Sterol synthesis. High-pressure liquid chromatography of C27 sterol precursors of cholesterol

Joan Ryan Thowsen and George J. Schroepfer, Jr.

Departments of Biochemistry and Chemistry, Rice University, Houston, TX 77001

Summary

Conditions have been described which permit the rapid (~3.5 hr) column chromatographic separation of the acetate derivatives of a number of C27 sterol precursors of cholesterol differing only in the number and position of double bonds in the sterol nucleus. On columns containing μPorasil with hexane–benzene 9:1 as the eluting solvent, acetate derivatives of the Δ², Δ⁴, Δ⁶, Δ⁸, Δ¹⁰, Δ¹², and Δ¹⁴ sterols were separated. — Thowsen, J. R., and G. J. Schroepfer, Jr. Sterol synthesis. High-pressure liquid chromatography of C27 sterol precursors of cholesterol. J. Lipid Res. 1979. 20: 681–685.

Supplementary key words sterol precursors of cholesterol

The enzymatic formation of cholesterol from lanosterol (4,4α-trimethyl-5α-cholesta-8,24-dien-3β-ol) involves a very large number of potential intermediates (1–4). A number of these potential intermediates include sterols that differ only in the number and position of nuclear double bonds. These variants include sterols with nuclear double bonds in the following positions: Δ², Δ⁴, Δ⁶, Δ⁸, Δ¹⁰, Δ¹², Δ¹⁴, and Δ¹⁶. The separation of these compounds from one another is a less than trivial matter. In the C27 series, the separation of cholesterol, cholesta-5,7-dien-3β-ol, and 5α-cholest-7-en-3β-ol has been achieved by chromatography on silicic acid–Super Cel columns (5–10). However, the resolution of these sterols was frequently less than complete and very long development times (2–3 days) were required using these columns. Complete separations of the above
Another by a combination of column chromatographic separation from each other by gas-liquid chromatography of suitable derivatives (8, 13, 14) or by alumina–Super Cel–silver nitrate column chromatography of the free sterols or their acetate derivatives (14–16). The results of research from several laboratories have indicated the possible intermediary role of sterols (4, 8). Chromatography of the acetylated sterols on columns of silica gel G-Super Cel-silver nitrate (4, 16–27). Exploration of the metabolism of those sterols required the development of chromatographic methods to allow the separation of these compounds from one another and from the sterols noted above. To this end we have described methodology that permits the resolution of these compounds from one another by a combination of column chromatographic techniques. Silicic acid–Super Cel column chromatography can be used to isolate the C_{27} monohydroxy-sterols (4, 8). Chromatography of the acetylated C_{27} sterols on columns of silica gel G–Super Cel–silver nitrate permitted the separation of the monounsaturated steryl acetates from diunsaturated steryl acetates and, in addition, permitted separation of the Δ^{7,14}, Δ^{5,14} and Δ^{5,7-} steryl acetates from one another (4, 25, 27, 28). Subsequent chromatography of the monounsaturated steryl acetate fraction (from the silica gel G–Super Cel–silver nitrate column) on columns of alumina–Super Cel–silver nitrate permitted separation of the acetates of 5α-cholest-8(14)-en-3β-ol, 5α-cholest-8-en-3β-ol, 5α-cholest-7-en-3β-ol, and cholesterol (4, 14, 25, 24, 25, 28). While this methodology has proved to be extremely valuable in the purification, isolation, and identification of various C_{27} sterols, a very serious defect of this approach has been the extraordinarily long time required to complete the three separate column chromatographic analyses.

Several laboratories have explored the use of reversed-phase high-pressure liquid chromatography for the separation of sterols through their derivatives (29–32). Rees, Donnayeh, and Goodwin (29) have reported on the chromatographic behavior of the acetate derivatives of a number of C_{27}, C_{28}, and C_{29} sterols upon reversed-phase high-pressure liquid chromatography. While 3β-acetoxy-5α-cholestanol could be resolved from the acetate derivatives of cholesterol and 5α-cholest-7-en-3β-ol, effective separations of the latter compounds from one another was not achieved. 3β-Acetoxy-5α-cholestan-7-one was cleanly separated from 3β-acetoxy-cholesta-5,24-diene and 3β-acetoxy-cholesta-5,7-diene while only partial separation of the latter two compounds was achieved. Trocha, Jasne, and Sprinson (30) recently reported the results of the study of the behavior of a number of yeast sterols upon reversed-phase high-pressure liquid chromatography. A number of 4- and 14-methylated sterols and ergosterol derivatives were studied. Separation of ergost-8-en-3β-ol from ergost-7-en-3β-ol, but not of ergosta-8,22,24(28)-trien-3β-ol from ergosta-7,22,24(28)-trien-3β-ol, was achieved in the chromatographic system employed. Hunter, Walden, and Heftmann (31) recently reported the resolution of a mixture of 5α-cholest-7-en-3β-ol, cholesterol, and 7-dehydrocholesterol by reversed-phase high-pressure chromatography. Hansbury and Scallen (32) have also recently reported the separations of desmosterol from cholesterol, lanosterol from dihydrolanosterol, and 7-dehydrocholesterol from cholesterol using reversed-phase high-pressure liquid chromatography. The utility of this form of chromatography to separate sterols differing only in the presence or absence of a Δ^5-double bond had also been found previously by Trocha et al. (30).

