Journal of Chemistry and Biochemistry
June 2016, Vol. 4, No. 1, pp. 9-22
ISSN 2374-2712 (Print) 2374-2720 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jcb.v4n1a2
URL: https://doi.org/10.15640/jcb.v4n1a2

Caroate (Oxone®) Oxidation of 3β-Substituted Δ^5-Steroids

Nida A McKee[1], Alyssa M Parish[2], Zhihai Qiu[3] & Edward J Parish[4*]

Abstract

We have previously described the utility of dioxiranes in the oxidation of 3β-substituted Δ^5-sterols. They can be generated in situ from caroate (Oxone®; $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) and a ketone. In the present report, we describe the oxidation of 3β-substituted Δ^5-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].

[1] Department of Chemistry and Biochemistry, Auburn University, Auburn, Alabama 36849 USA.
& Department of Chemistry, University of North Sumatra, Medan, Indonesia.
Email: parisej@auburn.edu; Phone: 334-844-6986; Fax; 334-844-6959
[2] Department of Chemistry and Biochemistry, Auburn University, Auburn, Alabama 36849 USA.
[3] Department of Chemistry and Biochemistry, Auburn University, Auburn, Alabama 36849 USA.
[4] Department of Chemistry and Biochemistry, Auburn University, Auburn, Alabama 36849 USA.

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].

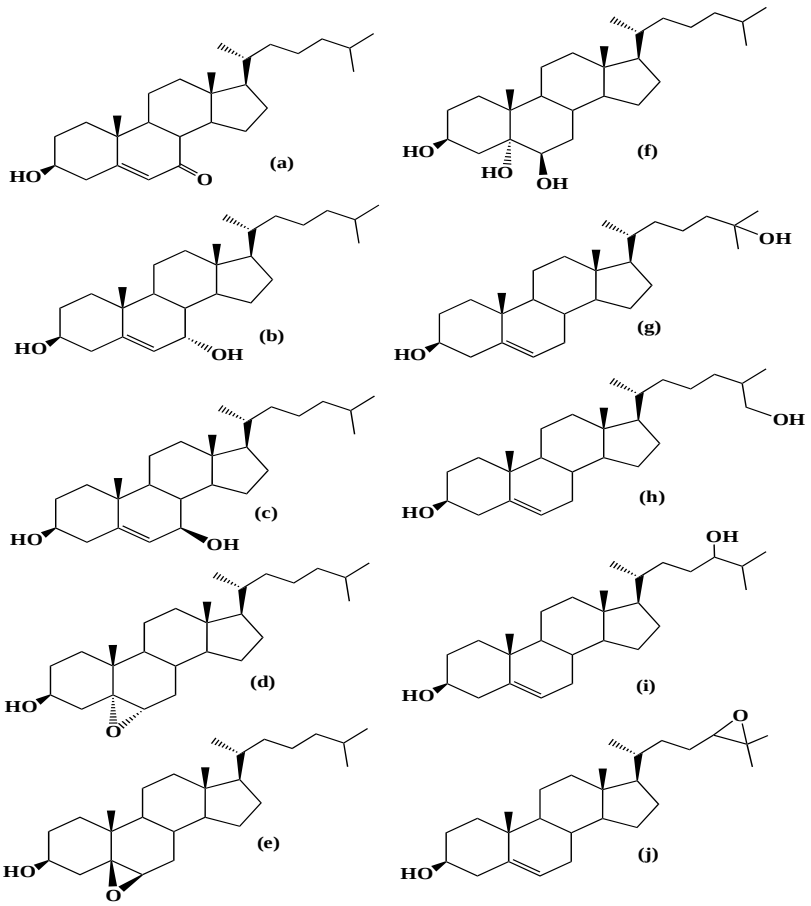


Figure 1: Examples of oxysterols

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 Δ^5-sterols. Dioxiranes are the smallest cyclic peroxides that contain a carbon atom. They can be generated *in situ* from caroate (Oxone®; $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) 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.

