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A facile synthesis of C-24 and C-25 oxysterols by *in situ* generated ethyl(trifluoromethyl)dioxirane

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ABSTRACT

Experiments were performed to compare the regioselective hydroxylation of the isopropyl C–H bond at C-25 in 5α-cholestan-3β-yl acetate by *in situ* generated dimethyldioxirane, methyl(trifluoromethyl)dioxirane, hexafluoro(dimethyl)dioxirane or ethyl(trifluoromethyl)dioxirane (ETDO). The dioxiranes were generated from the corresponding ketones and potassium peroxymonosulfate in *aq*. $NaHCO_3$, pH 7.5–8.0. Of the four dioxiranes examined, partially fluorinated, sterically bulky ETDO displayed the highest reactivity and regioselectivity. Using *in situ* generated ETDO, a facile, synthesis was developed for two naturally occurring oxysterols, *i.e.*, 25-hydroxycholesterol, as well as its 3-sulfate (overall yield of the sulfate, 24%) and 24-oxocholesterol (16%), starting from cholesterol.

1. Introduction

Oxysterols are oxygenated derivatives of cholesterol having a second oxygen function in addition to that at C-3 in the steroid nucleus and bearing an iso-octyl or modified iso-octyl side chain at C-17. Oxysterols are intermediates in bile acid biosynthesis in the hepatocyte [1] and also serve to transport cholesterol from brain to liver for conversion to bile acids [2]. These compounds have a variety of biological properties such as cytotoxicity, carcinogenicity, and mutagenicity. Moreover, oxysterols are present in human atherosclerotic plaque and are suggested to play an active role in plaque development. Although a number of structurally different oxysterols have been hitherto isolated from biological tissues, all of them have the 3β-hydroxy-Δ^5 steroid nucleus of cholesterol. For example, high levels of the oxysterol conjugates of (24*S*)-24-hydroxycholesterol 3-sulfate-24-glucuronide [3] and 5-cholesten-3β,25-diol 3-sulfate [4,5] have recently been found to be present in significant amounts in human tissues.

The remote-oxyfunctionalization of unactivated tertiary methine and/or secondary methylene C—H bonds in

Abbreviations: DMDO, dimethyldioxirane; EI, electron ionization; ETDO, ethyl(trifluoromethyl) dioxirane; EtOAc, ethyl acetate; FAB, fast atom bombardment; m.p., melting point; IR, infrared; HDDO, hexafluoro(dimethyl)dioxirane; ^{1}H NMR, proton nuclear magnetic resonance; ^{13}C NMR, carbon 13 nuclear magnetic resonance; LR-MS, low-resolution mass spectrum; HR-MS, high-resolution mass spectrum; PIM, positive ion mode; NIM, negative ion mode; TLC, thin layer chromatography; MTDO, methyl(trifluoromethyl)dioxirane; THF, tetrahydrofuran.

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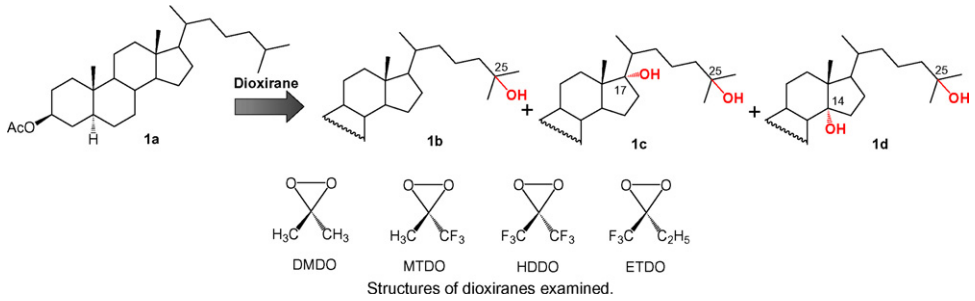

Fig. 1 – Chemical structures of dioxiranes examined and their oxidation products (1b–1d) with 5α-cholestan-3β-yl acetate (1a).

hydrocarbons by non-microbial or non-enzymatic method is of particular interest from the viewpoint of biomimetic chemistry. The hope is to develop facile syntheses of bioactive compounds from abundant natural sources using such "artificial enzymes" [6]. To this end, a variety of versatile oxygen-transfer reagents has been developed by many groups of workers.

Dioxiranes, the smallest three-membered cyclic peroxides that contain a carbon atom, are of particularly interest, because of their selective reactivity [7,8]. In addition, synthetic procedures based on dioxirane oxidations can be performed under extremely mild, nonhydrolytic conditions. The conversion of hydrophobic natural compounds to hydrophilic oxygen-containing derivatives would appear to be a highly useful achievement of dioxirane chemistry. For this purpose a variety of dialkyldioxiranes [9,10], their halogenated analogs [11–17] and chiral dioxiranes [8,18,19] have been employed.

