

Separation of sterol acetates by column and thin-layer argentation chromatography

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SUMMARY Column and thin-layer chromatographic systems employing silver nitrate-impregnated adsorbents are described for the separation of sterol acetates which differ in the number of double bonds in the steroid nucleus or side chain.

KEY WORDS chromatography · column · thin-layer · argentation · sterols · sterol acetates

STUDIES OF the sterol metabolism of insects made it necessary for us to separate cholesterol from 7-dehydrocholesterol¹ and from desmosterol. Since these separations involve sterols differing in degree of unsaturation, it was felt that chromatography with silver nitrate-impregnated adsorbents would give the desired separations. Preliminary experiments with the thin-layer systems of Avigan, Goodman, and Steinberg (1) indicated that better separations could be achieved with sterol acetates. This report describes the column and thin-layer systems used to separate a variety of sterol acetates.

Materials. All solvents were redistilled over sodium and stored over sodium until used. Sterols used as standards were obtained from commercial sources or synthesized in this laboratory. After purification, all showed single spots on TLC (1) and single peaks in three systems of GLC (2).

Column Chromatography. 25 g of silver nitrate was dissolved in 500 ml of distilled water. To this solution was added 100 g of silicic acid (Unisil, Clarkson Chemical Company, Inc., Williamsport, Pa.)²; the water was then

Abbreviations: TLC, thin-layer chromatography; GLC, gas-liquid chromatography.

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¹ The trivial names and the corresponding systematic names of sterols used in this report are as follows: brassicasterol, 24 β -methyl- $\Delta^5,22$ -cholestadien-3 β -ol; campesterol, 24 α -methyl- Δ^5 -cholesten-3 β -ol; cholesterol, Δ^5 -cholesten-3 β -ol; cholestanol, 5 α -cholestan-3 β -ol; $\Delta^8(14)$ -cholestenol, $\Delta^8(14)$ -cholesten-3 β -ol; Δ^{14} -cholestenol, Δ^{14} -cholesten-3 β -ol; desmosterol, $\Delta^5,24$ -cholestadien-3 β -ol; 22,23-dihydrobrassicasterol, 24 β -methyl- Δ^5 -cholesten-3 β -ol; 24,25-dihydrolanosterol, 4,4,14 α -trimethyl- Δ^8 -cholesten-3 β -ol; fucosterol, $\Delta^5,24(28)$ -stigmastadien-3 β -ol; lanosterol, 4,4,14 α -trimethyl- $\Delta^8,24$ -cholestadien-3 β -ol; lathosterol, Δ^7 -cholesten-3 β -ol; β -sitosterol, 24 α -ethyl- Δ^5 -cholesten-3 β -ol; stigmastanol, 24 α -ethyl-cholestan-3 β -ol; stigmasterol, 24 α -ethyl- $\Delta^5,22$ -cholestadien-3 β -ol; 7-dehydrocholesterol, $\Delta^6,7$ -cholestadien-3 β -ol; Δ^{22} -cholesterol, $\Delta^5,22$ -cholestadien-3 β -ol.

² Mention of a proprietary name or product does not necessarily imply endorsement by the U.S. Department of Agriculture.

TABLE 1 COLUMN CHROMATOGRAPHY OF STEROL ACETATES ON SILICIC ACID IMPREGNATED WITH SILVER NITRATE

Mixture	Sterols (as acetates)	Peak Eluent*	Purity†	Common Fraction‡
1	a) 24,25-Dihydrolanosterol	15	99+	20§
	b) Lanosterol	25	99+	
2	a) Cholesterol	20	99+	30§
	b) Desmosterol	50	99+	
3	a) Cholestanol	10	99+	15§
	b) Cholesterol	20	99+	
4	a) Cholesterol	20	99+	30§
	b) 7-Dehydrocholesterol	50	99+	

15 mg of each component applied to a 6 g column (1 × 10.5 cm). Column eluted with 0, 5, 10, 15, 20, 25, 30, 35, and 50% benzene in *n*-hexane.

