PYRROLES RELATED TO PORPHOBILINOGEN[1]

G. P. ARSENAULT[2] AND S. F. MACDONALD

ABSTRACT

A study of the decarboxylation of pyrroles related to 5-carboxyporphobilinogen has led to an improved synthesis of porphobilinogen, to isoporphobilinogen, and to other pyrroles required for the preparation of possible intermediates in the biosynthesis of porphyrins.

Among the porphobilinogen derivatives which have been considered as possible intermediates in the biosynthesis of uroporphinogens from porphobilinogen are isoporphobilinogen, 5-aminomethylporphobilinogen (2), the carbinols analogous to these and to porphobilinogen, and polypyrranes. The behavior of synthetic specimens of these with enzymes is pertinent to the biogenetic problem. Also, a study of isoporphobilinogen under conditions which convert porphobilinogen to uroporphinogens might clarify ill-defined assumptions relating the mechanisms of the enzymic and non-enzymic conversions of porphobilinogen.

Many projected syntheses of these postulated intermediates required pyrroles with one α-position free and the other bearing a potential aminomethyl group, carbinol group, or bridge. We had found only one route to such intermediates in the decarboxylation of 5-carboxyporphobilinogen lactam in boiling water, a method too dependent on structure for its applicability in analogous cases to be assessed other than empirically. Consequently, other methods were studied for decarboxylating 5-carboxypyrroles with appropriate substituents in the remaining positions.

The triethyl ester of I*a* had been converted into the ester of I*c* and thence into I*c* itself (3). This last was treated with iodine in bicarbonate to yield the iodo-aldehyde I*f*. Although this product was obtained only once analytically pure, the other specimens were otherwise undistinguished and were quite satisfactory as intermediates. The analytical difficulties could not be ascribed to an iodine–pyrrole complex (4). The iodo-aldehyde I*f* was reduced catalytically to the α-free aldehyde I*h*. The carbinol (I, R′ = H, R″ = CH_2OH) would be expected to be very unstable (5) and has not yet been isolated as a reduction product of I*h*. The oxime, I*i*, was obtained from I*h* but more conveniently by two unexpected routes. An attempt to prepare the oxime of I*f* led directly to I*i*, iodine being lost; the ready dehalogenation of some iodopyrroles has been reported (4). This dehalogenation with hydroxylamine is of limited applicability as the triethyl ester of I*g* survived the same treatment unchanged. An attempt to improve the conversion of the aldehyde I*c* into its oxime I*d* (3) led to the third and best preparation of I*i*: longer heating resulted in the expected oxime I*d* being decarboxylated to I*i*. The ease of this decarboxylation may be unrelated to that of 5-carboxy-alkylpyrroles and of 5-carboxy-porphobilinogen lactam; some 5-carboxypyrromethenes also decarboxylate readily (6).

Under carefully chosen conditions (see below and Experimental) the catalytic reduction of I*i* gave as the sole product porphobilinogen I*j*, identified by comparison with natural material as such, as the hydrochloride, and as the lactam. The yield of porphobilinogen from the triethyl ester of I*a* in four steps was 29%. On a much smaller scale our previous synthesis (7) had given 5% in seven steps after extensive purification.

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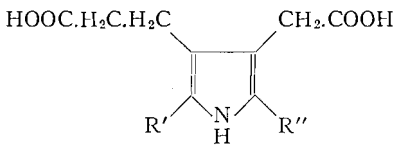
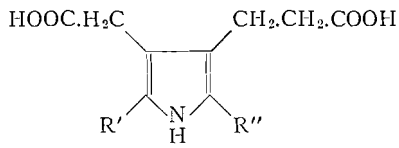
[2]*National Research Council of Canada Postdoctorate Fellow, 1958–60.*

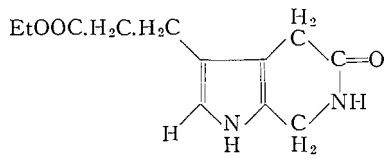


I

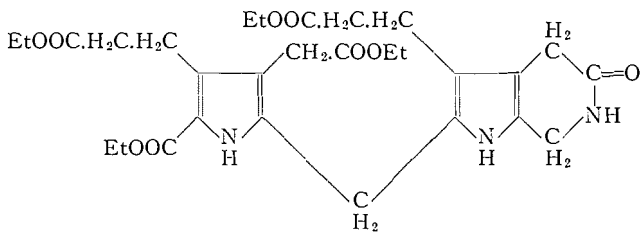


II

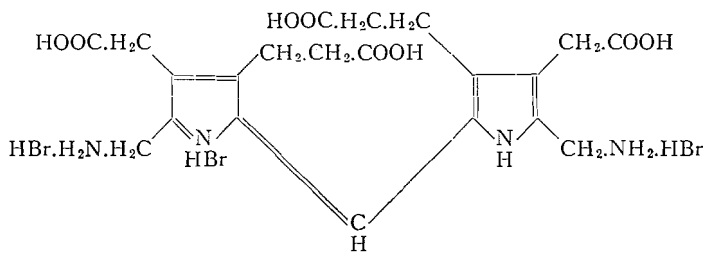
(*a*) R′ = COOH, R″ = CH_3
(*b*) R′ = COOH, R″ = CH_2Br
(*c*) R′ = COOH, R″ = CHO
(*d*) R′ = COOH, R″ = CH=N.OH
(*e*) R′ = COOH, R″ = $CH_2.NH_2$
(*f*) R′ = I, R″ = CHO
(*g*) R′ = I, R″ = COOH
(*h*) R′ = H, R″ = CHO
(*i*) R′ = H, R″ = CH=N.OH
(*j*) R′ = H, R″ = $CH_2.NH_2$
(*k*) R′ = H, R″ = CN
(*l*) R′ = COOH, R″ = H
(*m*) R′ = H, R″ = CH=C(CN)COOEt
(*n*) R′ = CHO, R″ = CN
(*o*) R′ = CHO, R″ = CH=C(CN)COOEt



III



IV



V

The carbinol (I, R′ = H, R″ = CH_2OH) was mentioned above, and routes to aminomethyl-porphobilinogen (I, R′ = R″ = CH_2NH_2) are also being explored. When the aldehyde group of I*h* was protected by condensation with cyanoacetic ester (8), the product, I*m*, could not be esterified. However, the aldehyde group of the ethyl ester of I*h* was similarly

protected and the resulting ester of I*m* was formylated to the ethyl ester of I*o* using dimethyl formamide and phosphorus oxychloride. The methyl ester of the oxime I*i*, which formed a stable hydrochloride, was converted into the methyl ester of the nitrile I*k* by dimethyl formamide and phosphorus oxychloride under mild conditions. Under more vigorous conditions the same reagents formylated the nitrile ester I*k* to the ester of the aldehyde-nitrile I*n*.

