phyrin 4 only in having a shallow minimum instead of a flat maximum at 920 cm.$^{-1}$; after heating to 170° its spectrum was similar but more diffuse.

Anal. Calcd. for $C_{40}H_{46}N_4O_8$: C, 67.59; H, 6.52. Found: C, 67.49; H, 6.39.

The copper complex of the methyl ester formed needles from chloroform–methanol, m.p. 230–233° (lit.[4] 216–217°), characterized by an X-ray powder photograph.

The ethyl ester of coproporphyrin 4, from the methyl ester by hydrolysis and esterification, separated from ethanol as sheaved prismatic needles, characterized by an X-ray powder photograph. It showed a solid phase change at 90°, traces melted at 145°, a phase change and melting at 156–160°, final melting at 165–168° (lit.[4] m.p. 152°).

Natural Coproporphyrin 3.—The methyl ester (no. 7, Table II) from *C. diphtheriae*[5,44] (lit. m.p. Table I; X-ray powder photograph[45] and infrared mull spectrum[25,26] also reported) was recrystallized from methanol. Its mixed m.p. with a synthetic specimen (1a, Table II) 153–155° and 178–182° also revealed the fibrous intermediate form.

Fischer's Synthetic Coproporphyrin 3. (a) Specimen No. 5[46] Table II (lit. m.p. 150–153°[47]).—Its m.p. as received was essentially unchanged after filtering a chloroform solution through alumina and crystallizing from chloroform–methanol. The m.p. is too high for irrationally synthesized material.[7]

(b) Specimen 6,[48] Table II.—Labeled "Coproporphyrin 3 methyl ester, m.p. 133°, Hiernis, presumably not quite pure" was presumably irrationally synthesized.[7] Recrystallization either once or twice from chloroform–methanol left 0.7 mg./ml. in the methanol.

The behavior of mixtures. (a).—The mixed m.p. of coproporphyrin 4 methyl ester and coproporphyrin 3 methyl ester (specimen 3a) was 145–160°. Equal weights of the two crystallized together from acetone–methanol gave homogeneous glistening prismatic needles (85% recovery, 0.3 mg./ml. remained in the methanol), m.p. 138–141° after sintering from 133°, remelt 134–147°. Except for some lines in common with coproporphyrin 4 methyl ester, the X-ray powder photograph was distinctive.

(b).—The methyl esters of the coproporphyrins 1,2,3 and 4 were crystallized together in the ratio 1:1:4:2 from chloroform–methanol giving various types of needles (90% recovery, 0.3 mg./ml. remained in the methanol), m.p. 152–240°.

(c).—Equal weights of the coproporphyrin 3 and 4 methyl ester copper complexes (mixed m.p. 190–215°) were crystallized together from chloroform–methanol giving homogeneous bent hair-like crystals sintering at 188° and melting at 191–204° (mostly 193–194°). The X-ray powder photograph was very similar to that of the pure coproporphyrin 3 derivative, the differences being chiefly in the intensities.

(44) We are grateful to Professor C. H. Gray for this specimen.
(45) O. Kennard and C. Rimington, *Biochem. J.*, **55**, 105 (1953).
(46) We are grateful to Professor C. Rimington for this specimen.
(47) C. Rimington, *Proc. Roy. Soc.* (*London*), **B127**, 106 (1939).
(48) We are grateful to Professor A. Treibs for this specimen.

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Pyrromethanes and Porphyrins Therefrom[1]

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The three 5,5′-free pyrromethanes Ic, IIc and IIIc related to porphobilinogen were synthesized. Their behavior did not support the assumption that pyrromethanes without stabilizing groups are two unstable to work with. At room temperature they condensed with the 5,5′-diformyl derivative Ie to the pure uroporphyrins 2, 4 and 3 (55–65%) the choice of the acid catalyst being critical. Formally this porphyrin synthesis is comparable to those from pyrromethenes; while less sensitive to the nature of the substituents, it is more limited by symmetry. Some related porphyrin syntheses were also investigated.

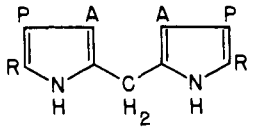
Among the intermediates in the biosynthesis of heme, those between porphobilinogen and uroporphinogen 3 appear least amenable to accumulation, isolation and characterization by current biochemical and analytical methods. It is natural to assume that these intermediates are pyrromethanes and polypyrranes, which are in the right state of oxidation. Their structures, like those of the porphyrins, could only be determined by synthesis. Comparable substances were unknown and thought to be very unstable. We synthesized some related pyrromethanes to clarify the analytical problem and to provide possible intermediates for testing in appropriate enzyme systems. Further study of these has been concerned with the development of porphyrin syntheses.

Dipyrrylmethane itself and derivatives with negative or hydroxyl groups on the nuclei are well known and stable; both N-[5] and *meso*-substituted derivatives[6,7] have also been described. However, the only known derivative without such groups, 3,3′-diethyl-4,4′-dimethylpyrromethane,[8] has been ignored in the belief that instability made work with such compounds impracticable.[6,9] Apparently the uncertain behavior of the stabilized pyrromethanes in porphyrin synthesis[10,11,12] discouraged interest in the unstabilized ones whose preparation had usually been attempted in acid media which minimized their stability.

