Possible Intermediates in the Biosynthesis of Porphyrins[1]

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Details of our synthesis of 5-aminomethyl-4,3′-dicarboxymethyl-3,4′-di-(2-carboxyethyl)-dipyrrylmethane (**1***b*), as a stable potassium salt are now reported. This synthetic product had been shown to be an intermediate in the biosynthesis of uroporphinogen 1. The analogous tripyrrane, **15**, should be available from the lactam of 2-(2-methoxycarbonylethyl)-3-methoxycarbonylmethyl-4,6-di-(2-ethoxycarbonylethyl)-5-ethoxycarbonylmethyl-7-carboxymethyl-8-aminomethyl-tripyrrane (**22**) which has also been synthesized.

5-Formyl-4,3′-di-ethoxycarbonylmethyl-3,4′-di-(2-ethoxycarbonylethyl)-5′-benzyloxycarbonyldipyrrylmethane (**24**), was obtained through the condensation of the bromomethyl pyrrole **2***b* with the hydrazone, **23**, from a 2-formyl-5-free pyrrole and Girard's "T" reagent. This depends on the supression of the deactivating effect of the aldehyde group in a hydrazone from which the aldehyde is easily regenerated.

Notre synthèse de l'aminométhyl-5 dicarboxyméthyl-4,3′ di(carboxy-2 éthyl)-3,4′ dipyrrylméthane (**1***b*), comme sel de potassium stable est maintenant rapportée de façon détaillée. On a montré que ce produit synthétique est un intermédiaire dans la biosynthèse de l'uroporphinogène 1. L'analogue tripyrrane **15** pourrait être disponible à partir de la lactame de la (méthoxycarbonyl-2 éthyl)-2 méthoxycarbonyl méthyl-3 di(éthoxycarbonyl-2 éthyl)-4,6 éthoxy carbonylméthyl-5 carboxyméthyl-7 aminométhyl-8 tripyrrane (**22**), qui a été également synthétisée. Le formyl-5 diéthoxycarbonylméthyl-4,3′ di(éthoxycarbonyl-2 éthyl)-3,4′ benzyloxycarbonyl-5′ dipyrridylméthane (**24**), a été obtenu par condensation du bromométhylpyrrole **2***b* avec l'hydrazone **23** du formyl-2 pyrrole libre en -5 et le réactif "T" de Girard. Cela dépend de la suppression de l'effet désactivant du groupe aldéhyde dans une hydrazone à partir de laquelle l'aldéhyde est facile à regénérer.

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Introduction

Some time ago we undertook the synthesis of compounds which might be intermediates between porphobilinogen and the uroporphinogens in the biosynthesis of the porphyrins. Although there was no evidence at the time that such intermediates existed, they would be identified if the synthetic materials were substrates for the enzymes involved. Another incentive arose because the value of labelling is minimized when four identical units of porphobilinogen condense to the uroporhinogen; a second substrate, not equivalent to porphobilinogen, would provide one solution to this difficulty.

After it was shown (1) that neither opsopyrrole-dicarboxylic acid (2) nor *iso*-porphobilinogen (3) were intermediates, we turned to dipyrrylmethanes. Neither **1***a* nor its symmetrical isomers were intermediates (1, 4). However, their synthesis (5, 6) showed that unsymmetrical 5,5′-dicarbethoxydipyrrylmethanes, free of symmetrical isomers, could be obtained by condensing 2-free and 2-bromomethyl pyrroles[9] in hot AcOH–NaOAc. More important, unstabilized dipyrrylmethanes such as **1***a* were easily obtained from their carboxylated derivatives, and their quite unexpected stability (*cf.* 7, 8) was a prerequisite for further work in this direction.

Attention was then directed to aminomethyl dipyrrylmethanes, first to methylene-bis-porphobilinogen (3) which was not an intermediate (1, 4), and then to **1***b*. A synthesis of **1***b* based on that of **1***a* would involve the condensation of **2***b*, presumably available by known general methods, with a 5-free pyrrole bearing a potential 2-aminomethyl group. Such groups, like

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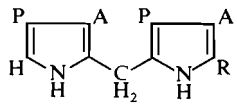
[9]In hot AcOH–NaOAc, bromomethyl pyrroles are equivalent to acetoxymethylpyrroles into which they are rapidly converted.



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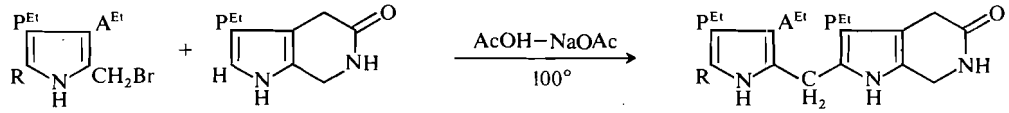
$A = CH_2COOH$ $P = CH_2CH_2COOH$
$A^{Me} = CH_2COOMe$ $P^{Me} = CH_2CH_2COOMe$
$A^{Et} = CH_2COOEt$ $P^{Et} = CH_2CH_2COOEt$
$A^{Bz} = CH_2COOCH_2Ph$ $P^{Bz} = CH_2CH_2COOCH_2Ph$

