Caroate (Oxone®) Oxidation of 3β-Substituted Δ⁵-Steroids

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Abstract

We have previously described the utility of dioxiranes in the oxidation of 3β-substituted Δ⁵-sterols. They can be generated in situ from caroate (Oxone®; 2KHSO₅·KHSO₄·K₂SO₄) and a ketone. In the present report, we describe the oxidation of 3β-substituted Δ⁵-steroids by caroate alone to form oxysterols.

Keywords: Caroate, Oxone®, Steroid, Oxysterol

1. Introduction

Oxysterols are a group of sterols which, on the sterol nucleus or on the side chain of the molecule, bear one or more additional oxygen function groups, such as hydroxyl group(s), ketone group(s), or epoxide group(s), other than that at carbon-3. Closely related compounds may also be called "ox sterols." They are oxygenated derivatives of cholesterol and versatile intermediates of steroid biosynthesis. These compounds may be absorbed into the blood circulation as contaminants of cholesterol-containing diets, or come from lipoprotein oxidation or intracellular cholesterol catabolism [1, 2-7].

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Some oxysterols, such as 7-ketocholesterol (Figure 1a), 7α-hydroxycholesterol (Figure 1b), 7β-hydroxycholesterol (Figure 1c), cholesterol 5α, 6α-epoxide (Figure 1d), cholesterol 5β, 6β-epoxide (Figure 1e), cholestane-3β, 5α, 6β-triol (Figure 1f), 25-hydroxycholesterol (Figure 1g), 26-hydroxycholesterol (Figure 1h), 24-hydroxycholesterol (Figure 1i) and 24, 25-epoxycholesterol (Figure 1j), etc. have been detected in plasma and aortic tissues of humans and in experimental animals [4, 8-12].

Figure 1: Examples of oxysterols
The most remarkable activities of oxysterols are the regulation of cholesterol homeostasis [1, 13, 14]. As mentioned above, maintenance of cholesterol homeostasis in cells is very important; any disturbances may result in serious consequences. Many oxysterols are extraordinarily potent regulators of cellular cholesterol metabolism.

Even at relatively low concentrations, oxysterols display the capabilities of modulating the biosynthesis and esterification of cholesterol, uptake of lipoprotein-associated cholesterol and efflux of membrane cholesterol to extracellular acceptors.

In general, oxysterols represent a class of potent regulatory molecules with a wide variety of significant biological activities, including effects on cholesterol biosynthesis, membrane function, DNA synthesis, cell growth and proliferation. Further studies are needed to clarify the roles and relative importance of oxysterols in many processes. Since naturally-occurring oxysterols are unfortunately very limited with regard to structural types, available quantities, and reasonable cost, new methods to synthesize oxysterols with high efficiency and selectivity have been continuously developed to provide these compounds for biological investigations.

2. Discussion and Results

In our previous work, we have described the utility of dioxiranes in the oxidation of 3β-substituted Δ5-sterols. Dioxiranes are the smallest cyclic peroxides that contain a carbon atom. They can be generated in situ from caroate (Oxone®; 2KHSO5·KHSO4·K2SO4) and a ketone. Dioxiranes are versatile oxidizing agents. The most common reaction of dioxiranes is epoxidation, with nearly 1:1 ratios of α/β isomer products in all cases.
$\Delta^5$-Steroids with different side chains were epoxidized by dioxiranes generated in situ from several commercially available ketones. Although ketones function as catalyst, they were used in about an equivalent amount or large excess to accelerate the reaction [15].