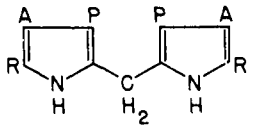
Many simple pyrroles lose their ring carboxyl groups on heating with aqueous sodium hydroxide, and 3,3′-diethyl-4,4′-dimethylpyrromethane (but not its symmetrical isomer[13]) had also been obtained thus from its 5,5′-dicarboxylic acid in low and uncertain yield.[8] We found that Ia was too insoluble to react with 10% sodium hydroxide at

(1) Issued as N.R.C. 5724. Brief accounts of this work have appeared.[3,4]
(2) National Research Council of Canada Postdoctorate Fellow.
(3) S. F. MacDonald, THIS JOURNAL, **79**, 2659 (1957).
(4) S. F. MacDonald and K. H. Michl, *Angew. Chem.*, **70**, 54 (1958).
(5) A. H. Corwin and W. M. Quattlebaum, THIS JOURNAL, **58**, 1081 (1936).
(6) A. Treibs and A. Scherer, *Ann.*, **577**, 139 (1952).
(7) H. Fischer, A. Schormuller and R. E. Windeckei, *ibid.*, **498**, 284 (1932).
(8) H. Fischei, P. Halbig and B. Wallach, *ibid.*, **452**, 268 (1927).
(9) H. Fischer and H. Orth, "Die Chemie des Pyrrols," Leipzig, 1934 and 1937, Vol. I, p. 333; II/1, p. 4.
(10) H. Fischer and J. Hiernis, *Z. physiol. Chem.*, **196**, 155 (1931). Note also the redistribution reactions of partially stabilized pyrromethanes in ethanolic hydrochloric acid, H. Fischer and H. J. Riedl, *ibid.*, **207**, 193 (1932).
(11) H. Fischer and C. E. Staff, *ibid.*, **234**, 97 (1935).
(12) H. Fischer and P. Halbig, *Ann.*, **447**, 123 (1926).
(13) H. Fischer and G. Stangler, *ibid.*, **459**, 53 (1927).

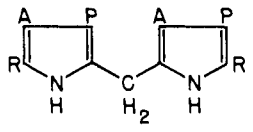
A = CH_2COOH P = CH_2CH_2COOH



I



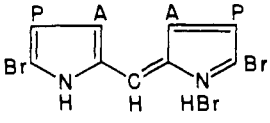
II



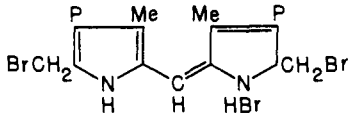
III

a, R = COOH (hexa-ethyl ester)
b, R = COOH
c, R = H

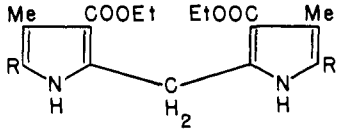
d, R = H (tetramethyl ester)
e, R = CHO (tetramethyl ester)



IV

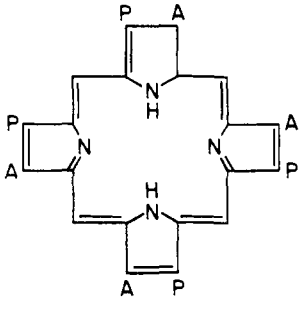


V

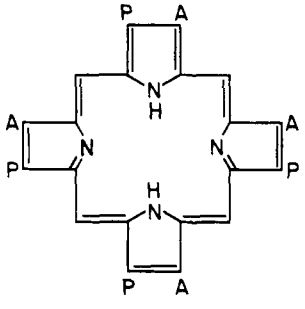


VI a, R = H

VI b, R = CHO



UROPORPHYRIN 2



UROPORPHYRIN 4

170° but Ib, usually in the presence of hydrazine as a stabilizer,[14] gave Ic which was precipitated by acid. The decarboxylations of IIb and IIIb were analogous, but the greater solubility of IIIc[15] and particularly of IIc necessitated the removal of sodium by ion exchange. A second route to Ic was found in the reduction of IV, obtained from Ib with bromine,[16] with sodium amalgam.

It remained to be shown that Ic, IIc and IIIc were amenable to further development, particularly that their bridges were sufficiently resistant to oxidation and redistribution reactions in acid media. Here the synthesis of uroporphyrins was appropriate, particularly that of uroporphyrin 2, the highest melting and otherwise the best characterized of the uroporphyrins, and that of uroporphyrin 3 which had been obtained in inadequate yield by Fischer's general methods.[15]

Following a familiar pyrromethene synthesis, uroporphyrin 2 (21%) was obtained from Ic with formic acid and hydrogen bromide at 100°. This was not studied further because, like related syntheses of other porphyrins employing a pyrromethane,[17] a tetrapyrrane[18] or a pyrromethene,[19] it would, couple unambiguously only identical symmetrical components.

