Tetrahedron, 1960, Vol. 8, pp. 266 to 270. Pergamon Press Ltd. Printed in Northern Ireland

THE CHEMISTRY OF PORPHYRIN *a*

POSITION OF THE FORMYL GROUP

M. PIATTELLI
Institute of Organic Chemistry, Naples, Italy

(*Received* 30 *July* 1959)

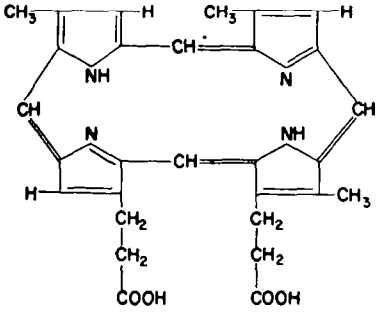
Abstract—Porphyrin *a* was transformed into the corresponding nitrile, which was oxidized with potassium permanganate. 3-Cyano-4-propionic-2,5-pyrroledicarboxylic acid was identified among the degradation products by chromatographic comparison with the same acid obtained by synthesis. This result shows that the formyl group is present in position 8.

A new synthesis of "cytopyrrolic acid" (III), a peculiar oxidation product of porphyrin *a*, is also described.

IDENTIFICATION of 3-methyl-2,4,5-pyrroletricarboxylic (I) 3-methyl-4-propionic-2,5-pyrroledicarboxylic (II) and 3-propionic-2,4,5-pyrroletricarboxylic (III) acids, degradation products from porphyrin *a* with $KMnO_4$, has been previously described.[1,2]

3-Propionic-2,4,5-pyrroletricarboxylic acid, not found before by degradation of natural porphyrins, was called "cytopyrrolic acid" and its structure was demonstrated by synthesis.[3] These results and the quantitative determination of carboxyl groups[4] (only two are present, obviously those of the propionic side chains), indicate that three readily oxidizable groups are present in porphyrin *a*.

At the same time, the structure of cytodeuteroporphyrin, first obtained by Warburg and Gewitz[5] from haemin *a* by the resorcinol melt, was proved by synthesis;[6] its formula is as follows:



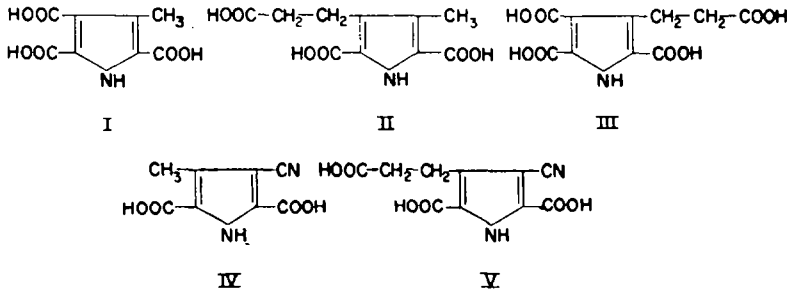
The sequence of pyrrole rings was therefore elucidated; but the nature of the substituents at positions 2, 4, and 8 still remained obscure.

Since there was strong evidence for a formyl group, based on the reaction of haemin *a* with cysteine,[7] hydroxylamine and hydrazine[8] and on spectroscopically

[1] M. Piattelli and R. A. Nicolaus, *Rend. Acc. Sc. fis. e mat.* (Ser. 4ª), XXVI 44 (1959).
[2] M. Piattelli and R. A. Nicolaus, *Rend. Acc. Sc. fis. e mat.* (in press).
[3] M. Piattelli, *Rend. Acc. Sc. fis. e mat.* (in press).
[4] O. Warburg and H. S. Gewitz, *Z. Physiol. Chem.* **288**, 1 (1951).
[5] O. Warburg and H. S. Gewitz, *Z. Physiol. Chem.* **292**, 174 (1953).
[6] G. S. Marks, D. K. Dougall, E. Bullock and S. F. McDonald, *J. Amer. Chem. Soc.* **81**, 250 (1959).
[7] W. A. Rawlinson and J. H. Hale, *Biochem. J.* **45**, 247 (1949).
[8] R. Lemberg and J. E. Falk, *Biochem. J.* **49**, 674 (1951).

observed conversion of an aldoxime to a nitrile,[9] attempts were made to ascertain the existence of the formyl by chemical methods and possibly to determine its position.