a R = H
b R = CH_2NH_2



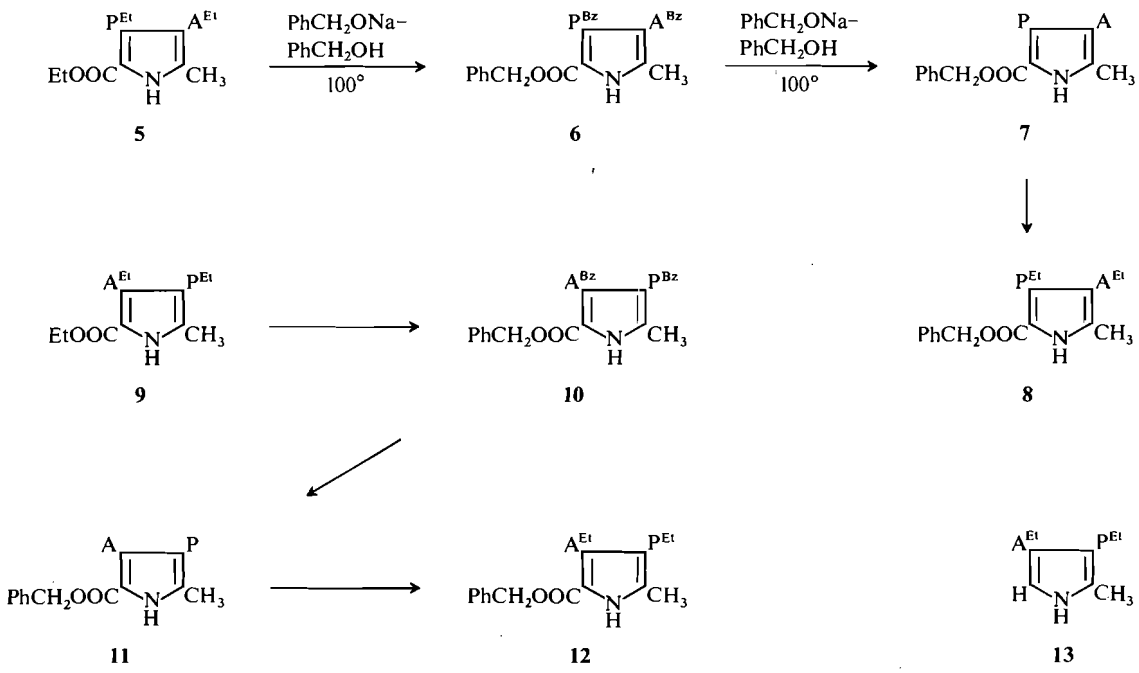
2 3 4

(*a*) R = COOEt; (*b*) R = $COOCH_2Ph$; (*c*) R = COOH; (*d*) R = I; (*e*) R = H

CN, frequently deactivate the 5-position, but a suitable component was found in the ethyl ester of porphobilinogen lactam, **3**, which condensed with **2***a* to **4***a* (3).

We have indicated the later steps in the synthesis of **1***b* (9), and the product, used as its potassium salt, was shown to be an intermediate in the biosynthesis of uroporphyrin 1, into which up to *ca.* 50% of **1***b* was incorporated by the enzyme system (22, 10). We have been reluctant to publish the synthetic work in detail because the product **1***b* has not been characterized in a way which would distinguish it from its isomers, and because it contained about 10% of an impurity. This limits the usefulness of **1***b*: its 10% incorporation would be ambiguous, and a natural intermediate could not be positively identified merely by comparison with it. In its critical steps, our synthesis does not differ in principle from those that have been described (11) or outlined (12).

In order to obtain the bromomethylpyrrole **2***b*, we first converted **5** to **8** by methods which were also applicable to the conversion of **9** into **12**; the latter would be required for isomers of **1***b*. We failed to obtain **8** from the diethyl ester



5 6 7 9 10 8 11 12 13

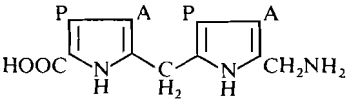
of hemopyrrole-dicarboxylic acid with phosgene, dimethylaniline, and benzyl alcohol, *cf.* (13). Although this method converted the diethyl ester of cryptopyrrole-dicarboxylic acid, **13**, into **12**, the overall yield from **9** was only 15%. Another general method involving successive transesterifications, *cf.* (14), also failed, but a modification in its first step was useful. When **5** or **9** were heated with sodium benzylate in benzyl alcohol, the tribenzyl esters **6** and **10** first formed were converted to **7** and **11**. These conversions occurred directly rather than during the work-up, *cf.* (14), and the formation of acids here was not unprecedented (15). Under modified conditions, **7** and **11** became the chief products, and these were esterified to **8** and **12**. The hydrogenation of **6**, **7**, or **8** removed the benzyl groups to yield the expected acids. Neither the partial hydrolysis of **6** to **7** nor the partial transesterification of **10** to **12** was promising.

The 2-methylpyrroles **6** and **8** were brominated to the corresponding 2-bromomethylpyrroles one of which, **2***b* from **8**, was condensed with **3** to **4***b*, *cf.* (3). The latter was hydrogenated to **4***c* which gave **4***d* with iodine in bicarbonate, and thence **4***e* by hydrogenation. It was later found that this last could be obtained by decarboxylating **4***c* directly in water at 100°, but the product then contained impurities which were only evident through t.l.c. and were difficult to remove.

It was essential to obtain the dipyrrylmethane **1***b*, an acid-labile acid, in a stable form. Further, a product containing excess salts could not be subjected to thin-layer electrophoresis. Many pyrrole-polycarboxylic acids are precipitated as their potassium salts when the corresponding esters are heated in ethanolic KOH, and **4***e* was thus converted into the tetrapotassium salt of **1***b*. Sodium and barium salts were obtained likewise but to no advantage.

The potassium salt was stable when dry and, in the absence of porphobilinogen, gave no uroporphinogen under the conditions of incubation (10), *cf.* however (11). The analysis of the first specimen obtained was satisfactory, but those of later specimens were less so, and their n.m.r. spectra showed that ethoxy was present. This was not due to ester groups for, after the products had been dissolved in water and freeze-dried, ethoxy was absent and the analyses were satisfactory. It was also evident that the lactam ring, in which the CH_2CO protons are easily exchanged, was not present in the product, *cf.* (9). Thus, when **4***e* was hydrolyzed in NaOH–EtOH or in NaOD–EtOD, the sodium salt isolated showed *ca.* 4 or 2 protons respectively at τ 6.7, before or after subsequent treatment with NaOD–D_2O or NaOH–H_2O. Evidently the hydrolyses were complete and ethoxy in the products was due to ethanol. Thin-layer electrophoresis of the potassium salt revealed the more troublesome faster running impurity mentioned above; subject to the uncertainty of the Ehrlich color, the amount of this was roughly 10%.[10]

When **4***a* was heated in ethanolic KOH a potassium salt again separated. The free acid, **14**, obtained from it, analyzed badly and



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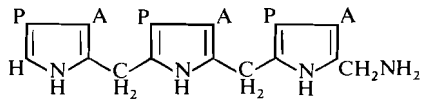
attempts to decarboxylate it were, as expected, unpromising.

