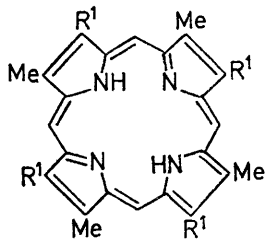
Porphyrins and Bile Pigments from Brominated Pyrromethenes [1]

By **K. M. Smith**, Robert Robinson Laboratories, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

Aetioporphyrin-I (1a) is obtained in 50—60% yield by heating 5-bromo-3,4'-diethyl-3',4,5'-trimethylpyrromethene perbromide (4a) under reflux in formic acid, surpassing the yields from the established syntheses using the less accessible 5-bromo-5'-bromomethylpyrromethene hydrobromides (2a, b). The 5-bromo-5'-methylpyrromethene hydrobromides and one equivalent of free bromine can be used as an alternative to the crystalline perbromides; in this way a 50% yield of coproporphyrin-I tetramethyl ester (1b) has been obtained.

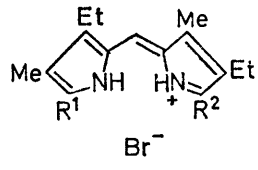
Aetiobiliverdin-IVγ (7a) is shown to be a by-product of the aetioporphyrin-I syntheses when 98% formic acid is used as the reaction medium, and the procedure has been modified to give preparatively acceptable yields of this bile pigment.

THE regular disposition of the peripheral side-chains in aetioporphyrin-I (1a) makes it an acceptable model for studying the properties of the macrocycle, and hence an attractive target for efficient synthesis. This paper describes improvements in the established syntheses of aetioporphyrin-I (1a) and an analogue, coproporphyrin-I



(1)

a; R^1 = Et
b; R^1 = P^{Me}
P^R = $CH_2CH_2CO_2R$



(2)

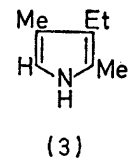
a; R^1 = Br; R^2 = CH_2Br
b; R^1 = CH_2Br; R^2 = Br
c; R^1 = Br; R^2 = Me

tetramethyl ester (1b), and also a modification of one of the procedures which enables the synthesis of a bile pigment model compound in reasonable yield.

Two similar procedures for the synthesis of aetioporphyrin-I have been described by Fischer and his co-workers; the first [2a,3] employed the heating of the dibrominated pyrromethene hydrobromide (2b) under reflux in formic acid (to give 16% yield) and the second [2a] the heating of the isomeric pyrromethene (2a) in formic acid, but more gently on a boiling water-bath (giving *ca.* 40% of aetioporphyrin-I).

The major disadvantage of these approaches is the relative inaccessibility of the dibrominated pyrromethenes (2a, b). For example, treatment of kryptopyrrole (3) with 2·5 equivalents of bromine in acetic acid furnishes [2b,4] a mixture of the required substance (2a), and the corresponding 5-bromo-5'-methylpyrromethene perbromide (4a). From 50 g of this mixture only 15 g of the pyrromethene (2a) can be isolated, whereas 30 g of the perbromide (4a) is obtained; [2c,4] the latter can only be converted into (2a) in disappointingly low yield.[2c,3,5]

Pyrromethene perbromides [*e.g.* (4)] do not appear to have been exploited in porphyrin synthesis and this may be due to the lack of success experienced by Fischer in such attempts.[4] It was, however, recognised that the perbromides [*e.g.* (4)] gave porphyrin when treated with



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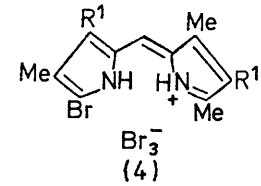
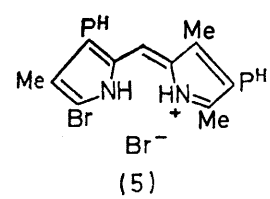


(4)

a; R^1 = Et
b; R^1 = P^H

formic acid.[3,4] Fischer made use [5] on occasions, of crude dibrominated pyrromethene–pyrromethene perbromide mixtures, and obtained yields of porphyrin far in excess of those he reported from the pure perbromides. He also obtained his best yield (35%) of octaethylporphyrin by heating the appropriately substituted pyrromethene perbromide in a melt of succinic acid.[2d,6] A re-examination of the earlier work has shown that when the perbromide (4a) is heated under reflux in formic acid yields between 50 and 60% of aetioporphyrin-I (1a) are obtained. This represents a considerable improvement over Fischer's existing procedures [2a] using the dibrominated pyrromethene hydrobromides (2a, b), even neglecting the greater availability of the perbromide (4a).