When the caroate oxidation of steroids 1-3 (Figure 2) was carried out under acidic:

**Table 1: Oxidation of 3β-hydroxyandrost-5-en-17-one 1, pregnenolone 2, and cholesterol 3 by caroate under acidic conditions at room temperature**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>KHSO$_3$ loading [equiv.]</th>
<th>Reaction time [h]</th>
<th>Isolated yield of triols</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>20</td>
<td>75%</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>24</td>
<td>76%</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>48</td>
<td>73%</td>
</tr>
</tbody>
</table>

Conditions, that is, no potassium bicarbonate was used to neutralize the acidic proton which is contained in Oxone® and is produced during the reaction, steroid-3β, 5α, 6β-triols were obtained as single isomers (Figure 2, Table 1). The structure
Figure 2: Oxidation of 3β-hydroxyl Δ5-steroids by caroate under acidic conditions and acetylation of the trial products and stereochemistry of products was determined by comparing their NMR spectra [16, 17, 19-26] and melting points [16-18,20,25,27] and those of their 3,6-diacetate derivatives with reported data. The corresponding 5, 6-epoxides are believed to be the intermediates which produce the final 3β,5α,6β-triols by opening the epoxide ring in the acidic aqueous medium, because their formation was observed on TLC (thin layer chromatography) plates during the reaction courses. Actually, when a mixture of 5α,6α-epoxycholestan-3β-ol\textbf{10a} and 5β,6β- epoxycholestan-3β-ol\textbf{10b} (about 1:1 molar ratio) was treated with 0.2 M Oxone® in aqueous solution, the cholestane-3β,5α,6β-triol\textbf{6} was isolated with 90% yield as the only product (Figure 3). This observation is also consistent with reports [28-31] that the
Figure 3: Acids ring-opening reaction of 5,6-epoxycholestan-3β-ol. Nucleophilic ring-opening reaction of the steroidal 5,6-epoxide tends to afford a 5,6-diaxial product whether the epoxide ring is α-configuration or β-configuration. In the case of hydrolysis, both steroidal 5α, 6α-epoxide and steroidal 5β, 6β-epoxide give the same product which possesses the 5α, 6β-diol configuration.

Apparently, to obtain such a product, steroidal 5α, 6α-epoxide should be cleaved with inversion at C6 while the 5β, 6β-epoxide would be cleaved at the bond extending to C5 (Figure 3). For the α-epoxide, the approach of the nucleophile has to be from the β-face, therefore, relatively unhindered C6 is favored over C5; the β-epoxide, on the other hand, forces the approach of the nucleophile from the α-face. The reason that the reaction occurs selectively at C5 appears to be the formation of the more stable Trans ring junction.

The reactions under acidic conditions were faster and required less caroate than the corresponding reactions under neutral conditions for two reasons. One is that caroate
Figure 4: Nucleophilic ring-opening of steroidal 5,6-epoxides is more stable at lower pH; the other is that under acidic conditions less salt was used than under neutral conditions, therefore the solubility of substrate in the reaction solution was increased.

3. Experimental

3.1. General Methods

Procedure for recording of melting points (M.P.) and infrared (IR), $^1$H NMR, and mass (MS) spectra were those used previously [32]. Similarly, details concerning the use of thin-layer (TLC) and column chromatography have been described [33]. Solvent systems for TLC analysis were: 10-50% ether or ethyl acetate in toluene (by volumes).

3.2. Chemical Synthesis

3.2.1 Preparation of 3β, 5α, 6β-Trihydroxyandrostan-17-one, 4
To a mixture of 80 mL of tetrahydrofuran and 80 mL of water was added 1.44 g (4.99 mmol) of 3β-hydroxyandrost-5-en-17-one 1. The reaction mixture was stirred vigorously at room temperature. Oxone® was added in portions (~1.23 g of Oxone® each portion) until the starting material was used up as monitored by TLC. The reaction mixture was then diluted with water and extracted with dichloromethane. The organic layer was washed with saturated NaCl solution and dried over anhydrous MgSO₄.

After removal of the solvent, the crude product was recrystallized from acetone-methanol to afford 1.21 g (75% yield) of 3β, 5α,6β-trihydroxyandrostan-17-one 4 as white crystals. mp 299-301 °C (lit. 293-294 °C [16]; 298-301 °C [17]); ¹H NMR (400 MHz, DMSO-d₆) δ 4.50 (d, J = 4.2 Hz, 1H, 6β-OH), 4.21 (d, J = 5.7 Hz, 1H, 5α-OH), 3.80 (m, 1H, H-3α), 3.80 (s, 1H, 5α-OH), 3.34 (br, 1H, H-6α), 2.35 (dd, J = 19.2, 8.3 Hz, 1H), 1.03 (s, 3H, H-19), 0.76 (s, 3H, H-18); ¹³C NMR (100 MHz, DMSO-d₆) δ 220.1 (C-17), 74.4 (C-5), 73.9, 65.7, 50.6, 47.2, 44.9, 40.9, 38.0, 35.4, 33.3, 32.1, 31.6, 31.1, 29.7, 21.5, 20.0, 16.3, 13.5.

