The Analytical Reduction of Porphyrins to Pyrroles[1]

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When porphyrins or related pigments are reduced by HI–AcOH, their meso-substituents (R = H or alkyl) appear as α-CH_2R groups in the resulting pyrroles. The identification of the pyrroles, as by g.l.p.c., defines and orders the meso-substituents flanking each pair of peripheral substituents in the porphyrin. If one meso-substituent differs from the others, all the substituents about the two adjacent pyrrole nuclei are then ordered.

The pyrroles can be reductively alkylated *in situ*, and symmetry then reduces their number when the aldehyde used (R.CHO) corresponds to a bridge and its meso-substituent. This may simplify the pyrrole mixtures, identify meso-substituents, or make pyrroles distinguishable by g.l.p.c.

The critical pyrrole – propionic acids are separable as methyl esters by g.l.p.c. Desoxophylloerythrin and pyrophaeophorbides (the latter first reduced with potassium borohydride) are reduced to cyclopenteno-pyrroles which reveal the isocyclic rings and the 5-substituents. The r.r.t.'s of relevant pyrroles are reported.

La réduction des porphyrines et des pigments du même genre par HI–AcOH fait que les substituants méso (R = H ou alkyle) apparaissent sous forme de groupements CH_2R en α des pyrroles résultants. L'identification des pyrroles par c.p.l.g., par exemple, définit et classe les substituants méso qui se trouvent à côté de chaque paire de substituants périphériques dans la porphyrine. Si un des substituants méso diffère des autres, tous les substituants autour des deux noyaux pyrroles adjacents sont alors classés.

L'alkylation réductive *in situ* des pyrroles peut réduire par symétrie le nombre de pyrroles lorsque l'aldéhyde utilisé (R.CHO) correspond au pont et au substituant méso de la porphyrine initiale. Ceci peut simplifier le mélange des pyrroles, identifier les substituant méso et différentier les pyrroles par c.p.l.g.

Les acides critiques pyrrole – propioniques sont séparables de leurs esters méthyliques par c.p.l.g. La désoxophylloérythrine et les pyrophaephorbides (ces dernières d'abord réduites par le borohydrure de potassium) sont réduites en cyclopenténo-pyrroles, ce qui révèle les noyaux isocycliques et les substituants en 5. Les données t.r.r. des pyrroles correspondants sont rapportées.

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The pairs of peripheral substituents on porphyrins and related pigments are now usually determined by identifying the maleinimides or the pyrrole-2,5-dicarboxylic acids resulting when the pigments are oxidized with chromic acid or with permanganate, respectively. The maleinimides have been separated and identified by g.l.p.c., by g.l.p.c. and mass spectra, t.l.c., or by t.l.c. and mass spectra (1–3, 60), the pyrrole-2,5-dicarboxylic acids by paper chromatography (4). Chromic acid is usually preferred because it gives much higher yields and these can be significant when two identical pairs of peripheral substituents are present. However, permanganate has advantages: while permanganate degrades isocyclic rings to carboxyl, chromic acid destroys the pyrrole ring bearing either of these groups. The reference pyrrole-2,5-dicarboxylic acids are also more generally available synthetically than are the corresponding maleinimides.

To the same end, the pigments had been reduced by HI–AcOH at 100° to mixtures of homologous pyrroles, meso-substituents being absent or ignored (Scheme 1, $R^1 = R^2 = H$). This method was abandoned for the yields were divided among homologues, complex mixtures of labile pyrroles had to be separated by fractionating the picrates, and some pyrroles do not form picrates. Although some of the purely alkyl pyrroles were recently separated by g.l.p.c., the chromic acid method was more suitable for more complex pigments (1). The chief disadvantage of the HI–AcOH reduction had been avoided by reducing the pigments and *C*-methylating the pyrroles in one step with KOMe—MeOH at 220° (5*a*, *b*). One tetra-alkyl pyrrole then replaced four homologues (Scheme 1), but propionic acid side-chains were partially replaced by methyl (6).[5]

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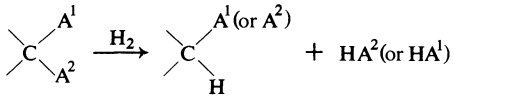
[5]This suggests a reversed Michael addition followed by methylation. The analogous thermal fission of 2,4-dimethyl-5-carbethoxy-3-(2,2-dicarboxyethyl)-pyrrole dihydrazide to 2,4-dimethyl-5-carbethoxy-pyrrole (7) is interesting because perhaps facilitated by the formation of a pyrazolidone.

Although chromic acid oxidation is unique in revealing the reduced pyrrole ring in chlorins and phaeophorbides as a succinimide (1, 8), the HI–AcOH reduction had potential advantages. It was unnecessary to reduce α-hydroxyethyl side-chains to prevent further degradations. The reagent was an excellent solvent, used in indefinite excess, and easily removed. The total yields were also competitive and the products more volatile. Three considerations (9) then made the reductive method more attractive: reference pyrroles were now more easily available, the pyrrole mixtures could be simplified by reductive methylation *in situ*, avoiding KOMe—MeOH at 220°, and it was possible that meso-substituents in the pigments might be retained on the pyrroles. The implications of these are developed in Scheme 1, where the rôle of alkylation is rationalized and extended. It should be noted that vinyl or α-hydroxyethyl groups are converted to ethyl, and that ring carbethoxy or acetyl groups are split off by HI–AcOH.

Scheme 1 assumes that all and only the indicated C—C fissions will take place. The only experimental evidence here lay in the pyrroles obtained by reducing porphyrins lacking meso-substituents, particularly hemin, and in the reduction of meso-methyl-dipyrrylmethanes to α-free and α-ethyl-pyrroles (10). Not all the expected pyrroles were obtained from coproporphyrin (5*b*), but the methods of isolation were presumably inadequate.

The assumption in Scheme 1 differs only in emphasis from what was apparently Küster's interpretation of the reduction of hemin: that it was reduced to monopyrroles because α,α-dipyrrylmethane bridges, but only these, would undergo reductive fission (11). Although this led him to the correct structure for the porphin nucleus in 1912, the central 16-membered ring was unacceptable at that time (12*a*), and only synthetic evidence for the structure (symmetry) of the nucleus was acceptable much later (5*f*).

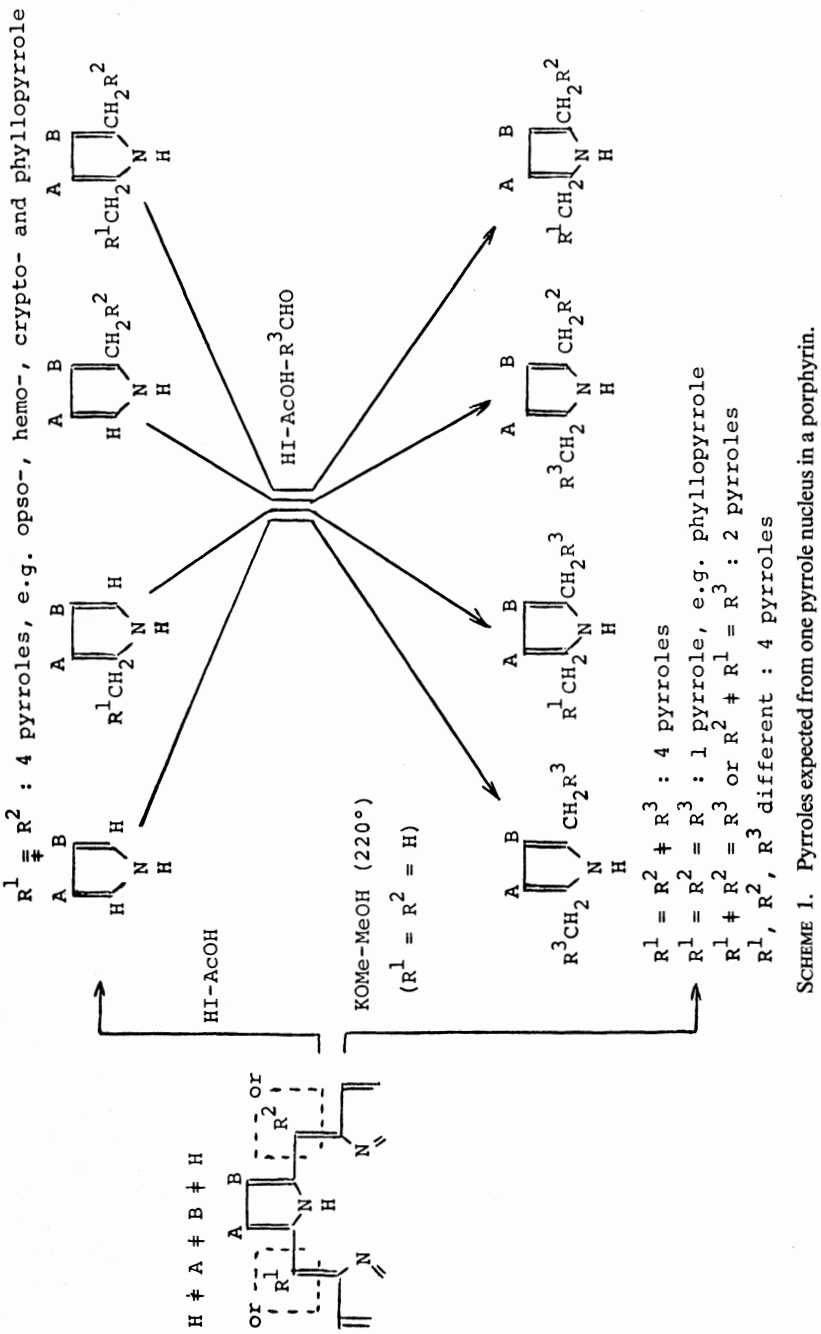
In the meantime Küster's idea was apparently regarded as an *ad hoc* assumption supporting an impossible structure. However, analogous reductions had previously been correctly interpreted in the same way by Boehm (13): the reductive fission of linear methylene-bis-phloroglucin derivatives in male fern by zinc and alkali. More generally, the reduction of porphinogens, the intermediates in the HI–AcOH reduction of porphyrins, is analogous to many reductive fissions of C—O, C—N, and C—C bonds:



where A^1 = or ≠ A^2, and both are such that A^- and $\diagdown\!\!\diagup C_+$ are usually regarded as stabilized; in porphinogens, $\diagdown\!\!\diagup C \diagdown\!\!\diagup$ here represents a bridge.

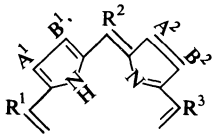
Küster's assumption was confirmed (although its reasonableness was denied) when a 1,2-di-(α-pyrryl)-ethane, unlike dipyrrylmethanes, proved stable to HI–AcOH (14). It was then concluded that the α-pyrryl residues in hemin could hardly be linked through two carbon atoms, but the result was not used to eliminate the two rival porphyrin structures (5*f*) and a third which arose later (5*g*). All three would require that the porphyrins reduce to derivatives of two pyrroles and a 1,2-dipyrrylethane. The confusion is further illustrated by an apparent attempt to find a hydrocarbon, representing the γ-substituent or the γ-bridge, among the reduction products of phylloporphyrin (15). However, in a return to Küster's interpretation and its implications, the absence of α-ethyl derivatives among the pyrroles was somewhat tentatively cited as evidence for the absence of meso methyl groups in hemin (16). Ironically, α-ethyl pyrroles from hemin had been reported (5*b*); although the methods then current were probably inadequate to decide their existence, hindsight had rejected them as unlikely (17).

