PAPER CHROMATOGRAPHY OF METHYL ESTERS OF PORPHYRINS*

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A micromethod for detecting and determining various porphyrins extracted from biological materials, especially nervous tissues (1), is needed. The technique of filter paper chromatography by Consden, Gordon, and Martin (2), with modification by Williams and Kirby's capillary ascent (3), has been applied to the problem in this study. The recent paper by Nicholas and Rimington (4) on partition chromatography of porphyrins prompts us to record our results on paper chromatography of the methyl esters of porphyrins. By using the double developing technique described below, we have been able to distinguish between esters of coproporphyrin I and III and those of protoporphyrin IX and mesoporphyrin IX respectively.

Procedure and Results

The apparatus was simply assembled from two beakers, one 250 ml. and one 1000 ml., set up concentrically, a piece of ordinary 6 mm. rubber tubing, and a piece of thick plate glass. The small beaker served as the container for the developing solvents and the larger one as the chamber for maintaining a saturated atmosphere of the desired solvent. The mouth of the liter beaker was trimmed with the rubber tubing, cut longitudinally and stapled end to end, to serve as a gasket between the beaker and the plate glass cover. Two sets of such apparatus were required for one complete development.

A great number of solvents were tested as the developing agents. All porphyrin esters tested traveled to the solvent front in most solvents and did not travel at all in other solvents. The following solvents were found to give a separation of methyl esters of uroporphyrin I, coproporphyrin, and protoporphyrin IX, but made no distinction between the isomers, coproporphyrin I and III: (a) n-butyl bromide, (b) benzene and kerosene, (c) ethylene bromide and kerosene, (d) bromobenzene and kerosene, (e)

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1 The authors are very much indebted to Dr. C. J. Watson and Dr. S. Schwartz, University of Minnesota, for their generous gift of methyl esters of coproporphyrin I and III, uroporphyrin I, and protoporphyrin IX.
chlorobenzene and kerosene. On the other hand, a mixture of oleic acid and kerosene differentiated between the methyl esters of coproporphyrin I and III, but gave no distinct separation of the methyl esters of uroporphyrin I and coproporphyrin I. It has been found that two successive developings with the following two solvent systems gave a satisfactory separation of the methyl esters of coproporphyrin I and III, protoporphyrin IX, mesoporphyrin IX, and uroporphyrin I. The first developing solvent system was composed of chloroform and kerosene, and the second system was composed of n-propyl alcohol and kerosene. The solvent pairs (d) and (e) mentioned above were also found to be satisfactory as the first developing agent, but with lesser intensity of the fluorescent spots.

The procedure for preparing the paper chromatogram was as follows: Whatman No. 1 filter paper was cut into sheets of 12.5 × 14.5 cm., and spotted along the basal line, drawn 2 cm. from one of the longer edges, with acetone solutions of the methyl esters of the porphyrins by capillary pipettes (5), which delivered about 0.3 μl. of solution per drop. As many as twelve spots were conveniently placed on the same sheet of paper. The paper was then made into a cylinder by means of two capillary glass hooks. In the meantime the first developing chamber was saturated with chloroform for 5 minutes. Then the developing solvents, 4 ml. of kerosene and 2.6 ml. of chloroform, were successively delivered into the small beaker by pipettes. The spotted paper cylinder was inserted immediately after the solvents were thoroughly mixed by gently tilting the covered chamber. The chromatogram was allowed to develop at room temperature (24°) for 25 minutes. By that time the solvent front was about 75 mm. from the basal line. Then the cylinder was taken out and placed on a sheet of paper to drain for a second. Meanwhile the solvent front was marked with a pencil. After being dried in an oven at 105-110° for about 4 minutes and cooled to room temperature, the cylinder was placed into the second developing chamber, which had been saturated with kerosene before the introduction of the developing solvent, consisting of 5 ml. of kerosene and 1 ml. of n-propyl alcohol. After the solvent ascended to the same front, the cylinder was taken out and dried again. The time needed for the second developing was about 40 minutes. The positions of the porphyrin esters were then located under ultraviolet light from a mineral light of 3660 A. Methyl esters of coproporphyrin III, protoporphyrin IX, and mesoporphyrin IX usually appeared as center-dense red fluorescent spots, while those of coproporphyrin I and uroporphyrin I were much more intense and evenly illuminated with sharp boundaries, although their

