A Sensitive Non-Destructive Method for the Demonstration of Bile Acids on Thin-Layer Chromatography

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(Received 21 August 1967)

This communication describes a spray for the demonstration of bile acids and some other compounds separated by thin-layer chromatography. Previous methods for the demonstration of bile acids on thin-layer chromatography are either destructive and somewhat selective, e.g. sulphuric acid or phosphomolybdic acid, or relatively insensitive, e.g. a water spray (Hofmann, 1964). In contrast with these, our spray is applicable where quantitative analysis is subsequently desired.

Fluorescent sprays have been used in the detection of neutral lipids on thin-layer chromatography (Morris, 1966) and a systematic investigation was made to find a similar spray suitable for bile acids for which these sprays are not useful. Many aromatic hydrocarbons fluoresce in ultraviolet light. A thin-layer plate with a resolved mixture of bile acids or salts was sprayed with a solution of an aromatic hydrocarbon in light petroleum and then examined under ultraviolet light. A Hanovia ultraviolet lamp with emission of 3650, 3021, 2972, 2650 and 2537 Å was used.

Acridine or anthracene causes a uniform fluores-

cence over the plate but does not detect the presence of bile acids. The fluorescence of decalin is completely inhibited by Kieselgel, but again bile acids are not demonstrated. The green fluorescence of pyrene is suppressed by the presence of Kieselgel. This effect is reversed by the presence of bile acids on the plate.

Pyrene is an extremely sensitive spray. About 5 ml. of a 0.05% solution of pyrene in light petroleum (b.p. 60–80°) is sprayed evenly over the surface of the plate. Table 1 lists the bile acids that can be detected in this manner, the sensitivity being to 0.2 µg. for bile acids and 1 µg. for bile salts. Table 1 shows that the sensitivity is unaffected by oxo groupings on the bile acid nucleus. There is a diminution of fluorescence within 1 hr. that is presumably due to the oxidation of the pyrene on the plate.

The spray is removed to the solvent front by running the plate in a non-polar system, e.g. diethyl ether–light petroleum (b.p. 60–80°) (2:3, v/v); the positions of the bile acids remain unchanged. The positions of the bile acids are marked before the spray is removed, and quantitative analysis is possible.

Pyrene is easily oxidized to more polar products that are less easily washed up the plate by the solvent system. It is necessary that the pyrene is pure and that the plate is re-run in the non-polar solvent system within ½ hr. of spraying with pyrene.

Table 1 also lists some other compounds that have been demonstrated. Those steroids that do not absorb in ultraviolet light are seen by fluorescence, whereas those steroids that have an absorption spectrum are seen as dark spots.

There is no evidence that pyrene has any tumour-producing activity, but contaminants may have carcinogenic activity (E. Boyland, personal communication), so that again the purity of the pyrene is essential.

This work was supported by a Temporary Research Grant from the Medical Research Council.

Hofmann, A. F. (1964). In *New Biochemical Separations*, p. 261. Ed. by James, A. T. & Morris, L. J. London: D. Van Nostrand Co Ltd.

Morris, L. J. (1966). *J. Lipid Res.* 7, 717.

Table 1. *Bile acids and other compounds that have been detected by the pyrene spray*

3 α -Hydroxycholanolic acid (lithocholic acid)
 3 β -Hydroxy-5 α -cholanolic acid
 3 α -Hydroxychol-6-enic acid
 3,12-Dioxocholanolic acid
 3 α ,6 α -Dihydroxycholanolic acid (hydoxycholic acid)
 3 α ,7 β -Dihydroxycholanolic acid (ursodeoxycholic acid)
 3 α ,7 α -Dihydroxycholanolic acid (chenodeoxycholic acid)
 3 α ,12 α -Dihydroxycholanolic acid (deoxycholic acid)
 3 α -Hydroxy-7-oxocholanolic acid
 3 α -Hydroxy-12-oxocholanolic acid
 3,12-Dioxocholanolic acid
 3 α -Hydroxy-12-oxochol-7-enic acid
 3 α ,7 α -Dihydroxy-12-oxocholanolic acid
 3 α ,12 α -Dihydroxy-7-oxocholanolic acid
 3 α ,6 α ,7 α -Trihydroxycholanolic acid (hyocholic acid)
 3 α ,7 α ,12 α -Trihydroxycholanolic acid (cholic acid)

Taurine and glycine conjugates of lithocholic acid, chenodeoxycholic acid, deoxycholic acid and cholic acid

Cholesterol; cholesteryl esters; glycerol tripalmitin; glycerol 1-mono-olein; stearic acid; butobarbitone

3 β -Hydroxyandrost-5-en-17-one; 3 α -hydroxy-5 α -androst-17-one; 3 α -hydroxy-5 β -androst-17-one