Coproporphyrin-I tetramethyl ester (1b) can be prepared [2e,7] in varying yields by heating the 5-bromo-5'-methylpyrromethene hydrobromide (5) in a variety



(5)

of organic acid melts (Table), followed by esterification. As an extension of Fischer's work, and for comparison with the work reported here, the pyrromethene (5) was

[1] A preliminary report of part of this work has been published: K. M. Smith, *Tetrahedron Letters*, 1971, 2325.

[2] H. Fischer and H. Orth, 'Die Chemie des Pyrrols,' Akademische Verlag., Leipzig, vol. III, 1937: (*a*) p. 193; (*b*) p. 106; (*c*) p. 73; (*d*) p. 188; (*e*) p. 484; (*f*) pp. 661, 709; (*g*) p. 114; (*h*) p. 705; (*i*) p. 109; (*j*) p. 86; (*k*) p. 483.

[3] H. Fischer and G. Stangler, *Annalen*, 1927, **459**, 53.

[4] H. Fischer, E. Baumann, and H. J. Riedl, *Annalen*, 1929, **475**, 205.

[5] W. Siedel and F. Winkler, *Annalen*, 1943, **554**, 162.

[6] H. Fischer and R. Bäumler, *Annalen*, 1929, **468**, 58.

[7] H. Fischer, H. Friedrich, W. Lamatsch, and K. Morgenroth, *Annalen*, 1928, **466**, 147.

heated under reflux in formic acid, and gave 19% of coproporphyrin-I tetramethyl ester after methanolysis. This compares favourably with many of Fischer's yields, the best of which are summarised in the Table.

TABLE

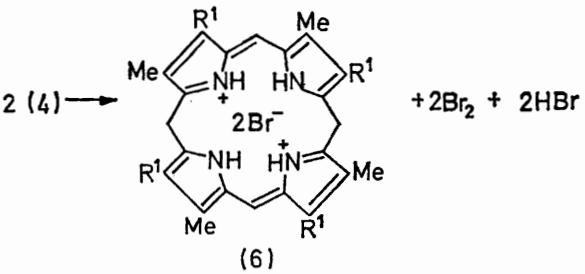
Yields of coproporphyrin-I tetramethyl ester (1b) obtained from (5)

Acid	Temp. (°C)	Time	Yield (%) of (1b)
Succinic	180—190	1 h	30—35
Tartaric	190—200	20 min	43
Oxalic	180	1·5 h	11
Malic	190	20 min	18
Phosphoric	230	10 min	23
[Formic †	*ca.* 100	2 h	19]

† This work.

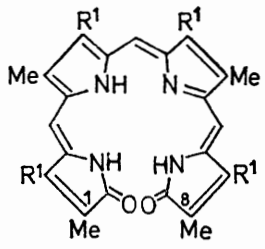
In order to obtain a direct comparison of the hydrobromide and perbromide methods, attempts were made to prepare the perbromide (4b) from the hydrobromide (5) so that the yields of porphyrin from the heating of each of these in formic acid could be related. However, the great insolubility of the pyrromethene hydrobromide (5) made it impossible to obtain the pure perbromide (4b). The best result was a mixture containing approximately equal amounts of the perbromide and hydrobromide (elemental analysis), and this gave a 41% yield of coproporphyrin-I tetramethyl ester (1b) when heated under reflux in formic acid and subsequently methanolysed. Since perbromides are a peculiar phenomenon of the crystalline state which undergo dissociation to the hydrobromide and one equivalent of bromine when taken into solution, and also because perbromide formation is unpredictable, the reaction of the 5-bromo-5′-methylpyrromethene hydrobromide (5) in formic acid was repeated, but in the presence of one equivalent of free bromine, in order to approximate to the use of the inaccessible perbromide (4b). A 50% yield of coproporphyrin-I tetramethyl ester (1b) was obtained after methanolysis, showing that considerably better yields of porphyrin are obtained when the perbromides rather than hydrobromides of 5-bromo-5′-methylpyrromethenes are heated under reflux in formic acid.

