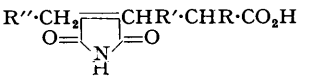
806. *The Syntheses of Pyrroles, a Porphyrin, and the Maleinimide related to the Uroporphyrins.*

By S. F. MacDonald.

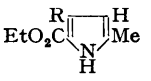
As already briefly reported (MacDonald, *Chem. and Ind.*, 1951, 759, 1092), representatives of the hitherto unknown pyrroles with both acetic acid and propionic acid residues in the β-positions have been synthesized, including one of the hypothetical reduction products of the uroporphyrins. A porphin-tetra-acetic-tetrapropionic acid has been synthesized and oxidized to a carboxylated hæmatinic acid identical with that from natural uroporphyrin I. Though the synthetic porphyrin appears to be a mixture of type isomers, its oxidation and other properties support Fischer's structures for the uroporphyrins.

The history and chemistry of the uroporphyrins have been summarized by Hans Fischer (*Ber.*, 1927, **60**, 2611; cf. Fischer and Orth, "Chemie des Pyrrols," Leipzig, 1937, Vol. II/i, pp. 504 *et seq.*). As these pigments have no apparent physiological function, general interest in them has depended on the prevailing views on their relation to hæmin. Recently earlier hypotheses have been reversed; the uroporphyrins and the related pyrroles are now postulated as intermediates in the biosynthesis of hæmin (cf. Falk and Rimington, *Ann. Reports*, 1950, 271).

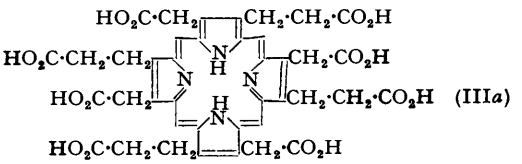
Uroporphyrin I, isolated from the urine of a patient with congenital porphyria, was shown to be an octacarboxylic acid, giving the tetracarboxylic acid coproporphyrin I (III*a*; Me for $CH_2{\cdot}CO_2H$) on partial decarboxylation. Its oxidation gave a carboxylated hæmatinic acid which decarboxylated to hæmatinic acid (I*a*) and to ethylmethylmaleinimide and was formulated as (I*b*, *c*, or *d*).



(I*a*; R = R′ = R″ = H)
(I*b*; R = CO_2H; R′ = R″ = H)
(I*c*; R′ = CO_2H; R = R″ = H)
(I*d*; R″ = CO_2H; R = R′ = H)



(II*a*; R = Me)
(II*b*; R = $CH_2{\cdot}CO_2Et$)
(II*c*; R = $CH_2{\cdot}CH_2{\cdot}CO_2Et$)



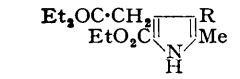
(III*a*)

Pyrroles corresponding to (I*b* and *c*), which could be synthesized from (II*a*), were each converted into the corresponding type-I and type-II porphyrins, tetramethylporphin-tetra(methylmalonic) acids and tetramethylporphintetrasuccinic acids. Neither of the

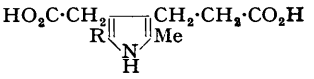
type-I porphyrins was identical with uroporphyrin I. Also, the more available type-II prophyrins were oxidized to the carboxylated hæmatinic acids (I*b* and *c*), which differed from that obtained from uroporphyrin I. Only the structure (I*d*) remained for the analytical carboxylated hæmatinic acid, and Fischer formulated uroporphyrin I as porphin-1 : 3 : 5 : 7-tetra-acetic-2 : 4 : 6 : 8-tetrapropionic acid (III*a*). The other uroporphyrin isomers are formally derived from it by reversing the order of the two β-substituents on two opposite pyrrole nuclei (type II; III*b*), on one pyrrole nucleus (type III; III*c*), or on two adjacent nuclei (type IV; III*d*).

The further development of this approach to the structure and synthesis of the uroporphyrins required the intermediates (II*b* or c) (preceding paper). The transformations of (II*b*) now described are completely analogous to one synthesis of coproporphyrins from (II*a*) (Fischer and Andersag, *Annalen*, 1926, **450**, 201; 1927, **458**, 117).

