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Improved separation of sterols by column chromatography

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SUMMARY Some closely related sterols have been separated on columns of neutral alumina–Super-Cel impregnated with silver nitrate. Most notable are the separations of some Δ^8-sterols from their Δ^7-isomers, separations which have been difficult, if not impossible, to achieve with other published column chromatographic methods.

KEY WORDS column chromatography · argentation · intermediates in cholesterol biosynthesis

We report here a new column chromatographic method for the separation of sterols that is an adaptation, for preparative purposes, of a previously described thin-layer chromatographic method (1). The method affords useful separations of several sterol mixtures that are inseparable or poorly separable by other reported thin-layer (1–2) and column (3–7) chromatographic techniques. Most notable in this regard are the separations Δ^8-cholesten-3β-ol from Δ^7-cholesten-3β-ol and $\Delta^{8,24}$-cholestadien-3β-ol from $\Delta^{7,24}$-cholestadien-3β-ol.

Materials. Neutral alumina AG7 without binder (particle size 2–44 μ) was purchased from Bio-Rad Laboratories, Richmond, Calif. Hyflo Super-Cel is a product of the Johns-Manville Corporation. Reagent grade chloroform, acetone, and silver nitrate were used without further purification. Cholesterol was purified by use of the dibromide (8). Δ^8-Cholesten-3β-ol[1] and 4-^{14}C-Δ^7-cholesten-3β-ol[2] were prepared by chemical synthesis in this laboratory. A mixture of $\Delta^{8,24}$-cholestadien-3β-ol, $\Delta^{7,24}$-cholestadien-3β-ol, Δ^8-cholesten-3β-ol, and Δ^7-cholesten-3β-ol was a gift from Doctors Ajit Sanghvi and Ivan D. Frantz, Jr. of the University of Minnesota. 7-Dehydrocholesterol was prepared from 7-dehydrocholesteryl benzoate (Aldrich Chemical Co., Inc., Milwaukee, Wis.). Gas–liquid

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[1] W. Lee, R. Kammereck, and G. J. Schroepfer, Jr., unpublished work.

[2] A. M. Paliokas and G. J. Schroepfer, Jr., unpublished work.

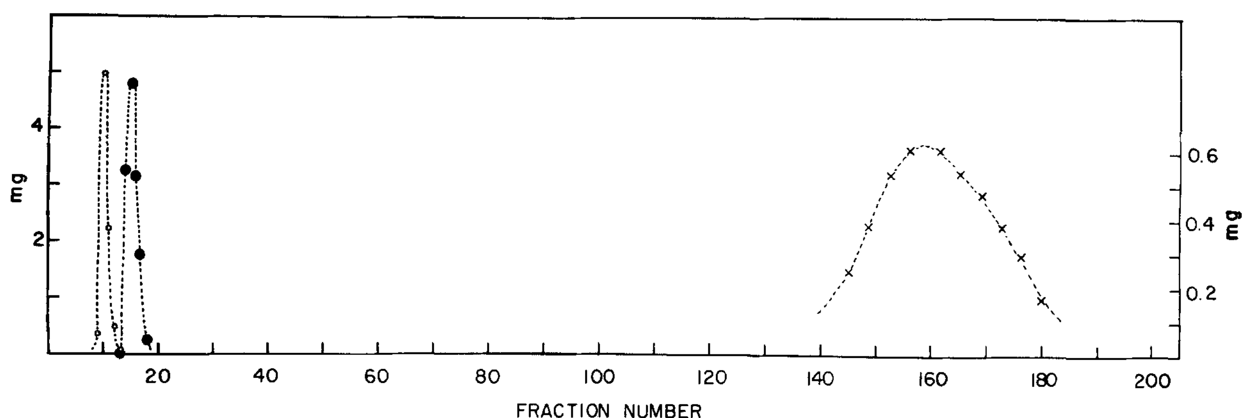

FIG. 1. Column chromatographic separation of Δ^7-cholesten-3β-ol (small circle), cholesterol (large circle), and 7-dehydrocholesterol (X). Column dimensions: 1.8 × 27.5 cm; fraction size, 6.8 ml; flow rate, 0.68 ml/min; solvent, chloroform–acetone 98:2. The amount of sterol in each fraction was assayed by colorimetric analysis with the Liebermann-Burchard reagent.