The structures of I*k* and I*n* were confirmed by conversion into known derivatives of II*a*: I*k* into II*l* and its ethyl ester, I*n* into II*c*. The sequence implies a formally general solution to the problem of interconverting isomeric 3,4-dialkylpyrroles such as I*a* and II*a*. Only particular solutions had been found to this problem which was once of some practical significance (9).

Both porphobilinogen and isoporphobilinogen (I*j* and II*j*) had been identified by paper chromatography among the products obtained by heating their 5-carboxy derivatives (I*e* and II*e*) with copper acetate in aqueous pyridine (10). However, neither had been isolated and in the case of porphobilinogen, our own experience with this synthesis had been discouraging (7). Accordingly, the synthesis of isoporphobilinogen originally projected depended on the decarboxylation of II*e* after acylating the primary amino group, a closer analogue of the easily decarboxylated 5-carboxyporphobilinogen lactam being hardly to be expected. To this end the ethyl ester of the aldehyde II*c*, obtained from II*a*, was converted to the ester of the oxime II*d* and thence to the ethyl ester hydrochloride of II*e*. We then turned to the following more attractive route analogous to the new synthesis of porphobilinogen. The aldehyde II*c*, obtained from its ester, was converted into isoporphobilinogen through II*f* and II*i*. We also converted II*f* into the α'-free aldehyde II*h*.

The reduction of the oxime I*i* to porphobilinogen, I*j*, had been carried out in relatively dilute solution to completely suppress the formation of a by-product of unknown structure and higher R_f (see Experimental). Although both products showed some reactions expected of porphobilinogen and gave similar analytical figures, the required product was identified through derivatives as a monohydrate corresponding to the structure I*j*, and through comparison with natural material, as porphobilinogen.

The reduction of the oxime II*i* to isoporphobilinogen, II*j*, had to be carried out at greater dilution than was that of I*i*, to completely suppress the formation of small amounts of an analogous by-product which again had a higher R_f. Isoporphobilinogen could be identified because it analyzed for anhydrous II*j*, had an R_f identical with that of porphobilinogen, and gave a well-characterized diethyl ester hydrochloride.

Of the pyrromethanes related to porphobilinogen, only the 5,5′-free compounds are known (11). In two approaches to others, the ethyl ester of porphobilinogen lactam, III, was condensed with the ester of I*b*, and 5-carboxyporphobilinogen, I*e*, was condensed with formic acid and hydrogen bromide to give compounds which may be provisionally formulated as IV and V, although we have not yet developed methods for checking the homogeneity of such pyrromethanes and pyrromethenes by paper chromatography. The methene V was reduced to a colorless substance which did not analyze well for the corresponding pyrromethane and which was not converted into uroporphyrins enzymically (12).

EXPERIMENTAL

The melting points are uncorrected as taken in capillaries. The infrared spectra in Nujol mull and X-ray powder photographs are by Dr. R. N. Jones and Mr. R. Lauzon,

and by Dr. Maria Przybylska. Paper chromatograms were ascending overnight runs in ethanol – concentrated ammonia – water (7:1:2), using an acidic permanganate spray (13) or, in the upper layer of *n*-butanol – glacial acetic acid – water (4:1:5), using an Ehrlich's spray. Such runs determining R_f's designated by "in ammonia" and "in acetic acid" respectively showed one spot only. Darco refers to Darco G-60 charcoal and ether, to anhydrous ether. The analyses on specimens dried to constant weight were by Mr. J. R. H. Séguin.

2-Formyl-5-carboxy-pyrrole-3-acetic acid-4-propionic acid (Ic)

The following method has given more consistent yields than did the earlier method (3). The triethyl ester of I*c* (16 g) (3) and 360 ml of absolute ethanol were added to 24 g of sodium hydroxide in 600 ml of water. Nitrogen was passed through while the mixture was warmed to solution and then kept at 50° for 1 hour. The orange solution was then concentrated to 100 ml in a rotary evaporator at <40°. The red concentrate was maintained below 10° while it was acidified to Congo red with 52 ml of concentrated hydrochloric acid. The temperature was then raised to 25°. The product crystallized as brownish-pink microneedles (12 g) which were filtered off after ¼ hour and washed with water, then with ether. It was purified by extracting into 800 ml of acetone (Soxhlet, 5 hours, 200 mg insoluble). The cooled acetone solution was forced by air pressure through a column (35 mm diam. × 60 mm of Darco/Celite 535 (1:1 by vol.) supported on a 15-mm bed of Celite 535) which was then thoroughly washed with acetone. When the pale yellow eluate was concentrated to 75 ml and cooled to 0°, 9.9 g (81%) of colorless needles separated; m.p. 241–243° (decomp., inserting at 235°), Ehrlich test dark red on heating; R_f "in ammonia": 0.19.

For analysis the product was recrystallized from acetone (Soxhlet) and dried at 56° and 5×10^{-2} mm. Calc. for $C_{11}H_{11}O_7N$: C, 49.07; H, 4.12; N, 5.20. Found: C, 49.33; H, 4.24; N, 5.32.

