Decarboxylation of Uroporphyrin by Heating at Atmospheric Pressure

By TORBEN K. WITH

*Department of Clinical Chemistry, Svendborg Hospital,
5700 Svendborg, Denmark*

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Free uroporphyrin is decarboxylated by heating in the air to 150–200°C. Complete decarboxylation occurs after heating to 200°C for 5 min, with a yield of 66–75%. Heptacarboxylic porphyrin can be prepared with a yield of 40% by heating uroporphyrin to 150°C for 60 min, and hexacarboxylic porphyrin with a yield of about 20% by heating to 150°C for 2 h.

Decarboxylation of uroporphyrin to coproporphyrin is a basic technique in the analysis of porphyrin isomers, and partial decarboxylation provides porphyrins with seven, six and five carboxyl groups. A simple method for preparation of these compounds in reasonable amounts would facilitate the study of porphyrin biosynthesis, i.e. the intermediary steps of the biosynthesis pathway to chlorophylls, cytochromes, haemoglobins and corrins.

Decarboxylation of uroporphyrin was first carried out by Fischer (1916) by heating the ester to 200°C with potassium methoxide for 4 h at a pressure of 2 MPa (20 atm). Later Fischer & Zerweck (1924) simplified the method by heating uroporphyrin dissolved in 1% HCl for 3 h to 180–185°C in Carius tubes. Edmonson & Schwartz (1953) improved the yield to nearly 100% by evacuating the tubes before heating. Chu & Chu (1959) found that increasing the HCl concentration to 3% resulted in partial decarboxylation, and With (1974) found that decarboxylation took place if the tubes were heated briefly at 180°C, with immediate cooling. Despite these improvements decarboxylation still required heating in Carius tubes, a time-consuming technique only suitable for work with a few mg. It was therefore an interesting discovery that free uroporphyrin, precipitated from HCl or NH₃ solutions, was partially decarboxylated by heating to 150°C in the air for 30–60 min and completely decarboxylated by heating up to 200°C for 5 min. Our observation is in agreement with some recent findings obtained from mass spectrometry of uroporphyrin ester, showing that formation of the molecular ion of heptacarboxylic porphyrin ester occurred by a thermal process before ionization of the sample (Møller & With, 1974).

Materials and Methods

Uroporphyrin I was prepared from 25–60 litres of bovine porphyria urine by adsorbing on to talc at

pH 3–4, extraction of porphyrins from the dried adsorbate with H₂SO₄–methanol (1:20, v/v) and extracting the ester with chloroform the next day. The crude ester solution was evaporated and the residue treated with conc. HCl for at least 48 h to effect hydrolysis. After addition of an equal volume of water and filtration, the porphyrin was precipitated at pH 3–4 from the filtrate and collected on a paper on a Büchner funnel by suction. This precipitate was extracted with boiling 1 M-HCl, dissolving most of the coproporphyrin, and leaving most of the uroporphyrin undissolved. After the filter had been washed with a little water the uroporphyrin was dissolved as a scarlet solution by sucking conc. NH₃ through (With, 1958, 1969b). The ammoniacal uroporphyrin solution was evaporated overnight in an open vessel. The pH was then 8–9 and the porphyrin was precipitated almost completely by adding a few drops of acetic acid. If uroporphyrin ester was used it was dissolved in conc. HCl and left for 48 h hydrolysis, filtered after dilution with an equal volume of water and precipitated by gradual addition of conc. NH₃; at approximately pH 1 a rapid and almost complete precipitation took place in concentrated uroporphyrin solutions. The precipitate was collected on a sintered glass funnel, porosity grade 3, dried in a vacuum desiccator, and heated on the funnel in a thermostatically controlled ($\pm 5^\circ\text{C}$) electric oven. Before and after heating, samples were taken for t.l.c. examination both as free porphyrins on talc (With, 1969a) and, after esterification, as methyl esters on silica gel (Rimington & With, 1974). For each experiment between 10 and 100 mg of uroporphyrin, freshly precipitated and dried, was used. Recovery was determined by weighing 0.2 mg samples, before and after heating, on an electronic microbalance (LM 500 RICC). The samples were dissolved in 0.5–1 ml of diluted NH₃ and diluted to 200 ml with 0.5 M-HCl, after which light absorption was determined at the

Soret maximum, 380 and 430nm, and the porphyrin content calculated by using the correction of Rimington & Sveinsson (1950) and the constants of Rimington (1960). After determination of the recovery the heated porphyrin was esterified with 5% (v/v) H_2SO_4 in methanol, extracted with chloroform in the usual way and subjected to t.l.c. on silica gel (Doss, 1970; Rimington & With, 1974), with elution of the spots of the various porphyrins with chloroform-methanol (4:1, v/v) and subsequent reading at the Soret maximum in chloroform. From these readings the relative amounts of the different porphyrins were calculated.

Results

Heating to 200°C for 2min (Plate 1a, sample C) caused almost complete decarboxylation to coproporphyrin, and heating to 200°C for 1min (Plate 1a, sample B) gave partial decarboxylation, with strong spots of uroporphyrin and heptacarboxylic porphyrin, and weak spots of hexacarboxylic and pentacarboxylic porphyrins and coproporphyrin

being observed. Heating to 200°C for 30min (Plate 1a, sample D) gave considerable loss of porphyrin, as most of the heated mass was insoluble in H_2SO_4 -methanol.

