Thin-layer chromatography of sterols on neutral alumina impregnated with silver nitrate

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SUMMARY Thin-layer chromatography for the rapid separation of several sterols on neutral alumina impregnated with silver nitrate is described. The method is particularly effective for sterols that differ in the number and location of olefinic bonds.

KEY WORDS thin-layer chromatography · sterols · alumina–silver nitrate · intermediates · cholesterol biosynthesis

Progress in the purification, isolation, and characterization of the enzymes involved in cholesterol formation from lanosterol has been restricted by the lack of simple, rapid methods of assay for most of the reactions. Silicic acid column chromatogaphy, while very effective in the separation of many of the sterol intermediates in the biosynthesis of cholesterol (1–4), is time-consuming (4–7 days for a single experiment). Gas–liquid chromatography of the sterols or their derivatives (5–8) represents a potentially rapid assay method but requires a substantial investment in equipment if a large number of assays are contemplated. A method is described here for the rapid separation of several intermediates in sterol biosynthesis. A large number of assays can be carried out with inexpensive equipment.

The sterols are separated on chromatoplates of alumina impregnated with silver nitrate; the separations are qualitatively similar to those reported by others (9–11) on silica gel impregnated with silver nitrate.

Materials. Neutral Alumina AG7 (labeled "2–44 microns") containing 5% by weight of $CaSO_4$ was purchased from Bio-Rad Laboratories, Richmond, Calif. One lot contained particles larger than 74 μ in diameter; such particles were removed by sieving. Reagent grade silver nitrate, chloroform, and acetone were used without further purification. Cholesterol was purified by way of the dibromide (12). Δ^7-Cholesten-3β-ol, Δ^8-cholesten-3β-ol, $\Delta^{8(14)}$-cholesten-3β-ol, cholestan-3β-ol, $\Delta^{8,14}$-cholestadien-3β-ol, Δ^7-ergosten-3β-ol, and $\Delta^{7,22}$-ergostadien-3β-ol were prepared by chemical synthesis in this laboratory. Lanosterol was isolated from commer-

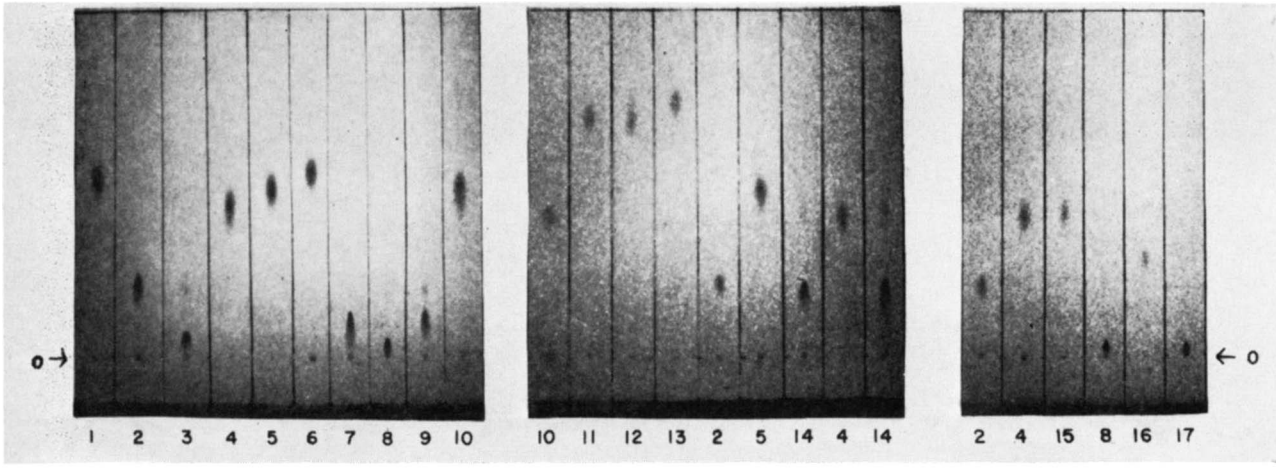

FIG. 1. Thin-layer argentation chromatography of sterols on alumina. Chloroform–acetone 95:5, 16 min at 22°C. Sprayed with H_3PO_4.

