A highly efficient, stereoselective oxyfunctionalization of unactivated carbons in steroids with dimethyldioxirane †

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Oxyfunctionalization of unactivated methine carbon atoms with dimethyldioxirane (DMDO) is studied for various substituted steroids related to the 5β-cholane and 5α-cholestane series. A highly efficient, stereoselective, one-step remote oxidation of specific methine carbons is attained by DMDO oxidation under mild conditions to give a variety of novel mono- and dihydroxylated steroids. The reactivity and site selectivity of remote oxyfunctionalization is influenced significantly by the structural and steric environments as well as by the degree of electron density of the target methine carbon atoms in the molecules. The non-enzymatic procedure may be usefully applied to effective and short-step syntheses of bioactive steroids.

Introduction

One topic of the recent major interest in organic synthesis is so-called 'biomimetic synthesis', which involves direct remote oxyfunctionalization of specific unactivated methine and methylene carbon atoms in alkanes,[1] in analogy with enzyme-controlled reactions *in vitro*. It is well known that the cytochrome P-450 oxidase-dependent system found in mammalian liver oxidizes regio- and stereoselectively unactivated carbon atoms of aliphatic and aromatic hydrocarbons to produce the corresponding hydroxylated compounds.[2] Such one-step oxyfunctionalization is specially attractive as an efficient and convenient chemical transformation to afford biologically and physiologically important compounds. Therefore, a variety of oxygen-transfer reagents and catalysts have been recently developed which include, for example, ozonation on silica gel,[3] photochemical functionalization of the esters of benzophenone carboxylic acid derivatives[1,4] or with peracids,[5] the $AgSbF_6$ system,[6] the Gif oxidation system [iron cluster–metallic zinc–pyridine–(aqueous) acetic acid–triplet oxygen],[7] the manganese(III) porphyrin–iodosylbenzene system,[8,9] ruthenium porphyrin–2,6-dichloropyridine *N*-oxide,[9,10] and perfluorodialkyloxaziridines.[11] Although these methods are of particular importance from the viewpoint of biomimetic synthesis, they still have several drawbacks such as low yields of desired compounds, less straightforward and selective processes, and/or the use of costly reagents and catalysts.

Recently, the successful use of dimethyldioxirane (DMDO) and its analogs as a new class of powerful and versatile oxygen-transfer reagents has been demonstrated for a variety of substrates.[12] In particular, DMDO, generated readily and inexpensively by the reaction of acetone with $KHSO_5$, inserts an oxygen atom into unactivated methine C–H bonds of aliphatic alkanes and cycloalkanes to give the corresponding tertiary alcohols and ketones, respectively. Such non-enzymatic remote oxyfunctionalization of unactivated carbon atoms at ring D and side chain in steroids seems to be useful in the synthesis of bioactive steroidal medicines, starting from abundantly available bile acid and sterol sources in Nature.[13]

Bovicelli *et al.*[14] have shown that DMDO oxidation of both 5α-cholestan-3-one and 3β-acetoxy-5α-cholestane causes direct one-step insertion of the hydroxy function into the unactivated tertiary methine C–H at C-25 site-selectively to give the corresponding 25-hydroxylated steroids. Subsequently, the same authors[15] and Dixon *et al.*[16] have reported independently that by a similar DMDO treatment of 5β-coprostane and 5β-cholane series of steroids as their methyl ester–peracetate derivatives, the methine carbon at C-5 is hydroxylated preferentially to afford the corresponding 5β-hydroxylated derivatives stereoselectively. In more recent work, Cérre *et al.*[17] have revealed that the methyl ester–peracetate derivatives of 5β-bile acids related to chenodeoxycholic and ursodeoxycholic acids containing a 7-acetoxy group gave a significant amount of 17α- or 14α-hydroxylated product in addition to the respective 5β-hydroxylated ones. These intriguing results in the DMDO oxidation products of 5α- and 5β-steroids prompted us to a more detailed study of what factors control the reactivity and site selectivity of direct oxyfunctionalization of unactivated carbons in steroids. This paper presents additional details and observations encountered in the DMDO oxyfunctionalization of structurally different 5β-cholane and 5α-cholestane series of steroids under strictly identical and controlled conditions.