The presence of meso-substituents in a pigment has been inferred from a missing methine C*H* signal in the n.m.r. spectrum, or by degrading the pigment to a porphyrin with a "phyllo-type" visible spectrum. The substituent was then identified and located by identifying the porphyrin with a synthetic one. Similarly, although the pairs of peripheral substituents in a pigment could be determined analytically, their further ordering depended ultimately upon the identification of an analytical with a synthetic porphyrin. In both cases the possible structures might be limited by empirical rules governing spectra etc. or by biogenetic ideas. However, the former could be misleading if based on an unforeseeably in appropriate model, *cf.* (18, 19), and the relevance of the latter may be in question, *cf.* petroporphyrins (20).



SCHEME 1. Pyrroles expected from one pyrrole nucleus in a porphyrin.

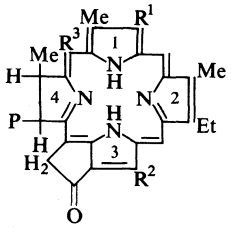
Scheme 1 implies that the identification of the pyrroles obtained by reduction will identify and locate the meso and peripheral substituents about a pyrrole nucleus in a pigment and, if any meso-substituent (R^2) is different from the others, all the substituents about the two adjacent pyrrole nuclei:



On the other hand, methylation will be fully effective in simplifying the pyrrole mixtures only in the absence of meso-substituents in the pigment. Some further implications of Scheme 1 are inescapable; these will be illustrated by the examples in Tables 1 and 2.

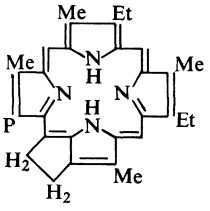
Tables 1 and 2 show the products expected from the pyrrole nuclei of typically substituted porphyrins. The products are those required by Scheme 1 but some represent special cases wherein a peripheral position is unsubstituted (A or B = H, becoming CH_2R^3 after alkylation), or one of the meso-substituents (X) is sufficiently large that the pyrroles bearing it do not appear with the others on g.l.p.c. The products from the reduced ring 4 of phorbins and chlorins should be unreduced pyrroles (15), the same as those from ring 4 of the corresponding porphyrins. Examples (*i*), (*j*), and (*k*) in Table 2 with large meso-substituents (X) will then represent the simplest model for ring 4 of pyrophaeophorbides and desoxophylloerythrin. The relative retention times (r.r.t.) in the tables were determined with reference pyrroles (see below), and the logarithmic scale suggests the separabilities, *e.g.* the pyrroles **2** and **3**, like **21** and **23**, could be distinguished by their r.r.t.'s but ran together under one peak.

Ignoring the r.r.t.'s in these examples, the following information could be obtained merely by counting the pyrroles and by noting whether or not the highest pyrrole homologue obtained by reduction was unchanged by alkylation, *i.e.* was a tetra-alkylpyrrole. A large meso-substituent would be recognized, but only as such, when reduction gave two pyrroles as in examples (*i*), (*j*), and (*k*). All other meso-substituents would be identified by the aldehyde used when the number of pyrroles was reduced from four to one as in

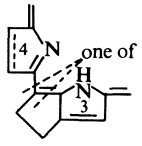


Pyrophaeophorbides

R^1 = $CH{:}CH_2$; R^2 = Me; R^3 = H:
Pyrophaeophorbide-*a*
R^1 = $CH(OH)CH_3$; R^2 = Et; R^3 = H:
Chlorobium phaeophorbide 650 fraction 4
R^1 = $CH(OH)CH_3$: R^2 = Et; R^3 = Me:
Chlorobium phaeophorbide 660 fraction 5



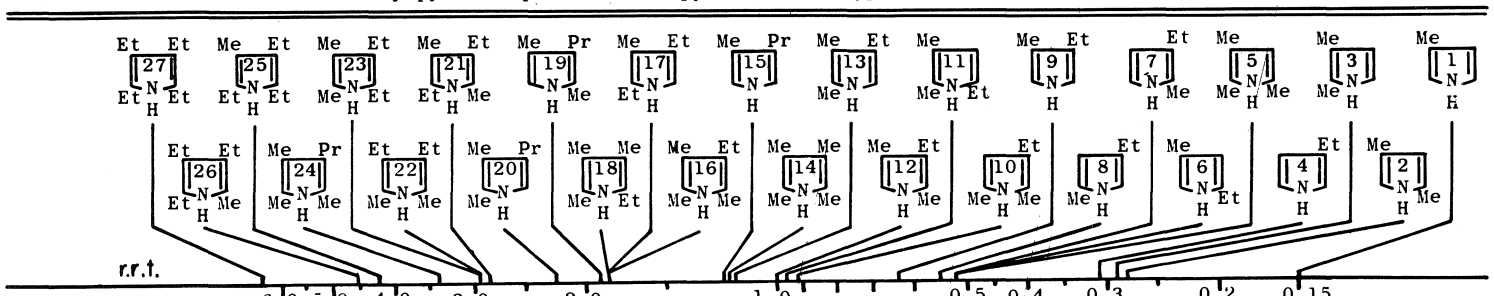
Desoxo-phylloerythrin



Assumed fissions

(*a*), (*b*), (*c*), (*d*), and (*g*), from three to one (see below), from four to two as in (*e*), (*f*), and (*h*), or from two to one as in (*i*) and (*j*). In the absence of a large meso-substituent (more than two pyrroles on reduction), no pyrrole common to the three chromatograms would show that one or both of the peripheral positions was unsubstituted as in (*c*), (*d*), and (*f*). The peripheral substituents would not be further defined unless the meso-substituents were identical. If they were, reduction would give three pyrroles if A = B (*cf.* Scheme 1; no examples in Tables 1 and 2); A and B would then be an alkyl group if there was a pyrrole common to the three chromatograms, H if not, and the aldehyde defining the meso-substituents here would convert the three pyrroles to one. If the meso-substituents were identical and A = H, B = alkyl as in (*c*) and (*d*), B would usually be identifiable. In (*d*), the general case, one aldehyde would identify the meso-substituents as usual by converting four pyrroles to one in which the 2- and 5-substituents were identical, and another aldehyde would identify B by converting four pyrroles to three in which the 3- and 4-substituents were B. In (*c*), the degenerate case wherein B (Me) is the next higher homologue of the meso-substituent (H), B could not be identified because the aldehyde identifying the meso-

TABLE 1. Alkyl pyrroles expected from the pyrrole nuclei of typical porphyrins, and their r.r.t.'s at 75°



Pr = n-C_3H_7

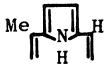
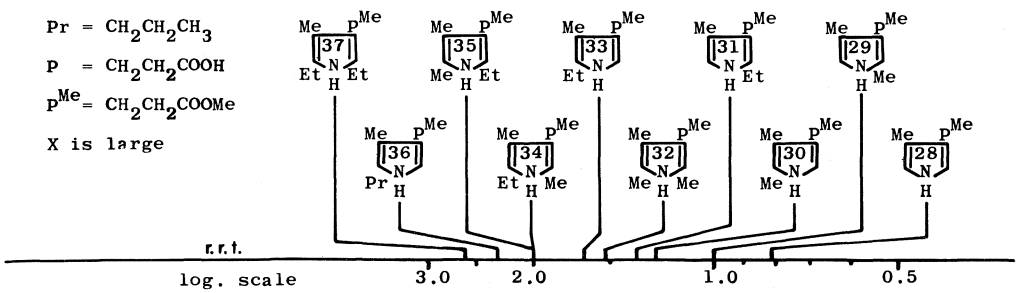
 (a)	HI							16		13	12		9											
	HI-H_2CO							16																
	HI-MeCHO			25		23,21		16																
 (b)	HI				24		20	19		15														
	HI-H_2CO				24																			
 (c)	HI													5		3,2		1						
	HI-H_2CO									14														
	HI-MeCHO			25		23,21		16																
 (d)	HI										10		8,7			4								
	HI-H_2CO							16																
	HI-MeCHO	27		26		22																		
 (e)	HI					21		17			12		9											
	HI-H_2CO					21		16																
	HI-MeCHO			25		21																		
 (f)	HI										11			6		3		1						
	HI-H_2CO							18		14														
	HI-MeCHO			25		23																		

TABLE 2. Pyrrole esters expected from the pyrrole nuclei of typical porphyrins, and their r.r.t.'s at 135°



		r.r.t.
(g)	HI	32 30 29 28
	HI-H_2CO	32
	HI-MeCHO	37 35,34 32
(h)	HI	35 31,30 28
	HI-H_2CO	35 32
	HI-MeCHO	37 35
(i)	HI	30 28
	HI-H_2CO	32
	HI-MeCHO	37 35
(j)	HI	33 28
	HI-H_2CO	34 32
	HI-MeCHO	37
(k)	HI	36 28

substituents would also make the 3- and 4-substituents on the pyrroles identical.

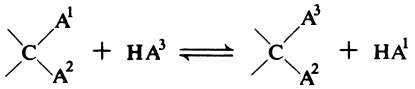
The practicability of the methods suggested here depends, although in varying degrees, on the extent to which all and only the expected pyrroles are formed and are distinguishable. The following was clear from the behavior of reference pyrroles (see below). All the pyrroles expected from naturally substituted porphyrins were sufficiently stable in HI–AcOH at 110°, although opsopyrrole–carboxylic acid, **28**, unlike phyllopyrrole–carboxylic acid, **32**, was reportedly destroyed at 130° (15). This is fortuitous, for the instability of some pyrroles expected from unnaturally substituted porphyrins would result in missing pyrroles or extra ones. There was also no evidence that the pyrroles were alkylated significantly in HI–AcOH through scavenging. It may be significant here that cryptopyrrole was unchanged in hot HI–HCOOH, although we had expected that it would be converted into the dipyrrylmethene and thence into the fully methylated phyllopyrrole, *cf.* dipyrrylmethenes from pyrroles and HBr–HCOOH (5*h*). The methylations and ethylations were also clean. On the other hand, it was clear from the r.r.t.'s of the reference pyrroles that overlapping peaks would limit the usefulness of counting methods, *e.g.* the situation in example (*c*) would be confused with that in (*d*) because the pyrroles **21** and **23** appear under one peak.

The assumption in Scheme 1 governing the reduction remained to be tested. Twelve typical porphyrins of known structure were reduced in HI–AcOH at 110° and the products were submitted to g.l.p.c. before and after alkylation, the latter being effected by adding paraformaldehyde or paraldehyde to the mixture and continuing the heating. Mass spectra were used only occasionally for they do not readily distinguish

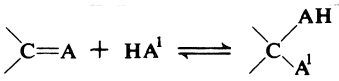
isomeric alkyl pyrroles with the same substituents (21). Chromatograms like those in Charts 1 and 2 were obtained only after several sources of extraneous peaks had been eliminated; this was our major effort. The peaks to the right of the ester peaks in Chart 2 correspond to incompletely removed alkyl pyrroles and become more obvious when shifted by alkylation. The numbers identifying the pyrroles correspond to those of the pyrroles in Tables 1 and 2.

The validity of the assumption (Scheme 1) may be judged by the extent to which the chromatograms represent the examples (Tables 1 and 2) or composites thereof. A subjective element exists here whenever the base lines depart from the horizontal, but the following general remarks apply equally to the chromatograms not reproduced here. There were no peaks in the chromatograms not attributable to pyrroles even when the yields were low, and although the g.l.p.c. detector is less specific than the diazonium salt or chlorine-benzidine used in the paper chromatography of pyrrole dicarboxylic acids or the t.l.c. of maleinimides. In particular, reduced pyrroles were not detected, *cf.* (12*b*). An unexpected pyrrole peak appeared among the esters from pyrophaeophorbides, and others appeared among the alkyl pyrroles if these pigments were first reduced with potassium borohydride (see below). Otherwise all the definite peaks were anticipated. This was surprising if only because any "scrambling" of the porphinogens in hot acid[6] would result in the loss of translocation of bridges.