The condensation of Ic with V in hot acetic acid–sodium acetate, conditions used in the synthesis of pyrromethanes,[15] led to 2,3-dimethylporphin-6,7-diacetic acid-1,4,5,8-tetrapropionic acid, isolated as the methyl ester (33%). Paper chromatography and degradation to coproporphyrin 2 showed that this was a pure hexacarboxylic acid of type 2. Related hexacarboxylic acids occur naturally.[20]

Although Ic was too insoluble, its ester Id was converted into the aldehyde Ie by hydrogen cyanide and hydrogen chloride. Experiments followed spectroscopically indicated that Ic or Id condensed with Ie in the presence of hydrobromic acid to a "pyrromethene" (presumably β,δ-dihydro-uropor-

(14) Compare A. Tiebs and R. Schmidt, *Ann.*, **577**, 105 (1952).

(15) This intermediate and uroporphyrin 3 are discussed in detail elsewhere: E. J. Tarlton, S. F. MacDonald and E. Baltazzi, THIS JOURNAL, **82**, 4389 (1960).

(16) S. F. MacDonald and K. H. Michl, *Can. J. Chem.*, **34**, 1768 (1956).

(17) J. S. Andrews, A. H. Corwin and A. G. Sharp, THIS JOURNAL, **72**, 491 (1950).

(18) A. H. Corwin and E. C. Coolidge, *ibid.*, **74**, 5196 (1952).

(19) H. Fischer and W. Lamatsch, *Ann.*, **462**, 240 (1928).

(20) T. C. Chu and Edith Chu, *J. Biol. Chem.*, **234**, 2741 ff. (1959).

phyrin 2) which was more slowly oxidized by air to pure uroporphyrin 2 isolated as the methyl ester ($\leqslant 45\%$).

In all the above syntheses, Ic behaved as a reliable intermediate. However, with formic acid and air at 40°, it gave a mixture of uroporphyrins (see below). As the other pyrromethanes were apparently less stable, we sought optimal conditions for the porphyrin synthesis from Ic and Ie so that the reliability of IIc and IIIc would be realistically assessed by analogous syntheses.

It was remarkable that no porphyrin had been obtained from VIa and VIb with perchloric acid, naturally one of the first porphyrin syntheses attempted.[21] Analysis of the product had suggested that the second phase of the condensation, ring closure, had not taken place. Perchloric acid is suitable for the analogous synthesis of pyrromethenes[21] and the reactivity of VIa is beyond question.[17] The contrasting results with hydrobromic and with perchloric acid suggested that the perchlorate ion was not sufficiently nucleophilic for these porphyrin syntheses and that hydrogen iodide should be the best reagent.

Conditions for this porphyrin synthesis at room temperature were finally standardized: the condensation being carried out in acetic acid containing 0.4% hydrogen iodide, the subsequent oxidation by aerating after adding sodium acetate. This minimized the influence of mineral acid which might promote side reactions[10] leading to isomeric uroporphyrins, and resulted in the pure porphyrins being precipitated completely. Under these standard conditions the uroporphyrins 2, 3 and 4 (65%, 60% and 55%) were obtained from Ie with Id, IIIc and IIc. Added perchloric acid did not decrease the yield of uroporphyrin 2. However, substituting other acids for hydrogen iodide led to a 7% yield of uroporphyrin 2(?) using perchloric acid and a 14% yield of uroporphyrin 3 using hydrogen chloride.

The importance of the choice of acid was confirmed when VIa and VIb were converted to 1,4,5,8-tetramethyl-2,3,6,7-tetracarbethoxyporphin (65%) under the standard conditions; when perchloric acid replaced hydrogen iodide the yield was 2% of a product containing only 30% of porphyrin. The condensation of Ie with VIa using hydrogen iodide gave 1,4-dimethyl-2,3-dicarbethoxyporphin-6,7-diacetic acid-5,8-dipropionic acid tetramethyl ester (40%). In the absence of direct evidence, the type purity of these latter porphyrins can only be inferred from that of the uroporphyrins prepared from more labile pyrromethanes.

It is interesting historically that the first synthetic porphyrin was recognized only spectroscopically in the product of the reaction between VIb and hydrochloric acid.[22] We repeated this to isolate the tetramethyltetracarbethoxyporphin (8%) and show that the reported spectrum[12] had demonstrated its presence. The earlier failure to isolate it is probably explained by its then unprecedented insolubility.

The porphyrin synthesis from a 5,5′-free and a 5,5′-diformylpyrromethane may be tentatively compared with other general methods. The more easily assessed factors are considered first. There had been only two general methods of porphyrin synthesis which consistently led to products which were not grossly contaminated with isomers: from the hydrobromides of a 5,5′-dimethylpyrromethene and a 5,5′-dibromopyrromethene or from the hydrobromides of two 5-bromo-5′-methylpyrromethenes, preferably at 160–200°, the yields being 0.1–45%. When the 5-methyl groups could be brominated, the resulting 5-bromomethylpyrromethenes sometimes gave higher yields and permitted variations employing 5-free or 5-carboxypyrromethenes as the other component. There were restrictions imposed by the substituents due to their lability or to their effect on the yield. The two methods employing pyrromethenes were restricted by symmetry in different ways but this restriction on the second of them, evident in the mesoporphyrins 7, 10 and 15,[23] might have been avoided by the synthesis of porphyrins with the side-chains initially modified to make the separation of by-products possible.[24] Two further syntheses from pyrromethenes had led to pure isomers but required 5-free pyrromethenes which were not available by general methods.[19,25]