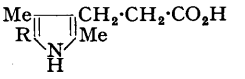
The propionic acid residue was introduced into (IV*a* = II*b*) as usual (Fischer and Orth, *op. cit.*, Vol. I, p. 270) *via* the aldehyde (IV*b*) and the acrylic acid (IV*c*). Catalytic reduction of the last in alkaline solution resulted in partial hydrolysis, and the product was isolated as (V*a*), or better as (IV*d*) after esterification. Hydrolysis of (IV*d*) gave (V*d*), which was stable when dry.



(IV*a*; R = H)
(IV*b*; R = CHO)
(IV*c*; R = CH:CH·CO_2H)
(IV*d*; R = CH_2·CH_2·CO_2Et)
(IV*e*; R = CH_2·CH_2·CO_2Et; CH_2Br for Me)



(V*a*; R = CO_2Et)
(V*b*; R = CO_2H)
(V*c*; R = H)

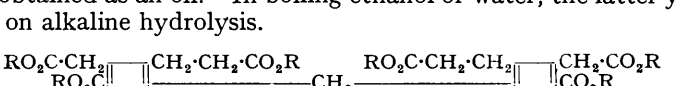


(VI*a*; R = CO_2Et)
(VI*b*; R = CO_2H)
(VI*c*; R = H)

The structures of these pyrroles were confirmed by decarboxylation of the free acetic acids under vigorous conditions to known dimethylpyrroles, as (IV*a*, CH_2·CO_2H for CH_2·CO_2Et) had given ethyl 2 : 4-dimethylpyrrole-5-carboxylate (preceding paper). Thus (V*a*), obtained as above or by the partial hydrolysis of (IV*d*), was decarboxylated to (VI*a*). Similarly, (V*b*) gave (VI*c*). These degradations do not distinguish the relative positions of the methyl and the acetic acid groups. However, the conversion of (IV*d*) into (IV*e*) and thence into the dipyrrylmethane (VII*a*) without the loss of a carbethoxy-group requires a methyl group in the α-position.

Like analogous pyrrole-α-carboxylic acids, (V*b*) was rapidly decarboxylated in water at 100°. The resulting dicarboxylic acid must be (V*c*). In view of the resistance of the propionic acid group to decarboxylation, the only alternative formulation is as the known (VI*b*), which is much less soluble in water and is decarboxylated to (VI*c*) under the above conditions. The acid (V*c*), which might be termed cryptopyrroledicarboxylic acid, is probably one component of the mixture which had been obtained by reducing uroporphyrin I with hydrogen iodide, for extensive decarboxylation did not accompany the reduction (Fischer, *Z. physiol. Chem.*, 1916, **98**, 78; Fischer and Zerweck, *ibid.*, 1924, **137**, 243; Fischer and Andersag, *Annalen*, 1927, **458**, 117). Like the reduction products, (V*c*) is very soluble in water and forms no picrate in wet ether and its methyl ester has been obtained only as an oil. Further work on the characterization of such pyrroles is necessary. With hydrogen iodide and acetic acid at 100°, both (V*b*) and (V*c*) gave high-melting, ether-insoluble products containing iodine (cf. the behaviour of an isomeric pyrrolesuccinic acid, Fischer and Holt, *Z. physiol. Chem.*, 1934, **229**, 94).

With bromine in acetic acid, or better in carbon disulphide, (IV*d*) was converted into (IV*e*), obtained as an oil. In boiling ethanol or water, the latter yielded (VII*a*) which gave (VII*b*) on alkaline hydrolysis.

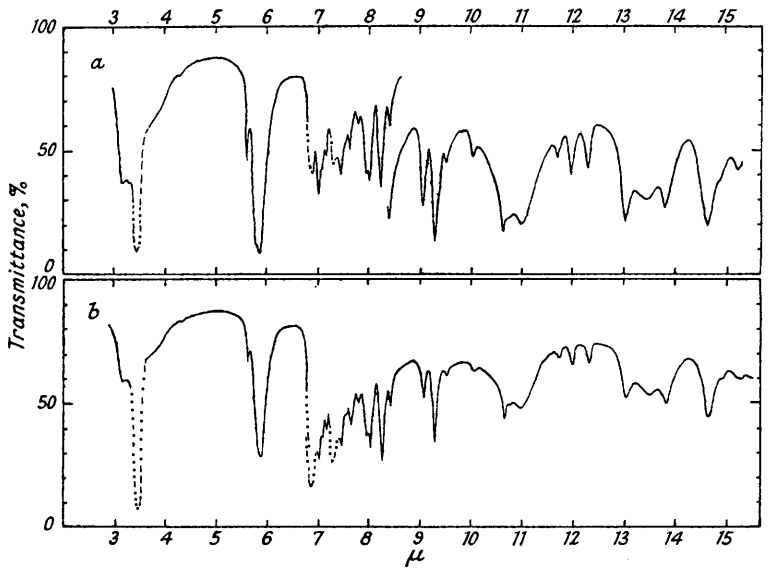


(VII*a*; R = Et)
(VII*b*; R = H)