[6]"Scrambling" refers to intermolecular redistribution reactions of porphinogens in hot acid leading to new porphinogens in which the pyrrole nuclei are randomized (22) and added formaldehyde may form the bridges (23). Analogous reactions have been observed in dipyrrylmethanes (24) and in their meso-alkyl derivatives (25); the latter may also lose their bridges with KOMe—MeOH at 220° (6, 26). It will be clear from the following expressions that the reductive C—C fissions and "scrambling" have the same structural requirements, and that the latter is analogous to many more familiar reactions:



a special case of which is



where A, A^1, A^2, A^3 are defined as above. Syntheses of unsymmetrical dipyrrylmethanes and dipyrrylmethenes may give symmetrical products in the same way.

The chromatograms also showed significant peaks corresponding to all the expected pyrroles. Although this was meaningless when a peak represented more than one pyrrole, some components appeared alone when other porphyrins were reduced, and some others could be identified unambiguously after the pyrroles were alkylated (see comments on the chromatograms below). In the absence of evidence to the contrary, it may reasonably be assumed that all the expected pyrroles will appear on the chromatograms, and some significance may be attributed to their absence. Incidentally, stoichiometry requires equal amounts of 2,5-dialkylpyrroles and 2,5-free pyrroles except from phaeophorbides, and the somewhat larger amounts of the former may reflect their greater stability.

Aside from the extra peaks mentioned above, the assumption underlying Scheme 1 appears to be justified, and the chromatograms can be inferred from the structures of the porphyrins. However, the structural information available from the chromatograms is limited by overlapping or ambiguous peaks and by other factors. When less than four differently substituted nuclei are found, qualitative data will not show when this is because two or more are identically substituted, and awkward possibilities then arise unless it can be assumed that large substituents are absent and all the products appear on the chromatograms. For this reason, and because the peripheral substituents would not all be ordered unless there were four different meso-substituents, the structures of more complex porphyrins will be more completely determined. The following discussion of the chromatograms will illustrate this and introduce some variations in the method.

The chromatograms of the alkyl pyrroles and pyrrole esters from hemin (Charts 1 and 2) and the r.r.t.'s of the reference pyrroles (Tables 1 and 2) define all the substituents about two pyrrole nuclei as those in examples (*a*) and (*g*). Ignoring the r.r.t.'s in each case, the patterns before and after methylation indicate unambiguously two different peripheral substituents, neither H, and no meso alkyl substituents. Only two differently substituted pyrrole nuclei are identified but the peripheral substituents on a third are also as in example (*a*) because the yields of the alkyl pyrroles were 64% of 2 mol before methylation and 70% after; the yield of the esters was 42%, 35% after methylation and 18% after

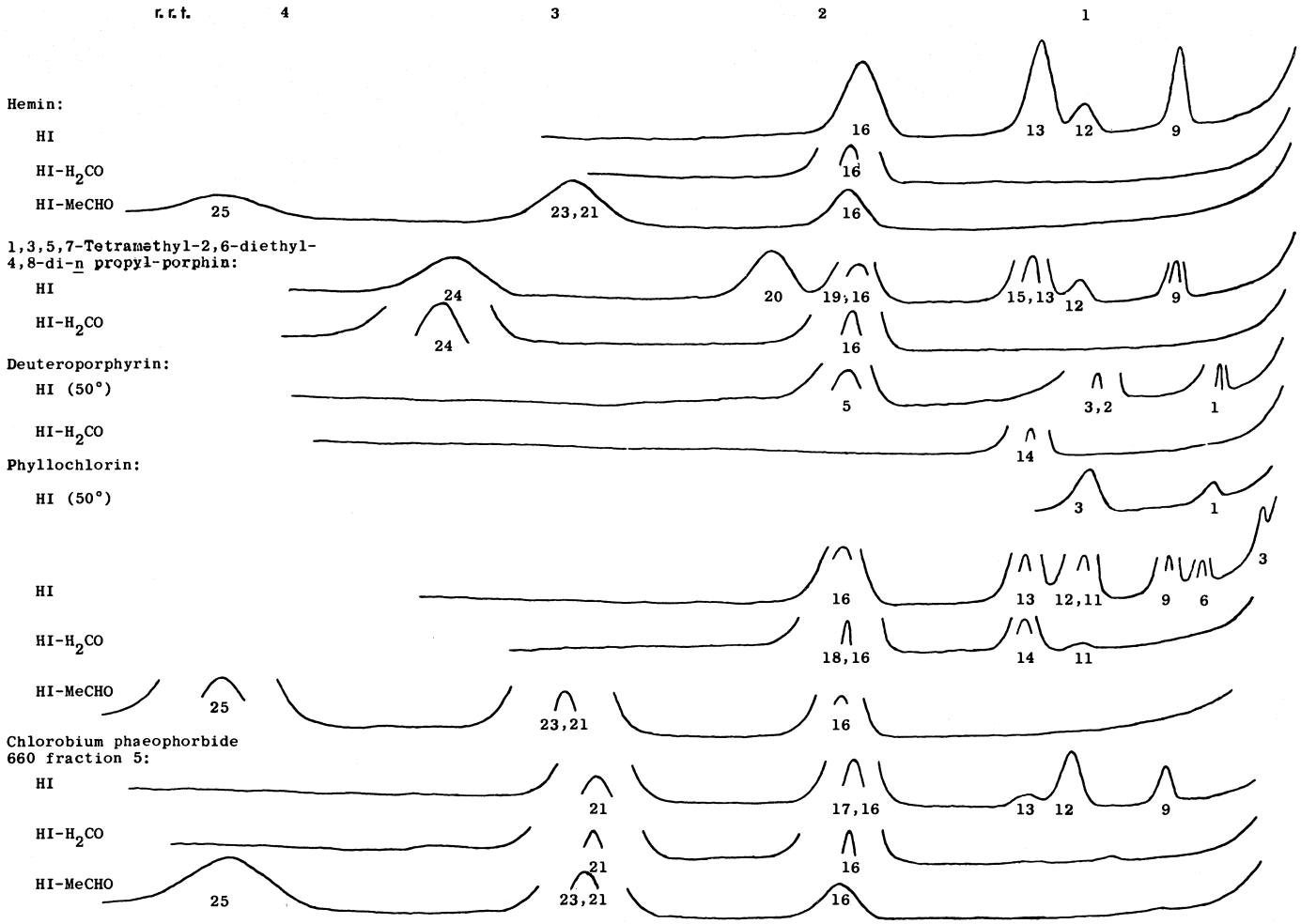


CHART 1. The g.l.p.c. of alkyl pyrroles from porphyrins, at 75° unless specified.

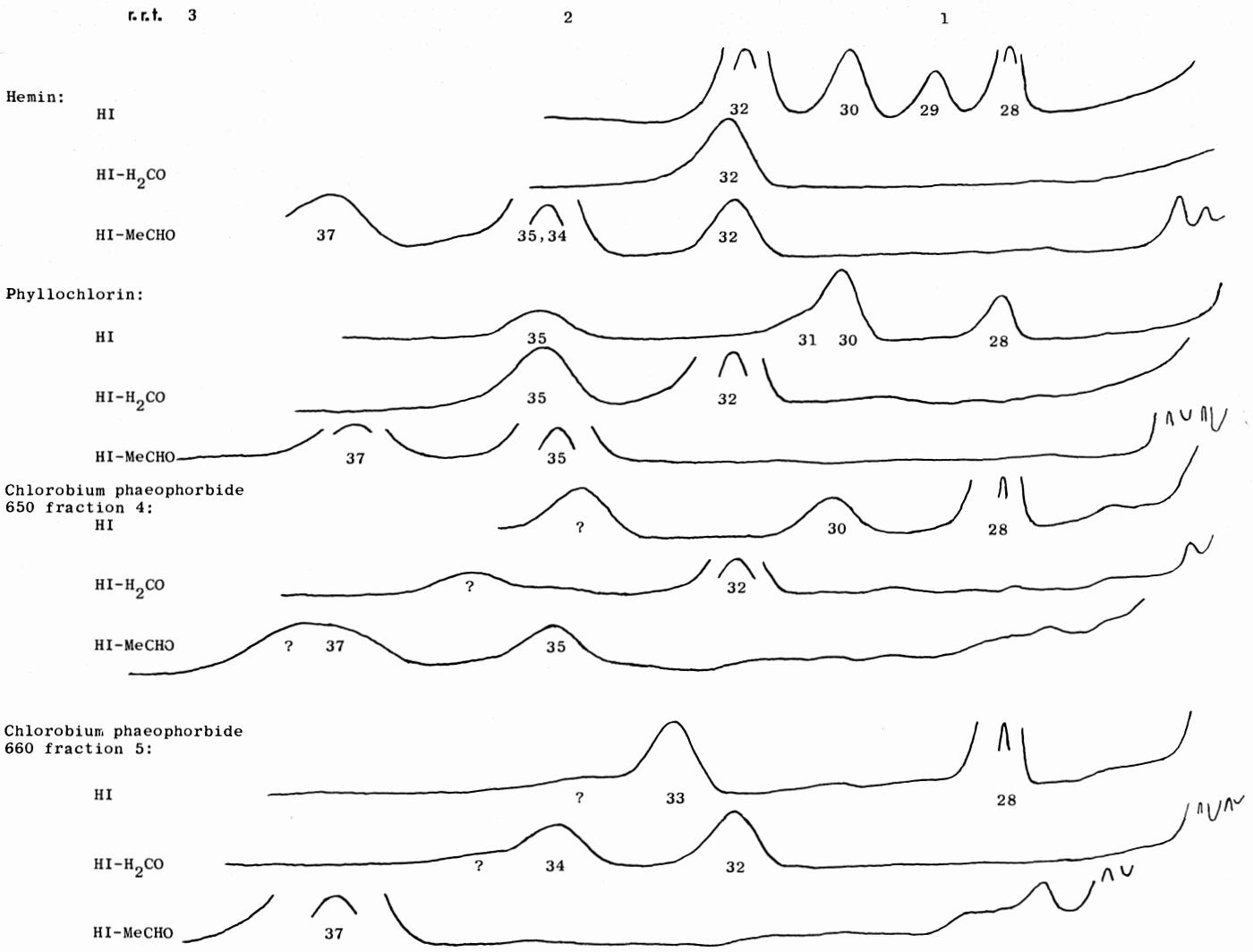


CHART 2. The g.l.p.c. of pyrrole esters from porphyrins at 135°.

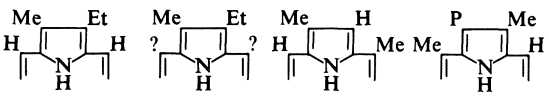
ethylation. Largely because only two differently substituted nuclei are identified, the chromatograms would not exclude a structure with three nuclei as in (*a*) and one as in (*g*), or a large substituent, but a meso methyl or ethyl group is excluded. The chromatograms of the alkyl pyrroles from hemin, etioporphyrin *1*, pyrophaeophorbide-*a*, and Chlorobium chlorophyll 650 fraction 4 were essentially the same, as were those of the pyrrole esters from hemin, coproporphyrin *3*, and deuteroporphyrin *9*.