The synthesis from a 5,5′-free and a 5,5′-diformylpyrromethane is formally comparable in generality to the first of the above methods. Both require one symmetrical component to avoid ambiguity and are thus restricted to type 2 (two routes), type 3 (four routes) and type 4 (two routes) porphyrins. However, the synthesis from pyrromethenes requires either one of two isomeric pyrroles for a type 4 porphyrin but both isomers for a type 2 porphyrin; in this synthesis from pyrromethanes the reverse is true. The limited data suggest that higher and more consistent yields may be expected by the pyrromethane method, particularly in the presence of labile or negative side-chains. Thus the uroporphyrins 2, 3 and 4 were obtained from pyrromethenes (at 135° to avoid partial decarboxylation) in yields of only 4,[16] 0.9[15] and 3%,[16] respectively. Similarly, porphyrins with one ring carboxyl group had been synthesized from pyrromethenes under Fischer's conditions ($\leqslant 6\%$) and the above 1,4,5,8-tetramethyl-2,3,6,7-tetracarbethoxyporphin (7%) under a modification of these conditions.[26]

In comparing these as general synthetic methods the purity of the products is perhaps the more important and certainly the more difficult question. Although the properties of the uroporphyrins 3 and 4 are not characteristic (see Experimental and the discussion of uroporphyrin 3[15]), the uroporphyrins 2, 3 and 4 (from Ie with Id, IIIc and IIc) as well as the derived coproporphyrins appear to be as pure as any synthesized from pyrromethenes,[15,16,27] particularly as judged by the consistently high m.p.'s of the coproporphyrin methyl esters and copper

(21) H. Fischer and H. Orth, ref. 9, II/1, p. 2.

(22) H. Fischer and H. Orth, ref. 9, II/1, pp. 163, 178.

(23) For the nomenclature of mesoporphyrins see H. Fischer and H. Orth, ref. 9, II/1, p. 434.

(24) H. Fischer and A. Rothhaas, *Ann.*, **484**, 85 (1930).

(25) H. Fischer and H. Berg, *ibid.*, **482**, 189 (1930).

(26) G. G. Kleinspehn, A. E. Briod and W. S. Caughey, Abstracts of the 135th Meeting of the American Chemical Society, April, 1959, p. 35-O.

(27) F. Morsingh and S. F. MacDonald, THIS JOURNAL, **82**, 4377 (1960).

complexes. We have discussed the syntheses from dipyrrylmethenes in general and all intermediates except unstabilized pyrromethanes.[15,27] In general, unstabilized pyrromethanes may be expected to be less stable than pyrromethenes but more easily purified and characterized and converted to porphyrins under milder conditions. Particularly in the case of uroporphyrin 2 there are grounds for believing that no isomers arose through side-reactions of the pyrromethanes. Neither Ic, Id nor Ie alone gave any porphyrin under the standard conditions for porphyrin synthesis although IIIc gave 0.8%. Further, when these uroporphyrin syntheses were carried out under less favorable conditions in yields of about 10%, essentially pure isomers were again obtained directly. However work on uroporphyrin 4 indicated that IIc was much less stable than its isomers in warm acetic acid. This suggests that some analogous pyrromethanes may be even less stable in acid. If so, the generality of this synthesis would be limited by the decreasing yield or purity of the porphyrins. It would appear that dipyrryl-ketones should be more reliable intermediates than either pyrromethanes or pyrromethenes.

The synthesis from pyrromethanes and their aldehydes would not be generally reliable if the β-substituents rather than the synthetic method determined the purity of the product. Among all the methods which led directly from pyrroles or pyrromethanes to porphyrins, Fischer found one which did not always lead to mixtures of isomers. This was the "cold method," the action of formic acid and air on a 5,5′-dicarboxypyrromethane at 40°, which gave either type 2 porphyrins[11] or mixtures of isomers[10] depending on the β-substituents. The β-substituents characteristic of the uroporphyrins, however, did not exert any such favorable influence. The "cold method" gave low-melting mixtures of uroporphyrin esters: 3% of m.p. 275–290° from Ib, 55% of m.p. 256–258° from IIb,[16] and 43% of m.p. 257–261° when modified by using Ic. In these and the following uroporphyrin syntheses from pyrromethanes the nature of the product was unambiguous and determined by the method of synthesis (which defined the 5,5′-substituents on the pyrromethanes as well as other conditions). Thus Ib and Ic with formic acid and hydrogen bromide at 100° gave, respectively, a uroporphyrin mixture (22%, m.p. <260°) and uroporphyrin 2 (21%, m.p. 307–312°) as noted above. The heterogeneity of the low-melting specimens was confirmed by the recovery and m.p. after recrystallization from chloroform–acetone.

In connection with uroporphyrin 2 we attempted to clear up the polymorphism of coproporphyrin 2 methyl ester. This ester has only one m.p. although solid phase changes are visible on heating it and, except in one instance,[16] it has given the expected infrared mull spectrum and X-ray powder photograph. However, a normal specimen after heating gave X-ray photographs too complex to be rationalized.