2-Formyl-5-iodopyrrole-3-acetic acid-4-propionic acid (If)

A suspension of 4.0 g of I*c* in 60 ml of water and 3.0 g of $KHCO_3$ was added to a solution of 9.0 g KI and 3.8 g of iodine in 60 ml of water. After the mixture was shaken to solution on the steam bath, heating was continued for 1 hour while carbon dioxide was evolved and the color lightened considerably. A cold finger, occasionally washed down with a few drops of absolute ethanol, prevented the loss of iodine. The solution was cooled to 20° and scratched. After ¼ hour the crude product (2.9 g, m.p. 196–197° decomp.) was filtered off and washed with water. After two recrystallizations from 65 ml of water, flesh-colored needles resulted (2.6 g, 50%); m.p. 199–200° (decomp., iodine evolved, inserted at 185°); Ehrlich test slowly positive cold; R_f "in ammonia": 0.40.

For analysis it was dried (60°, 1×10^{-4} mm). Calc. for $C_{10}H_{10}O_5NI$: C, 34.21; H, 2.87; N, 3.99; I, 36.15. Found: C, 34.28; H, 2.94; N, 4.17; I, 36.47, 36.04 (Schöniger), 35.97 (Carius).

2-Formylpyrrole-3-acetic acid-4-propionic acid (Ih)

The iodopyrrole I*f* (2.0 g) in 60 ml of absolute ethanol was added to 25 ml of water containing 776 mg of sodium acetate trihydrate and 250 mg of palladium black. The mixture was shaken under hydrogen (20°, 1 atm) until absorption ceased after the uptake of 1 mole during 1 hour. The filtrate and aqueous washings were taken to dryness in a rotary evaporator (bath at 50°) and 20 ml of water was twice added and removed in the same way. The residue was extracted with 5 ml of water and the insoluble product

filtered off and then washed (2×1 ml). This product was dried and then extracted into 200 ml of ether (Soxhlet, 6 hours). After the ether solution had been concentrated to 50 ml, yellow needles separated (970 mg, 76%); m.p. 176–180° (decomp., inserting at 160°); Beilstein test for halogen negative; Ehrlich test (magenta) fast in the cold; R_f "in ammonia": 0.33; in ethanol, λ_{max} 302 mμ ($\epsilon = 16\times10^3$). The aldehyde CH and the pyrrole ring CH were both confirmed by the N.M.R. spectrum.*

For analysis the product in acetone was decolorized by passage through a small column of Darco. The precipitate obtained from the eluate by adding ether was recrystallized three times from acetone–ether: colorless, flat needles, m.p. unchanged. Calc. for $C_{10}H_{11}O_5N$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.61; H, 4.94; N, 6.12.

2-Formylpyrrole-3-acetic acid-4-propionic acid Diethyl Ester

The aldehyde I*h* (351 mg) was warmed to solution in 5 ml of absolute ethanol, and diazoethane (from 3 g of nitrosoethylurea) in 75 ml of ether was added to the cooled solution. Evaporation left an oil which was dried *in vacuo* overnight and then crystallized by rubbing with ether. After sublimation (135°, 5×10^{-4} mm) it was obtained as pale yellow crystals (353 mg, 79%); m.p. 47–52°, softening from 37°; Ehrlich test red in the cold. The product of only one run crystallized and was not analyzed.

2-Formylpyrrole-3-acetic acid-4-propionic acid Oxime (Ii)

Method (a)

Hydroxylamine hydrochloride (500 mg) was dissolved in 3 ml of water, and 2 ml of 10% sodium hydroxide was added. The aldehyde I*h* (128 mg) and 1.4 ml of this solution were heated on the steam bath for ½ hour. The cooled solution was acidified to Congo red with 2 drops of concentrated hydrochloric acid and cooled at 0° for ½ hour; then the product (128 mg, 94%) was separated and washed successively with water, ethanol, and ether. It formed colorless microcrystals; m.p. 210–212° (decomp., inserted at 200°); Ehrlich test slowly violet cold. The R_f "in ammonia" (0.29) and the X-ray powder photograph were identical with those of the products of methods (*b*) and (*c*) below.

For analysis it was recrystallized four times (70% recovery) from 0.01 *N* NaOH/0.01 *N* HCl and dried at 60°, 0.02 mm. Calc. for $C_{10}H_{12}O_5N_2$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.82; H, 5.11; N, 11.55.

Method (b)

The iodopyrrole I*f* (3 g), 6 g of hydroxylamine hydrochloride, 36 ml of water, and 24 ml of 10% sodium hydroxide were heated for 1 hour on the steam bath (gases evolved). The cooled solution was passed through a column of Darco (22 mm diam.×10 mm), the column being washed with 15 ml of water. The yellow filtrate and washings were made acid to Congo red with ca. 18 ml of 2 *N* HCl, then cooled to 0° for 1 hour. The product (1.7 g, 81%) was separated and washed successively with water, ethanol, and ether to give cream-colored crystals; m.p. 212–213.5° (decomp., inserted at 200°); Beilstein halogen test negative, Ehrlich test slowly violet cold; in ethanol, λ_{max} 287 mμ ($\epsilon = 19\times10^3$).

For analysis it was recrystallized from acetone (Soxhlet) and dried (60°, 0.1 mm), giving cream-colored needles, m.p. 211–212° (decomp., inserted at 200°). Found: C, 49.89; H, 5.05; N, 11.51.

Preparative Method (c)

Hydroxylamine hydrochloride (24 g) in 144 ml of water was brought to pH 6–7 with

*We are grateful to Dr. H. J. Bernstein for this interpretation.

96 ml of 10% NaOH. The 5-carboxylpyrrole I*c* (9 g) was added and the solution was refluxed (mantle) under nitrogen for 7 hours in the dark. After being left at 20° in the dark overnight (important), the solution was filtered through a column of Darco (18 mm diam. × 40 mm, prewashed with water) and the column washed with water until the eluate was colorless. The purple eluate was acidified to Congo red with ca. 95 ml of 2 *N* HCl, scratched, and left at 0° for 1½ hours. The nearly colorless diamond-shaped crystals (6.02 g) were separated and washed successively with water, ethanol, and ether. A colorless product (5.29 g, 66%) was obtained after two recrystallizations from 0.1 *N* NaOH/2 *N* HCl (to pH 3); m.p. 210–211° (decomp., inserted at 200°); R_f "in acetic acid": 0.67.

