Functionalization of Steroid Side Chains: Conversion of Cholesterol to Chol-5-ene-3β,24-diol

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Cholesterol has been converted to chol-5-ene-3β,24-diol in a 14% overall yield. The three-step process involves protection of the double bond of cholesterol as 6β-acetamido-5α-cholestan-3β-ol acetate (1), elimination of the three terminal carbons of the side chain by a novel oxidative cleavage with trifluoroperoxyacetic acid, and elimination of acetamide to restore the 5,6 double bond.

The utilization of cholesterol as a starting material in the synthesis of the pharmaceutically important steroids has been of relatively little importance. This is due principally to the lack of an efficient method for selective functionalization of the cholesterol side chain. The classic method has been to oxidize the 5,6-dibromo derivative of cholesterol with chromic acid. After regeneration of the 5,6 double bond with zinc, the major product isolated is 3β-hydroxyandrost-5-en-17-one. Despite extensive investigation, the overall yield is only about 7%.[1] In the present work, a method is described for the conversion of cholesterol to purified, crystalline chol-5-ene-3β,24-diol (3) in a 14% overall yield.

This method introduces a new reaction in steroid chemistry, oxidation with trifluoroperoxyacetic acid. This reagent shows selectivity for attacking positions remote from electronegative groups.[2] With tertiary hydrogens such as in isopentane, hydroxylation is followed by a carbon–carbon bond cleavage and loss of the isopropyl group as acetone.[2] An analogous sequence was observed in the present work.

Results and Discussion

Scheme I outlines the reaction pathway used for the conversion of cholesterol to chol-5-ene-3β,24-diol (3).

Cholesterol is converted to 6β-acetamido-5α-cholestan-3β-ol acetate (1) in a two-step process. Cholesterol acetate is nitrated by using either the method of Fieser[3] or that of Rowlands[4] to yield 6-nitrocholesterol acetate. Yields as high as 90% were obtained by the Fieser procedure. Presumably the 90% yields could be made reproducible by further work and study. However, the yields of apparently identical runs varied from 0 to 90%, and this problem has been discussed by Rowlands.[4] The Rowlands procedure gave reproducible yields of 65%.

6-Nitrocholesterol acetate is hydrogenated under 3 atm of hydrogen at room temperature to yield 6β-acetamido-5α-cholestan-3β-ol acetate (1). The use of 3 atm of hydrogen in place of 50 atm, Pt in place of Pd, and 25 °C in place of 150 °C gave improved yields over those reported.[5] GC analysis indicates that the yield was even better than the 70% isolated.

The key step in the reaction sequence is the trifluoroperoxyacetic acid oxidation of the acetamide (1). Work with model systems[2,6-9] has shown that trifluoroperoxyacetic acid will selectively oxidize alkyl positions remote from existing electronegative groups. Also, the reagent will selectively attack tertiary positions. Frommer and Ullrich[7] reported a t/s ratio of 33:1 in the oxidation of isopentane. Scheme II outlines the mechanism for the formation of the C-24 alcohol after the initial attack at C-25. This scheme is analogous to that proven for isopentane oxidation.[2]

The removal of the 6β-acetamido protecting group and the restoration of the 5,6 double bond use an elimination reaction reported for other steroidal acetamides.[10,11] This step was first checked on the unoxidized material, 6β-acetamido-5α-cholestan-3β-ol acetate (1). Refluxing 1 with toluene and acetic anhydride in the presence of a catalytic amount of 5-sulfosalicylic acid (3-carboxy-4-hydroxybenzenesulfonic acid) gave cholesterol acetate in 95% yield as estimated by GC. The identical procedure was applied to the crude product from the oxidation of 1 with trifluoroperoxyacetic acid.