Experimental[28]

5,5′-Dicarboxypyrromethane-3,3′-diacetic Acid-4,4′-dipropionic Acid Hexaethyl Ester (Ia).[16]—The following method gave a better yield. The triethyl ester of 2-methyl-5-carboxy-pyrrole-3-acetic acid-4-propionic acid (20 g.) was dissolved in 100 ml. of carbon tetrachloride. Bromine (9.5 g.) in 50 ml. of carbon tetrachloride was added and the mixture warmed to a clear solution while protected from moisture. After exposure to an ultraviolet lamp and sunlight for 20 min., the solvent was removed *in vacuo* below 50°. The crystalline residue was refluxed for 2 hours with 400 ml. of absolute ethanol. Most of the product separated after two days, the mother liquor was evaporated and the residue crystallized from 50 ml. of ethanol; yield 16.3 g. (84%), m.p. 148.5–150°.

5,5′-Dicarboxypyrromethane-3,3′-dipropionic acid-4,4′-diacetic acid hexaethyl ester (IIa)[29] was prepared (87%) just as was the above isomer except that both crystallizations from ethanol were at 0° and the second utilized only 20 ml. of ethanol.

Pyrromethane-3,3′-diacetic acid-4,4′-dipropionic acid (Ic). (**a**).—The hexacarboxylic ester Ia (10 g.) was heated in the steam-bath for 4.5 hr. in an open flask with 100 ml. of ethanol and 50 ml. of 10% aqueous sodium hydroxide. The residue was diluted to 50 ml. with water and heated under pressure with 25 ml. of 10% aqueous sodium hydroxide and 10 drops of hydrazine for 4.5 hr. (air-bath temperature 171–173°) in a 110-ml. Teflon lined screw-capped metal tube. The product was precipitated with sulfur dioxide below 10°, filtered off and washed with water. It was crystallized immediately by dissolving in 250 ml. of de-aerated 80% aqueous acetone under nitrogen and swirling off the acetone under vacuum (bath temp. 20°). It formed nearly colorless microprisms (5.6 g., 92%), m.p. 195–200° dec. depending on the rate of heating, much more soluble in aqueous acetone than in water or acetone, Ehrlich reaction positive cold.

Anal. Calcd. for $C_{19}H_{22}N_2O_8$: C, 56.15; H, 5.46; N, 6.89; neut. equiv., 101.6. Found: C, 56.35; H, 5.57; N, 6.80; neut. equiv., 103.2.

(**b**).—The pyrromethane IV[16] (175 mg. recrystallized from formic acid) in 1.5 ml. of water and 0.5 ml. of 10% aqueous sodium hydroxide was vigorously shaken for 20 min. with sodium amalgam giving a pale yellow solution without visible absorption. The product (74 mg., 64%) was precipitated by sulfur dioxide as colorless micro-needles, m.p. about 200°, Ehrlich reaction positive cold, Beilstein test for halogen positive; identity confirmed by conversion to the methyl ester Id, m.p. and mixed m.p. 106–108°, Beilstein test for halogen negative.

Pyrromethane-3,3′-diacetic acid 4,4′-dipropionic Acid Tetramethyl Ester (Id).—The acid Ic (5.62 g. obtained as under (a) above) was slurried with 25 ml. of methanol, and ethereal diazomethane from 20 g. of nitrosomethylurea added under nitrogen. When the solid had dissolved, the solvent was removed (bath temp. 20°). The residue was dried, then crystallized twice from 500 ml. of hexane (thimble, a second thimble below the first contained Darco). The product (5.12 g., 80%) formed very pale yellow prismatic needles, m.p. 103.5–105°. It is stable in evacuated sealed tubes.

A colorless analytical specimen, m.p. 104.5–105.5°, was obtained from ether.

Anal. Calcd. for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54; N, 6.06. Found: C, 59.58; H, 6.59; N, 6.10.

Pyrromethane-4,4′-diacetic Acid-3,3′-dipropionic Acid (IIc).—The hexacarboxylic acid IIb[16] (750 mg.) was heated under pressure at 170–180° for 4 hours with 7.5 ml. of 10% sodium hydroxide and one drop of hydrazine in a 10-ml. Teflon lined brass tube. The contents of the tube were diluted with water, 8 g. of Amberlite IR-120 (hydrogen form)[30] was added, and carbon dioxide was removed by a stream of nitrogen. The whole was poured onto a column of 11 g. of fresh resin which was then eluted with 500 ml. of water. The removal of water in a rotary evaporator below 20° left an oil which was dried to a glass. This was dissolved under nitrogen in 2 ml. of warm water from which the product (202 mg.) separated as gray micro-prisms, m.p. about 157° dec. Vacuum concentration of the mother liquor gave 78 mg. (total 45%).

(28) Melting points were determined as previously noted.[27] Infrared spectra were by Dr. R. N. Jones and Mr. R. Lauzon; X-ray powder photographs were by Dr. Maria Przybylska.

(29) S. F. MacDonald, *J. Chem. Soc.*, 4184 (1952).

(30) The resin had been shaken with water at 10 mm. and the water was de-aerated likewise.

Anal. Calcd. for $C_{19}H_{22}N_2O_8$: C, 56.15; H, 5.46; N, 6.89; neut. equiv., 101.6. Found: C, 56.41; H, 5.47; N, 6.57; neut. equiv., 100.1.