Our previous papers have reported the utility of dimethyldioxirane (DMDO) as an effective oxygen-donor to the specific tertiary and/or secondary C–H bonds in a variety of structurally different bile acids and steroids [20–22]. In these studies, a concentrated DMDO/$CHCl_3$ solution (see below) [23–27] was prepared from acetone and potassium peroxymonosulfate (Oxone®, $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$). A concentrated solution of methyl(trifluoromethyl)dioxirane (MTDO), generated from 1,1,1-trifluoro-2-propanone (TFP) [11–16] has been shown to be superior to DMDO in reactivity and selectivity [7,15] but to prepare this reagent, a large amount of expensive TFP is required.

As part of our ongoing program to develop an improved, biomimetic oxidant system [28,29], we report here the use of ethyl(trifluoromethyl)dioxirane (ETDO), which *in situ* is generated efficiently from commercially available 1,1,1-trifluoro-2-butanone and Oxone® in a reaction flask. We compared the efficiency of hydroxylation at C-25 of 5α-cholestan-3β-yl acetate (**1a**) by DMDO, MTDO, ETDO, and hexafluoro(dimethyl)dioxirane (HDDO) (Fig. 1). We were able to develop a facile synthesis of two naturally occurring oxysterols, 25-hydroxycholesterol as such and its 3-sulfate (**2c**) and 24-oxocholesterol (**3b**), from cholesterol (**4**) by using ETDO generated *in situ*. Previously reported chemical syntheses of these oxysterols are either multistep procedures with an extremely low-yield from **4** or alternatively, use an expensive natural product as the starting material.

2. Experimental

2.1. General procedure

Melting points (m.p.) were determined on a micro hot-stage apparatus and are uncorrected. IR spectra were obtained in KBr discs on a JASCO FT-IR 460 plus spectrometer (Tokyo, Japan). ^1H and ^{13}C NMR spectra were obtained on a JEOL JNM-EX 270 FT instrument at 270 and 68.8 MHz, respectively. Low-resolution mass (LR-MS) spectra were recorded with a JEOL GCmate gas chromatograph/mass spectrometer at 70 eV electron ionization (EI) or fast atom bombardment (FAB) probe using the positive (PIM) or negative ion mode (NIM). High-resolution mass (HR-MS) spectra were also measured using a JEOL GCmate with an EI probe under the PIM. Capillary gas chromatographic (CGC) analysis was carried out by Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) equipped with a flame ionization detector using temperature programming (from 260 to 300 °C, 2 °C/min); a chemically bonded fused-silica capillary column (25QC3/BPX5; 25 m × 0.32 mm i.d.; film thickness, 0.25 μm; SGE, Yokohama, Japan) was fitted with the instrument. Normal phase TLC for nonsulfated compounds was performed on pre-coated silica gel plates (0.25 mm layer thickness; E.Merck, Darmstadt, Germany) using hexane–ethyl acetate mixtures (95:5–60:40, v/v) as the developing solvent. Reversed-phase TLC for the sulfated compounds was carried out on pre-coated RP-$18F_{254S}$ plates using methanol–water–acetic acid mixtures (90:10:1, v/v/v) as the developing solvent. A Sep-Pak Vac tC_{18} cartridge (5 g; Waters, Milford, MA, USA) was used for solid-phase extraction.

Oxone® was purchased from Sigma–Aldrich Com. (St. Louis, MO, USA). Acetone, trifluoro-2-propanone, 1,1,1-trifluro-2-butanone, and hexafluoroacetone were available from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan).

2.2. General oxidation procedure of 5α-cholestan-3β-yl acetate (1a) with dioxiranes generated in situ

To a solution of **1a** (0.23 mmol) in CH_2Cl_2 (1 mL) in a three-necked reaction flask fitted with a dry-ice/acetone condenser, a pressure equalizing graduated separatory funnel and a silicone rubber septum, Oxone® (2.3 mmol) and water (3 mL) were added. To the resulting two-layer system, the different ketones (23 mmol) were added by a syringe through the septum. The

resulting suspension was vigorously stirred in an ice-bath and adjusted to mildly alkaline conditions of pH 7.5–8.0 with 1 M *aq*. $NaHCO_3$. As the reaction proceeded, the suspension changed gradually to a clear solution. After stirring at room temperature for 12 h, the reaction product was extracted with CH_2Cl_2. The combined extract was washed with water, dried with Drierite®, and evaporated *in vacuo*. The above procedure was repeated several times until most of **1a** was exhausted; the reaction was monitored by TLC and CGC.