At this point in the synthesis, chol-5-ene-3β,24-diol (3) exists as the 3,24-diacetate 2. The crude diacetate 2 is chromatographed on a prepacked silica Lobar column, eluting with a 65:35 hexane–ether solvent system. The diacetate 2 is isolated in a 23% yield from the acetamide 1. This represents a 14% overall yield from cholesterol based on a 90% yield in the nitration of cholesterol acetate and a 70% yield in the hydrogenation of the nitro compound to 1.

The melting points of both the diol and the diacetate are in agreement with literature values.[12] The diacetate was compared to an authentic sample with respect to GC retention time, NMR spectrum, IR spectrum, and R_f value on silica gel thin-layer plates.

The diol was converted to the bis(trimethylsilyl ether) and examined by electron-impact mass spectrometry. The mass spectrum was compared to the spectrum of the trimethylsilyl ether of an authentic sample. The fragmentation pattern was in agreement with the proposed structure and compared well with the spectrum of an authentic sample. The peak at m/e 504 is the parent ion, agreeing with the molecular weight of $C_{30}H_{56}O_2Si_2$. The peak at m/e 414 results from the loss of trimethylsilanol (mol wt 90) and confirms the molecular weight of 504. The dominant peak at m/e 129 is characteristic of trimethylsilyl ethers of 3β-hydroxy-5-ene steroids and is prominent in the mass spectrum of the trimethylsilyl ethers of cholesterol and sitosterol.[13]

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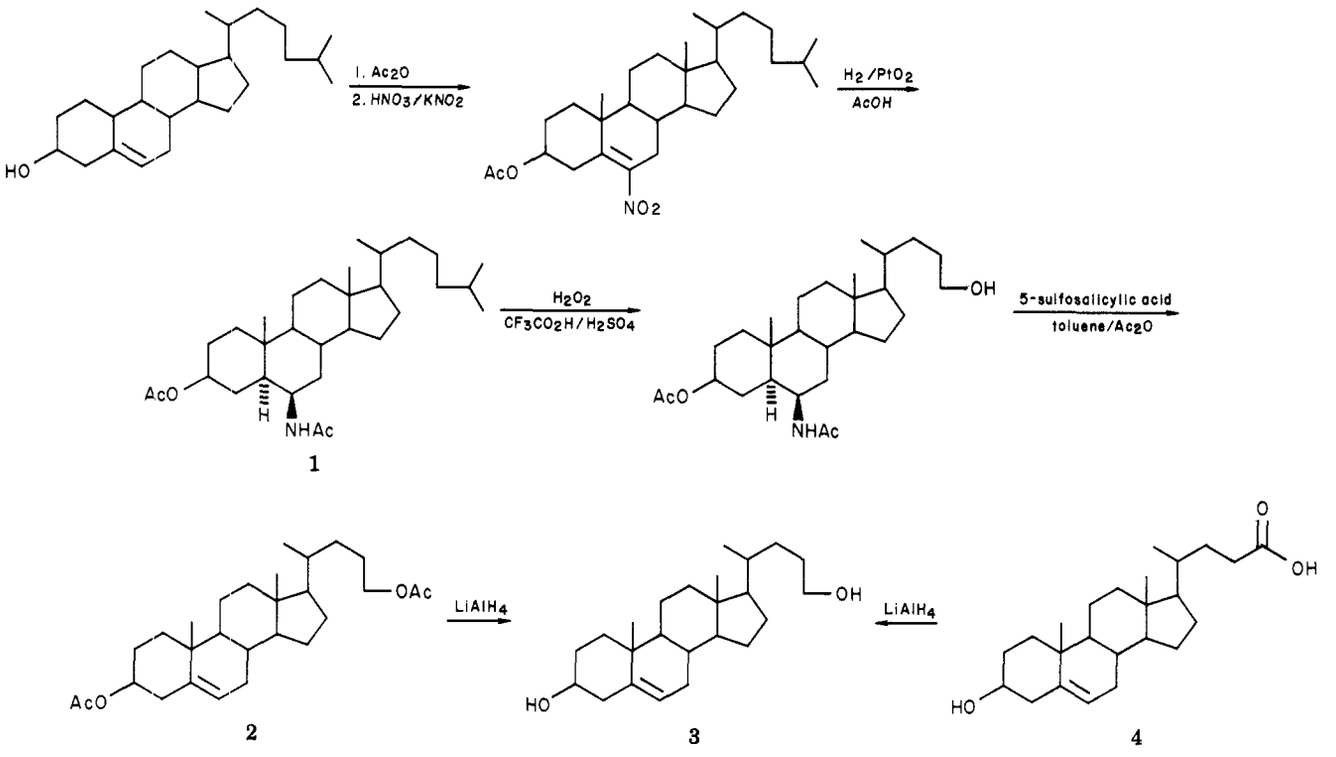
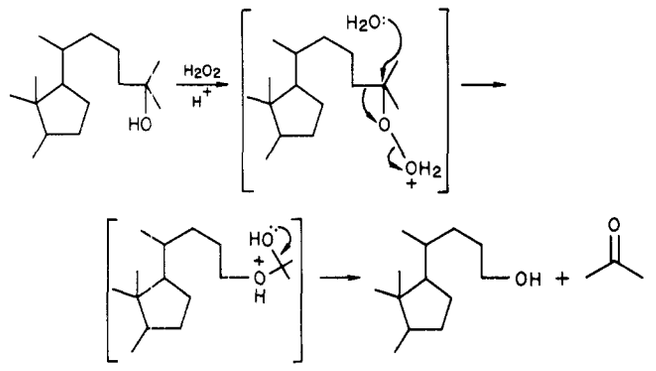
Scheme I