The peaks in the chromatograms from 1,3,5,7-tetramethyl-2,6-diethyl-4,8-di-*n*-propyl-porphin are identified by their r.r.t.'s as the pyrroles from (*a*) and (*b*). The composite pattern alone is ambiguous but it illustrates the simplification of pyrrole mixtures and one method for the resolution of composite peaks by alkylation.

The chromatograms of the alkyl pyrroles from deuteroporphyrin *9* and the r.r.t.'s of the reference pyrroles identify a nucleus substituted as in (*c*). The unmethylated pyrroles were run at a lower temperature here to reveal 3-methylpyrrole. After ethylation, the chromatogram (not shown) was like that of the ethylated pyrroles from hemin. The pattern alone correctly shows the unsubstituded meso positions and peripheral hydrogen. However, as the pyrroles **2** and **3** run together as do **21** and **22** (see the chromatograms from hemin), the pattern is that of a nucleus without meso or peripheral substituents. The chromatograms of the alkyl pyrroles from deuteroporphyrin *9* and from methyl 1,4,5,8-tetramethylporphin-2,3,6,7-tetracarboxylate were essentially the same.

The chromatograms of the alkyl pyrroles from diacetyl-deuteroporphyrin *9* were like those from deuteroporphyrin *9*, the acetyl groups being lost. However, their presence was evident when diacetyl-deuteroporphyrin was first reduced with potassium borohydride to hematoporphyrin. When the latter was reduced with HI–AcOH, the chromatogram showed the peaks of **12**, **13**, **16** together with unidentified peaks with higher r.r.t.'s, but no peaks of **2**, **3**, or **5**.

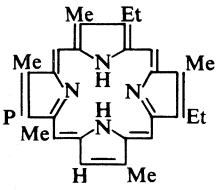
The r.r.t.'s of the alkyl pyrrole peaks from phyllochlorin except those marked **1**, **3**, **6**, and **14**, identify a nucleus substituted as in (*a*). Their yield was 49% of 2 mol as **9**, **13**, and **16**, aside from the contribution of **12**. This requires a second nucleus with peripheral substituents as in (*a*) wherein one meso-substituent might be a large alkyl group but not methyl. The remaining alkyl peaks and the ester peaks then each represent a single nucleus. The pyrrole **1** is identified unambiguously, the distinctive peak of 2-ethyl-3-methylpyrrole (r.r.t. 0.46) is absent, and alkyl groups moderately higher than ethyl would be evident after alkylation. The other set of alkyl pyrrole peaks is then either **1**, **3**, **6**, and **11** as indicated, or **1**, **2** with **3**, and **5**; these represent nuclei substituted as in (*f*) or (*c*), respectively. In principle, the two sets could be distinguished by propylation: were **5** present, it would appear among the products as the distinctive peak of 2,4,5-trimethyl-3-*n*-propylpyrrole; the data are in Table 3. The method is not yet practicable because a small peak coinciding with this one has not yet been eliminated from the propylation blank. Another way of distinguishing the two sets appears to have been found. When **11** and **12** are methylated to **18** and **16**, respectively, **11** disappears in 70 min, **12** in 25 min; **6** is equivalent to **11** here, to which it is even more rapidly methylated. The persistence of the **12**/**11** peak after methylation (1 h but not much longer) can then be attributed to **11** and **6**, and there is a nucleus substituted as in (*f*). A fourth nucleus is identified as in (*h*) by the chromatograms of the esters. As the latter represent only one nucleus, a favorable situation typical of many chlorophyll derivatives, their pattern alone would identify the meso-substituents. The following nuclei are then determined.



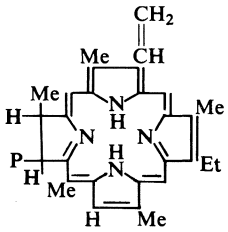
(*a*) (*f*) (*h*)

The meso-substituents on the second of these must be H, the only one allowed (see above) which has counterparts on the other three nuclei. The nuclei may be assembled in only four ways. One represents the skeleton of phyllochlorin, and the other three differ in the order of the peripheral substituents (Me and Et) on rings 1 and 2. The chromatograms from γ-phylloporphyrin *15* differed from these only in being cleaner, in having the shoulder of **31** better developed, and in the higher yields (alkyl pyrroles 70%, esters 39%).

Chromic acid oxidation will reveal the reduced ring 4 in phyllochlorin as a succinimide but it will not distinguish between 120 skeletons, *i.e.* 96



γ-Phylloporphyrin **15**



Phyllochlorin

phylloporphyrins, and 24 pyrroporphyrins which lack the meso methyl group. Not only is the meso methyl group easily identified and located by reduction, but its presence allows the structure of the porphyrin to be more completely determined, for the substituents on the two flanking nuclei can then be ordered.

From pyrophaeophorbides (see above), Scheme 1 requires the usual number of pyrroles from rings 1 and 2 but not from rings 3 and 4. One mode of fission between the latter would yield only four non-volatile derivatives of 1-(3-pyrryl)-3-(2-pyrryl)-propanone-1. The other fission would yield two pyrrole esters from ring 4, as in examples (*i*), (*j*), (*k*) (Table 2), and two ketocyclopenteno-pyrroles from ring 3. Presumably the higher r.r.t.'s of the last two would prevent their appearance with the alkyl pyrroles, and no serious attempt was made to detect them. Despite the threat of fissions about the carbonyl group, the chromatograms from the pyrophaeophorbides conform to this model except that an extra peak (r.r.t. 1.91 before alkylation) appears with the esters. As the same peak appeared whether the 5-position on ring 3 bore methyl or ethyl groups, it presumably represents ring 4. However, it has not yet been identified with any of several pyrrole – 2-propionic acids and pyrrole – 3-propionic acids made for the purpose. In the interpretation of the chromatograms, it can only be ignored in the hope that its ultimate identification will be revealing rather than confusing, *cf.* other extra peaks discussed below. It was most obvious among the peaks of the pyrrole esters from pyrophaeophorbide-*a*, least so among those from Chlorobium phaeophorbide 660 fraction 5.

We have studied only three pyrophaeophorbides, the Chlorobium phaeophorbides 660 fraction 5 and 650 fraction 4, and pyrophaeophorbide-*a*; no phaeophorbides bearing the 10-carbomethoxy group were examined. Chlorobium phaeophorbide 660 fraction 5 provides a model for the identification of the meso alkyl groups in the higher Chlorobium phaeophorbides 660, and for the confirmation of their location on the δ-positions. The structures of the lower members, fractions 5 and 6, had been determined, in part, by degrading them to δ-phylloporphyrins. This method was impracticable with the higher members because their degradations involved progressively lower yields, and some of these fractions occurred in relatively small amounts. The comparison of the results from the Chlorobium phaeophorbides 660 fraction 5 and 650 fraction 4, or from the latter and pyrophaeophorbide-*a*, were instructive because the two former differ only on the δ-position and the latter two differ significantly only on ring 3. The results from Chlorobium 650 fraction 4 and from pyrophaeophorbide-*a* suffice for these comparisons, but they are limited by the availability of the former, and by the yield of pyrroles from the latter: alkyl pyrroles 45% of 2 mol, esters 9% before methylation and 7% after.

As expected, the chromatograms from Chlorobium phaeophorbide 650 fraction 4 and from pyrophaeophorbide-*a* were the same. The 2-substituents on both were reduced to ethyl, and the pyrroles from ring 3 were not represented. The chromatograms of the alkyl pyrroles, the same as those of the alkyl pyrroles from hemin, identified one nucleus substituted as in example (*a*), but the yields were not high enough to reveal the second. It is typical of this method that no such difficulty would arise were rings 1 and 2 differently substituted as in all the higher Chlorobium phaeophorbides. Provided the anomalous peaks are ignored, the chromatograms of the esters and the r.r.t.'s of the reference pyrroles require a nucleus substituted as in (*i*), and the absence of a meso-substituent on the δ-position is revealed by the pattern alone.

The chromatograms of the alkyl pyrroles from Chlorobium phaeophorbide 660 fraction 5 (yield 75% of 2 mol) require a nucleus as in example (*a*) giving rise to the pyrroles **9**, **12**, **13**, **16**, and a second nucleus with the same peripheral substituents giving rise to **21** (as indicated) or **23**. All the cognates of **21**, *i.e.* **9**, **12**, and **17** from a nucleus as in (*e*), would be accommodated under the other peaks. All those of **23** would not, for 2,3-diethyl-4-methylpyrrole would be a distinct peak of r.r.t. 1.62. In the absence of this pyrrole, propylation confirmed **17** unambiguously (Chart 3 and the r.r.t.'s in Table 3), and thus the second

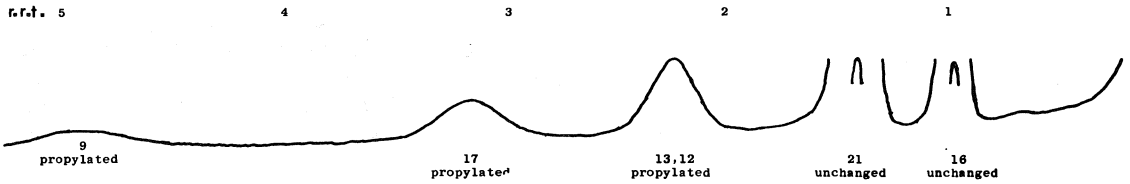


CHART 3. The g.l.p.c. of propylated alkyl pyrroles from Chlorobium phaeophorbide 660 fraction 5 at 100°.

TABLE 3. The r.r.t.'s of pyrroles*

Temperature (°C)	Substituents 5	4	3	2 H	2 Me	2 Et	2 n-C_3H_7
50	H	H	H	0.28	0.49 (43)	0.97[a]	
	H	H	Me	0.53[i] (44)	1.07 (45)		
	Me	”	”	*1.00* (5*a*)	2.21[d] (9)		
	H	Me	Et	2.74 (5*a*)	4.50[e] (46)		
	Me	”	”	5.56[b] (33)			
75	Me	H	H		0.28 (28)	0.49[c] (33)	
	Et	”	”			0.85[c,a]	
	H	H	Me	0.15[i] (44)	0.29 (45)	0.46[e] (33)	
	Me	”	”	0.28[f] (5*c*)	0.52[d] (9)	0.83[d] (46)	
	Et	”	”	0.52[f] (5*c*)	0.96[d,a]	1.46[d] (28)	
	H	H	Et	0.31[f] (47)	0.52[a]	1.10[e,a]	
	Me	”	”	0.55[d] (33)	0.92[d] (28)	1.48[d] (9)	
	Et	”	”	0.99[f] (5*c*)	1.69[d] (33)	2.50[d] (33)	
	H	Me	Me	0.32[h] (28)	0.62[e] (9)	0.98[e] (33)	
	Me	”	”		1.18[a] (5*d*)	1.86[c,a]	
	Et	”	”			2.76[b] (33)	
	H	Me	Et	0.64 (5*a*)	*1.00*[e] (46)	1.62[e] (28)	2.81[e] (36)
	Me	”	”	1.16[b] (33)	1.85[e] (46)	2.95[j] (28)	5.04[c] (33)
	Et	”	”	1.85[b] (33)	2.84[e] (46)	4.27[c] (28)	7.11[b] (33)
	H	Et	Et	1.26[h] (48)	1.96[e,a]	2.96[e,a]	
	Me	”	”		2.92[k] (5*d*)	4.60[d] (9)	
	Et	”	”			6.94[l,a]	
	H	Me	n-C_3H_7	1.21[h,a]	1.90[e] (28)	2.92[e,a]	4.98[e,a]
	Me	”	”	2.22[b,a]	3.41[b,a]	5.24[e,a]	8.77[e,a]
	Et	”	”	3.44[b,a]			
	n-C_3H_7	”	”	5.76[b,a]			
	H	Me	i-C_3H_7		1.61[e] (28)		
	Me	”	”		2.99[e] (28)		
	H	Me	n-C_4H_9		3.72[e] (28)		
	H	Me	i-C_4H_9	1.76[h,a]	2.73[e] (9)	4.14[e,a]	
	Me	”	”	3.10[b] (28)	4.86[e] (9)		
	Et	”	”	4.61[b,a]			
	H	Me	*sec*-C_4H_9		2.63[e,a]		
	Me	”	”		4.68[e,a]		
	—$CH_2CH_2CH_2$—		Me	2.00[b] (49)	3.74[b] (49)		
	—$CH_2CH_2CH_2$—		Et	3.84[b,a]	6.27[b,a]		
100	Me	Me	Et		*1.00*[e] (46)		2.35[e] (36)
	Et	”	”		1.43[e] (46)		3.15[b] (33)
	n-C_3H_7	”	”		2.26[e] (46)		4.95[e] (36)
	Me	Me	n-C_3H_7		1.68[e] (28)	2.48[e,a]	3.91[e,a]
	n-C_3H_7	”	”		3.80[b,a]	5.10[b,a]	8.00[e,a]
135	H	Me	Et		0.62[e] (46)		1.33[e] (36)
	Me	”	”	0.71[b] (33)	*1.00*[e] (46)		
	Et	”	”		1.38[e] (46)		
	n-C_3H_7	”	”	1.50[b,a]	2.00[b,a]		
	H	Me	n-C_3H_7		1.00[e] (28)	1.38[e,a]	2.00[e,a]
	Me	”	”	1.12[b,a]	1.58[e] (28)	2.12[e,a]	2.96[e,a]
	H	Me	i-C_3H_7		0.92[e] (28)		
	Me	Me	i-C_3H_7		1.38[e] (28)		
	H	Me	n-C_4H_9		1.67[e] (28)		