2.2.1. 5α,6β-Dibromocholestan-3β-yl acetate (5)

This compound was prepared by the usual method. m.p. 109–111 °C. (lit. 112–113 °C [30]). IR (KBr), ν_{max} cm^{-1}: 1741 (C=O). ^1H NMR ($CDCl_3$), δ: 0.71 (3H, s, 18-CH_3), 0.85 and 0.88 (each 3H, s, 26- and 27-CH_3), 0.91 (3H, d, *J* 5.4, 21-CH_3), 1.46 (3H, s, 19-CH_3), 2.05 (3H, s, $OCOCH_3$), 4.83 (1H, m, 6α-H), 5.48 (1H, br. m, 3α-H). LR-MS (FAB^+), *m/z*: 611 (M+Na, 4%), 529 (M−AcOH, 26%), 447 (M−Br, 53%), 415 (M−S.C.–AcOH, 6%), 385 (11%), 367 (M−Br_2–AcOH, 100%).

2.2.2. 5α,6β-Dibromo-25-hydroxycholestan-3β-yl acetate (6) (large-scale preparation)

The reaction was carried out by an essentially identical procedure mentioned above. Thus, a solution of the 5α,6β-dibromo-3β-acetate (**5**; 5 g, 8.5 mmol) in CH_2Cl_2 (25 mL) was added in a three-necked reaction flask. A vigorously stirred suspension of 1,1,1-trifluoro-2-butanone (83 mL, 850 mmol) was added to 100 mL Oxone® (50 g, 85 mmol) in distilled water (100 mL) and stirring was continued. The pH of the suspension was kept at 7.5–8.0 with 1 M *aq*. $NaHCO_3$ during the period of reaction. After stirring at room temperature for 12 h, the reaction product was extracted with CH_2Cl_2 and the combined extract was washed with water, dried with Drierite®, and evaporated *in vacuo*. The procedure was repeated three times (total reaction time, 27 h). The reaction product was purified by passage through a column of silica gel (100 g). Elution with hexane–EtOAc (1:9, v/v) afforded the starting compound **5**; 1.1 g (20%). Continued elution with hexane–EtOAc (2:8, v/v) gave the desired 3β-acetoxy-5α,6β-dibromo-25-hydroxy compound **6**, which was recrystallized from EtOAc–methanol as colorless prisms: yield, 2.3 g (45%). m.p. 120–122 °C (lit. 127–128 °C [12]). IR (KBr) ν_{max} cm^{-1}: 1723 (C=O), 3521 (OH). ^1H NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 5.4, 21-CH_3), 1.21 (6H, s, 26- and 27-CH_3), 2.05 (3H, s, $OCOCH_3$), 4.83 (1H, m, 6α-H), 5.48 (1H, br. m, 3α-H). LR-MS (FAB^+), *m/z*: 627 (M+Na, 12%), 527 (M−AcOH, 100%), 505 (M−Br–H_2O, 28%), 465 (M−AcOH–Br, 36%), 445 (M−AcOH–H_2O–Br, 92%), 415 (M−S.C.–AcOH, 20%), 367 (M−AcOH–H_2O–Br_2, 68%).

2.2.3. 3β,25-Dihydroxycholest-5-ene (2b)

To a solution of the 5α,6β-dibromo-25-hydroxy-3β-acetate **6** (300 mg, 0.5 mmol) dissolved in ether (9 mL) and acetic acid (100 μL), zinc powder (300 mg) was added in one portion and the suspension was vigorously stirred at room temperature for 1 h. Excess zinc was removed by filtration, and the mother liquor was washed with brine, dried with Drierite® and evaporated to dryness. Recrystallization of the residue from EtOAc–methanol afforded the unsaturated 3β-acetoxy-25-hydroxy-Δ^5 compound **2a** in the form of colorless prisms: yield, 200 mg (90%). m.p. 139–140 °C. (lit. 139–140 °C [31]). IR (KBr) ν_{max} cm^{-1}: 1731 (C=O), 3313 (OH). ^1H NMR ($CDCl_3$), δ: 0.68 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 5.4, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.21 (6H, s, 26- and 27-CH_3), 2.03 (3H, s, $OCOCH_3$), 4.61 (1H, br. m, 3α-H), 5.38 (1H, d, *J* 2.7, 6-H). LR-MS (FAB^+), *m/z*: 467 (M+Na, 19%), 384 (M−AcOH, 44%), 367 (M−AcOH–H_2O, 100%), 289 (M−S.C.–part of ring D, 9%), 255 (M−S.C.–AcOH, 19%), 213 (M−S.C.–AcOH–part of ring D–CH_3, 16%).