Δ^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

Substrate	$KHS0_5$ loading [equiv.]	Reaction time [h]	Isolated yield of triols
1	4	20	75%
2	6	24	76%
3	12	48	73%

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

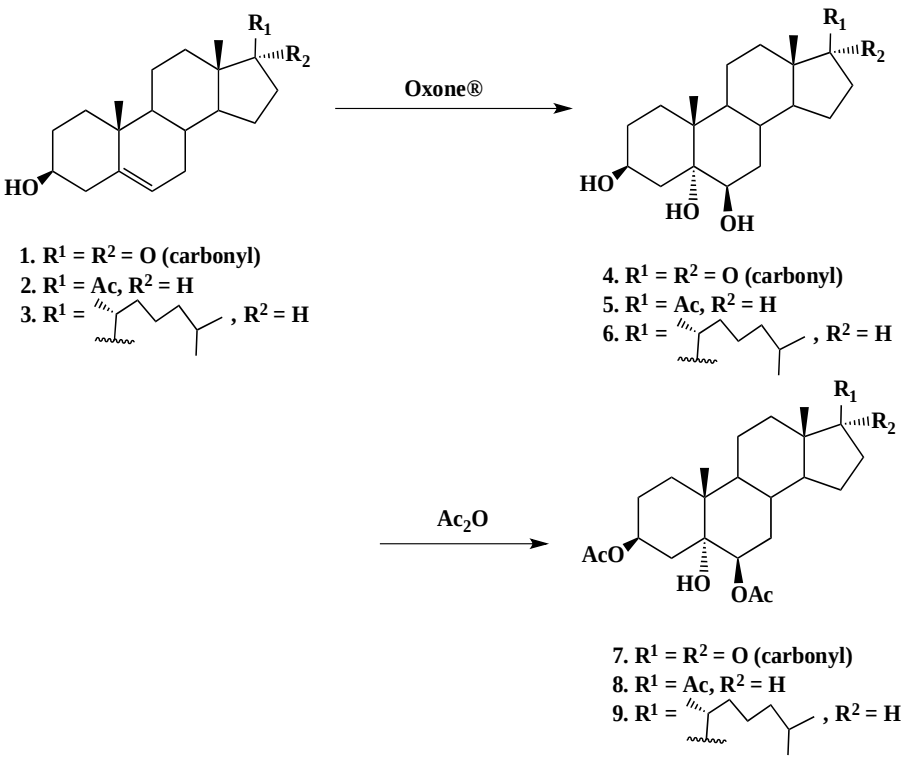
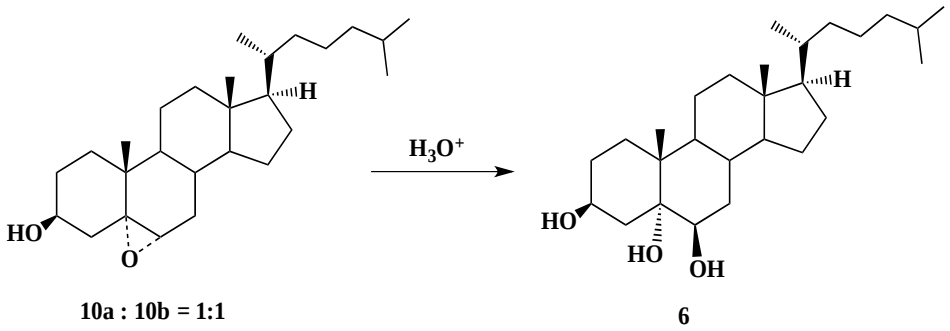


Figure 2: Oxidation of 3β-hydroxyl Δ^5-steroids by caroate under acidic conditions and acetylation of the trial products and stereochemistry of products was determined by comparing their NMR spectra [16, 17, 19-26] and melting points [16-18,20,25,27]and those of their 3,6-diacetate derivatives with reported 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**10a** and 5β,6β- epoxycholestan-3β-ol**10b** (about 1:1 molar ratio) was treated with 0.2 M Oxone® in aqueous solution, the cholestane-3β,5α,6β-triol**6** was isolated with 90% yield as the only product (Figure 3). This observation is also consistent with reports [28-31] that the



