)

The reported yield of a stable product was only obtained when *freshly* distilled cryptopyrrole was brominated; the resulting mixture of pyrromethenes was washed with a little acetic acid and then thoroughly with

much petrol ether (30–60°), and dried over KOH at 0.02 mm; then the methenes were separated. More of the desired product was obtained by boiling "methene II" with 15 parts of acetic acid for 5 min.

5-Carboxy-4,3′,5′-trimethyl-3-ethyl-4′-n-propyl-pyrromethene Hydrobromide (Vc, $R^1 = CH_2CH_2CH_3$) (44)

2-Formyl-3-ethyl-4-methyl-5-carboxy-pyrrole (1.25 g) was finely ground in an agate mortar and then mixed with 0.95 g of freshly distilled 2,4-dimethyl-3-*n*-propyl-pyrrole in a 10 ml beaker which was cooled in ice. Acetic acid (0.7 ml) was added, with stirring, followed by 0.7 ml of hydrobromic acid (48%). The syrup was stirred and rubbed (5 to 15 min) until the whole solidified to orange prisms (ca. 70%). These were filtered on sintered glass and washed with ether until the washings were light yellow.

5-Bromo-4,3′-dimethyl-5′-bromomethyl-3-ethyl-4′-n-propyl-pyrromethene Hydrobromide (Vd, $R^1 = CH_2CH_2CH_3$) (44)

The intermediate 5-carboxy-pyrromethene (above) (1 g) was powdered and stirred with 0.5 ml of acetic acid, the solution being cooled with ice, while bromine (1 g) in 1 ml acetic acid was added dropwise. After the reaction had subsided, the mixture was heated for 40 min in the steam bath. Next day the product was filtered off, and washed with a little acetic acid and then with ether until the washings were colorless. It formed purple prisms (50–60%) which decomposed above 190°. For analysis it was recrystallized as violet rods from acetic acid.

Anal. Calcd. for $C_{17}H_{23}N_2Br_3$: Br, 48.44. Found: Br, 46.89.

5-Carboxy-4,3′,5′-trimethyl-3-ethyl-4′-isobutyl-pyrromethene Hydrobromide (Vc, $R^1 = CH_2CHMe_2$)

This was obtained from 1.165 g of freshly redistilled 2,4-dimethyl-3-isobutyl-pyrrole, 1.42 g of 2-formyl-3-ethyl-4-methyl-5-carboxy-pyrrole, 0.8 ml of acetic acid, and 0.8 ml of 48% hydrobromic acid exactly as was the 4′-*n*-propyl homologue (above). It formed yellow crystals (66%).

Anal. Calcd. for $C_{19}H_{27}O_2N_2Br$: C, 57.70; H, 6.89; N, 7.09. Found: C, 57.34; H, 6.88; N, 7.16.

5-Bromo-4,3′-dimethyl-5′-bromomethyl-3-ethyl-4′-isobutyl-pyrromethene Hydrobromide (Vd, $R^1 = CH_2CHMe_2$)

This was obtained from the above 5-carboxy-pyrromethene (2.52 g), just as was the 4′-*n*-propyl homologue (above), by using 1.3 ml of acetic acid and then 1.05 ml of bromine in 2.6 ml of acetic acid, except that heat was applied for 1.5 h. It formed red crystals (69%).

Anal. Calcd. for $C_{18}H_{25}N_2Br_3$: C, 42.25; H, 4.95; N, 5.50. Found: C, 42.38; H, 4.89; N, 5.37.

3-Bromo-4,4′,5′-trimethyl-5-carboxy-pyrromethene-3′-propionic Acid Hydrobromide (Vg, $R^2 = Me$) (11)

Hemopyrrole-carboxylic acid (1.67 g) and 2.32 g of 2-formyl-3-bromo-4-methyl-5-carboxy-pyrrole were ground together in an agate mortar, transferred to a 10 ml beaker, and stirred without cooling with 1 ml of acetic acid and then 2 ml of 48% hydrobromic acid. The orange product (96%) which rapidly separated was filtered off, washed with 150 ml dry ether, and dried.

3-Bromo-4′,5′-dimethyl-4-ethyl-5-carboxy-pyrromethene-3′-propionic Acid Hydrobromide (Vg, $R^2 = Et$)

This was obtained, exactly as was its 4-methyl-5′-ethyl isomer (above), from hemopyrrole-carboxylic acid (1.42 g), 2-formyl-3-bromo-4-ethyl-5-carboxy-pyrrole (2.09 g), 0.25 ml of acetic acid, and 2 ml of 48% hydrobromic acid. It formed orange prisms (70%) which decomposed above 200° without melting.

Anal. Calcd. for $C_{17}H_{20}O_4N_2Br_2$: C, 42.87; H, 4.25; N, 5.89; Br, 33.56. Found: C, 42.79; H, 4.25; N, 5.82; Br, 33.38.

3-Bromo-4,4′-dimethyl-5′-ethyl-5-carboxy-pyrromethene-3′-propionic Acid Hydrobromide (VIb, $R^2 = R^3 = Me$)

2-Ethyl-3-methyl-pyrrole-4-propionic acid (0.91 g) and 2-formyl-3-bromo-4-methyl-5-carboxy-pyrrole (1.16 g) were ground in an agate mortar. Acetic acid (0.25 ml) was added and rubbing continued. In this case the intermediate may crystallize. Hydrobromic acid (1 ml of 48%) was added slowly, and rubbing gave a syrup from which the product crystallized, on further vigorous rubbing, as orange prisms (99%). These were filtered off on sintered glass, thoroughly washed with much dry ether, and dried *in vacuo* over sodium hydroxide.

Anal. Calcd. for $C_{17}H_{20}O_4N_2Br_2$: C, 42.87; H, 4.25; N, 5.89; Br, 33.56. Found: C, 42.73; H, 4.03; N, 5.51; Br, 33.41.

