Tetrahedron Letters No. 30, pp 2637 - 2640. 0040-4039/78/0722-2637$02.00/0

THE CHEMICAL OXIDATION OF PORPHOBILINOGEN

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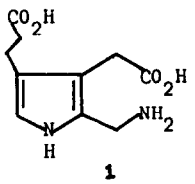
(Received in USA 19 December 1977; received in UK for publication 29 May 1978)

Porphobilinogen (2-aminomethyl-3-carboxymethyl-4-carboxyethylpyrrole) **1** is the universal metabolic precursor of heme, chlorophylls, porphyrins, and the corrin nucleus[1]. It is the only known monopyrrole in primary metabolism and has no other biological analogues. It is oxidized



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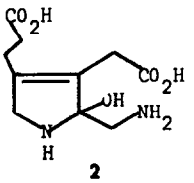

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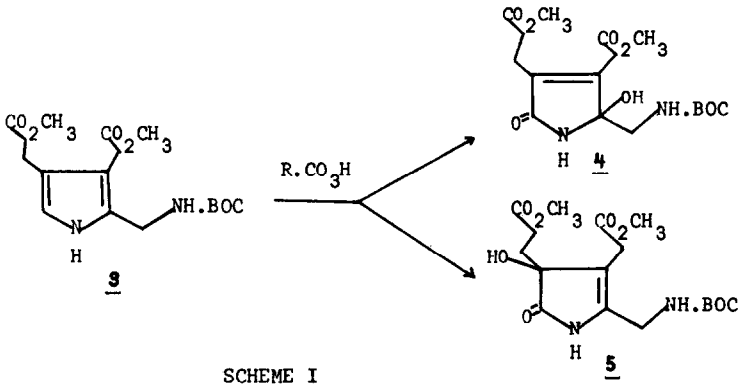
by porphobilinogen oxygenase affording 3-carboxyethyl-4-carboxymethyl-5-aminomethyl-5-hydroxy-3-pyrrolin-2-one (oxoporphobilinogen) **2**[2]. We have recently shown that the amides of γ-keto-α, β-unsaturated acids exist entirely in the cyclic form (as 5-hydroxy-3-pyrrolin-2-ones)[3]. Hence the structure of **2** is now secured by spectroscopic evidence[2], by degradation reactions[2], and by the obtention of synthetic analogues[3]. Oxoporphobilinogen **2** was found in the urines of porphyric rats excreting urinary porphobilinogen when the rats were treated with pregnenolone to induce hepatic porphobilinogen oxygenase[4]. Since 5-hydroxy-3-pyrrolin-2-ones were detected in urine and blood samples of patients with neurological disorders[5], and since these disorders are often associated with the various types of porphyrias[6], it is of interest to obtain **2** in sufficient amounts for pharmacological testing. The chemical oxidation of porphobilinogen **1** appears as the most simple procedure.

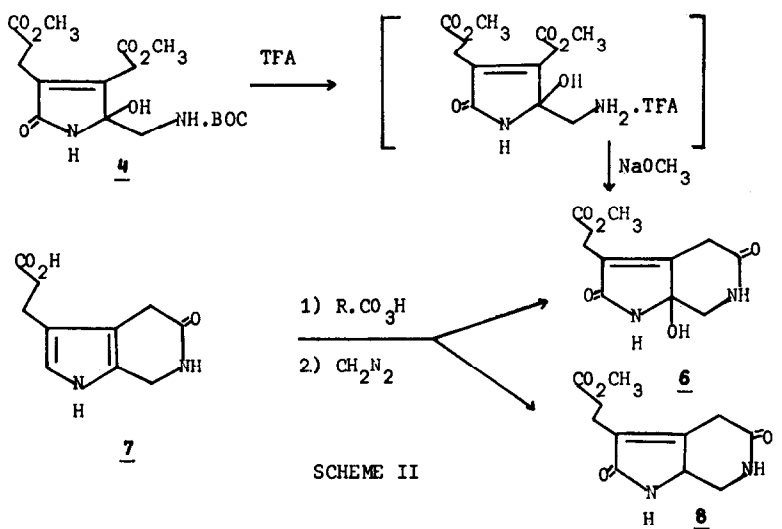
Porphobilinogen **1** can be obtained by a preparative synthesis[7]. We have shown that the peracid oxidation of alkylpyrroles afforded hydroxy-3-pyrrolin-2-one in fair yields[8]. Porphobilinogen **1** was protected prior to the oxidation by treatment with t-butyloxycarbonyl azide (50% yield)[9], followed by esterification of the BOC derivative with diazomethane [10]. The BOC dimethyl ester **3** (2g) dissolved in methylene chloride (100ml), was oxidized with two equivalents of m-chloroperbenzoic acid at 10° during 2 hs. The solution was washed with a 2% sodium bisulfite solution, then with 5% sodium bicarbonate, dried (Na_2SO_4), evaporated to dryness, and the residue was chromathographed on a tlc silica-gel column packed with 5% methanol in chloroform and was eluted with the same solvent. Two definite oxidation products were obtained (Scheme I).