Scheme II



The overall yield of 14% is largely limited by the step involving oxidation with trifluoroperoxyacetic acid. Despite optimization of yield with respect to temperature and added sulfuric acid, the highest yield was only 23%. After oxidation of 1, only 60–80% of the material can be extracted from aqueous solution by CH_2Cl_2, showing considerable loss to more polar products. Such polar products could result from oxidation at a less remote tertiary hydrogen such as C-17. Ring opening to hydroxy acids would generate four polar groups and the loss of a side chain, both changes enhancing water solubility.

After removal of the acetamido group, the side products continue to be relatively more polar. They elute with more difficulty, and NMR integrations indicate three acetoxy groups instead of the two present in 2. A nitrogen analysis indicated that over 80% of the acetamido group had been eliminated, so this was not a major problem.

Experimental Section

Instrumental Analysis. All melting points are uncorrected. Gas–liquid chromatography (GLC) was conducted on a Barber-Coleman Model 5000 gas chromatograph using a flame-ionization detector. All separations were performed on a 6-ft column with a 3-mm inside diameter containing 3% SE-30 on Gas Chrom Q (80–100 mesh) support (Applied Science, State College, PA). The operating temperature was 265 °C, and a N_2 carrier gas flow of 40 mL min^{-1} was used. NMR spectra were recorded on a Varian A-60 spectrometer using $CDCl_3$ as solvent and tetramethylsilane (Me_4Si) as internal standard. The mass spectra were recorded on a Finnigan 3200 GC-MS instrument. IR spectra were recorded on a Perkin-Elmer Model 580 infrared spectrometer.

6β-Acetamido-5α-cholestan-3β-ol Acetate (1). A mixture of 10 g of 6-nitrocholesterol acetate, 150 mL of acetic acid, 10 mL of acetic anhydride, and 0.20 g of PtO_2 was shaken under 3 atm of hydrogen for 48 h at 25 °C. After removal of the platinum by filtration, the solvent was removed at 100 °C under vacuum. The residue was dissolved in CH_2Cl_2 and washed with 5% Na_2CO_3 solution. The CH_2Cl_2 phase was dried over anhydrous Na_2SO_4, and the solvent was removed at 30 °C under vacuum. The residue was crystallized from methanol to yield 7.2 g (70%) of fine white rhombic crystals, mp 172–174 °C (lit.[5] mp 167 °C).