* Composition expressed as per cent benzene in *n*-hexane.

† Determined by GLC.

‡ Fraction in which both components appear.

§ The ratio of component a to component b in each of these common fractions was <0.05.

removed from the slurry by means of a rotary evaporator, and the adsorbent was dried overnight in an oven at 110°C. 6 g of the adsorbent was packed as a slurry in hexane to form a column 1 × 10.5 cm, and some anhydrous sodium sulfate was added to the top of the column to protect the surface. When 7-dehydrocholesterol was chromatographed, the column was wrapped in black paper, and the intensity of light in the laboratory was reduced; in no other case did the latter precaution seem necessary. Binary mixtures of sterol acetates (15 mg of each) dissolved in hexane were placed on the column and eluted stepwise with 5% increments (0–35%) of benzene in *n*-hexane, 50 ml per fraction, and then with 50 ml each of benzene-hexane 1:1 and benzene. The fractions were monitored by GLC on a column of 0.75% neopentyl glycol succinate polyester.

TLC. Five glass plates (20 × 20 cm) were coated to a thickness of 250 μ with a slurry of 25 g of Silica Gel H (Brinkmann Instruments Inc., Westbury, N.Y.) in 65 ml of aqueous silver nitrate (12.5% w/v), air-dried for 1 hr, and activated in an oven for 30 min at 110°C. The coated plates were stored in a light-tight desiccator over anhydrous calcium chloride. The acetates (10–20 μ g of each) were applied about 1.5 cm from the bottom of the plate, and the plate was placed in an unlined chromatographic tank containing 200 ml of benzene-hexane 3:5. The solvent front was allowed to rise 15 cm from the origin. The spots were made visible by spraying with 50% sulfuric acid and charring at 110°C.

Results. The following binary mixtures of sterol acetates were separated (Table 1) by column chromatography: 24,25-dihydrolanosterol-lanosterol, cholesterol-desmosterol, cholestanol-cholesterol, and cholesterol-7-dehydrocholesterol.

TABLE 2 RECOVERIES OF CHOLESTEROL AND 7-DEHYDRO-CHOLESTEROL FROM COLUMN

	Cholesterol		7-Dehydrocholesterol	
	<i>cpm</i>	%	<i>mg</i>	%
Applied	3.64×10^5	100	2.11	100
Cholesterol fraction	3.56×10^5	97.8	0	0
7-Dehydrocholesterol fraction	4.48×10^3	1.2	2.06	97.6
Recovered	3.60×10^5	99.0	2.06	97.6

7-Dehydrocholesterol was determined by the extinction at 282 $m\mu$ on a Spectronic 505 recording spectrophotometer. $E_{282} = 12,000$ in *n*-hexane.

To confirm the results obtained by the gas chromatographic monitoring of the column fractions we chromatographed the acetates of cholesterol-4- ^{14}C and of unlabeled 7-dehydrocholesterol on the column; the cholesterol was monitored by radioactivity and the 7-dehydrocholesterol by UV absorption. This procedure also enabled us to determine whether cholesterol or the highly labile 7-dehydrocholesterol decomposed appreciably on the column. The radioactivity recovered was 99% of that applied to the column, and the cholesterol fraction accounted for 98% of the applied radioactivity; 98% of the 7-dehydrocholesterol was recovered, with none detected in the cholesterol fraction (Table 2).

