Porphyrins Found in Urine of Patients with Symptomatic Porphyria

S. G. SMITH

Department of Medical Biochemistry, Welsh National School of Medicine, Cardiff CF4 4XW, Wales, U.K.

The exact structure of the type-III isomer penta-, hexa- and hepta-carboxylic porphyrinogens that are involved as intermediates in the conversion of uroporphyrinogen III into coproporphyrinogen III have been elucidated (Jackson et al., 1976). The corresponding porphyrins are to be found in the urine of patients suffering from the porphyrin-overproduction diseases symptomatic porphyria and congenital erythropoietic porphyria, together with the type-I isomers of these porphyrins. The faecal and urine extracts from symptomatic porphyric patients also contain isocoproprorphyrins (Elder, 1974), which are not in the direct biosynthetic pathway and whose role, if any, is uncertain.

It is possible, by using t.l.c. (Smith, 1975; system A), to separate completely a porphyrin methyl ester band running between coproporphyrin and pentacarboxylic porphyrin methyl esters. This is the characteristic position for isocoproporphyrin methyl ester. This band was found in all urine extracts from 12 untreated cases of symptomatic porphyria. The RF value of this band did not coincide exactly with that of isocoproporphyrin methyl ester prepared from faecal extracts or by chemical synthesis, the RF value being slightly lower in all cases. Mass-spectrometry results also differed, giving a peak at 682 instead of the expected 710 mass units for isocoproporphyrin methyl ester. The visible and u.v. spectra were almost identical. By using a technique which decarboxylates acetate groups (Cornford & Benson, 1963) both porphyrins were converted into tricarboxylic porphyrins, suggesting that the porphyrin in urine also belonged to the isocoproporphyrin series. A mol.wt. of 682 suggested a loss of an ethyl group by the isocoproporphyrin molecule to give desethylisocoproporphyrin. The suggested structure is shown in Fig. 1(a).

A second band was found on t.l.c. plates from eight of the twelve urine extracts from patients with symptomatic porphyria running between the penta- and hexa-carboxylic methyl ester bands. It showed a deep cherry-red fluorescence in u.v. light with a Soret

![Fig. 1. Proposed structure of porphyrins](image)

M, Methyl; P, propionyl; A, acetyl. For the first porphyrin (a), X = H; for the second porphyrin (b), X = −CO−CH₃ or −CH₂−CHO.
band at 409 nm when dissolved in chloroform. The spectrum in visible light was four-banded and rhodo-type, giving peaks at 637 nm (band I), 575 nm (band II), 546 nm (band III) and 504 nm (band IV). A small peak was also noted at 623 nm. Mass spectrometry gave a peak indicating a mol.wt. of 724, 14 mass units greater than coproporphyrin or isocoproporphyrin methyl esters. The simplest interpretation of this evidence was that the porphyrin was a formyl or oxo derivative of one of these porphyrins. To test this hypothesis the porphyrin was subjected to reduction with NaBH₄ (Clezy & Barrett, 1959), which should reduce a carbonyl group to a hydroxyl group. The reduced porphyrin methyl ester gave a Soret band at 399 nm in chloroform and a four-banded aetio-type spectrum with bands at 623 nm (band I), 568 nm (band II), 530 nm (band III) and 498 nm (band IV). On t.l.c., the reduced porphyrin methyl ester ran immediately above uroporphyrin methyl ester. The changes are typical of the conversion of a carbonyl group into a hydroxyl group. Hydroxylated porphyrins may be acetylated to produce derivatives which run chromatographically with increased \( R_F \) values (Barrett, 1959). This proved to be the case with the acetylated product, which ran to a position between coproporphyrin and penta-carboxylic porphyrin methyl esters. Finally, part of the original porphyrin methyl ester was subjected to a decarboxylation procedure which removes acetic acid carboxyl groups and methyl ester groups (Cornford & Benson, 1963). This resulted in a free porphyrin that ran in a tricarboxylic position on t.l.c. (Smith, 1976), indicating that the parent porphyrin possessed an acetate group and was therefore related to isocoproporphyrin and not coproporphyrin. The proposed structure is shown in Fig. 1(b).

It is concluded that the two porphyrins desethylisocoproporphyrin and oxoisocoproporphyrin are characteristically found in urine of patients with symptomatic porphyria. They have not been seen in urine extracts from five patients with congenital erythropoietic porphyria.


The Effect of Certain Anaesthetic Agents on the Activity of Rat Hepatic δ-Aminolaevulinate Synthase

MICHAEL R. MOORE and RANJIT K. PARikh

*University of Glasgow Department of Materia Medica and Therapeutics, and Division of Anaesthesia, Stobhill General Hospital, Glasgow G21 3UW, Scotland, U.K.*

It is now accepted that the initial and rate-controlling enzyme of haem biosynthesis, δ-aminolaevulinate synthase (EC 2.3.1.37), shows increased activity in each of the porphyrias (Moore & Goldberg, 1974). Various drugs, chemicals and endogenous compounds may induce acute attacks of porphyria, but it is difficult to assess, on the basis of retrospective clinical analysis, whether or not a specific drug may be porphyrinogenic. Studies have shown that compounds that increase the activity of δ-aminolaevulinate synthase are porphyrinogenic, and this criterion has been used, together with retrospective clinical analysis, in implicating the barbiturates as ubiquitously porphyrinogenic compounds. This study examines the effect of various commonly used anaesthetic agents on the activity of rat hepatic δ-aminolaevulinate synthase.

Groups of male Sprague–Dawley rats weighing between 200 and 250 g were used. The animals were kept in cages during the experiments in groups of three with suitable