When **1***b* proved to be an intermediate in the biosynthesis of uroporphyrin 1, we undertook the synthesis of the corresponding tripyrrane **15**. This has been carried to the lactam ester **22** analogous to **4***e*. However, in view of the impurity found in **1***b*, the hydrolysis of **22** as well as the biochemical work on the product, are being studied in cooperation with Bogorad, so the hydrolysis of **22** can be monitored by thin-layer electrophoresis.

The synthesis of **22** by a repetition of the reactions which had converted **3** into **4***e* would probably involve more labile intermediates. In particular, the tripyrrane from **2***b* and **4***e* might not be stable in the acid medium in which it was formed, and some preliminary experiments were unpromising. We turned to the condensation of 2-formylpyrroles with **4***e* because the resulting tripyrrenes should be more stable than tripyrranes in acid, and because pyrrole-2-aldehydes, unlike 2-bromomethylpyrroles, could be obtained without blocking groups (benzyloxycarbonyl) in the 5-position. In preliminary experiments, the aldehydes **16***a* and *b* were condensed with **4***e* to give **17***a* and *b*; the latter was hydrogenated to **18**. When the aldehydes **19***a* or *b* were used, the products were not

[10]L. Bogorad. Private communication.



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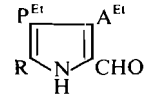

16

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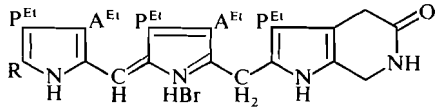
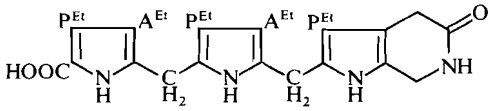
b R = $COOCH_2Ph$
c R = H



17

a R = COOEt
b R = $COOCH_2Ph$



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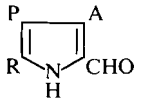
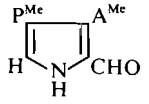


19

a R = H
b R = I
c R = COOH



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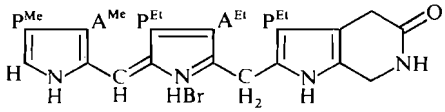

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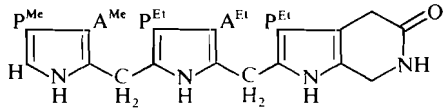
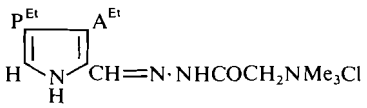


22

crystalline; the aldehyde **16***c*, which we have not obtained pure, also failed to give a crystalline product. However, **20** condensed with **4***e* to give the beautifully crystalline tripyrrene **21**, and this was hydrogenated with **22**.

We had explored other approaches to aminomethyl derivatives of dipyrrylmethanes, tripyrranes, and tetrapyrranes. One such, which we have not followed up, might depend on the interconversion (19) of 5-chloro- and 5-hydroxydipyrrylmethenes, and the latter (unlike the former) undergoing typical pyrrole reactions. Another might have been based on the conversion of 5-methyl- to 5-formyl-dipyrrylmethenes (20) had this been sufficiently general. A third approach, now attractive in connection with tetrapyrranes, was to condense **2***b* with pyrroles other than **3**, avoiding the limitations imposed by the use of the latter. 2-Formyl pyrroles, and to a lesser extent their condensation products with malondinitrile, etc., are strongly deactivated at the 5-position. The corresponding oximes, however, condensed with bromomethyl pyrroles to oximes of 5-formyl-dipyrrylmethanes. These condensations are not reported here because the results were erratic, but they did suggest the use of **23**, for Girard hydrazones are stable in AcOH but are easily split by dilute HCl. The condensation of **2***b* with **23** in hot AcOH–NaOAc gave the water-soluble hydrazone from which the aldehyde **24** was obtained with dilute HCl. Other 5-formyl-5′-benzyloxy-



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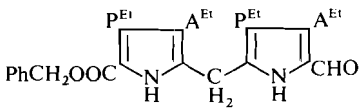

24

carbonyl- dipyrrylmethanes, with difierent β-substituents, have been obtained from the corresponding 5-*t*-butoxycarbonyl-dipyrrylmethanes (21), a route which would involve more intermediates in the present case. The benzyl ester **24** was hydrogenated to the corresponding acid, to which we referred some time ago (20); but we have recently had difficulty in repeating this hydrogenation.

Experimental

2-Methyl-3-carboxymethyl-4-β-carboxyethyl-5-benzyloxycarbonylpyrrole (7), and 2-Methyl-3-benzyloxycarbonylmethyl-4-β-benzyloxycarbonylethyl-5-benzyloxycarbonylpyrrole (6)

2-Methyl-3-ethoxycarbonylmethyl-4-β-ethoxycarbonylethyl-5-carbethoxypyrrole (**5** (16), 10.8 g) was added to a warm solution of 2.2 g of sodium in 260 ml of pure benzyl alcohol. The mixture was heated for 1 h on the steam bath with frequent swirling while a gel formed, then for 3 h under water-pump vacuum. Benzyl alcohol was then removed (rotary, 0.05 mm, bath 100°). The solution of the residue in 150 ml of water was extracted with ether (2 × 100 ml), then slowly acidified to congo red with 10% HCl. The precipitate was recrystallized from acetone (thimble) as colorless needles of the monobenzyl ester (7.2 g, 65%), m.p. 244°. Ehrlich's reaction, weakly positive on warming.

