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COPROPORPHYRINS FROM MONOPYRROLIC UNITS. A PMR ANALYSIS OF THE PRODUCT

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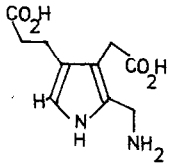
The problem of the exclusive[1] formation of uroporphyrin-III in the natural biosynthetic sequence from the single monopyrrole unit porphobilinogen (PBG) (1)has received much attention[2].

Contrasting with the enzymatically controlled sequence, synthetic condensation of PBG produces mixtures of the four uroporphyrin isomers[3]. The same is to be expected from the condensation of PBG analogues, but pyrrole (2) was at one time said[4] to produce exclusively coproporphyrin-III tetramethyl ester (3-III) when cyclized in acetic acid-ethanol at 100°C. However, under more moderate conditions, pyrrole (2) could possibly polymerize and cyclise in an exclusive head-to-tail fashion producing the type-I coproporphyrin tetramethyl ester isomer (3-I). We attempted an adaptation of our method[5] for pyrromethane and tripyrrane synthesis and in a typical experiment a solution of pyrrole (2)(556mg) in CH_2Cl_2(862ml) was treated with toluene p-sulphonic acid hydrate (56mg) in methanol (283ml) and heated under a stream of air at 35-40°. After 24 hours the reaction mixture was washed with water, dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. Chromatography on neutral alumina (Brockman, grade III), eluting with CH_2Cl_2, gave pure porphyrinic material which was crystallized from CH_2Cl_2-hexane (yield 73mg).



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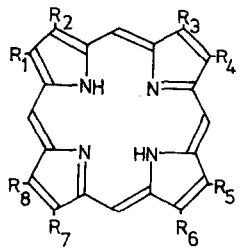

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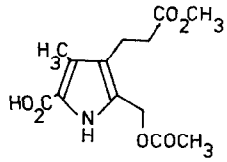

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	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
I	CH_3	P	CH_3	P	CH_3	P	CH_3	P
II	CH_3	P	P	CH_3	CH_3	P	P	CH_3
III	CH_3	P	CH_3	P	CH_3	P	P	CH_3
IV	CH_3	P	P	CH_3	P	CH_3	CH_3	P

P = $CH_2CH_2CO_2CH_3$

Attempting to circumvent difficulties[3b,6,7] with the analysis of porphyrin type isomers we found that the paramagnetically shifted proton magnetic resonance spectrum of the iron (III) cyanide complex is an extremely convenient method, proving once again[8] the great sensitivity of this kind of spectrum to minor structural differences in the porphyrins. Using established procedures[9] we prepared the iron complexes and looked at the well-resolved β-methyl porphyrin resonances[8]. Eight signals are well distinguished in our sample and these coincide with those of the four coproporphyrin isomers (I-11.03; II-11.90; III-11.10, 11.26, 11.66, 12.00; IV-11.36, 11.71)‡. The same eight resonances can be seen in the spectrum of the porphyrin obtained in hot acetic acid-ethanol[4] and this proves that it is also a mixture. The difference between the two cases is that in the mixture from the hot acetic acid cyclization all the eight β-methyl resonances have the same intensity proving the statistical distribution 1/8 type I, 1/8 type II, 1/2 type III, and 1/4 type IV, while in the sample obtained under more moderate conditions the resonance due to the methyls of coproporphyrin-I tetramethyl ester is noticeably stronger.

The sensitivity of the paramagnetic PMR spectra as a purity criterion for porphyrin samples is likely to be of far wider applicability. Melting points and chromatographic separation methods can lead to very disappointing results in some cases[4]. The paramagnetic PMR technique also offers some advantage relative to the carbon-13 NMR alternative[10].

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‡ Shifts are measured from an internal standard of TMS(δ=0) and are concentration independent. The spectra were obtained in a Varian T60A machine at standard probe temperature and identified using pure samples. Solutions were made by adding the hemes to a solution of 25mg of KCN in a mixture of 0.4ml pyridine and 0.1ml of water.

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