notes on methodology

Sterol synthesis. Chemical synthesis of 5α-cholest-7-en-3β,14α-diol

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Summary Reported herein is the chemical synthesis of 5α-cholest-7-en-3β,14α-diol by mild Wolff-Kishner reduction of 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7-one. The preparation of 5α-cholest-7-en-14α-ol-3-one from 5α-cholest-7-en-3β,14α-diol by the use of cholesterol oxidase is also described. These compounds were fully characterized by the results of infrared, nuclear magnetic resonance, and high and low resolution mass spectral studies.—**Pascal, R. A., Jr. and G. J. Schroepfer, Jr.** Sterol synthesis. Chemical synthesis of 5α-cholest-7-en-3β,14α-diol. *J. Lipid Res.* 1980. **21:** 118–122.

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As a part of a program of research designed to explore the mechanisms involved in the enzymatic removal of carbon atom 32 of 14α-methyl substituted sterol precursors of cholesterol we sought the preparation of the previously undescribed 5α-cholest-7-en-3β,14α-diol. The availability of this sterol would provide a key intermediate for the chemical synthesis of 14α-formoyloxy sterols. The latter type of sterol has been proposed as a potential intermediate in the overall enzymatic removal of carbon atom 32 of 14α-methyl substituted sterol precursors of cholesterol (1, 2).[3] We also sought the preparation of 5α-cholest-7-en-3β,14α-diol for investigation of its possible inhibitory action on sterol biosynthesis and for investigation of its possible intermediary role in the formation of α-ecdysone (2β,3β,14α,22R,25-pentahydroxy-5β-cholest-7-en-6-one) or its analogs from cholesterol.

The purpose of this paper is to describe a simple, one-step, high yield synthesis of 5α-cholest-7-en-3β,14α-diol from the known compound 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7-one (**Fig. 1**). 5α-Cholest-7-en-14α-ol-3-one was also prepared from the former compound through the action of cholesterol oxidase.

EXPERIMENTAL PROCEDURES AND RESULTS

General procedures

The recording of melting points and IR spectra were performed as described previously (5). NMR spectra were determined on $CDCl_3$ solutions of the sterols at 90 MHz on a Varian EM-390 spectrometer using tetramethylsilane (TMS) as an internal standard. Peaks are reported as ppm (δ) downfield from the TMS standard. Low resolution MS analyses were recorded using an LKB-9000S spectrometer under operating conditions described previously (6). High resolution mass spectral analyses were made on a Varian CH-5 spectrometer (courtesy of Professor C. C. Sweeley). TLC was performed on precoated silica gel G plates (Analtech, Newark, DE) and components on the plates were detected as described previously (7). GLC analyses were made using a Hewlett-Packard Model 402 unit using either 3% QF-1 or 3% OV-17 on Gas Chrom Q (100–120 mesh) columns under conditions described previously (8). The preparation of trimethylsilyl derivatives was carried out as described previously (9).

Materials

3β-Acetoxy-5α-cholest-7-ene (I; mp 116–117°C; purity in excess of 96% on the basis of GLC analyses on a 3% QF-1 column) was prepared from 5α-cholest-7-en-3β-ol by treatment with acetic anhydride in pyridine. The latter sterol was prepared by hydrogenation of 7-dehydrocholesterol over a Raney nickel catalyst (10). An authentic sample of 5α-cholesta-7,14-dien-3β-ol was prepared as described previously (11).

3β-Acetoxy-8α,14α-epoxy-5α-cholestan-7α-ol (II)

Compound II was prepared by the following modification of the method of Fieser, Nakanishi, and Huang (12). To *m*-chloroperbenzoic acid (6.4 g; 31.3 mmol peracid) in dry acidified $CHCl_3$ (dried over anhydrous $MgSO_4$) was added compound I (6.2 g; 14.5 mmol). The resulting solution was kept in the dark at 6°C for 12 days. Ether (500 ml) was added and the resulting mixture was washed twice with a

Abbreviations: IR, infrared; NMR, nuclear magnetic resonance; MS, mass spectra; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; MPLC, medium-pressure liquid chromatography.