Although the free acetic acids are sufficiently resistant to decarboxylation, these reactions have been carried out with their ethyl esters because (IV*b*; CH_2·CO_2H for

$CH_2 \cdot CO_2Et$) was inconveniently soluble in water. It is unlikely that this choice was always appropriate. Working with the α-carbethoxy-derivatives of the free propionic or acetic acids would avoid difficulties in handling their esters, which easily undergo hydrolysis and trans-esterification and frequently have low melting points and high solubilities in organic solvents; α-carbethoxy-groups are not inconvenient in these respects.

Prolonged treatment with formic acid at 40° converted (VII*b*) into a porphintetra-acetic-tetrapropionic acid, isolated as the methyl ester in 37% yield. In analogous cases, this method of porphyrin synthesis has been used to avoid difficulties in obtaining unsymmetrical dipyrromethenes, and the decarboxylation or very low yields which may complicate the conversion of these into porphyrins (Fischer and Hofmann, *Z. physiol. Chem.*, 1937, **246**, 15). However, it rarely proceeds rationally to type-II porphyrins, and mixtures of the type isomers usually result. In the present case there was no evidence of partial decarboxylation of the side-chains. Analysis indicated an octacarboxylic ester, and paper chromatography (Nicholas and Rimington, *Biochem. J.*, 1951, **48**, 306) of the free porphyrin,



a, The synthetic ester (I*d*). *b, Carboxylated hæmatinic acid from uroporphyrin I. Both in Nujol.*

obtained from the unrecrystallized methyl ester by acid hydrolysis, gave no indication of heptacarboxylic or lower acids. When the synthesis was carried out under conditions more favourable to decarboxylation, in boiling formic acid rather than at 40°, most of the ester was extracted by boiling methanol and gave analyses for a heptacarboxylic ester. However, there is little doubt that the product obtained at 40° is a mixture of type isomers (III*a*, *b*, *c*, and *d*) in unknown proportions, rather than pure (III*b*). The melting point of the methyl ester was not altered by repeated crystallization, but prolonged extraction of the amorphous free acid with boiling acetic acid gave fractions from which methyl esters with lower melting points were obtained. Also, a low-melting mixture of coproporphyrin esters was obtained after partial decarboxylation at 190°.

The synthetic porphintetra-acetic-tetrapropionic acid, obtained by alkaline hydrolysis of its methyl ester, was oxidized to (I*d*), shown by m. p., mixed m. p., and infra-red spectra (Figure) to be identical with the analytical carboxylated hæmatinic acid, which was obtained in the same yield by oxidizing uroporphyrin I. This confirms Fischer's structure (I*d*) for the carboxylated hæmatinic acid and provides the first direct evidence that acetic acid side chains are present in uroporphyrin I.

A synthetic tetramethylporphintetra-acetic acid differed from uroporphyrin I spectroscopically and was partially decarboxylated even on alkaline hydrolysis of its ester (Fischer and Mueller, *Z. physiol. Chem.*, 1937, **246**, 31); this made it difficult to account for the

properties of uroporphyrin I in terms of (III*a*). This difficulty is removed by the similarity in properties between the synthetic porphintetra-acetic-tetrapropionic acid and uroporphyrin I. The solubilities are all of the same order, those of the synthetic porphyrin being usually somewhat greater, as would be expected in a mixture of isomers. The absorption spectra in various solvents showed no significant differences. Both free porphyrins, though stable at 100° in alkali or acetic acid, are partially decarboxylated to coproporphyrins at 190°. They were indistinguishable by paper chromatography (Nicholas and Rimington, *loc. cit.*; Nicholas and Comfort, *Biochem. J.*, 1949, **45**, 208), both giving characteristically two spots.