SCHEME I

The main product (350 mg, 16%) had Rf, 0.50 (tlc on silica-gel; 5% methanol in chloroform) and was the pyrrolinone 4; ir (Cl_3CH)[11]: 3450 cm^{-1} (OH); 1740 cm^{-1} (ester CO); 1690 cm^{-1} (amide CO); (nmr; Cl_3CD; δ=0; TMS); 1.4 (s, 9H, $C(CH_3)_3$), 2.5 (b, 4H, CH_2CH_2); 3.5 (b. 3H, OH, $\underline{CH_2}NH$; reverted to a s 30 min after the addition of D_2O); 3.6 (s, 2H, CH_2CO), 3.7 (s, 6H, OCH_3), 5.2 (t, 1H, $CH_2\underline{NH}$); 6.7 (b, 1H, NH); ms (m/e); 386 (M^+, 18%), 368 (M-H_2O, 63%); 369 (M-OH, 42%). The second oxidation product had Rf, 0.60 (320 mg, 15%), and was the pyrrolinone 5; ir (Cl_3CH), 3450 cm^{-1} (broad, bridged OH), 1740 cm^{-1} (ester CO), 1690 cm^{-1} (amide CO); nmr ($CDCl_3$); 1.4 (s, 9H, $C(CH_3)_3$), 2.45, 2.57 (m, 4H, CH_2CH_2), 3.5 (b, 1H, OH; erased after D_2O addition); 3.6 (s, 2H, CH_2CO); 3.64, 3.72 (s, 6H, OCH_3); 4.3 (d, 2H, J=6HZ, $\underline{CH_2}NH$; reverted to a s 30 min after the addition of D_2O); 5.05 (b, 1H, $CH_2\underline{NH}$); 6.75 (b, 1H, NH); ms (m/e); 386 (M^+, 4%), 369 (M-OH, 44%).

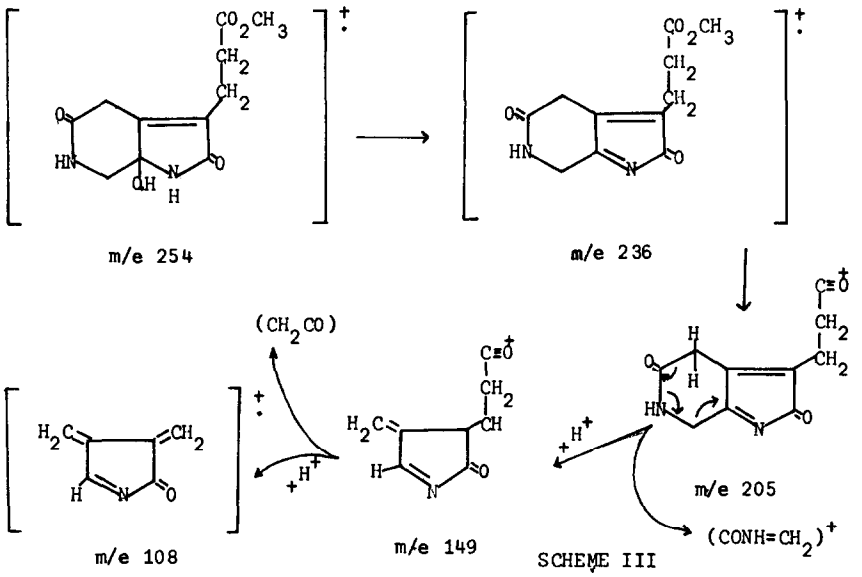
Further confirmation for structure 4 was obtained by converting it into the hydroxylactam 6, obtained in turn by the peracid oxidation of porphobilinogen lactam 7 (Scheme II).