A solution of the 3β-acetate **2a** (200 mg, 0.45 mmol) in 5% methanolic KOH (20 mL) was refluxed for 1 h. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2, and the solution was washed with water, dried with Drierite® and evaporated *in vacuo*. Recrystallization of the oily residue from *aq*. methanol gave the 3β,25-dihydroxy-5-ene **2b** as colorless needles: yield, 163 mg (90%). m.p. 178–180 °C. (lit. 178–180 °C [32]). IR (KBr), ν_{max} cm^{-1}: 3301 (OH). ^1H NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 5.4, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.21 (6H, s, 26- and 27-CH_3), 3.50 (1H, br. m, 3α-H), 5.34 (1H, br. s, 6-H). ^{13}C NMR ($CDCl_3$) δ: 11.9 (C-18), 18.7 (C-21), 19.4 (C-19), 20.7 (C-23), 21.1 (C-11), 24.3 (C-15), 28.2 (C-16), 29.2 and 29.3 (C-26 and C-27), 31.6 (C-2), 31.9 (C-7), 31.9 (C-8), 35.7 (C-20), 36.4 (C-22), 36.5 (C-10), 37.2 (C-1), 39.8 (C-12), 42.3 (C-4 and C-13), 44.4 (C-24), 50.1 (C-9), 56.0 (C-17), 56.7 (C-14), 71.1 (C-25), 71.8 (C-3), 121.7 (C-6), 140.7 (C-5). LR-MS (EI), *m/z*: 402 (M^+, 65%), 384 (M−H_2O, 100%), 369 (M−H_2O–CH_3, 53%), 366 (M−$2H_2O$, 21%), 351 (M−$2H_2O$–CH_3, 34%), 317 (M−H_2O–ring A–part of ring B, 13%), 299 (M−$2H_2O$–ring A–part of ring B, 29%), 273 (M−S.C., 75%), 255 (M−H_2O–S.C., 31%), 245 (24%), 231 (M−S.C.–ring D, 22%), 213 (M−H_2O-CH_3–S.C.–part of ring D, 43%). HR-MS (EI), calculated for $C_{27}H_{46}O_2$ [M^+], 402.3498; found *m/z*: 402.3498.

2.2.4. 3β-Sulfooxy-25-hydroxycholest-5-ene (2c, as sodium salt)

To a solution of the 3β,25-dihydroxy-5-ene (30 mg, 0.07 mmol) in dry pyridine (2 mL), sulfur trioxide–trimethylamine complex (30 mg, 0.22 mmol) was added, and the suspension was stirred at room temperature for 1 h [33]. The reaction mixture was poured onto ice-cooled petroleum ether (20 mL) and the precipitated solid was collected by filtration. After being washed with petroleum ether, the solid product was dissolved in methanol (1 mL). The resulting solution was adjusted to pH 8 by adding 1N NaOH, diluted with water (10 mL), and then loaded onto a preconditioned Sep-Pak Vac tC_{18} cartridge. The cartridge was successively washed with water (20 mL) and then with 20% methanol (20 mL), and the desired 3β-sulfooxy-25-hydroxy-5-ene (**2c**) was eluted with methanol (20 mL). After evaporation of the solvent, recrystallization of the residue from methanol–EtOAc gave the analytically pure **2c** in the form of colorless amorphous solids: yield, 25 mg (70%). m.p. 164–165 °C. IR (KBr) ν_{max} cm^{-1}: 3451 (OH). ^1H NMR (CD_3OD) δ: 0.72 (3H, s, 18-CH_3), 0.96 (3H, d, *J* 5.4, 21-CH_3), 1.03 (3H, s, 19-CH_3), 1.17 (6H, s, 26- and 27-CH_3), 4.13 (1H, br. m, 3α-H), 5.38 (1H, br. s, 6-H). LR-MS (FAB^-), *m/z*: 481 (M^-, 71%), 306 (10%), 199 (12%), 168 (15%), 153 (100%), 122 (22%), 97 (HSO_4^-, 68%), 80 (SO_3^-, 38%). HR-MS (FAB^-), calculated for $C_{27}H_{45}O_5S$, 481.2987; found *m/z*: 481.2992.

2.2.5. 5α,6β-Dibromocholest-24-en-3β-yl acetate (8)

A mixture of the 5α,6β-dibromo-25-hydroxyl-3β-acetate **6** (2 g, 3.3 mmol) in 1,4-dioxane (50 mL) and conc. H_2SO_4 (4 mL) was stirred at 0 °C for 2 h. Ice-water was added to the mixture and