5,5'-Diformylpyrromethane-3,3'-diacetic Acid-4,4'-dipropionic Acid Tetramethyl Ester (Ie).—A solution of the ester Id (1.03 g.) in 10 ml. of chloroform–ether–hydrogen cyanide (6:6:2 by vol., the solvents being free of ethanol), cooled in an ice–salt-bath and protected from moisture, was saturated with hydrogen chloride and a slow stream of the gas then continued for 2.5 hr. The solvent was removed by a stream of dry air and twice replaced by ether which was removed in the same way. After drying to 10 mm. the residue was dissolved in 70 ml. of ice-water, and 11 g. of $Na_2HPO_4 \cdot 7H_2O$ in 50 ml. of water added. Some of the product separated overnight at 0°, the rest after bringing the mother liquor to boiling then cooling. It was recrystallized from 30 ml. of 60% ethanol (Darco), then by extraction with acetone. It formed nearly colorless plates (830 mg., 72%), m.p. 207–208° after softening from 204°.

Anal. Calcd. for $C_{25}H_{30}N_2O_{10}$: C, 57.91; H, 5.83; N, 5.40 Found: C, 57.85; H, 5.73; N, 5.34.

Porphin-1,4,5,8-tetraacetic Acid-2,3,6,7-tetrapropionic Acid Octamethyl Ester (Uroporphyrin 2 Methyl Ester).[16] (a).—The pyrromethane Ic (35 mg.) was heated for 1 hour on the steam-bath with 1/3 ml. of 98% formic acid and 1/6 ml. of hydrogen bromide in acetic acid. The solution was made alkaline, heated, and acidified with acetic acid. The precipitate was esterified and worked up in the usual way giving an amorphous product (9.5 mg.), m.p. 305–310° from chloroform–methanol. Recrystallized from 1 ml. of chloroform and 10 ml. of acetone it formed the characteristic crystals (8.7 mg., 21%), m.p. 307–312° (uncor.) with decomposition (lit.[16] 312–315°). Its X-ray power photograph was identical with those previously obtained and degradation led to coproporphyrin 2 methyl ester, m.p. 284–286° (uncor.) (lit.[19] 288°).

(b).—The esters Id (46 mg.) and Ie (52 mg.) were separately dissolved in warm acetic acid and the cooled solutions combined and diluted to 50 ml. with acetic acid. Fifteen ml. of acetic acid containing 0.3 ml. of 56% hydriodic acid (colorless, stabilized with hypophosphorous acid) was added. After 10 min., 1 g. of anhydrous sodium acetate dissolved in 15 ml. of acetic acid was added, and air was passed in through a sintered glass disk for 24 hr., light being excluded. The product was filtered off, washed with acetic acid, dried, and left overnight in 5% methanolic hydrogen chloride. The solution was poured into chloroform and ice-water. The lower layer was well washed with water, dried, and filtered through a short column of alumina (Grade V), eluting with chloroform containing 5% of methanol. The eluate was filtered and concentrated. The chloroform was then boiled off while adding methanol to precipitate the product (62 mg.) as curly needles, which were recrystallized by dissolving in 5 ml. of hot chloroform, adding 50 ml. of hot acetone and cooling slowly to obtain the characteristic sheaves of hair-like needles (61 mg., 65%), m.p. 310–313° (uncor., solid phase changes apparent at about 220° and near the m.p.). The X-ray powder photograph and infrared mull spectrum were identical with those of the product synthesized from pyrromethenes.[16]

Anal. Calcd. for $C_{48}H_{54}N_4O_{16}$: C, 61.14; H, 5.77; N, 5.94; OMe, 26.33; C–Me, 0.00. Found: C, 60.89; H, 5.52; N, 6.09; OMe, 26.42; C–Me, 0.00.

Degradation to Coproporphyrin 2 Methyl Ester.—Acetic acid (1 ml.) and 1% hydrochloric acid (5 ml.) were added to 15 mg. of the uroporphyrin II ester of method (b) in a bomb-tube. The mixture was frozen and the tube sealed off under vacuum (0.01 mm.). The tube was warmed, shaken, then heated for 4 hours at 185–190°. The contents of the tube were washed out and the product precipitated hot using sodium hydroxide then dibasic sodium phosphate. It was centrifuged off, dried, esterified, and worked up using an alumina column as was the uroporphyrin. The crystals (9.2 mg. 81%), m.p. 285–287°, which separated from methanol displacing chloroform, were recrystallized from the same solvents giving 8.6 mg., m.p. 287–289° (uncor.) after phase changes at about 90° (questionable), 170° and 270° as expected.[16] Its X-ray powder photograph was identical with those of the two specimens previously obtained from uroporphyrin 2.[16] The photographs of this specimen after heating to 220° or to 283° were too complex to be rationalized in terms of distinct polymorphic forms.

(c).—When the reaction was carried out using hydrogen iodide as under (b) but in acetic acid–acetic anhydride (4:1), the product (6%), m.p. 308–315° (uncor.), could be recrystallized from chloroform–acetone with >90% recovery.

(d).—The esters Id (11 mg.) and Ie (12.7 mg.) in 12 ml. of acetic acid were treated with 4 ml. of acetic acid containing 0.04 ml. of 70% perchloric acid. After 20 min., 0.25 g. of sodium acetate in 4 ml. of acetic acid was added, and the solution aerated as under (b) above. Filtration gave 1.5 mg. (7%) of the product determined spectroscopically.

