15-Oxygenated Sterols by *m*-Chloroperbenzoic Acid Oxidation of 3β-Acetoxy-5α-cholesta-8,14-diene

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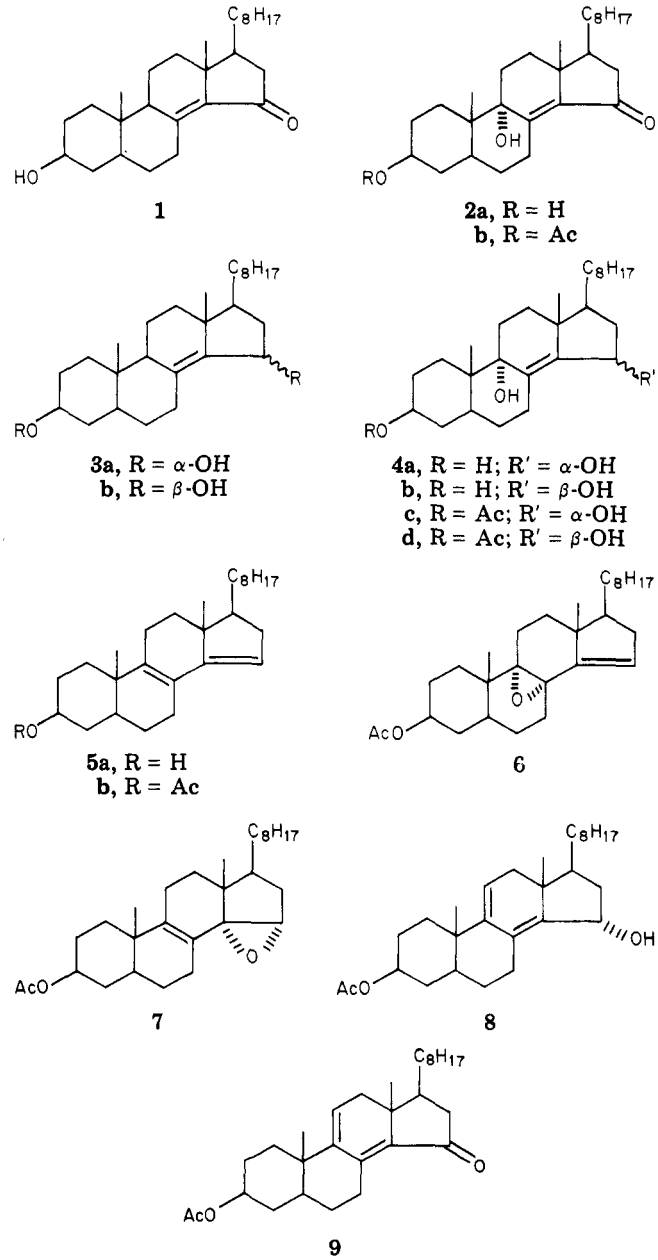
The epoxidation of 3β-acetoxy-5α-cholesta-8,14-diene with 1 molar equiv of *m*-chloroperbenzoic acid has been found useful for the synthesis of 15-oxygenated sterols. The major product of the reaction, 3β-acetoxy-14α,15α-epoxy-5α-cholest-8-ene, is decomposed on silica to a (1:1) mixture of 3β-acetoxy-5α-cholesta-8(14),9-(11)-dien-15α-ol and 3β-acetoxy-5α-cholest-8(14)-ene-9α,15α-diol. The minor product obtained in the epoxidation is 3β-acetoxy-8α,9α-epoxy-5α-cholest-14-ene.

A number of oxygenated sterols act as inhibitors of cholesterol synthesis in a wide variety of cell systems.[1] Particularly noteworthy are 15-oxygenated sterols such as 3β-hydroxy-5α-cholest-8(14)-en-15-one (**1**)[2] and 3β,9α-dihydroxy-5α-cholest-8(14)-en-15-one (**2a**)[3] and the epimeric compounds 5α-cholest-8(14)-ene-3β,15α-diol (**3a**) and 5α-cholest-8(14)-ene-3β,15β-diol (**3b**)[2] (see Chart I). As a consequence it was reasonable to think that also epimeric 5α-cholest-8(14)-ene-3β,9α,15α-triol (**4a**) and 5α-cholest-8(14)-ene-3β,9α,15β-triol (**4b**) could possess an inhibitory action of sterol synthesis.