There are two obvious explanations for the enhancement of porphyrin yields from perbromide preparations: (*a*) the presence of an *in situ* dehydrogenation agent in exactly the required proportion serves to convert the



(6)

macrocycle (6) (which is probably formed initially) into porphyrin before it can be decomposed in the boiling formic acid, or (*b*) heating of the pyrromethene (5) with bromine in formic acid is a peculiarly efficient method for the transformation of the 5-bromo-5′-methylpyrromethene (5) into its 5′-bromomethyl derivative. The former explanation seems the more likely, since the pure bromomethylpyrromethene hydrobromides (2a, b) do not give porphyrin in yields as high as those from the corresponding perbromides in formic acid. During the course of the reaction, free bromine can be seen to be refluxing from the medium only in the first 30 min of its 2-h duration. Explanation (*a*) requires a continual reserve of bromine to be available throughout the whole



(7)

a; R^1 = Et
b; R^1 = P^{Me}

of the reaction time. (The reaction is clearly incomplete after 30 min, as attested by the continued emanation of hydrogen bromide.) The isolation of certain by-products (see later) suggests that quantities of the

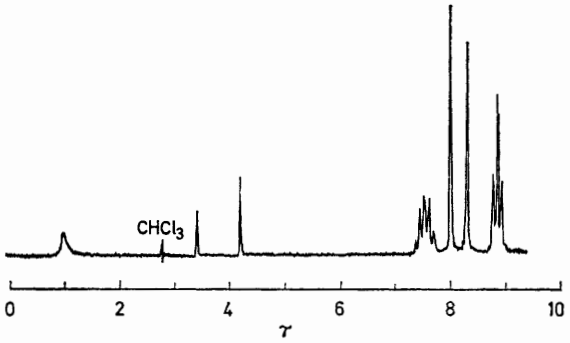


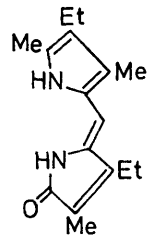
FIGURE N.m.r. spectrum (HA 100) of (7a) in $CDCl_3$

5-bromo-5′-bromomethylpyrromethene hydrobromides [*e.g.* (2a, b)] are generated in the course of the reaction, and hence a complex combination of both (*a*) and (*b*) may be responsible for the enhanced yields.

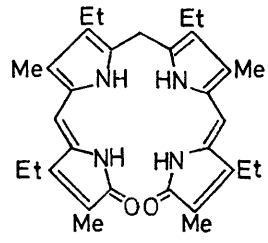
Chromatography of the crude products from the reaction of the dibrominated pyrromethene (2a) in refluxing 98% formic acid gave a 15% yield of aetioporphyrin-I (1a) [literature yield from this method,[3] but using the isomeric pyrromethene (2b) is 16%]. The porphyrin was easily eluted from Brockmann Grade III alumina using methylene chloride, but it left a well-defined blue band on the column which could be removed with further quantities of methylene chloride. Spectroscopic evidence (see Experimental section) suggested that the blue substance was aetiobiliverdin-IVγ (7a), obtained in 7% yield. The high degree of symmetry indicated by the n.m.r. spectrum (Figure) was particularly helpful

in the assignment of structure (7a); the upfield shift of the 1- and 8-methyl resonances was as expected from earlier work of Bonnett and McDonagh.[8] Mechanistic considerations, which will be discussed later, were also of assistance in the allocation of the structure. Confirmation was obtained from a melting-point comparison with the published data [2g,9] for aetiobiliverdin-IVγ which has been prepared earlier by Fischer from treatment of (8) with two equivalents of bromine; this material was in turn obtained [2g,9] in poor yield by hydrolysis of the corresponding 5-bromo-5′-methylpyrromethene (2c). Dearden and Jackson [10] have independently obtained aetiobiliverdin-IVγ (7a) from Fischer's alternative synthesis [2a] of aetioporphyrin-I.

The verdin (7a) doubtless arises by ' tail-to-tail ' self-condensation of the pyrromethene (2a) in contrast to the ' head-to-tail ' mode of condensation which generates aetioporphyrin-I. Loss of the elements of methylene bromide (or its hydrolysed equivalent) from two molecules of (2a), preceded or followed by hydrolysis of the terminal bromo-functions would lead to aetio-



(8)



(9)

bilirubin-IVγ (9) which would be transformed *in situ* to the verdin (7a); rubins are frequently converted into verdins when heated in formic acid.[2h] The two other steps in the proposed path are also well documented, *i.e.* the self-condensation of bromomethylpyrroles to symmetrical pyrromethanes [11] and the hydrolysis of bromopyrroles and bromopyrromethenes to the corresponding pyrrolinones.[2i,12]

