SC 2322

Separation of nuclear saturated from unsaturated sterols

The separation of sterols which differ from each other by only a double bond in the nucleus has proven quite difficult in many instances. Even the use of newer methods of separation such as gas–liquid chromatography has not accomplished the complete separation of such sterols as cholesterol from β-cholestanol (dihydrocholesterol). However, the separation of cholesterol from β-cholestanol has been achieved by various types of chromatography after oxidizing the cholesterol to a more polar compound[1–3]. These oxidations have been done with perbenzoic acid[1] or performic acid[2,3]. These acids are unstable and must be freshly synthesized frequently. Recently, MICHALEC[4] has separated these compounds by forming cholesteryl bromide during chromatography. A method will be presented for the rapid separation of a cholestene series of sterols from their dihydro analogues by thin-layer chromatography on silicic acid following peroxidation with *m*-chloroperbenzoic acid.

Sterol standards* were dissolved in 1–2 ml chloroform and epoxidized with a 5-fold molar excess (5–20-fold is satisfactory) of *m*-chloroperbenzoic acid** for 30 min at room temperature. After the addition of 4 ml diethyl ether the reaction mixture was washed with 10% $NaHCO_3$ until bubbling ceased. It was then washed with

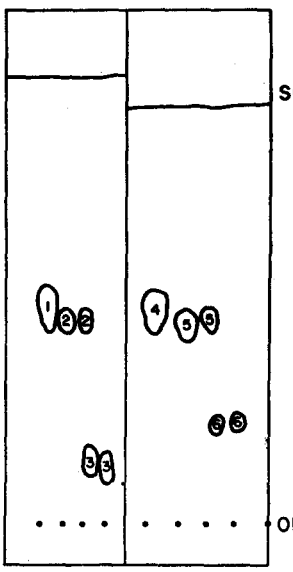

Fig. 1. Thin-layer chromatographic separation of sterols. Silica gel G (20 g + 40 ml water) was spread 250 μ thick and activated at 110° for 60 min. Chromatography of sterols (10–40 μg) was done in benzene–butyl acetate–butanone (75:25:10, v/v) with a running time of 60–70 min at room temperature. 1, cholesterol; 2, β-cholestanol; 3, cholesterol epoxide; 4, stigmasterol; 5, stigmastanol; 6, stigmasterol epoxide.

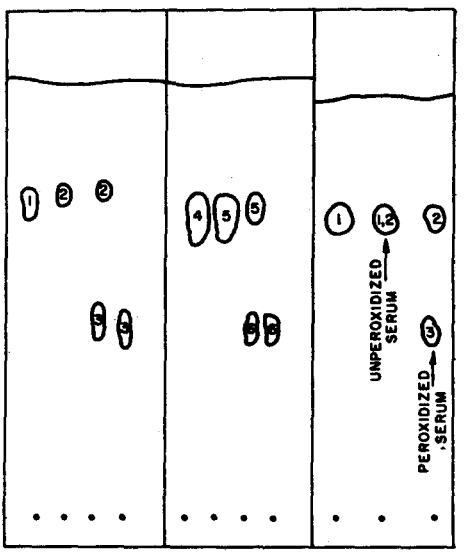

Fig. 2. Chromatographic separation of sterols. Adsorbasil No. 1 (20 g + 30 ml water) was spread 250 μ thick and activated at 110° for 60 min. The sterols (10–40 μg) were chromatographed in benzene–butyl acetate–butanone (75:25:10, v/v) with a running time of 27–35 min at room temperature. The numbers refer to compounds recorded in Fig. 1.

* The stigmasterol and dihydrostigmasterol were gifts from Dr. H. NICHOLAS.

** Generously supplied by R. J. GALL, Buffalo Electro Chemical Company, Division Food, Machinery, and Chemical Corp., Buffalo, N.Y. (U.S.A.).

aq. satd. NaCl and finally dried over anhydrous Na_2SO_4. The chloroform was evaporated off under a stream of nitrogen and the sterol residue dissolved in benzene (4 mg/ml). The entire process can be carried out in a test tube with removal of the aqueous washes by aspiration. Serum sterols were isolated after saponification by digitonin precipitation. The sterols were recovered from the digitonide as described by SCHOENHEIMER[5] and peroxidized as above. The sera containing β-cholestanol were obtained by feeding rats 500 mg β-cholestanol per 100 g diet for 5 days.

The sterols were chromatographed on silica gel spread approx. 250 μ thick on 5 × 20 cm glass plates. The support was activated at 110° for 60 min. The solvent system was benzene–butyl acetate–butanone (75:25:10, v/v) and was allowed to ascend for 16–18 cm past the origin. The separation of the sterols on silica gel G (Merck, Darmstadt) and Adsorbasil No. 1 (Applied Science Co., College Park, Pa.) is recorded in Figs. 1 and 2. The spots were visualized by spraying with 20 % antimony pentachloride followed by heating at 110° for 3 min. By incorporating 0.1 % Rhodamine 6G into the water used to slurry the silica gel, the sterols could be visualized with an ultraviolet light and eluted with chloroform.

It was demonstrated by the addition of [4-^{14}C]cholesterol to the β-cholestanol that less than 1 % cholesterol contaminated the β-cholestanol following chromatography and that 98 % of the radioactivity could be recovered from the epoxidized cholesterol spot. When other ring unsaturated sterols such as desmosterol or lanosterol were chromatographed following peroxidation, separation was obtained from the unsaturated compound. Desmosterol epoxide had a R_F similar to cholesterol epoxide suggesting that the 24,25 double bond was not oxidized under these conditions. However, the peroxidation of lanosterol yielded two spots with a R_F different from lanosterol suggesting oxidation at both the 24,25 double bond and the 8,9 ring double bond.

Thus, excellent separation of ring saturated sterols from their unsaturated analogues can be obtained by peroxidation of the unsaturated compound with *m*-chloroperbenzoic acid. With this oxidizing agent virtually complete oxidation of the ring double bond is obtained under very mild conditions. Another advantage of *m*-chloroperbenzoic acid is its relative stability which allows it to be stored for long periods of time without marked decomposition.

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