An unusual property common to the two porphyrins was noted when crystallization of the free synthetic porphintetra-acetic-tetrapropionic acid by Fischer and Hilger's method (*Z. physiol. Chem.*, 1925, **149**, 65) proved unsatisfactory. Irreversible changes in spectra and colour showed that both the free synthetic porphyrin and the free uroporphyrin I are unstable in hot pyridine–acetic acid, even in the dark. They are also unstable in hot N-sodium carbonate but are stable in stronger alkali such as 2N-ammonia or -sodium hydroxide. The methyl esters of both these porphyrins are stable in hot pyridine–acetic acid, as are both free coproporphyrin I and its methyl ester. This reaction is suggestive of the oxidations brought about by light, to which vinylporphyrins are particularly sensitive (cf. Fischer and MacDonald, *Annalen*, 1939, **540**, 211), and by the action of air and reducing agents on hæmin (Lemberg, *Biochem. J.*, 1935, **29**, 1322).

On this evidence, Fischer's uroporphyrin I structure can be regarded as proved only if it be assumed that all four pyrrole nuclei bear the same substituents; this assumption has been discussed from an earlier point of view by Fischer and Siebert (*Annalen*, 1930, **483**, 1) and by Fischer and Hofmann (*loc. cit.*). Though the easily decarboxylated methylmalonic acid residues may be excluded (Fischer and Zischler, *Z. physiol. Chem.*, 1937, **245**, 124), there has been no positive evidence against the presence of one pyrrole nucleus bearing methyl and succinic acid groups. More than one such is very unlikely for, though uroporphyrin I has never given more than two mols. of (I*d*) on oxidation, no isomer of it has been detected though (I*c*) is produced in very high yield on oxidation of tetramethylporphintetrasuccinic acid (Fischer and Staff, *Z. physiol. Chem.*, 1935, **234**, 97). Also (I*d*) was obtained in the same yield by oxidizing either uroporphyrin I or the synthetic porphintetra-acetic-tetrapropionic acid under identical conditions. As *meso*-substituents profoundly alter the spectra of porphyrins, the above evidence appears to exclude other alternative structures.

Proof of the absence of *C*-methyl groups would completely establish Fischer's uroporphyrin I structure (III*a*) except in the question of type. Though *C*-methyl bands appeared to be absent in the infra-red spectrum of uroporphyrin I, this evidence was treated with reserve (MacDonald, *loc. cit.*). However, it is now clear from the examination of the infra-red spectra of other porphyrins, that conclusions as to the presence or absence of *C*-methyl bands in those of the uroporphyrins must await further comparative data. Though the Kuhn–Roth *C*-methyl determinations have given apparently decisive results in favour of Fischer's structure, their validity will have to be established empirically. Further synthetic evidence could only be obtained by the synthesis of the natural uroporphyrins; these are being sought in the synthetic porphintetra-acetic-tetrapropionic acid and through further syntheses from the pyrroles (II*b* and *c*).

Experimental

M. p.s are uncorrected.

5-Carbethoxy-4-carbethoxymethyl-2-methylpyrrole-3-aldehyde (IV*b*).—A slow current of dry hydrogen chloride was passed, for 2 hours, into a solution of the pyrrole (IV*a*) (20 g.) in dry ether (60 c.c.), dry chloroform (60 c.c.), and anhydrous hydrogen cyanide (20 c.c.), the solution being protected from moisture, cooled in an ice-salt bath, and frequently shaken. Gas was passed in at an increased rate while the crystals separated and for 2 hours thereafter. The aldimine hydrochloride was filtered off, washed with ether, and dried *in vacuo*. It was dissolved in ice-water and quickly filtered, and the pH was brought to 4. The *aldehyde* (21·6 g.,

97%) soon separated; it was filtered off, washed with water, and dried *in vacuo* (m. p. 151—152°). Colourless needles, m. p. 154°, were obtained after recrystallization from ether (thimble), then from water (Found: C, 58·5; H, 6·6; N, 5·6. $C_{13}H_{17}O_5N$ requires C, 58·4; H, 6·4; N, 5·2%).