10a : 10b = 1:1 **6**

Figure 3: Acidic 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

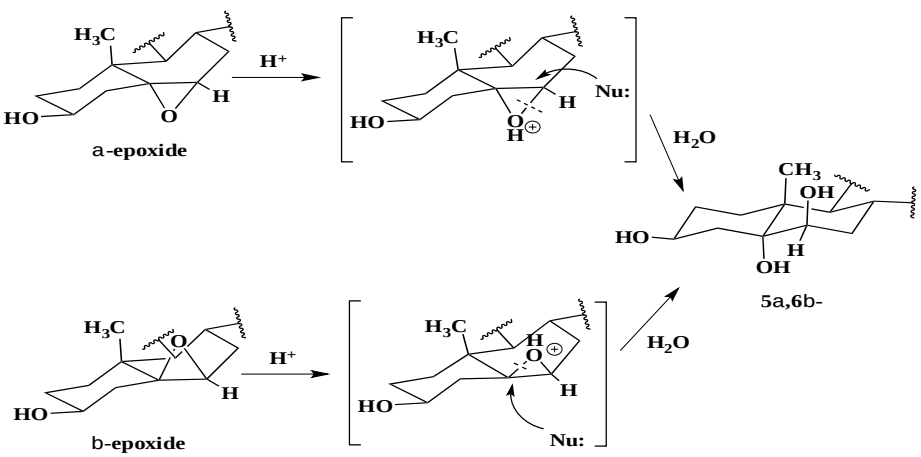


Figure 4: Nucleophilicring-opening of steroidal 5,6-epoxides is more stable at lower pH; the other is that under acidic conditions less salt was used than under neutral conditions, therefore the solubility of substrate in the reaction solution was increased.

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 $MgSO_4$.

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 ºC (lit. 293-294 ºC [16]; 298-301 °C [17]); ^{1}H NMR (400 MHz, DMSO-*d6*) δ4.50 (d, *J* = 4.2 Hz, lH, 6β-OH), 4.21 (d, *J* = 5.7 Hz, lH, 3β-OH), 3.80 (m, l H, H-3α), 3.73 (s, lH, 5α-OH), 3.34 (br, lH, H-6α), 2.35 (dd, *J* = 19.2, 8.3 Hz, lH), 1.03 (s, 3H, H-19), 0.76 (s, 3H, H-18); ^{13}C NMR (100 MHz, DMSO-*d6*) δ220.1 (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-Trihydroxypregnan-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-trihydroxypregnan-20-one **5** (white crystals, 1.33 g, 76% yield). mp 252-254 ºC (lit. 249-252 ºC[20]); ^{1}H NMR (250 MHz, DMSO-*d6*) δ4.42 (d, *J* = 3.8 Hz, lH, 6β-OH), 4.18 (d, *J* = 5.6 Hz, lH, 3β-OH), 3.80 (m, lH, H-3α), 3.68 (s, lH, 5α-OH), 3.30 (br, lH, H-6α), 2.56 (t, *J* = 8.7 Hz, lH, H-17α), 2.04 (s, 3H, H-21), 1.01 (s, 3H, H-19), 0.50 (s, 3H, H-18); ^{13}C NMR (62.5 MHz, DMSO-*d6*) δ208.6 (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-ol **3** (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-*d6*) δ4.40 (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-*d6*) δ74.3, 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$0), 384 (M^+-2$H_2$0), 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, $CDCl_3$) δ5.12 (m, lH, H-3α), 4.74 (br, lH, H-6α), 2.66 (s, lH, OH), 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, $CDCl_3$) δ221.2 (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-trihydroxypregnan-20-one **5** (0.351 g, 1.00 mmol) to 3β,6β-bis(acetoxy)-5α-hydroxypregnan-20-one **8 [**20] (white crystals, 0.352 g, 81% yield). mp 209-211 °C (lit. 207-209 °C [20]); ^{1}H NMR (250 MHz, $CDCl_3$) δ5.14 (m, lH, 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); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ209.8 (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** (0.421g, 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]); ^{1}H NMR (250 MHz, $CDCl_3$) δ5.15 (m, lH, H-3α), 4.70 (br, lH, H-6α), 2.07 (s, 3H, acetyl-H), 2.02 (s, 3H, acetyl-H), 1.15 (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); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ170.9 (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 Δ^5-sterols to epoxides.

