5β,6β-Epoxidation of 3β-Cholesteryl Acetate and its Analogues

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The treatment of acetylated Δ5-steroids with chromyl diacetate at low temperature afforded the 5β,6β-epoxy derivatives with stereoselectivity greater than 90 per cent.

The incorporation of oxygen containing groups into the steroid structure is of considerable interest in connection with the synthesis of corticosteroids and cardioactive substances.

Oxidations with reagents containing hexavalent chromium have been widely used either to functionalize non-activated positions of polycyclic hydrocarbons [1], or to obtain through allylic oxidations keto derivatives from unsaturated hydrocarbons [2, 3].

Treatment of olefins with oxidative reagents such as chromyl chloride [4], chromic acid [5] and chromyl acetate [6] yields a variety of complex mixtures. Evidence of epoxide formation as intermediates in such oxidations has been reported [7, 8], as well as epoxidation of allylic alcohols with chromium trioxide [9].

We wish to report a stereoselective 5β,6β-epoxidation on Δ5-steroids using chromyl diacetate under specific conditions (see below). Previous approaches to β-epoxide formation involved the preparation of trans-halohydrins followed by treatment with base [10], treatment of an olefin with organic peracids [11] followed by separation of the isomeric epoxides, or treatment of an unsaturated steroid with hydrogen peroxide in acetonitrile containing ferric acetylacetonate [12].

Results and Discussion

Our first attempts to oxidise 3β-acetoxycholesterol (1a) using chromium trioxide in acetic acid: acetic anhydride (1:1) as the reaction solvent produced a complex mixture of products which could not be avoided either by lowering the temperature or the oxidant-substrate ratio. In all cases the initial main product was the 7-oxo-derivative (3a) which was immediately overoxidized to the final complex mixture.

To minimize the overoxidation reactions we selected dichloromethane as solvent, which proved to be inert to the oxidant and a good solvent for both substrate and reagent, even at low temperatures. This solvent provided a completely different medium from the acetic acid: acetic anhydride mixture being non-polar, less viscose and aprotic. Accordingly, it should not participate in the reaction in the same way that the former solvent did. The most important aspect is that inorganic species are better defined in dichloromethane. If the reagent was prepared with CrO3/ACOH the active species AcO-CrO3H was only partially soluble in dichloromethane and almost no reaction occurred. However, if the reagent was prepared with CrO3/AC2O in an equimolar ratio, the active species (AcO)2-CrO2 was readily soluble in dichloromethane. This reagent was applied to the oxidation of acetylated derivatives of: cholesterol (1a),

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>others</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0</td>
<td>70</td>
<td>19</td>
<td>0</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>b, c</td>
<td>4</td>
<td>73</td>
<td>9</td>
<td>0</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>d</td>
<td>3</td>
<td>65</td>
<td>20</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>e</td>
<td>3</td>
<td>74</td>
<td>3</td>
<td>0</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>f</td>
<td>0</td>
<td>82</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18**</td>
</tr>
</tbody>
</table>

* The reactions were conducted at −78 °C with a 1:5 substrate: reagent ratio. Values were obtained by capillary CG analysis.
** 8% of 3β-acetoxy-5β,6β-16α,17α-diepoxypregnan-20-one, 5% of 3β-acetoxy-5α-hydroxy-16α,17α-epoxy pregnan-6,20-dione and 5% of 3β-acetoxy-16α,17α epoxypreg-5-en-7,20-dione.

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a mixture (54:37%) of sitosterol (1b), campesterol (1c), stigmasterol (1d), pregnenolone (1e), and 16-dehydroprogrenolone (1f). Using this reagent at 
-78°C the starting materials were readily oxidized to a mixture containing more than 65% of the respective 5β,6β-epoxy derivative, as it is shown in 
Table I. No change in the product pattern was observed, at the same temperature, when acetic acid was added to the reaction vessel. However, when the 
reaction was performed at 0°C the presence of acetic acid favoured the opening of the β-epoxide ring yielding the 5α-hydroxy-6-keto derivatives (5a-f).

Since cholesteryl acetate underwent a rapid oxidation in the above medium we studied the effect of substrate/reagent ratio and of the temperature. Oxidation experiments were carried out by using several ratios from 1:1 to 1:10 of substrate:oxidant concentrations.