Results and discussion

In order to prevent the simultaneous oxidation of hydroxy groups present in starting compounds to carbonyl, all the substrates examined were derivatized to their completely acetylated derivatives (**1**–**8**) prior to DMDO reaction. A concentrated DMDO solution (0.33–0.35 M) in $CHCl_3$,[17] instead of conventional DMDO solution (up to 0.11 M) in acetone,[16] was used, because the reaction rate of *O*-insertion by DMDO is accelerated.

† Electronic supplementary information (ESI) available: ^{13}C NMR chemical shifts for starting compounds **1**–**8** and oxyfunctionalization products **9**–**36** (Table S1). See http://www.rsc.org/suppdata/p1/b1/b104938k/

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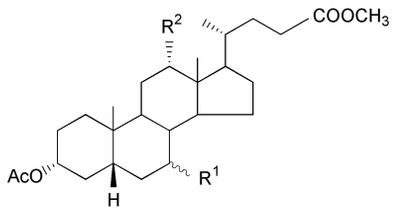
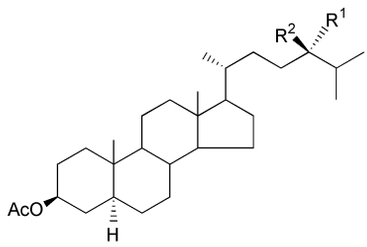
The results are compiled in Table 1. Treatment of substrates **1**–**8** with the concentrated DMDO solution in $CHCl_3$ usually resulted in the formation of several oxyfunctionalization products, and their composition changed significantly upon repeating the DMDO reaction (see Experimental section). Prolonged exposure of the substrates to DMDO usually caused an increasing amount of the formation of dioxygenated products, along with monooxygenated ones. For isolation purposes, two to four runs of the repeating reaction (total reaction times 24–48 h) were undertaken, because the ratios of the starting compounds : total monooxyfunctionalized products were minimized under the experimental conditions employed.

Oxidation of the peracetate derivatives of methyl 5β-cholanoates **1**–**5** differing from one another in the number, position, and stereochemical configuration of acetoxy groups at the C-3, -7 and/or -12 positions in the 5β-steroid nucleus

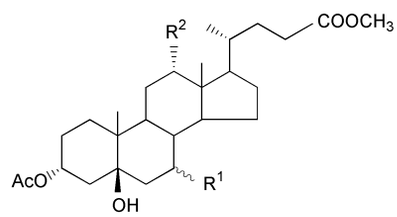


	R^1	R^2
1	H	H
2	α-OAc	H
3	β-OAc	H
4	H	OAc
5	α-OAc	OAc

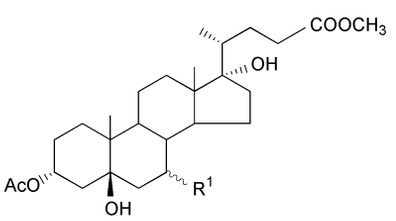
Ac = $-COCH_3$



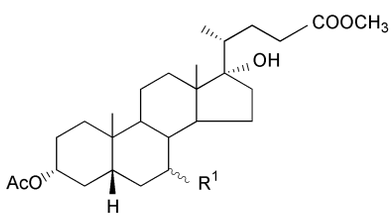
	R^1	R^2
6	H	H
7	CH_3	H
8	H	C_2H_5
30	CH_3	OH
34	OH	C_2H_5



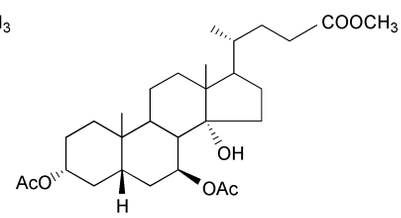
	R^1	R^2
9	H	H
11	α-OAc	H
17	β-OAc	H
20	H	OAc
23	α-OAc	OAc