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[3] The same possibility was suggested by one of us at two symposia lectures (3, 4).

saturated solution of Na_2CO_3 and once with water. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was evaporated under reduced pressure. The resulting white residue, which showed numerous components on TLC (solvent, 20% ether in benzene), was subjected to chromatography on a silica gel column (125 g; 100 cm × 2 cm; 60–200 mesh; Baker) using benzene to apply the sample and 10% ether in benzene as the eluting solvent. Fractions 24 ml in volume were collected. The contents of fractions 19 through 40 were pooled and, after evaporation of the solvent under reduced pressure, subjected to MPLC (80 psi) on an activated silica gel column (100 cm × 2.5 cm; 0.032–0.063 mm; Woelm). The sample was applied using 5% ether in benzene (23 ml) and the column was eluted with the same solvent mixture. After 350 ml had been eluted from the column, fractions 20 ml in volume were collected (flow rate, 10 ml per min). At fractions 130 and 150 the eluting solvent was changed to 10% ether in benzene and 30% ether in benzene, respectively. The contents of fractions 17 through 50 were pooled to give essentially pure II. The contents of fractions 51 through 185 (containing a mixture of the 8α, 14α- and 8α,9α-epoxy isomers) were pooled and rechromatographed on a MPLC column in a similar fashion to give additional pure 8α,14α-epoxysteryl ester. The combined purified product was crystallized from chloroform–methanol to give compound II (2.6 g; 39% yield) melting at 123–124°C (literature: 122–123°C (12, 13)); $[\alpha]_D$ + 3.6° (c., 1.77) (literature: +5.5° (12) and +6.1° (13)); IR, 3562, 2945, 1730, 1470, 1372, 1243, 1034, and 904 cm^{-1}; NMR, 0.91 (s, 3H, C-18 or C-19 CH_3), 0.93 (s, 3H, C-18 or C-19 CH_3), 1.98 (s, 3H, methyl of acetoxy function), 2.38 (br s, 1H, 7α-OH), 3.53 (m, 1H, C-7β-H), 4.70 (m, 1H, C-3α-H); MS, 460 (6%; M), 445 (5%; M-CH_3), 442 (23%: M-H_2O), 427 (12%; M-CH_3-H_2O), 426 (14%), 349 (11%), 329 (100%; M-H_2O-side chain), 313 (16%), 311 (21%), 306 (18%), 251 (13%), 250 (13%), 249 (33%), and 236 (21%). The product showed a single component on TLC (solvent, 20% ether in benzene; R_f 0.39). The corresponding 8α,9α-epoxy isomer has an R_f of 0.36 in the same system.

3β-Acetoxy-8α,14α-epoxy-5α-cholestan-7-one (III)

Compound III was prepared from the corresponding 7α-hydroxy compound by treatment with chromium trioxide in acetic acid by the following modification of the procedure of Fieser et al. (12). To compound II (2.38 g; 5.17 mmol) in acetic acid (50 ml) was added a solution of chromium trioxide (0.73 g; 7.3 mmol) in 80% acetic acid (63 ml). After 23 hr at room temperature, water (500 ml) was added and the resulting mixture was extracted three times with ether (300 ml portions). The combined extracts were washed twice with a saturated solution of Na_2CO_3 and once with water and dried over anhydrous $MgSO_4$. The residue obtained upon evaporation of the solvent under reduced pressure was subjected to chromatography on a silica gel column (40 g; 45 cm × 1.3 cm; 60–200 mesh; Baker) using benzene to apply the sample to the column and 10% ether in benzene as the eluting solvent. Fractions 20 ml in volume were collected. The contents of fractions 5 through 9 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from chloroform–methanol to give compound III (2.02 g; 85% yield) melting at 141.5–143.0°C (literature: 139–140°C (12) and 139.5–140.0°C (13)); $[\alpha]_D$ −75.6° (c., 0.49) (literature: −73.5° (12) and −75.7° (13)); IR, 2945, 1724, 1715, 1470, 1368, 1248, 1183, 1026, and 900 cm^{-1}; NMR, 0.94 (s, 3H, C-18-CH_3), 1.10 (s, 3H, C-19-CH_3), 2.00 (s, 3H, methyl of acetoxy function), 2.30 (m, 2H, C-6-H_2), 4.70 (m, 1H, C-3α-H); MS, 458 (12%; M), 443 (17%; M-CH_3), 442 (25%), 440 (14%; M-H_2O), 346 (12%), 345 (48%; M-side chain), 327 (100%; M-H_2O-side chain), 317 (20%), 277 (27%), 276 (18%), 267 (17%), 252 (19%), 239 (18%), 220 (19%), 219 (23%), and 207 (38%). The product showed a single component on TLC (solvent, 20% ether in benzene; R_f 0.49).