When a 1:1 molar ratio was used more than half of the starting material was recovered. In order to oxidize more than 90% of the substrate at least three molar equivalents of oxidant were needed. When more than 5 molar equivalents of oxidant were used the epoxide initially formed was converted to the 5α-hydroxy-6-keto derivative and finally to a complex mixture of products that was not analyzed further. Analysis of the influence of the reaction temperature showed that at -78°C the best results were obtained (those presented at Table I), while at 0°C a complex mixture of compounds in similar proportions were obtained.

The reaction gave comparable results with all the Δ4-steroids used. In each case the 5β,6β-epoxide was the main product with yields between 62 and 82%, the by-product ordinarily found was the 7-keto derivative (3a, 3d) and the 5α-hydroxy-6-keto derivative (5b, c and 5e), as it is shown in Table I. In those cases in which more than a double bond was present (1d and 1f) the optimum conditions led to the desired 5β,6β-epoxide without formation of significant amounts of by-products. The α-epoxy derivative was only obtained in the case of the oxidation of stigmasteryl-3β-acetate (1d).

In every case the reaction products were purified by chromatographic procedures and characterized by 1H and 13C NMR and gc-mass spectrometry. The main differences observed in the NMR spectra are indicative of the changes produced in the A/B ring system of the steroids. Typical data are presented in Table II and III.

Table II. 1H NMR chemical shifts of relevant protons of the A/B rings of compounds 1d–5d in deuterochloroform*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>H-3</th>
<th>H-6</th>
<th>H-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>1d</td>
<td>4.60  (m)</td>
<td>5.35 (d)</td>
<td>1.05 (s)</td>
</tr>
<tr>
<td></td>
<td>J=4 Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2d</td>
<td>4.75  (m)</td>
<td>3.09 (d)</td>
<td>1.00 (s)</td>
</tr>
<tr>
<td></td>
<td>J=2 Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3d</td>
<td>4.75  (m)</td>
<td>5.70 (s)</td>
<td>1.20 (s)</td>
</tr>
<tr>
<td>4d</td>
<td>5.00  (m)</td>
<td>2.95 (d)</td>
<td>0.80 (s)</td>
</tr>
<tr>
<td></td>
<td>J=4 Hz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5d**</td>
<td>5.00  (m)</td>
<td>–</td>
<td>0.90 (s)</td>
</tr>
</tbody>
</table>

* In ppm downfield from TMS; s: singlet, d: doublet, m: multiplet.
** At 3.30 ppm appears a signal exchangeable in D2O corresponding to the 5α-OH, and at 2.7 ppm a triplet with J=12 Hz corresponding to the H-7α.
Although the mechanism of the above reaction to afford the 5β,6β-epoxy steroids has not been disclosed, it seems that the 3β-acetoxy group does not participate in the reaction. It is already known that Δ4-steroids give a 1:1 mixture of α- and β-epoxides when treated with peracids. In the present case, our results suggest a steric course in which the α-face is more hindered than the opposite one. This may be explained considering an α-face attack of the Cr(VI) reagent on the π-bond of the olefin due, in part, to the shielding effect on the β-face by the angular methyl group. This new linkage could be formed either with the oxygen atom or with the transition metal atom [4], since the oxo moiety A (in Fig. 1) implies polarization as indicated in resonance structures B and C, and the transition metal atom is an electron acceptor.

After the α-face of the steroid has been blocked, a nucleophilic oxygen may approach to the β-face rendering stereospecifically the epoxide derivative as it is shown in Fig. 2. This fact would also account for the larger than equimolecular oxidant/substrate ratio needed for the completion of the reaction.

![Resonance structures of chromyl diacetate.](image)

**Experimental**

*General:* 1H and 13C NMR spectra were recorded in the FT mode on a Varian XL-100-15 spectrometer. Mass spectra (70 eV, direct inlet) were registered on a Varian-MAT CH-7A mass spectrometer with a Data System 166. GC analysis were performed on a fused silica capillary column (SP-2100, 10 m; temp. 200–280 °C, rate 8 °C/min).

**Reagent:** Finally powdered chromium anhydride (500 mg, 5 mmol) was treated with acetic anhydride (2 ml) or with a mixture of acetic acid–acetic anhydride 1:1 (1.5 ml) at 0 °C during 1 h. The brown solution was diluted with dichloromethane (free from ethanol) (23 ml) and used immediately.

