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[17]: 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.

(31) H. Fischer, H. Friedrich, W. Lamatsch and K. Morgenroth, *Ann.*, **466**, 147 (1928).

(32) Through the kindness of Dr. T. C. Chu.

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

Uroporphyrin 3[1]

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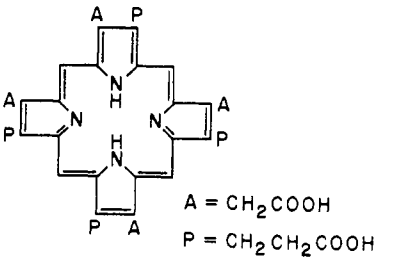
Uroporphyrin 3 was rationally synthesized both from pyrromethanes (60%) and conventionally from pyrromethenes (≤0.9%). Degradation to coproporphyrin 3 established its type purity. Uroporphyrin 3 methyl ester, synthetic or from turacin, was obtained in two crystalline forms neither of which was clearly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

(1) Issued as N.R.C. No. 5725. This work was reported at the 133rd Meeting of the American Chemical Society, San Francisco, Calif., April, 1958.

(2) National Research Council of Canada Postdoctorate Fellow.

(3) H. Fischer and H. J. Hofman, *Z. physiol. Chem.*, **246**, 15 (1937).

(4) S. F. MacDonald and R. J. Stedman, *Can. J. Chem.*, **32**, 896 (1954).

(5) S. F. MacDonald and K. H. Michl, *ibid.*, **34**, 1768 (1956).

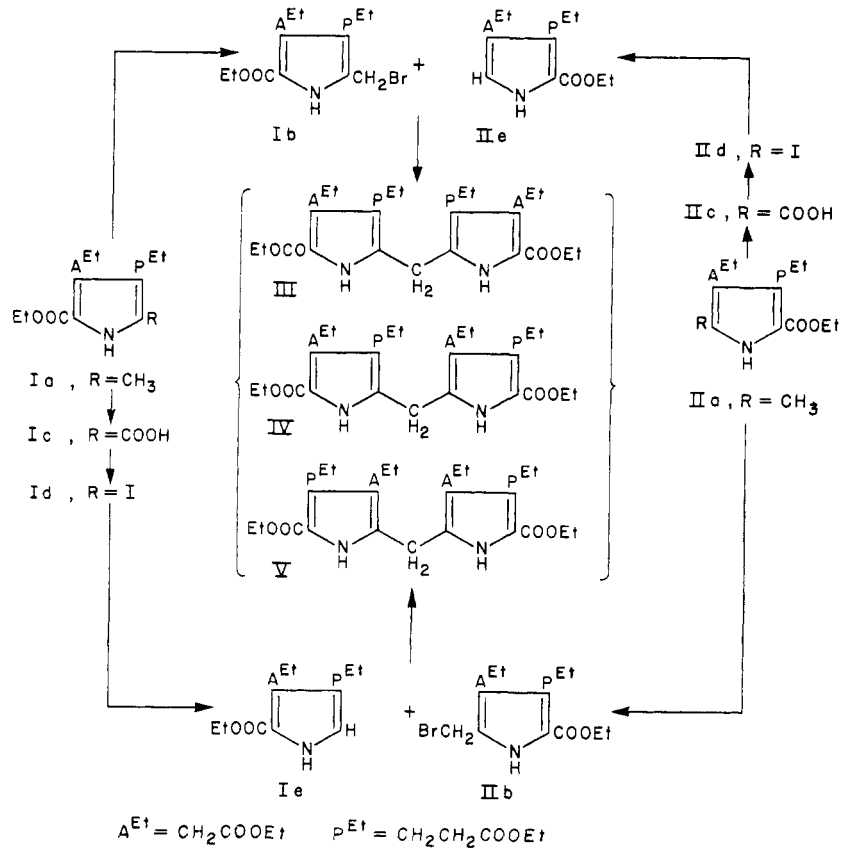
(6) G. P. Arsenault, E. Bullock and S. F. MacDonald, THIS JOURNAL, **82**, 4384 (1960).

(7) A. Treibs and W. Ott, *Naturwissenschaften*, **40**, 476 (1953); *Ann.*, **615**, 137 (1958).

(8) H. Fischer, K. Platz and K. Morgenroth, *Z. physiol. Chem.*, **182**, 265 (1929).

(9) H. Fischer and J. Hiernis, *ibid.*, **196**, 155 (1931).

CHART 1



We had already brominated Ia to Ib which gave III in boiling ethanol, and converted IIa into IIb and V analogously.[5,6] The methyl group of Ia had also been removed by conversion to carboxy using sulfuryl chloride, followed by thermal decarboxylation to Ie.[10] The last step was improved by converting Ic to Id with iodine, followed by catalytic hydrogenation to Ie[11] and the same sequence was used to convert IIa through IIc and IId into IIe. Alternatively Ie was obtained from Ic over the bromo analog of Id.

Experiments preliminary to the synthesis of the unsymmetrical pyrromethane IV showed that the symmetrical isomer V resulted when IIb and IIe were refluxed together in benzene. Iodide was not promising as a catalyst because both the bromomethylpyrroles Ib and IIb were rapidly converted into the corresponding symmetrical pyrromethanes III and V, respectively, by potassium iodide in refluxing acetone.

When either Ib and IIe or IIb and Ie were refluxed together in benzene, IV resulted together with small amounts of the symmetrical isomers III and V. The resulting mixture, like artificial ones, could be separated on alumina; V, IV and III were eluted in that order. Although this defined the pure isomer IV it was unsatisfactory as a preparative method, much being lost on the column in larger runs. The rate of elution, as opposed to the order, may be greatly influenced by other impurities and consequently we have not found standard conditions for the separation of the components from the crude mixture by chromatography.