the reaction product was extracted with EtOAc. The combined organic layer was washed successively with 5% *aq*. $NaHCO_3$ and brine, dried with Drierite®, and evaporated to dryness. The brown residue was chromatographed on a column of silica gel (60 g), eluting with hexane–EtOAc (95:5, v/v). Recrystallization of a homogeneous fraction from EtOAc–methanol gave 5α,6β-dibromocholest-24-en-3β-yl acetate (**8**) as colorless needles: yield, 1.4 g (70%). m.p. 102–103.5 °C. IR (KBr) ν_{max} cm^{-1}: 1735 (C=O). ^1H NMR ($CDCl_3$) δ: 0.71 (3H, s, 18-CH_3), 0.93 (3H, d, *J* 5.4, 21-CH_3), 1.46 (3H, s, 19-CH_3), 1.59 and 1.60 (each 3H, s, 26- and 27-CH_3), 2.08 (3H, s, –$OCOCH_3$), 4.83 (1H, m, 6α-H), 5.09 (1H, t, *J* 5.4, 24-H), 5.48 (1H, br. m, 3α-H). ^{13}C NMR ($CDCl_3$) δ: 12.2 (C-18), 17.6 (C-27), 18.6 (C-21), 20.1 (C-19), 21.3 (C-11), 24.0 (C-15), 24.7 (C-23), 25.7 (C-26), 26.2 (C-7), 28.1 (C-16), 30.8 (C-6), 35.6 (C-20), 36.0 (C-22), 36.5 (C-2), 37.2 (C-1), 39.6 (C-4), 41.9 (C-10), 41.9 (C-12), 42.7 (C-13), 47.2 (C-8), 55.1 (C-9), 56.0 (C-17), 56.1 (C-14), 74.0 (C-3), 88.1 (C-5), 125.1 (C-24), 131.0 (C-25), 170.4 (–O<u>C</u>OCH$_3$). LR-MS (FAB$^+$) *m*/z: 609 (M+Na, 9%), 585 (M^+, 6%), 527 (M−AcOH, 78%), 505 (19%), 473 (M−S.C., 19%), 447 (M−AcOH–Br, 69%), 365 (M−Br_2–AcOH, 100%), 329 (25%), 307 (44%). HR-MS (FAB$^+$), calculated for $C_{29}H_{46}O_2NaBr_2$ [M+Na]$^+$, 607.1762; found, *m*/z: 607.1764.

2.2.6. *24ξ-Hydroxycholest-5-en-3β-yl acetate (**9b**)*

To a magnetically stirred solution of the 3β-acetoxy-5α,6β-dibromo-5-ene **8** (1.2 g, 2.0 mmol) in dry tetrahydrofuran (THF) (20 mL), 1.0 M BH_3/THF solution (20 mL) was added dropwise with ice-bath cooling. The mixture was stirred at room temperature for 1 h under a nitrogen stream. NaOH (3N, 10 mL) and then 30% H_2O_2 (10 mL) were slowly added with ice-bath cooling, and the resulting mixture was stirred at 0 °C for 30 min. The product was extracted with $CHCl_3$, and combined extract was washed with water, dried with Drierite®, and evaporated to dryness. The oily residue, which is consisted of an epimeric mixture (**9a**) of 24R- and 24S-hydroxy-5α,6β-dibromides, was dissolved in ether (35 mL) and acetic acid (0.4 mL). Zinc powder (1.0 g) was added to the mixture with vigorous stirring and the resulting suspension was stirred at room temperature for 1 h. Excess zinc was removed by filtration, and the mother liquor was washed with brine, dried with Drierite®, and evaporated to dryness. Chromatography of the oily residue on a column of silica gel (50 g) and elution with benzene-EtOAc (96:4, v/v) afforded an epimeric mixture of the title compound **9b**, which recrystallized from aqueous methanol as colorless amorphous solids: yield, 540 mg (60%). m.p. 133–134 °C. IR (KBr) ν_{max} cm^{-1}: 1737 (C=O), 3348 (OH). ^1H NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.89–0.94 (9H, m, 21-, 26- and 27-CH_3), 1.02 (3H, s, 19-CH_3), 2.03 (3H, s, –$OCOCH_3$), 3.33 (1H, m, 24ξ-H), 4.62 (1H, br. m, 3α-H), 5.38 (1H, br. s, 6-H). LR-MS (FAB$^+$), *m*/z: 467 (M+Na, 15%), 385 (M−AcOH, 100%), 367 (85%), 283 (17%), 255 (M−AcOH–S.C., 27%), 213 (M−AcOH–S.C.–part of ring D-CH_3, 19%). HR-MS (FAB$^+$), calculated for $C_{29}H_{48}O_3Na$ [M+Na]$^+$: 467.3501; found, *m*/z: 467.3500.

2.2.7. *24-Oxocholest-5-en-3β-ol (**3b**)*

Jones reagent (1 mL) was added dropwise to a solution of the 24ξ-hydroxy-Δ^5-3β-acetate **9b** (500 mg, 1.1 mmol) in acetone (25 mL) under 0 °C, and the mixture was stirred for 30 min at room temperature. Isopropanol (2 mL) was added, and the oxidation product was extracted with CH_2Cl_2 (50 mL). The combined organic layer was washed with water, dried over Drierite®, and evaporated to dryness. Recrystallization of the oily residue from EtOAc gave analytically pure 24-oxocholest-5-en-3β-yl acetate (**3a**) as colorless needles: yield, 450 mg (90%). m.p. 128–130 °C (lit. 127–128 °C [34]; 130–132 °C [35]). IR (KBr) ν_{max} cm^{-1}: 1703 (C=O, ketone), 1730 (C=O, ester). ^1H NMR ($CDCl_3$) δ: 0.67 (3H, s, 18-CH_3), 0.92 (3H, d, *J* 8.1, 21-CH_3), 1.02 (3H, s, 19-CH_3), 1.08 and 1.10 (each 3H, s, 26- and 27-CH_3), 2.03 (3H, s, $OCOCH_3$), 4.60 (1H, br. m, 3α-H), 5.38 (1H, d, *J* 2.7, 6-H). LR-MS (FAB$^+$), *m*/z: 465 (M+Na, 13%), 383 (M−AcOH, 100%), 365 (10%), 255 (M−H_2O–S.C., 12%), 213 (M−AcOH–CH_3–S.C.–part of ring D, 10%).