Anal. Calcd. for $C_{18}H_{19}O_6N$: C, 62.60; H, 5.55; N, 4.06; OEt, 0.00. Found: C, 62.81; H, 5.82; N, 3.47; OEt, 0.00.

Ether and benzyl alcohol were removed from the ether washings, finally at 100° and 0.1 mm, and the residue was crystallized from hexane (thimble) as colorless hairs of the tribenzyl ester (0.4 g), m.p. 108°. The yield of this is increased by using less sodium.

Anal. Calcd. for $C_{32}H_{31}O_6N$: C, 73.12; H, 5.95; N, 2.67. Found: C, 72.91; H, 5.91; N, 3.08.

Both the monobenzyl ester and the tribenzyl ester were converted into the corresponding tricarboxylic acid, m.p. 151–152° dec. (lit. (16), 155–156° dec.), by hydrogenation in ethanol over palladium black.

When the tribenzyl ester (400 mg) was refluxed for 1.5 h in 65 ml of tetrahydrofuran and 15 ml of *N*/10 NaOH, the unchanged tribenzyl ester (147 mg) and the monobenzyl ester (76 mg) were recovered.

2-Methyl-3-ethoxycarbonylmethyl-4-β-ethoxycarbonylethyl-5-benzyloxycarbonylpyrrole (8)

The monobenzyl ester (**7**, 2.8 g) was left in the dark overnight in 80 ml of 5% absolute ethanolic HCl at 20°. Ethanol was evaporated at $<50°$, more ethanol was added and evaporated, and the residue was recrystallized from hexane (thimble) as colorless needles (2.6 g, 79%), m.p. 97–98°.

Anal. Calcd. for $C_{22}H_{27}NO_6$: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.89; H, 6.83; N, 3.67.

2-Methyl-3-ethoxycarbonylmethyl-4-β-ethoxycarbonylethyl-5-carboxypyrrole

The corresponding 5-carbobenzoxypyrrole, **8**, was hydrogenated in ethanol over palladium black. The catalyst and solvent were removed, and the residue was crystallized from cold acetone–hexane as colorless plates, m.p. 126–127°, Ehrlich's reaction, strongly positive cold.

Anal. Calcd. for $C_{15}H_{21}O_6N$: C, 57.85; H, 6.80; N, 4.50. Found: C, 58.01; H, 6.84; N, 4.63.

2-Bromomethyl-3-ethoxycarbonylmethyl-4-β-ethoxycarbonylethyl-5-benzyloxycarbonylpyrrole (2b)

Method a; Using Bromine

The corresponding 2-methylpyrrole (**8**, 204 mg) in 5 ml of carbon tetrachloride, was treated with a solution of bromine (0.025 ml) in carbon tetrachloride (2 ml), and the solution was irradiated for 20 min under a u.v. lamp. The solvent was removed *in vacuo* at 20°, more was added and removed in the same way, and the residue was crystallized from *n*-hexane (thimble) as colorless needles (45–55%), m.p. 119–120°.

Anal. Calcd. for $C_{22}H_{26}NO_6Br$: C, 55.00; H, 5.46; N, 2.92; Br, 16.64. Found: C, 54.78; H, 5.39; N, 3.04; Br, 16.49.

To scale up this preparation, the crude products from small runs were crystallized together.

Method b; Using N-Bromosuccinimide

The 2-methylpyrrole (**8**, 4 g) in 105 ml of CCl_4 under dry N_2 was heated (bath 89–90°) and stirred for 20 min under reflux with 1.78 g of NBS and a trace of benzoylperoxide. The filtrate and washings from the cooled mixture were evaporated and the crystalline residue was crystallized from 45 ml of benzene–*n*-hexane (1:2) using Darco, then recrystallized twice from the same solvents; yield 40–50%.

2-Acetoxymethyl-3-ethoxycarbonylmethyl-4-β-ethoxycarbonylethyl-5-benzyloxycarbonylpyrrole

The bromomethylpyrrole **2***b* (500 mg) in 15 ml of acetic acid containing 50 mg of anhydrous sodium acetate, was warmed for 10 min at 70°. Water was then added and the turbid solution was left at 10°. The colorless product was separated and recrystallized from aqueous ethanol as needles, m.p. 105–106°.

Anal. Calcd. for $C_{24}H_{29}NO_8$: C, 62.73; H, 6.36; N, 3.05. Found: C, 62.55; H, 6.51; N, 2.89.

2-Bromomethyl-3-benzyloxycarbonylmethyl-4-β-benzyloxycarbonylethyl-5-benzyloxycarbonylpyrrole

The corresponding 2-methyl pyrrole (**6**, 988 mg) was dissolved in 10 ml CCl_4, 0.1 ml of bromine was added, and the solution was irradiated by a u.v. lamp for 20 min. The solution, from which some of the product had precipitated, was evaporated below 50°; more solvent was added and removed in the same way. The residue was crystallized from ether–hexane (thimble), dissolved in ether, and the ether solution was filtered through Darco. When the solution was concentrated, the product separated. It was recrystallized twice from ether–hexane as colorless hairs, m.p. 140–141°.

Anal. Calcd. for $C_{32}H_{30}NO_6Br$: C, 63.57; H, 5.01; N, 2.32; Br, 13.23. Found: C, 63.43; H, 5.00; N, 2.40; Br, 13.02.

2-Methyl-3-β-carboxyethyl-4-carboxymethyl-5-benzyloxycarbonyl-pyrrole (11)

Sodium (0.52 g) was warmed to solution in 35 ml of pure benzyl alcohol and 2-methyl-3-β-ethoxycarbonylethyl-4-ethoxycarbonylmethyl-5-carbethoxypyrrole (**9** (17), 2.59 g) in 40 ml of benzyl alcohol was added. The solution was heated on the steam bath for 5 1/4 h, cooled, poured into 400 ml of ether, and the whole was extracted with water.