More recently IIb and Ie were condensed in acetic acid–sodium acetate and IV purified by crystallization, recrystallizing if necessary until the crystals developed from only 1 or 2 centers. It was shown that this would have removed III or V from the product if these had been present. No V was then detected by chromatography on alumina although it was recovered after 1% of it had been added; here again standard chromatographic conditions were not found but undue losses were avoided. The solubilities decreased in the order III > IV > V, and IV was easily separated from an equal weight of III by crystallization. The three isomers III, IV and V were also characterized by their infrared spectra in carbon disulfide and as mulls. There is no evidence that they form mixed crystals (solid solutions).

As shown in Chart 2, IV was hydrolyzed to VI which could be brominated to the required unsymmetrical pyrromethene VII although the usual conditions were ineffective. Uroporphyrin 3 ($\leqslant 0.9\%$) was obtained from VII with either of the known symmetrical pyrromethenes VIII or IX[12] in methylsuccinic acid at 135°. The relatively low temperature was necessary to avoid partial decarboxylation of the product. It may be noted that Fischer's general methods imply six pairs of such porphyrin syntheses: one pair for type 1 porphyrins, two for type 2, two for type 3 and one for type 4. All but the above pair had, in principle, already been used. A convenient preparation of methylsuccinic acid is recorded.

The two syntheses from pyrromethenes, particularly that using VII and IX, were impracticable as preparative methods and the yields were still lower in larger runs. However, uroporphyrin 3 was obtained in 60% yield by the rational synthesis from pyrromethanes[6]: the decarboxylation of VI in alkali led to X which with XI in acetic acid containing hydriodic acid gave uroporphyrin 3 after oxidation.

The purity of the uroporphyrin 3 synthesized from the pyrromethenes should depend largely on that of VII; that of an analogous intermediate has been discussed.[13] As the pyrromethanes were

(10) D. M. MacDonald and S. F. MacDonald, *Can. J. Chem.*, **33**, 573 (1955).

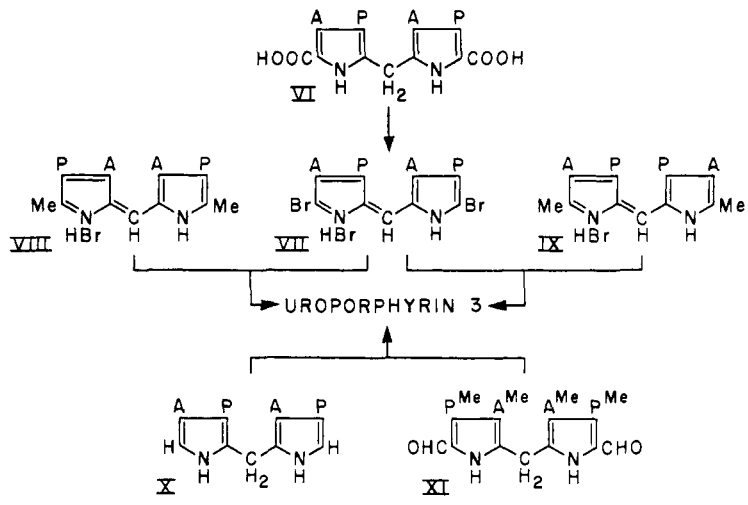
(11) Compare G. G. Kleinspehn and A. H. Corwin, THIS JOURNAL, **76**, 5641 (1954).

(12) S. F. MacDonald and R. J. Stedman, *Can. J. Chem.*, **33**, 458 (1955).

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

apparently pure, any isomers in the uroporphyrin 3 from X and XI should be irrational products of the porphyrin synthesis. The pyrromethane X alone, unlike XI,[6] gave 0.9% by weight of uroporphyrin under our standard conditions for the porphyrin synthesis. The effect of this might be halved because random synthesis favors type 3 and again halved by the competition of the rational synthesis. When X and XI were condensed to uroporphyrin 3 using hydrogen chloride instead of hydrogen iodide the yield was 21% crude, 14% pure. Thus insoluble by-products, apparently favored by the less nucleophilic hydrogen chloride, contaminated the crude product. The coproporphyrin 3 ester from the uroporphyrin, although definitely identified as such, melted about 3° too low. It is uncertain whether the isomeric uroporphyrins responsible for this arose during the condensation or during the esterification, through the action of methanolic hydrogen chloride on the by-products.

CHART 2



A = CH_2COOH, A^{Me} = CH_2COOMe, P = CH_2CH_2COOH, P^{Me} = CH_2CH_2COOMe

The uroporphyrin 3 methyl ester from X and XI using hydrogen iodide was thoroughly characterized. It separates in one form (partly amorphous) from acetone–methanol or chloroform–methanol and in another form from benzene–heptane, the two forms having similar m.p.'s but giving different infrared mull spectra and X-ray powder photographs. However, these criteria (see below) can give little indication of its purity which was established by paper chromatography and more definitely by comparison of the derived coproporphyrin 3 with rationally synthesized reference material.[13] The coproporphyrin 3 from this uroporphyrin was comparable with the presumably best reference specimens, particularly in view of its consistently high m.p.'s and the consistent behavior of the polymorphic ester. The uroporphyrin 3 synthesized from pyrromethenes, VII with either VIII or IX, was not thoroughly characterized but it degraded to coproporphyrin 3 comparable with reference specimens.