3-Bromo-4′-methyl-4,5′-diethyl-5-carboxy-pyrromethene-3′-propionic Acid Hydrobromide (VIb, $R^2 = Et$, $R^3 = Me$)

This was obtained, exactly as was its 4-methyl-5′-ethyl homologue (above), from 2-ethyl-3-methyl-pyrrole-4-propionic acid (1.45 g), 2-formyl-3-bromo-4-ethyl-5-carboxy-pyrrole (1.97 g), 0.25 ml of acetic acid, and 2 ml of 48% hydrobromic acid, with rubbing for about 15 min to induce crystallization. The product (75%) formed orange prisms which decomposed at about 200°. It decomposed when its recrystallization was attempted.

Anal. Calcd. for $C_{18}H_{22}O_4N_2Br_2$: C, 44.11; H, 4.53; N, 5.72; Br, 32.60. Found: C, 44.16; H, 4.86; N, 5.89; Br, 32.97.

3-Bromo-4′-methyl-4-ethyl-5′-n-propyl-5-carboxy-pyrromethene-3′-propionic Acid Hydrobromide (VIb, $R^2 = R^3 = Et$)

This was obtained, exactly as was its 4-methyl-5′-ethyl homologue (above), from 2-*n*-propyl-3-methyl-pyrrole-4-propionic acid (0.9 g), 2-formyl-3-bromo-4-ethyl-5-carboxy-pyrrole (1.2 g), 0.85 ml of acetic acid,

and 1.5 ml of 48% hydrobromic acid, with rubbing for 0.5 h; then seed was obtained by rubbing a portion on a glass plate. The product (80%) formed orange prisms.

Anal. Calcd. for $C_{19}H_{24}O_4N_2Br_2$: C, 45.25; H, 4.79; N, 5.56. Found: C, 44.98; H, 4.55; N, 5.61.

5-Carboxy-3,4'-dimethyl-4,3',5'-triethyl-pyrromethene Hydrobromide (VIIc)

This was obtained, exactly as were its analogues above, from 1 g of 4-methyl-3-ethyl-2-carboxy-pyrrole-5-aldehyde, 0.75 g of 3-methyl-2,4-diethyl-pyrrole, 0.7 ml of acetic acid, and 0.7 ml of 48% hydrobromic acid in a cooled 10 ml beaker, the product being washed with 10% methanol in ether and then with ether. A sample of the red crystals (1.2 g, 57%) was recrystallized from methanol–ether as orange-red needles, m.p. 120–124° (decomp.).

5-Bromo-3,4'-dimethyl-4,3'-diethyl-5'-bromoethyl-pyrromethene Hydrobromide (VIId) (5)

The corresponding 5-carboxy-pyrromethene above (VII*c*, 1.2 g) was suspended in 2 ml of acetic acid, 0.4 ml of bromine added, and the mixture heated for 5 min in the steam bath. The red crystalline product (1.4 g, 89%) was filtered off from the cooled mixture and washed with ether. A sample for analysis was dissolved in acetone, heated for 10 min, filtered off the next day, and crystallized twice from acetic acid to give red prisms which decomposed above 280°.

Anal. Calcd. for $C_{17}H_{23}N_2Br_3$: C, 41.19; H, 4.68; N, 5.66; Br, 48.46. Found: C, 41.05; H, 4.16; N, 5.50; Br, 48.29.

5,3'-Dibromo-4,4',5'-trimethyl-pyrromethene-3-propionic Acid Hydrobromide (VIIh)

This was obtained from 2,3-dimethyl-pyrrole (VII*f*, see above) and 2-formyl-4-methyl-5-carboxy-pyrrole-3-propionic acid (VII*e*, see above) over the 5-carboxy-pyrromethene VII*g*, as reported (5); it decomposed above 220°.

Anal. Calcd. for $C_{15}H_{17}O_2N_2Br_3$: C, 36.23; H, 3.42; N, 5.63; Br, 48.24. Found: C, 36.57; H, 3.50; N, 5.37; Br, 48.04.

Porphyrins (Ordered as the Corresponding "650" Fractions 1–5 and then "660" Fractions 3–6)

4-Isobutyl-5-ethyl-4-desethyl-5-desmethyl-pyrroporphyrin 15 Methyl Ester (1,3,8-Trimethyl-2,5-diethyl-4-isobutyl-porphin-7-propionic Acid Methyl Ester (IIIa, $R^1 = CH_2CHMe_2$, $R^2 = Et$))

5-Bromo-4,3'-dimethyl-5'-bromomethyl-3-ethyl-4'-isobutyl-pyrromethene hydrobromide (V*d*, $R^1 = CH_2CHMe_2$, 2.1 g), 3-bromo-4',5'-dimethyl-4-ethyl-5-carboxy-pyrromethene-3'-propionic acid hydrobromide (V*g*, $R^2 = Et$, 2.93 g), and 5 g of methylsuccinic acid were ground together, dried at 20° in high vacuum, and then heated, with stirring, in a test tube at 160° and then (10 min after melting) at 180° for 2 min. The partially cooled melt was poured into 1.5 l of warm water. The pH was brought to 5 with disodium phosphate, and the mixture was heated for 1 h on the steam bath and then allowed to cool overnight. The solid was filtered off, well washed with water, dried, powdered, and extracted with 3 l of ether for 3 days (thimble). The ether was repeatedly extracted with 100 ml portions of 0.5% hydrochloric acid until neither colored impurities were extracted nor precipitates formed. The acid porphyrins were precipitated as their sodium salts by shaking the ether with 10% sodium hydroxide (4 × 25 ml). These were filtered off, dissolved in concentrated hydrochloric acid, and brought into 1.5 l of ether with sodium acetate. The pyrroporphyrin was extracted from the washed ether solution with 4% hydrochloric acid, leaving bromoporphyrins in the ether. The pyrroporphyrin was returned to fresh ether by using sodium acetate. Its ether solution was washed, dried, concentrated, and chromatographed on a column of 6% hydrochloric acid on Celite 545, development being carried out with equilibrated ether (45). The large lower band was cut out, leaving three small red, yellow, and brown ones. The pigment in it was eluted with 2% hydrochloric acid in acetone and brought into ether by using water. When this ether solution was washed, dried, and concentrated, most of the product (13 mg) crystallized; visible spectrum in ether (relative density in parentheses): maxima at 622 (0.46), 595 (0.09), 568 (0.48), 526 (0.70), 497 (1.00), and 493 (0.98) mμ, with shoulders at 613, 573, and 471 mμ.