TABLE 3.—*Concluded*

Temperature (°C)	Substituents 5	4	3	2 H	2 Me	2 Et	2 n-C_3H_7
	H	Me	i-C_4H_9	0.96[h,a]	1.38[e] (9)	1.83[e,a]	2.59[e,a]
	Me	"	"	1.50[b] (28)	2.00[b] (28)	2.71[e,a]	3.79[e,a]
	H	Me	sec-C_4H_9		1.25[e,a]		
	Me	"	"		1.92[e,a]		
135	Me	H	P^{Me}	0.75[f] (50)			
	H	Me	P^{Me}	0.81[h] (35)	*1.00*[e] (34)	1.35[e] (36)	1.93[e,a]
	Me	"	"	1.25[b] (35)	1.52[e] (34)	2.02[e] (36)	2.83[e,a]
	Et	"	"	1.65[b] (51)	2.00[b] (51)	2.59[b] (51)	3.56[e,a]
	n-C_3H_7	"	"	2.29[b] (51)	2.73[b] (51)		
	H	Me	P	1.24[h] (35)	1.33[e] (34)		
	Me	"	"	1.86[b] (35)			

*The substituents on a pyrrole are defined by the position of its r.r.t. in the Table. In each temperature group the r.r.t.'s are relative to the R_f of the pyrrole whose r.r.t. (1.00) is in italics. A single note or reference appended to an r.r.t. refers to the preparation of the pyrrole in quantity. The first of two indicates the precursor from which the pyrrole was prepared *in situ* as described in the Experimental; the second gives the source of the precursor. (*a*) See Experimental, (*b*) from the corresponding 2-carbethoxypyrrole, (*c*) from the 3-carbethoxypyrrole, (*d*) from the 4-carbethoxypyrrole, (*e*) from the 5-carbethoxypyrrole, (*f*) from the 2,4-dicarbethoxypyrrole, (*g*) from 2,4-dimethyl-3-carbethoxypyrrole, (*h*) from the 2-carboxy-5-carbethoxypyrrole, (*i*) from 2-carbethoxy-3-methyl-4-bromo-5-carboxypyrrole, (*j*) from 2,3-dimethyl-5-carbethoxypyrrole, (*k*) from 2,5-dimethyl-3-carbethoxypyrrole, (*l*) from 2,5-diethyl-3-carbethoxypyrrole. P = CH_2CH_2COOH, P^{Me} = CH_2CH_2COOMe.

nucleus is as in (*e*). Mass spectra across the **17**/**16** peak gave no indication that it was even partially resolved. The anomalous peak is much reduced in the chromatograms of the esters here. These (yield 25%), with the r.r.t.'s of the reference pyrroles, identify a third nucleus as in (*j*); the pattern alone would indicate the meso-substituents. The pyrrole ester **33** was further identified under its g.l.p.c. peak by its mass spectrum, in which the mass peaks were those calculated and in the ratio found with the reference pyrrole: M = 195, $M - CH_3$, $M - CH_2COOMe$, $M - CH_2$-CH_2COOMe. As the **33** peak was undiminished when the HI–AcOH used had previously been heated with cryptopyrrole, it did not arise through scavenging (see under Reference Pyrroles below).

In connection with these chromatograms, it should be noted that propylation also confirmed **16** and **21** as tetraalkyl pyrroles by a method which, unlike methylation or ethylation, could not produce them from lower homologues. Further, if the analytical problem is only that of the meso alkyl group, the identification of **16** excludes its presence on the α-position. Finally, the δ-methyl group here is even more favorably situated than the γ-methyl group in phyllochlorin, for the derived pyrrole esters are better separated by g.l.p.c. Presumably there should be no difficulty in identifying and locating the meso alkyl groups in small amounts of the higher Chlorobium phaeophorbides 660.

Analytically, the isocyclic ring in chlorophylls has been identified only indirectly, and its presence prevents the identification of ring 3 by chromic acid oxidation. The indirect methods, except successive degradations, may be capriciously invalidated by other structural features. For example, the characteristic visible spectrum associated with the isocyclic ring in phylloerythrin is shared by cyclic lactones, but is not evident in phylloerythrin derivatives if the carbonyl group is reduced or if there is a δ-methyl substituent. *A priori*, the identification of the isocyclic ring and ring 3 as a cyclopentenopyrrole, after HI–AcOH reduction, appeared more promising with desoxo-phylloerythrin than with pyrophaeophorbides wherein the isocyclic ring might be labilized by the keto group. As other analytical methods take advantage of the keto group in one way or another, the reduction of desoxo-phylloerythrin was also interesting because its analogues among the petroporphyrins involve the same analytical problem.

The chromatograms of the alkyl pyrroles from desoxo-phylloerythrin showed the peak of **39** before (Chart 4 and the r.r.t.'s of the reference pyrroles in Table 3) and after methylation, as well as the usual alkyl pyrroles from rings 1 and 2. To make the pyrophaeophorbides amenable to this method, conveniently and on the same scale, their keto groups were first reduced with potassium borohydride to carbinole groups (27) which would be reduced to methylene by HI–AcOH, *cf.* the reduction of 9-hydroxy-desoxo-phyllo-

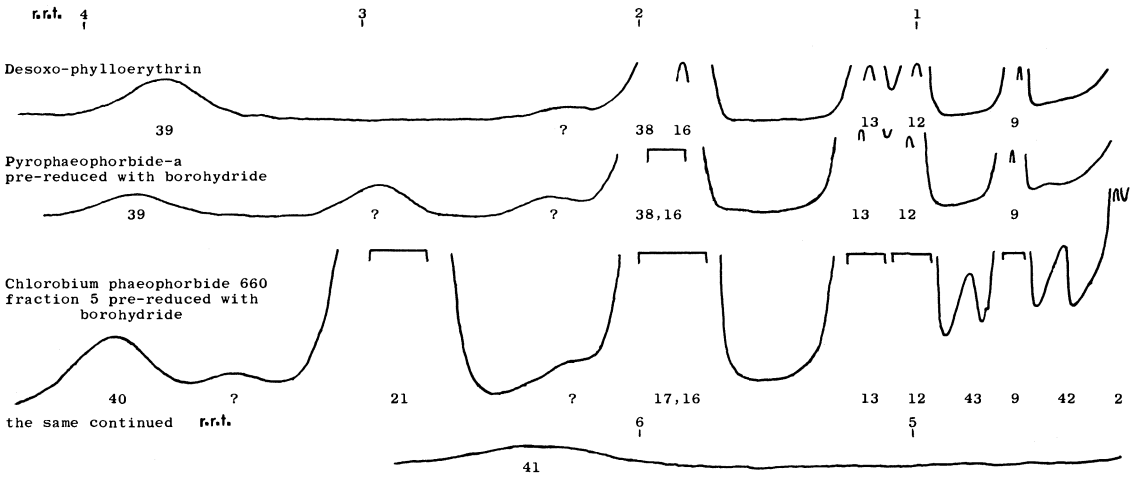
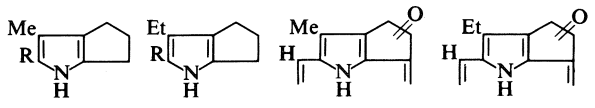


CHART 4. The g.l.p.c. of cyclopenteno-pyrroles and alkyl pyrroles from desoxo-phylloerythrin and from pre-reduced pyrophaeophorbides at 75°.

erythrin (*5i*). The chromatograms of the alkyl pyrroles from the modified pyrophaeophorbide-*a* then showed the peak of **39** before and after methylation. Those from the modified Chlorobium phaeophorbide 660 fraction 5 showed the peaks of **40** and **41** before methylation, and **41** after; this distinguishes **40** from **39**. Two of these pyrroles were also identified under their g.l.p.c. peaks by their mass spectra, in which the mass peaks were those calculated and in the ratios found with the reference pyrroles: **39** from desoxo-phylloerythrin ($M = 135$, $M-1$, $M-15$); **40** from the phaeophorbide 660 ($M = 135$, $M-1$, $M-15$, $M-29$). When the alkyl pyrroles from both the modified and unmodified Chlorobium phaeophorbide 660 fraction 5 were chromatographed at 135°, minimal amounts of **40** and **41** were also apparent in the latter, about 1/40 of the amounts in the former, and not enough to be detected in chromatograms run at 75°. In view of the contrasting results before and after borohydride reduction, but ignoring the possibility of other labile groups on the isocyclic rings, the nucleus (1) is determined in pyrophaeophorbide-*a* and (*m*) in Chlorobium phaeophorbide 660 fraction 5.

The remaining peaks (Chart 4), like the anomalous peak among the esters, differ from all others previously discussed. Although definitely located, they are not always present, and their absence may then have no significance. Those identified below arise through side-reactions not contemplated in Scheme 1, without firm precedents, and apparently dependent on structural factors remote from the groups involved. Further, they represent by-products and thus a second set of pyrroles from one nucleus.

The r.r.t.'s of three peaks in the chromatogram from Chlorobium phaeophorbide 660 fraction 5 (Chart 4 and Table 3) identify the pyrroles **43**,

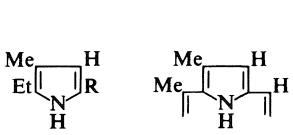


38 R = H
39 R = Me

40 R = H
41 R = Me

(*l*)

(*m*)



42 R = H
43 R = Me

(*n*)

(*o*)

42, and thus **2** rather than **3**. A second reduction showed the peak of **42** better developed but neither **43** nor **2**; a third confirmed these as **14** after methylation. If the pigment was not first reduced with potassium borohydride, these peaks were barely noticeable or absent. The three pyrroles require the nucleus (*n*), and this must result from the partial loss of a labile C_2 group (the α-hydroxy-ethyl group on ring 1) from a precursor of (*e*). If two carbons of the isocyclic ring were lost, pyrroles isomeric with the above would be formed from ring 3 but their r.r.t.'s would be different. The identification of (*n*) through a set of peaks here is more convincing than it would be through a single peak, *e.g.* of a maleinimide.