Both forms of the synthetic uroporphyrin 3 methyl ester were compared with those of uroporphyrin 4 and of the following mixtures of uroporphyrin isomers, crystallized from chloroform–methanol and from benzene–heptane (see Experimental): (1) our synthetic uroporphyrin mixture from 5,5′-dicarboxypyrromethane-3,3′-dipropionic acid-4,4′-diacetic acid with formic acid at 40°,[5,14] (2) a synthetic uroporphyrin 3 from 2-hydroxymethyl-5-carboxypyrrole-3-propionic acid-4-acetic acid with hot dilute hydrochloric acid,[7] (3) a uroporphyrin from pathological urine ("McCawl"[15]), (4) the uroporphyrins 1, 2, 3 and 4 crystallized together in the ratio 1:1:4:2 (that of random synthesis), (5) the same in the ratio 1:1:2:4. The m.p.'s 250–262°, did not clearly distinguish any of the above pure porphyrin esters and mixtures nor a mixture of the uroporphyrin 3 and 4 methyl esters. Infrared spectra in chloroform did not distinguish the uroporphyrin 3 and 4 methyl esters. The infrared spectra of all these specimens in Nujol mull were diffuse and differed consistently and significantly in only one respect: those which had been crystallized from chloroform–methanol or acetone–methanol and which contained uroporphyrin 3 [uroporphyrin 3, (4) and (5)] or were believed to contain it [(1), (2) and (3)] showed a pronounced broad maximum or shoulder at 1245 cm.$^{-1}$.[16] These last six specimens from chloroform-methanol and the mixtures (3) and (4) from benzene–heptane, like one specimen of uroporphyrin 4,[6] also differed from the others in showing stronger C–O maxima at 1170 cm.$^{-1}$ than at 1200 cm.$^{-1}$. Among all the mull spectra the other differences were small and inconsistent, and no significance could be ascribed to them. They may reflect uncertainty in the crystal form (uroporphyrin 4[6]) or in the proportion of crystalline material. Thus the infrared maximum at 1245 cm.$^{-1}$ was better developed in those specimens of pure uroporphyrin 3 from chloroform–methanol which contained a higher proportion of obviously crystalline material. This maximum was particularly well developed in mixtures such as (4) which was obtained completely crystalline from chloroform–methanol, gave a clearer X-ray powder photograph than did pure uroporphyrin 3, and crystallized more obviously from its melt. The value of X-ray powder photographs in distinguishing the uroporphyrin 3 and 4 methyl esters is uncertain because useful photographs of the latter, although different, were only obtained after heating.[6] The methyl esters of uroporphyrin 3 and of the mixtures (1), (2), (3), (4) and (5) when crystallized from chloroform–methanol gave identical X-ray photographs except for the relative intensity of one extra

(14) S. F. MacDonald, *J. Chem. Soc.*, 4184 (1952); *Chemistry & Industry*, 1092 (1951).

(15) O. Kennard and C. Rimington, *Biochem. J.*, **55**, 105 (1953).

(16) A relationship between uroporphyrin 3 and the maximum at 1245 cm.$^{-1}$ had been suggested previously by the infrared mull spectra of other synthetic mixtures; these had been crystallized from chloroform–methanol.[5]

line. This variation was not related to purity. The esters of uroporphyrin 3 and of the mixtures (1), (2) and (3) when crystallized from benzene–heptane gave identical photographs differing from the first; (4) and (5) from the same solvent both gave unique photographs similar to that of the uroporphyrin 4 ester after it had been heated to 260°.

Clearly uroporphyrin 3 methyl ester cannot be distinguished from some mixtures containing it or believed to contain it by m.p.'s, mixed m.p.'s, infrared spectra or X-ray powder photographs, and the latter show that such mixtures may exist in the same two crystal forms as does uroporphyrin 3. Paper chromatography distinguishes neither the uroporphyrins 3 and 4 nor the coproporphyrins 3 and 4,[17] and may be misleading otherwise (see below). Consequently the synthetic uroporphyrin 3 has not the usual value of a reference specimen despite its purity. Conversely, in the absence of any other definite evidence, uroporphyrin 3 may be tentatively identified as a component in mixtures such as (1), (2) and (3) where the ester from chloroform–methanol gave the X-ray photograph and the 1245 cm.$^{-1}$ max. of that form of uroprophyrin 3; any considerable amount of uroporphyrin 4 might have been revealed, as in (4) and (5), by the photograph of the benzene–heptane form. As a mixture of the uroporphyrin 3 and 4 esters crystallizes from chloroform–methanol better than either isomer alone, their mixture might more effectively conceal other isomers in solid solution.

It is uncertain whether or not any purpose is served by distinguishing the mixtures we studied from "Waldenstrom esters" which frequently melt higher. The latter had been compared to mixtures of the uroporphyrin 1 and 3 esters[15] before the question of polymorphism was raised and before the other isomers were available. Other uroporphyrins obtained from porphobilinogen with acid had been shown to be mixtures of isomers by the m.p.'s of the derived coproporphyrin esters.[18,19] A specimen obtained similarly[5] was examined only as crystallized from chloroform–methanol; its m.p., infrared mull spectrum and X-ray photograph indicated the expected apparent identity with uroporphyrin 3. In the first specimen from porphobilinogen[18] and in another mixture[20] paper chromatography of the uroporphyrin and of the coproporphyrin had indicated a lower proportion of the high melting type 1 and 2 isomers than that demanded by the m.p.'s of the coproporphyrin esters. The relevance of the composition of mixtures synthesized chemically, from porphobilinogen or such as (1) and (2), to the problem of the biosynthesis of uroporphyrins from porphobilinogen may be a matter of opinion. However, the problem of determining their composition is unsolved, and the study of simplified models involves further uncertainty due to the influence of the β-substituents in irrational porphyrin syntheses.[5,6]

Both forms of the natural uroporphyrin methyl ester from turacin agreed with the corresponding ones of the synthetic uroporphyrin 3. However, their properties are not sufficiently distinctive to establish the identity of the two porphyrins and thus prove the structure of the former. To confirm previous work[21] the uroporphyrin from turacin was degraded to the coproporphyrin which was shown to be coproporphyrin 3 by comparison with a synthetic specimen. As the turacin uroporphyrin is an octacarboxylic acid[21] degrading to coproporphyrin 3, the complete formal proof that it is uroporphyrin 3 would require in addition only a C-methyl determination.[4]

The polymorphism of the uro- and coproporphyrin 3 methyl esters, the resemblance of the former to mixtures, and the lack of suitable reference specimens of coproporphyrin 3 had caused some confusion. Our first synthetic uroporphyrin 3 (from VII and VIII and crystallized from acetone) had differed from the turacin uroporphyrin (from benzene–heptane) and was apparently identical with the mixture (1) above (from chloroform–methanol). Further, it degraded to a coproporphyrin methyl ester which melted higher than did any synthetic coproporphyrin 3 of Fischer.[13] Although the uroporphyrin mixtures (1) and (3) above had been correctly distinguished[15] from the turacin uroporphyrin by X-ray powder photographs and infrared mull spectra, this was only because corresponding forms were not compared.