Alkaline hydrolysis of **3a** (500 mg, 1.1 mmol) with 5% methanolic KOH, as described for the preparation of **2b**, gave 24-oxochol-5-en-3β-ol (**3b**), which recrystallized from *aq*. methanol as colorless prisms: yield, 440 mg (99%). m.p. 131–133 °C (lit. 133–134 °C [34]). IR (KBr) ν_{max} cm^{-1}: 1714 (C=O), 3381 (O–H). ^1H NMR ($CDCl_3$) δ: 0.68 (3H, s, 18-CH_3), 0.92 (3H, d, *J* 8.1, 21-CH_3), 1.01 (3H, s, 19-CH_3), 1.09 (each 3H, s, 26- and 27-CH_3), 3.52 (1H, m, 3α-H), 5.35 (1H, br. s, 6-H). ^{13}C NMR ($CDCl_3$) δ: 11.9 (C-18), 18.3 (C-21), 18.4 and 18.5 (C-26 and C-27), 19.4 (C-19), 21.1 (C-11), 24.2 (C-15), 28.1 (C-16), 29.8 (C-2), 31.6 (C-7), 31.9 (C-8 and C-22), 35.4 (C-20), 36.5 (C-10), 37.2 (C-1 and C-23), 39.7 (C-12), 40.8 (C-25), 42.3 (C-4 and C-13), 50.1 (C-9), 55.9 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-6), 140.7 (C-5), 215.5 (C-24). LR-MS (EI) *m*/z: 400 (M^+,100%), 385 (M−CH_3, 22%), 382 (M−H_2O, 72%), 367 (M−CH_3–H_2O, 35%), 357 (M−part of S.C.<$CH(CH_3)_2$>, 9%), 339 (M−H_2O–part of S.C., 10%), 314 (52%), 299 (29%), 289 (M−H_2O–ring A–part of ring B, 34%), 271 (43%), 255 (M−H_2O–S.C., 47%), 229 (M−H_2O–S.C.–part of ring D, 29%), 213 (M−H_2O–CH_3–S.C.–part of ring D, 78%). HR-MS (EI), calculated for $C_{27}H_{44}O_2$ [M^+]: 400.3341; found *m*/z: 400.3337.

3. Results and discussion

As mentioned above, dioxiranes are powerful and versatile oxidants, particularly for the remote-oxyfunctionalization of unactivated tertiary and/or secondary C–H bonds in substrates, in analogy with cytochrome P450 enzymes *in vivo*. Volatile and labile dioxiranes can be generated by a large excess of an appropriate ketone and Oxone® under conditions where pH is controlled using $NaHCO_3$ as buffer. The generated dioxirane was collected either as an isolated stock solution in the parent ketone [9–15,23–27] or in its concentrated form by extraction into $CHCl_3$ [20–22,26] (or CCl_4 [27]). The dioxirane solution can then be stored in a −20 °C freezer to prevent decomposition. Otherwise, dioxiranes are generated *in situ* in a reaction flask [25,27]. For example, DMDO could be generated to a concentration of *ca*. 0.10 M as an acetone solution [23,24], which could still be further concentrated (to *ca*. 0.18 M) by extraction with $CHCl_3$ [20–22,26] or CCl_4 [27]. Using the isolated (or concentrated) solution of dioxirane allows the oxidation to be performed under extremely mild, strictly neutral, nonhydrolytic conditions. However, the isolation method has a practical drawback, with the exception of DMDO, since a large excess amount of expensive ketone precursor (*e.g.*, TFP) is required. The *in situ* generation method of dioxiranes using the expensive ketones has been shown to be feasible particularly in preparative, large-scale reactions, but

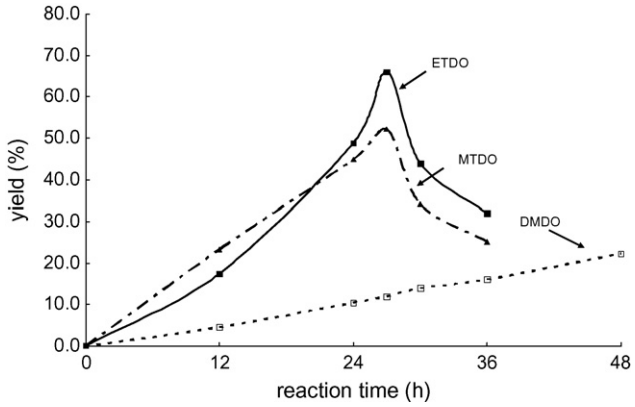

Fig. 2 – Time-course of the conversion (%) of 5α-cholestan-3β-yl acetate (1a) to 25-hydroxy-5α-cholestan-3β-yl acetate (1b) by dioxiranes generated *in situ*.

a detailed study has not yet been reported except for DMDO [8,25,27].