chromatography of the trimethyl silyl ether derivatives was carried out on a column of 5% diethylene glycol succinate on acid-washed Chromosorb W (60–80 mesh) which was 8 ft in length and 1/4 inch in diameter. The column temperature was 188°C and the inlet pressure was 25 psi. Under these conditions the retention times (relative to cholestane) for trimethyl silyl ether derivatives of Δ^8-cholesten-3β-ol, Δ^7-cholesten-3β-ol, $\Delta^{8,24}$-cholestadien-3β-ol, and $\Delta^{7,24}$-cholestadien-3β-ol were 3.15, 3.74, 4.25, and 5.09, respectively.

Preparation and Development of Column. The following procedure has been found convenient for the preparation of a column of adsorbent 1 cm in diameter and 100–110 cm in length. Neutral alumina AG-7 without binder (30 g) was thoroughly mixed with Hyflo Super-Cel (15 g) in a 1 liter, round-bottomed flask. Silver nitrate (9 g) in water (75 ml) was added and the flask was swirled to allow complete mixing of the slurry (which rapidly darkened). The mixture was frozen in an acetone–Dry Ice bath and lyophilized for 24 hr. The resulting dark gray powder was stored in a vacuum desiccator over Drierite overnight. The solvent (in most cases chloroform–acetone 98:2) was added to the dry powder, and the resulting slurry was thoroughly mixed. The slurry was poured into glass columns and the column was packed under a pressure of approximately 5 psi in a manner similar to that described by Frantz (5) for the preparation of silicic acid columns. The sterols were applied to the column in a small volume (~1 ml) of solvent. The sterols were eluted from the column with the same solvent mixture.

Results. Figs. 1–3 show successful resolution of the following mixtures: cholesterol, Δ^7-cholesten-3β-ol, and 7-dehydrocholesterol (Fig. 1); Δ^8-cholesten-3β-ol and Δ^7-cholesten-3β-ol-4-^{14}C (Fig. 2); a mixture of Δ^8-cholesten-3β-ol, Δ^7-cholesten-3β-ol, $\Delta^{8,24}$-cholestadien-3β-ol, and $\Delta^{7,24}$-cholestadien-3β-ol (Fig. 3). In the latter case the percentage of acetone in the eluting solvent

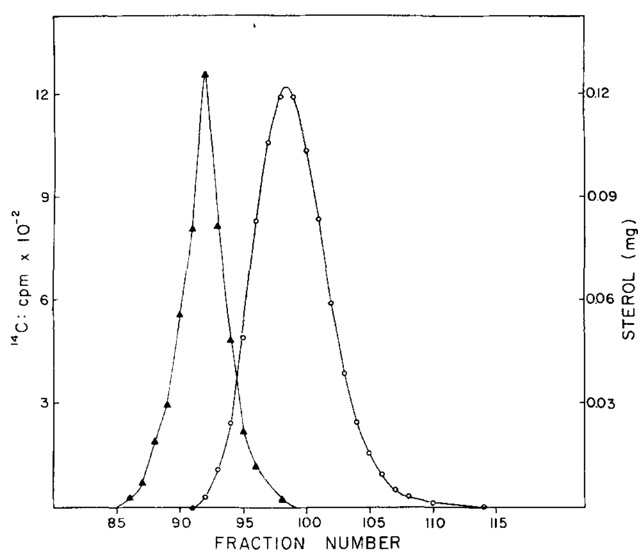

FIG. 2. Column chromatographic separation of Δ^8-cholesten-3β-ol (1.2 mg) and Δ^7-cholesten-3β-ol (~2 μg). ▲, Δ^8-cholesten-3β-ol, measured colorimetrically; ○, Δ^7-cholesten-3β-ol-4-^{14}C. Column dimensions, 1.0 × 100 cm; fraction size, 0.7 ml; flow rate, 0.035 ml/min; solvent, chloroform–acetone 98:2.