	R^1
10	H
13	α-OAc
19	β-OAc



	R^1
12	α-OAc
14	β-OAc



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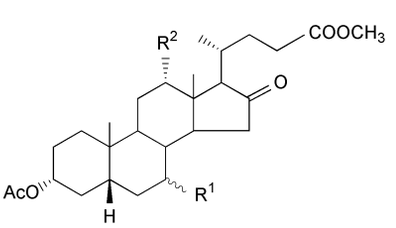
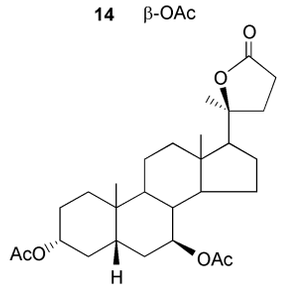


	R^1	R^2
16	β-OAc	H
21	H	OAc
24	α-OAc	OAc



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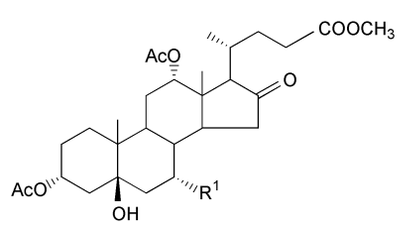
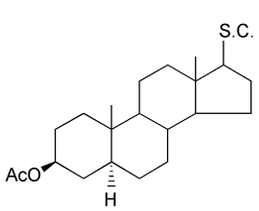
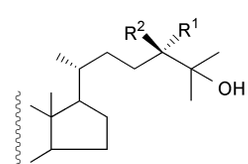


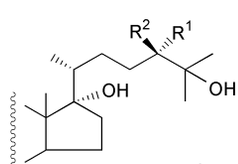
	R^1
22	H
25	OAc



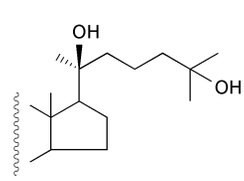
side chain (S.C.) :



	R^1	R^2
26	H	H
31	CH_3	H
35	H	C_2H_5



	R^1	R^2
27	H	H
36	H	C_2H_5



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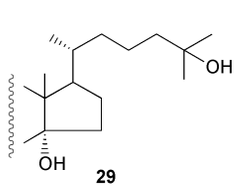

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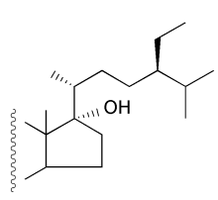

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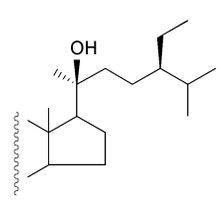

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Table 1 Oxidation products of various steroids with DMDO

Substrate	Reaction time (*t*/h)	Products (yield, %)[a]		
		Starting compound recovery (%)	Monooxygenated compounds[b]	Dioxygenated compounds[b]
1	36	(9)	**9** (48)	**10** (36), uk 1 (7)
2	12	(67)	**11** (21), **12** (10)	**13** (2)
2	24	(44)	**11** (35), **12** (14)	**13** (7)
2	36	(29)	**11** (39), **12** (18)	**13** (14)
2	48	(21)	**11** (45), **12** (16)	**13** (18)
3	12	(82)	**14** + **17** (2), **15** (6), **16** (6), **18** (4)	
3	24	(69)	**14** + **17** (4), **15** (10), **16** (11), **18** (6)	
3	36	(46)	**14** + **17** (7), **15** (15), **16** (16), **18** (11)	**19** (5)
3	48	(21)	**14** + **17** (14), **15** (20), **16** (22), **18** (15)	**19** (8)
4	36	(23)	**20** (36) **21** (26)	**22** (15)
5	12	(89)	**23** (11)	
5	24	(78)	**23** (20), **24** (2)	
5	36	(58)	**23** (33), **24** (5)	**25** (3), uk 1 (1)
5	48	(51)	**23** (40), **24** (2)	**25** (4), uk 1 (3)
5	60	(42)	**23** (44), **24** (4)	**25** (6), uk 1 (4)
6	12	(42)	**26** (37), uk 1 (13)	**27** (3), **28** + **29** (5)
6	24	(20)	**26** (45), uk 1 (15)	**27** (8), **28** + **29** (12)
6	36	(5)	**26** (39), uk 1 (8)	**27** (19), **28** + **29** (29)
7	24	(11)	**30** (31), **31** (40)	uk 1 (3), uk 2 (5), uk 3 (7), uk 4 (3)
7	36	(6)	**30** (16), **31** (25)	uk 1 (7), uk 2 (15), uk 3 (21), uk 4 (10)
8	12	(53)	**33** (5), **34** (10), **35** (22), uk 1 (7)	**36** (3)
8	24	(16)	**32** (5), **33** (8), **34** (18), **35** (32), uk 1 (5)	**36** (10), uk 2 (2), uk 3 (64)