* The time may be reduced to 15 minutes if only the low RF value members are to be determined, or it may be increased to 35 minutes if a better separation of proto- and mesoporphyrin is desired. The RF values are essentially the same.
Fig. 1. Paper chromatograms of methyl esters of porphyrins. Section A, complete paper chromatogram obtained from double development with chloroform-kerosene and n-propyl alcohol-kerosene systems. Sections B and C, partial chromatograms obtained by single development respectively with the first and the second solvent system alone. The numeral I stands for methyl ester of uroporphyrin I, 2 for that of coproporphyrin I, 3 for that of coproporphyrin III, 4 for that of protoporphyrin IX, 5 for that of mesoporphyrin IX, and M for an artificial mixture of the five esters; H, spaces for the hooks.

**TABLE I**

R$_F$ Values of Methyl Esters of Porphyrins in Chloroform-Kerosene and n-Propyl Alcohol-Kerosene at 24°

<table>
<thead>
<tr>
<th>Methyl ester of</th>
<th>R$_F$</th>
<th>Minimum detectable amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uroporphyrin I</td>
<td>0.17</td>
<td>0.005</td>
</tr>
<tr>
<td>Coproporphyrin I</td>
<td>0.47</td>
<td>0.05</td>
</tr>
<tr>
<td>&quot; III</td>
<td>0.67</td>
<td>0.005</td>
</tr>
<tr>
<td>Protoporphyrin IX</td>
<td>0.84</td>
<td>0.04</td>
</tr>
<tr>
<td>Mesoporphyrin IX</td>
<td>0.89</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**TABLE II**

R$_F$ Values of Porphyrin Esters in Chloroform-Alkane and n-Propyl Alcohol-Alkane at 24°

<table>
<thead>
<tr>
<th>Methyl ester of</th>
<th>n-Decane</th>
<th>n-Dodecane</th>
<th>n-Tetradecane</th>
<th>n-Hexadecane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uroporphyrin I</td>
<td>0.14</td>
<td>0.20</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>Coproporphyrin I</td>
<td>0.42</td>
<td>0.52</td>
<td>0.45</td>
<td>0.47</td>
</tr>
<tr>
<td>&quot; III</td>
<td>0.70</td>
<td>0.76</td>
<td>0.74</td>
<td>0.66</td>
</tr>
<tr>
<td>Protoporphyrin IX</td>
<td>0.86</td>
<td>0.92</td>
<td>0.92</td>
<td>0.89</td>
</tr>
<tr>
<td>Mesoporphyrin IX</td>
<td>0.92</td>
<td>0.96</td>
<td>0.95</td>
<td>0.93</td>
</tr>
</tbody>
</table>
fluorescence was invisible or barely visible before the chromatogram was dried after the second dip. The paper chromatograms obtained from the double development and from the single development with either solvent system alone are shown in Fig. 1. It is obvious that only the combination of the two solvent systems gave the satisfactory chromatogram. The very faint streaks above coproporphyrin I methyl ester may be due to a trace of contamination, although this has not yet been conclusively verified.

The $R_p$ value, the ratio of the distance traveled by porphyrin to that traveled by solvent, was calculated for each porphyrin ester and listed together with the corresponding minimum detectable amount in Table I.

Kerosene from several companies has been tried and found to give the same result. In place of kerosene a few pure alkanes have also been tried with successful results, but again with lesser fluorescent spot intensity. Their $R_p$ values are tabulated in Table II.

In the place of $n$-propyl alcohol, isopropyl alcohol may also be used.

Variations in temperature and concentration of spots affect the $R_p$ values but do not change the relative chromatographic sequence of porphyrin esters.

Free porphyrins do not move in these double developing solvent systems.

The quantitative determination of porphyrins by paper chromatography and the application of this method to the study of porphyrins from biological materials are under investigation.

**SUMMARY**

A micromethod for detecting and separating methyl esters of coproporphyrin I and III, protoporphyrin IX, mesoporphyrin IX, and uroporphyrin I by paper chromatography has been described. The satisfactory developing solvent system was composed of two mixtures, the first being chloroform and kerosene and the second $n$-propyl alcohol and kerosene. The $R_p$ values for these esters were determined.

**BIBLIOGRAPHY**

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