SCHEME II

The hydroxypyrrolinone 4 was dissolved in trifluoroacetic acid, the solution was kept

at 25° during 20 min; the trifluoracetic acid was evaporated under nitrogen, and the residue was dissolved in a sodium methoxide solution in methanol and kept at 25° during 24 hs. After evaporation of the solvent, and purification of the residue by chromathography through a tlc silica-gel column using 20% methanol in chloroform, the lactam **6** was obtained as the only product (40% yield). Structure **6** (3-(β-methoxycarbonylmethyl)-2,5-dioxo-7a-hydroxy-2,4,5,6,7,7a-hexahydro-1H-pyrrolo [2,3-c] pyridine) was secured on the basis of its ir (KBr), 1675 cm^{-1} (amide CO, six-membered ring); nmr (Cl_3CD-20% CD_3OD, δ=0, TMS), 2.6 (b, 4H, CH_2CH_2), 3.5 (m, 3H, CH_2CO, OH), 3.7 (s, 3H, OCH_3), 4.0 (b, 2H, $\underline{CH_2}NH$); ms (m/e), 254 (M^+, 10%), 236 (50%), 205 (55%), 207 (30%), 149 (base peak), 108 (40%). The most characteristic fragmentation of the ms spectrum of lactam **6** is depicted in Scheme III and is characteristic for the oxidized lactams of type **6**.



SCHEME III

Lactam **6** was obtained independently by the chemical oxidation of porphobilinogen lactam **7**[7]. The oxidation was carried out in acetic acid using two equivalents of m-chloroperbenzoic acid. The oxidation products were esterified with diazomethane and separated by column chromathography on tlc silica-gel using ethyl acetate: methanol, 3:1 as eluant. A substance with Rf, 0.57 was isolated and found to be identical with lactam **6** (31%). The second oxidation product was found to be lactam **8** (47%); ir (KBr), 1750 cm^{-1} (ester CO), 1675 cm^{-1} (amide CO); nmr (TFA, δ=0 for TMS), 2.75 (m, 4H, CH_2CH_2), 3.8 (b, 2H, CH_2CO), 4.0 (s, 3H, OCH_3), 3.8-4.3 (m, 3H, $NHCH_2CH$); ms (m/e), 238 (M^+, 90%), 209 (238-CH_2NH, 45%), 149 (238-OCH_3-$CONHCH_2$, base peak), 108 (70%), (see SCHEME III) No 1,2-oxidation product was isolated in the peracid oxidation of **7** or of analogous lactams.

The butyloxycarbonyl group of **4** was cleaved by treatment with trifluoracetic acid at

room temperature during 20 min. After evaporation of the acid the residue was dissolved in a 2N sodium hydroxide solution which was kept at 20° during 72 hs. The solution was adjusted to pH 5 with acetic acid and filtered through a Dowex-1X4 resin column. Elution with 0.8M acetic acid, and evaporation of the eluates afforded oxoporphobilinogen **2** (42% yield). Its was identical (tlc chromathography) with the product obtained in the enzymatic oxidation of porphobilinogen **1**[2]; ir (Nujol); 3900 cm^{-1} (OH), 3200 cm^{-1} (NH); 1680 (CO pyrrolinone); nmr (D_2O, δ=0 for sodium DSS); 2.06 (b, 4H, CH_2CH_2), 2.54 (s, 2H, CH_2COOH); 3.39 (t, 2H, $\underline{CH_2}NH_2$), 3.25 (b, 1H, OH); 8.4 (b, 1H, NH); ms (direct inlet, 60°), 258 (M^+, 40%). The analogous treatment of **5** with trifluoroacetic acid followed by sodium hydroxide did not afford an isolable product. Oxoporphobilinogen **2** was also obtained by saponification of the lactam **6** following a similar procedure to the described for **4**.

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11. All nuclear magnetic resonance spectra were run on a Perkin-Elmer R-12 instrument; infrared spectra were recorded on a Perkin-Elmer 21 instrument.

Acknowledgement. This work was generously supported by grants from the National Institutes of Health (GM-11973) and the Consejo Nacional de Investigaciones (Argentina).