For analysis it was recrystallized twice more in the same way and dried (60°, 0.01 mm). Found: C, 49.85; H, 5.33; N, 11.80.

2-Formylpyrrole-3-acetic acid-4-propionic acid Dimethyl Ester Oxime

A solution of 2 g of the oxime I*i* in 60 ml of 5% hydrogen chloride in methanol was kept at 0° overnight and then taken to dryness *in vacuo* below 20° in a rotary evaporator. To the residue dissolved in 100 ml of water, 4 ml of 2 *N* NaOH was added. After ½ hour the red-brown precipitate was separated, washed with water, dried (weight: 2 g), and extracted into 750 ml of ether (Soxhlet, 2 hours). The light brown extract was filtered through a column of Darco (18 mm diam. × 16 mm). The colorless eluate and ether wash were concentrated to 15 ml, and 50 ml of *n*-pentane was added with scratching. The colorless needles which separated (1.86 g, 83%) were washed with pentane; m.p. 130–133°; Beilstein halogen test negative; Ehrlich test slowly red cold; in ethanol, λ_{max} 285 mμ ($\epsilon = 20\times10^3$).

For analysis it was recrystallized twice from methanol–water (65% recovery) and dried (20°, 0.01 mm), m.p. 131–135°. Calc. for $C_{12}H_{16}O_5N_2$: C, 53.72; H, 6.01; N, 10.44; OMe, 23.14. Found: C, 53.86; H, 5.84; N, 10.53; OMe, 22.88.

2-Formylpyrrole-3-acetic acid-4-propionic acid Dimethyl Ester Oxime Hydrochloride

A solution of 201 mg of the oxime I*i* in 6 ml of 5% hydrogen chloride in methanol was kept at 0° for 3 hours and then taken to dryness at 20°; 6 ml of methanol was thrice added and removed in the same way. The residue was slurried with 10 ml of chloroform and the pink crystals (181 mg, 71%) filtered off, m.p. 160–164° (decomp., inserted at 150°); Beilstein halogen test positive; Ehrlich test very slowly violet cold.

For analysis it was twice precipitated from methanol by ether at 20° and dried (25°, 1×10^{-4} mm), giving colorless needles (35% recovery), m.p. 162–164° (decomp., inserted at 150°). Calc. for $C_{12}H_{16}O_5N_2$.HCl: C, 47.29; H, 5.62; N, 9.19; Cl, 11.64; OMe, 20.37. Found: C, 47.43; H, 5.65; N, 9.05; Cl, 11.44; OMe, 20.22.

2-Aminomethylpyrrole-3-acetic acid-4-propionic acid (Porphobilinogen, Ij)

A suspension of 5 g of the oxime I*i* and 2 g of palladium black in 100 ml of water was shaken under hydrogen at room temperature and pressure until absorption ceased after the uptake of 2 moles in about 2 hours. After the addition of 5 ml of concentrated ammonia to dissolve the product, filtration through paper (Whatman No. 5) gave a yellow solution. After bringing its pH to 7 with glacial acetic acid this solution was filtered through a column of alumina (Woelm, neutral, prewashed with water, 18 mm diam. × 50 mm) and the column washed with water until the Ehrlich reaction of the eluate was faint. The eluate (400 ml) was concentrated to 100 ml in a rotary evaporator (oil pump, baths 25–30° and ice–salt). The concentrate was acidified to pH 4.5 with glacial acetic acid with scratching, quickly cooled, and kept at 0° for 4 hours. The product, after being washed successively with water, ethanol, and ether, formed buff-colored, barrel-shaped

plates (3.2 g, 63% as the monohydrate); m.p. 174–177° (decomp., inserting at 167°); Ehrlich reaction rapidly positive cold. Its R_f "in acetic acid" (0.49) and its infrared mull spectrum were identical with those of natural porphobilinogen.

The X-ray powder photograph was identical with that of porphobilinogen synthesized over the lactam (7). Between these photographs and that of natural material there were differences in the relative intensities of 3 lines among 30. These differences are not considered significant because the natural porphobilinogen was photographed only as received, when its infrared mull spectrum showed a gross anomaly, unlike the spectrum of the same material after recrystallization as the hydrochloride and then as the base.

The synthetic porphobilinogen also agreed with natural material in the following respects:* (1) the quantitative Ehrlich reaction; (2) paper electrophoresis at pH 2.4, 4.4, 6.6, and 10.4; (3) the uroporphyrin mixtures (examined by paper chromatography after conversion to coproporphyrins) formed by the self-condensation of the porphobilinogen at various pH's; (4) the enzymic conversion to uroporphyrin.

For analysis it was recrystallized from 0.7 *N* ammonia – glacial acetic acid and dried (20°, 0.01 mm). Calc. for $C_{10}H_{14}O_4N_2.H_2O$: C, 49.17; H, 6.60; N, 11.47. Found: C, 48.88; H, 6.87; N, 11.41.

Porphobilinogen Lactam

This synthetic porphobilinogen was converted into the lactam (52%) by the method of Cookson and Rimington (14) as colorless hexagonal plates, Ehrlich test fast cold.

For analysis it was recrystallized from water (Soxhlet) and dried (20°, 0.01 mm), m.p. 281–283° (decomp., inserted at 270°). It was identical with the lactam from natural porphobilinogen by the following criteria: infrared mull spectrum, X-ray powder photograph, and R_f (0.63 "in acetic acid"). Calc. for $C_{10}H_{12}O_3N_2$: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.43; H, 5.77; N, 13.35.

Porphobilinogen Hydrochloride

This synthetic porphobilinogen was converted into the monohydrate of the hydrochloride (59%) by the method of Cookson and Rimington (14).