Our desire to study the biological activity of **4a** and **4b** prompted us to synthesize them. Since by reduction of **2a** only **4b** was obtained in useful yield, we thought of an alternative method to synthesize **4a** based on our previous work showing that sterols containing an 8,14-diene system are good starting materials for the introduction of oxygenated functions by chromic acid oxidation in both the 15- and 9α-positions.[4] With the most reasonable mechanism for this reaction (Scheme I) in mind, we treated 3β-acetoxy-5α-cholesta-8,14-dien-7-one with *m*-chloroperbenzoic acid (MCPBA) and were able to introduce, under mild conditions, oxygenated functions in 15α- and 9α-positions.[5]

These results have prompted us to study the action of MCPBA on 3β-acetoxy-5α-chloesta-8,14,-diene (**5b**) in order to obtain 15-oxygenated sterols. Initial experiments with **5b** using 1 molar equiv of MCPBA in chloroform or in dichloromethane indicated the formation of a complex mixture in which only minor amounts of epoxides were present (preparative TLC and mass spectra). In order to minimize epoxide opening by MCBA produced during the reaction, diethyl ether was used as the solvent; in fact, it is well-known that less attack by *m*-clorobenzoate anion occurs in this solvent.[6] However, the best results were obtained by using a two-phase buffered system.[7] In this case, examination of the reaction mixture by TLC showed the presence of two monoepoxidation compounds: 3β-acetoxy-8α,9α-epoxy-5α-cholest-14-ene (**6**) and 3β-acetoxy-14α,15α-epoxy-5α-cholest-8(9)-ene (**7**) in approximately a 3:7 ratio. The observed ratio of the two epoxides shows that steric factors outweigh electronic ones and the trisubstituted double bond is epoxidized at a faster rate

Chart I



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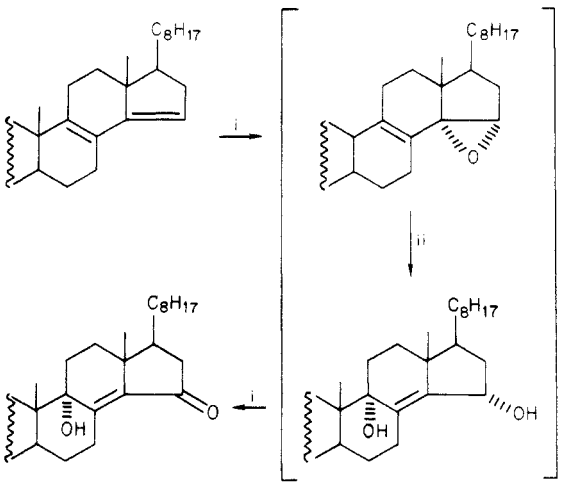
Scheme I[a]



[a] i, Jones reagent; ii, H_2O, H^+.

than the tetrasubstituted one. The elemental analysis and mass spectra of **6** and **7** are in keeping with the molecular formula $C_{29}H_{46}O_3$. The IR spectra of both compounds show only the band of the acetate group in the carbonyl region. Compound **6** survives chromatography on silica[8] and can be crystallized. The ^1H NMR information is consistent with the assigned structure for **6**, showing a signal at δ 5.8 for the olefinic proton at C-15, a signal at δ 4.7 for the 3α-proton, and no signals for epoxidic protons. Finally, the positions of the C-18 and C-19 methyl signals are in agreement with the calculated values.[9]

The ^1H NMR spectrum (CCl_4) of **7** exhibits a signal at δ 3.5 for the epoxide proton at C-15 and no signals attributable to olefinic protons. Compound **7** decomposed in chloroform-d_1 solution, and thus it was impossible to obtain its ^1H NMR spectrum in this solvent to confirm the proposed structure by the positions of the C-18 and C-19 methyl signals.

The epoxide **7** is rather unstable; it survives brief contact with basic alumina (TLC) but can be recovered only in part after rapid chromatography[8] (on basic alumina), necessary to separate it from **6**. The amorphous compound could not be crystallized. It was stable for a few days at low temperature, but polar or acidic solvents, moisture, or silica decomposed it to a mixture of 3β-acetoxy-5α-cholest-8(14)-ene-9α,15α-diol (**4c**) and 3β-acetoxy-5α-cholesta-8(14),9(11)-dien-15α-ol (**8**).

