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STEROL SYNTHESIS. CHEMICAL SYNTHESIS OF 3β-BENZOYLOXY -14α, 15α-EPOXY-5α-CHOLEST-7-ENE, A KEY INTERMEDIATE IN THE SYNTHESIS OF 15-OXYGENATED STEROLS *

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3β-Benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene was obtained in 96% yield upon treatment of 3β-benzoyloxy-5α-cholesta-7, 14-diene with *m*-chloroperbenzoic acid. The Δ^7-14α, 15α-epoxy-steryl ester provides a useful intermediate for the syntheses of sterols with an oxygen function at carbon atom 15. For example, treatment of 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene with methanolic hydrochloric acid gave 3β-benzoyloxy-5α-cholest-8(14)-en-15-one in 82% yield.

I. Introduction

The biological formation of 15-oxygenated steroids has been reported [1–13]. Moreover, 15-hydroxylated derivatives of sterols have been suggested as potential intermediates in the biosynthesis of cholesterol [14–21] and several 15-hydroxy sterols have been shown to be efficiently converted to cholesterol upon incubation with rat liver homogenate preparations [15–18, 22]. A number of 15-oxygenated sterols have recently been shown to be extremely potent inhibitors of sterol biosynthesis in L-cells and mouse liver cells in culture [23]. For example, 5α-cholest-8(14)-en-3β-ol-15-one causes a 50% inhibition of sterol synthesis from [1-^{14}C] acetate in L-cells at a concentration of 10^{-7} M and a 50% reduction in the activity of HMG-CoA reductase at a concentration of 3×10^{-7}M [23].

A classic approach for the introduction of a 15-keto function in a sterol was developed by Barton and his associates [24, 25] and involves treatment of a $\Delta^{7,14}$-diene with a peracid followed by acid treatment of the resulting reaction mixture. This approach was utilized in the total synthesis of lanosterol [26] and in the synthesis of 14α-methyl-5α-cholest-7-en-3β-ol [27]. While Barton and Laws [25] postulated the formation of a $\Delta^{7,22}$-14,15-epoxide as a product of the action of

* A preliminary account of this work has been presented (E.J. Parish and G.J. Schroepfer, Jr. Cholesterol Biosynthesis. A New Chemical Synthesis of 5α-Cholest-8(14)-en-3β, 15α-diol, Abstracts, 27th Southeast-31st Southwest Regional A.C.S. Meeting, A194, 1975).

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perphthalic acid upon 3β-acetoxy-ergosta-7,14,22-triene, in no case has the potential epoxide intermediate been isolated and characterized.

The purpose of the present communication is to describe the chemical synthesis of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene and the transformation of this compound to 3β-benzoyloxy-5α-cholest-8(14)-en-15-one upon treatment with acid.

II. Experimental

A. General

Melting points were recorded on a Thomas Hoover melting point apparatus and are uncorrected. Analytical thin-layer chromatographic (TLC) analyses were performed using 250 μm layers of silica gel G (Merck, Darmstadt) using benzene or 35% ethyl acetate in chloroform as the developing solvents. Silica gel PF–silver nitrate (10% silver nitrate) plates were 250 μm in thickness and were developed with benzene or a mixture of chloroform and acetone (95 : 5). Components on the plate were visualized after spraying with molybdic acid [28] followed by brief heating at 80°C. Gas–liquid chromatographic (GLC) analyses were performed using a Hewlett–Packard Model 402 instrument equipped with dual flame ionization detectors. Three columns (6 ft X 0.25 in., o.d.) were used: SE-30 (1%), OV-1 (3%), and OV-17 (3%) on Gas-Chrom Q (100/120 mesh). Helium (66 ml/min) was employed as the carrier gas and the column temperature was maintained at 240–280°C depending on the type of column in use. Mass spectral (MS) analyses were made on a CEC Model 21-110B spectrometer using a perfluorokerosene internal mass marker. High resolution mass spectral measurements were made using peak matching techniques. Nuclear magnetic resonance (NMR) spectra were determined in $CDCl_3$ solution on a Perkin–Elmer HR-12 spectrometer. Tetramethyl silane (TMS) was used as an internal standard. Peaks are reported as ppm (δ) downfield from the TMS standard. Infrared (IR) spectra were recorded on a Beckman IR-9 spectrometer using KBr pellets. Optical rotations were recorded using a Rudolph polarimeter with approximately 1% solutions of the sterols in chloroform. Ultraviolet (UV) spectra were recorded using ethanol solutions of the sterols. Cholesta-5,7-dien-3β-ol was purchased from Sigma Chemical Company (St. Louis, Mo.). 3β-Acetoxy-5α-cholesta-7,14-diene (m.p. 85–86°C [literature: 84–85°C, 84.0–85.5°C and 86–87°C [29]]; IR: 3380, 1641 cm^{-1}; NMR: 1.2 (m, methylene envelope), 2.03 (s, 3H, C-3-acetate), 4.70 (m, 1H, C-3-H), 5.56 (m, 1H, C-15-H), 5.81 (m, 1H, C-7-H); single component on analyses by TLC (silica gel G and silica gel PF–silver nitrate) and by GLC) was prepared by pyrolysis of 3β, 7α-diacetoxy-5α-cholest-8(14)-ene as described previously [29]. 3β, 7α-Diacetoxy-5α-cholest-8(14)-ene (m.p. 140.0–141.5°C [literature: 137.0–138.5°C [29]]; IR: ν_{max} 1728 cm^{-1}; NMR: 1.2 (m, methylene envelope), 2.03 (s, 6H, diacetoxy), 4.78 (m, 1H, C-3-H), 5.64 (m, 1H, C-7-H); single component on analyses by TLC (silica gel G and silica gel PF–silver nitrate)) was

