Hepta-, Hexa-, and Pentacarboxylic Porphyrins of Porphyria Cutanea Tarda*

II. PREPARATION OF THE PORPHYRINS BY STEPWISE DECARBOXYLATION OF UROPORPHYRINS

T. C. Chu and Edith Ju-Hwa Chu

From the Department of Chemistry, Immaculate Heart College, Los Angeles, California

(Received for publication, April 8, 1959)

Hepta-, hexa-, and pentacarboxylic porphyrins have been detected in porphyrin as well as normal urine samples. From the former they have been isolated and converted into pure methyl esters (1). For a better understanding of the chemistry of these porphyrins, pure uroporphyrin I and uroporphyrin III were separately subjected to decarboxylation under controlled conditions. The decarboxylation products are described and their relations with those isolated from the porphyric urine are discussed in this report.

EXPERIMENTAL

Pure octamethyl esters of Uro I* (m.p. 295-296°) and Uro III (m.p. 261-262°) isolated from the urine samples of a porphyrin patient (2) were used as the starting materials. They were hydrolyzed and decarboxylated according to the procedure of Edmondson and Schwartz (3) with some modifications. A Pyrex ignition tube, 1.8 X 15 cm, containing a porphyrin sample in 5 to 10 ml of HCl solution was sealed under vacuum. The concentration of HCl was varied between 0.1 to 10%, and the temperature of the reaction oven was maintained at 175 ± 2°. The size of a sample varied from 50 µg to a few milligrams. The total yield of the main decarboxylation products, ranging from 90 to 95%, was determined colorimetrically (4). Several unidentified porphyrins amounting to 1 to 5% were obtained as by-products. There was also a trace of greenish pigment, especially when the reaction mixture contained very dilute HCl.

The results of the decarboxylation of a typical series of Uro I are given in Fig. 1. Uro III was not studied as extensively as Uro I due to a limited supply of the material. However, the results so far obtained have shown that it follows a course of decarboxylation similar to Uro I. For comparison, samples of Uro I obtained from Dr. Watson and Uro III (turacin) from Dr. Rimington were also decarboxylated to various intermediates. In Fig. 1, especially lD, it can be seen that the decarboxylation of Uro I in dilute HCl solution takes a course of stepwise decarboxylation of the acetic acid groups. The first acetic acid group was decarboxylated within 10 minutes of heating and thus 7 I was formed. On further heating other acetic acid groups were successively decarboxylated with the formation of 6 I and 5 I porphyrins. At the end of 1½ hours, only 5 I remains as the potential precursor of Copro I. Separate experiments with 7 I, 6 I, and 5 I porphyrins gave similar results of a stepwise decarboxylation.

However, in a more diluted hydrochloric acid solution such as 0.2%, another reaction besides decarboxylation occurs. At the end of 1 hour, only 75% of the sample was accounted for by Copro I, and a green pigment of unknown nature was obtained. With 0.1% HCl, the yield of Copro I was further lowered to 33% and more green pigment was produced. The nature of the green pigment is being studied.

It is evident from Fig. 1 that the optimal condition for decarboxylation of Uro I to Copro I is to heat the sample in 1% HCl solution for 2 hours. A similar result has been obtained in the case of decarboxylating Uro III to Copro III. If a sample is heated in a 5% HCl solution for 1 hour, a mixture of 7 I, 6 I, and 5 I is obtained. When 1% HCl solution is used, Copro I can be prepared from either 5 I, 6 I, or 7 I in 1½ hours. The time of heating should be reduced to 30 minutes for the preparation of 6 I and 5 I from 7 I, and 45 minutes for that of 5 I and Copro I from 6 I.

After decarboxylation, each sample was extracted, esterified and chromatographed as usual (1). Near the top of every chromatogram, there were found some weak fluorescent bands of unknown nature. The main zones were further purified as described (1).

By comparison of methyl esters of these intermediates with those obtained from a porphyrin patient (1), it has been found that they are similar in many properties, but definitely different in their melting points.