The results of IR, ^1H NMR, and mass spectral analysis are in accordance with the assigned structure for **4c** and **8**. In particular, the chemical shifts observed for the C-18 and C-19 methyl groups show good agreement with the calculated values.[9]

The enediol **4c** was oxidized by chromium trioxide to **2b**. Compound **4c** was identical with the more polar 15-alcohol prepared, in low yield, by sodium borohydride reduction of 3β-acetoxy-9α-hydroxy-5α-cholest-8(14)-en-15-one (**2b**) and differs from its less polar epimer of assigned structure **4d** notably in the ^1H NMR spectral position of the C-18 methyl signal. In fact, the C-18 methyl signal of **4c** resonates at δ 0.83 (calcd[9] 0.84); the corresponding methyl group of **4d** appears at δ 1.03 (calcd[9] 1.06) in accordance with the deshielding effect of the 15β-hydroxy group.

The UV absorption maximum of **8** (λ_{max} = 270 nm) differs from the calculated[10] value (λ_{max} = 293 nm) but agrees with the value observed for a similar 8(14),9(11)-diene.[11]

Reaction of **8** with 4-phenyl-1,2,4-triazoline-3,5-dione yields a Diels–Alder adduct diagnostic for a cis diene.[12] Chromium trioxide oxidation of **8** affords 3β-acetoxy-5α-cholesta-9(11),8(14)-dien-15-one (**9**) identical with that prepared by dehydration of **2b** with *p*-toluenesulfonic acid.[4]

Both **4c** and **8** are obtained (30% yield) together with **6** (20% yield) when the reaction of **5b** with MCPBA was followed by rapid chromatography on silica; under these conditions no trace of **7** can be recovered.

These results represent a new mild method for the functionalization of C and D steroid rings from an 8,14-diene steroid.

Experimental Section

All melting points are uncorrected. Infrared spectra were taken as Nujol mulls, and absorptions are reported as reciprocal centimeters; NMR spectra were taken on a Varian HA-100 as chloroform-*d* solutions when not otherwise indicated and are reported in δ units relative to Me_4Si. Optical rotations were taken as chloroform solutions. The mass spectra were determined on a Varian MAT 112 S spectrometer by direct-inlet methods. The progress of all reactions and column chromatographs (basic alumina grade III, or silica gel 60) was monitored by TLC on alumina (F_{254}) microplates. Hexane–ethyl acetate–triethylamine (95:5:0.1) and hexane–ethyl acetate (70:30) were used as developing solvents, and spots were detected by spraying with 70% sulfuric acid, followed by heating or by exposure to iodine vapor.

Reaction of 3β-Acetoxy-5α-cholesta-8,14-diene (5b) with *m*-Chloroperbenzoic Acid. A solution of *m*-chloroperbenzoic acid (500 mg) in diethyl ether (5 mL) was added to a stirred solution of **5b** (960 mg) in diethyl ether (60 mL) and 0.5 M aqueous sodium bicarbonate (25 mL). The mixture was stirred for 2 h and then separated. The organic layer was washed with water, dried, and evaporated to give a solid residue (1 g). The residue (two components on alumina TLC) was chromatographed[8] on basic alumina eluted with hexane–ethyl acetate (95:5 v/v) to afford the following: (a) 3β-Acetoxy-8α,9α-epoxy-5α-cholest-14-ene (**6**): 185 mg; mp 129–131 °C (from hexane); $[\alpha]^{20}_D$ +23°; IR 1730 cm^{-1}; ^1H NMR δ 5.8 (s, 1 H, 15–H), 4.7 (m, 1 H, 3α-H), 2.0 (s, 3 H, CH_3COO), 1.05 (s, 3 H, 19-CH_3; calcd[9] 1.06), 0.8 (s, 3 H, 18-CH_3; calcd[9] 0.8); mass spectrum, *m/z* (relative intensity) 442 (20, M^+), 427 (2, M – CH_3), 382 (22, M – AcOH), 367 (38, M – AcOH – CH_3), 329 (10, M – C_8H_{17}), 311 (22, M – H_2O – C_8H_{17}), 251 (M – H_2O – AcOH – C_8H_{17}), 108 (100).

Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.7; H, 10.5. Found: C, 78.6; H, 10.4.

(b) A mixture of **6** and **7**, 105 mg.

(c) 3β-Acetoxy-14α,15α-epoxy-5α-cholest-8(9)-ene (**7**): 100 mg; mp 120–121 °C (amorphous, slightly impure); IR 1730 cm^{-1}; ^1H NMR (CCl_4) δ 4.7 (m, 1 H, 3α-H), 3.5 (s, 1 H, 15β-H), 2.0 (s, 3 H, CH_3COO), 0.98 (s, 3 H, 19-CH_3), 0.81 (s, 3 H, 18-CH_3); mass spectrum, *m/z* 442 (15, M^+), 424 (7, M – H_2O), 367 (22, M – AcOH – CH_3), 329 (11, M – C_8H_{17}), 311 (78, M – C_8H_{17} – H_2O, 275 (100), 251 (33), 213 (22).

Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.7; H, 10.5. Found: C, 78.8; H, 10.6.

(d) 3β-Acetoxy-5α-cholesta-8(14),9(11)-dien-15α-ol (**8**): 230 mg; mp 129–130 °C (from aqueous acetone); $[\alpha]^{20}_D$ +12.5°; IR 3350, 1720 cm^{-1}; UV λ_{max} 270 nm (ϵ 5150); ^1H NMR δ 5.45 (dd, 1 H, 11-H, J_{AX} = 3 Hz, J_{BX} = 7 Hz), 4.65 (overlapping, m, 2 H, 3α- and 15β-H), 2.00 (s, 3 H, CH_3COO), 0.87 (s, 3 H, 19-CH_3; calcd[9] 0.86), 0.72 (s, 3 H, 18-CH_3; calcd[9] 0.78); mass spectrum, *m/z* (relative intensity) 442 (7, M^+), 311 (49, M – C_8H_{17} – H_2O), 275

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(100), 251 (21, M − H_2O − AcOH), 213 (21).

Anal. Calcd for $C_{29}H_{46}O_3$: C, 78.7; H, 10.5. Found C, 78.6; H, 10.5.

(e) 3β-Acetoxy-5α-cholest-8(14)-ene-9α,15α-diol (**4c**): 270 mg; mp 147–148 °C (from diisopropyl ether); $[\alpha]^{20}_D$ +43°; IR 3350, 1720 cm^{-1}; ^1H NMR δ 4.70 (m, 1 H, 3α-H), 4.60 (m, 1H, 15β-H; $W^1/_2 \simeq 8$ Hz), 2.00 (s, 3 H, CH_3COO), 0.83 (overlapping; 2 s, 6 H, 18- and 19-CH_3; calcd[9] 0.86 for both); mass spectrum, *m/z* (relative intensity) 460 (23, M^+), 442 (7, M − H_2O), 424 (4, M − $2H_2O$), 400 (68, M − AcOH), 382 (52, M − AcOH − H_2O), 367 (18, M − AcOH − H_2O − CH_3), 311 (45, M − C_8H_{17} − $2H_2O$), 251 (34, M − C_8H_{17} − $2H_2O$ − AcOH), 109 (100).

Anal. Calcd for $C_{29}H_{48}O_4$: C, 75.6; H, 10.5. Found: C, 75.4; H, 10.3.

When a similar crude reaction residue (two spots on alumina TLC) was chromatographed on silica, the following products were obtained: **6** (200 mg), **8** (290 mg), **4c** (300 mg).

Apparently **7** is completely transformed into **8** and **4c** by silica and partially by alumina.

Reduction of 3β-Acetoxy-9α-hydroxy-5α-cholest-8(14)-en-15-one (2b) with Sodium Borohydride. The 15-ketone **2b** (0,5 g) in isopropyl alcohol (20 mL) and methanol (10 mL) was reduced with sodium borohydride (0.65 g dissolved in 10 mL of methanol and 1 mL of water) at 0 °C to afford a mixture of 15β-alcohol **4d** (350 mg) and 15α-alcohol 4c (30 mg) which were isolated and purified by chromatography. Compound **4c** was identical with the product described above. Compound **4d** shows the following: mp 116–177 °C (from diisopropyl ether); $[\alpha^{20}_D$ + 13°; IR 3350, 1720 cm^{-1}; ^1H NMR δ 4.64 (overlapping, m, 2 H, 3α- and 15α-H), 2.00 (s, 3 H, CH_3COO), 1.03 (s, 3 H, 18-CH_3; calcd[9] 1.06), 0.85 (s, 3 H, 19-CH_3; calcd[9] 0.85); mass spectrum *m/z* (relative intensity) 460 (5, M^+), 442 (14, M − H_2O), 424 (5, M − $2H_2O$), 400 (100, M − AcOH), 382 (56, M − AcOH − H_2O), 367 (19, M − AcOH − H_2O − CH_3), 311 (26, M − C_8H_{17} − $2H_2O$), 251 (14, M − C_8H_{17} − $2H_2O$ − AcOH), 109 (100).

Anal. Calcd for $C_{29}H_{48}O_4$: C, 75.6; H, 10.5. Found: C, 75.7; H, 10.5.