Chol-5-ene-3β,24-diol 3,24-Diacetate (2). 6β-Acetamido-5α-cholestan-3β-ol acetate (1; 0.50 g) was dissolved in 20.1 mL of trifluoroacetic acid and 10.7 mL of 96% H_2SO_4. The solution was cooled to 0 °C. To the solution was added with stirring 1.0 mL of 50% aqueous H_2O_2. The solution was stirred at 0 °C for 4 h. The reaction mixture was quenched by slow addition to a cold, dilute solution of aqueous NaOH. The aqueous, basic mixture was extracted with CH_2Cl_2. The CH_2Cl_2 phase was dried over anhydrous Na_2SO_4, and the solvent was removed at 35 °C under vacuum.

The residue was dissolved in 25 mL of toluene and 5 mL of acetic anhydride. To the solution was added 0.050 g of 5-sulfosalicylic acid. The reaction mixture was stirred at reflux for 18 h. The reaction mixture was then washed successively with 10% Na_2SO_4 solution and 5% Na_2CO_3 solution. The toluene phase was dried over anhydrous Na_2SO_4, and the solvent was removed under vacuum at 70 °C.

The crude diacetate was chromatographed on a prepacked silica Lobar column, eluting with a 65:35 mixture of hexane and ether. Fractions of 15 mL were collected. The diacetate 2 appeared in fractions 10, 11, and 12. The purified diacetate was recrystallized from methanol to yield 0.105 g (23%) of white needles: mp 127–129 °C (lit.[12] mp 127–129 °C); NMR ($CDCl_3$) δ 0.68 (s, 3 H, CH_3 at C-18), 1.02 (s, 3 H, CH_3 at C-19), 2.03 (s, 6 H, acetates), 4.05 (m, 2 H, CH_2OAc at C-24), 4.53 (m, 1 H, CH at C-3), 5.38 (m, 1 H, vinyl H at C-6); IR (KBr) 1735 (s), 1245 (s), 1040 cm^{-1} (m). Anal. Calcd for $C_{28}H_{44}O_4$: *m/e* 444.3239. Found: 444.3235.

Chol-5-ene-3β,24-diol (3). The diacetate 2 (0.100 g) was dissolved in 25 mL of dry tetrahydrofuran (THF). $LiAlH_4$ (0.100

g) was added slowly to the THF solution. The reaction mixture was refluxed for 2 h. Excess $LiAlH_4$ was destroyed by careful addition of wet THF. The reaction mixture was filtered, and the solvent was removed under vacuum at 50 °C. The diol crystallized upon removal of the solvent. The diol was recrystallized from methanol to yield crystals, mp 191–193 °C (lit.[12] mp 193–195 °C).

Chol-5-ene-3β,24-diol 3,24-Bis(trimethylsilyl ether). The diol **3** (0.100 g) was dissolved in 2 mL of dry pyridine. To this solution was added 0.5 mL of a 9:1 mixture of *N,O*-bis(trimethylsilyl)acetamide and trimethylchlorosilane. The solution was maintained at 25 °C for 24 h. Solvent and excess reagent were removed at 2 mmHg and 50 °C: mass spectrum (70 eV), *m/e* (rel intensity) 504 (4), 414 (18), 285 (7), 255 (3), 129 (100), 95 (65), 75 (53), 73 (96), 69 (76).

Authentic Samples. Chol-5-ene-3β,24-diol. 3β-Hydroxychol-5-en-24-oic acid (**4**; 0.100 g) was dissolved in 20 mL of anhydrous THF. $LiAlH_4$ (0.100 g) was added slowly. The reaction mixture was stirred at reflux for 18 h. Wet THF was added slowly to destroy excess $LiAlH_4$. The reaction mixture was filtered, and THF was removed under vacuum at 50 °C. The product was recrystallized from methanol to yield crystals, mp 190–193 °C (lit.[12] mp 193–195 °C).