The present work describes the utility of caroate alone to produce steroids-3β, 5α, 6β-triols as single isomers from Δ^5-sterols.

5. References

Schroepfer, G. J. Jr. (2000). Oxysterols: Modulators of Cholesterol Metabolism and Other Processes. Physiol. Rev. 80, 361-554.

Nourooz-Zadeh, J., Appelqvist, L. A. (1988). Cholesterol Oxides in Swedish Foods and Food Ingredients: Milk Powder Products. J. Food Sci. 53, 74-79.

Fontana, A., Antoniazzi, F., Ciavaita, M. L., Trivellone, E., Cimino, G. (1993). ^{1}H-NMR Study of Cholesterol Autooxidation in Egg Powder and Cookies Exposed to Adverse Storage. J. Food Sci. 58, 1286-1290.

Babiker, A., Diczfalusy, U. (1998). Transport of side-chain oxidized oxysterols in the human circulation. Biochim. Biophys. Acta 1392, 333-339.

Axelson, M., Larsson, O. (1996). 27-Hydroxylated Low Density Lipoprotein (LDL) Cholesterol Can Be Converted to 7α,27-Dihydroxy-4-cholesten-3-one (Cytosterone) before Suppressing Cholesterol Production in Normal Human Fibroblasts. J. Biol. Chem. 271, 12724-12736.

Bergstrom, S., Lindstredt, S., Samuelson, B., Corey, E. J., Gregoriou, G. A. (1958).The Stereochemistry of 7α-hydroxylation in the biosynthesis of Cholic acid from cholesterol. J. Am. Chem. Soc. 80, 2337-2338.

Watabe, T., Kanai, M., Isobe, M., Ozawa, N. (1981). The hepatic microsomal biotransformation of Δ^5-steroids to 5 α, 6 β-glycols via α - and β-epoxides. J. Biol. Chem. 256, 2900-2907.

Breuer, O., Björkhem, I. (1990). Simultaneous quantification of several cholesterol autoxidation and monohydroxylation products by isotope-dilution mass spectrometry. Steroids 55, 185-192.

Kudo, K., Emmons, G. T., Casserly, E. W., Via, D. P., Smith, L. C., Pyrek, J. St., Schroepfer, G. J. Jr. (1989). Inhibitors of sterol synthesis. Chromatography of acetate derivatives of oxygenated sterols. J. Lipid Res. 30, 1097-1111.

Mol, M. J. T. M., de Rijke, Y. B.,Demacker, P. N. M.,Stalenhoef, A. F. H. (1997). Plasma levels of lipid and cholesterol oxidation products and cytokines in diabetes mellitus and cigarette smoking: effects of vitamin E treatment. Atherosclerosis 129, 169-176.

Smith, L. L., Van Lier, J. E. (1970). Sterol metabolism Part 9.26-hydroxycholesterol levels in the human aorta. Atherosclerosis 12, 1-14.

Hodis, H. N., Crawford, D. W., Sevanian, A. (1991). Cholesterol feeding increases plasma and aortic tissue cholesterol oxide levels in parallel: further evidence for the role of cholesterol oxidation in atherosclerosis. Atherosclerosis 89, 117-126.

Lund, E., Bjoerkhem, I. (1995). Role of Oxysterols in the Regulation of Cholesterol Homeostasis: A Critical Evaluation. Acct. Chem. Res. 28, 241-249.

Morel, D. W., Lin, C. Y. (1996). Cellular biochemistry of oxysterols derived from the diet or oxidation in vivo. J. Nutr..Biochem. 7, 495-506.

Parish, E. J., Qiu, Z. (2004). Dioxirane oxidation of 3β-substituted Δ^5-steroids. Lipids 39, 805-809.

Kieslich, K. (1969). Mikrobiologischeumwandlung von 3β-hydroxy-5,6-epoxy-steroiden.Tetrahedron 25, 5863-5868.