Saponification with methanolic KOH of **4c** affords the triol **4a**: mp 175–176 °C (from diisopropyl ether); $[\alpha]^{20}_D$ +62°; IR 3300 cm^{-1}; ^1H NMR ($CDCl_3$–Me_2SO-d_6) δ 4.43 (m, 1 H, 15β-H), 3.70 (m, 1 H, 3α-H), 0.78 (s, overlapping, 6 H, 18- and 19-CH_3).

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.4; H, 11.1. Found: C, 77.5; H, 11.0.

Saponification of **4d** with methanolic KOH affords the triol **4b**: mp 187–188 °C (from diisopropyl ether); $[\alpha]^{20}_D$ +29°; IR 3300 cm^{-1}; ^1H NMR δ 4.5 (m, 1 H, 15α-H 3.6 (m, 1 H, 3α-H), 1.0 (s, 3 H, 18-CH_3; calcd[9] 1.03), 0.81 (s, 3 H, 19-CH_3; calcd[9] 0.83).

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.4; H, 11.1. Found: C, 77.4; H, 11.2.

The triols **4a** and **4b** were obtained also in 2:8 ratio (75% yield) by lithium aluminum hydride reduction of **2b**.

Oxidation of 3β-Acetoxy-5α-cholest-8(14)-ene-9α,15α-diol (4c). The alcohol **4c** (200 mg) was oxidized with Sarett reagent[13] (or with Jones reagent[14]) to yield the corresponding ketone **2b**: 180 mg; mp 194–195 °C; $[\alpha]^{20}_D$ +15.3°; the compound had the same IR, ^1H NMR, and mass spectra as those reported previously.[4]

Oxidation of 3β-Acetoxy-5α-cholesta-8(14),9(11)-dien-15α-ol (8). The dienol **8** (200 mg) was oxidized with chromium trioxide (100 mg) in pyridine to afford after usual work up the unsaturated ketone **9**: 180 mg; mp 144–145 °C (from methanol); $[\alpha]^{20}_D$ +44.3°; IR 1730 1690, 1640 cm^{-1}; UV λ_{max} 329 nm (ε 8700); ^1H NMR δ 5.7 (dd, 1 H, 11-H; J_{AX} = 3 Hz, J_{BX} = 7 Hz), 4.7 (m, 1 H, 3α-H), 4.0 (dd, 1 H, 7β-H, $J \simeq 18$ Hz), 2.0 (s, 3 H, CH_3COO), 0.94 (s, 3 H, 19-CH_3; calcd[4,9] 0.94), 0.90 (s, 3 H, 18-CH_3; calcd[4,9] 0.92).

Anal. Calcd for $C_{29}H_{44}O_3$: C, 79.0 H, 10.1 Found: C, 79.0; H, 10.1.

Reaction of 3β-Acetoxy-5α-cholesta-8(14),9(11)-dien-15α-ol (8) with 4-Phenyl-1,2,4-triazoline-3,5-dione. A solution of 4-phenyl-1,2,4-triazoline-3,5-dione in acetone was added dropwise to a stirred solution of **8** (0.2 g) in dichloromethane (40 mL) until a pink coloration persisted. After the usual workup the adduct was obtained: 200 mg: glass; IR 1760, 1730, 1705 cm^{-1}; ^1H NMR δ 7.5 (s, 5 H, aromatic H), 4.5–5.0 (overlapping, 3 H, 11β-H, 15β-H, 3α-H), 4.5–5.0 (overlapping, 3 H, 11β-H, 15β-H), 0.9 (s, overlapping, 6 H, 18- and 19-CH_3).

Anal. Calcd for $C_{37}H_{51}N_3O_5$: C, 73.8; H, 8.5; N, 7.0. Found C, 74.0; H, 8.4; N, 6.9.

Treatment of 3β-Acetoxy-9α-hydroxy-5α-cholest-8(14)-en-15-one (2b) with *p*-Toluenesulfonic Acid. *p*-Toluenesulfonic acid (100 mg) in benzene (80 mL) was refluxed, and part of the solvent (40 mL) was distilled off. The enone **2b** (300 mg) was added and the solution refluxed for 10 min. After the usual workup, 3β-acetoxy-5α-cholesta-9(11),8(14)-dien-15-one (**9**) was obtained (mp 144–145 °C), identical with that obtained above.

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Registry No. 2b, 72584-37-3; **4a**, 77825-86-6; **4b**, 77825-87-7; **4c**, 77825-88-8; **4d**, 77825-89-9; **5b**, 5226-33-5; **6**, 77825-90-2; **7**, 77825-91-3; **8**, 77846-59-4; **8** 4-phenyl-1,2,4-triazoline-3,5-dione adduct, 77825-92-4; **9**, 77825-93-5.

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