Porphyrin *a* was, therefore, transformed into oxime and this was dehydrated to nitrile by boiling with acetic anhydride; the product was oxidized with alkaline $KMnO_4$ and comparison of the mixture of degradation products with samples of acids I, II, III, IV, and V was made by paper chromatography.



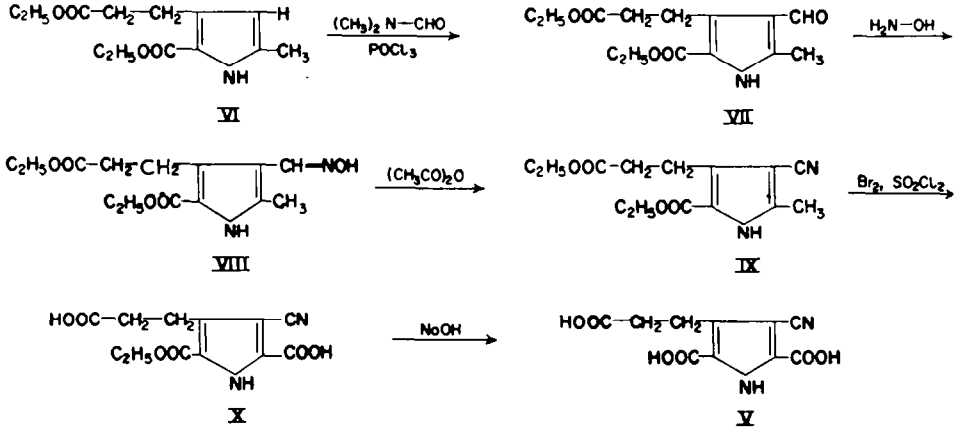
I II III

IV V

Only the acids I, II, III and V were actually identified.

This result shows that a formyl group is really present in porphyrin a, *and is in position* 8.

The acid V was synthesized as follows:



VI VII

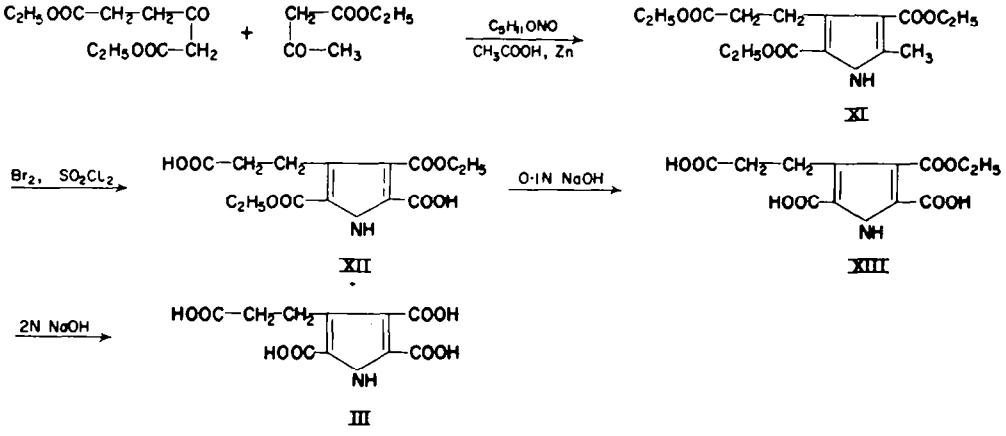
VIII IX

X V

The pyrrole VI, prepared according to MacDonald,[10] was formylated with dimethylformamide and phosphorus oxychloride to give the aldehyde VII, m.p. 120–121°, this procedure being easier than that of Gattermann. By reaction of the aldehyde VII with hydroxylamine, the oxime VIII, m.p. 141–142° was obtained, which was dehydrated to the nitrile IX, m.p. 128–129° by boiling with acetic anhydride. This compound reacted with bromine and sulphuryl chloride in acetic acid, affording the ester X, m.p. 245–246°. The hydrolysis of X to the acid V, m.p. 272–273°, was achieved by boiling with 2 N sodium hydroxide.