prepared from 3β-acetoxy-5α-cholest-7-ene as described previously [29]. 3β-Acetoxy-5α-cholest-7-ene (m.p. 117.5–119.5°C [literature: 118–119° [30]]; single component on analyses by TLC (silica gel G and silica gel PF–silver nitrate) and by GLC) was prepared from 5α-cholest-7-en-3β-ol by treatment with acetic anhydride in pyridine. 5α-Cholest-7-en-3β-ol (m.p. 122.0–123.5°C [literature: 122.0–122.5°C [30], 121.5–122.0°C [31]]; single component on analyses by TLC (silica gel G and silica gel PF–silver nitrate) and GLC) was prepared by catalytic hydrogenation (Raney nickel) of cholesta-5,7-dien-3β-ol.

B. 5α-Cholesta-7,14-dien-3β-ol

3β-Acetoxy-5α-cholesta-7,14-diene (3.5 g; 8.2 mmol) was dissolved in anhydrous ether (250 ml) and lithium aluminium hydride (5.0 g) was slowly added. After stirring at room temperature for 2 hr, the mixture was cooled to 0°C and ice was cautiously added to destroy the unreacted hydride. The mixture was poured into a 2% aqueous sodium chloride solution and thoroughly extracted with ether containing methylene chloride (5%). The combined extracts were dried over anhydrous magnesium sulfate and the solvent was evaporated under reduced pressure to yield a white residue (2.8 g) which was recrystallized from methanol to give 5α-cholesta-7,14-dien-3β-ol (2.7 g; 85%) melting at 104.0–105.5°C [literature: 104–105°C [29]]; IR: ν_{max} 3380 and 1641 cm^{-1}; NMR: 1.20 (m, methylene envelope), 3.65 (m, 1H, C-3-H), 5.59 (m, 1H, C-15-H), and 5.85 (m, 1H, C-7-H); MS: 384 (M; 100%), 369 (M–CH_3; 10%), 366 (M–H_2O; 2%), 271 (M–side chain; 39%), 257 (10%), and 253 (M–H_2O–side chain; 2%); high resolution MS: 384.3387 (calc. for $C_{27}H_{44}O$: 384.3392). The compound showed a single component on TLC analyses on silica gel PF–silver nitrate plates (solvent system: 5% acetone in chloroform) and on GLC analyses on the three systems noted above.