Experimental[22]

2-Bromomethyl-5-carboxypyrrole-3-acetic Acid-4-propionic Acid Triethyl Ester (IIb).—Bromine (2.5 g. in 15 ml. of carbon tetrachloride) was added to a solution of 5 g. of IIa in 30 ml. of carbon tetrachloride and the resulting oil dissolved by gentle warming. After exposure to an ultraviolet lamp and sunlight for 20 min., the solvent was removed *in vacuo* below 50°. The residue was warmed to solution in 45 ml. of carbon tetrachloride which was then removed in the same way. After drying at 0.1 mm. the residue was crystallized from 400 ml. of *n*-hexane giving 5.6 g. (92%), m.p. 95–96° (lit.[5] 92–93°).

2-Bromomethyl-5-carboxypyrrole-3-propionic Acid-4-acetic Acid Triethyl Ester (Ib).—This more soluble isomer, m.p. 63–64° (lit.[5] 65–66°), was prepared like the one above (35%).

2-Carboxy-3-carbethoxymethyl-4-(2-carbethoxyethyl)-5-carbethoxy-pyrrole (IIc).—The preparation of this from IIa was completely analogous to that of its isomer Ic[10] from Ia. The analytical sample, m.p. 159–160°, was obtained as colorless plates by recrystallizing the crude product (51%, m.p. 157–158°) from acetone–hexane.

Anal. Calcd. for $C_{17}H_{23}O_8N$: C, 55.28; H, 6.28; N, 3.79. Found: C, 55.25; H, 6.19; N, 3.92.

2-Bromo-5-carboxypyrrole-3-propionic Acid-4-acetic Acid Triethyl Ester.—Bromine (0.5 g. in 1 ml. of acetic acid) was added to 1.09 g. of the carboxypyrrole Ic[10] in 10 ml. of acetic acid at 40–45°. After 35 min., 8 ml. of water was added over 45 min. all at 40–45°. The solution was poured into 200 ml. of ice-water. The precipitate was collected next day, washed with water, dried, and crystallized from

(17) J. E. Falk, E. I. B. Dressel, A. Benson and B. Knight, *Biochem. J.*, **63**, 87 (1956).

(18) G. H. Cookson and C. Rimington, *ibid.*, **57**, 476 (1954).

(19) J. Waldenstrom and B. Vahlquist, *Z. physiol. Chem.*, **260**, 189 (1939).

(20) See footnote 25.

(21) R. E. H. Nicholas and C. Rimington, *Biochem. J.*, **50**, 194 (1951).

(22) The melting points were determined as previously noted.[13] The reference specimen of coproporphyrin 3 methyl ester was specimen 1a of reference 13. We are grateful to Dr. W. H. Barnes and Dr. Maria Przybylska for the X-ray powder photographs, to Dr. R. N. Jones and Mr. R. Lauzon for the infrared spectra and to Professor C. Rimington, Dr. D. Mauzerall and Dr. T. C. Chu for the paper chromatography. We are indebted to Professor C. Rimington for specimens of the uroporphyrin 3 methyl ester from turacin[21] and of the "McCawl" uroporphyrin ester,[15] and to Professor A. Treibs for a specimen of his synthetic uroporphyrin 3.[7]

hexane to give colorless needles (722 mg., 66%), m.p. 86–87° unchanged after recrystallization.

Anal. Calcd. for $C_{16}H_{22}O_6NBr$: C, 47.53; H, 5.48; N, 3.47; Br, 19.77. Found: C, 47.67; H, 5.40; N, 3.52; Br, 19.64.

2-Iodo-5-carboxypyrrole-3-propionic Acid-4-acetic Acid Triethyl Ester (Id).—During 15 min., 1.39 g. of iodine in 20 ml. of ethanol was added to a stirred solution of 2 g. of the carboxy-pyrrole Ic[10] and 2 g. of potassium bicarbonate in 20 ml. of ethanol and 20 ml. of water. The solution was heated to boiling to discharge the iodine color then poured into 400 ml. of ice-water. After 2 hours, the precipitate was collected, washed, dried, and recrystallized from ether–hexane to give 2.13 g. (87%) of colorless needles, m.p. 97–98°. The analytical sample, m.p. 98–99°, was prepared by recrystallization.

Anal. Calcd. for $C_{16}H_{22}O_6NI$: C, 42.58; H, 4.92; N, 3.10; I, 28.12. Found: C, 42.62; H, 4.87; N, 3.08; I, 28.30.

2-Iodo-5-carboxypyrrole-3-acetic Acid-4-propionic Acid Triethyl Ester (IId).—The preparation of IId from IIc was completely analogous to that of Id from Ic. The yield was 83%, m.p. 109–110°. The analytical specimen, m.p. 111–112°, had been recrystallized from ether–hexane as colorless prismatic needles.

Anal. Calcd. for $C_{16}H_{22}O_6NI$: C, 42.58; H, 4.92; N, 3.10; I, 28.12. Found: C, 42.78; H, 5.09; N, 3.14; I, 27.96.

5-Carboxypyrrole-3-propionic Acid-4-acetic Acid Triethyl Ester (Ie). (a).—The α-bromopyrrole (209 mg.) in 20 ml. of ethanol was shaken overnight with 100 mg. of 5% palladium–charcoal and 100 mg. of magnesium oxide under hydrogen (19 lb./in.²). The filtered solution was concentrated *in vacuo*, water added, and extracted with ether. The ether layer was washed, dried, and evaporated. The residue was crystallized from ether–hexane giving colorless needles (143 mg., 85%), m.p. 52–53° (lit.[10] 51–52°).