This reasoning led us to examine the reactivity and regioselectivity of dioxiranes generated *in situ* under identical experimental conditions. Our initial effort was directed to define optimal conditions for the *in situ* generation of dioxiranes. We tested the previously unreported HDDO and ETDO as well as the known DMDO and MTDO. We used 5α-cholestan-3β-yl acetate as the target molecule (**1a**).

As described in detail in Section 2, each of the four variants of dioxiranes was generated *in situ* at room temperature from acetone (or 1,1,1-trifluoroacetone, hexafluoroacetone, and 1,1,1-trifluro-2-butanone) and Oxone® in water. To the suspension was added a solution of **1a** in CH_2Cl_2. It was essential to maintain the pH of the two-layer system (CH_2Cl_2/water) at 7.5–8.0 by adding *aq*. $NaHCO_3$ during the period of reaction with vigorous stirring. Fig. 2 shows the time-course of the conversion (%) of **1a** to 25-hydroxy-5α-cholestan-3β-yl acetate (**1b**). According to a previous report [12], **1a** was converted to **1b** in 78% yield at 0 °C for 3 h with an isolated stock solution of MTDO/TFP (*ca*. 0.8 M) but in 30% yield at 20°C for 24 h with a DMDO/acetone solution (0.1 M), indicating that the former is more effective than the latter. Under the conditions examined in our experiments, **1a** was transformed into **1b** regioselectively except for one case (see below), but yields and optimal reaction times were significantly influenced by the structures of the oxidants. Thus, when **1a** was subjected to the DMDO oxidation, the formation of **1b** gradually increased until the maximum conversion of *ca*. 40% was obtained after 72 h (six times). Unexpectedly, the hydroxylation of **1a** with fully fluorinated HDDO did not proceed at all. On the contrary, partially fluorinated MTDO [12,36] and ETDO, both of which have trifluoromethyl and alkyl groups, showed a similar reactivity and inserted an oxygen atom at C-25 effectively to give **1b** in the maximum conversion of *ca*. 52% and 66%, respectively, with a considerable reduction in the reaction time (27 h, three times). The prolonged reaction with MTDO (or ETDO) decreased rapidly the conversion to **1b** and caused a second-order reaction forming increasing amounts of the double-functionalized 17α,25- and 14α,25-diols (**1c** and **1d**, respectively) [20]. A comparison of the *in situ* generated MTDO and ETDO reactions also revealed that after 27 h, yields of **1a**, **1c**, **1d**, and unknowns by MTDO were 39%, 4%, 4%, and 1%, respectively, but those by ETDO were 13%, 6%, 8%, and 7%, respectively. The result evidently suggests that a more bulky ETDO is superior to MTDO in reactivity and attacks preferentially a less sterically hindered C–H bond at C-25 in **1a**. Although a mechanism of the observed differences in the reactivity among the four dioxiranes examined is unclear, the coexistence of both an electron-withdrawing fluoro group and an electron-donating alkyl group in dioxirane molecules may be one of the essential factors.

Based on above the results, our next effort was to develop a facile synthesis of naturally occurring 25-hydroxycholesterol and its 3-sulfate (**2c**) as well as 24-oxocholesterol (**3b**) starting from cholesterol (**4**). The synthetic routes are shown in Fig. 3. A key step in the syntheses involves the hydroxylation at C-25 of 5α,6β-dibromocholestan-3β-yl acetate (**5**) [12] with *in situ* generated ETMO. In order to prevent simultaneous oxidation of the 3β-hydroxyl group and the Δ^5-bond in **4** by ETDO, it was converted to the 3β-acetoxy-5α,6β-dibromide **5** in two steps [37]. Treatment of **5** with *in situ* generated ETDO at room temperature for 27 h afforded the expected 25-hydroxy-5α,6β-dibromide **6** in 45% isolated yield after chromatographic purification. Procedures and product isolation are quite straightforward, since ETDO is quite volatile and easily removed. The ^1H and ^{13}C NMR spectral data of **6** were in good agreement with those reported in the literature [12]. Debromination of **6** with zinc powder in ether/acetic acid, followed by alkaline hydrolysis of the resulting product with methanolic KOH gave 25-hydroxycholesterol (**2b**, 90% yield), which is a key intermediate useful in the synthesis of various oxysterols.

