PAPER CHROMATOGRAPHIC SEPARATION OF MESOPORPHYRINS I AND IX

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INTRODUCTION

The separation of porphyrins by column or paper chromatography has been reviewed by FALK[1,2]. Under suitable conditions, porphyrins with differing numbers of carboxylic functions may be separated from each other. The separation of position isomers by paper chromatography is also possible in certain instances. Thus, in the method of CHU, GREEN AND CHU[3] coproporphyrin tetramethyl esters I and III are separable. With the system introduced by FALK AND BENSON[4], the uroporphyrin octamethyl esters are similarly separable into pairs. Isomers I and II run together as do also isomers III and IV. Using the unesterified coproporphyrins, and a 2,6-lutidine–water system, the coproporphyrin isomers I, II and III may be separated from each other, but isomers III and IV run together[5–7]. No chromatographic separation of dicarboxylic porphyrin isomers has yet been described.

Free coproporphyrins and uroporphyrins occur in small amounts in normal biological materials, the III series isomers usually predominating, but in certain pathological conditions, for example, congenital porphyria, the proportion of isomer I far exceeds that of III. Naturally occurring haemoproteins such as haemoglobin, myoglobin, catalase, peroxidase and cytochrome *b* contain as their prosthetic group a ferroprotoporphyrin which is considered, on present evidence, to be haem derived entirely from the protoporphyrin designated IX by FISCHER[8]. This isomer is theoretically derivable from aetioporphyrin III and thus belongs also to the isomeric series III. No series I type of protoporphyrin has been found in nature despite search and some false reports[9,10]. The difficulty of detecting a series I protoporphyrin admixed with protoporphyrin IX is very considerable without the aid of chromatography and would inevitably demand much material.

Protoporphyrins may be easily converted to the more stable mesoporphyrins without alteration of isomeric type and we have now devised a paper chromatographic method which separates mesoporphyrin I from mesoporphyrin IX.

EXPERIMENTAL

Materials

Synthetic dimethyl esters of mesoporphyrin I and of mesoporphyrin IX were available from the collection of Professor H. FISCHER. A specimen of mesoporphyrin IX

dimethyl ester, which had been prepared by one of us (C. RIMINGTON) from the protoporphyrin of rats' faeces, was also used.

For hydrolysis, the esters are left in contact with 7 *N* HCl at room temperature in the dark for 42 hours. Excess acid is then removed in a vacuum desiccator containing NaOH.

Apparatus and procedure

The apparatus and general procedure are similar to those used by ERIKSEN[7]. The porphyrins (about 0.3 μg) *freshly* dissolved in pyridine (AnalaR*) are applied to Whatman No. 1 paper for chromatography and an ascending chromatogram is developed overnight with 2,6-lutidine–water (5:1) in an atmosphere of 0.88 s.g. NH_4OH and at a temperature of approximately 20°. After about 20 min in a chromatography drying oven at 50°, the porphyrin spots are located by their red fluorescence under ultra-violet light. The lower limit of detectability is about 0.06 μg and not more than 0.5 μg should be applied.

RESULTS

The R_F values found are recorded in Table I.

Mixtures were prepared of mesoporphyrins I and IX as indicated in Fig. 1, the total being 0.3 μg in each case.

TABLE I

POSITIONS OF MESOPORPHYRINS I AND IX ON PAPER CHROMATOGRAPHY

Material	R_F
Mesoporphyrin I (H. FISCHER's collection)	0.3
Mesoporphyrin IX (H. FISCHER's collection)	0.41
Mesoporphyrin IX (prepared from protoporphyrin of rats' faeces)	0.425
Mixture of mesoporphyrins I and IX	0.3 and 0.425

These were chromatographed with the results shown. 10% of isomer I was clearly distinguishable, there being two spots connected by faint fluorescence. 5% of isomer I was just detectable by tailing of fluorescence towards the I position when compared with the clear spot of pure mesoporphyrin IX.

DISCUSSION

The technique described offers the possibility of detecting, as mesoporphyrin, 5% or more of protoporphyrin I admixed with the ubiquitous isomer IX. It is still uncertain whether or not any protohaem or protoporphyrin of the I series occurs in either normal or pathological conditions.

* High purity reagent as supplied by Hopkin & Williams, Ltd., England.

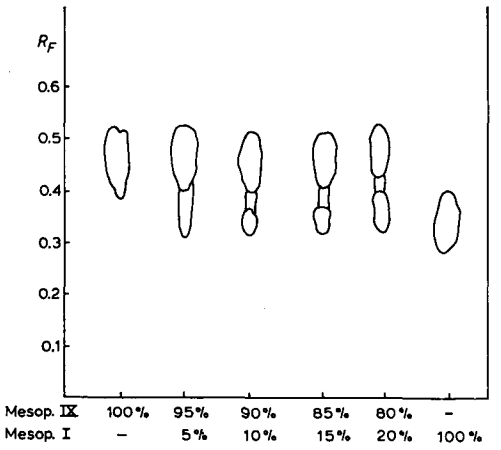

Fig. 1. Chromatographic separation of artificially prepared mixtures of mesoporphyrins I and IX. For details, see text.

The method has been applied to the investigation of a protoporphyrin present in unusually large amount in the erythrocytes of a patient suffering from a hitherto undescribed type of erythropoietic porphyria (MAGNUS, JARRETT, PRANKERD AND RIMINGTON[11]). Only protoporphyrin IX was detected.

SUMMARY

Using a 2,6-lutidine–water (5:1) system in an atmosphere of 0.88 s.g. ammonia, it is possible to separate mesoporphyrins I and IX. Five parts of the former is detectable when admixed with 95 parts of the latter. Since haems and protoporphyrins may be converted to mesoporphyrins without change in isomer type the technique renders possible the detection of this proportion of protoporphyrin I in haem or porphyrin-containing biological materials.

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