Sterols: *1*, cholestan-3β-ol; *2*, cholesterol; *3*, Δ^6-cholesten-3β-ol; *4*, Δ^7-cholesten-3β-ol; *5*, Δ^8-cholesten-3β-ol; *6*, $\Delta^{8(14)}$-cholesten-3β-ol; *7*, desmosterol; *8*, 7-dehydrocholesterol; *9*, $\Delta^{8,14}$-cholestadien-3β-ol; *10*, lanosterol; *11*, 24,25-dihydrolanosterol; *12*, 4,4-dimethyl-Δ^7-cholesten-3β-ol; *13*, 4,4-dimethyl-Δ^8-cholesten-3β-ol; *14*, mixture of $\Delta^{8,24}$-cholestadien-3β-ol (55%), $\Delta^{7,24}$-cholestadien-3β-ol (29%), Δ^8-cholesten-3β-ol (3%), and Δ^7-cholesten-3β-ol (12%); *15*, Δ^7-ergosten-3β-ol; *16*, $\Delta^{7,22}$-ergostadien-3β-ol; and *17*, ergosterol.

cially available material "lanosterol," C. P. (Mann Research Laboratories, Inc., New York) by a modification of the method of Bloch and Urech (13). 24, 25-Dihydrolanosterol (4,4,14α-trimethyl-Δ^8-cholesten-3β-ol), 4,4-dimethyl-Δ^8-cholesten-3β-ol (containing approximately 9% of the Δ^7-isomer), and 4,4-dimethyl-Δ^7-cholesten-3β-ol (containing approximately 3% of the Δ^8-isomer) were generous gifts from Dr. James Gaylor, Cornell University. A mixture of $\Delta^{8,24}$-cholestadien-3β-ol (55%), $\Delta^{7,24}$-cholestadien-3β-ol (29%), Δ^8-cholesten-3β-ol (4%), and Δ^7-cholesten-3β-ol (12%) was a generous gift from Doctors Ivan D. Frantz, Jr., and Ajit Sanghvi, University of Minnesota. 7-Dehydrocholesterol was prepared from 7-dehydrocholesteryl benzoate (Aldrich Chemical Co., Inc., Milwaukee, Wis.). Ergosterol, purchased from Mann Research Laboratories, Inc., New York, was recrystallized several times from acetone–water. Desmosterol ($\Delta^{5,24}$-cholestadien-3β-ol) was a product of Organon Inc. (West Orange, N. J.). Squalene, purchased from Mann Research Laboratories, Inc., was purified via the thiourea adduct (14). Farnesol, nerolidol, nerol, and geraniol were purchased from Aldrich Chemical Co., Inc. Calciferol and cholecalciferol were purchased from Mann Research Laboratories, Inc.

Preparation and Development of Chromatoplates. Neutral Alumina AG7 (35 g) containing 5% binder was thoroughly mixed with water (25 ml) in a porcelain evaporating dish for approximately 1 min with the aid of a spatula. Silver nitrate (10.5 g), dissolved in water (10 ml), was added to the slurry and the mixture was thoroughly stirred with a spatula (time of mixing about 1 min). This amount of material is convenient for the preparation of two 20 × 20 cm. chromatoplates, each about 250 μ thick. The chromatoplates were allowed to dry in air for 3–4 hr at room temperature. The plates began to darken after about 5 min and were dark gray or black after standing for about an hour; they were stored in a desiccator over Drierite overnight. No precautions were taken to avoid exposure to light. About 20 μg of each sample in ether was applied to the plate, which was then developed in chloroform–acetone 95:5. Development time was 15–18 min or 26–28 min for the solvent to move up the plate 12 or 15 cm, respectively. The plates were dried in air, sprayed with phosphoric acid, and heated on an aluminum block at ~170°C for about 5 min. Upon heating, the chromatoplate turns white and dark brown spots corresponding to the sterols appear. The minimum amount of cholesterol that can be detected under these condtions is 2–5 μg.

Results. Fig. 1 shows typical chromatograms. Cholestanol and the sterols containing a relatively hindered olefinic bond such as in Δ^8-, $\Delta^{8(14)}$-, and Δ^7-cholestenol are completely separated from cholesterol but are incompletely separated from one another. While the Δ^7-sterols have consistently shown a slightly lower R_f than the corresponding Δ^8-sterols (note spots 4 and 5, and 12 and 13), useful separations of these isomers have not been achieved. Δ^6-Cholestenol, containing an unhindered olefinic bond, and the dienols such as desmosterol, 7-dehydrocholesterol, and $\Delta^{8,14}$-cholestadienol are also widely separated from cholesterol but are incompletely separated from each other in this solvent system (spots 3, 7, 8, 9). Other compounds remaining at or near the origin under these conditions (but not shown in the figures) are squalene, farnesol, nerolidol, geraniol, nerol, calciferol, and cholecalciferol.