Bensasson, C. M.; Hanson, J. R.; Hunter, A. C. (1998)The hydroxylation of Δ^5-androstenes by Cephalosporiumaphidicola.Phytochemistry49, 2355-2358.

Ehrenstein, M. (1941). Investigations on Steroids. V. Acetolysis of the Stereoisomeric5,6-Oxides and Preparation of the Acetates of 4-Androstene-3,17-Dione-6(α)-ol and 6(α)-Hydroxy-11-Desoxycorticosterone.J. Org. Chem. 6, 626-646.

Kovganko, N. V., Ananich, S. K. (2000). Reaction of pregnenolone and 3α,5-cyclo-5α-pregnan-6β-ol-20-one with trifluoroperacetic acid. Chem.Nat. Compd. 36, 381-383.

Caspi, E.,Balasubrahmanyam, S. N. (1963). Oxidation of Steroidal Ketones. III. Selenium Dioxide-Catalyzed Hydrogen Peroxide Oxidation of 4-En-3-ones.J. Org. Chem. 28, 3383-3386.

Tori, K., Komeno, T. (1965). NMR Studies on steroids—VII: Substituent effects due tosulfur-containing groups in ring a of 5α-steroids.Tetrahedron 21, 309-328.

Narayanan, C. R.,Sarma, M. R. (1968). Deshielding effect on neighboring protons on the esterification of a hydroxyl group.Tetrahedron Lett. 9, 1553-1556.

Konno, C., Hikino, H. (1976). ^{13}C nuclear magnetic resonance spectra of ethers and glycols.Tetrahedron 32, 325-331.

Meyer, W., Spiteller, G. (1997). Oxidized phytosterols increase by ageing inphotoautotrophic cell cultures of Chenopodiumrubrum.Phytochemistry 45, 297-302.

Li, S., Pang, J., Wilson, W. K., SchroepferJr, G. J. (1999). Sterol synthesis. Preparation andcharacterization of fluorinated and deuterated analogs of oxygenated derivatives of cholesterol. Chem. Phys. Lipids 99, 33-71.

Coxon, J. M., Hartshorn, M. P., Lane, G. A. (1970). NMR spectra of some 3,6-disubstituted-5α-oxygenated cholestanes.Tetrahedron 26, 841-844.

Witiak, D. T., Parker, R. A., Brann, D. R., Dempsey, M. E., Ritter, M. C., Connor, W. E.,Brahmankar, D. M. (1971).Biological evaluation in vivo and in vitro of selected 5α-cholestane-3β,5α,6β-triols analogs as hypocholesterolemic agents. J. Med. Chem. 14, 216-222.

Fieser, L. F.,Fieser, M. (1959). Steroids. Reinhold Publishing Corporation, New York, (pp 193-202).

Bowers, A., Denot, E.,Urquiza, R., Sanchez-Hidalgo, L. M. (1960).Some fission reactions of steroid 5,6-epoxides induced by boron trifluorideetherate. Tetrahedron 8, 116-125.

Mincione, E., Lanciano, F. (1980).Thallium nitrate as a reagent for the conversion of epoxides into α-hydroxynitrate esters and for the cleavage of aliphatic ethers.Tetrahedron Lett. 21, 1149-1150.

Korde, S. S., Baig, M. H. A., Desai, U. R., Trivedi, G. K. (1996).Differential behavior of (25R)-5,6-epoxyspirostan-22α-O-3β-ol and (25R)-5,6-epoxyspirostan-22α-O-3β,4β-diol toward Dowex.Steroids61, 290-295.

Cassidei, L., Fiorentino, M., Mello, R., Sciacovelli, O., Curci, R. (1987). Oxygen-17 and carbon-13 identification of the dimethyldioxirane intermediate arising in the reaction of potassium caroate with acetone.J. Org. Chem. 52, 699-700.

Murray, R. W., Jeyaraman, R., Pillay, M. K. (1987). Chemistry of dioxiranes. 6. Electronic effects in the oxidation of sulfides and sulfoxides by dimethyldioxiraneJ. Org. Chem. 52, 746-748.