was increased since the use of the solvent mixture chloroform–acetone 98:2 with a column of these dimensions resulted in an impractically slow elution of the $\Delta^{7,24}$- and $\Delta^{8,24}$-dienols. However, the change in solvent composition was accompanied by an incomplete separation of Δ^8-cholesten-3β-ol from Δ^7-cholesten-3β-ol. The recoveries of added sterols have, in general, been good. For example, when 2.2 μg of cholesterol-4-^{14}C was subjected to chromatography on a column (35 × 1.5 cm) prepared as described above, the recovery of added ^{14}C was 99.6%. However, on several occasions we have been unable to elute 7-dehydrocholesterol from columns of the dimensions used in Figs. 2 and 3 with the solvent mixtures described above.

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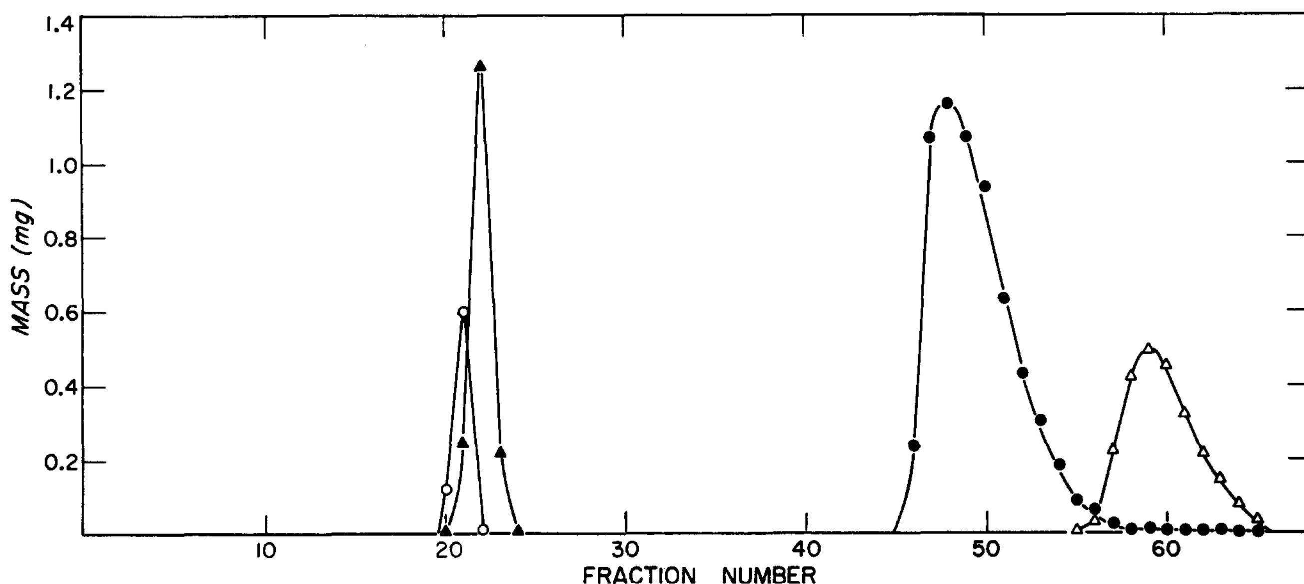

FIG. 3. Column chromatographic separation of a mixture (~14 mg) of Δ^8- and Δ^7-sterols. △, Δ^8-cholesten-3β-ol; ▲, Δ^7-cholesten-3β-ol; ●, $\Delta^{8,24}$-cholestadien-3β-ol; ○, $\Delta^{7,24}$-cholestadien-3β-ol. Column dimensions, 1 × 110 cm; fraction size, 4.0 ml; flow rate, 0.2 ml/min; solvent, chloroform–acetone 97:3. The amount of sterol in each fraction was determined by a combination of colorimetric analysis with the Liebermann-Burchard reagent and gas–liquid chromatographic analysis.

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