The product (68%), m.p. 211–215°, was precipitated when the aqueous layer was acidified. It was recrystallized from acetone–hexane then from acetone as colorless prisms, m.p. 217–218°.

Anal. Calcd. for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; N, 4.06. Found: C, 63.20; H, 5.87; N, 3.86.

The ether layer was evaporated and the residue was crystallized from hexane to give the tribenzyl ester (see below) as colorless needles (67 mg), m.p. 89–92°.

*2-Methyl-3-β-benzyloxycarbonylethyl-4-benzyloxycarbonylmethyl-5-benzyloxycarbonylpyrrole (**10**)*

2-Methyl-3-β-ethoxycarbonylethyl-4-ethoxycarbonylmethyl-5-carbethoxypyrrole (**9**, 4.99 g) was dissolved in 25 ml of pure benzyl alcohol, 0.105 g of sodium were added, and the mixture was heated in the steam bath first for 1 h excluding moisture, then for 2 h at 12 mm. The solvent was removed *in vacuo* at 100°, water was added to the residue, and the mixture was extracted with ether. The product which crystallized from the ether at 0° was recrystallized from *n*-hexane as colorless needles (31%), m.p. 92–93°.

Anal. Calcd. for $C_{32}H_{31}NO_6$: C, 73.12; H, 5.95; N, 2.67. Found: C, 72.84; H, 5.88; N, 2.96.

When the tribenzyl ester was refluxed with sodium (1.5 atoms) in ethanol for 20 min, it was reconverted into the triethyl ester (50%), m.p. and mixed m.p. 63°.

*2-Methyl-3-β-ethoxycarbonylethyl-4-ethoxycarbonylmethyl-5-benzyloxycarbonylpyrrole (**12**)*

2-Methyl-3-β-carboxyethyl-4-carboxymethyl-5-benzyloxycarbonylpyrrole, **11**, was left overnight in 30 parts of 5% ethanolic HCl in the dark. The solvent was removed at 50°, more ethanol was added and removed in the same way, and the residue (98%) was recrystallized from *n*-pentane as colorless needles, m.p. 88–89°.

Anal. Calcd. for $C_{22}H_{27}NO_6$: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.74; H, 6.72; N, 3.46.

*Lactam of 5-Aminomethyl-4-carboxymethyl-3,4′-di-(β-ethoxycarbonylethyl)-3′-ethoxycarbonylmethyl-5′-benzyloxycarbonyldipyrrylmethane (**4b**)*

The ethyl ester of porphobilinogen lactam (**3** (3), 118 mg) and the 2-bromomethylpyrrole (**2***b*, 240 mg) in 5 ml of acetic acid containing 50 mg of anhydrous sodium acetate were heated for 40 min on the steam bath. Water (9 ml) was then slowly added, and the mixture was heated until clear. When crystals appeared in the cooled solution, 50 ml of hot water were added, and the mixture was allowed to cool, finally at 10°. The product was separated and recrystallized from 35% ethanol (60 ml) as pale yellow micro-crystals (55–75%), m.p. 142–142.5°; other forms melting up to 180° may appear directly or from the melt. T.l.c.[11] in $CHCl_3$–MeOH (9:1): R_f 0.60 and usually a small spot on the start line. Larger runs are equally satisfactory. The analogous trimethyl-benzyl ester has been described (11).

Anal. Calcd. for $C_{34}H_{41}N_3O_9$: C, 64.24; H, 6.50; N, 6.62. Found: C, 64.11; H, 6.60; N, 6.49.

*Lactam of 5-Aminomethyl-4-carboxymethyl-3,4′-di-(β-ethoxycarbonylethyl)-3′-ethoxycarbonylmethyl-5′-carboxydipyrrylmethane (**4c**)*

The corresponding 5′-carbobenzoxydipyrrylmethane (**4***b*, 1.91 g) in 1 l of 95% ethanol was shaken with 400 mg of palladium black for 24 h under hydrogen. The mixture was then warmed to dissolve the product and filtered. The filtrate was concentrated and then cooled. The product which separated was recrystallized from 250 ml of 95% ethanol as a gray micro-crystalline powder (60–75%), m.p. 192.5–193.5 (dec.). T.l.c. in $CHCl_3$–MeOH (1:1): R_f 0.6 only.

Anal. Calcd. for $C_{27}H_{35}N_3O_7$: C, 59.43; H, 6.46; N, 7.70. Found: C, 59.29; H, 6.39; N, 7.66.

*Lactam of 5-Aminomethyl-4-carboxymethyl-3,4′-di-(β-ethoxycarbonylethyl)-3′-ethoxycarbonylmethyl-5′-iododipyrrylmethane (**4d**)*

The corresponding 5′-carboxy-dipyrrylmethane (**4***c*, 347 mg) was warmed to solution in 17.5 ml of 95% ethanol and 7 ml of water containing 165 mg of sodium bicarbonate. Iodine (164 mg) in ethanol (7 ml) was added to the cooled solution, which was heated briefly then refrigerated overnight. The product (55–70%) was washed with water then with ethanol. For analysis, it was recrystallized from ethanol–acetone (1:2) as small light yellow crystals, m.p. 149–150° (dec.). T.l.c. in $CHCl_3$–MeOH (1:1): R_f 0.8 only.

Anal. Calcd. for $C_{26}H_{34}N_3O_7I$: C, 49.76; H, 5.57; N, 6.70; I, 20.23. Found: C, 49.98; H, 5.50; N, 6.81; I. 19.99.