For analysis it was recrystallized from 2 *N* hydrochloric acid, dried (2 hours, 20°, 0.01 mm), and then exposed to air overnight to give faintly pink microneedles giving an I.R. mull spectrum and X-ray powder photograph identical with those of the natural hydrochloride. Calc. for $C_{10}H_{14}O_4N_2.HCl.H_2O$: C, 42.78; H, 6.10; N, 9.98; Cl, 12.63. Found: C, 43.01; H, 5.98; N, 9.89; Cl, 12.44.

A *by-product* of the reduction of the oxime Ii to porphobilinogen was initially very troublesome. The formation of this by-product, like that of porphobilinogen, was accompanied by the uptake of 2 moles of hydrogen. The ratio of the two products was independent of the hydrogen pressure but the by-product was favored by higher oxime concentrations and by reduction in dilute ammonia rather than in water. For example, 100 mg of the oxime with the catalyst in 1 ml of 1.5 *N* ammonia (1 atm of hydrogen) gave the by-product and a trace of porphobilinogen; in 25 ml of 0.7 *N* ammonia (1 atm or 120 atm of hydrogen) or in 1 ml of 0.7 *N* acetic acid (1 atm of hydrogen), porphobilinogen and a trace of by-product resulted.

The by-product analyzed much like the porphobilinogen monohydrate, gave a positive Ehrlich test cold, gave uroporphyrins with hot 2 *N* hydrochloric acid, and formed a hydrochloride. However, it gave no lactam by the method of Cookson and Rimington (14)

*We are indebted to Dr. J. J. Scott and to Professor L. Bogorad for the tests with enzymes, and to Dr. D. Mauzerall for the other tests.

and it differed from porphobilinogen in its R_f (0.66 "in acetic acid"), in its X-ray powder photograph, and in the infrared mull spectra of both the base and hydrochloride.

For analysis it was recrystallized from 1.5 *N* ammonia – acetic acid and dried (20°, 1×10^{-4} mm) to give colorless hair-like microcrystals, m.p. 178–181° (decomp., inserted at 166°). Calc. for $C_{10}H_{14}O_5N_2$: C, 49.58; H, 5.83; N, 11.57. Found: C, 49.68; H. 6.31; N, 11.05.

2-(ω-Cyano-ω-carbethoxy-vinyl)-pyrrole-3-acetic acid-4-propionic acid Diethyl Ester (Ethyl Ester of Im)

The ethyl ester of the aldehyde I*h* (the crude oily product from 351 mg of I*h* and ethereal diazoethane) was heated for ½ hour on the steam bath with 200 mg of ethyl cyanoacetate, 5 ml of absolute ethanol, and 2 drops of 25% aqueous methylamine. The crude product, which separated on cooling and scratching, was recrystallized from ethanol, giving 297 mg (51%), m.p. 108–110°.

For analysis it was recrystallized four times from ethanol (80% recovery) and dried (60°, 0.01 mm), giving yellow microneedles; m.p. 111–112° (softening from 105°); Ehrlich's test blue hot; insoluble in cold ethanol. Calc. for $C_{19}H_{24}O_6N_2$: C, 60.62; H, 6.43; N, 7.44. Found: C, 60.91; H, 6.50; N, 7.34.

The acid I*m* was prepared analogously from I*h* using aniline as the catalyst. It formed yellow crystals from ethanol; m.p. 191–194° (decomp., inserted at 180°); soluble in hot acetone; insoluble in refluxing ether. Attempts to esterify it with 5% ethanolic hydrogen chloride and with ethereal diazoethane failed.

2-(ω-Cyano-ω-carbethoxy-vinyl)-5-formylpyrrole-3-acetic acid-4-propionic acid Diethyl Ester (Ethyl Ester of Io)

The ethyl ester of I*m* (247 mg), suspended in 0.428 ml of dimethyl formamide, was treated with 0.070 ml of phosphorous oxychloride (10% excess) and heated to solution on the steam bath, then for 10 minutes longer. Water (15 ml) containing 573 mg of sodium acetate trihydrate was added to the cooled solution. After the mixture was scratched and allowed to stand ½ hour, the brownish-yellow product separated. Its solution in 10 ml of chloroform was filtered through a column of alumina (Woelm neutral grade IV, 8 mm diam. × 20 mm). The residue left on evaporating the eluate was recrystallized from ether – *n*-pentane and then from ethanol–water, giving yellow needles (137 mg, 52%), m.p. 107–109°.

For analysis it was twice recrystallized from ethanol–water (65% recovery) and dried (56°, 5×10^{-4} mm), m.p. 110–111°. Calc. for $C_{20}H_{24}O_7N_2$: C, 59.40; H, 5.98; N, 6.93. Found: C, 59.35; H, 5.79; N, 6.99.

2-Cyanopyrrole-3-acetic acid-4-propionic acid Dimethyl Ester (Methyl Ester of Ik)

The dimethyl ester of I*i* (500 mg, 1.87 mmoles) was warmed to solution in 0.67 ml of dimethyl formamide (previously distilled over phosphorus pentoxide). This solution was shaken vigorously at −15° while 0.19 ml (2.06 mmoles) of phosphorus oxychloride was added slowly, then left at 20° for ¼ hour. The mixture was treated with 1.125 g (8.3 mmoles) of sodium acetate trihydrate in 5 ml of water, diluted to 25 ml with water, and scratched; light brown crystals (394 mg) then separated. These were extracted into 200 ml of ether (Soxhlet, 1 hour), the solution was filtered through a column of Darco (8 mm diam. × 15 mm), and the column was washed with ether. The colorless eluate was concentrated to 10 ml. Crystallization was induced by scratching and completed by evaporating more ether while adding *n*-pentane. The product (344 mg, 74%) formed colorless

glittering crystals, Ehrlich test faintly rose hot. It exists in two forms, m.p. 81–83° and 103–106°, either of which might separate from solution or melt and which gave identical infrared mull spectra (nitrile band at 2240 cm^{-1}). The U.V. spectrum in ethanol, λ_{max} at 256 mμ ($\epsilon = 12\times10^3$) and 235 mμ ($\epsilon = 8\times10^3$), with no absorption at 280–290 mμ, showed that the oxime was absent.