Ethyl 3-Carbethoxymethyl-4-2′-carboxyvinyl-5-methylpyrrole-2-carboxylate (IV*c*).—The aldehyde (IV*b*) (9·5 g.), 90% ethanol (40 c.c.), malonic acid (5 g.), and aniline (4 c.c.; freshly distilled) were refluxed on a steam-bath for 20 hours, then filtered hot (charcoal), and the solvent was distilled off. The crystalline residue was made into a slurry with toluene (10 c.c.), filtered off, and washed with toluene, 40% ethanol, and with light petroleum, leaving the *ester* (5·6 g., 51%), m. p. 205—207°. From 40% ethanol (charcoal), colourless prisms, m. p. 205—206° (Found: C, 59·0; H, 6·4; N, 4·9. $C_{15}H_{19}O_6N$ requires C, 58·2; H, 6·2; N, 4·5%), were obtained.

Ethyl 4-2′-Carbethoxyethyl-3-carbethoxymethyl-5-methylpyrrole-2-carboxylate (IV*d*).—The acrylic acid (IV*c*) (5·6 g.) in water (25 c.c.) and sodium hydroxide (10 c.c. of 10%) was filtered (charcoal) and shaken at 15° under hydrogen (1 atm.) with Raney nickel (2·5 c.c.). Absorption ceased after the theoretical uptake in 24 hours. The mixed esters (V*a*) and (V*a*; $CH_2{\cdot}CO_2Et$ for $CH_2{\cdot}CO_2H$) (4·6 g.), m. p. 130—160°, were precipitated with sulphur dioxide at 0°. Part (4·1 g.) of them was dissolved in hot ethanolic hydrogen chloride (25 c.c. of 5%) and poured into water after 24 hours at 20°. The *product* (4·1 g., 75%), filtered off and washed with water, had m. p. 60—63°. From light petroleum (b. p. 40—60°; thimble), colourless needles, m. p. 63—63·5° (Found: C, 60·3; H, 7·4; N, 4·2. $C_{17}H_{25}O_6N$ requires C, 60·2; H, 7·4; N, 4·1%), were obtained; Ehrlich's reaction was negative in the cold but strongly positive on heating.

The same product was obtained in lower yield by reduction of the acrylic acid in dilute sodium hydroxide with sodium amalgam at 8°, followed by esterification.

Ethyl 4-2′-Carboxyethyl-3-carboxymethyl-5-methylpyrrole-2-carboxylate (V*a*).—The acrylic acid (IV*c*) was reduced as above but for 2 days. The crude *product* was precipitated with acid, crystallized twice from water (30 parts), and sublimed (187°/2 × 10^{-3} mm.), as colourless prisms, m. p. 178° (decomp.); Ehrlich's reaction was positive in the hot (Found: C, 55·1; H, 6·05; N, 5·1. $C_{13}H_{17}O_6N$ requires C, 55·1; H, 6·0; N, 4·9%).

4-2′-Carboxyethyl-3-carboxymethyl-5-methylpyrrole-2-carboxylic Acid (V*b*).—The tri-ester (IV*d*) (1·7 g.) in ethanol (20 c.c.) and 10% aqueous sodium hydroxide (20 c.c.) was heated in an open flask on the steam-bath until dry (2·5 hours). The crystalline residue was well washed with ethanol, dissolved in water (20 c.c.), filtered (charcoal), and made acid to Congo-red with sulphur dioxide at 0°. The precipitated *acid* (1·2 g., 92%) was filtered off, washed with water, and dried *in vacuo* at 20° (m. p. 144°). After two recrystallizations from dry acetone (thimble) it formed colourless prisms, m. p. 145—146° (decomp.), Ehrlich's reaction being positive in the cold (Found: C, 52·0; H, 5·7; N, 5·2. $C_{11}H_{13}O_6N$ requires C, 51·8; H, 5·1; N, 5·5%). When dry, it slowly becomes violet, but is again obtained pure and colourless by extraction with acetone.

3-2′-Carboxyethyl-4-carboxymethyl-2-methylpyrrole (V*c*).—The tricarboxylic acid (V*b*) (460 mg.) with water (2 c.c.) was heated to effect solution at 100° (*ca.* 5 minutes) and freeze-dried. After two crystallizations from dry ether–light petroleum (b. p. 40—60°; thimble), the product was obtained as colourless needles, m. p. 126° (decomp.) (Found: C, 57·2; H, 6·4; N, 6·7. $C_{10}H_{13}O_4N$ requires C, 56·9; H, 6·2; N, 6·6%). Ehrlich's reaction was positive in the cold.