3.2.2 Preparation of 3β, 5α, 6β-Trihydroxypregn-20-one, 5

The method described above for the synthesis of triol 4 was used to convert 3β-hydroxypregn-5-en-20-one 2 (1.58 g, 4.99 mmol) to 3β, 5α, 6β-trihydroxypregn-20-one 5 (white crystals, 1.33 g, 76% yield). mp 252-254 °C (lit. 249-252 °C[20]); ¹H NMR (250 MHz, DMSO-d₆) δ 4.42 (d, J = 3.8 Hz, 1H, 6β-OH), 4.18 (d, J = 5.6 Hz, 1H, 3β-OH), 3.80 (m, 1H, H-3α), 3.68 (s, 1H, 5α-OH), 3.30 (br, 1H, H-6α), 2.56 (t, J = 8.7 Hz, 1H, H-17α), 2.04 (s, 3H, H-21), 1.01 (s, 3H, H-19), 0.50 (s, 3H, H-18); ¹³C NMR (62.5 MHz, DMSO-d₆) δ 208.6 (C-17), 74.3, 74.0, 65.7, 62.8, 55.8, 44.5, 43.7, 40.9, 37.8, 34.4, 32.0, 31.2, 31.1, 30.0, 24.1, 22.2, 20.7, 16.3, 13.2.
3.2.3 Preparation of Cholestane-3β, 5α, 6β-triol, 6

The method described above for the synthesis of triol 4 was used to convert cholest-5-en-3β-ol3 (1.93 g, 4.99 mmol) to cholestane-3β,5α,6β-triol 6 (white crystals, 1.53 g, 73% yield). mp 232-234 °C (lit. 233.5-235 °C [25]); 242-244 °C [27]); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 4.40 (d, \(J = 4.1\) Hz, lH, 6β-OH), 4.18 (d, \(J = 5.7\) Hz, lH, 3β-OH), 3.81 (m, lH, H-3α), 3.63 (s, lH, 5α-OH), 3.31 (br, lH, H-6α), 1.04 (s, 3H, H-19), 0.89 (d, \(J = 6.3\) Hz, 3H, H-21), 0.86 (d, \(J = 6.5\) Hz, 3H, H-26H or H-27), 0.85 (d, \(J = 6.5\) Hz, 3H, H-26 or H-27), 0.64 (s, 3H, H-18); \(^{13}\)C NMR (62.5 MHz, DMSO-\(d_6\)) \(\delta\) 74.3, 74.1, 65.7, 55.8, 44.5, 42.2, 40.9, 39.0, 37.7, 35.7, 35.3, 34.5, 32.0, 31.1, 30.0, 27.8, 27.4, 23.9, 23.3, 22.6, 22.4, 20.7, 18.5, 16.2, 11.9; MS (EI) \(m/z\) 420 (M\(^+\)), 402 (M\(^+\)-H\(_2\)O), 384 (M\(^+\)-2H\(_2\)O), 348, 262, 248, 244, 229, 211, 141, 107, 81.