(e).—The esters Id (19.2 mg.) and Ie (21.8 mg.) in 22 ml. of acetic acid were treated with 6 ml. of acetic acid containing 0.06 ml. of 70% perchloric acid and 0.12 ml. of 56% hydriodic acid. The subsequent steps as under (b) gave 29 mg. (75%), m.p. 306–311° (uncor.), 80% of which was recovered after crystallization from chloroform–acetone.

Porphin-1,4,6,7-tetraacetic Acid-2,3,5,8-tetrapropionic Acid Octamethyl Ester (Uroporphyrin 4 Methyl Ester).[16]—The aldehyde Ie (100 mg.) was warmed to solution in 30 ml. of acetic acid and cooled. The pyrromethane IIc (78 mg.) was dissolved in 100 ml. of acetic acid *at room temperature*. The two solutions were mixed and 30 ml. of acetic acid containing 0.6 ml. of colorless 56% hydriodic acid added. After 30 min., 2 g. of anhydrous sodium acetate in 30 ml. of acetic acid was added. Subsequent operations followed method (b) for uroporphyrin 2. The amorphous product (100 mg., 55%) separated from chloroform–methanol, m.p. 255–258° after sintering and largely changing to flat crystals from 252° (lit.[16] 255.5–259.5°). From benzene–heptane it separated either amorphous or as hair-like micro-crystals both melting at 254–257° after sintering and largely changing to flat crystals from 248°. It was also characterized by its infrared spectrum in chloroform.

The crystal forms from both solvent pairs are uncertain. The product from pyrromethenes (from acetone[16]) and the above two from chloroform–methanol and benzene–heptane gave indistinct X-ray powder photographs and diffuse infrared mull spectra (max. at 1165 >, =, and < max at 1200 $cm.^{-1}$, respectively). After being heated to 245° the above specimen from chloroform–methanol and that from pyrromethenes[16] gave identical X-ray powder photographs.

Degradation to Coproporphyrin 4 Methyl Ester.—Uroporphyrin 4 methyl ester (10 mg.) was degraded exactly as the uroporphyrin 2 ester except for the crystallization of the coproporphyrin ester. After the chloroform was thoroughly displaced, the methanol was concentrated to 1 ml. and left one day. Coproporphyrin 4 methyl ester (72% after recrystallization) was obtained as glistening needles. The methyl ester, m.p. 185.5–188.5° after sintering at 177°, and its copper complex, m.p. 233–235°, melted consistently higher than the reference specimens (m.p. 182–186° and 230–233°[27]), mixed m.p.'s 183–188° and 233–235° after sintering from 231°. The X-ray powder photographs of the methyl ester and its copper complex were identical with those of the reference specimens. The infrared mull spectra of the methyl esters differed only in the absence of a small modular maximum at 1245 $cm.^{-1}$ in that of the reference specimen.

When the uroporphyrin 4 synthesis was carried out in the same way except that the pyrromethane IIc was *warmed* to solution in acetic acid, the yield was consistently 10 to 12%. The coproporphyrin 4 methyl ester obtained on degradation had the expected m.p., undepressed by the reference specimen, but its X-ray powder photograph was unique.

Mixtures of Uroporphyrins. (a).—The pyrromethane Ib (50 mg.) was heated on the steam-bath for 1 hour with 0.5 ml. of the solution obtained from two volumes of 98% formic acid and one volume of 30% hydrogen bromide–acetic acid. Water was then added and the porphyrin was precipitated at *p*H 4–5. The usual esterification and isolation gave needles mixed with amorphous material (10 mg. 22%), m.p. about 250–260°. Recrystallization from chloroform–acetone gave 3.3 mg., m.p. 302–307°.

(b).—The pyrromethane Ic (100 mg.) was slurried with 98% formic acid and maintained at 40° for 1 week while passing in air. The crude porphyrin was isolated, esterified, and worked up as usual giving 50 mg. (43%), m.p. 257–261°. Recrystallization from chloroform–acetone gave 7 mg., m.p. 290–300°.

1,4,5,8-Tetramethylporphin-2,3,6,7-tetracarboxylic Acid Tetraethyl Ester.[17,26] (a).—Acetic acid (8 ml.) containing 0.16 ml. of 56% hydriodic acid was added to a solution of 21 mg. of the pyrromethane VIa and 25 mg. of the corresponding aldehyde VIb[12] in 25 ml. of acetic acid. After 30

min., 0.5 g. of sodium acetate in 10 ml. of acetic acid was added and air passed in for 24 hr. to precipitate the crystalline product (29 mg., 66%). Its solution in 250 ml. of chloroform was filtered through alumina (Grade V) and the filtrate concentrated to 30 ml. The crystals which separated were dissolved in 60 ml. of hot chloroform. After concentrating to 30 ml. and cooling, flat needles (23 mg.), m.p. >350°, separated from the solution. The visible spectrum of a chloroform solution recorded spectrophotometrically was of the "etio" type in contrast to that noted visually[12]: maxima (and ϵ) at 521 mμ (13×10^3), 558 mμ (7.1×10^3), 598 mμ (5.1×10^3) and 655 mμ (4.5×10^3).