Decarboxylation of (V*a*) *to Carbethoxycryptopyrrolecarboxylic Acid* (VI*a*).—The mono-ester (V*a*) was heated for a few minutes at 250°, then sublimed *in vacuo* (185°/2 × 10^{-3} mm.). The product formed colourless crystals [from ether–light petroleum (b. p. 40—60°)], m. p. 154° [lit., 152°, 154°, and 159° (Fischer and Andersag, *loc. cit.*; Siedel and Winkler, *Annalen*, 1943, **554**, 167, 193) (Found: C, 60·4; H, 7·3; N, 6·2. Calc. for $C_{12}H_{17}O_4N$: C, 60·2; H, 7·2; N, 5·9%).

Degradation of (IV*d*) *to Carbethoxycryptopyrrolecarboxylic Acid* (VI*a*).—The tri-ester (IV*d*) (545 mg.) was heated under reflux for 4 hours in ethanol containing sodium hydroxide (150 mg.). The solvent was distilled off, the residue dissolved in water, and the solution acidified. The impure mono-ester (V*a*) was extracted from the precipitate with ether (thimble) and decarboxylated to (VI*a*), which melted at 154° after successive crystallizations from ether, dilute ethanol, and water. Ehrlich's reaction was negative in the cold, positive in the hot.

Degradation of the Tricarboxylic Acid (V*b*) *to Cryptopyrrolecarboxylic Acid* (VI*c*).—The tricarboxylic acid (V*b*) (220 mg.) was heated with glycerol (bath, 210°) until no more gas was evolved; water was added and the product (VI*c*) extracted with ether. It formed pale tan crystals (from water), m. p. 136°, mixed m. p. 136—137° with authentic material of m. p. 138°; Ehrlich's reaction was positive in the cold.

Ethyl 2-Bromomethyl-3-2′-carbethoxyethyl-4-carbethoxymethylpyrrole-5-carboxylate (IV*e*) *and Diethyl 5 : 5-Dicarbethoxy-3 : 3′-bis-2″-carbethoxyethyl-4 : 4′-biscarbethoxymethyldipyrrylmethane* (VII*a*).—(*a*) The tri-ester (IV*d*) (1 g.) in acetic acid (0·3 c.c.) was treated with bromine in acetic acid (1 c.c. containing 0·5 g. of bromine) at 30°. After some hours, the solvent was removed *in vacuo*, leaving the α-bromomethyl derivative (IV*e*) as an oil. This was boiled for 3 hours with water (500 c.c.), and the tar obtained on cooling was crystallized from 50% aqueous ethanol (8 c.c.), to give the *dipyrrylmethane* (VII*a*) (0·17 g., 17%), m. p. 95—97° (halogen absent; Beilstein test). For analysis it was recrystallized twice from hexane (thimble), forming large pale tan-coloured plates, m. p. 100·5° (Found: C, 60·1; H, 7·2; N, 4·4. $C_{33}H_{46}O_{12}N_2$ requires C, 59·8; H, 7·0; N, 4·2%).

(*b*) The tri-ester (IV*d*) (1·25 g.) in carbon disulphide (1 c.c.) was treated with bromine in carbon disulphide (0·6 g. in 2·5 c.c.) at 20°. After a few minutes, the solvent was removed *in vacuo*, and the oily bromomethyl derivative (IV*e*) washed twice with water by decantation, and dried *in vacuo*. It was heated under reflux with ethanol (5 c.c.) for 4 hours, most of the solvent was distilled off, and the residue seeded and left at 0°. The product (VII*a*) (0·45 g., 37%) remained after draining on a porous tile. It was pure enough for the next step.

Better yields of a mixed ester (60—80%) have since been obtained by brominating the dimethyl ester of (V*a*) and boiling the product with methanol.

5 : 5′-Dicarboxy-3 : 3′-bis-2″-carboxyethyl-4 : 4′-biscarboxymethyldipyrrylmethane (VII*b*).—The dipyrrylmethane (VII*a*) (0·8 g.) in ethanol (4·5 c.c.) and aqueous sodium hydroxide (4·5 c.c. of 10%) was refluxed for 2 hours, the ethanol distilled off, and the residue cooled and diluted to 10 c.c. After filtration (charcoal) and acidification with sulphur dioxide at 0°, the *product* (0·51 g., 86%) soon separated and was dried *in vacuo* [m. p. 146° (decomp.)]. Recrystallized from dry acetone (thimble), it formed somewhat hygroscopic, colourless crystals, m. p. 157° (decomp.) (Found: C, 50·8; H, 4·8; N, 5·55. $C_{21}H_{22}O_{12}N_2$ requires C, 51·0; H, 4·5; N, 5·7%).