For analysis it was twice recrystallized from ether – *n*-pentane (74% recovery) and dried (20°, 0.01 mm), m.p. 80–82° (softening from 75°). Calc. for $C_{12}H_{14}O_4N_2$: C, 57.59; H, 5.64; N, 11.20; OMe, 24.80. Found: C, 57.40; H, 5.62; N, 11.35; OMe, 24.64.

2-Carboxypyrrole-3-acetic acid-4-propionic acid (IIl)

The dimethyl ester of the nitrile I*k* (102 mg) in 4 ml of 10% sodium hydroxide and enough ethanol (about 2 ml) to effect solution was heated on the steam bath for 6 hours while nitrogen was bubbled through it and its volume maintained at 4 ml by the occasional addition of water. The cooled solution was passed through a column of Amberlite IR-120 (wet hydrogen form, 16 g) and the column was washed with water until the eluate gave a negative Ehrlich test cold. The residue left on taking the eluate to dryness in a rotary evaporator (bath at 45°) was extracted by 5×15-ml portions of boiling acetone. The extract was filtered and concentrated to 5 ml, 20 ml of ether was added, and amorphous mauve-colored impurities were filtered off at once. When the filtrate was scratched, the product (55 mg, 56%) crystallized as nearly colorless rectangular plates; m.p. 183–184° (decomp., inserted at 165°), undepressed by authentic material derived from the ester of II*a* (lit. 178° (3)); Ehrlich test quickly bluish-red cold. The R_f "in ammonia" (0.16) was identical with that of authentic material.

2-Carboxypyrrole-3-acetic acid-4-propionic acid Triethyl Ester

The acid (from the ester of I*k*) with diazoethane in ether gave the product as colorless needles; Ehrlich test slowly bluish-red cold; m.p. 51–52.5°, undepressed by authentic material (lit. 51–52° (3)). The two specimens also gave identical infrared mull spectra and X-ray powder photographs.

2-Cyano-5-formylpyrrole-3-acetic acid-4-propionic acid Dimethyl Ester (Ester of In)

The ester of the nitrile I*k* (200 mg, 0.8 mmole) was dissolved in 0.4 ml of dimethyl formamide (previously distilled over phosphorus pentoxide), and 0.1 ml (1.1 mmole) of phosphorus oxychloride was added. The solution was heated for 25 minutes on the steam bath in an open vessel and cooled to 20°, and 592 mg (4.4 mmoles) of sodium acetate trihydrate in 2.5 ml of water was added. The suspended oil crystallized after the solution was scratched. After the volume of the mixture was made up to 8 ml with water, the buff-colored precipitate was filtered off and washed with water. The dried precipitate (134 mg) was dissolved in 125 ml of boiling ether and the cooled solution filtered through a column of Darco (8 mm diam. × 10 mm). The colorless eluate was concentrated to 2 ml, crystallization was induced by scratching, and the product (112 mg, 50%) was filtered off and washed with 1 ml of ether and then with *n*-pentane. It formed colorless plates; m.p. 113–116° (softening from 90°); Ehrlich test slowly brown hot; infrared band at 2255 cm^{-1} in Nujol mull; in ethanol, λ_{max} 294 mμ ($\epsilon = 19\times10^3$). It can be distilled at 160°, 1×10^{-5} mm.

For analysis it was recrystallized from ether and twice dried (20° and 1×10^{-2} mm, then 100° and 1×10^{-3} mm), giving colorless plates, m.p. 113–115°. Calc. for $C_{13}H_{14}O_5N_2$: C, 56.11; H, 5.07; N, 10.07. Found: C, 55.87; H, 4.98; N, 9.99. Specimens dried at 20°, softened from about 100°, and analyzed as a hydrate containing $\frac{1}{3}H_2O$.

2-Carboxy-5-formylpyrrole-3-acetic acid-4-propionic acid (IIc)

(a) From the Triethyl Ester of Ia via the Dimethyl Ester of In

This was carried out before the ester of I*n* had been obtained crystalline. The crude oily ester of I*n* (obtained as above by adding aqueous sodium acetate after reacting the ester of I*k* with dimethyl formamide and phosphorus oxychloride) was hydrolyzed for 6 hours on the steam bath in 10% sodium hydroxide. After passage through Amberlite IR-120 (hydrogen form), the solution was evaporated to dryness. The solution of the residue in acetone was decolorized by filtration through a column of Darco and was then taken to dryness. Adding ether to the residue induced the product to crystallize. This showed a single spot on paper chromatography and gave an infrared mull spectrum, X-ray powder photograph, and R_f ("in ammonia" but descending for 70 hours to ensure the absence of II*l*) identical with those of authentic material obtained ultimately from the ester of II*a* by method (*b*) below.

(b) Preparative Method (from the Triethyl Ester of IIa via the Triethyl Ester of IIc)

The triethyl ester of II*c* (3) (16 g) was hydrolyzed to II*c* just as the ester of I*c* was to I*c*. The crude product (red crystals, 9.36 g) was extracted into acetone and passed through Darco to give 7.34 g (60%) of cream-colored crystals; m.p. 234–238° (decomp., inserted at 225°); Ehrlich test slowly brownish-red cold; R_f "in ammonia" identical with that of I*c*.

For analysis it was recrystallized from acetone (Soxhlet) and then at <50° from water and then dried at 25° and 1×10^{-2} mm to give pink radiating needles. Calc. for $C_{11}H_{11}O_7N$: C, 49.07; H, 4.12; N, 5.20. Found: C, 48.97; H, 4.38; N, 5.14.

2-Formyl-5-iodopyrrole-3-propionic acid-4-acetic acid (IIf)

The preparation of II*f* from 4 g of II*c* followed that of I*f* from I*c*. The buff-colored crystalline crude product (1.89 g), m.p. 221–222° (decomp., introduced at 200°), was twice recrystallized from 110 ml of water. The product (1.46 g, 28%) formed yellow needles; m.p. 221–224° (decomp., iodine evolved, introduced at 200°); Ehrlich test slowly positive cold; R_f "in ammonia" identical with that of I*f*.