*Lactam of 5-Aminomethyl-4-carboxymethyl-3,4′-di-(β-ethoxycarbonylethyl)-3′-ethoxycarbonylmethyldipyrrylmethane (**4e**) (J. M. Osgerby)*

A suspension of the 5′-iodo-dipyrrylmethane (**4***d*, 232 mg) in 500 ml of ethanol, to which had been added 35 mg of anhydrous sodium acetate in 3 ml of water, was shaken in the dark overnight with palladium black under hydrogen. The colorless solution was filtered and the solvent removed below 50°. The residue was recrystallized from aqueous ethanol as small grey prismatic rods (70–80%), m.p. 180°; Ehrlich's reaction, strongly positive cold. For analysis, it was recrystallized from ether (thimble). N.m.r. ($CDCl_3$) τ 8.78 (m, 9.4, OCH_2CH_3), 7.38 (sym. m, 7.9, CH_2CH_2CO), 6.68, 6.50, 6.21, 5.92, and 5.66 (poorly resolved t, J = 2.5–3.0 Hz, s, s, m, broad s, 13.6, CH_2CONH, CH_2COO, bridge CH_2, OCH_2CH_3, CH_2NHCO), 3.58 and 3.19 (d, J = 2.5 Hz, broad s, 2.4, pyrrole C*H*, CON*H*), 0.94 and 0.64 (poorly resolved d, J = 2.0–2.5 Hz, s, 1.7, two pyrrole N*H*). T.l.c. ($CHCl_3$–MeOH, 4:1): R_f 0.74 only. The analogous trimethyl ester has been described (11).

Anal. Calcd. for $C_{26}H_{35}N_3O_7$: C, 62.26; H, 7.04; N, 8.38. Found: C, 62.20; H, 7.07; N, 8.44.

*Tetrapotassium Salt of 5-Aminomethyl-4,3′-di-carboxymethyl-3,4′-di-β-carboxyethyldipyrrylmethane (**1b**), cf. (11) (J. Pluscec)*

The lactam ester (**4***e*, 113 mg) in 5 ml of absolute ethanol containing 560 mg of KOH was refluxed on the steam bath for 80 min; the product began to separate after 5 min. The product was washed by decantation with absolute ethanol, with ether, then with acetone. It was transferred to a tube using acetone, then dried for 15 h in the dark (1×10^{-4} mm, 20°). The product was a light brown powder (75%), very hygroscopic before it was dried; Ehrlich's reaction, strongly positive cold. For the second analysis and for the n.m.r. spectrum, a specimen was dissolved in water and then freeze-dried; n.m.r. (D_2O) τ 7.48 (m, 8, CH_2CH_2CO), 6.68 (s, 3.9, CH_2COO), 6.27 (s, 1.9, bridge CH_2), 5.92 (s, 1.7, CH_2NH_2), 3.50 (s, 0.8, pyrrole C*H*); u.v. no strong absorption 650–300 mμ.

[11]All t.l.c. on silica gel H coated plates.

Anal. Calcd. for $C_{20}H_{21}N_3O_8K_4$: C, 40.87; H, 3.60; N, 7.15; K, 26.61. Found: C, 40.52, 40.48; H, 3.97, 3.97; N, 7.32, 6.92; K, 26.23, 26.39.

A sample was heated for 20 min at 100° in 0.5 *N* HCl. The product was a mixture of isomeric uroporphyrins (40%), methyl ester m.p. 250°.

*5-Aminomethyl-4,3′-di-carboxymethyl-3,4′-di-(β-carboxyethyl)-5′-carboxydipyrrylmethane (**14**), cf. (11)*

The tetra-ethyl ester of the corresponding lactam (**4***a* (3), 460 mg) was refluxed for 1 3/4 h in 22 ml of absolute ethanol containing 2.25 g of KOH. The light-brown precipitate was washed by decantation, first with ethanol then with ether. It was dissolved in 4 ml of water and the product (90%) was precipitated by dilute HCl. It was obtained as a pinkish amorphous powder, m.p. 193–195° (reddening at 125°) by precipitating three times from 0.5 *N* ammonia with *N*-acetic acid, using de-aerated water; Ehrlich's reaction, positive after standing in the cold.

Anal. Calcd. for $C_{21}H_{25}N_3O_{10}$: C, 52.61; H, 5.26; N, 8.77. Found: C, 52.64; H, 6.51; N, 9.72.

In an attempt to decarboxylate the product, it was heated with water for 2 h at 100° in a tube which had been sealed under vacuum. From the dark red product, only uroporphyrin (2%) was isolated. This contained some uroporphyrin 2 for the m.p. of its methyl ester was raised from 265–275° to 295–305° by recrystallizing it from chloroform–acetone, *cf.* (18).

*2-Formyl-3-ethoxycarbonylmethyl-4-β-ethoxycarbonylethyl-5-benzyloxycarbonylpyrrole (**16***b*)

Sulfuryl chloride (0.43 ml) was slowly added to a stirred solution of the 2-methyl pyrrole (**8**, 1g) in 15 ml of absolute ether at 0–4°. After 5 min, the ice bath was removed and stirring was continued for 30 min. The ether was removed *in vacuo* below 20°, and 3 × 10 ml of ether were added and removed in the same way. The oily residue was boiled for 3 min with 40 ml of water containing 2.5 g of sodium acetate. The mixture was quickly cooled to 0°. The product which separated was crystallized from 40 ml of ethanol and 190 ml of water, then recrystallized from hexane (thimble) as colorless needles (80%), m.p. 89–91°. For analysis it was twice recrystallized from hexane, m.p. then 90–92°.

Anal. Calcd. for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.38; H, 6.24; N, 3.56.

2-Formyl-3-β-ethoxycarbonylethyl-4-ethoxycarbonylmethyl-5-benzyloxycarbonylpyrrole

The preparation of this from the 2-methyl pyrrole **12** and sulfuryl chloride followed that of its isomer with the 3- and 4-substituents interchanged. The colorless needles of the product (80%), m.p. 98–100°, were recrystallized twice from hexane for analysis, m.p. then 99–101°.

Anal. Calcd. for $C_{22}H_{25}NO_7$: C, 63.60; H, 6.07; N, 3.37. Found: C, 63.43; H, 6.00; N, 3.51.