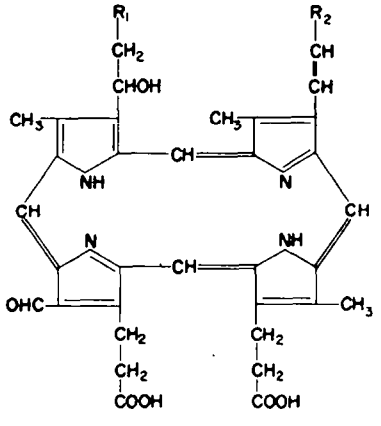
[9] H. Dannenberg and M. Kiese, *Biochem. Z.* **322**, 395 (1952).
[10] S. F. MacDonald, *J. Chem. Soc.* 4176 (1952).

Further proof of the structure of V was also obtained. This compound was subjected to prolonged alkaline hydrolysis, giving "cytopyrrolic acid" III. This acid was previously synthesized;[3] a new easier method has been worked out which is summarized in the following scheme:



Ethyl β-ketoadipate, prepared according to Eisner *et al.*[11] and MacDonald,[10] was converted into the oximine derivative which was condensed with ethyl acetoacetate to give the pyrrole XI, m.p. 94–95°. After reaction of XI with bromine and sulphuryl chloride, the diester XII, m.p. 198–199°, was obtained. The selective saponification of one of the ester groups, achieved with 0·1 N sodium hydroxide, gave a monoester, which melted at 227–228° alone or admixed with an authentic specimen of XIII. Further hydrolysis of the ester XIII with 2 N sodium hydroxide yielded the acid III.

On the basis of the most recent information on the structure of porphyrin a[12,13] and on the evidence that the formyl group and the unsaturated side chains (postulated by Warburg and Gewitz,[4] relying upon the hydrogen uptake in the catalytical reduction of haemin a in borate buffer) are very likely on opposite pyrroles,[8] the formula of porphyrin a can be written as follows:



[11] U. Eisner, J. A. Elvidge and R. P. Linstead, *J. Chem. Soc.* 2223 (1950).
[12] P. S. Clezy and J. Barrett, *Biochim. Biophys. Acta* **33**, 584 (1959).
[13] J. Barrett, *Nature, Lond.* **183**, 1185 (1959).

where R_1 and R_2 are alkyl groups (or eventually hydrogen). Our present work calls for definite proofs that structures of the substituents at positions 2 and 4, as formulated above, are really correct (this is highly probable but yet uncertain) and to elucidate the distribution between them of about twelve more carbon atoms, resulting from elementary analysis.

Preliminary experiments, performed by catalytical reduction of porphyrin *a* followed by permanganate oxidation, have not shown the presence, in the mixture of degradation products, of 4-ethyl-3-methyl-2,5-pyrroledicarboxylic acid: this is a normal oxidation product of mesoporphyrin IX, phaephorbide *a* and all related compounds having a β-methyl-β'-ethyl substituted pyrrolic nucleus.[14] This result shows that R_2 at least is not hydrogen.

EXPERIMENTAL

M.p. are not corrected.

Paper chromatograms were run on Whatman No. 1 filter paper (descending development); after drying, they were sprayed with the diazonium salt of sulphanilic acid and with N sodium hydroxide.

5-Carbethoxy-4,2'-carbethoxyethyl-2-methylpyrrole-3-aldehyde (VII). Phosphorus oxychloride (4 ml) was added at 20° to a stirred solution of the pyrrole VI (5 g) in dimethylformamide (2 ml). The mixture was heated for 2 hr at 100°, then cooled, poured into water (100 ml) and sodium acetate trihydrate was added until pH 4 was reached. After 4 hr the light brown precipitate was filtered off, washed and crystallized from water (charcoal) to give 2·15 g (38·6%) of VII, m.p. 120–121°.

5-Carbethoxy-4,2'-carbethoxyethyl-2-methylpyrrole-3-aldoxime (VIII). Aldehyde VII (1·45 g), hydroxylamine hydrochloride (3·5 g), 10% sodium hydroxide solution (14 ml) and just sufficient ethanol to give a clear solution were heated under reflux for 10 min. After cooling the product was filtered off, washed with water and dried. Crystallized from ethanol, it formed colourless prisms (1·22 g, 79·9%), m.p. 141–142° (Found: C, 56·84; H, 6·72. $C_{14}H_{20}O_5N_2$ requires: C, 56·74; H, 6·80%). Ehrlich's reaction was positive in the hot.