For analysis an acetone solution was decolorized by filtration through a small column of Darco. The precipitate, obtained from the eluate by adding hexane, was recrystallized four times from water and dried (56°, 1×10^{-4} mm), giving colorless needles, m.p. 215–220° (decomp., iodine evolved, introduced at 200°). Calc. for $C_{10}H_{10}O_5NI$: C, 34.21; H, 2.87; N, 3.99; I, 36.15. Found: C, 34.35; H, 3.12; N, 3.99; I, 36.24.

2-Formylpyrrole-3-propionic acid-4-acetic acid (IIh)

The iodopyrrole II*f* (500 mg) in 24 ml absolute ethanol was added to 194 mg sodium acetate trihydrate in 16 ml of water, and the solution was shaken for 1 hour under hydrogen (20°, 1 atm) with 125 mg of palladium black. The filtrate and aqueous washings from the catalyst were taken to dryness in a rotary evaporator (bath temp. 45°), then 5 ml of water was twice added and removed in the same way. The residue was extracted into 75 ml of ether (Soxhlet, 6 hours). The residue left on evaporating the ether was dissolved in 15 ml of boiling acetone. This solution was filtered through a column of Darco, the eluate concentrated to 5 ml, and the colorless product (175 mg, 55%) precipitated by the addition of 20 ml of ether.

For analysis it was recrystallized twice from water (56% recovery) and dried (56°, 1×10^{-4} mm) to give colorless needles; m.p. 178–182° (decomp., inserted at 160°); Beilstein test for halogen negative, Ehrlich test rapidly red cold; R_f "in ammonia" identical with that of its isomer I*h*. Calc. for $C_{10}H_{11}O_5N$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.50; H, 4.82; N, 6.25.

This preparation could presumably be improved by using the method later developed for I*h*, that is, by washing the crude product with water before extracting into ether.

*2-Formylpyrrole-3-propionic acid-4-acetic acid Oxime (II*i*)*

Hydroxylamine hydrochloride (2 g) was dissolved in 12 ml of water and 8 ml of 10% NaOH added (pH 6–7), then 1 g of the iodopyrrole II*f*. The solution was heated on the steam bath for $1\frac{1}{2}$ hours (gas evolved), cooled to 20°, brought to pH 3 with concentrated hydrochloric acid, and then left overnight at 0°. The precipitate (403 mg) was separated, washed with a little water, then pentane, and dissolved in 4 ml of boiling water. Left at 20° overnight, the solution deposited buff-colored crystals (366 mg, 54%); m.p. 178–180° (decomp., inserted at 160°); Ehrlich test very slowly blue cold; Beilstein halogen test negative. The R_f "in ammonia" (0.33) was exceptional in that it differed from that of the isomeric I*i* (0.29).

For analysis it was recrystallized from water and dried, giving buff-colored crystals, m.p. unchanged. Calc. for $C_{10}H_{12}O_5N_2$: C, 50.00; H, 5.04; N, 11.66. Found: C, 49.76; H, 4.89; N, 11.56.

This product appears to remain in supersaturated aqueous solutions more than does its isomer I*i* and to be more strongly retained on Darco. Paper chromatography indicated that it was the sole product when II*c* was treated as in the conversion of I*c* to I*i* by method (*c*), but the isolation of the II*i* resulting by this method has not been worked out.

*2-Aminomethylpyrrole-3-propionic acid-4-acetic acid (isoporphobilinogen, II*j*)*

The oxime II*i* (125 mg) and 250 mg of palladium black were shaken under hydrogen (20°, 1 atm) with 250 ml of redistilled water until absorption ceased after the uptake of 2 moles in 2 hours. The catalyst was filtered off (Whatman No. 1 paper) and the filtrate concentrated to 20 ml in a rotary evaporator (baths at 30° and 0°). The pink solution, after the pH was brought to 7, was filtered through an alumina column (Woelm, neutral, 8 mm diam. × 20 mm, prewashed with water), which was then washed with water until the eluate gave a very faint Ehrlich test in the cold. The eluate (50 ml) was concentrated in a rotary evaporator (baths at 30° and 0°) to about 0.5 ml. After the addition of 0.7 *N* ammonia, the pH was brought to 4–5 with glacial acetic acid (volume of solution now <2 ml). After the solution was scratched, it was left at 0° for 3 hours. The product (83 mg, 70%) was separated and washed with a little water to give colorless needles; m.p. 192–195° (decomp., inserted at 180°); Ehrlich test rapidly red cold; R_f "in acetic acid" identical with that of porphobilinogen. It was also characterized by its X-ray powder photograph and infrared mull spectrum.

For analysis it was recrystallized from 0.7 *N* ammonia – glacial acetic acid and dried (20°, 0.01 mm). Calc. for $C_{10}H_{14}O_4N_2$: C, 53.09; H, 6.24; N, 12.38. Found: C, 52.92; H, 6.29; N, 11.96.

Isoporphobilinogen Diethyl Ester Hydrochloride

A solution of 51 mg of isoporphobilinogen, II*j*, in 2 ml of 5% hydrogen chloride in ethanol was left at 0° overnight. The colorless solution was taken to dryness *in vacuo* below 20° and 1 ml of ethanol was three times added and removed as above. The product crystallized on adding ether and seeding, giving 66 mg (92%) of colorless needles; m.p. 97–98.5°; Ehrlich test rapidly red cold; soluble in water and in ethanol. It was also characterized by its X-ray powder photograph and infrared mull spectrum.

For analysis it was recrystallized from ethanol–ether (recovery 88%) and dried (20°, 0.01 mm), m.p. 99–100°. Calc. for $C_{14}H_{23}O_4N_2Cl$: C, 52.74; H, 7.27; N, 8.79; Cl, 11.12. Found: C, 52.68; H, 7.24; N, 8.63; Cl, 11.03.