*Lactam of 1-Ethoxycarbonyl-2,4,6-tri-β-ethoxycarbonylethyl-3,5-di-ethoxycarbonylmethyl-7-carboxymethyl-8-aminomethyltripyrrene-12-hydrobromide (**17***a*)

The 5-carbethoxypyrrole aldehyde (**16***a* (2), 35 mg) and the dipyrrylmethane (**4***e*, 50 mg, prepared by decarboxylating its 5′-carboxylic acid directly in water at 100°) in 5 ml of ethanol were treated with 2 drops of 48% HBr, cooling, and scratching. After the product began to separate, the mixture stood at 10° overnight. The solvent was evaporated and the crystalline residue was washed with ethanol then recrystallized from the same solvent (3 ml) as small red crystals (31%), m.p. 130–132°. For analysis, it was recrystallized again as small red rods, m.p. unchanged; t.l.c. in $CHCl_3$–MeOH (9:1): R_f 0.74 only; u.v. in ethanol λ_{max} (ε): 493 (32 000), 277 mμ (17 400).

Anal. Calcd. for $C_{43}H_{57}N_4O_{13}Br$: C, 56.27; H, 6.21; N, 6.11; Br, 8.72. Found: C, 55.99; H, 6.17; N, 6.21; Br, 8.79.

*Lactam of 1-Benzyloxycarbonyl-2,4,6-tri-β-ethoxycarbonylethyl-3,5-di-ethoxycarbonylmethyl-7-carboxymethyl-8-aminomethyltripyrrene-12-hydrobromide (**17***b*)

This was obtained like the 1-ethoxycarbonyl derivative as small red rods (45%), m.p. 137–139° (dec.), from the 5-benzyloxycarbonylpyrrole aldehyde **16***b* and the dipyrrylmethane **4***e*. Here the mixture was not left overnight, nor was the residue washed before being recrystallized. U.v. in ethanol λ_{max}(ε): 493 (29 800), 277 mμ (17 300).

Anal. Calcd. for $C_{48}H_{59}N_4O_{13}Br$: C, 58.90; H, 6.03; N, 5.72; Br, 8.18. Found: C, 58.76; H, 6.13; N, 5.67; Br, 8.24.

*Lactam of 1-Carboxy-2,4,6-tri-β-ethoxycarbonylethyl-3,5-di-ethoxycarbonylmethyl-7-carboxymethyl-8-aminomethyltripyrrane (**18**) (F. Boyer)*

The 1-benzyloxycarbonyl derivative (**17***b*, 100 mg) in 40 ml of absolute ethanol was converted into the yellow base with ammonia vapor then shaken with 100 mg of palladium black under hydrogen. The absorption was theoretical in about 1 h. The catalyst was separated and the solvent was removed. The residue was crystallized from 10 ml of ethanol as tiny colorless crystals (48 mg), m.p. 194–196° (dec.), unchanged on recrystallization.

Anal. Calcd. for $C_{41}H_{54}N_4O_{13}$: C, 60.74; H, 6.66; N, 6.91. Found: C, 60.58; H, 6.70; N, 6.85.

Lactam of 2-β-Carboxyethyl-3,7-di-carboxymethyl-4,6-di-β-ethoxycarbonylethyl-5-ethoxycarbonylmethyl-8-aminomethyltripyrrene-12-hydrobromide

3-Carboxymethyl-4-β-carboxyethylpyrrole-2-aldehyde (**19***a*) (3) and the dipyrrylmethane **4***e* were condensed as above. After the solvent was removed, ethanol was twice added to the residue and evaporated. The residue was dissolved in hot absolute ethanol, the solution was cooled and dry ether was added to turbidity. The dark red partially crystalline product, m.p. 140° (dec.), was re-precipitated for analysis.

Anal. Calcd. for $C_{36}H_{45}N_4O_{11}Br$: C, 54.76; H, 5.73; N, 7.10; Br, 10.26. Found: C, 54.71; H, 5.64; N, 7.19; Br, 10.30.

*2-Formyl-3-methoxycarbonylmethyl-4-β-methoxycarbonylethylpyrrole (**20**), cf. (11)*

To the corresponding pure decarboxylic acid (**19***a* (3), 128 mg) in 2 ml of absolute ethanol and 5 ml of dry ether, ethereal diazomethane (from 3 g of nitrosomethylurea) was slowly added. When the solvent was evaporated below 20°, the residue crystallized at once. It was recrystallized from 85% ethanol as flattened rods (70%), m.p. 99–100°; t.l.c. in chloroform–methanol (9:1): R_f 0.70 only (iodine or Ehrlich's). This had not been obtained crystalline (11).

Anal. Calcd. for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 56.81; H, 6.10; N, 5.69.

*Lactam of 2-β-Methoxycarbonylethyl-3-methoxycarbonylmethyl-4,6-di-(β-ethoxycarbonylethyl)-5-ethoxycarbonylmethyl-7-carboxymethyl-8-aminomethyltripyrrene-12-hydrobromide (**21**)*

The aldehyde **20** (32 mg) and the dipyrrylmethane **4e** (55 mg, obtained from its 5′-iodo derivative) were gently warmed to solution in 3 ml of absolute ethanol. To the cooled solution, two drops of 48% HBr were added, and crystallization was induced in the deep red solution by scratching, first in the ice-bath then at room temperature (*ca.* 1/2 h). After the mixture had stood 2 h in the cold, the product was separated on a sintered glass funnel and washed with 2 ml of ether. It formed small red rods (64%), m.p. 117–119°. For analysis, it was recrystallized from ethanol as red rods, m.p. 119–120°, t.l.c. in acetone–methanol (2:1): R_f 0.70 only, a yellow spot turned brown by iodine, or light grey with Ehrlich's reagent and HCl vapor; u.v. in ethanol λ_{max} 482 mμ (26 400).

Anal. Calcd. for $C_{38}H_{49}N_4O_{11}Br$: C, 55.81; H, 6.04; N, 6.85; Br, 9.77. Found: C, 55.83; H, 6.19; N, 6.80; Br, 9.59.