C. 3β-Benzoyloxy-5α-cholesta-7,14-diene

5α-Cholesta-7,14-dien-3β-ol (1.5 g; 3.9 mmol) was dissolved in a mixture of pyridine (20 ml) and benzoyl chloride (15 ml) and heated on a steam bath for 2 hr. The solution was poured into ice (200 g) and, after standing for 4 hr, the separated product was collected by filtration and recrystallized from acetone–water and chloroform–methanol to yield 3β-benzoyloxy-5α-cholesta-7,14-diene (1.4 g) melting at 154.0–154.5°C [literature: 152°C [29], 151–152° [32]]; IR: ν_{max} 1724, 1688, 1605, 1588, 1281, and 717 cm^{-1}; MS: 488 (M; 40%), 473 (M–CH_3; 6%), 375 (M–side chain; 83%), 366 (M–benzoic acid; 16%), 361 (37%), 351 (21%), 253 (M–side chain–benzoic acid; 100%); high resolution MS: 488.3589 calc. for $C_{34}H_{48}O_2$: 488.3609); NMR: 1.30 (m, methylene envelope), 4.95 (m, 1H, C-3-H), 5.58 (m, 1H, C-15-H), 5.84 (m, 1H, C-7-H), 7.75 (m, 5H, aromatic). The compound showed a single component on TLC analysis on silica gel G plates (solvent systems, 35% ethylacetate in chloroform and 10% ether in benzene) and on silica gel GF–silver nitrate plates (solvent system, benzene).

D. 3β-Benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene

3β-Benzoyloxy-5α-cholesta-7,14-diene (0.75 g; 1.48 mmol) was dissolved in anhydrous ether (30 ml) and, after cooling to 18°C, *m*-chloroperbenzoic acid (0.64 g) in anhydrous ether (5 ml) was added. The stirred solution was maintained at 0°C for 5 hr and then placed in a freezer at −15°C for 24 hr. The product was collected by filtration and recrystallized from acetone–water to yield 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene (0.72 g; 96% yield) melting at 200–202°C; IR: 1718, 1604, 1586, and 1281 cm^{-1}; MS *: 504 (M; 8%), 489 (M–CH_3; 3%), 386 (M–H_2O; 7%), 391 (M–side chain; 3%), 382 (M–benzoic acid; 5%), 367 (M–benzoic acid–CH_3; 45%), 364 (M–benzoic acid–H_2O; 25%), 349 (M–benzoic acid–CH_3–H_2O; 39%), 337 (12%), 269 (12%), and 251 (44%); high resolution MS: 504.3614 (calc. for $C_{34}H_{48}O_3$: 504.3603); $[\alpha]_D^{24}$ − 24.8°; NMR: 1.20 (m, methylene envelope), 3.69 (s, 1H, C-15-H), 4.95 (m, 1H, C-3-H), 5.64 (s, 1H, C-7-H), and 7.75 (m, 5H, aromatic). The compound showed a single component on TLC analyses on silica gel G plates (solvent systems, 35% ethyl acetate in chloroform and 10% ether in benzene).

E. Large-scale preparation of 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene

While the solid establishment of the reaction detailed above required the availability of pure 3β-benzoyloxy-5α-cholesta-7,14-diene, we explored an alternative method for the preparation of 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene on a large scale. The preparation of large amounts of pure 3β-benzoyloxy-5α-cholesta-7,14-diene via the reactions outlined above requires 5 separate reactions, one of which (the pyrolysis of the diester) requires the use of very pure material. Treatment of 3β-benzoyloxy-cholesta-5,7-diene with HCl gas in chloroform according to Knight et al. [27] gave a product (70% yield) which melted at 148–150°C and contained a mixture of the $\Delta^{7,14}$ and $\Delta^{8,14}$ isomers. Analysis by NMR (C-7 vinylic proton present in the $\Delta^{7,14}$ isomer but not in the $\Delta^{8,14}$ isomer) indicated the sample to contain approx. 73% 3β-benzoyloxy-5α-cholesta-7,14-diene. This material (75 g; 148 mmol) was dissolved in anhydrous ether (3000 ml) with gentle warming on a steam bath. The solution was placed in an ice bath and cooled to 18°C at which time a solution of *m*-chloroperbenzoic acid (63.6 g) in ether (400 ml) was added. The stirred mixture was allowed to stand at 0° for 5 hr and then at −15°C for 24 hr. The material which precipitated was collected on a filter, washed with cold ether, and recrystallized from acetone–water to give 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene (40.2 g; 52% yield) melting at 200–201°C; IR: ν_{max} 1718, 1604, 1586, 1281 cm^{-1}; MS: 504 (M; 100%), 489 (M–CH_3; 8%), 391 (M–side chain;

* This mass spectrum was recorded on a Finnegan Model 3300 spectrometer.