The crystalline acid was left overnight in 5% hydrogen chloride in methanol, and the product brought from this into chloroform by using water. The washed and dried (sodium sulfate) chloroform solution was filtered through a column of alumina (grade V, made up with the 5% methanol in chloroform that was also used for the elution), and concentrated. The chloroform was removed by boiling it off while methanol was being added. Part of the product (4 mg, m.p. 223–226°) crystallized. The rest (5.6 mg, m.p. 222.5–225.5°) was obtained by evaporating the mother liquors and crystallizing the residue from ether (final volume 2 ml) at 0°. The whole was recrystallized slowly from ether to give 5 mg of needles; visible spectrum in ether (relative density in parentheses): maxima at 622 (0.47), 596 (0.09), 568 (0.49), 526 (0.72), 496 (1.00), and 493 (1.00) mμ, with shoulders at 613, 573, and 470 mμ.

Anal. Calcd. for $C_{35}H_{42}O_2N_4$: C, 76.33; H, 7.63. Found: C, 75.95; H, 7.43.

It melted at 225.5–227.5°. The mixed melting point with the "pyrroporphyrin" ester from *Chlorobium* pheophorbide 650, fraction 1 (m.p. 229.5–231° (2)), was 226–230° with softening from 224°; the X-ray powder photographs of these two specimens were identical.

Copper Complex of the Methyl Ester

About 0.2 mg of the ester in 0.5 ml of chloroform was boiled in a 3 × $\frac{3}{8}$ inch test tube; copper acetate (0.5 mg), in methanol (0.5 ml) containing a trace of acetic acid, was added. Half the solvent was boiled off (a glass boiling stick made from melting point tubing being used), and replaced by methanol; this was

repeated three times as the product crystallized out. After a final concentration to ca. ¼ ml, the product was separated and washed with methanol (centrifuge). It formed a felt of hair-like crystals, m.p. 229–231.5°, mixed m.p. with the "pyrroporphyrin" ester copper complex from *Chlorobium* pheophorbide 650, fraction 1 (m.p. 230–232.5° (2)), 229–232.5°; the X-ray powder photographs of these two specimens were identical.

4-n-Propyl-5-ethyl-4-desethyl-5-desmethyl-pyrroporphyrin 15 Methyl Ester (1,3,8-Trimethyl-2,5-diethyl-4-n-propyl-porphin-7-propionic Acid Methyl Ester (IIIa, $R^1 = CH_2CH_2CH_3$, $R^2 = Et$))

5-Bromo-4,3′-dimethyl-5′-bromomethyl-3-ethyl-4′-*n*-propyl-pyrromethene hydrobromide (V*d*, $R^1 = CH_2CH_2CH_3$, 2.62 g), 3-bromo-4′,5′-dimethyl-4-ethyl-5-carboxy-pyrromethene-3′-propionic acid hydrobromide (V*g*, $R^2 = Et$, 2.5 g), and 5 g of methylsuccinic acid were heated together and the melt worked up exactly as in the synthesis of the 4-isobutyl homologue above. The Celite column was eluted for 4 h to separate the single slow-moving porphyrin band from dark material at the top. The intermediate free acid (40 mg) crystallized when its ether solution (3 ml) was allowed to stand; visible spectrum in ether (relative densities in parentheses): maxima at 622 (0.47), 597 (0.09), 567 (0.47), 525 (0.71), 497 (1.00), and 493 (0.99) mμ, with shoulders at 613, 573, and 471 mμ.

The methyl ester (30 mg) was prepared exactly as was the 4-isobutyl homologue above. It crystallized from methanol displacing chloroform, as long glistening needles; visible spectrum in ether (relative density in parentheses): maxima at 622 (0.48), 595 (0.09), 568 (0.47), 525 (0.71), 497 (1.00), and 493 (1.00) mμ, with shoulders at 613, 573, and 470 mμ.

Anal. Calcd. for $C_{34}H_{40}O_2N_4$: C, 76.09; H, 7.51. Found: C, 75.93; H, 7.59.

It had m.p. 210–213°. The mixed melting point with the "pyrroporphyrin" ester from *Chlorobium* pheophorbide 650, fraction 2 (m.p. 207–210° (2)), was 207–211°. The X-ray powder photographs of these synthetic and analytical specimens were identical.

The copper complex of the ester formed needles, m.p. 211–213°, mixed m.p. with the "pyrroporphyrin" ester – copper complex from *Chlorobium* pheophorbide 650, fraction 2 (m.p. 208.5–211.5° (2)), 209–213°; the X-ray powder photographs of these two specimens were identical.

The synthetic porphyrin has also been identified (3) with the pyrroporphyrins from *Chlorobium* pheophorbide 660, fractions 3 and 4.

The Desoxo-phylloerythrin Methyl Ester from Chlorobium Pheophorbide 650 Fraction 2 (4-n-Propyl-5-ethyl-4-desethyl-5-desmethyl-desoxo-phylloerythrin Methyl Ester (IIb, $R^1 = CH_2CH_2CH_3$, $R^2 = Et$))

The corresponding phylloerythrin methyl ester (cf. II*a*) (38 mg, m.p. 249–251° from chloroform–methanol after chromatography on sucrose; this had been obtained from *Chlorobium* pheophorbide 650, fraction 2, with hydrogen iodide in acetic acid (46)) was reduced by the Wolf–Kishner method as in the reduction of phylloerythrin itself (9). The crude product was extracted with ether, purified on a column of Celite – 3.5% hydrochloric acid, esterified, and crystallized as usual. It formed needles (15 mg), m.p. 221–222°, unchanged after hydrolysis, refractionation, and reesterification; visible spectrum in tetrahydrofuran–ether (relative density in parentheses): maxima at 620 (0.52), 594 (0.08), 566 (0.38), 531 (0.22), and 498 (1.0) mμ, with shoulders at 613 and 572 mμ.