The purpose of the present report is to summarize the results of preliminary investigations of the behavior of the acetate derivatives of a number of C_{27} sterols on both reversed-phase and absorption high-pressure liquid chromatography. Described herein is a system that permits the resolution of the acetates of C_{27} sterols with double bonds in the Δ^5, Δ^7, Δ^8, Δ^8(14), Δ^6(14), Δ^7,14, and Δ^5,7 positions from one another in a relatively short period of time. A preliminary account of this work has been presented (33).

MATERIALS AND METHODS

Thin-layer chromatography was performed on plates (250 μm in thickness) of silica gel G (Analtech, Inc., Newark, DE; solvent, benzene–ether 1: 1) or 15% silver nitrate–silica gel G (34), (solvents, ether–hexane 9:1 or chloroform). Components on the plate were visualized after spraying with a molybdic acid solution (35) followed by heating at 100–120°C for 10 min. Gas–liquid chromatography was performed using a Hewlett-Packard Model 402 unit equipped with flame ionization detector. A silanized glass column (6 ft × 4 mm) of 3% QF-1 on Gas Chrom Q (100–120 mesh) was used with a column temperature of 240°C. Gas–liquid chromatography–mass spectral analyses were made using an LKB 9000S unit equipped with a 3% OV-17 on Gas Chrom Q
Fig. 2. Chromatographic separation of 3β-acetoxy-5α-cholestan-3β-ol (A), 3β-acetoxy-5α-cholestan-5-ene (B), 3β-acetoxy-5α-cholestan-8(14)-ene (C), 3β-acetoxy-5α-cholestan-8,14-diene (D), 3β-acetoxy-5α-cholestan-14-ene (E), and 3β-acetoxy-5α-cholestan-7,14-diene (F) on four columns of μPorasil (30 cm x 4 mm each) using chloroform–hexane 4: 6 as the eluting solvent at a flow rate of 1.0 ml per min. The amounts of the steryl acetates applied to the column varied from ~75 to 170 μg.

Fig. 3. Chromatographic separation of 3β-acetoxy-5α-cholestan-3β-ol (A), cholesteryl acetate (B), 3β-acetoxy-5α-cholestan-8(14)-ene (C), 3β-acetoxy-5α-cholestan-8,14-diene (D), and 3β-acetoxy-5α-cholestan-7,14-diene (E) on four columns of μPorasil (30 cm x 4 mm each) using hexane–benzene 8: 2 as the eluting solvent at a flow rate of 2.5 ml per min. The amount of each steryl acetate injected was ~180–240 μg.

Fig. 4. Chromatography of cholesteryl acetate (A), 3β-acetoxy-5α-cholestan-8(14)-ene (B), 3β-acetoxy-5α-cholestan-8,14-diene (C), 3β-acetoxy-5α-cholestan-7,14-diene (D), 3β-acetoxy-5α-cholestan-5,7-diene (E), and 3β-acetoxy-5α-cholestan-7,14-diene (F) on four columns of μPorasil (30 cm x 4 mm each) using hexane–benzene 9: 1 as the eluting solvent at a flow rate of 1.0 ml per min. The loads of the steryl acetates varied from ~170 μg to 470 μg.
acetates of cholest-5,7-dien-3β-ol and 5α-cholesta-
8,14-dien-3β-ol were not achieved under the same
conditions. In view of the latter situation, we system-
atically explored other solvent mixtures for the
eletion of the steryl acetate from the μPorasil
columns. Hexane–benzene 8:2 gave encouraging
results. Fig. 3 shows the partial resolution of the
acetate derivatives of 5α-cholestan-3β-ol, cholesterol,
5α-cholestan-8(14)-en-3β-ol, 5α-cholestan-8-en-3β-ol, and
5α-cholestan-7-en-3β-ol. Decreasing the polarity of the
eluting solvent mixture (hexane–benzene 9:1) per-
mitted the separation of the acetate derivatives
of the Δ9, Δ814, Δ8, Δ7, Δ5, Δ8,14, and Δ7,14 sterols as
shown in Fig. 4.

DISCUSSION

The results presented herein indicate that HPLC
provides very rapid and useful separations of a number
of steryl acetates differing only in the number
and position of double bonds in the sterol nucleus.
While we have not as yet systematically investigated
such important matters as the column capacity and
detector response for each of the concerned steryl
acetates, a presentation of our results at this state
permits the very rapid ( ~3.5 hr) separation of steryl
acetates which can be achieved only in a minimum of
7–10 days using previously described methodology.[4]
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