6%), 382 (M–benzoic acid; 10%), 367 (M–CH_3-benzoic acid; 8%) *; high resolution MS: 504.3613 (calc. for $C_{34}H_{48}O_3$: 504.3603); NMR: 1.20 (m, methylene envelope), 3.69 (s, 1H, C-15-H), 4.95 (m, 1H, C-3-H), 5.64 (s, 1H, C-7-H), 7.75 (m, 5H, aromatic); $[\alpha]_D^{24} - 24.3°$. The compound showed a single component on TLC analyses on silica gel G plates (solvent systems, 35% ethyl acetate in chloroform and 10% ether in benzene).

F. Conversion of 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene to 3β-benzoyloxy-5α-cholest-8(14)-en-15-one

3β-Benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene (5.0 g; 9.91 mmol) was heated under reflux for 15 min with a solution consisting of chloroform (100 ml), methanol (300 ml), and concentrated hydrochloric acid (20 ml). The solution was concentrated to half its volume under reduced pressure, diluted with water, and extracted thoroughly with ether containing methylene chloride (5%). The combined extracts were washed successively with an aqueous $NaHCO_3$ solution and water, dried over anhydrous magnesium sulfate, and evaporated to dryness under reduced pressure to yield a pale yellow oil which solidified on standing. The residue was subjected to chromatography on a silica gel column (375 g; 100 cm X 2.0 cm; 60–200 mesh; Baker Chemical Company) using benzene as the eluting (flow rate 1.5 ml per min) solvent. Fractions 24 ml in volume were collected. The contents of fractions 1–25 were pooled, and yielded, upon evaporation of the solvent, a yellow solid (0.84 g) which was found to be a mixture of relatively non-polar compounds on TLC analysis on a silica gel PF–silver nitrate plate (solvent system, benzene). The contents of fractions 30–60 were pooled and gave, upon evaporation of the solvent and recrystallization from chloroform–methanol, 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (4.08 g; 82% yield) melting at 157–158°C [literature: 157–158°C [16], 156°C [26], 156–158°C [26]]; IR: ν_{max} 1710, 1612, 1580, 710 cm^{-1}; UV: λ_{max} 258 nm (ϵ = 14,500) and 232 nm (ϵ = 15,300); NMR: 1.2 (m, methylene envelope), 4.22 (m, 1H, C-7β-H), 5.01 (m, 1H, C-3-H); MS: 504 (M; 69%). The compound showed a single component on analyses by TLC on silica gel G plates (solvent systems, 10% ether in benzene and 35% ethyl acetate in chloroform).

III. Discussion

The results described above demonstrate the formation, in high yield (96%), of 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene upon treatment of 3β-benzoyloxy-5α-cholesta-7,14-diene with *m*-chloroperbenzoic acid. The NMR, IR, and low and high resolution mass spectra were fully compatible with the assigned structure. Un-

* The mass spectrum of this sample was identical in all respects to the other preparation of the epoxy-steryl benzoate when recorded on the Finnegan spectrometer.

equivocal determination of the configuration and location of the epoxide function (and the complete structure of the compound) was established by X-ray structural analysis of 3β-*p*-bromobenzoyloxy-14α, 15α-epoxy-5-cholest-7-ene which was prepared by the action of *m*-chloroperbenzoic acid upon 3β-*p*-bromobenzoyloxy-5α-cholesta-7,14-diene [33].

Conditions are also described for the efficient preparation of 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene on a large scale, a point of considerable importance since the compound in question is an extremely useful starting material for the preparation of various 15-oxygenated sterols * (vide infra; [34–36]).

Treatment of 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene with methanolic hydrochloric acid gave 3β-benzoyloxy-5α-cholest-8(14)-en-15-one in high (82%) yield. The latter compound had the identical melting point as the product previously described [16, 26] from methanolic hydrochloric acid treatment of the reaction mixture obtained by the action of peracids on 3β-benzoyloxy-5α-cholesta-7,14-dien-3β-ol. In addition, the UV, IR, NMR, and low and high resolution mass spectra were fully compatible with the assigned structure.

The high yields obtained upon formation of the 3β-benzoyloxy-14α, 15α-epoxy-5α-cholest-7-ene and its conversion to the $\Delta^{8(14)}$-15-keto steryl ester strongly suggest that the Δ^{7}-14α, 15α-epoxy steryl ester is an intermediate in the previously described overall conversion of $\Delta^{7,14}$-dienes to the corresponding $\Delta^{8(14)}$-15-ketones [15, 16, 24–27].

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