(b) The α-iodopyrrole Id (2.15 g.) was reduced as under (a) above with 850 mg. of 5% palladium–charcoal, 850 mg. of magnesium oxide and 50 ml. of ethanol. The product, m.p. 52–53° (1.37 g., 87%), was worked up in the same way.

5-Carboxypyrrole-3-acetic Acid-4-propionic Acid Triethyl Ester (IIe).—The preparation of IIe from IId was completely analogous to that of Ie from Id. The product (89%), m.p. 53.5–54.5°, was recrystallized several times from ether–pentane to obtain the analytical sample as colorless needles, m.p. 54–55°.

Anal. Calcd. for $C_{16}H_{23}O_6N$: C, 59.06; H, 7.13; N, 4.31. Found: C, 59.10; H, 6.97; N, 4.42.

5,5′-Dicarboxypyrromethane-3,3′-dipropionic Acid-4,4′-diacetic Acid Hexaethyl Ester (III).[6,14]—The pyrrole Ia (500 mg.) was converted into Ib in carbon tetrachloride at 45–50° with 260 mg. of bromine. After removing the solvent, the residue was twice dissolved in ether which was removed in the same way. The oily residue was dissolved in 5 ml. of acetone containing 243 mg. of sodium iodide; sodium bromide precipitated at once. The mixture was treated with 1 ml. of water and boiled for 10 minutes. Ether was added and the crude product isolated as was its isomer below. It was chromatographed on 20 g. of alumina on a 20-mm. column, the only crystalline material being eluted with 25% ether in benzene. This fraction was recrystallized from ether–hexane to yield 170 mg. (35%) of colorless prisms and plates, m.p. 99–100° (lit.[14] 100.5°).

5,5′-Dicarboxypyrromethane-3,3′-diacetic Acid-4,4′-dipropionic Acid Hexaethyl Ester (V).[5,6] (a).—The pyrroles IIb (258 mg.) and IIe (200 mg.) were refluxed together in 10 ml. of benzene for one hour with exclusion of moisture. The solvent was removed *in vacuo* leaving a crystalline residue which was recrystallized from acetone–hexane to yield colorless needles (369 mg., 88%), m.p. 145–146°.

(b).—The pyrrole IIb (100 mg.) was boiled for 5 minutes with 40 mg. of sodium iodide and 2 ml. of acetone. Sodium bromide appeared immediately. Ether was added to the mixture and the solution washed with aqueous sodium thiosulfate, then with water. The residue left on evaporating the ether was crystallized from ether–pentane to give 34 mg. (43%), m.p. 145–146°.

5,5′-Dicarboxypyrromethane-3,4′-diacetic Acid-4,3′-dipropionic Acid Hexaethyl Ester (IV). From Ie and IIb. (a) **In Benzene.**—The pyrroles Ie (1.0 g.) and IIb (1.29 g.) were refluxed for 1 hour in dry benzene with exclusion of moisture. The cooled solution was chromatographed on 40 g. of alkaline alumina Grade II/III, 100-ml. fractions being collected. Elution with 10% ether in benzene gave an initial fraction, m.p. 95–115°, probably containing V. The remaining fractions eluted with 10–25% ether in benzene were combined and recrystallized from acetone–hexane to give 1.39 g. (68%) of IV as colorless needles, m.p. 102–104°. Recrystallized from the same solvents it melted at 104–105°,[23] mixed m.p.'s with III and V, 85–93° and 100–132°, respectively.

Anal. Calcd. for $C_{33}H_{46}O_{12}N_2$: C, 59.80; H, 7.00; N, 4.23. Found: C, 60.01; H, 7.01; N, 4.13.

The residue from the eluates with 50–75% ether–benzene was recrystallized from ether–hexane to give 78 mg. (4%) of III as colorless prisms, m.p. 95–99°. After two recrystallizations it melted at 100–101°, undepressed by an authentic specimen of III.

(b) **In Acetic Acid–Sodium Acetate.**—A solution of Ie (4.40 g.), IIb (5.64 g.) and 1.4 g. of anhydrous sodium acetate in 40 ml. of acetic acid was refluxed for 30 minutes. The crude product was isolated by pouring the solution into ice-water. Its solution in 100 ml. of benzene was rapidly filtered through 160 g. of alumina (Woelm grade IV) in a 5.5-cm. column eluting with 1500 ml. of benzene then with 1500 ml. of ethyl acetate. Removing the solvent left a residue which was dissolved in 40 ml. of warm acetone to which 200 ml. of warm *n*-hexane was added. The undisturbed solution deposited 5.6 g. (63%) of colorless needles radiating from a single center, m.p. 104.5–105.5° after softening from 103.5°.

From Ib and IIe. (c).—The pyrroles Ib (1.227 g.) and IIe (990 mg.) were refluxed together in benzene then chromatographed as under (a) above. The residue from the 10–25% ether-in-benzene eluates was crystallized from acetone–hexane to give 1.448 g. of IV (72%), m.p. 103–105°, undepressed with the specimen obtained as under (a) above.

The material eluted by 50–75% ether-in-benzene was recrystallized from ether–hexane to give colorless prisms of III (15 mg., 0.7%), identified by m.p. 99–100° and mixed melting point.

Chromatographic Separation of the Pyrromethanes III, IV and V.—A benzene solution of the three pure pyrromethanes (200 mg. each) was chromatographed on 24 g. of alkaline alumina (grade II/III) in a 22-mm. column eluting with ether–benzene. The fractions were 50 ml.: No. 1–2, benzene; no. 3–25, 5% ether; no. 26–38, 10% ether; no. 39–45, 25% ether; no. 46–48, 50% ether; no. 49, 75% ether. On evaporation, no. 1–7 and 49 left no residue; no. 8–28 were crystalline; no. 29–48 were oils which crystallized readily from ether–hexane. The softening and clearing points showed minima in no. 31 and 40.

The combined fractions 9–24 (174 mg.) were recrystallized three times from acetone–hexane to give 98 mg. (49% recovery) of V, m.p. 145–146°. Crystallized four times from acetone–hexane, no. 31–38 (99 mg.) gave 40 mg. (20%) of IV, m.p. 103–104°. Fractions 42–48 (107 mg.) from acetone–hexane gave 86 mg. (43%) of III, m.p. 100–101°.