*Lactam of 2-β-Methoxycarbonylethyl-3-methoxycarbonylmethyl-4,6-di-(β-ethoxycarbonylethyl)-5-ethoxycarbonylmethyl-7-carboxymethyl-8-aminomethyltripyrrane (**22**) (N. Stojanac)*

The tripyrrene hydrobromide **21** (126 mg) was dissolved in 100 ml of 95% ethanol and converted to the yellow base by adding 3 drops of NH_4OH. Palladium black (80 mg) was added, and the solution was shaken for 2 h under hydrogen (1 atm, 21°); the uptake ceased at the theoretical amount in 15 min. The catalyst was separated and washed with hot ethanol. Ethanol was evaporated (rotary, bath 35°) and the residue was crystallized from 12 ml of ethanol as a grey micro-crystalline powder (68%), m.p. 169–171° (dec.); Beilstein test for halogen negative, Ehrlich's reaction, positive cold. For analysis, it was recrystallized from ether (thimble, 7 h) as a light grey powder, m.p. 171–173° (dec.); t.l.c. (crude product or pure) in acetone–methanol (2:1): R_f 0.69 only; n.m.r. ($CDCl_3$, TMS) τ 8.83 (m, 13.1, OCH_2CH_3), 7.40 (sym. m, 12.3 CH_2CH_2CO), 6.66 and 6.57 (singlets, 5.2, CH_2CONH and CH_2COOR), 6.35 and 6.27 (singlets, 8.4, OCH_3 and bridge CH_2), 5.94 (m, 4.7, OCH_2CH_3), 5.65 (broad s, 1.2, CH_2NH), 3.73 and 3.61 (singlets, 2.7, pyrrole CH and CH_2NH), 1.40 (s, 0.9, pyrrole NH), 0.83 (s, 1.40, pyrrole NH).

Anal. Calcd. for $C_{38}H_{50}N_4O_{11}$: C, 61.75; H, 6.82; N, 7.58. Found: C, 61.81; H, 6.67; N, 7.40.

*Hydrazone (**23**) from 2-Formyl-3-ethoxycarbonylmethyl-4-(2-ethoxycarbonylethyl)-pyrrole and Girard's "T" Reagent*

A solution of the aldehyde (**16c**, 0.8 g) and Girard's "T" reagent (0.55 g) in 10 ml of 95% ethanol and 0.9 ml of AcOH was refluxed on the steam-bath for 30 min. The solvents were removed from the cooled solution (rotary, finally at 0.1 mm). The residue was dissolved in *n*-propanol, ether was added to turbidity, and some Girard's reagent which separated at once was removed. When more ether was added to the solution, the product crystallized. It was recrystallized until t.l.c. showed it to be pure (about four times) by dissolving it in a little *n*-propanol, filtering from some undissolved Girard's reagent, and adding ether to turbidity. It formed colorless plates (50%), m.p. 212–214°; t.l.c. (ethanol–ammonia–water, 7:1:2) R_f 0.3 only.

Anal. Calcd. for $C_{19}H_{31}N_4O_5Cl$: C, 52.95; H, 7.25; N, 13.00; Cl, 8.23. Found: C, 52.81; H, 7.08; N, 13.09; Cl, 8.14.

*5-Formyl-4,3′-di-ethoxycarbonylmethyl-3,4′-di-(2-ethoxycarbonylethyl)-5′-benzyloxycarbonyl-dipyrrylmethane (**24**)*

A solution of **2b** (165 mg), **23** (150 mg), and anhydrous NaOAc (34 mg) in 3.4 ml of acetic acid was heated 30 min on the steam-bath. The solvent was then removed, finally at 1×10^{-4} mm. The residual hydrazone was dissolved in 7.5 ml of water by gentle warming and concentrated HCl (1.6 ml) was added to the filtered solution. After 10 min, the precipitated oily aldehyde was washed with water by decantation until it was neutral. Its dried (Na_2SO_4) solution in benzene was poured onto a column of alumina (grade 3, made up in benzene), from which the product was eluted with ether. The eluate was evaporated and the residue was crystallized from aqueous ethanol to give the product (35%), m.p. 94–97°. For analysis it was recrystallized twice as colorless needles, m.p. 102.5–104°; Ehrlich's reaction, negative cold; t.l.c. (chloroform–ether, 3:1) R_f 0.2 only; n.m.r. ($CDCl_3$) 8.82 (m, 11, OCH_2CH_3), 7.32 (m, 7.1, CH_2CH_2COO), 6.52 and 6.38 (singlets, 3.7, CH_2COO), 6.06 and 5.96 (m and s, 9, bridge CH_2 and OCH_2CH_3), 4.86 (s, 1.7, Ph CH_2), 2.86 (s, 4.5, C_6H_5), 0.64 (s, 0.9, CHO), 0.06 (broad s, 0.7, NH), −0.4 (broad s, 0.8, NH).

Anal. Calcd. for $C_{36}H_{44}N_2O_{11}$ (mol. wt. 680.7): C, 63.53; H, 6.52; N, 4.12. Found (681 (mass spectrum)): C, 63.35; H, 6.64; N, 4.30.

2-Formyl-4,3′-di-ethoxycarbonylmethyl-3,4′-di-(2-ethoxycarbonylethyl)-5′-carboxydipyrrylmethane (J. Pluscec)

The corresponding 5′-benzyloxycarbonyl derivative (**24**, 485 mg) was hydrogenated in 10 ml of 95% ethanol over palladium black (150 mg). After the theoretical uptake of hydrogen, the catalyst was separated and the solution was concentrated to 2 ml. The product (56%), m.p. 154–156° (dec.) separated at 10°.

For analysis, it was recrystallized twice as colorless prisms turning red in air, m.p. 156–158° (dec.), Ehrlich's reaction, positive cold.

Anal. Calcd. for $C_{29}H_{38}N_2O_{11}$ (neut. equiv. 591): C, 58.96; H, 6.49; N, 4.74. Found (589): C, 59.13; H, 6.66; N, 4.88.

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