At least one, and possibly two hydrolytic reactions upon each pyrromethene unit (2a) are required for its conversion into the verdin (7a), and hence the presence of water in the reaction medium is obligatory. It seemed that it might be possible to adapt Fischer's porphyrin synthesis to give quantities of bile pigments [such as (7a) *] at the expense of the porphyrin yield by use of aqueous reaction media. Accordingly, a series of experiments were carried out, and the solvent system which gave the greatest overall recovery of tetrapyrrolic pigments and the best bile pigment to porphyrin product ratio was found to be 10% aqueous formic acid. In this solvent mixture the pyrromethene (2a) gave a 22% yield of aetiobiliverdin-IVγ (7a) and only 13% of aetioporphyrin-I, which was easily separated from the former compound by chromatography. Higher proportions of water in the medium resulted in precipitation of the starting material, while prolonged reactions in aqueous formic acid at reduced temperatures gave a good recovery of tetrapyrroles, but the product ratio was in favour of the porphyrin. An experiment in which the pyrromethene (2a) was refluxed in methanol before treatment at 100° in aqueous formic acid gave a good ratio of verdin to porphyrin, but the overall recovery was only *ca.* 20%.

In his most efficient aetioporphyrin-I synthesis, Fischer reported [2a] a yield of *ca.* 40%, presumably using absolute formic acid. Since the best recoveries of tetrapyrroles obtained in this work were in the region of 35% it is unlikely that Fischer's experiments produced any aetiobiliverdin-IVγ and therefore one can confidently expect that the verdin is produced at the expense of the porphyrin in the work reported here using Fischer's procedure. The situation may be different with Fischer's less efficient method; [3] the aetioporphyrin-I yield reported is 16%, whereas this work gave a 15% yield of porphyrin together with a further 7% of the verdin (7a). Again, it is not certain whether or not the earlier workers used absolute formic acid. The work-up procedures used by Fischer and his contemporaries made use either of the basicity of the macrocycle (' acid-number ' extraction [13]) or else Soxhlet extraction of the crude products with a chosen sequence of solvents. These methods may not have been efficient enough to demonstrate the presence of verdin in the crude mixtures.

The aetioporphyrin-I (1a) and coproporphyrin-I tetramethyl ester (1b) preparations from perbromides also yielded the verdins (7a; 3%) and (7b; <1%) respectively. The quantities of these were much smaller than was isolated from the dibrominated pyrromethene (2a), and this is understandably so since the perbromide 5′-methyl group has no activation towards self-condensation. However, the isolation of the verdins (7) from these perbromide reactions does imply the intermediacy of at least small quantities of the 5-bromo-5′-bromomethylpyrromethene hydrobromides (2a, b), no doubt generated by bromination of the 5′-methyl group with bromine liberated from the perbromides once dissolved in formic acid.

EXPERIMENTAL

M.p.s were measured on a hot-stage apparatus. Neutral alumina (Merck, Brockmann Grade III) was used for all chromatographic separations. Formic acid was obtained from B.D.H., 98—100% purity, s.g. 1·22. Electronic

* The symmetry of (7a) about the central methine carbon atom renders it a particularly useful bile pigment model compound.

[8] R. Bonnett and A. F. McDonagh, *Chem. Comm.*, 1970, 237.
[9] H. Fischer and E. Adler, *Z. physiol. Chem.*, 1932, **206**, 187.
[10] Personal communication from Professor A. H. Jackson (Cardiff).
[11] H. Fischer and H. Orth, ' Die Chemie des Pyrrols,' Akademische Verlag., Leipzig, vol. I, 1934, p. 333.
[12] W. Siedel, *Annalen*, 1943, **554**, 144.
[13] R. Willstätter and W. Mieg, *Annalen*, 1906, 350, 1; J. E. Falk, ' Porphyrins and Metalloporphyrins,' Elsevier, Amsterdam, 1964, p. 122.

absorption spectra were determined with a Unicam SP 800 spectrophotometer and n.m.r. spectra were measured with a Varian HA-100 instrument in deuteriochloroform using tetramethylsilane as internal standard. Mass spectra were determined with an A.E.I. MS12 spectrometer (at 50 μA and 70 eV).