Pairs of these isomers were separated either in the same way or on grade IV neutral alumina with successively benzene–petroleum ether, benzene, ether–benzene.

Detection of V in IV.—The pure methane IV (200 mg., obtained by method b) was chromatographed on 16 g. of alumina (Woelm, grade IV) in a 2-cm. column. Benzene (600 ml.) eluted nothing, then dry ether (300 ml.) eluted *ca.* 1 mg. of IV, m.p. 100–106°.

When a mixture of the same IV (200 mg.) and V (2 mg.) was chromatographed in the same way, the ether eluted *ca.* 1 mg., m.p. 70–138°, recrystallized from acetone–hexane to give V, m.p. and mixed m.p. 147–150°.

Separation of III and IV by Crystallization.—Two-hundred mg. each of the pure pyrromethanes was crystallized together from acetone (1.6 ml.)–hexane (8 ml.) to give 114 mg. of IV as needles, m.p. 106–107° after sintering from about 100°. Recrystallization gave 81 mg., m.p. and mixed m.p. 106–107.5°, shrinking from 105°.

5,5′-Dicarboxypyrromethane-3,4′-diacetic Acid-4,3′-dipropionic Acid (VI).—The unsymmetrical pyrromethane IV (500 mg.) was heated on the steam-bath with 3 ml. of 10%

(23) The substance is dimorphic. When rapidly heated it melts at about 102°, the higher m.p. is found by heating very slowly from 90°.

sodium hydroxide and 6 ml. of ethanol in an open flask for 3 hours. The residue was diluted with 10 ml. of water and the product (344 mg., 92%), m.p. 157–158° dec., precipitated at 0° with sulfur dioxide, washed, and dried *in vacuo*. For analysis it was thrice precipitated from dilute ammonia by sulfur dioxide at 0°, collecting and washing by centrifuging, and dried *in vacuo* for 16 hours at 80°.

Anal. Calcd. for $C_{21}H_{22}O_{12}N_2$: C, 51.01; H, 4.49; N, 5.67. Found: C, 50.86; H, 4.70; N, 5.52.

5,5'-Dibromopyrromethene-3,4'-diacetic Acid-4,3'-dipropionic Acid Hydrobromide (VII).—Bromine (10 g.) was made up to 25 ml. with 98% formic acid and the mixture shaken to solution. The pyrromethane VI (500 mg.) in a 25-ml. conical flask was treated at 0° with 6 ml. of the solution. After standing 1 hour at room temperature protected from moisture, the solution was filtered through a coarse sintered glass funnel into a 50-ml. conical flask. The filtrate was concentrated to a viscous oil in a desiccator over sodium hydroxide and calcium chloride for 1 hour at 50 mm. then at 0.2 mm. for about 45 min. On dissolving the oil in 3 ml. of acetic acid and scratching, the product separated. After 2 hours, it was filtered off, washed with acetic acid and dried *in vacuo* over potassium hydroxide, m.p. 155–180° dec. For analysis the bright red microcrystals (230 mg., 35%) were dried for 13 hours in high vacuum.

Anal. Calcd. for $C_{19}H_{19}O_8N_2Br_3$: C, 35.48; H, 2.98; N, 4.36; Br, 37.28. Found: C, 35.65; H, 3.12; N, 4.45; Br, 37.06.

Larger runs gave poorer yields. When light was largely excluded through the operations, the yields were as high as 59%.

Pyrromethane-3,4'-diacetic Acid-4,3'-dipropionic Acid (X).—The ester IV (1 g.) was heated for 2 hours in an open flask with 10 ml. of ethanol and 4 ml. of 10% sodium hydroxide on the steam-bath. The residue, 0.45 g. of sodium hydroxide and one drop of hydrazine were made up to 4.5 ml. with water and heated under pressure in a Teflon lined tube for 4 hours at 170–173°. The contents of the tube were deionized as in the preparation of the isomer[6] using a total of 20 g. of Amberlite IR-120. Evaporation of the eluate in the same way left boat-shaped prisms which were washed onto a filter with 2.5 ml. of water and dissolved in 8 ml. of de-aerated 80% acetone. The nearly colorless micro-prisms (403 mg., 66%) which separated on removing the acetone *in vacuo* were filtered and washed with 1 ml. of water, m.p. about 170° dec. depending on the rate of heating.

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, 55.90; H, 5.43; N, 7.01; neut. equiv., 101.7.

Pyrromethane-3,4'-diacetic Acid-4,3'-dipropionic Acid Tetramethyl Ester.—The acid X(150 mg.) was treated with ethereal diazomethane. Ether was removed *in vacuo* and the residue washed with heptane then dissolved in ether. The solution was warmed to precipitate dark material and filtered at 0°. Ether was removed from the filtrate (3 ml.) by a stream of nitrogen leaving the nearly colorless granular product (116 mg.), m.p. 53.5–54.5°, remelt 74–75°.

Anal. Calcd. for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54. Found: C, 60.15; H, 6.60.

Methylsuccinic Acid.—Commercial itaconic acid (100 g.) was slurried with 200 ml. of water and hydrogenated (50 lb./in.^{2}, 20°) over platinum oxide. The residue obtained after filtration, evaporation and drying, was crystallized from chloroform giving 95 g., m.p. 115–116°.

