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Note

Resolution of epimeric (25*R*)- and (25*S*)-26-hydroxycholesterol by high-pressure liquid chromatography

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We have reported previously[1,2] the synthesis of two polar metabolites of cholecalciferol, 24,25-dihydroxycholecalciferol and 25,26-dihydroxycholecalciferol. The poor stereoselectivity led to mixtures of diastereoisomers (epimers), 24*R* and 24*S* in the case of the former metabolite and 25*R* and 25*S* in the case of the latter. Recently, we have achieved[3,4] the resolution of the epimers by high-pressure liquid chromatography (HPLC) on high-surface-area silica at the intermediate stage of 24,25-dihydroxycholesterol 3,24-diacetate and 25,26-dihydroxycholesterol 3,26-diacetate, respectively. The separation was performed by following a recycling mode.

We now report a similar resolution of a mixture of (25*R*)- and (25*S*)-26-hydroxycholesterol 3β,26-diacetate by HPLC, leading to the still unknown pure (25*S*)-26-hydroxycholesterol[5].

The transformation of (25*R*)- and (25*S*)-26-hydroxycholesterol into (25*R*)- and (25*S*)-26-hydroxycholecalciferol, respectively, will be reported subsequently.

EXPERIMENTAL

Instrumental

High-pressure liquid chromatography was performed with a Waters Assoc. chromatograph equipped with a 6000A pump, a U6K injector, a refractometric detector and two columns (30 cm × 7.9 mm I.D.) packed with Microporasil. The mobile phase was *n*-hexane containing 2.5% (v/v) of ethyl acetate at a flow-rate of 2 ml/min and a pressure of 31 kg/cm^2.

NMR spectra were obtained on a Cameca 250-MHz spectrometer in 10^{-2} *M* carbon tetrachloride solution with tetramethylsilane as internal standard.

Melting points were measured in open capillary tube and are uncorrected.

Optical rotations were measured for chloroform solutions.

Cholesterol derivatives

A mixture of (25*R*)- and (25*S*)-26-hydroxycholesterol was obtained according

according to Varma *et al.*[5] by hydroboration of cholesta-5,25-dien-3β-ol tetrahydropyranyl ether with either (+)- or (—)-diisopinocampheylborane. The corresponding diol 3β,26-diacetates were prepared by acetylation with acetic anhydride and pyridine at room temperature. Saponification of diacetates after resolution was accomplished in 5% methanolic potassium hydroxide solution at ambient temperature.

RESULTS

The hydroboration of cholesta-5,25-dien-3β-ol-tetrahydropyranyl ether with either (+)- or (—)-diisopinocampheylborane[5] led to a *ca.* 1:1 mixture of (25*R*)- and (25*S*)-26-hydroxycholesterol. Varma *et al.*[5] obtained nearly pure (*ca.* 83% optical purity) 25*S* epimer with (—)-diisopinocampheylborane.

The resolution of (25*R*)- and (25*S*)-26-hydroxycholesterol 3β,26-diacetate was accomplished at the 25-mg level; ten recycles were necessary in order to achieve complete separation.

The less polar epimer

M.p. 99–100°; $\alpha_D^{25°} = -34.3°$ ($c = 1.1$). Authentic (25*S*)-26-hydroxycholesterol-3β,26-diacetate is unknown. Saponified: m.p. 173°; $\alpha_D^{25°} = -38.7°$ ($c = 1.5$). Reported for (25*S*)-26-hydroxycholesterol ("optical purity *ca.* 83%"): m.p. 171–172 (ref. 5); $\alpha_D^{25°} = -38.0 \pm 1.16°$ ($c = 1.72$)[5].

The more polar epimer

M.p. 128–129°; $\alpha_D^{20°} = -37.0°$ ($c = 0.9$). Reported for (25*R*)-26-hydroxycholesterol 3β,26-diacetate (derived from kryptogenin): m.p. 128–129° (ref. 6); $\alpha_D^{20°\cdot} = -35°$ ($c = 1$)[6]. Saponified: m.p. 172–173°; $\alpha_D^{0°\cdot} = -30.3°$ ($c = 1.2$). Reported for (25*R*)-26-hydroxycholesterol: m.p. 177–178 (ref. 6) or 172–173 (ref. 5); $\alpha_D^{20°} = -30°$ ($c = 1$)[6]; ($\alpha_D^{21°\cdot} = -33.5 \pm 1.3°$ ($c = 1.5$)[5].

We conclude that the less polar epimer is 25*S* and the more polar epimer is 25*R*.

NMR spectrometry

Mixture of (25*R*)- and (25*S*)-26-hydroxycholesterol 3β-26-diacetate: CH_3—18, 0.67 (s); CH_3 — 21 and 27, 0.92 (d); CH_3 — 19, 1.01 (s); OAc — 26, 1.96 (s); OAc — 3, 2.0 (s); H — 26, between 3.7 and 3.9 (AB part of an ABX); H — 3, 4.5 (m); H — 6, 5.35 (d) ppm.

25*R* and 25*S* diastereomers can be characterized by their CH_2 — 26 signal. The latter appears (AB part of an ABX) in the case of 25*S* as δ_A 3.86, δ_B 3.74 ppm, $J_{AX} = 5$ Hz, $J_{BX} = 7$ Hz, $J_{AB} = 11$ Hz, and in the case of 25*R* as δ_A 3.92, δ_B 3.82 ppm, $J_{AX} = 6$ Hz, $J_{BX} = 2.5$ Hz and $J_{AB} = 11$ Hz (according to a first-order analysis).

DISCUSSION

HPLC is a highly efficient method for separating a mixture of (25*R*)- and (25*S*)-26-hydroxycholesterol 3,26-diacetate and provides pure epimers, as confirmed by NMR spectrometry. The melting point and optical rotation of the more polar epimer are in good agreement with (25*R*)-26-hydroxycholesterol 3,26-diacetate derived

from kryptogenin[5,6] and this identity is confirmed after saponification to (25*R*)-26-hydroxycholesterol[5,6].

The less polar epimer, (25*S*)-26-hydroxycholesterol 3,26-diacetate, never prepared before by chemical methods, was converted by saponification into (25*S*)-26-hydroxycholesterol with a melting point and optical rotation similar to those of the material of 83% optical purity prepared by Varma *et al.*[5],

Schubert *et al.*[7] reduced 26-hydroxycholest-4-en-3-one, obtained by microbial oxidation of cholesterol, to 26-hydroxycholesterol (m.p. 171–173°) and its 3,26-diacetate (m.p. 119–123°). X-ray studies[8] have shown this hydroxyenone to be of 25*S* configuration. The melting point of the latter diacetate is different from the m.p. (99–100°) found for our (25*S*)-26-hydroxycholesterol-3,26-diacetate. This disagreement is at present under investigation.

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