2-Formyl-5-carboxypyrrole-3-propionic acid-4-acetic acid Triethyl Ester Oxime (Ethyl Ester of IId)

Hydroxylamine hydrochloride (122 mg) in 0.6 ml of water was added to 42 mg of sodium in 10 ml of ethanol and the precipitate was filtered off and washed with ethanol. The filtrate and 500 mg of the ester of II*c* (3) were refluxed for $\frac{3}{4}$ hour. The cooled solution was poured into 175 ml of ice water. After being left overnight at 0°, the product (435 mg, 83%) was filtered off and washed with water, leaving colorless needles, m.p. 104–109°.

For analysis it was twice recrystallized from aqueous ethanol (65% recovery) and dried (57°, 1×10^{-4} mm), m.p. 107–112° softening from 103° (lit. (10), 88°). Calc. for $C_{17}H_{24}O_7N_2$: C, 55.43; H, 6.56; N, 7.61. Found: C, 55.31; H, 6.38; N, 7.79.

2-Aminomethyl-5-carboxypyrrole-3-propionic acid-4-acetic acid Triethyl Ester Hydrochloride (Ester Hydrochloride of IIe)

The ethyl ester of II*d* (476 mg) was shaken under hydrogen (20°, 1 atm) with 100 mg of palladium black, 50 ml of absolute ethanol, and 0.25 ml of concentrated hydrochloric acid until the uptake of hydrogen ceased ($3\frac{1}{2}$ hours, 2 moles taken up). The filtered solution was concentrated to 5 ml *in vacuo*, and 50 ml of ether was added. Scratching induced the separation of the product (386 mg, 76%) as colorless crystals; m.p. 147.5–149°; soluble in 2 *N* HCl; Ehrlich reaction positive hot.

For analysis it was recrystallized from ethanol–ether (recovery 93%) and dried *in vacuo* at 90°, m.p. 147.5–149.5°. Calc. for $C_{17}H_{27}O_6N_2Cl$: C, 52.33; H, 6.96; N, 7.17; Cl, 9.07. Found: C, 52.44; H, 6.93; N, 6.99; Cl, 8.73. The m.p. of the free ester has been reported as 185° (10).

Porphobilinogen Lactam Ethyl Ester (III)

Porphobilinogen lactam (100 mg) (7) was left for 5 hours in 8% ethanolic hydrogen chloride at 20°. The solvent was removed *in vacuo*, then more ethanol was added and removed in the same way. When the residue was crystallized from 3 ml of hot ethanol, two crops of the product were obtained as tan irregular plates (66 mg, 58%), m.p. 239–244° (decomp., inserting at 230°). Calc. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.99; H, 7.23; N, 12.01. The ester was also prepared from the lactam with diazoethane in ether–ethanol.

5-Aminomethyl-5′-carboxypyrromethane-4,3′-diacetic acid-3,4′-dipropionic acid Lactam Tetraethyl Ester (IV)

The ethyl ester of porphobilinogen lactam (III, 47.6 mg) and the ethyl ester of I*b* (83.7 mg) (15) were heated for 40 minutes on the steam bath with 1.7 ml of anhydrous sodium acetate in acetic acid (100 mg in 10 ml). Water (4 ml) was added slowly and the mixture heated nearly to a clear solution, then cooled slowly. When a few crystals had separated, the volume was brought to 20 ml with hot water. The nearly colorless amorphous product (81 mg, 70%) was separated from the cooled solution. At about 170° it either melted or changed to crystals, m.p. 181–186°.

For analysis it was extracted by ether (thimble) in which it is very slightly soluble, giving colorless microprisms which at 170° sintered and changed to needles, m.p. 185–189° (hot stage). Calc. for $C_{29}H_{39}N_3O_9$: C, 60.72; H, 6.85; N, 7.33; Found: C, 60.93; H, 6.85; N, 7.21.

The assigned structure is consistent with its U.V. spectrum in ethanol: minimum at 254 mμ and $\epsilon = 15.5\times10^3$ at the 281 mμ maximum. Compare the spectrum of the hexaethyl ester of 5,5′-dicarboxypyrromethane-3,3′-diacetic acid-4,4′-dipropionic acid

($\epsilon = 30.8 \times 10^3$ at 283 mμ (maximum); no minimum) and of the ethyl ester of porphobilinogen lactam (no maximum but a rise toward 210 mμ).

Condensation of 5-Carboxyporphobilinogen with Formic Acid

5-Carboxyporphobilinogen (7) (500 mg), I*e*, was heated on the steam bath for $\frac{1}{2}$ hour with 5 ml of 98% formic acid and 2.5 ml of hydrogen bromide (30% in acetic acid). The cooled solution was scratched after the addition of 10 ml of acetic acid then kept at 0° for 1 hour. The crystalline product, formulated as V (472 mg, 72%), was washed with acetic acid, then ether, and twice recrystallized from formic acid – acetic acid (1:4). After being dried overnight at 20° *in vacuo* over sodium hydroxide, the product (57% recovery) formed orange microneedles, decomposing on heating. Calc. for $C_{21}H_{29}O_8N_4Br_3$: C, 35.76; H, 4.14; N, 7.95; Br, 34.00. Found: C, 36.07; H, 3.94; N, 7.49; Br, 33.08.

Adding three equivalents of sodium hydroxide to a solution of the hydrobromide in water precipitated yellow plates, which did not analyze for the free base of the pyrromethene; m.p. about 275° (decomp.); Beilstein halogen test negative. Calc. for $C_{21}H_{26}O_8N_4$: C, 54.54; H, 5.67; N, 12.12. Found: C, 52.08; H, 6.26; N, 14.68.

After the hydrobromide was reduced in dilute sodium hydroxide with sodium amalgam, acetic acid precipitated initially colorless crystals which, after recrystallization, did not analyze well for the pyrromethane. Calc. for $C_{21}H_{28}O_8N_4$: C, 54.30; H, 6.08; N, 12.06. For $C_{21}H_{28}O_8N_4.C_2H_4O_2$: C, 52.67; H, 6.15; N, 10.68. Found: C, 52.84; H, 6.30; N, 10.33.

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