**Typical oxidation experiment**

The reagent was added dropwise into a cooled (0 °C or −78 °C) solution of cholesteryl acetate (428 mg, 1 mmol) in dichloromethane (150 ml) with continuous stirring. When the addition was finished the reaction mixture was poured into 8% aqueous NaOH soln and stirred for 30 min. The organic layer was separated, washed successively with 8% aqueous NaOH soln and water, and dried over SO2Mg. The residue obtained after removal of the solvent was purified by flash chromatography (silica gel, Cl2CH2–hexane 9:1) monitoring by TLC (silica gel, Cl2CH2). Pure compounds (GC) were identified by their respective 1H NMR [13, 14], 13C NMR [15, 16] and mass spectra.

![Plausible mechanism for the 5β,6β-epoxidation reaction of Δ4-steroids.](image)

5β,6β-Epoxy-cholestan-3β-yl acetate (2a)

M.p.: 130–132 °C (lit. [12]: 130–133.5 °C). MS: m/z (%): 444 (M+, 2), 384(100), 377(46), 356(24), 355(30), 362(17). 1H NMR: 0.64 (s, 3H, H-18), 0.85 (d, J = 6 Hz, 6-H, H-26 and H-27), 1.00 (s, 3H, H-19), 2.04 (s, 3H, acetate), 3.08 (d, J = 2 Hz, 1H, H-6), 4.75 (m, 1H, H-3).
3\(\beta\)-Acetoxy-cholest-5-en-7-one (3a)

M.p.: 157–158 °C (lit. [2]: 157–159 °C). MS: \(m/z\) (%): 442 (M\(^{-}\), 6), 382(100), 367(42), 187(33), 174(60), 161(26). \(^1\)H NMR: 0.7 (s, 3H, H-18), 0.85 (d, \(J = 6\) Hz, 6H, H-26 and H-27), 1.22 (s, 3H, H-19), 2.08 (s, 3H, acetate), 4.7 (m, 1H, H-3), 5.7 (s, 1H, H-6).

3\(\beta\)-Acetoxy-5\(\alpha\)-hydroxy-cholestan-6-one (5a)

M.p.: 155–156 °C (lit. [17]: 230–231 °C). MS: \(m/z\) (%): 460 (M\(^{-}\), 3), 400(100), 382(38), 367(15), \(^1\)H NMR: 0.64 (s, 3H, H-18), 0.80 (s, 3H, H-19), 0.85 (d, \(J = 6\) Hz, 6H, H-26 and H-27), 2.00 (s, 3H, acetate), 3.25 (s, 1H, \(5\alpha\)-OH), 5.00 (m, 1H, H-3).

3\(\beta\)-Acetoxy-sitost-5-en-7-one and 3\(\beta\)-Acetoxy-campestan-6-one

\(^1\)H NMR: 0.64 (s, 3H, H-18), 0.80 (s, 3H, H-19), 2.02 (s, 3H, acetate), 4.70 (m, 1H, H-3), 5.1 (t, \(J = 6\) Hz, 2H, H-22 and H-23).

5\(\alpha\),6\(\beta\)-Epoxy-sitost-5-en-3\(\beta\)-yl acetate and 5\(\alpha\),6\(\beta\)-Epoxy-campest-5-en-3\(\beta\)-yl acetate

\(^1\)H NMR: 0.68 (s, 3H, H-18), 0.72–0.90 (m, 9H, H-26, H-27, H-28 and H-29), 1.22 (s, 3H, H-19), 2.08 (s, 3H, acetate), 4.75 (m, 1H, H-3), 5.1 (t, \(J = 6\) Hz, 2H, H-22 and H-23).

5\(\beta\),6\(\beta\)-Epoxy-stigmast-22-en-3\(\beta\)-yl acetate (4d)

Detected by \(^1\)H NMR in samples containing the compound 2d: 0.8 (bs, 6H, H-18 and H-19), 0.7–0.88 (m, 9H, H-26, H-27 and H-29), 2.02 (s, 3H, acetate), 2.95 (d, \(J = 4\) Hz, H-6), 5.00 (m, 1H, H-3).

3\(\beta\)-Acetoxy-5\(\alpha\)-hydroxy-stigmast-22-en-6-one (5d)

Detected by GC-MS analysis. MS: \(m/z\) (%): 486 (M\(^{-}\), 3), 426(100), 408(18), 393(7).