2 : 3 : 6 : 7-Tetrabis-2″-carbomethoxyethyl-1 : 4 : 5 : 8-tetrabiscarbomethoxymethylporphin and Type Isomers (III*b*, *a*, *c*, *and d*) (*Octamethyl Porphintetra-acetate-tetrapropionate*).—A stream of air was passed through a suspension of the dipyrrylmethane (VII*b*) (2·08 g.) in 98% formic acid (50 c.c.) at 40° for 5 days, the volume being maintained by adding acid. The porphyrin was then precipitated by pouring the mixture into saturated brine (500 c.c.) and adjusting the pH to about 3 with sodium hydroxide. It was filtered off and washed successively with brine, water, and methanol; a little acetic acid had been added to each of these to prevent peptization. The product was dissolved in methanolic hydrogen chloride (100 c.c.; saturated at 0°), and the solution left for 1 day, diluted with chloroform (200 c.c.), and poured into ice-water. The chloroform layer was washed with water, very dilute sodium hydroxide, and again water. After being dried (Na_2SO_4), the solution was filtered through a column of neutral alumina and the pigment washed through with more chloroform. The filtrate and washings were filtered and concentrated. On addition of hot methanol the *product* (732 mg., 37%) separated as hair-like needles, m. p. 256—258° (block), m. p. unchanged after four recrystallizations from chloroform–methanol (66% recovery) (Found: C, 61·15; H, 5·8; N, 6·1; OMe, 27·9; *C*-Me 0·0. $C_{48}H_{54}O_{16}N_4$ requires C, 61·1; H, 5·8; N, 5·9; OMe, 26·3%).

The ester is soluble in hot ethyl acetate, but insoluble in ether and methanol. When solutions in acid are neutralized, ether extracts about five times as much of this ester as it does of uroporphyrin I ester. In 25% hydrochloric acid, the spectra of the synthetic ester and of uroporphyrin I methyl ester could not be distinguished with a comparison spectroscope. The spectra were measured in chloroform (dried with sulphuric acid and then potassium hydroxide, stabilized with 5% v/v of dry ether) (Unicam) (figures in parentheses are $10^{-4}\varepsilon$): Synthetic ester (80 mg./l.), max. at 626 (0·377), 572 (0·669), 536 (0·897), 502 (1·356), 406 (20·65), inflexion at 595—597 (0·139), min. at 609 (0·084), 554 (0·148), 522 (0·330), 459 mμ (0·186). Uroporphyrin ester (70 mg./l.), max. at 626 (0·383), 572 (0·680), 537 (0·912), 502 (1·568), 406 (20·03), inflexion at 596—598 (0·146), min. at 607 (0·077), 554 (0·167), 522 (0·352), 458 (0·193). Here, as elsewhere unless otherwise indicated, uroporphyrin I methyl ester refers to products, m. p. *ca.* 283°, obtained from the urine of a case of congenital porphyria by the method of Fischer and Orth (*op. cit.*, p. 514, method a).

Porphintetra-acetic-tetrapropionic Acid (*Mixed Isomers*).—The synthetic ester (80 mg.) was refluxed with N-sodium hydroxide (10 c.c.) for 5 hours, then diluted with water, and acetic acid was added. The dried precipitate was dissolved in pyridine and the *acid* (53 mg.) precipitated by addition of hot acetic acid to the filtered solution; the microscopic stout needles, still contaminated with amorphous material after a second crystallization, were dried at 80°/5 × 10^{-4}

mm. (Found: C, 56·3; H, 4·6; N, 6·9. $C_{40}H_{38}O_{16}N_4$ requires C, 57·8; H, 4·6; N, 6·75%). The acid hydrolysis of the ester gave a product which was no better.

On extraction of its solutions in alkali with ether and acetic acid, traces of the porphyrin can be detected in the ether spectroscopically. When alkaline solutions were acidified with mineral acid to pH 3, ethyl acetate extracted about 15 times as much of this porphyrin as it did of uroporphyrin I (measured by ultra-violet fluorescence). The amorphous synthetic porphyrin is very slightly soluble in boiling acetic acid, being incompletely dissolved after 6 hours' extraction (thimble).