Pyrromethenes

5-Bromo-5′-bromomethyl-3,4′-diethyl-3′,4-dimethylpyrromethene Hydrobromide (2a).—A rapidly stirred solution of t-butyl 3-ethyl-2,4-dimethylpyrrole-5-carboxylate (20 g; recrystallised three times from methanol–water) in dry ether (600 ml) was treated with a solution of bromine (4·8 ml) in dry ether (400 ml), added during 2 or 3 min. After stirring for a further 30 min the solution was added dropwise during 1 h to a stirred solution of bromine (9·6 ml) in dry ether (800 ml) and then stirred for an additional 30 min before being left overnight in a refrigerator. The pyrromethene was separated by filtration and washed with a little ice-cold ether before being dried thoroughly *in vacuo*. The product (12—15 g, 56—70%), m.p. >300° was stored in a vacuum desiccator and used as soon as possible. N.m.r. spectrum τ −3·0 (3N*H*), 2·85 (methine-*H*), 5·21 (2H, s, CH_2Br), 7·0—7·8 (4H, m, CH_2CH$_3$), 7·69, 7·95 (both 3H and s, CH_3), 8·82 and 8·85 (both 3H and t, CH$_2$CH_3).

Attempted Preparation of 5-Bromo-3,4′-bis-(2-carboxyethyl)-3′,4,5′-trimethylpyrromethene Perbromide (4b).—5-Bromo-3,4′-bis-(2-carboxyethyl)-3′,4,5′-trimethylpyrromethene hydrobromide [2j,14] (2 g) was suspended in glacial acetic acid (20 ml) and treated with bromine (0·2 ml) before being heated on a boiling water-bath for 2 min. The resulting suspension was stirred until it reached room temperature (*ca.* 30 min) and the product was separated by filtration, washed with acetic acid, and ether, and then dried *in vacuo*. Elemental analysis (Found: C, 39·7; H, 4·1; N, 4·6%) indicated that the product was a mixture of the starting pyrromethene hydrobromide (Calc. for $C_{18}H_{22}Br_2N_2O_4$: C, 44·1; H, 4·5; N, 5·7%) and the required perbromide ($C_{18}H_{22}Br_4N_2O_4$ requires C, 33·3; H, 3·4; N, 4·3%). A similar result was obtained when the reaction was carried out in chloroform or methylene chloride. When the above mixture was heated under reflux in formic acid (see later) it gave 41% of coproporphyrin-I tetramethyl ester (1b) after methanolysis and chromatography.

Porphyrins

2,4,6,8-Tetraethyl-1,3,5,7-tetramethylporphin (*'Aetioporphyrin-I'*) (1a).—(a) *From the dibrominated pyrromethene* (2a).[2a,3] 5-Bromo-5′-bromomethyl-3,4′,diethyl-3′,4-dimethylpyrromethene hydrobromide (3·2 g) was suspended in formic acid (80 ml) and heated under reflux during 4 h before being reduced in volume (to *ca.* 20 ml) by distillation at atmospheric pressure. The residual dark brown solution was poured into water (1 l) and neutralised with dilute aqueous ammonia. The precipitate was collected, washed with water, dried *in vacuo*, and then chromatographed on alumina (300 g) in methylene chloride. The eluates were evaporated and the porphyrin was crystallised from methylene chloride–methanol to give deep purple prisms (228 mg, 15%), m.p. >300°. Elution with further quantities of methylene chloride furnished *aetiobiliverdin*-IVγ (119 mg, 7%) which was obtained as dark blue prisms, m.p. 263—264° (lit.,[2f,9] 263—265°) from methylene chloride–methanol (Found: C, 74·7; H, 7·6; N, 11·0. Calc. for $C_{31}H_{38}N_4O_2$: C, 74·7; H, 7·7; N, 11·2%), $\lambda_{max.}$ in CH_2Cl_2 366 nm (ε 52,000) and 638 (16,000); n.m.r. spectrum (Figure) τ 1·0 (3N*H*, broad and conc. dependent), 3·37 (1 methine-*H*), 4·12 (2 methine-*H*), 7·42 and 7·52 (both 4H and q, CH_2CH$_3$), 7·95 and 8·22 (both 6H and s, CH_3), 8·80 and 8·82 (both 6H and t, CH$_2$CH_3), *m/e*(%) 498 (100) P^+, 374 (5).