3.2.4 Preparation of 3β, 6β-Bis (acetoxy)-5α-hydroxyandrostan-17-one, 7

In 10 mL of acetic anhydride was added 0.323g (1.00 mmol) of 3β,5α,6β-trihydroxyandrostan-17-one 4. The mixture was stirred and heated in a 120-140 °C oil bath for 2 h. It was then cooled and poured into 25 mL of ice water. The precipitate was collected by filtration, washed with cold water, and recrystallized from acetone-water to afford 0.326 g (80% yield) of 3β,6β-bis (acetoxy)-5α-hydroxyandrostan-17-one 7 as white crystals. mp 215-217 °C (lit. 216.5-217 °C [18]); \(^1\)H NMR (250 MHz, CDCl\(_3\)) \(\delta\) 5.12 (m, lH, H-3α), 4.74 (br, lH, H-6α), 2.66 (s, lH, O-H), 2.41 (dd, \(J = 19.0, 8.3\) Hz, lH), 2.07 (s, 3H, acetyl-H), 1.99 (s, 3H, acetyl-H), 1.14 (s, 3H, H-19), 0.86 (s, 3H, H-18); \(^{13}\)C NMR (62.5 MHz, CDCl\(_3\)) \(\delta\) 221.2 (C-17), 171.0 (acetyl, C=O), 170.5 (acetyl, C=O), 76.0, 74.7 (C-5), 70.8, 50.9, 48.0, 45.1, 38.7, 36.7, 36.0, 31.9, 31.6, 30.6, 30.4, 26.7, 21.8, 21.6, 21.5, 20.4, 16.4, 14.1.
3.2.5 Preparation of 3β, 6β-Bis (acetoxy)-5α-hydroxypregnan-20-one, 8

The method described above for the acetylation of triol 4 was used to convert 3β,5α,6β-trihydroxypregn-20-one 5 (0.351 g, 1.00 mmol) to 3β,6β-bis(acetoxy)-5α-hydroxypregn-20-one 8 [20] (white crystals, 0.352 g, 81% yield). mp 209-211 °C (lit. 207-209 °C [20]); 1H NMR (250 MHz, CDCl3) δ 5.14 (m, 1H, H-3α), 4.71 (br, lH, H-6α), 2.53 (t, J = 8.8 Hz, lH, H-17α), 2.11 (s, 3H, H-21), 2.07 (s, 3H, acetyl-H), 2.01 (s, 3H, acetyl-H), 1.14 (s, 3H, H-19), 0.62 (s, 3H, H-18); 13C NMR (62.5 MHz, CDCl3) δ 209.8 (C-20), 171.0 (acetyl, C=O), 170.5 (acetyl, C=O), 76.2, 74.9, 70.8, 63.8, 56.1, 45.0, 44.5, 39.1, 38.7, 36.9, 31.9, 31.7, 31.5, 30.9, 26.8, 24.5, 22.9, 21.6, 21.2, 16.5, 13.7.

3.2.6 Preparation of Cholestane-3β, 5α,6β-triol,3,6-diacetate, 9

The method described above for the acetylation of triol 4 was used to convert cholestane-3β,5α,6β-triol 6 (0.421 g, 1.00 mmol) to cholestane-3β, 5α, 6β-triol 3, 6-diacetate 9 [21,22,26] (white crystals, 0.452 g, 89% yield). mp 166-167 °C (lit. 165-166 °C [27]); 1H NMR (250 MHz, CDCl3) δ 5.15 (m, 1H, H-3α), 4.70 (br, lH, H-6α), 2.07 (s, 3H, acetyl-H), 2.02 (s, 3H, acetyl-H), 1.14 (s, 3H, H-19), 0.90 (d, J = 6.8 Hz, 3H, H-21), 0.86 (d, J = 6.4 Hz, 6H, H-26 and H-27), 0.68 (s, 3H, H-18); 13C NMR (62.5 MHz, CDCl3) δ 170.9 (acetyl, C=O), 170.4 (acetyl, C=O), 76.4, 75.1, 71.0, 56.4, 55.9, 45.2, 42.9, 40.1, 39.7, 38.7, 37.0, 36.3, 36.0, 32.0, 31.6, 30.9, 28.4, 28.2, 26.9, 24.3, 24.1, 23.0, 22.8, 21.7, 21.6, 21.2, 18.8, 16.5, 12.4.

4. Conclusion

In our previous studies, we have described the use of dioxiranes, generated in situ from caroate (Oxone®) and a ketone, for the oxidation of Δ5-sterols to epoxides.
The present work describes the utility of caroate alone to produce steroids-3β, 5α, 6β-triols as single isomers from Δ⁵-sterols.

5. References