The compound **2b** was sulfated cleanly at C-3 with sulfur trioxide-trimethylamine complex to afford the desired 25-hydroxycholesterol 3-sulfate (**2c**) [33]. The analytically pure **2c** was efficiently obtained by solid-phase extraction using a Sep-Pak Vac tC_{18} cartridge; the total yield from cholesterol (**4**) was 24%. The sulfate **2c** has recently been identified in primary rat hepatocytes [4] and also detected in the nuclei of normal human liver tissue [5].

When the 25-hydroxy-5α,6β-dibromide **6** was subjected to the dehydration reaction with conc. H_2SO_4, it was converted to the Δ^{24}-unsaturated compound **8** exclusively; the $\Delta^{25(26)}$-isomer was not formed at all. The trisubstituted Δ^{24}-structure in **8** was characterized by the olefinic carbon signals appearing at 125.1 (C-24) and 131.0 (C-25) ppm, respectively, in the ^{13}C NMR and the proton signal resonating at 5.09 ppm (H-24) as triplet (*J* 5.4 Hz) in the ^1H NMR. In addition, the ^{13}C chemical shifts of the C_{20}–C_{27} in the side chain were in good agreement with those reported for desmosterol (3α-hydroxycholest-5,24-diene) [38].

Hydroboration of **8** with BH_3/THF complex, followed by oxidative cleavage of the resulting alkylborane with alkaline hydrogen peroxide gave an anti-Markovnikovs' type regioselective formation of the 24ξ-hydroxy compound **9a**. Without isolating each of the 24*R*- and 24*S*-hydroxy epimers at this stage, debromination of **9a** with zinc powder in ether–acetic acid afforded the 24ξ-hydroxy-Δ^5 compound **9b** in isolated yield of 60%, which showed a single spot on TLC and a sin-

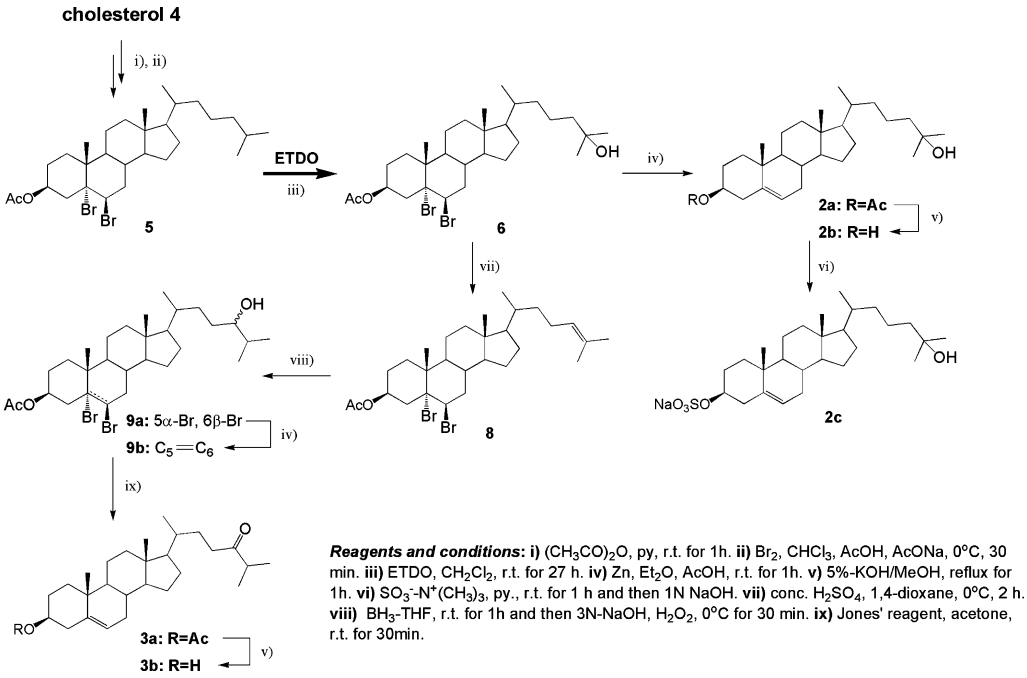

Fig. 3 – Synthetic route to 3β-sulfooxy-25-hydroxycholest-5-ene (2c) and 24-oxocholesterol (3b) from cholesterol (4).

gle peak by CGC. Finally, oxidation of **9b** with Jones reagent, followed by the alkaline hydrolysis of the resulting oxidation product **3a** gave the desired 24-oxocholesterol (**3b**) in an excellent yield of 99%. Thus, **3b**, which has been isolated from various biological fluids [34,39,40] was obtained from cholesterol (**4**) in eight steps with overall yield of 16%. The present method for syntheses of oxysterols starting from cholesterol (**4**) is very simple and inexpensive.

In conclusion, ETDO generated *in situ* from 1,1,1-trifluoro-2-butanone/Oxone® system is a new, attractive synthetic reagent that can be used for regioselective oxyfunctionalization of readily available natural compounds such as cholesterol. Further applications of ETDO to the synthesis of bioactive molecules are now being conducted in our laboratory.

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