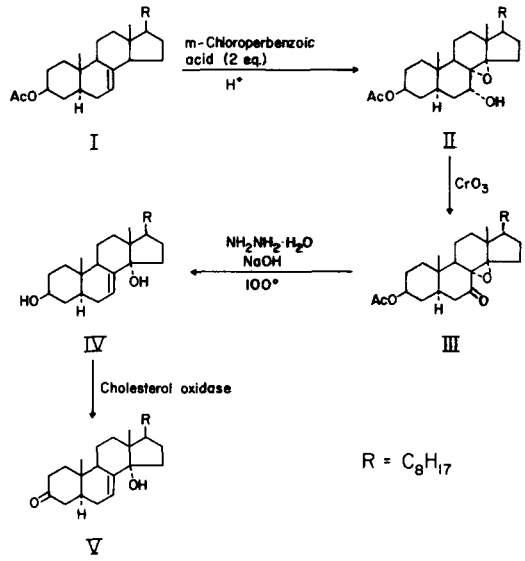

Fig. 1. Synthetic scheme for the synthesis of 14α-hydroxy sterols. (I, 3β-acetoxy-5α-cholest-7-ene; II, 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7α-ol; III, 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7-one; IV, 5α-cholest-7-en-3β,14α-diol; V, 5α-cholest-7-en-14α-ol-3-one).

5α-Cholest-7-en-3β,14α-diol (IV)

Compound III (1.85 g; 4.05 mmol), sodium hydroxide (6.0 g), and hydrazine hydrate (60 ml) were

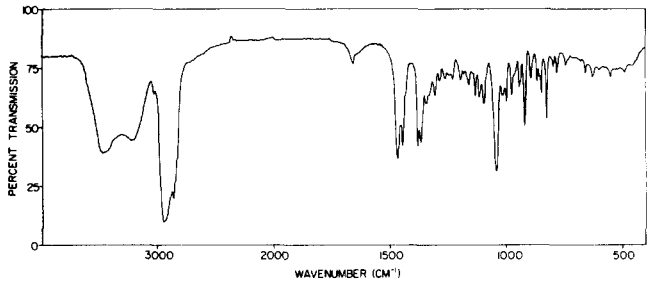

Fig. 2. Infrared spectrum of 5α-cholest-7-en-3β,14α-diol (IV).

heated under reflux for 15 min. At this point vigorous foaming began and heating of the mixture was terminated. After stirring for 2 hr, the mixture was heated, with stirring, at 80°C for 2 hr and finally for 2 hr under reflux. After cooling to room temperature, water (500 ml) was added and the resulting mixture was extracted three times with ether (200 ml portions). The combined extracts were washed twice with a saturated NaCl solution, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. The resulting yellow crystalline residue was subjected to chromatography on a column (45 cm × 2.0 cm) of basic alumina AG-10 (150 g; 100–200 mesh; Bio-Rad; packed as a slurry in benzene) using a 1:1 mixture of ether and benzene to apply the sample to the column and as the eluting solvent. Fractions 20 ml in volume were collected. The contents of fractions 12 through 18 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from acetone to give compound IV (1.16 g; 72% yield) which showed unusual melting point behavior, i.e., 109°C, crystals began to bend and twist; 133–134°C, melted to give a cloudy liquid; and 138–139°C, clearing; IR (**Fig. 2**), 3480, 3225, 2960, 1470, 1382, 1045, 920, and 826 cm^{-1}; NMR (**Fig. 3**), 0.65 (s, 3H, C-18-CH_3), 0.78 (s, 3H, C-19-CH_3), 3.57 (m, 1H, C-3α-H), 5.50 (m, 1H, C-7-H); MS (**Fig. 4**), 402 (4%, M), 384 (85%, M-H_2O), 369 (25%; M-H_2O-CH_3), 271 (100%; M-H_2O-side chain), 257 (29%), and 253 (19%); high resolution MS, 402.3483 (calculated for $C_{27}H_{46}O_2$: 402.3498). The product showed a single component on TLC (solvent, 40% ether in benzene; R_f 0.18).

To compound IV (~0.5 mg) in hexane (5 ml) was added 9N H_2SO_4 (3 ml). After thorough shaking for 30 sec the hexane layer was removed and immediately dried over anhydrous $MgSO_4$ and Na_2CO_3. The product showed a λ_{max} at 242 nm (ϵ = 10,200) [literature: 242 nm, ϵ = 9,870 (11), 242 nm, ϵ = 9,700 (14) 242 nm, ϵ = 9,440 (15)]. The trimethylsilyl ether of the product showed one major component (93.5%) on GLC (3% OV-17; 260°C) with the same retention time as that of the trimethylsilyl derivative of authentic 5α-cholesta-7,14-dien-3β-ol.