5-Carbethoxy-4,2'-carbethoxyethyl-2-methylpyrrole-3-nitrile (IX). The oxime VIII (1 g) was refluxed for 4 h with acetic anhydride (35 ml) and anhydrous sodium acetate (1 g). Acetic anhydride was removed under reduced pressure and the brown residue was suspended in water, filtered off, washed and dried. The crude product, crystallized from light petroleum (b.p. 80–100°; 250 ml), afforded pure IX (0·75 g, 79·8%) in long, colourless needles, m.p. 128–129° (Found: C, 60·41; H, 6·34. $C_{14}H_{18}O_4N_2$ requires: C, 60·42; H, 6·52%). Ehrlich's reaction was positive in the hot.

5-carbethoxy-3-cyano-4-propionic-2-pyrrolecarboxylic acid (X). The nitrile IX (0·5 g) was dissolved in acetic acid (5 ml) containing acetic anhydride (0·3 ml); bromine (0·1 ml) and sulphuryl chloride (0·7 ml) were added to the solution, at about 15°. The mixture was allowed to stand at 4° overnight, and after addition of water (4 ml) heated at 60° for 20 min. After cooling, more water was added (100 ml) and the solution was extracted with ether (100 ml in four portions). The extracts were dried over magnesium sulphate, and the ether was removed by distillation under reduced pressure, leaving the crude product. After three recrystallizations from ethyl acetate, colourless needles (0·175 g, 34·8%), m.p. 245–246°, were obtained, which did not give Ehrlich's reaction (Found: C, 51·42; H, 4·33. $C_{12}H_{12}O_6N_2$ requires: C, 51·43; H, 4·32%). Analytical data revealed that this compound was a monoester, not the expected diester; with the diazonium salt of sulphanilic acid a yellow colour was obtained, which indicated that an α-carboxylic group was still esterified;[15] therefore it was formulated as X.

3-Cyano-4-propionic-2,5-pyrroledicarboxylic acid (V). The ester X (0·1 g) was heated under reflux for 1 hr with 2N NaOH (2 ml). After cooling at 0°, the clear solution was acidified and extracted with ether (40 ml in four portions). The extracts were dried over magnesium sulphate, and the ether removed *in vacuo*. The residual crystalline material (78 mg) was analyzed by paper chromatography: beside the acid V, it contained traces of 3-propionic-2,4,5-pyrroletricarboxylic acid III and little amounts of another pyrrole derivative (probably the corresponding amide). Crystallization from acetic acid gave the pure acid V (60 mg, 66·7%) as colourless needles, m.p. 272–273° (Found: C,

[14] R. A. Nicolaus, L. Mangoni and L. Caglioti, *Annali di Chim.* **46**, 793 (1956).
[15] R. A. Nicolaus and L. Mangoni, *Annali di Chim.* **46**, 847 (1956).

47·41; H, 3·25. $C_{10}H_8O_6N_2$ requires: C, 47·62; H, 3·20%). Ehrlich's reaction was negative; with the diazonium salt of sulphanilic acid in alkaline solution it gave a bright red colour. Prolonged hydrolysis (12 hr under reflux) of V with 2N NaOH gave 3-propionic-2,4,5-pyrroletricarboxylic acid, identified by paper chromatography.

3,5-*Dicarbethoxy*-4,2′-*carbethoxyethyl*-2-*methylpyrrole* (XI). Ethyl β-ketoadipate (12 g) containing conc HCl (0·2 ml) was treated with amyl nitrite (6·5 g) during 1 hr at 30–35°. The resulting solution was set aside at room temp overnight and then run during 1 hr into a vigorously stirred mixture of acetic acid (65 ml), ammonium acetate (8 g), ethyl acetoacetate (7·15 g) and zinc dust (3·2 g) at 60–65°; at the same time zinc dust (8·5 g) was added in small portions. The mixture was then heated for 3 hr at 100°, the hot solution decanted from zinc into stirred ice-water (600 ml), and the zinc washed with hot acetic acid. The product was filtered off, washed with water and crystallized from dilute ethanol, giving 10 g (55·4%) of colourless needles, m.p. 94–95° (Found: C, 58·90; H, 7·16. $C_{16}H_{23}O_6N$ requires: C, 59·06; H, 7·13%). Ehrlich's reaction was positive in the hot.