The behavior of pyrophaeophorbide-*a* suggests that vinyl groups may also be partially lost. About half the chromatograms from this pigment, whether or not it was prereduced with borohydride, showed very small but sometimes sharp peaks of **5** and **2** or **3**. These might arise from ring 1 if the vinyl group were lost, or from ring 3 if two carbons of the isocyclic ring were lost; the latter is unlikely, particularly in view of the behavior of Chlorobium phaeophorbide 660 fraction 5.

Presumably vinyl groups and α-hydroxy-ethyl groups would behave similarly here in the strong acid and, in contrast to acetyl groups (*cf.* diacetyldeuteroporphyrin *9*), they are both largely reduced to ethyl if not completely so (*cf.* hemin). More evidence is required, but it seems that these side-chains will be identified but not distinguished by their partial loss, provided that other structural factors are favorable. If this be so, the nuclei in Chlorobium phaeophorbide 660 fraction 5 identified as (*e*) and (*n*) are derived from (*o*); the other nuclei present were defined as (*a*), (*m*), and (*j*). The assembly of these yields the skeleton of the pigment except for the order of the substituents on ring 2. It also identifies a modified ethyl group on ring 1 and a carbonyl group in the isocyclic ring (see Fig. 1).

Aside from the question of the meso-substituent in Chlorobium chlorophyll 660 fraction 5, purely analytical methods had not ordered the substituents on rings 1, 2, or 4, and the evidence for the isocyclic ring was more indirect than usual. However, although the reductive method would have shown to its best advantage here, it would not have solved the essential analytical problem: the unspecified order of the substituents on ring

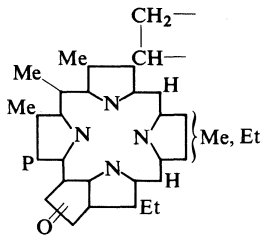


FIG. 1. The skeleton of Chlorobium phaeophorbide 660 fraction 5 derived by reductive degradations.

2 would have necessitated the degradation of the pigment to a phylloporphyrin or, the meso-substitution being known, a pyrroporphyrin for comparison with synthetic material.

These reductions suggest an alternative to a second component, in addition to porphobilinogen, in some studies of the biosynthesis of porphyrins. The abundance of the parent ions in the mass spectra of the pyrroles from a pigment would show which, if either, of the nuclei adjacent to a bridge was derived from the porphobilinogen contributing that bridge. This would require that the pigment was formed from a mixture of natural and doubly labelled porphobilinogen (*e.g.* from δ-aminolevulinic acid-5-^{13}C), and that the pyrroles were sufficiently distinguished by their β-substituents.

The Reference Pyrroles and Their Relative Retention Times

Table 3 gives the r.r.t.'s of most of the pyrroles expected from the chlorophylls, of some isomers from which these pyrroles should be distinguished, and of all the *C*-methyl, *C*-ethyl, and *C*-methyl-ethyl pyrroles. The pyrrole – propionic acids were chromatographed as their methyl esters; a few of the free acids were run for comparison but they had longer retention times and their peaks showed tailing. The pyrrole esters from uroporphyrin, and higher alkyl pyrroles such as the 2,3- and 3,5-dimethyl-4-*n*-octadecylpyrroles can be chromatographed at 180–210° but are not reported here.

We had made few alkyl pyrroles on a preparative scale, and these had usually been stored as their more stable carbethoxy derivatives. Consequently, with few exceptions, the alkyl pyrroles were prepared from carbethoxy derivatives (1 mg) by decarboxylating the latter in HI–AcOH at 110°. If the product was to be alkylated, an aldehyde was added and the mixture was heated again

before being worked-up for chromatography. The same procedure was used to reduce the porphyrins and chromatograph their pyrroles. It should be noted that β-alkyl pyrroles may be obtained from their tricarboxylic acid-diesters in HI–AcOH at the dilution used here. We had first decarboxylated the latter to dicarboxylic esters in the belief that tricarboxylic acids would not be decarboxylated (see Table 3).

The general reliability of decarboxylations in HI–AcOH is implicit in much of Fischer's work, *cf.* (*5b*), and the alkylations were restricted to types which had offered no difficulty (28). However, the usual controls on the identities of the products were impracticable here and it was assumed that the chromatograms would reveal any anomalous products. In some cases the identities of the pyrroles were confirmed chromatographically by comparison with bulk products, or with the same pyrroles from different precursors; all the important pyrroles were also obtained by reducing porphyrins.

The only apparent failures obviously arose from the instability of the pyrroles in HI–AcOH. Pyrrole itself is decomposed in HI–AcOH, and its 2,5-dialkyl derivatives are not sufficiently stable in the acid at temperatures above 90°; in neither case do the decomposition products appear on the chromatograms. In other cases only the side-chains are degraded. As *t*-butyl groups are easily split off from aromatic nuclei (29, 30), it was not surprising that HI–AcOH converted 2,4-dimethyl-3-*t*-butyl-5-carbethoxypyrrole into 2,4-dimethylpyrrole. Neither *i*-propyl nor *sec*-butyl groups were lost from analogous pyrroles, but the *sec*-butyl group was degraded to ethyl etc. though to a negligible extent. As noted above, none of these unstable pyrroles would be expected from natural porphyrins.

In about 75% of the decarboxylations only one pyrrole appeared on the chromatogram and its r.r.t. was consistent with those of its homologues. In the remaining decarboxylations, which usually involved 3- or 4-carbethoxypyrroles, there were in addition one or two unidentified peaks which usually represented <5% of the product. However, a second peak representing 20% of the product accompanied that of 2-ethyl-5-methylpyrrole.

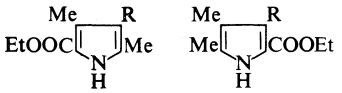
When precautions were taken (see Experimental), neither methylation nor ethylation introduced further complications but extra peaks were often noted after propylations, and these also appeared in the blank.

The purification of the ether used eliminated some extra peaks. Others arose during the esterification of the pyrrole – propionic acids with diazomethane. Some of the latter were eliminated when the diazomethane was prepared from *N*-nitroso-*p*-toluenesulfonylmethylamide rather than from *N*-nitroso-methylurea, *cf.* (31), but two further peaks of r.r.t. 2.0 and 2.2 were only eliminated when the ethereal diazomethane was purified by washing it with concentrated aqueous ammonia; ammonia does not interfere with the esterification, *cf.* (32). Other extra peaks were finally traced to the *C*-methylation of the pyrrole – propionic acids (but not their esters) by ethereal diazomethane when catalyzed by traces of water or, more effectively, the solution from which the acids had been extracted. This was most obvious when the peak of methyl phyllopyrrole-carboxylate accompanied the expected peak of methyl cryptopyrrole-carboxylate. This *C*-methylation was prevented by drying the ether solution of the acids with $MgSO_4$, an excess of which lowered the yield. The esterifications were also slower in the absence of water. A possible source of extra peaks was eliminated when it was found that diazomethane would not bring about the base catalyzed trans-esterification of the pyrrole methyl esters in the presence of ethanol or ethyl acetate.

Although we had anticipated the alkylation of pyrroles in HI–AcOH through scavenging, it was demonstrated in only one case. When crystalline opsopyrrole-carboxylic acid was esterified by diazomethane in dry ether, the chromatogram showed only the one expected peak of **28**. If it was esterified in wet ether, a small peak of the *C*-methyl derivative **30** was also evident. If it was first heated in HI–AcOH, a third and smaller peak corresponding to **33** appeared in heavily loaded chromatograms; this peak did not appear if the HI–AcOH used had first been heated with cryptopyrrole. Evidently this alkylation is both insignificant and easily eliminated.

Some of the carbethoxypyrroles were obtained in roundabout ways merely because the materials were at hand. Aside from this, the more important ones were obtained as follows, *cf.* (33).

The Knorr synthesis gave **44** (R = COOEt, $COCH_3$, or CH_2CH_2COOEt (34)), thence successively **44** (R = COOEt → COOH → H →



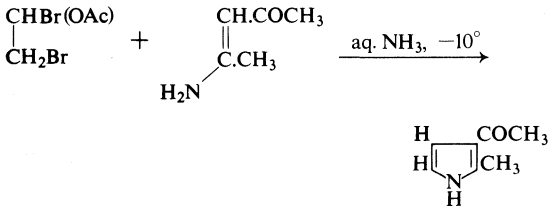
44 **45**

alkyl), but **44**, (R = $COCH_3$ → Et). If higher acylacetates or diethyl β-ketoadipate were used in the ring synthesis, the methyl groups were replaced by others.

The pyrroles **45** (R = H or Et) were obtained via the Hantzsch synthesis, thence **45** (R = H → alkyl) (33). If a higher acylacetate was used in the ring synthesis, the α-methyl group was replaced by another.

The α-methyl groups in **44** and **45** were converted successively to COOH, halogen or H (35), and alkyl; alternatively to CH_2Br then CH_2R (36). Pyrroles with higher α-alkyl groups were thus obtained without the use of higher acylacetates in the ring synthesis.

The only unconventional pyrrole ring synthesis used represents a further extension of the Hantzsch synthesis wherein acetylacetone (as its imine) replaces the acylacetate



A list of Hantzsch syntheses given earlier (33) did not include that of 2-methyl-3-cyanopyrrole from diacetonitrile (37) or that of 2-(α-pyridyl)-3-carbethoxypyrrole from α-pyridoylacetic ester (38). A third such synthesis (39) had given a low-melting 2,5-dimethyl-4-ethyl-3-carbethoxypyrrole, *cf.* (28), from questionable 3-chloropentanone-2 (40). We regret that our demonstration (33) that the 2,3-dimethyl-5-carbethoxypyrrole of Fischer and Fink (41) was contaminated with the 4-methyl derivative had been anticipated (42).

Experimental

The ether used was reagent grade anhydrous ether, washed twice with acidified aqueous ferrous sulfate, three times with water, then stirred 15 min with anhydrous $MgSO_4$; it was then distilled and the center 60% was stored < 10 days in the dark at 0°.

Diazomethane: distilled ethereal diazomethane, from *N*-nitroso-*p*-toluenesulfonylmethylamide using Carbitol (52), was stirred magnetically at 0° with 1/2 volume of concentrated aqueous ammonia for 20 min. The ether layer was stored at 0° in the dark over KOH (1 pellet/2 ml) for less than 1 week, but > 24 h if it was essential to avoid *C*-alkylation. Solutions < *ca.* 0.5 *M* gave lower yields of esters.

The paraformaldehyde gave no trouble but some lots of paraldehyde were rejected because they gave peaks in the blank; no specimens of propionaldehyde or parapropionaldehyde were consistently satisfactory in this respect. Hemin (Eastman) was converted into deuterohemin, thence into deuteroporphyrin, or into diacetyldeuterohemin and diacetyl-deuteroporphyrin (5). Etioporphyrin *I* (51), 1,3,5,7-tetramethyl-2,6-diethyl-4,8-di-*n*-propylporphin (51), desoxo-phylloerythrin (51), methyl 1,4,5,8-tetramethylporphin-2,3,6,7-tetracarboxylate (53), and coproporphyrin *3* (35) had been made previously. Pyrophaeophorbide-*a*, phylloporphyrin (54), and phyllochlorin (55) were from H. Fischer's collection, the Chlorobium phaeophorbides (56, 57) were from A. S. Holt's.