Chol-5-ene-3β,24-diol 3,24-Bis(trimethylsilyl ether). The trimethylsilyl ether was prepared as previously described: mass spectrum (70 eV), *m/e* (rel intensity) 504 (5), 414 (18), 285 (35), 255 (14), 129 (63), 95 (43), 75 (70), 73 (100), 69 (58).

Chol-5-ene-3β,24-diol 3,24-Diacetate. The diacetate was prepared as previously described: mp 127–129 °C (lit.[12] mp 127–129 °C).

Conclusion

The preparation of **3** from cholesterol offers a new and relatively efficient method for the utilization of cholesterol as a starting material in steroid synthesis. Although **3** itself is of no pharmaceutical importance, conversion of this compound to 25-hydroxycholesterol is a very straightforward synthetic problem compared with the existing method for synthesizing this compound.[14-16] Further degradation of **3** to the 20-keto steroids is also possible. The method described for the oxidation of cholesterol is being extended to sitosterol and campesterol, the two most abundant sterols.

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Registry No. **1**, 17398-36-6; **2**, 71129-66-3; **3**, 54668-67-6; **3** bis-(Me_3Si) derivative, 71129-67-4; **4**, 5255-17-4; 6-nitrocholesterol acetate, 1912-54-5; cholesterol, 57-88-5.

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Isolation, Structure Elucidation, and Partial Synthesis of Xestosterol, a Biosynthetically Significant Sterol from the Sponge *Xestospongia muta*

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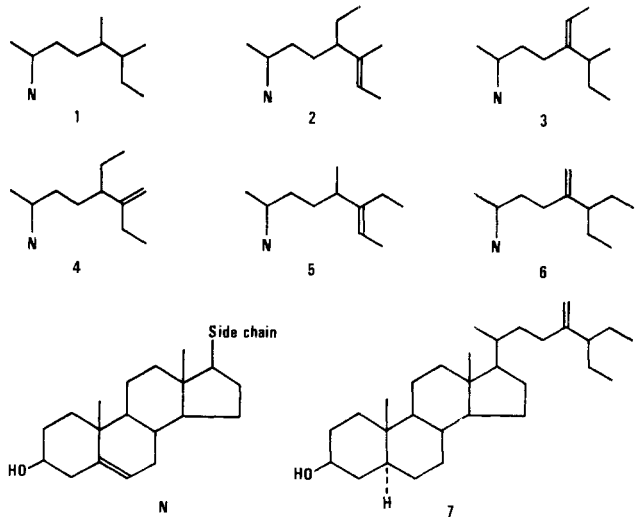
The major component of the sterol constituents of the Carribean sponge *Xestospongia muta* is a new C_{30} sterol—xestosterol—characterized by the "elongated" side chain depicted by structure **6**, as was confirmed by partial synthesis. The probable biological function of **6** or similar sterols in sponges is emphasized.

Marine organisms have been the source of numerous sterols possessing side chains with unusual alkylation patterns.[1] Sterols with "extended" side chains, which have been encountered in sponges, are of particular interest. Aplysterol (**1**) and its 24(28)-dehydro[2] and 25-dehydro[6] analogues stelliferasterol (**2**),[3,4] isostelliferasterol (**3**),[3] and strongylosterol (**4**)[4,5] are all characterized by an extra carbon atom attached to C-26. Such compounds were found in several cases[2,3,6] to be the major sterol components of a sponge, and strongylosterol is even the sole sterol of the sponge *Strongylophora purissima*.[5] This suggests that they play a functional role, most likely in cell membrane stabilization. The reason why these special sterols—which have no terrestrial counterparts—are used instead of cholesterol remains an open question,[7,8] but it is clear that the elucidation of this problem could shed new light on the functional mechanism of membranes in general.

More recently, verongulasterol (**5**), a *minor* sterol from the sponge *Verongia cauliformis*,[6] was found to possess the unique feature of being alkylated at *both* C-26 and C-27



positions. However, it is present in such small amounts that it could not possibly play a functional (i.e., membrane)

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