Separation of cholesterol and desmosterol by thin-layer chromatography

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STUDIES OF INHIBITORS of cholesterol biosynthesis and the recognition of 24-dehydrocholesterol (desmosterol) as a potential intermediate in this biosynthetic pathway have stimulated attempts to devise a suitable technique for the separation of these sterols (1, 2). However, a simple, rapid method for the separation of free cholesterol and free desmosterol has not been described.

Kaufmann and Makus (3) have proposed a method for the separation of fatty acids, which involves the coating of the silica gel layer with undecane, enabling the separation to proceed primarily via partition rather than adsorptive chromatography. The separation of the fatty acids appeared to be related to the number of double bonds present as well as the number of methyl groups. It occurred to us that this technique might be applicable to the separation of desmosterol and cholesterol, which differ only by the presence of a double bond. We have applied this basic technique to the separation of these sterols and have achieved excellent results. The procedure follows:

Glass plates, 20 x 20 cm, are coated with a 275 μ layer of silica gel[1] (proportion of gel to water, 1:1.5). The plates are activated for 1 hr at 120°, and stored in a desiccator until used. The coated plate is developed in a 15% solution of undecane in petroleum ether to the

[1] Adsorbil 1, purchased from Applied Science Laboratories, Inc., State College, Pennsylvania.

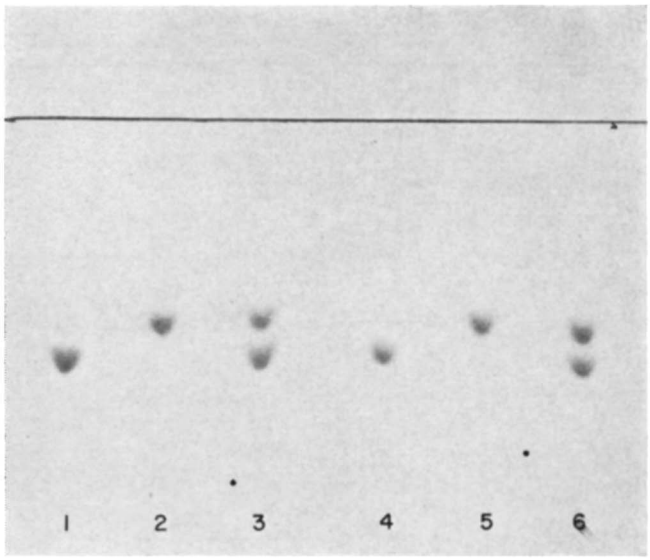

FIG. 1. Separation of cholesterol and desmosterol by thin-layer chromatography. Cholesterol (1,4); desmosterol (2,5); mixture of cholesterol and desmosterol (3,6).

approximate height of 15 cm. The plates are air-dried for 5 min and the samples spotted. After further air-drying for 1 hr, the plates are developed to the 10-cm mark in a mixture of acetic acid–acetonitrile 1:1, 70% saturated with undecane. This solution is prepared by saturating acetic acid–acetonitrile 1:1 with undecane in a separatory funnel and diluting 70 cc of the saturated solution to 100 cc with the original acetic acid–acetonitrile mixture. Again, the plates are air-dried for a few minutes, then heated at 150° for 1 hr to remove the undecane. If the undecane is not completely removed, background discoloration develops after spraying and heating with the visualizing agent. To minimize this effect, we have modified the heating procedure as follows: The oven is turned off, the door opened, and the plate allowed to cool to 100–110°. The plates are then removed from the oven and immediately sprayed with a 10% solution of phosphomolybdic acid in ethanol. The sterols usually become visible without any additional heating and with a minimum of background color. If further heating is necessary, the time (10–20 min) and temperature (100–120°) must be carefully controlled to prevent excessive background discoloration.

The separation of desmosterol[2] and cholesterol[3] is shown in Fig. 1. Desmosterol has an R_F value of 0.52 and cholesterol of 0.42. The R_F values of other sterols tested include lanosterol, 0.38, dihydrocholesterol, 0.39; and 5,7-cholestadiene-3β-ol, 0.49. The appearance of desmosterol in the serum of 7 patients given an inhibitor of cholesterol biosynthesis, 20,25-diazacholesterol (4), and its disappearance following cessation of the drug, was clearly demonstrable with this technique.

The method described is rapid, convenient, and reproducible and should find many applications in studies of lipid metabolism.

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[2] Mann Research Laboratories, chromatographically pure.

[3] California Foundation for Biochemical Research, recrystallized via the dibromide.

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