(b) *From the perbromide* (4a). 5-Bromo-3,4′-diethyl-3′,4,5′-trimethylpyrromethene perbromide [2b,4] (5·6 g) was suspended in formic acid (40 ml) and heated under reflux during 2 h. The mixture was evaporated to dryness at atmospheric pressure using an oil-bath at *ca.* 140° (45 min); the residue was kept briefly under high vacuum to remove last traces of formic acid before chromatography on alumina (300 g) using methylene chloride as eluant. The porphyrin fractions were collected and evaporated, the residue being crystallised from methylene chloride–methanol to give aetioporphyrin-I (1·25 g, 52%), identical in all respects with the material obtained in (*a*) (Found: C, 80·6; H, 8·1; N, 11·6. Calc. for $C_{32}H_{38}N_4$: C, 80·3; H, 8·0; N, 11·7%). Elution with further quantities of methylene chloride gave aetiobiliverdin-IVγ (69 mg; 3%), identified with the material obtained in (*a*). Repetition of the experiment on a smaller scale (using 1-g of perbromide) gave 59% of aetioporphyrin-I and no detectable amount of the verdin (7a).

2,4,6,8-Tetrakis-(2-methoxycarbonylethyl)-1,3,5,7-tetramethylporphin (*'Coproporphyrin-I Tetramethyl Ester'*) (1b).—(a) *From the pyrromethene hydrobromide* (5). 5-Bromo-3,4′-bis-(2-carboxyethyl)-3′,4,5′-trimethylpyrromethene hydrobromide [2j,14] (1 g) was suspended in formic acid (10 ml) and heated under reflux during 2 h before the solvent was removed by distillation at atmospheric pressure. The residue dissolved in 5% (v/v) sulphuric acid in methanol (100 ml) was left at room temperature in the dark for 12 h. The solution was poured into water and extracted with several portions of methylene chloride which were re-extracted with water. The organic phase was dried ($MgSO_4$) and evaporated to dryness, the deep purple residue then being chromatographed on alumina (200 g) using methylene chloride as eluant. The porphyrin fractions were evaporated and crystallised from methylene chloride–methanol to give coproporphyrin-I tetramethyl ester (140 mg, 19%), m.p. 255—256° (lit.,[2k,7] 248—252° corr.).

(b) *From the pyrromethene hydrobromide and free bromine.* 5-Bromo-3,4′-bis-(2-carboxyethyl)-3′,4,5′-trimethylpyrromethene hydrobromide [2j,14] (2 g) was suspended in formic acid and treated with bromine (0·65 g); the mixture was then heated under reflux during 2 h. The reaction was worked-up as described above and gave coproporphyrin-I tetramethyl ester (733 mg, 50%), m.p. 254—256°. The product was identical in all respects with the material from (*a*) (Found: C, 67·5; H, 6·6; N, 7·8. Calc. for $C_{40}H_{46}N_4O_8$: C, 67·6; H, 6·5; N, 7·9%), τ 0·06 (4 *meso*-*H*), 5·73 (8H, t, CH_2CH$_2$CO), 6·37 (12H, s, OCH_3), 6·48 (12H, s, CH_3), 6·83 (8H, t, CH$_2$CH_2CO), and 14·0 (2N*H*). Further elution of the column with methylene chloride furnished coprobiliverdin-IVγ (7 mg, <1%). It is likely that this material was formed in a yield greater than that reported, but it is extremely polar and suffered decomposition during its removal from the column. The material was also very soluble in methanol, and in view of the small amount obtained, it was not satisfactorily crystallised. N.m.r. spectrum τ 3·16 (1 methine-*H*); 4·20 (2 methine-*H*), 6·40

[14] H. Fischer and H. Andersag, *Annalen*, 1926, **450**, 212.

and 6·44 (both 6H and s, OCH_3), 7·0—7·6 (16H, m, CH_2CH_2), and 7·96 and 8·23 (both 6H and s, CH_3).

Modification of the Aetioporphyrin-I Synthesis to Give a Majority of Aetiobiliverdin-IVγ.—5-Bromo-5′-bromomethyl-3,4′-diethyl-3′,4-dimethylpyrromethene hydrobromide (2·6 g) in 10% aqueous formic acid (20 ml) was heated on a boiling water-bath for 4 h with the flask being swirled occasionally. The green-brown solution was poured into water (1 l) and neutralised with dilute aqueous ammonia. The precipitate was collected, washed with water, dried *in vacuo*, and then chromatographed with methylene chloride on alumina (200 g). Aetioporphyrin-I was first eluted and was crystallised from methylene chloride–methanol to give purple prisms (168 mg, 13%) identical with the material described earlier. Further elution furnished aetiobiliverdin-IVγ (298 mg, 22%), which crystallised from methylene chloride–methanol and was found to be identical with the material characterised earlier.

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