Heating to 90–100°C for 1h (Plate 1a, samples F and G) did not affect the uroporphyrin, whereas heating to 120°C for 1h (Plate 1a, sample H) gave a small increase in hepta- and hexa-carboxylic porphyrin; heating to 150°C for 1h (Plate 1a, sample I) gave a series of almost identical spots of uroporphyrin, hepta-, hexa- and penta-carboxylic porphyrin and coproporphyrin. Heating to 150°C for 15min (Plate 1b, sample C) resulted in spots of uroporphyrin, hepta- and hexa-carboxylic porphyrin, for 30–60min (Plate 1b, samples D–F) spots of uroporphyrin, hepta-, hexa- and penta-carboxylic porphyrin and coproporphyrin, and for 1½–4h (Plate 1b, samples G–J) weak uroporphyrin spots and a corresponding increase in the hepta- to copro-porphyrin spots.

Recovery experiments (Table 1) showed close to 100% recovery after heating to 200°C for 1min and above 80% after 2min. After 5min at 200°C one-quarter to one-third of the porphyrin was lost. Heating to 150°C gave a recovery of above 90% for heating for 30min or less, heating for 2h gave approx. 25% loss, and for 4h approx. 50% loss. There was no consistent difference between uroporphyrin precipitated from HCl and that from NH_3 .

Quantitative observations on the porphyrin distribution are given in Table 2. The highest yield of heptacarboxylic porphyrin was obtained after heating at 150°C for 1h, and the highest yield of hexacarboxylic porphyrin was obtained after heating at 150°C for 2h. Crystallization from chloroform-methanol was performed on the eluates from the isolated spots, and the eluates and crystals both showed high chromatographic purity (Place 1c). By heating 100mg quantities of uroporphyrin we have prepared 10mg quantities of hepta- and hexacarboxylic porphyrin. We have also studied the behaviour of uroporphyrin III from turacin and

Table 1. *Recovery of uroporphyrin after heating*

Total porphyrins recovered after heating uroporphyrin I, calculated as molar percentage of original porphyrin.

Heating conditions		From uroporphyrin prepared by precipitation from NH_3 (%)	From uroporphyrin I prepared by precipitation from HCl (%)
°C	min		
200	1	98	97
200	2	81	95
200	5	76.5	66
150	5		91.5
150	15		94
150	30		91.5
150	60		78.5
150	120		74
150	240		46.5

Table 2. *Distribution of tetra- to octa-carboxylic porphyrins after heating of uroporphyrin I to 150°C for 0–240min, as a percentage of total porphyrin*

The porphyrins were determined by t.l.c. after esterification. By re-esterification and chromatography, suburoporphyrins were shown to be mainly incompletely esterified uroporphyrin.

Porphyrin	% of total porphyrin								
	Time (min)	...	0	5	15	30	60	120	240
Uroporphyrin	81		87	73	63	43	29	19	
Heptacarboxylic porphyrin	0		7	12	21	41	32	15	
Hexacarboxylic porphyrin	0		0	0	6	3	18	16	
Pentacarboxylic porphyrin	0		0	0	0	2	5	17.5	
Coproporphyrin	0		0	0	0	2	8	19.5	
'Suburoporphyrin'	19		6	15	10	9	8	13

EXPLANATION OF PLATE I

(a) Heating of uroporphyrin I precipitated from NH_3 solution

T.l.c. of methyl esters prepared from free uroporphyrin I heated to 200°C, 150°C, 120°C and 90°C. Technique: silica gel run in benzene-ethyl acetate-methanol (17:2:1, by vol.) for 60 min at 20–22°C. (A) Before heating; (B) after heating for 1 min at 200°C; (C) after 2 min at 200°C; (D) after 30 min at 200°C; (E) markers; from top downwards meso-, copro- and uro-porphyrin, and below uroporphyrin, suburoporphyrins, consisting mainly of partly esterified material; (F) after heating to 90°C for 60 min; (G) after heating to 100°C for 60 min; (H) after heating to 120°C for 60 min; (I) after heating to 150°C for 60 min.

(b) T.l.c. of methyl esters prepared from free uroporphyrin I from NH_3 solution by heating to 150°C

Technique as in Plate 1(a). (A) Markers (as in Plate 1a); (B) before heating; (C) after heating for 15 min; (D) after heating for 30 min; (E) after heating for 45 min; (F) after heating for 60 min; (G) after heating for 90 min; (H) after heating for 120 min; (I) after heating for 180 min; (J) after heating for 240 min.

(c) T.l.c. of the ester fractions, prepared by heating free uroporphyrin I, after purification by preparative t.l.c. and crystallization

(A) Uroporphyrin marker with a trace of coproporphyrin; (B) coproporphyrin fraction from preparative t.l.c.; (C) pentacarboxyl fraction from preparative t.l.c.; (D) crystals from (C); (E) hexacarboxyl fraction; (F) crystals from (E); (G) heptacarboxyl fraction; (H) crystals from (G); (I) uroporphyrin fraction; (J) crystals from (I).

found that it behaved similarly to uroporphyrin I on heating.

Discussion

As pointed out by Chu & Chu (1959) mixtures of isomers are formed during decarboxylation, especially for hexacarboxylic porphyrin. But in the case of the symmetric uroporphyrin I only one hepta- and one penta-carboxylic porphyrin is formed. For the hexacarboxylic porphyrin of the I series there are two isomers, one formed by decarboxylation of two acetic acid groups on two adjacent pyrroles, and one by decarboxylation of two acetic acid groups on two opposite pyrroles. For the asymmetric uroporphyrin III molecule a considerable number of isomers are possible, i.e. four different heptacarboxylic porphyrins III, six different hexacarboxylic porphyrins III and four different pentacarboxylic porphyrins III. On thermic decarboxylation, either by the classic method or at atmospheric pressure, a random mixture of isomers is most likely to result. What happens during enzymic biosynthetic decarboxylation of porphyrinogens is not known. Here one compound or mixtures of the four possible heptacarboxylic porphyrins III and one or more of the six possible hexacarboxylic porphyrins III could be preferred.

Elucidation of this problem would require methods for separation of these isomers.

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