With the TLC system, we were able to separate mixtures of compounds that differed by a nuclear double bond, e.g., cholesterol-cholestanol and lathosterol-cholestanol, but not cholesterol-lathosterol (Fig. 1). A Δ^{24} -double bond permitted a wide separation from the corresponding dihydrosterol, e.g. cholesterol-desmosterol, 24,25-dihydrolanosterol-lanosterol. The effect of a Δ^{22} -double bond was not so pronounced; the separation

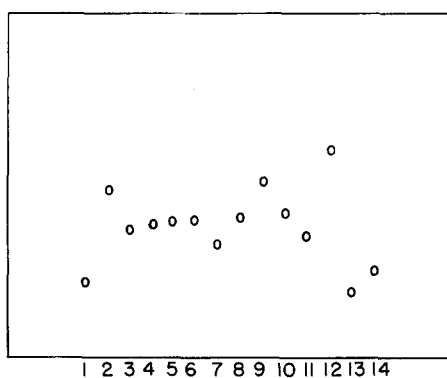


FIG. 1. Thin-layer separation of sterol acetates on silver nitrate-impregnated silica gel. Solvent system: benzene-hexane 3:5 (no chamber lining). 1, Fucosteryl acetate; 2, stigmastanyl acetate; 3, stigmasteryl acetate; 4, cholesteryl acetate; 5, β -sitosteryl acetate; 6, campesteryl acetate; 7, brassicasteryl acetate; 8, 22,23-dihydrobrassicasteryl acetate; 9, cholestanyl acetate; 10, lathosteryl acetate; 11, lanosteryl acetate; 12, 24,25-dihydrolanosteryl acetate; 13, desmosteryl acetate; 14, fucosteryl acetate.

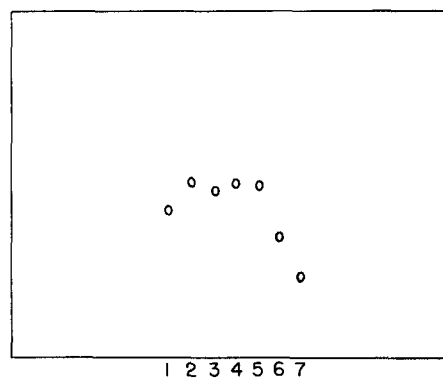


FIG. 2. Influence of double bond and alkyl substituents in side chain of sterol acetates chromatographed on silver nitrate-impregnated silica gel. Same system as Fig. 1. 1, Brassicasteryl acetate; 2, 22,23-dihydrobrassicasteryl acetate; 3, stigmasteryl acetate; 4, β -sitosteryl acetate; 5, cholesteryl acetate; 6, Δ^{22} -cholesteryl acetate; 7, desmosteryl acetate.

between cholesterol and 22-dehydrocholesterol was less than that between cholesterol and desmosterol. In addition, alkyl substituents at C_{24} influenced the effect of the Δ^{22} -double bond (Fig. 2): a methyl group at C_{24} reduced the separation (22,23-dihydrobrassicasterol-brassicasterol) and an ethyl group reduced the separation even more (β -sitosterol-stigmasterol). Also lathosterol could be separated from $\Delta^{8(14)}$ -cholestenol, and Δ^{14} -cholestenol was widely separated from these two sterols. Fucosterol was separated very well from stigmasterol (Fig. 1). A number of sterols of the coprostane series (A/B rings *cis*) were tested, but the separations obtained by the present system were unsatisfactory.

Discussion. The use of silver nitrate-impregnated silicic acid for the separation of cholesterol and cholestanol was reported previously by de Vries (3) and Shefer, Milch, and Mosbach (4); de Vries reported no conditions. Separation of 7-dehydrocholesterol from cholesterol (as the acetates on alumina) was reported by Kaplanis, Robbins, and Tabor (5). We find that the batch elution procedure described here is simpler and faster than the reported methods. Although several changes of eluent are needed, elution of a 6 g column is completed in little more than an hour. Acetylation methods are simple and rapid, and the sterols tested are stable during saponification.

Avigan et al. (1) described some TLC systems for the separation of a number of sterols and sterol acetates, but their systems required 40-cm plates, specially prepared adsorbent, and as much as 36 hr for chromatography. Other TLC systems have been reported (6, 7), but these do not adequately separate the compounds separable by our system.

Both the column and TLC systems have been used in this laboratory for separation of sterols isolated from biological material. The thin-layer system is also useful in

monitoring column chromatography or the synthesis of certain sterols.

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