Anal. Calcd. for $C_{36}H_{42}O_2N_4$: C, 76.83; H, 7.52; N, 9.96. Found: C, 76.41; H, 7.85; N, 10.14.

The copper complex formed needles, m.p. 233–235°.

This was compared with the synthetic desoxo-phylloerythrin (see below) corresponding to fraction 5.

4-Isobutyl-4-desethyl-pyrroporphyrin 15 Methyl Ester (1,3,5,8-Tetramethyl-2-ethyl-4-isobutyl-porphin-7-propionic Acid Methyl Ester (IIIa, $R^1 = CH_2CHMe_2$, $R^2 = Me$))

5-Bromo-4,3′-dimethyl-5′-bromomethyl-3-ethyl-4′-isobutyl-pyrromethene hydrobromide (V*d*, $R^1 = CH_2CHMe_2$, 2 g), 3-bromo-4,4′,5′-trimethyl-5-carboxy-pyrromethene-3′-propionic acid hydrobromide (V*g*, $R^2 = Me$, 2 g), and 5 g of methylsuccinic acid were heated together and the melt worked up exactly as in the synthesis of the 5-ethyl homologue above. On the Celite column, the pyrroporphyrin was in a dense bluish red band which was easily separated from two slower and weaker ones. The free acid did not crystallize from ether (3 ml), so the ether was evaporated and the residue esterified and crystallized from methanol displacing chloroform, as was its 5-ethyl homologue. The methyl ester formed prisms (2 mg); visible spectrum in ether (relative density in parentheses): maxima at 623 (0.47), 596 (0.09), 568 (0.47), 525 (0.71), 497 (1.00), and 493 (1.00) mμ, with shoulders at 614, 573, and 470 mμ. It melted at 224–226° after changing to needles at 220–221.5°. The mixed melting point with the "pyrroporphyrin" ester from *Chlorobium* pheophorbide 650, fraction 3, of m.p. 220–222° which has been analyzed (2) was 220–222.5°; the X-ray powder photographs of these two specimens were identical.

The copper complexes of these two esters, synthetic and of natural origin (2), both formed sheaves of needles, m.p. and mixed m.p. 222–223.5°; their X-ray powder photographs were identical.

The mixed melting point with synthetic 1,3,8-trimethyl-2,5-diethyl-4-isobutyl-porphin-7-propionic acid methyl ester (m.p. 225.5–227.5°) was 217 to ca. 223°.

5-Ethyl-5-desmethyl-pyrroporphyrin 15 Methyl Ester (1,3,8-Trimethyl-2,4,5-triethyl-porphin-7-propionic Acid Methyl Ester (IIIa, $R^1 = R^2 = Et$))

5-Bromo-4,3′-dimethyl-5′-bromomethyl-3,4′-diethyl-pyrromethene hydrobromide (V*d*, $R^1 = Et$, 1.5 g) and 3-bromo-4′,5′-dimethyl-4-ethyl-5-carboxy-pyrromethene-3′-propionic acid hydrobromide (V*g*, $R^2 = Et$, 1 g) were heated with 5 g of methylsuccinic acid, etc., as usual to yield the washed ether extract of the melt. Part of the product was extracted from the ether with 2% hydrochloric acid and worked up as under *a*; the

balance was precipitated as the sodium salt and worked up as under *b*; etioporphyrin I (145 mg) remained in the ether.

(*a*) The pigment was returned to ether, and the product was separated from a faster band on Celite – 5% hydrochloric acid (5 × 12 cm), then esterified, and crystallized from methanol displacing chloroform. The product formed purple prisms (20 mg), m.p. 185–205°; visible spectrum in chloroform (relative intensities in parentheses): maxima at 619 (0.336), 566 (0.466), 534 (0.685), and 498 (1.00) mμ, with shoulders at 591 and 532 mμ and inflexions at 573, 526, 501, and 572 mμ. This ester (2 mg) was chromatographed three times in 4% chloroform – petrol (30–60°) on icing sugar (m.p. then 186–190°) and then in ether on successively Celite – 6.5% hydrochloric acid and then Celite – 7% hydrochloric acid (m.p. then 188–191°, remelt 208–210°). The balance was recrystallized for analysis, prisms m.p. 187–210°, remelt 205–210°.

Anal. Calcd. for $C_{33}H_{38}O_2N_4$: C, 75.83; H, 7.33; N, 10.72. Found: C, 75.91; H, 7.48; N, 10.61.

(*b*) The sodium salts were dissolved in hydrochloric acid. The pigment was brought from this into ether, extracted with 4% hydrochloric acid (the bromo-pyrroporphyrin being left in the ether), returned to ether, and re-precipitated as the sodium salt; these steps were repeated. The residue left when the final ether solution had been evaporated was esterified and crystallized from methanol displacing chloroform to give prisms (19 mg), m.p. 187–189°, resolidifying to needles, m.p. 205–211°. The mixed melting point with the analytical pyrroporphyrin from *Chlorobium* pheophorbide 650, fraction 4 (see below), was 210–216.5°, remelt 212–217°; the X-ray powder photographs of these analytical and synthetic materials differed only in that the former had a few extra lines.

The high-melting form of the synthetic porphyrin ester was obtained by heating it, in a tube sealed off at 1×10^{-4} mm, from 180 to 205° over 3 h and then 1 h at 205°; m.p. 213–216.5°, mixed m.p. with the pyrroporphyrin ester from fraction 4 (as originally crystallized, see below) 212.5–217°. After both specimens had been heated *in vacuo* to 205°, their X-ray powder photographs were consistent, with each being a mixture of two forms, with one predominant form in common.