5α-cholest-7-en-14α-ol-3-one (V)

To component 1 (200 ml) of the BioDynamics (BMC Division) cholesterol Auto Test was added cholesterol oxidase (6.4 ml; component 3 of the cholesterol Auto Test) and the resulting mixture was diluted with water (200 ml). Compound IV (82.5 mg; 0.205 mmol) in isopropanol (20 ml) was added and the resulting mixture was stirred at room temperature for 6 hr. The mixture was extracted three times with ether (300 ml portions) and the combined extracts were dried over anhydrous $MgSO_4$. The yellowish oil, obtained upon evaporation of the solvent under reduced pressure, was subjected to chromatography on a basic alumina (100–200 mesh; Bio-Rad) column (45 cm × 1.3 cm; packed as a slurry in benzene) using 30% ether in benzene to apply the sample to the column and as the eluting solvent. Fractions ~13 ml in volume were collected. The contents of fractions 18 through

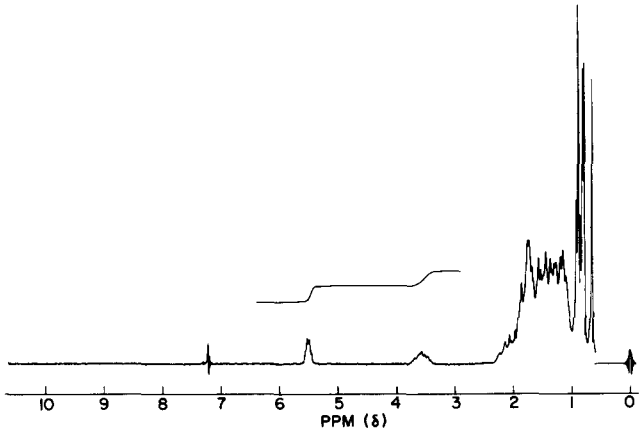

Fig. 3. Nuclear magnetic resonance spectrum of 5α-cholest-7-en-3β,14α-diol (IV).

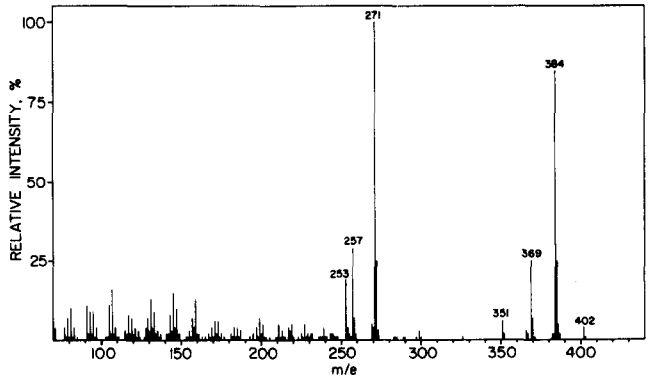

Fig. 4. Low resolution mass spectrum of 5α-cholest-7-en-3β,14α-diol (IV).

33 were pooled. The crystalline residue, obtained upon evaporation of the solvent, showed three components on TLC (solvent, 50% ether in hexane). Accordingly, the material was subjected to MPLC (40 psi) on a column (100 cm × 1.5 cm) of basic alumina (100–200 mesh; Bio-Rad) which had been wet with benzene and subsequently equilibrated with 30% ether in benzene. The crude sterol was applied to the column in the same solvent mixture (8 ml) which was also used as the eluting solvent. Fractions 20 ml in volume were collected (flow rate, 5 ml per min). The contents of fractions 28 through 65 were pooled and the residue obtained upon evaporation of the solvent was recrystallized from acetone to yield compound V (28.6 mg; 35% yield) which showed the following melting point behavior: 164°C, crystals bend and soften; 167–168°C, melt; IR, 3530, 2956, 1716 (ketone in 6-membered ring), 1441, 1385, and 1246 cm^{-1}; NMR, 0.68 (s, 3H, C-18-CH_3), 1.01 (s, 3H, C-19-CH_3), 2.22 (m, 4H, C-2-H_2 and C-4-H_2), 5.51 (m, 1H, C-7-H); MS, 400 (2%; M), 382 (48%; M-H_2O), 367 (13%; M-H_2O-CH_3), 269 (100%; M-H_2O-side chain), 255 (27%), and 145 (12%); high resolution MS, 400.3342 (calculated for $C_{27}H_{44}O_2$: 400.3341). The product showed a single component on TLC (solvent, 50% ether in hexane; R_f 0.24).