It is noted from Table I that some of the products particularly methyl esters of 6 I and 6 III, possess a rather wide melting range. By means of a long Hyflo column, somewhat similar to that used for the separation of Uro I and III (2), a batch of the purified ester of 6 I of m.p. 190-240° was spread into a wide band. The upper half of the band consisted of about 60% of the porphyrin which crystallized in needles (Fig. 2, a) and melted at 190-200°, and the lower half yielded crystals (Fig. 2, b) melting at 229-260°. Both of these fractions were subjected to decarboxylation under identical conditions and gave excellent yields of Copro I (methyl ester, m.p. 250-252°).
When the esters of 7 I, 7 III, and 5 I were likewise chromatographed, they yielded no similar fractions of widely different melting points. A sample of 5 III (methyl ester, m.p. 150–170°) was decarboxylated, before chromatographic fractionation, to Copro III in an excellent yield.

The photomicrographs of the crystalline forms of these products together with those isolated from urine samples (1) are given in Fig. 2 for comparison.

The results of the methoxyl determinations of the products are listed in Table I. They agreed very well with the calculated values for methyl esters of 7 I, 6 I, and 5 I. Other analytical data, including C, H, and N determinations of the heptacarboxylic porphyrin isolated from a patient, have already been reported (1).

Paper chromatographic study of methyl esters of those isomeric porphyrins has shown some differences in the solvent systems, respectively, designed for the differentiation of Uro I and III, and for Copro I and III. When the size of a sample spot is about 0.3 μg, the difference between each pair of isomers I and III is apparent on both paper chromatograms (Fig. 3, A and B). With a smaller sample, e.g. 0.2 μg, the difference is almost unnoticeable in the solvent system A, but can be seen in the solvent system B.

**DISCUSSION**

It is evident from Fig. 1 that uroporphyrins are more stable toward heat in a relatively concentrated HCl solution. For instance, a considerable quantity of Uro I remained unchanged after heating for 15 minutes in a 5% HCl solution, whereas only a negligible amount was recovered from the more dilute HCl. In a very dilute HCl solution, uroporphyrin was even transformed into a green pigment after a brief heating at a much lower temperature. At HCl concentrations from 0.5 to 10%, uroporphyrin was successively decarboxylated into various porphyrins. A variation in the HCl concentration, temperature or heating time would, therefore, affect the composition of the decarboxylation product.

Our results have led us to believe that the decarboxylation product "224" of the "284" uroporphyrin reported by Grinstein et al. (5) might be a mixture of 5 I and III, and their "183" porphyrin obtained from decarboxylation of their "208" might also be an impure pentacarboxylic porphyrin. The unidentified narrow band above the Copro zone, described more recently by Edmondson and Schwartz (3) in their decarboxylation experiment, might also be a pentacarboxylic porphyrin.

The identity of the decarboxylation products of uroporphyrins with those porphyrins isolated from a porphyric patient (1) has been established by their absorptions in various regions including infrared (6), their decomposition products, the paper chromatographic behavior, etc. The isolation of these intermediate porphyrins from cows with congenital porphyria2 constitutes additional evidence for the existence in nature of all these porphyrins, particularly the 7 I and 6 I porphyrins. Methyl esters of these porphyrins from porphyric cows have crystalline forms almost unnoticeable in the solvent system A, but can be seen in the solvent system B.

Similarly, a sample of methyl ester of 6 III (m.p. 105–190°) was spread on a long Hyflo chromatogram, the fraction extracted from the upper 2/3 of the band melting at 150–190° and that from the lower portion melting at 200–240°. Secondary chromatography of the upper portion gave two fractions, one melting at 145–150° (Fig. 2, 7) and the other melting at 165–195°, while the lower portion was also separated into two fractions m.p. 250° (Fig. 2, 8) and 254°. The fractions with m.p. 190–205° (Fig. 2, 8) and 254°, about 50 μg each, were again subjected to decarboxylation and both gave a total yield of 70% Copro III (methyl ester, m.p. 151/165°).
FIG. 2. Photomicrographs of crystals of methyl esters of porphyins; 1 to 8, prepared from decarboxylation of uroporphyrin I and III; 9 to 12, isolated from porphyric urine samples. 1, Pentacarboxylic porphyrin I, or 5 I, m.p. 232°; 2, 5 III, m.p. 160-160°; 3, heptacarboxylic porphyrin I, or 7 I, m.p. 255°; 4, 7 III, m.p. 205°; 5, hexacarboxylic porphyrin I, or low melting 6 I, m.p. 200°; 6, high melting 6 I, m.p. 290°; 7, low melting 6 III, m.p. 150°; 8, high melting 6 III, m.p. 205°; 9, 5 I III, m.p. 224°; 10, 6 I III, m.p. 196°; 11, 7 I III, m.p. 216°; and 12, an unidentified octacarboxylic porphyrin, m.p. 238°. Magnification: 11, X 200; others, X 100.
A.