The synthetic and analytical (from fraction 4) esters were recrystallized again in the same way as prisms, and now gave identical X-ray powder photographs (synthetic, m.p. 189–193°; analytical, m.p. 191–194°). These changed to, or when they were cooled resolidified to, needles (synthetic, m.p. 207–214°; analytical, m.p. 211–214°) or sheets (synthetic, m.p. 213–216°; analytical, m.p. 213–216.5°). The analytical specimen sometimes resolidified, when it was cooled, to crystals of uncertain form which changed to the above needles at 185–190°; here a fourth form sometimes appeared as prismatic needles, m.p. 215.5–216.5°. The mixed melting point of the synthetic and analytical prisms was 189–193°, resolidifying to needles, m.p. 206–214.5°.

The synthetic specimen – copper complex formed fine red needles, m.p. 220–226°, mixed m.p. with the analytical complex (m.p. 220–226°) 219–227°. The X-ray powder photographs of these two specimens were identical.

The mixed melting point with the copper complex of synthetic 1,3,5,8-tetramethyl-2-ethyl-4-isobutylporphin-7-propionic acid methyl ester (m.p. 222–223.5°) was 201 to ca. 213°.

The "Pyrroporphyrin" Methyl Ester from Chlorobium Pheophorbide 650 Fraction 4 (1,3,8-Trimethyl-2,4-5-triethyl-porphin-7-propionic Acid Methyl Ester (IIIa, $R^1 = R^2 = Et$))

The "pyrroporphyrin" free acid (2) in ether was chromatographed on Celite – 4.8% hydrochloric acid (2 × 10 cm). The single broad red band was eluted with ether. The pigment was esterified, its chloroform solution filtered through alumina, and the product crystallized from chloroform–methanol as purple prisms (4.6 mg), m.p. 214.5–216.5°, with some signs of a solid phase change at 180°; visible spectrum in chloroform (relative intensities in parentheses): maxima at 618 (0.335), 566 (0.461), 534 (0.672), and 497 (1.00) mμ, with shoulders at 592 and 532 mμ and inflexions at 573, 525, 500, and 472 mμ.

The copper complex formed fine red needles, m.p. 220–226°.

4-n-Propyl-4-desethyl-desoxo-phylloerythrin Methyl Ester (IIb, $R^1 = CH_2CH_2CH_3$, $R^2 = Me$)

Essentially as in the analogous synthesis of desoxo-phylloerythrin itself (7), 18 g of 5-bromo-4,3′-dimethyl-5′-bromomethyl-3-ethyl-4′-*n*-propyl-pyrromethene hydrobromide (V*d*, $R^1 = CH_2CH_2CH_3$) and 18 g of 4,4′,5′-trimethyl-3-(ω-bromovinyl)-5-carboxy-pyrromethene-3′-propionic acid hydrobromide (IV*b*) were heated in succinic acid at 190–220°. The 18 melts, freed of succinic acid with hot water at pH 4 (disodium phosphate), were powdered, dried, and extracted for 3 days with 1 l of chloroform (thimble). The extract was poured into 20 volumes of ether, and the acidic porphyrins (1.66 g) were precipitated by shaking the filtered solution with 2% sodium hydroxide. A portion (0.5 g) was dissolved in acetic acid – hydrochloric acid and the pigments were brought into 3.5 l of ether by using sodium acetate. The washed and clarified ether solution was concentrated to 800 ml, shaken with 1% hydrochloric acid, and applied to a column of Celite 545 (9.5 × 42 cm, made up with 3.5% hydrochloric acid). Elution with ether separated the main band from much slower and much faster ones. It was extruded and eluted with 50% acetone, from which the pigment was brought into ether. The washed ether solution was evaporated and the residue esterified, crystallized, and recrystallized in the usual way. The product (48 mg, 0.8%) formed plates (occasionally needles), m.p. 235–237.5° after sintering and apparently a solid phase change from 234°; visible spectrum in tetrahydrofuran–ether (relative density in parentheses): maxima at 621 (0.54), 595 (0.07), 567 (0.37), 531 (0.22), and 498 (1.0) mμ, with shoulders at 613 and 573 mμ.

Anal. Calcd. for $C_{35}H_{40}O_2N_4$: C, 76.61; H, 7.35; N, 10.21. Found: C, 77.02; H, 7.15; N, 10.55.

Its mixed melting points with the desoxo-phylloerythrin esters from *Chlorobium* pheophorbide 650,

fractions 2 (see above) and 5 (see below), were respectively 213–216° and 235–237.5°. Its X-ray powder photograph was identical with that of the latter desoxo-phylloerythrin ester from fraction 5, and these two specimens had identical visible spectra in chloroform: λ_{max} (relative density in parentheses) 619 (0.41), 567 (0.40), 536.7 (0.24), 502 (1.0) mμ.

Its copper complex formed needles, m.p. 245–247.5°.

Anal. Calcd. for $C_{35}H_{38}O_2N_4Cu$: C, 68.89; H, 6.28; CuO, 13.04. Found: C, 68.56; H, 6.34; CuO, 12.39.

Its mixed melting points with the copper complexes of the desoxo-phylloerythrin esters from *Chlorobium* pheophorbide 650, fractions 2 and 5, were respectively 220–228° and 245.5–247°. Its X-ray powder photograph was identical with that of the latter complex from fraction 5.

1,3,5,7-Tetramethyl-2,6-diethyl-4,8-di-n-propyl-porphin

The chloroform–ether solution, from which the above synthetic desoxo-phylloerythrin had been precipitated as its sodium salt, was evaporated. An aliquot was dissolved in chloroform and passed through an alumina column (alkaline, grade II, made up with 5% methanol in chloroform). The eluate was concentrated and the pigment passed through a similar column. It was crystallized from chloroform–methanol as long prisms (17%) which decomposed at about 310°.

Anal. Calcd. for $C_{34}H_{42}N_4$: C, 80.59; H, 8.36; N, 11.06. Found: C, 80.72; H, 8.55; N, 11.54.