3,5-*dicarbethoxy*-4-*propionic*-2-*pyrrolecarboxylic acid* (XII). Bromine (1·1 ml) and sulphuryl chloride (6·8 ml) were added to a solution of the pyrrole XI (6 g) in acetic acid (40 ml) containing acetic anhydride (2 ml), at 15°. The mixture was allowed to stand overnight at 4°, water was added until it became slightly turbid and then heated at 60° for 20 min. After cooling, water was added to a total volume of 300 ml; the crude product was filtered off, washed and crystallized from dioxane–water mixture, yielding 3·7 g (61·3%) of m.p. 198–199° (colourless plates). Ehrlich's reaction was negative (Found: C, 51·56; H, 5·18. $C_{14}H_{17}O_8N$ requires: C, 51·37; H, 5·24%). Analysis proved that the compound obtained was a diester instead of the expected triester. With the diazonium salt of sulphanilic acid, a yellow colour was obtained: this demonstrated that an α-carboxylic group was still esterified. On the other hand, its partial hydrolysis with a slight excess of 0·1N NaOH for 15 min at 100° afforded a monoester which, after crystallization from acetic acid, had m.p. 227–228°, undepressed when mixed with an authentic specimen of 3-carbethoxy-4-propionic-2,5-pyrroledicarboxylic acid (XIII). The identity of these two compounds was confirmed by paper chromatography. The structure of the ester XII was thus proved. Hydrolysis of the monoester XIII with excess of 2N NaOH as described elsewhere,[3] gave 3-propionic-2,4,5-pyrroletricarboxylic acid.

Action of hydroxylamine on porphyrin a, *dehydration with acetic anhydride and subsequent oxidation*

Porphyrin *a*, prepared according to Lemberg[16,17] (15 mg) was added to a solution of hydroxylamine hydrochloride (15 mg) in pyridine (1 ml). After 10 min, ether was added (200 ml), and pyridine removed by washing first with N HCl and then with water. The washed ether solution, dried over sodium sulphate, was evaporated to dryness under reduced pressure. Acetic anhydride (10 ml) and anhydrous sodium acetate (0·2 g) were added to the solid residue, and the mixture was refluxed for 30 min. Acetic anhydride was removed by heating on a steam-bath *in vacuo* and the residue suspended in water, filtered off and washed. The product was oxidized without any further purification: it was suspended in 2N K_2CO_3 (1 ml) and oxidized by gradual addition of a saturated solution of potassium permanganate. When the colour of the permanganate persisted for 10 min (1·5 ml were needed), the resulting mixture was briefly boiled, the manganese dioxide filtered off and washed twice with hot distilled water (2 ml). Combined filtrate and washings were adjusted to pH 4–4·5 with HCl and 20% calcium chloride solution (0·2 ml) was added. After 1 hr, the precipitate was removed by filtration, the clear filtrate made acidic to Congo red and extracted with ether (20 ml in five portions). The extracts, dried over magnesium sulphate, were evaporated to dryness. Distilled water (0·1 ml) was added to the residue and the filtered solution was used for paper chromatography.

Chromatograms were run with n-butanol: acetic acid: water (60 : 15 : 25) (R_f value for V is 0·70) or ethanol: 33% NH_3: water (80 : 4 : 16) (R_f value for V is 0·46) as solvents, and the acids I, II, III and V were detected.

3-Propionic-2,4,5-pyrroletricarboxylic acid was always present in remarkable amounts: this is difficult to explain and could be due either to incomplete reaction of porphyrin *a* with hydroxylamine or to previous oxidation of the formyl group of porphyrin *a*. It cannot be accounted for by hydrolysis of the cyano group of V in alkaline solution, because this reaction is very slow at room temperature.

The same results were obtained with haemin *a*, prepared according to Warburg and Gewitz.[18]

[16] R. Lemberg, *Nature, Lond.* **172**, 619 (1953).
[17] R. Lemberg, D. B. Morell, W. H. Lockwood, M. Stewart and B. Bloomfield, *Chem. Ber.* **89**, 309 (1956).
[18] O. Warburg, H. S. Gewitz and V. Völker, *Z. Naturf.* **10b**, 541 (1955).