Porphin-1,3,5,8-tetraacetic Acid-2,4,6,7-tetrapropionic Acid Octamethyl Ester (Uroporphyrin 3 Methyl Ester). (a).—The pyrromethenes VIII (1.260 g.) and VII (1.578 g.) were dried and fused with 11.4 g. of methyl succinic acid at 135° (boiling ethylbenzene-bath) in three lots as in the synthesis of the other uroporphyrins[4,5] and worked up in the same way except as noted. After diluting the melt with water, the mixture stood overnight before centrifuging for 1 hour at 1500 r.p.m. The concentrated chloroform solution of the crude methyl ester was put through a column of grade IV alumina (60 g. packed with chloroform containing 5% methanol). The concentrated eluate, after washing with 50% aqueous resorcinol,[24] was put through 6 g. of deactivated alumina. The eluate was concentrated and the chloroform boiled off while methanol was added. When red needles began to separate the solution was allowed to cool slowly. The product (20 mg., 0.9%), m.p. 252–255°, was twice recrystallized by extracting into dry acetone and displacing the acetone with methanol giving 16 mg., m.p. 253–259° after sintering and changing form from 244°. It was further characterized by its infrared mull spectrum (broad shoulder at 1245 cm.$^{-1}$) and by its X-ray powder photograph which showed no trace of the extra line.

The uroporphyrin 3 ester (4.8 mg.) was partially decarboxylated as was uroporphyrin 2.[6] The resulting coproporphyrin 3 methyl ester formed needles (2.7 mg., 75%), m.p. 175–178° after sintering and changing to plates at 155°. After one recrystallization from methanol and two from chloroform–methanol, 1.7 mg. remained, m.p. 176–179°, not depressed when mixed with the high-melting form of the reference specimen which gave an identical X-ray powder photograph. Paper chromatography[17] using lutidine or dioxane showed only the coproporphyrin 3 or 4 spot except on overloading when there was a very faint trace as of coproporphyrin 2. The uroporphyrin from another run degraded to coproporphyrin 3 methyl ester, m.p, 176–179°, giving an X-ray powder photograph identical with that of the low-melting form of the reference specimen.

(b).—The pyrromethenes VII (485 mg.) and IX (389 mg.) were fused with 3.6 g. of methylsuccinic acid and worked up as under (a) above. The product, m.p. 255–259°, was degraded as usual to coproporphyrin 3 methyl ester, m.p. 176–179°, the X-ray powder photograph of which was identical with that of the low melting form of the reference specimen.

(c) **(Preparative Method).**—Except as noted this followed the synthesis of uroporphyrin 2 from the di-aldehyde XI.[6] The pyrromethanes XI (105 mg.) and X (82.4 mg.) in 100 ml. of acetic acid were treated with 0.6 ml. of hydriodic acid in 30 ml. of acetic acid. After 20 min., 2 g. of anhydrous sodium acetate in 30 ml. of acetic acid was added and air passed through for 24 hours to precipitate about 120 mg. of the product. After esterification, etc., the chloroform was slowly displaced by methanol and the solution then boiled 1 hour to increase the proportion of crystalline material. The ester (116 mg., 60%) separated as a mixture of needles, m.p. 255–258°, and amorphous material, m.p. 258–260° after changing to flat crystals from 250°.

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, 61.22; H, 5.77; N, 5.98; OMe, 26.13; C-Me, 0.00.

The products of several runs gave X-ray powder photographs identical with that of the product of method (a) except that the extra line was either absent or present in varying intensity in the former. Small and inconsistent differences, similar to those brought about by recrystallization, were apparent among the infrared mull spectra. Otherwise these were identical with that of the product of method (a) except that they showed either a broad shoulder or a maximum at 1245 cm.$^{-1}$. The product was also characterized by its infrared spectrum in chloroform. A second form crystallized completely from benzene–heptane as tiny bent hair-like crystals, m.p. 255–260° after sintering and changing to flat crystals from 247°; its infrared mull spectrum had a minimum at 1255 cm.$^{-1}$. The X-ray powder photograph was also distinct from that of the first form, both having fifteen measurable lines. Paper chromatography by the lutidine method showed only the uroporphyrin 3 or 4 spot. By the dioxan method it behaved like the standard (from turacin[21]), both showing some tailing of the spot, except that one of five synthetic specimens showed a very faint trace running like "pseudouroporphyrin."

The uroporphyrin of method (c) was degraded to coproporphyrin 3 methyl ester. As crystallized once, leaving 0.05 mg./ml. in the methanol, this formed clusters of needles (80%) which at 153–156° melted or changed to fibrous crystals melting or changing to plates at 165–170°, leaving plates, m.p. 178–182°. When mixed with the reference specimen the behavior on heating was unchanged. The X-ray powder photographs of the low and the high melting forms as well as the infrared mull spectra of the low-melting form were identical with those of the reference specimen except that in one of four specimens the first of these had the extra line also noted in specimen 3b of reference 13. The copper complex melted at 216–219° sintering from 213°. Its X-ray powder photograph was also identical with that of the reference specimen complex. Paper chromatography showed the coproporphyrin 3 or 4 spot only.

(24) Before use, the aqueous resorcinol solution was washed several times with benzene, then chloroform.

(**d**).—The pyrromethanes X and XI were treated as under (c) except that an equivalent amount of hydrogen chloride in acetic acid was used instead of hydrogen iodide. The precipitate obtained after aeration (21%) gave the uroporphyrin ester (14%), m.p. 253–257°, after esterification, etc.

It was degraded to coproporphyrin 3 methyl ester, the X-ray powder photograph and infrared mull spectrum of which were identical with those of the low-melting form of the reference specimen. Its m.p.'s were 149–153°, 161–165° (sparkling crystals) and 174–178°, copper complex 208–214° sintering from 205°.

The Behavior of X under the Conditions of Porphyrin Synthesis.—The pyrromethane was treated with hydrogen iodide in acetic acid followed by sodium acetate and air. The solution was evaporated and the residue esterified with diazomethane in pyridine–ether (avoiding the action of methanolic hydrogen chloride on starting material, etc.). After washing colored impurities out of the chloroform solution with aqueous resorcinol, the uroporphyrin (0.9%) was determined spectroscopically.

