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Note

An improved method for the separation of coproporphyrins I and III

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Some of the procedures in the literature[1–3] for the separation of coproporphyrins I and III do not seem to give clear-cut results in our hands. This note describes a method which gives reproducible separations and utilizes commercially available cellulose polygram sheets. The chromatograms are developed for a long period of time (18 to 24 h) and a clear separation of the isomers is achieved. "Distance ratio" (cm/h) is used to characterize the movement of sample spots because the solvent front has been lost. Commercial preparations of coproporphyrins I and III and the mixture of isomers obtained by decarboxylation of uroporphyrins I and III were used. Their mobilities were compared with two dicarboxylic porphyrins, mesoporphyrin IX and deuteroporphyrin IX, and octacarboxylic porphyrins, uroporphyrins I and III. The "distance ratios" of the decarboxylated uroporphyrins correspond to those of standard coproporphyrins I and III, respectively. The dicarboxylic porphyrins moved far ahead of the coproporphyrins and the uroporphyrins remained at the origin.

EXPERIMENTAL

Polygram cel 300, plastic sheets pre-coated with cellulose, 20 × 20 cm (Brinkmann, Westbury, N.Y., U.S.A.) were used.

Polygram sheets were heated at 100° for 15 min before and after spotting samples.

Coproporphyrin I-tretramethyl ester, coproporphyrin III-tetramethyl ester, uroporphyrin I-octamethyl ester, and uroporphyrin I,III ester (Waldenstrom Type) were purchased from Sigma, St. Louis, Mo., U.S.A.

Deuteroporphyrin IX was prepared by the method of Falk[4] and purified by the method of Alben *et al.*[5].

Mesoporphyrin IX was prepared by the method of Falk[4], catalytic hydrogenation over Pd in formic acid.

The porphyrins were prepared from their corresponding methyl esters by hydrolysis in concentrated HCl in the dark for at least 24 h.

Decarboxylation of uroporphyrin I and uroporphyrin I,III to coproporphyrin I and coproporphyrin I,III was carried out by the method of Edmondson and Schwartz[6].

The porphyrins were spotted from a solvent mixture suggested by Eriksen[7] but with one drop of 0.1 *M* EDTA added to 10 ml ammonium hydroxide (30% NH_3)–water–acetone (1:2:7).

The porphyrins were detected by observing their fluorescences in a BLE Spectroline (Black Light Eastern) viewing cabinet by excitation at 366 nm.

A mixture of 2,6-lutidine–ammonia–water–0.1 *M* EDTA (10:4.2:2.8:0.02) as suggested by Pluscec and Bogorad[8] was placed in the developing chamber at 25° for equilibration for 1 h. The polygram sheet was heated for 15 min, spotted, and heated for 15 min. Development took place at 25° in the dark for different time periods: 18 h, 21 h, 24 h, and 48 h. Such long time periods of development result in the solvent front going off the chromatogram, but the coproporphyrin isomers remain on the polygram and are continually being separated. However, the 48-h run was too long and extensive diffusion of spots occurred. The optimum time in our experience is 18 to 21 h. Under these conditions, uroporphyrins I and III do not leave the origin. R_F values cannot be used to characterize the sample spots. Instead, we would like to use the term "distance ratio" which is defined as the distance in cm travelled per unit time (cm/h). The D_R values obtained are listed in Table I. Fig. 1 shows the variation of

TABLE I
DISTANCE RATIOS OF PORPHYRIN ISOMERS

Porphyrin	D_R			
	18 h	*21 h*	*24 h*	*48 h*
Copro I	0.306	0.329	0.350	Diffused
Decarboxylated uro I	0.306	0.329	0.346	Diffused
Copro III	0.394	0.400	0.425	Diffused
Decarboxylated uro I	0.306	0.333	0.346	Diffused
Decarboxylated uro III	—	0.405	0.426	Diffused
Deutero IX	—	0.786	—	—
Meso IX	—	0.800	—	—

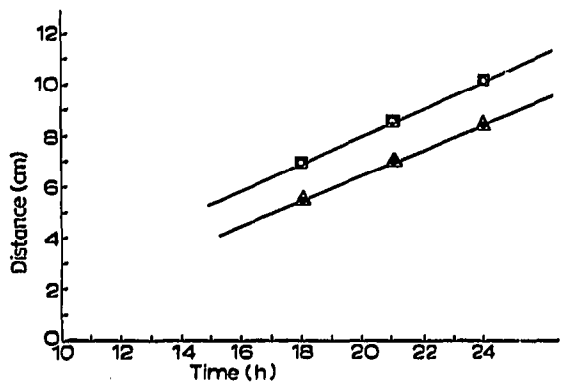

Fig. 1. Variation of distance with time for copróporphyrins and decarboxylated uroporphyrins. ●, Coproporphyrin I; ○, Coproporphyrin III; △, decarboxylated uroporphyrin I; □, decarboxylated uroporphyrin III.

distance travelled with time for the isomers of coproporphyrin and decarboxylated uroporphyrin.

The separation of coproporphyrin I and coproporphyrin III by allowing the Polygram to develop for 18 to 21 h, even though the solvent front goes off the upper edge of the chromatography sheet, results in a distinct separation and is reproducible. The availability of commercial cellulose polygram sheets renders a greater convenience for the separation of coproporphyrin I and coproporphyrin III.

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