DISCUSSION

Described herein are chemical procedures which effect the net allylic hydroxylation of 5α-cholest-7-en-3β-ol at the 14α-position. While more direct methods for this purpose would be desirable, the extreme acid lability of the 14α-hydroxy-Δ^7-ene system precludes their use. For example, Fieser and Ourisson (14) have suggested that the oxidation of Δ^7-cholestenyl acetate by selenium dioxide in acetic acid proceeds via an initial allylic hydroxylation at C-14. However, the formation of this initial product appears to be followed by an allylic rearrangement and acetylation to yield the observed product, 3β,7α-diacetoxy-5α-cholest-8(14)-ene.

Wolff-Kishner reduction of α,β-epoxy ketones to give allylic alcohols was first described by Wharton and Bohlen (15). The high yields of this reaction and the stability of the resulting allylic alcohols to the basic conditions of the reaction provided very favorable features for the synthesis of the desired tertiary allylic alcohol, 5α-cholest-7-en-3β,14α-diol (IV), as did the fact that the required precursor α,β-epoxy ketone, 3β-acetoxy-8α,14α-epoxy-5α-cholestan-7-one (III), was a known compound. This reaction is a variation of the Kishner reduction–elimination of α-substituted ketones. Leonard and Gelfand (16) have concluded that the most probable mechanism of such reactions involves the initial formation of the hydrazone, isomerization of the hydrazone to a diimine intermediate, and decomposition of the diimine in a four-centered elimination reaction to yield an olefin. In the case of α,β-epoxy ketones, the elimination reaction results in the opening of the epoxide to give an allylic alcohol.

In initial attempts at the synthesis of IV by this approach, relatively impure compound III, contaminated with the corresponding 8α,9α-epoxy isomer, was employed. However, the resulting mixture of products, 5α-cholest-7-en-3β,14α-diol and 5α-cholest-7-en-3β,9α-diol, proved impossible to resolve by chromatography. It was therefore necessary to obtain compound III in pure form. 3β-Acetoxy-8α,14α-epoxy-5α-cholestan-7α-ol (II) was prepared by a modification of the procedure of Fieser et al. (12) with careful purification by MPLC to remove the contaminating 8α,9α-epoxy isomer. The pure product was characterized by melting point and optical rotation and by the results of IR, NMR, and MS studies. Oxidation of II to the desired α,β-epoxy ketone III was carried out by a modification of the procedure of Fieser et al. (12) and full characterization of the carefully purified product was made.

Mild Wolff-Kishner reduction of compound III gave the desired diol IV in high (72%) yield. The new compound was characterized by its melting point behavior and by the results of IR, NMR, and high and low resolution MS studies. The presence of an olefinic proton resonance at 5.50 ppm was compatible with the presence of a Δ^7-double bond. The high abundances of ions corresponding to M-H_2O and M-H_2O-side chain in the MS of compound IV were compatible with the presence of a tertiary allylic alcohol. In addition, treatment of IV with acid under mild conditions gave 5α-cholesta-7,14-dien-3β-ol as judged by its characteristic absorbance in the ultraviolet and by the chromatographic behavior of its trimethylsilyl ether derivative.

Oxidation of compound IV with cholesterol oxidase gave 5α-cholest-7-en-14α-ol-3-one (V) which was fully characterized. The results of NMR and MS studies were compatible with the presence of a tertiary allylic alcohol. The presence of a ketone at position 3 was indicated by the absorption, in its IR spectrum, at 1716 cm^{-1} due to a ketone in a 6-membered ring, by the appearance of a resonance at 2.22 ppm in the NMR spectrum of compound V, and by the disappearance of the C-3α-H resonance at 3.57 ppm in the NMR spectrum of compound IV. In addition, the C-18 and C-19 methyl resonances in the NMR

spectrum of compound V were shifted 0.03 ppm and 0.23 ppm, respectively, from the positions of the corresponding methyl resonances in the NMR spectrum of compound IV. These latter findings are also indicative of the location of the ketone function in compound V at C-3.

It is important to note that basic alumina is best employed for the chromatographic purification of compounds IV and V since some dehydration and rearrangement of these compounds take place during silica gel column chromatography.

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