The Desoxo-phylloerythrin Methyl Ester from Chlorobium Pheophorbide 650 Fraction 5 (4-n-Propyl-4-desethyl-desoxo-phylloerythrin Methyl Ester (IIb, $R^1 = CH_2CH_2CH_3$, $R^2 = Me$))

The mesopyropheophorbide (2) (24 mg from *Chlorobium* pheophorbide 650, fraction 5), 0.4 g of sodium in 6 ml of absolute ethanol, and 0.3 ml of 95% hydrazine were frozen in a bomb tube, which was then sealed after it had been evacuated to 0.1 mm. The tube was warmed, shaken, and heated for 5 h at 170° in a rocking oven. The pigment was brought into ether by using acetic acid and the desoxo-phylloerythrin extracted with 2% hydrochloric acid. Evaporating the ether layer left the corresponding meso-desoxo-pyrropheophorbide, which was heated with 4 ml of acetic acid and 2 ml of 3% hydrochloric acid in a sealed tube, which had been deaerated as above, for 5 h at 180°. The resulting pigment was brought into ether and more of the product extracted with 2% hydrochloric acid. The pigment in the hydrochloric acid extracts was brought into ether with sodium acetate, and purified on a column of Celite – 5% hydrochloric acid, the single band being eluted with acetone after extrusion and the pigment being returned to ether. When the washed ether solution was concentrated, the product separated as needles of the free acid (7.7 mg). This was esterified and crystallized to give the product as plates, m.p. 235–237° after sintering and an apparent solid phase change at 234–235°.

The copper complex formed needles, m.p. 246–247°.

4-n-Propyl-5-ethyl-4-desethyl-5-desmethyl-δ-phylloporphyrin Methyl Ester (1,3,8,δ-Tetramethyl-2,5-diethyl-4-n-propyl-porphin-7-propionic Acid Methyl Ester (IIIb, $R^1 = CH_2CH_2CH_3$, $R^2 = Et$, $R^3 = Me$))

5-Bromo-4,3′-dimethyl-5′-bromomethyl-3-ethyl-4′-*n*-propyl-pyrromethene hydrobromide (V*d*, $R^1 = CH_2CH_2CH_3$, 2.75 g), 3-bromo-4′-methyl-4,5′-diethyl-5-carboxy-pyrromethene-3′-propionic acid hydrobromide (VI*b*, $R^2 = Et$, $R^3 = Me$, 2.6 g), and 5 g of methylsuccinic acid were heated together and stirred at 160–170° for 15 min and then at 180° for 5 min. The crude product was isolated and extracted with ether as in the case of the 4-isobutyl-5-ethyl-pyrroporphyrin (above). The ether was washed with 0.1% hydrochloric acid, and the acidic porphyrins were then separated as their sodium salts by shaking the ether with 10% sodium hydroxide. They were returned to fresh ether, extracted with 4% hydrochloric acid, again brought into ether, and passed through a column of Celite – 2% hydrochloric acid. Elution for 5 h removed a little porphyrin, leaving a blue band and, near the top, a black one. The blue band was separated, and the pigment was eluted with 2% hydrochloric acid in acetone and then transferred to ether. The residue that was left when the ether was evaporated was esterified and brought into chloroform. The solution was passed through a column of alumina, filtered, and then concentrated to 2 ml while methanol was being added to displace the chloroform.

The product (5 mg) separated overnight; visible spectrum in ether: λ_{max} (relative density in parentheses): 629 (0.10), 576 (0.35), 534 (0.35), and 501 (1.00) mμ. Recrystallization from methanol gave prisms melting from 173° to 190°, with partial solidification at 180° followed by intervening remelting at 183–187°. Recrystallization from ether then gave needles, m.p. 191–194°, with the suggestion of a phase change at 190°.

X-ray powder photographs showed that the forms from methanol and from ether were different. The photograph of the latter was almost identical with that of the δ-phylloporphyrin ester (3), m.p. 196–199°, from *Chlorobium* pheophorbide 660, fraction 3, there being small differences in the intensities of a few lines. The photographs of the synthetic ester had nothing in common with those of the δ-phylloporphyrin ester from fraction 4.

1,3,8-Trimethyl-2-5,δ-triethyl-4-n-propyl-porphin-7-propionic Acid Methyl Ester (IIIb, $R^1 = CH_2CH_2CH_3$, $R^2 = R^3 = Et$)

5-Bromo-4,3′-dimethyl-5′-bromomethyl-3-ethyl-4′-*n*-propyl-pyrromethene hydrobromide (V*d*, $R^1 = CH_2CH_2CH_3$, 1.88 g) and 3-bromo-4′-methyl-4-ethyl-5-*n*-propyl-5-carboxy-pyrromethene-3′-propionic acid hydrobromide (VI*b*, $R^2 = R^3 = Et$, 2.3 g) were fused with methylsuccinic acid, and the product was isolated exactly as was the δ-methyl analogue above; a single blue band appeared on the Celite column. The product (3 mg) formed poorly defined prisms, m.p. 140–156°; visible spectrum in ether (relative density in parentheses): λ_{max} 628 (0.10), 574 (0.34), 534 (0.32), and 502 (1.0) mμ.

It was recrystallized from methanol as prisms, m.p. 151–170°, with intervening darkening at 120° and partial phase changes to glistening prisms at 130–131°, to small needles from 151°, and to long needles at 164°.

The analytical δ-phylloporphyrin from *Chlorobium* pheophorbide 660, fraction 4 (3), had m.p. 195–197°, with the suggestion of phase changes at 164–190°. X-ray powder photographs showed that it existed in two (or more) forms, neither of which was identical with that of the synthetic material. The analytical and synthetic copper complexes, m.p. 174–179° and 172–180° respectively, mixed m.p. 172–180°, did not give identical X-ray powder photographs.