Uroporphyrin 3 Methyl Ester from Turacin.[21]—One recrystallization from chloroform–methanol gave homogeneous needles; a second gave needles mixed with amorphous material, m.p. 254–257° sintering and changing to flat crystals from 250°. There was a trace of the extra line in the X-ray powder photograph. From benzene–heptane it formed tiny bent hairs, m.p. 254–257° sintering and changing to compact crystals from 245°, mixed m.p. with the same form of the synthetic material of method (c) 254–257° after sintering from 248°. The two forms of the natural material gave X-ray powder photographs identical with those of the corresponding forms of synthetic material; with the qualifications mentioned above the same was true of the infrared mull spectra (first form: shoulder at 1245 cm.$^{-1}$, max. at 1170 cm.$^{-1}$ >1200 cm.$^{-1}$).

This natural uroporphyrin was degraded to coproporphyrin 3 methyl ester (70%, 0.04 mg./ml. remained in the methanol) obtained as clusters of needles, X-ray powder photograph identical with that of the low-melting form of the reference specimen. The needles either melted or changed to fibrous crystals at 153–155°. The latter either melted between 163–172° usually with previous sintering or changed to plates, m.p. 178–182°. When the needles were mixed with the reference specimen, their behavior on heating was unchanged. The copper complex melted at 216–219° after sintering from 213°.

Mixtures of Uroporphyrin Methyl Esters. (**1**).—The uroporphyrin mixture from 5,5′-dicarboxypyrromethane-3,3′-dipropionic acid-4,4′-diacetic acid with formic acid at 40°[5,14] crystallized completely from chloroform–methanol in long needles, m.p. 256–258° after sintering, undepressed on admixture with the turacin uroporphyrin. From benzene–heptane it formed hairs, m.p. 255–260° after changing to flat crystals from 245°; the X-ray photograph had twenty-five measurable lines. It had been degraded to a mixture of coproporphyrin methyl esters, m.p. 135–183°. Uroporphyrin 2, the only proven component, had been obtained from the fraction separating from pyridine at room temperature.[25]

(**2**).—The uroporphyrin 3 from 2-hydroxymethyl-5-carboxypyrrole-3-propionic acid-4-acetic acid with hot dilute hydrochloric acid[7] crystallized completely from chloroform–methanol in needles, m.p. 253–257°, and from benzene–heptane as curled hairs, m.p. 250–254° after changing to flat crystals from 245°. There was a trace of the extra line in the X-ray photograph of the form from chloroform–methanol. It degraded to a coproporphyrin methyl ester, m.p. 133–210°. The only component we identified was uroporphyrin 2 methyl ester, m.p. 313–315° after sintering from 300°, isolated by crystallization from pyridine, thrice from acetone, then from chloroform–acetone.

(**3**).—The urinary uroporphyrin from a case ("McCawl") of *porphyria cutanea tarda*[15] crystallized completely from chloroform–methanol in needles and hairs, m.p. 254–258°, undepressed on admixture with the turacin uroporphyrin. There was no extra line in the X-ray powder photograph. From benzene–heptane it formed bent hairs, m.p. 254–257°. Both forms sintered and changed to flat crystals from 245°. Crystallization from pyridine did not raise the m.p. above 265°.

(**4**).—A mixture of the pure synthetic uroporphyrin 1,2,3 and 4 methyl esters in the ratio 1:1:4:2 was crystallized from chloroform–methanol as homogeneous hair-like crystals, m.p. 255–259° after sintering and changing to flat crystals from 250°. There was a trace of the extra line in the X-ray powder photograph which had 25 measurable lines. From benzene–heptane homogeneous fine hairs separated, m.p. 255–259° after changing to flat crystals from 245°. The corresponding coproporphyrin mixture behaved otherwise.[13]

(**5**).—A mixture of the pure synthetic uroporphyrin 1,2,3 and 4 methyl esters in the ratio 1:1:2:4 was crystallized from chloroform–methanol as needles together with some hair-like crystals and amorphous material, m.p. 255–259° after sintering from 248°. From benzene–heptane fine curled hairs separated, m.p. 255–262° after partly changing to flat crystals from 245°.

A mixture of the synthetic uroporphyrin 3 and 4 methyl esters, mixed m.p. 252–255° after sintering from 245°, crystallized from chloroform–methanol completely as long needles, m.p. 254–257° after sintering and changing to flat crystals. The losses on crystallizing all the above mixtures were insignificant.

(25) The fraction remaining in pyridine at 0°, m.p. 252–256°, behaved as uroporphyrin 3 or 4 on paper chromatography with 2,6-lutidine or dioxane. It degraded to a coproporphyrin which behaved on paper with 2,6-lutidine–ammonia as coproporphyrin 3 or 4 with tailing suggesting 5% of coproporphyrin 1 but no coproporphyrin 2. However, the solubility (0.5 mg./ml. in methanol) and m.p. (half at 140–165°, half at 215–225°) of the coproporphyrin methyl ester showed that it was a mixture and contained much of the type 1 or type 2 isomers.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

Alkaloids of *Balfourodendron riedelianum*. Balfourodine and Isobalfourodine

BY HENRY RAPOPORT AND KENNETH G. HOLDEN[1]

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The structures of two alkaloids of *Balfourodendron riedelianum*, balfourodine and isobalfourodine, as well as two of their isomeric transformation products, ψ-balfourodine and ψ-isobalfourodine, have been determined. These compounds represent the four possible isomeric linear and angular dihydrofuro- and dihydropyrano-quinolones corresponding to 2-alkoxy-4-quinolones and 4-alkoxy-2-quinolones. Through the use of synthetic compounds of unquestioned structure, a general spectral method for distinguishing these various isomeric ring systems has been developed. In addition, four known alkaloids have been isolated, bringing to seven the number of characterized alkaloids from this plant source.

In a previous publication[2] the isolation of alkaloids from *Balfourodendron riedelianum*, a rutaceous plant indigenous to Brazil and Argentina, was described, and structural assignments were made to the two alkaloids present in the plant in highest concentration. Balfourolone (III) was shown to be an artifact of this isolation procedure. It arose from alkali attack on the O^4-methylbalfourodinium

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(2) H. Rapoport and K. G. Holden, THIS JOURNAL, **81**, 3738 (1959).