The Borohydride Reduction of Chlorobium Phaeophorbide 660 Fraction 5

The pigment (1 mg) was dissolved in 2 drops of pyridine. Methanol (50 ml) was added then potassium borohydride (100 mg) in methanol (1 ml) was dropped in. The solution was stirred for 1 h then shaken with ether and 4% HCl. The pigment in the acid layer was brought into fresh ether. This was washed with water, dried ($MgSO_4$), and evaporated.

The Reduction of Porphyrins or the Decarboxylation of Carbethoxypyrroles

As the chromatograph was not programmed, separate reductions were carried out to furnish the alkyl pyrroles and the pyrrole esters from the pigments. Here and in subsequent steps the specified heating times and temperatures must not be exceeded, and the pyrroles should be protected as far as possible from light; otherwise the yields suffer.

Acetic acid (Anachemia reagent, 1.0 ml) then hydriodic acid (AnalaR 66%, stored at 0° but not decolorized, 0.5 ml) were added to the pigment (1 mg, higher concentrations lower the yield) or the carbethoxypyrrole (1 mg) in a 1.5 ml flask fitted with a stirring bar and a reflux condenser. The flask was then placed in a pre-heated oil-bath and stirred magnetically. Carbethoxy derivatives of the sensitive 2,5-dialkylpyrroles were heated 45 min at 90°; pyrophaeophorbides, desoxo-phylloerythrin, and diacetyl-deuteroporphyrin were heated 3 h at 105–110°; all other compounds 1.5 h at 105–110°.

The Alkylation of the Products

If the products were to be alkylated, 3 mg of paraformaldehyde or 0.01 ml of paraldehyde (or other aldehyde) were added to the cooled mixture; if paraformaldehyde was added before the pigments were reduced, extra peaks appeared in the chromatograms. The mixture was then heated again at 105–110° for 30 min or, when β-free pyrroles present were difficult to alkylate, 1 h. However, if methylated pyrrole esters were to be chromatographed, the mixture was heated for 30 min at 50°; a higher temperature resulted in lower yields and sometimes in an extra peak (r.r.t. 2.25) in the chromatograms.

The Extraction of the Alkyl Pyrroles

This procedure was the same whether or not the pyrroles were alkylated and whether alkyl pyrroles or esters were to be chromatographed.

The solution was decolorized with the minimum amount of phosphonium iodide (a few mg) (9), starting the reaction by returning the flask to the oil-bath for a few seconds. The solution was then colorless, very pale orange, or green (hemins); the turbidity, if any, was very slight.

The flask was fitted with a reflux condenser modified to trap the distillate, cooled, and placed in a 20° oil-bath. The solvent was removed at 5–10 mm with magnetic stirring as the bath was raised to 65–70° over 30 min. Only a film or a very little oil should remain on the walls of the flask or the yield will be lowered. When the products had been propylated, some extra peaks were minimized when the residue at this stage was kept 1 h at 65° and 0.05 mm. The flask was cooled in ice, and NaOH (1 ml of 10%) was added. The alkyl pyrroles were then extracted with 3 × 1 ml of ether at p*H* > 10.

If the alkyl pyrroles were to be chromatographed, the ether extracts were placed in a chilled tube and centrifuged (5 min), transferred to a 10 ml conical flask, and swirled at intervals over 15 min with anhydrous $MgSO_4$ (40 mg). The ether was transferred to a pear-shaped flask and removed at room temperature under a partial vacuum; unless care is taken, the more volatile pyrroles may be lost here. The residue was dissolved in ether: 1.0 ml if the product was derived from a carbethoxypyrrole, 0.10 ml if from a pyrophaeophorbide or desoxophylloerythrin, 0.25 ml if from another porphyrin and the products had been ethylated, 0.5 ml in all other cases. In every case, 2 μl of the ether solution was applied to the column.

The Preparation of the Pyrrole Esters

If the pyrrole esters were to be chromatographed, the flask containing the alkaline aqueous layer was placed in an ice-bath and stirred magnetically. Phosphoric acid (*ca.* 1 ml of 1 vol 85%:4 vol of water) was added until the mixture just began to turn congo red to black, and the pyrrole acids were extracted with 3 × 1 ml ether at p*H* 4.0. The extract was centrifuged for 10 min then transferred to a 10 ml conical flask.

When it was not essential to minimize *C*-methylation, 1 ml of ethereal diazomethane was added to the extract which was then kept in the dark for 45 min. To minimize *C*-methylation, the extract was stirred for 15 min in the dark with 40 mg of anhydrous $MgSO_4$, transferred to another flask, and esterified as above. In either case, the evaporation of the ether solution followed that of the alkyl pyrroles. The residue was dissolved in ether: 0.10 ml if the esters were derived from a pyrophaeophorbide or desoxo-phylloerythrin, or if the pyrroles had been ethylated; otherwise 0.5 ml. In all cases 5 μl were applied to the column.

Chromatography

A Pye Argon Chromatograph was used with glass columns packed with 0.5 × 117 cm of Chromosorb G (acid washed and treated with DMCS) with 5% SE-30. Unlike other columns (45), these do not separate the 2- and 3-methylpyrroles. Low-temperature column: 50 ± 0.1°, flow rate 171 ml/min, *ca.* 10.5 p.s.i., R_t of the 2,4-dimethylpyrrole standard 6.8 min; 75°, 171 ml/min, *ca.* 12.1 p.s.i., R_t of the cryptopyrrole standard 8.0. High-temperature column: 100°, 158 ml/min, *ca.* 16 p.s.i., R_t of the phyllopyrrole standard 5.4; 135°, 80 ml/min, *ca.* 10.1 p.s.i., R_t of the phyllopyrrole standard for alkyl pyrroles 2.4, R_t of the methyl cryptopyrrole-carboxylate standard for pyrrole esters 11.0. The sample was injected quickly through the open top of the column and as close to the packing as possible while the gas flow was interrupted; sophisticated methods gave poorer results. The R_t's were measured from the start of the ether peak. Yields were calculated from the areas under the peaks, assuming that the response of the detector was molar; bulk samples of cryptopyrrole and of methyl cryptopyrrole-carboxylate were used in calibrating for alkyl pyrroles and pyrrole esters, respectively, immediately before every determination of a yield.

2-Methyl-3-acetylpyrrole

Crude dibromovinyl acetate (from 43 g of vinyl acetate and 27.5 ml of bromine (43)) and 50 g of acetylacetoneimine were stirred into 400 ml of 28% aqueous ammonia at −10°. Stirring was continued for 18 h while the temperature rose slowly to 20°. The mixture was heated to 90°, cooled, and evaporated (rotary, 25 mm, 50° bath). The residue was mixed with 100 ml of water and made acid to congo red with hydrochloric acid then extracted with ether (4 × 200 ml). The extract was evaporated, the residue distilled (to 135°, 0.01 mm), and the distillate was crystallized from 100 ml of water (Darco) giving 6.1 g of colorless prisms, m.p. 95.5–96.5°. For analysis it was recrystallized from pentane (thimble) as colorless prismatic rods, m.p. 94.5–95.5° (lit. (58) 94–95°).

Anal. Calcd. for C_7H_9ON: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.43; H, 7.48; N, 11.40.

To confirm its structure, it was methylated in HI–AcOH–paraformaldehyde to 2,4,5-trimethyl-3-acetylpyrrole (9), identified by m.p. and mixed m.p.

2-Methyl-3-ethylpyrrole

2-Methyl-3-acetylpyrrole (0.03 mol) was reduced with $LiAlH_4$ by the method given for 3-carbethoxy-pyrroles (33). The product was distilled (65°, 15 mm) as a colorless oil (78%).

Anal. Calcd. for $C_7H_{11}N$: C, 77.01; H, 10.16; N, 12.83. Found: C, 77.18; H, 10.32; N, 12.98.

Ethyl α-Oximinopropionylacetate

The crude oil (59) crystallized at 0° overnight. It was dried on tile, sublimed (60°, 1 × 10^{-4}), crystallized from pentane (thimble), and resublimed. Yield 7 g, m.p. 60–63° (lit. (49) 62°).

Anal. Calcd. for $C_7H_{11}O_4N$: C, 48.55; H, 6.40; N, 8.09. Found: C, 48.21; H, 6.58; N, 8.27.

2-Carbethoxy-3-ethyl-4,5-cyclopentenopyrrole

This was obtained like the 3-methyl analogue (49) using 6 g of ethyl α-oximinopropionylacetate, 10 g of cyclopentanone, 40 ml of AcOH, and 12 g of zinc dust. After the supernatant was diluted with 400 ml of water, the crude product separated at 0°. It was dried on tile, sublimed (85°, 1 × 10^{-4} mm), and crystallized thrice from absolute ethanol as colorless plates (138 mg), m.p. 113–115°.

Anal. Calcd. for $C_{12}H_{17}O_2N$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.51; H, 8.43; N, 6.87.

2-n-Propyl-4-methyl-5-carbethoxypyrrole

2-*n*-Propyl-4-methyl-3,5-dicarbethoxypyrrole was obtained by the Knorr synthesis from 11 g of crystalline ethyl α-oximinoacetoacetate, 11 g of ethyl *n*-butyrylacetate, AcOH, and zinc dust at 65°. It formed colorless needles (38%), m.p. 115–118°, from ethanol. This ester (2 g) was converted into the 3-carboxylic acid in 20 ml concentrated H_2SO_4 at 40° for 35 min (*cf.* 2,4-dimethyl-5-carbethoxypyrrole (5*e*)). The acid was decarboxylated in refluxing ethanolamine (15 ml) for 40 min and the mixture was poured into ice-water. The product was extracted into ether, the extract was washed with water and the ether was evaporated. The residue was sublimed as colorless needles (44%), m.p. 55–57°. It was analyzed after conversion to the 3-*n*-propyl derivative (see below).

2,4-Di-n-propyl-5-carbethoxypyrrole

2,4-Di-*n*-propyl-3,5-dicarbethoxypyrrole (5*c*) was converted to the 3-carboxylic acid in concentrated H_2SO_4 as usual. The acid was decarboxylated in refluxing ethanolamine, and the product was precipitated by water. After sublimation it melted at 47–50°; yield from the diester 58%. It was analyzed as the 4-methyl derivative (see below).

2,3-Dimethyl-5-ethyl-4-carbethoxypyrrole

The Knorr synthesis was carried out with 2,3-butanedione monoxime, ethyl propionylacetate, and zinc dust in AcOH at 65°. The product was precipitated by water and crystallized from ethanol as needles, m.p. 102–103.5° (lit. (33) 99–101°).

2-Ethyl-5-acetyl-3-carbethoxypyrrole

Acetyl chloride (1.2 ml) in 2 ml of CS_2 was slowly added to a stirred mixture of 2-ethyl-3-carbethoxypyrrole (1 g (33)), powdered anhydrous $AlCl_3$ (0.8 g), and CS_2 (20 ml) at 0°. The mixture was then stirred for 4 h at 20° then poured into ice-water. This was extracted with ether and the extract was washed with 5% aqueous NaOH and with water. The extract was evaporated and the residual product was sublimed (105°, 1×10^{-4} mm), m.p. 119–122°; yield 70%.

2,5-Diethyl-3-carbethoxypyrrole

The corresponding 5-acetylpyrrole (above) was hydrogenated in ethanol over Raney nickel (120°, 1700 p.s.i., 4 h). The product was distilled as an oil (140–150°, 15 mm). The structure was confirmed by reductive alkylation to 2,5-diethyl-4-methyl-3-carbethoxypyrrole, m.p. and mixed m.p. 101–103° (lit. (28) 101–103°).