5-Ethyl-5-desmethyl-δ-phylloporphyrin 15 Methyl Ester (1,3,8,δ-Tetramethyl-2,4,5-triethyl-porphin-7-propionic Acid Methyl Ester (IIIb, $R^1 = R^2 = Et$, $R^3 = Me$))

Methylsuccinic acid (6.6 g), 3-bromo-4′-methyl-4,5′-diethyl-5-carboxy-pyrromethene-3′-propionic acid hydrobromide (VI*b*, $R^2 = Et$, $R^3 = Me$, 1 g), and 5-bromo-4,3′-dimethyl-5′-bromomethyl-3,4′-diethyl-pyrromethene hydrobromide (V*d*, $R^1 = Et$, 5.6 g) were ground together and heated in six portions at 160° for 20 min. The melt was freed of methylsuccinic acid, dried, powdered as usual, and then heated under reflux for 2 h in 200 ml of 5% methanolic hydrogen chloride. The pigment in the filtered solution was then brought into chloroform. The chloroform solution was filtered through deactivated alumina, washed with 45% aqueous resorcinol (10 × 50 ml subsequently backwashed), washed with water, dried, and again filtered through alumina; then the solvent was evaporated. The residue was stirred for 2 h with 500 ml of ether, and undissolved etioporphyrin separated. The pigments in the ether were chromatographed twice on Celite – 2% hydrochloric acid and once on Celite – 1.8% hydrochloric acid, in each case the blue band of the product being eluted with ether. The pigment in the final eluate was extracted with 2% hydrochloric acid and returned to ether. The solution was concentrated to 5 ml and left at 10° overnight. The product separated as needles, m.p. 209–211°, raised to 210–211.5° and then 214–215.5° (7.4 mg) when recrystallized from chloroform–methanol and then from ether; visible spectrum in ether (relative density in parentheses): maxima at 629.5 (0.11), 577 (0.39), 534 (0.40), and 502 (1.00) mμ.

Anal. Calcd. for $C_{34}H_{40}O_2N_4$: C, 76.09; H, 7.51; N, 10.44. Found: C, 76.27; H, 7.21; N, 10.61.

The mixed melting point with the phylloporphyrin ester from *Chlorobium* pheophorbide 660, fraction 5 (see below), was 214–215°, and the X-ray powder photographs of the two specimens were identical. The mixed melting point of the synthetic ester with synthetic δ-phylloporphyrin 15 methyl ester (m.p. 212–213.5°) (see below) was 202 to ca. 208°.

The copper complex formed needles, m.p. 212.5–215°, mixed m.p. with the copper complex of the above analytical phylloporphyrin 213–215°. The X-ray powder photographs of these two specimens were identical. The mixed melting point of the copper complex of the analytical phylloporphyrin with that of synthetic δ-phylloporphyrin 15 methyl ester (m.p. 206–209.5°) was 199 to ca. 206°.

The "Phylloporphyrin" Methyl Ester from Chlorobium Pheophorbide 660 Fraction 5 (1,3,8,δ-Tetramethyl-2,4,5-triethyl-porphin-7-propionic Acid Methyl Ester)

The analytical "phylloporphyrin" (free acid) (3) was left overnight in 5% methanolic hydrogen chloride, the ester brought into ether, and the solution chromatographed on Celite – 2% hydrochloric acid (2 × 12 cm). Two narrow brown bands followed a blue one. The pigment in the latter was eluted and its ether solution concentrated to 0.5 ml. The product (1.3 mg) separated as needles, m.p. 215–215.5°; visible spectrum in ether (relative density in parentheses): maxima at 629 (0.10), 578 (0.38), 535 (0.39), and 502 (1.00) mμ.

The copper complex formed needles, m.p. 213.5–215.5°.

δ-Phylloporphyrin 15 Methyl Ester (1,3,5,8,δ-Pentamethyl-2,4-diethyl-porphin-7-propionic Acid Methyl Ester (IIIb, $R^1 = Et$, $R^2 = R^3 = Me$)) (5)

5-Bromo-3,4′-dimethyl-4,3′-diethyl-5′-bromoethyl-pyrromethene hydrobromide (VII*d*, 4 g), 5,3′-dibromo-4,4′,5′-trimethyl-pyrromethene-3′-propionic acid hydrobromide (VII*h*, 4.01 g), and 16 g of methylsuccinic acid were heated together in portions, and the crystalline δ-phylloporphyrin free acid (40 mg) was isolated exactly as reported (5). This was dissolved in concentrated hydrochloric acid, brought into ether by using sodium tartrate, and chromatographed as usual on Celite – 2% hydrochloric acid. The pigment in the single red slow-moving band was eluted with acetone – 2% hydrochloric acid, and brought into ether; then the ether was evaporated. The residue was esterified and crystallized as usual from methanol displacing chloroform to give red needles (35 mg) of the ester, m.p. 210–212° (lit. (5) m.p. 214°), 212–213.5° after one or more recrystallizations from methanol displacing chloroform.

Anal. Calcd. for $C_{33}H_{38}O_2N_4$: C, 75.83; H, 7.33; N, 10.72. Found: C, 75.59; H, 7.54; N, 10.54.

X-ray powder photographs showed that it crystallized in either of two forms or in mixtures of the two. The usual pure form was obtained from both boiling and cold (20°) solutions; the other form was obtained pure only once, from a boiling solution. When the usual form was heated, a solid phase change at about 200° was sometimes apparent.

The phylloporphyrin ester from *Chlorobium* pheophorbide 660, fraction 6 (3) (m.p. 211–214°, mixed m.p. with the above 211–213°), separated sometimes as a mixture of these two forms and sometimes in a form giving an X-ray powder photograph identical with that of the usual form of the synthetic ester. The visible spectra of the two specimens in chloroform were identical; λ_{max} (relative density in parentheses): 628 (0.08), 576 (0.42), 539.5 (0.39), and 505 (1.0) mμ.

The copper complexes of these esters, synthetic and of natural origin, formed needles, m.p. 206–209.5° and 205.5–208° after sintering from 205.5 and 205° respectively (lit. (5) m.p. 189°), mixed m.p. 206–209.5° after sintering from 205.5°. The X-ray powder photographs of these two specimens were identical.