The synthetic ester was heated under reflux with 10% aqueous sodium hydroxide for 10 hours, and the free porphyrin precipitated and extracted with boiling acetic acid for 6 hours (thimble). The porphyrin in solution was precipitated, esterified with methanolic hydrogen chloride, and crystallized from chloroform–methanol (Found: C, 61·3; H, 5·9%).

In 10% aqueous sodium hydroxide, the spectra of the synthetic porphyrin and of uroporphyrin I were identical (comparison spectroscope), as were those of their copper complexes.

The Carboxylated Hæmatinic Acid (Id).—The synthetic octamethyl porphintetra-acetate-tetrapropionate (150 mg.) was hydrolysed by 5 hours' refluxing with N-sodium hydroxide (5 c.c.). After evaporation, the residue was dissolved in 50% (v/v) sulphuric acid (9 c.c.), and chromic acid (220 mg. in 1 c.c. of water) was dropped in at 0° during 1 hour. After being kept overnight at 0°, the filtered solution was diluted with water (4 c.c.) and repeatedly extracted with pure ether. The ether was removed from the extract, and the residue dissolved in 0·75N-sodium carbonate (4 c.c.) and extracted with ether. The carbonate solution was brought to pH 1 with sulphuric acid, saturated with ammonium sulphate, and repeatedly extracted with ether. This extract was dried (Na_2SO_4) and the ether evaporated off, leaving the crystalline product; recrystallized from dry ether (5 c.c.; thimble) and dried (60°/3 × 10^{-4} mm.), this formed colourless prisms (25 mg.), m. p. 179° (decomp.) (Found: C, 47·7; H, 4·25; N, 6·2. Calc. for $C_9H_9O_6N$: C, 47·6; H, 4·0; N, 6·2%). Infra-red spectrum in Nujol: Fig. (*a*).

The analytical carboxylated hæmatinic acid (24 mg.), obtained by oxidizing uroporphyrin I methyl ester (151 mg.) in the same way, had m. p. 178° (decomp.) alone or mixed with the synthetic product. In all cases sintering began at about 165° but was never considerable, The m. p.s depend on the rate of heating (here 1°/min.), and so were determined in pairs (lit., 188°, 179—180°, 178°, 184° or 176°, 168° for the analytical product; Fischer, *Z. physiol. Chem.*, 1916, **98**, 78; Fischer and Holt, *loc. cit.*; Fischer and Staff, *Z. physiol. Chem.*, 1934, **234**, 97; Fischer and Hofmann, *loc. cit.*; Fischer, Hartmann, and Riedl, *Annalen*, 1932, **494**, 246). Infra-red spectrum in Nujol: Fig. (*b*).

Partial Decarboxylation of the Synthetic Porphintetra-acetic-tetrapropionic Acid to Coproporphyrins (cf. Fischer and Zerweck, *loc. cit.*).—The porphyrin ester (44 mg.) was hydrolysed in concentrated hydrochloric acid overnight at 20°, the acid diluted to 1%, and the mixture heated for 4 hours at 185—195°. The resulting porphyrin was fractionated between hydrochloric acid and ether, esterified with diazomethane, and again fractionated. The porphyrin left on evaporation of the ether was dissolved in chloroform and filtered through a column of alumina, then crystallized by addition of hot methanol to the concentrated solution giving a mixture (13 mg.) of prismatic needles, m. p. *ca.* 180°, and radiating clusters of needles, m. p. *ca.* 135°. Recrystallization from chloroform–methanol (thimble) gave irregular aggregates of prismatic needles (6 mg.), m. p. 183° (block) (Found: C, 67·6; H, 6·8. Calc. for $C_{40}H_{46}O_8N_4$: C, 67·6; H, 6·5%). The mother-liquors deposited radiating clusters of needles (2 mg.), m. p. 134—135° (block); this m. p. has been recorded for coproporphyrin III ester but the infra-red spectra preclude identity.

No products other than coproporphyrins were detected; the crude free porphyrin was not extracted by ether from 0·2% hydrochloric acid, and the ester was extracted from ether by 2% hydrochloric acid. The esters were spectroscopically identical with coproporphyrin I in chloroform (comparison spectroscope).

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