Anal. Calcd. for $C_{36}H_{38}N_4O_8$: C, 66.04; H, 5.85; N, 8.56; OEt, 27.53. Found: C, 66.24; H, 5.90; N, 8.63; OEt, 27.23.

(b).—When this synthesis was carried out in the same way except that an equivalent amount of 70% perchloric acid was used instead of the hydriodic acid, aeration precipitated about 1 mg. (2%) of a product shown spectroscopically to contain 0.27 mg. of porphyrin.

(c).—The aldehyde VIb (102 mg.) was heated with concentrated hydrochloric acid on the steam-bath. The precipitate was dissolved in chloroform, the solution filtered through a column of deactivated alumina and concentrated. The product which crystallized (7 mg.) had the same visible spectrum in chloroform as did that of method (a) above. In acetic acid–hydrochloric acid both had strong bands at 626 and 575 mμ, a very weak one at 530 mμ. Evidently Fischer observed the first two bands and a third band due to an impurity.[12]

1,4-Dimethyl-2,3-dicarbethoxyporphin-5,8-dipropionic Acid-6,7-diacetic Acid Tetramethyl Ester.—The pyrromethanes VIa (16 mg.) and Ie (26 mg.) were dissolved in 25 ml. of acetic acid, and acetic acid containing 0.16 ml. of 56% hydriodic acid was added. After 20 min., 0.5 g. of anhydrous sodium acetate was added. Aeration precipitated 16 mg. (40%) of the crystalline product, m.p. 250.5–251.5°.

Anal. Calcd. for $C_{42}H_{46}N_4O_{12}$: C, 63.15; H, 5.80; N, 7.01; alkoxy (as OMe), 23.31. Found: C, 62.90; H, 5.77; N, 6.89; alkoxy (as OMe), 22.38.

2,3-Dimethylporphin-6,7-diacetic Acid-1,4,5,8-tetrapropionic Acid Hexamethyl Ester.—A solution of 175 mg. of the pyrromethene hydrobromide V,[31] 122 mg. of the pyrromethane Ic and 300 mg. of anhydrous sodium acetate in 200 ml. of acetic acid was heated on the steam-bath for 1 hour. The cooled solution was then aerated for 1 hour. The crystalline but impure product (100 mg.) was separated and washed with acetic acid, then with ether. It was esterified with methanolic hydrogen chloride, brought into chloroform, the solution filtered through deactivated alumina and the chloroform replaced by methanol. Crystallized again from chloroform–methanol it formed tiny bent hairs (82 mg., 33%), m.p. 233–238° after sintering and a solid phase change from 230°. Paper chromatography[32] of the free acid showed only the hexacarboxylic acid spot. It was degraded to coproporphyrin 2 methyl ester, m.p. 285–287° (uncor.).

Anal. Calcd. for $C_{44}H_{50}N_4O_{12}$: C, 63.91; H, 6.10; N, 6.78; OMe, 22.52; C-Me, 3.64. Found: C, 64.09; H, 6.19; N, 6.87; OMe, 22.34; C-Me, 3.19.

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[CONTRIBUTION FROM THE DIVISION OF PURE CHEMISTRY, NATIONAL RESEARCH COUNCIL OF CANADA, OTTAWA 2, CANADA]

Uroporphyrin 3[1]

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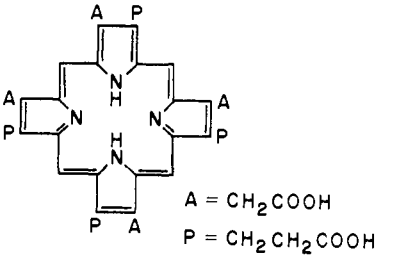
Uroporphyrin 3 was rationally synthesized both from pyrromethanes (60%) and conventionally from pyrromethenes (≤0.9%). Degradation to coproporphyrin 3 established its type purity. Uroporphyrin 3 methyl ester, synthetic or from turacin, was obtained in two crystalline forms neither of which was clearly distinguished as such from the corresponding forms of some mixtures of uroporphyrin isomers.

The structure of uroporphyrin 1,[3] proved analytically and by synthesis,[4] defines that of uroporphyrin 3. The naturally occurring isomer of uroporphyrin 1 which decarboxylates to coproporphyrin 3 is considered to be uroporphyrin 3, and its role in the biosynthesis of porphyrins from porphobilinogen supports this assumption as to the nature of its side-chains. The uroporphyrins 2 and 4 had also been rationally synthesized[5,6] and a non-rational synthesis of uroporphyrin 3[7] is discussed below.

Because natural sources of uroporphyrin 3 are inadequate or unreliable, synthetic uroporphyrin 3 was necessary both as reference material and as a substrate in biochemical work. It was also desirable to confirm formally the structure of natural uroporphyrin 3 by comparison with the synthetic product.



UROPORPHYRIN 3

We first attempted a synthesis of uroporphyrin 3 analogous to that of coproporphyrin 3,[8,9] but we failed to obtain the necessary unsymmetrical 5,5′-dimethylpyrromethene.[5] The only alternative suggested by Fischer's general methods was to use the 5,5′-dibromopyrromethene VII as the unsymmetrical component. Chart 1 shows the synthesis of the unsymmetrical pyrromethane intermediate IV.

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(2) National Research Council of Canada Postdoctorate Fellow.

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