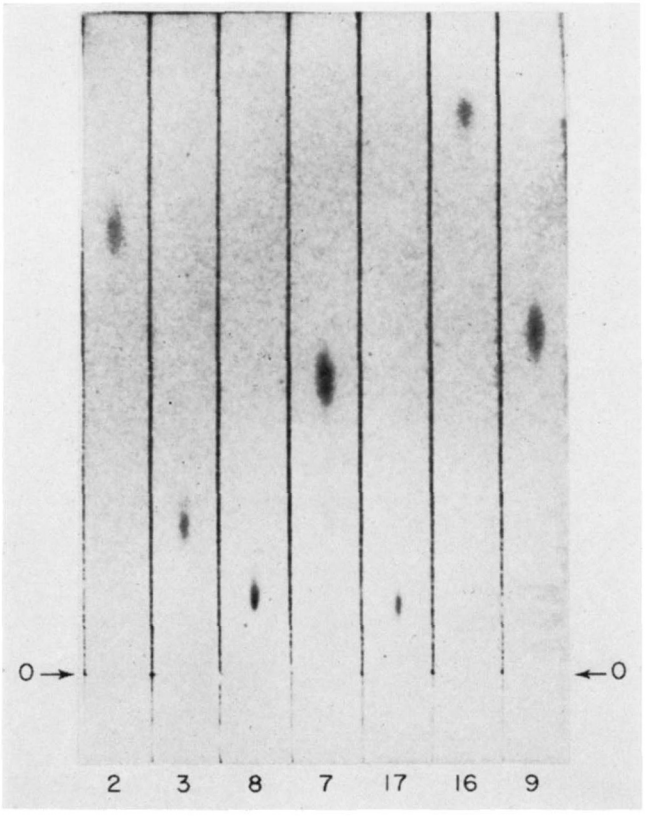

FIG. 2. Thin-layer chromatography of sterols in a more polar solvent: chloroform–acetone 65:35, for 26 min. Temperature, spray, and sterols as in Fig. 1.

An increase in the polarity of the developing mixture resulted in the resolution of a number of the more strongly adsorbed compounds (Fig. 2). Desmosterol and 7-dehydrocholesterol, essentially inseparable by silicic acid column chromatography (4) or by gas–liquid chromatography of their 3β-methoxyl (4,5) or trimethylsilyl (7) derivatives, are completely separated under these conditions. The separations illustrated in the figures were obtained with about 20 μg of sterol per spot. The capacity of the plates for each of the sterols has not been studied. Complete separation of cholesterol and Δ^7-cholestenol, each applied in 200 μg quantities to a single spot, was achieved on a layer 250 μ thick.

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REFERENCES

1. Frantz, I. D., Jr., E. Dulit, and A. G. Davidson. 1957. *J. Biol. Chem.* **226:** 139.
2. Kandutsch, A. A., and A. E. Russell. *J. Biol. Chem.* 1959. **234:** 2037.
3. Schroepfer, G. J., Jr., and I. D. Frantz, Jr. *J. Biol. Chem.* 1961. **236:** 3137.
4. Clayton, R. B., A. N. Nelson, and I. D. Frantz, Jr. *J. Lipid Res.* 1963. **4:** 166.
5. Clayton, R. B. 1962. *Biochemistry*. **1:** 357.
6. Brooks, C. J. W., and L. Hanaineh. 1963. *Biochem. J.* **87:** 151.
7. Wells, W. W., and M. Makita. 1962. *Anal. Biochem.* **4:** 204.
8. Horning, E. C., and W. J. A. VandenHeuvel. 1963. *Ann. Rev. Biochem.* **32:** 709.
9. Truswell, A. S., and W. D. Mitchell. 1965. *J. Lipid Res.* **6:** 438.
10. Copius Peereboom, J. W., and H. W. Beekes. *J. Chromatog.* 1965. **17:** 99.
11. Ditullio, N. W., C. S. Jacobs, Jr., and W. L. Holmes. 1965. *J. Chromatog.* **20:** 354.
12. Fieser, L. F. 1953. *J. Am. Chem. Soc.* **75:** 5421.
13. Bloch, K., and J. Urech. 1958. *Biochem. Prepn.* **6:** 32.
14. Goodman, DeW. S., and G. Popjak. 1960. *J. Lipid Res.* **1:** 286.