2-Ethylpyrrole and 2-Ethyl-5-carbethoxypyrrole

2-Ethyl-3-carbethoxypyrrole (33) was heated with 10% aqueous NaOH at 170° for 20 h in a Teflon lined screw-capped metal vessel. The resulting 2-ethylpyrrole (5*a*) was an oil (79%), b.p. 12 mm 59–61°. Using phosgene, just as with 2-methylpyrrole (43), this was converted to 2-ethyl-5-carbethoxypyrrole (70%), b.p. 15 mm 130°, m.p. 50–51° (lit. (5*e*) 48°).

Anal. Calcd. for $C_9H_{13}O_2N$: C, 64.65, H, 7.84; N, 8.38. Found: C, 64.60; H, 7.77; N, 8.38.

2-Ethyl-3-acetyl-5-carbethoxypyrrole

A solution of 2-ethyl-5-carbethoxypyrrole (2 g) and anhydrous stannous chloride (2 g) in 10 ml of acetic anhydride was heated to the boiling point, filtered hot, cooled, and mixed with excess 5% aqueous NaOH. The precipitate was distilled (120°, 1×10^{-4} mm), yield 47%, m.p. 100–101°.

2,3-Diethyl-5-carbethoxypyrrole

The corresponding 3-acetylpyrrole (above) was hydrogenated in ethanol over Raney nickel (170°, 1800 p.s.i., 6 h) and the product was distilled (60°, 1×10^{-4} mm); yield 53%, m.p. 60–62°.

Anal. Calcd. for $C_{11}H_{17}O_2N$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.83; H, 8.94; N, 7.33.

2-Carboxy-3-n-propyl-4-methyl-5-carbethoxypyrrole

The corresponding 2-methyl pyrrole (28) in dry ether was treated with sulfuryl chloride (3.3 mol) and the solution was then refluxed 1 h. The ether was evaporated at 20°, more ether was added and then evaporated, and hot aqueous sodium acetate was added to the residue. The mixture was boiled 10 min, cooled, and extracted with ether. The product was extracted from the ether into aqueous Na_2CO_3, and precipitated from the latter with acid. It formed needles (35%) from ethanol, m.p. 208–210°.

Anal. Calcd. for $C_{12}H_{17}O_4N$: C, 60.24; H, 7.16; N, 5.85. Found: C, 59.97; H, 7.04; N, 5.72.

2-Carboxy-3-i-butyl-4-methyl-5-carbethoxypyrrole

The corresponding 2-methyl pyrrole (9) was treated with sulfuryl chloride like the 3-*n*-propyl pyrrole above. The product was sublimed (130°, 1×10^{-4} mm) and crystallized twice from aqueous ethanol; yield 12%, m.p. 198–199°.

Anal. Calcd. for $C_{13}H_{19}O_4N$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.82; H, 7.44; N, 5.42.

2,4-Dimethyl-3-t-butyl-5-carbethoxypyrrole

A solution of 0.5 g of isobutylene in 0.5 g of concentrated H_2SO_4 was added to a stirred solution of 2,4-dimethyl-5-carbethoxypyrrole in 5 ml of acetic acid at 75° over 1/2 h. After a further 2 h at 75°, the mixture was poured into ice-water which was then brought to p*H* 8 with aqueous NaOH. The precipitated product was sublimed. The cubic crystals in the sublimate were separated manually and recrystallized twice from aqueous methanol, m.p. 108–110°, yield 6%.

Anal. Calcd. for $C_{13}H_{21}O_2N$: C, 69.92; H, 9.48; N, 6.27. Found: C, 69.73; H, 9.31; N, 6.45.

2-Carbethoxy-3-n-propyl-4,5-dimethylpyrrole

Hydriodic acid (*d* 1.95, 10 ml) was slowly added to 10 ml of stirred and cooled acetic acid; hypophosphorous acid (50%, 2 ml) was then added. 2-Carbethoxy-4,5-dimethylpyrrole (0.004 mol (28)) was added to the mixture at room temperature followed by propionaldehyde (0.008 mol). The solution was stirred 2½ h at *ca.* 25° then poured into 200 ml of water. This was made alkaline with ammonia, extracted with ether, and the ether was evaporated to leave the crude product. This was an oil which solidified after distillation (1×10^{-4} mm). It was recrystallized from aqueous ethanol then from ethanol, m.p. 101–104° (lit. (5*e*) 102°), yield 40%.

Anal. Calcd. for $C_{12}H_{19}O_2N$: C, 68.86; H, 9.15; N, 6.69. Found: C, 68.79; H, 9.02; N, 6.54.

2-Carbethoxy-3-n-propyl-4-methyl-5-ethylpyrrole

The crude product, obtained as above but using 2-carbethoxy-4-methyl-5-ethylpyrrole (33), was an oil which largely solidified after distillation (75°, 1×10^{-4} mm). It was dried on tile then crystallized from ethanol as long colorless needles (19%), m.p. 73–74°.

Anal. Calcd. for $C_{13}H_{21}O_2N$: C, 69.91; H, 9.48; N, 6.27. Found: C, 69.77; H, 9.32; N, 6.42.

2-Carbethoxy-3-i-butyl-4-methyl-5-ethylpyrrole

The crude product, obtained as above but using 2-carbethoxy-4-methyl-5-ethylpyrrole and isobutyraldehyde, solidified at 0° after distillation (70°, 1×10^{-4} mm). It was recrystallized twice from methanol, m.p. 53–55° (12%).

Anal. Calcd. for $C_{14}H_{23}O_2N$: C, 70.85; H, 9.77; N, 5.90. Found: C, 70.83; H, 9.86; N, 6.06.

2-Carbethoxy-3-ethyl-4-methyl-5-n-propylpyrrole

The crude product, obtained as above but using 2-carbethoxy-4-methyl-5-*n*-propylpyrrole (33) and paraldehyde, was crystallized from aqueous ethanol, sublimed, recrystallized and resublimed, m.p. 58–59.5°.

Anal. Calcd. for $C_{13}H_{21}O_2N$: C, 69.92; H, 9.48; N, 6.27. Found: C, 69.75; H, 9.50; N, 6.45.

2-n-Propyl-3-(2-carbethoxy-ethyl)-4-methyl-5-carbethoxypyrrole

3-(2-Carbethoxy-ethyl)-4-methyl-5-carbethoxypyrrole (0.4 g (35)), 5 ml of hydriodic acid, 5 ml of acetic acid, 1 ml of hypophosphorous acid and 0.23 ml of propionaldehyde were reacted as above. The solution was poured into 60 ml of water which was then brought to p*H* 5 with ammonia. The precipitate (of the partially hydrolyzed product) was esterified in 5 ml of 5% ethanolic HCl. The solvent was evaporated and the residue was distilled then recrystallized from pentane (0.1 g), m.p. 61.5–63.5° (lit. (36) 63–64°).

Anal. Calcd. for $C_{16}H_{25}O_4N$: C, 65.08; H, 8.53; N, 4.74. Found: C, 65.19; H, 8.40; N, 4.90.

2-Methyl-3,4-diethyl-5-carbethoxypyrrole

Acetic anhydride (20 ml) was stirred into 5 ml of concentrated hydrochloric acid with cooling. 2-Methyl-4-ethyl-5-carbethoxypyrrole ((5*e*), 0.004 mol), paraldehyde (0.008 mol), and zinc amalgam (20 mesh, 10 g) were added at 25° and the mixture was stirred 1/2 h. The solution was decanted into 200 ml of water, this was made alkaline with ammonia and the crude product was filtered off. It was purified by sublimation, m.p. 76–77° (lit. (5*e*) 75°). Yield 20%.

Anal. Calcd. for $C_{12}H_{19}O_2N$: C, 68.86; H, 8.96; N, 6.67. Found: C, 68.83; H, 9.15; N, 6.87.

2-Ethyl-3-n-propyl-4-methyl-5-carbethoxypyrrole

The crude product, obtained as above but using 2-ethyl-4-methyl-5-carbethoxypyrrole (5*e*) and propionaldehyde, was sublimed (80°, 1×10^{-4} mm), m.p. 86–87° (50%).

Anal. Calcd. for $C_{13}H_{21}O_2N$: C, 69.92; H, 9.48; N, 6.27. Found: C, 70.14; H, 9.31; N, 6.48.

2-Ethyl-3-i-butyl-4-methyl-5-carbethoxypyrrole

The crude product, obtained as above but using 2-ethyl-4-methyl-5-carbethoxypyrrole and isobutyraldehyde, was sublimed (80°, 1×10^{-4} mm) and recrystallized from aqueous ethanol, m.p. 94–96° (44%).

Anal. Calcd. for $C_{14}H_{23}O_2N$: C, 70.85; H, 9.77; N, 5.90. Found: C, 70.95; H, 9.59; N, 6.07.

2,4-Di-n-propyl-3-methyl-5-carbethoxypyrrole

The crude product, obtained as above but using 2,4-di-*n*-propyl-5-carbethoxypyrrole (above) and paraformaldehyde, was extracted with ether then sublimed (65°, 1×10^{-4} mm), m.p. 78–80° (17%).

Anal. Calcd. for $C_{14}H_{23}O_2N$: C, 70.85; H, 9.77; N, 5.90. Found: C, 70.72; H, 9.74; N, 5.98.

2,3-Di-n-propyl-4-methyl-5-carbethoxypyrrole

The crude product, obtained as above but using 2-*n*-propyl-4-methyl-5-carbethoxypyrrole (above) and propionaldehyde, was distilled (80°, 1×10^{-4} mm). The partially solidified distillate was dried on tile then recrystallized twice from aqueous ethanol as colorless needles (20%), m.p. 84–87°.

Anal. Calcd. for $C_{14}H_{23}O_2N$: C, 70.85; H, 9.77; N, 5.90. Found: C, 70.68; H, 9.53; N, 6.05.

2-n-Propyl-3-i-butyl-4-methyl-5-carbethoxypyrrole

The crude product, obtained as above using 2-*n*-propyl-4-methyl-5-carbethoxypyrrole (0.65 g) and isobutyraldehyde (0.5 g), was repeatedly sublimed (65°, 1×10^{-4} mm) and recrystallized to remove starting material which was evident in the g.l.p.c.; yield, 30 mg, m.p. 78–79°.

Anal. Calcd. for $C_{15}H_{25}O_2N$: C, 71.67; H, 10.03; N, 5.57. Found: 71.49; H, 10.21; N, 5.68.

2,4-Dimethyl-3-sec-butyl-5-carbethoxypyrrole

The crude product, obtained as above but using 2,4-dimethyl-5-carbethoxypyrrole with 2-butanone and stirring at 30–35°, was sublimed then thrice recrystallized from ethanol as colorless plates (130 mg), m.p. 102–104°.

Anal. Calcd. for $C_{13}H_{21}O_2N$: C, 69.92; H, 9.48; N, 6.27. Found: C, 70.08; H, 9.31; N, 6.26.

2,3,4-Triethyl-5-carbethoxypyrrole

The crude product, obtained as above but using 2,4-diethyl-5-carbethoxypyrrole (5*e*) with paraldehyde, was this time extracted by ether. The ether was evaporated and the residue was distilled. The distillate partially solidified at 0°. It was dried on tile at 0°, recrystallized from aqueous methanol and again distilled (30%), m.p. 47–49°.

Anal. Calcd. for $C_{13}H_{21}O_2N$: C, 69.92; H, 9.48; N, 6.27. Found: C, 70.00; H, 9.42; N, 6.12.

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