It has been reported (5) that the spectrum of the synthetic δ-phylloporphyrin 15 is identical with that of γ-phylloporphyrin 15, in which the intensity of band 2 is significantly greater than that of band 3. Elsewhere (47) these two bands in the spectrum of δ-phylloporphyrin 15 are reported to be nearly equal in intensity, as they are in the spectra of all the synthetic and degradational δ-phylloporphyrins mentioned above.

δ-Phylloporphyrin 15 (Free Acid)

Some of the synthetic ester was hydrolyzed at 20° overnight in acetic acid – hydrochloric acid (1:1) (alkaline hydrolysis apparently resulted in decomposition). The acid was brought into ether, precipitated by 5% sodium hydroxide as the sodium salt, and returned to ether with acid. Concentrating the ether gave the product as needles. The X-ray powder photographs of this and of the phylloporphyrin free acid (3) derived from the above *Chlorobium* pheophorbide 660, fraction 6, were identical.

ACKNOWLEDGMENTS

The authors thank Dr. A. S. Holt and his colleagues for their generous cooperation, and Dr. Maria Przybylska for the interpretation of the X-ray powder photographs.

REFERENCES

1. J. L. Archibald, S. F. MacDonald, and K. B. Shaw. J. Am. Chem. Soc. **85**, 644 (1963).
2. J. W. Purdie and A. S. Holt. Can. J. Chem. In press.
3. A. S. Holt, J. W. Purdie, and J. W. F. Wasley. Can. J. Chem. In press.
4. J. W. Mathewson, W. R. Richards, and H. Rapoport. J. Am. Chem. Soc. **85**, 346 (1963).
5. H. Fischer, W. Siedel, and L. LeThierry d'Ennequin. Ann. **500**, 137 (1933).
6. H. Fischer and E. Strobel. Ann. **531**, 251 (1937). E. Strobel. Dissertation, Technische Hochschule, Munich, Germany. 1936.
7. H. Fischer and J. Riedmair. Ann. **490**, 91 (1931).
8. H. Fischer and J. Riedmair. Ann. **499**, 288 (1932).
9. H. Fischer, O. Moldenhauer, and O. Süs. Ann. **585**, 1 (1931).
10. H. Fischer and H. Gibian. Ann. **550**, 208 (1942); **552**, 153 (1942).
11. H. Fischer, H. Berg, and A. Schormüller. Ann. **480**, 109 (1930).
12. F. Morsingh and S. F. MacDonald. J. Am. Chem. Soc. **82**, 4377 (1960).
13. S. F. MacDonald and A. Markovac. Can. J. Chem. In press.
14. R. F. Bacon. Am. Chem. J. **33**, 79 (1905).
15. B. R. Baker, R. E. Schaub, and J. H. Williams. J. Org. Chem. **17**, 116 (1952).
16. S. F. MacDonald and R. J. Stedman. Can. J. Chem. **33**, 458 (1955).
17. H. Lund. Ber. **67**, 935 (1934).
18. B. Riegel and W. M. Lilienfeld. J. Am. Chem. Soc. **67**, 1273 (1945).
19. F. Bohlmann, H. J. Mannhardt, and H. G. Viehe. Chem. Ber. **88**, 364 (1955).
20. D. S. Breslow, E. Baumgarten, and C. R. Hauser. J. Am. Chem. Soc. **66**, 1286 (1944).
21. A. Brandstrom. Acta Chem. Scand. **5**, 1413 (1951).
22. E. E. Blaise. Compt. Rend. **132**, 978 (1901).
23. S. F. MacDonald. J. Chem. Soc. 4176 (1952).
24. E. Bulloch, A. W. Johnson, E. Markham, and K. B. Shaw. J. Chem. Soc. 1430 (1958).
25. H. Fischer and O. Süs. Ann. **484**, 118 (1930).
26. H. Fischer, E. Sturm, and H. Friedrich. Ann. **461**, 244 (1928).
27. F. K. Signaigo and H. Adkins. J. Am. Chem. Soc. **58**, 709 (1936).
28. W. Siedel and F. Winkler. Ann. **554**, 162 (1943).
29. H. Fischer and H. Orth. Chemie des pyrrols. Vol. I. Akademische Verlagsgesellschaft, Leipzig. 1934.
30. A. Treibs and R. Schmidt. Ann. **577**, 111 (1952).
31. H. Fischer and M. Bertl. Z. Physiol. Chem. **229**, 52 (1934).
32. G. Dupont. Compt. Rend. **148**, 1522 (1909).
33. J. Ellis, A. H. Jackson, A. C. Jain, and G. W. Kenner. J. Chem. Soc. 1935 (1964).
34. R. A. Nicholaus and R. Nicholetti. Ann. Chem. **47**, 167 (1957).
35. A. Wahl. Compt. Rend. **152**, 95 (1911).
36. H. Musso and K. Figge. Ann. **668**, 1 (1963).
37. W. Siedel. Z. Physiol. Chem. **231**, 167 (1935).
38. G. M. Badger, R. L. N. Harris, and R. A. Jones. Australian J. Chem. **17**, 987 (1964).
39. H. Fischer and H. Andersag. Ann. **458**, 117 (1927).
40. A. Treibs and W. Ott. Ann. **615**, 156 (1958).
41. R. L. Hinman and S. Theodoropulos. J. Org. Chem. **28**, 3052 (1963).
42. A. Hayes, G. W. Kenner, and N. R. Williams. J. Chem. Soc. 3779 (1958).
43. H. Fischer and H. Orth. Chemie des pyrrols. Vol. II. Akademische Verlagsgesellschaft, Leipzig. 1937. Part I. p. 106.
44. H. Fischer and A. Rothhaas. Ann. **484**, 102 (1930).
45. D. W. Hughes and A. S. Holt. Can. J. Chem. **40**, 171 (1962).
46. A. S. Holt and D. W. Hughes. J. Am. Chem. Soc. **83**, 499 (1961).
47. F. Pruckner. Z. Physik. Chem. **190A**, 101 (1942).