5\(\beta\),6\(\beta\)-Epoxy-pregnan-20-oxo-3\(\beta\)-yl acetate (2e)

M.p.: 134–135 °C. MS: \(m/z\) (%): 374 (M\(^{-}\), 1), 314(100), 299(60), 297(60), 286(27), 271(10), 269(11).

\(^1\)H NMR: 0.60 (s, 3H, H-18), 1.00 (s, 3H, H-19), 2.03 (s, 3H, acetate), 2.13 (s, 3H, H-21), 3.09 (d, \(J = 2\) Hz, 1H, H-6), 4.7 (m, 1H, H-3).

3\(\beta\)-Acetoxy-5\(\alpha\)-hydroxy-pregnan-6,20-dione (3e)

Detected by GC-MS analysis and by \(^1\)H NMR in samples containing compound 5d. MS: \(m/z\) (%): 372 (M\(^{-}\), 1), 312(100), 187(45), 174(50), 161(28).

\(^1\)H NMR: 0.64 (s, 3H, H-18), 1.20 (s, 3H, H-19), 2.02 (s, 3H, acetate), 2.13 (s, 3H, H-21), 4.75 (m, 1H, H-3), 5.7 (s, 1H, H-6).

3\(\beta\)-Acetoxy-5\(\alpha\)-hydroxy-pregnan-6,20-dione (5e)

M.p.: 145–146 °C. MS: \(m/z\) (%): 390 (M\(^{-}\), 4), 360(100), 330(28), 312(14). \(^1\)H NMR: 0.6 (s, 3H, H-18), 0.8 (s, 3H, H-19), 2.02 (s, 3H, acetate), 2.13 (s, 3H, H-21), 2.85 (s, 1H, H-5, –OH), 5.00 (m, 1H, H-3).

3\(\beta\)-Acetoxy-5\(\alpha\)-hydroxy-pregnan-20-oxo-3\(\beta\)-yl acetate

M.p.: 146–147 °C. MS: \(m/z\) (%): 372 (M\(^{-}\), 7), 312(100), 294(14), 297(91), 297(56), 269(76), 251(13). \(^1\)H NMR: 0.88 (s, 3H, H-18), 1.05 (s, 3H, H-19), 2.06 (s, 3H, acetate), 2.24 (s, 3H, H-21), 3.12 (d, \(J = 2\) Hz, 1H, H-6), 4.7 (m, 1H, H-3), 5.68 (t, \(J = 2\) Hz, H-16).

5\(\beta\),6\(\beta\)-Epoxy-pregnan-6,20-dione

M.p.: 197–198 °C. MS: \(m/z\) (%): 388 (M\(^{-}\), 1), 328(100), 313(20), 295(6), 285(8), 267(17).
\( ^1H \) NMR: 1.00 (s, 6H, H-18 and H-19), 2.02 (s, 3H, acetate), 2.06 (s, 3H, H-21), 3.10 (d, \( J = 2 \) Hz, 1H, H-6), 3.70 (s, 1H, H-16), 4.70 (m, 1H, H-3).

3\( \beta \)-Acetoxy-5\( \alpha \)-hydroxy-16\( \alpha \),17\( \alpha \)-epoxy-pregnan-6,20-dione

M.p.: 176–177 °C. MS: \( m/z \) (%): 404 (M\(^+\), 2), 344(100), 373(15), 360(58), 300(27), 285(10), 267(14). \( ^1H \) NMR: 0.80 (s, 3H, H-19), 1.00 (s, 3H, H-18), 2.00 (s, 3H, acetate), 2.02 (s, 3H, H-21), 2.80 (t, \( J = 12 \) Hz, 1H, H-7\( \alpha \)), 3.70 (s, 1H, H-16), 5.00 (m, 1H, H-3).

3\( \beta \)-Acetoxy-16\( \alpha \),17\( \alpha \)-epoxypregn-5-en-7,20-dione

Detected by \( ^1H \) NMR in a sample containing the previous compound: \( ^1H \) NMR: 1.08 (s, 3H, H-18), 1.22 (s, 3H, H-19), 2.04 (s, 3H, acetate), 2.06 (s, 3H, H-21), 3.70 (s, 1H, H-16), 4.75 (m, 1H, H-3), 5.75 (s, 1H, H-6).