KC-KD

B.

KC-KP

5'5''6'6''7'7''U'U''

C' C''5'5''6'6''7'7''

Fig. 3. Paper chromatograms of porphyrin esters. C and U, for copro- and uroporphyrins; 5, 6, and 7, for penta-, hexa-, and heptacarboxylic porphyrins of I or III series as specified. The solvent mixture KC-KD, consisted of 3:3 ml of kerosene-chloroform, followed by 4:4:1:8 ml of kerosene-dioxane; KC-KP, 3:3 ml kerosene-chloroform, 5:0.9 mL kerosene-n-propyl alcohol (2).

Miles (7) have also reported the separation of a heptacarboxylic porphyrin (m.p. 242°) from a patient with congenital porphyria.

Variations in the melting point ranges of the 6 I and III porphyrin esters, and the fact that both low and high melting portions of a preparation gave the same coproporphyrin on decarboxylation, indicate the possible existence of more isomers, as do the properties of the products isolated from porphyric urine.

On the basis of the established structure of Uro I (8), 7 I and 6 I can be assigned, respectively, the structures of 7-methylporphin-1,3,5-triacetic-2,4,6,8-tetrapropionic acid and 3,5,7-trimethyl-porphin-1-acetic-2,4,6,8-tetrapropionic acid. There are, however, two possible structures for the hexacarboxylic porphyrins, namely 5,7-dimethyl-porphin-1,3-diacetic-2,4,6,8-tetrapropionic acid and 3,7-dimethyl-porphin-1,5-diacetic-2,4,6,8-tetrapropionic acid. The low and high melting ester fractions of 6 I may well correspond to these two isomers, but which isomer has the low and which the high melting point, remains to be determined. Since more isomers are possible in the III series, additional data are needed for the establishment of structures of the decarboxylation products of Uro III.

In this connection we would like to mention that the “208°” porphyrin of Grinstein et al. (9) and the “pseudouro,” m.p. 211–216°, of Canivet and Rimington (9) might be the 7 I III porphyrins with different proportions of the isomers. The paper chromatographic difference observed by Falk et al. (10) might be similar to one of those shown in Fig. 3, although another possibility, namely the presence of more than one isomeric form of 7 III, is not completely ruled out.

SUMMARY

1. The stepwise decarboxylation of uroporphyrins I and III has been studied, and hepta-, hexa-, and pentacarboxylic porphyrins have been prepared.
2. The properties of these products have been compared with those isolated from a porphyric patient. The results support the view that the naturally occurring hepta-, hexa-, and pentacarboxylic porphyrins are mixtures of isomers of the I and III series.
3. The hexacarboxylic porphyrins of both the I and the III series have been found to be mixtures.
4. It is suggested that Watson’s “208,” Rimington’s “pseudouro,” and the heptacarboxylic porphyrins of the I and III series described here may be mixtures containing the isomers in different proportions.

REFERENCES

Hepta-, Hexa-, and Pentacarboxylic Porphyrins of Porphyria Cutanea Tarda: II. PREPARATION OF THE PORPHYRINS BY STEPWISE DECARBOXYLATION OF UROPORPHYRINS
T. C. Chu and Edith Ju-Hwa Chu


Access the most updated version of this article at http://www.jbc.org/content/234/10/2747.citation

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/234/10/2747.citation.full.html#ref-list-1