[a] Determined by capillary GC; for conditions, see Experimental section. [b] uk ('unknown') means that the compound could not be isolated in a pure form and its structure remained uncertain.

(*cis* A/B-ring junction) furnished useful information about factors governing the oxyfunctionalization of unactivated methine carbons in the molecules. Thus, the major reaction product of methyl 3α-acetoxy-5β-cholan-24-oate **1** with DMDO for 36 h was identified as the corresponding 5β-hydroxylated compound **9** in a yield of 48%, in accord with a previous finding (Table 1).[15,17] Compound **1**, however, also suffered simultaneous double oxyfunctionalization at the C-5 and C-17 positions to produce compound **10** in a reasonable yield (36%). Both the 5β- and 17α-hydroxylation proceeded stereoselectively and the configuration of the resulting C–OH was the same as that of the original methine C–H bond.[15] Interestingly, the expected 17α-monohydroxylated compound was not formed.

When methyl 3α,12α-diacetoxy- and 3α,7α,12α-triacetoxy-5β-cholan-24-oate **4** and **5** possessing an axially oriented 12α-substituent were subjected to DMDO oxidation for 36 h, respective 5β-hydroxylated compounds (**20** and **23**) were similarly isolated as the main reaction products (36 and 33% yields, respectively).[15–17] In these cases, however, 16-oxo compounds (**21** and **24**) and their doubly oxyfunctionalized 5β-hydroxy-16-oxo derivatives (**22** and **25**) were formed concurrently in 3–26% yield, indicating that DMDO also oxidizes unactivated methylene carbons to give the corresponding ketones. So far as this reaction has been examined, the ketogenesis observed for the methylene carbon at C-16 in steroids was the sole example of this, supporting the generalization[12b,c] that tertiary methine C–H bonds are attacked by DMDO more readily than are secondary methylene C–H bonds.

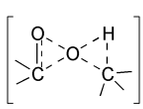
On the other hand, methyl 3α,7α-diacetoxy-5β-cholan-24-oate **2**, having an axial 7α-acetoxy group, was hydroxylated site selectively at both the C-5 and C-17 methine carbons to give the corresponding 5β- and 17α-monohydroxylated (**11** and **12**)[17] and their dihydroxylated (**13**) compounds in 39, 18 and 14% yield, respectively. Of further interest was the 7β-epimer **3** of compound **2**, which on treatment with DMDO (36 h) afforded three major components, *i.e.*, 14α-, 16- and 20*S*-oxygenated compounds (**15**, 15%; **16**, 16%; and **18**, 11% yield, respectively), along with three minor components, *i.e.*, 5β- and 17α-monohydroxylated and 5β,17α-dihydroxylated compounds (**17**, **14** and **19**, respectively). The results were very different from previous findings,[17] in which only compounds **14**, **15** and **17** were isolated as the DMDO reaction products, probably owing to a difference in the experimental conditions. In compound **18**, the formation of the γ-lactone at the C-17 position may occur by intramolecular esterification between the 20β-hydroxy group and the side chain carboxy group. The above observations apparently imply that the presence or absence of an acetoxy function at the C-7 position in the substrate and its stereochemical nature effect significantly both the reactivity and the site selectivity of the DMDO oxyfunctionalization.

Under identical experimental conditions, the regioselectivity of oxyfunctionalization of the 5α-cholestane series of steroids (**6**–**8**) having a *trans* A/B-ring junction and a C_8–C_{10} alkyl side chain at the C-17 position differed much from those observed for the 5β-cholane series of steroids mentioned above. Oxidation of 5α-cholestan-3β-yl acetate **6** by DMDO for 24 h led to the formation of several oxidized products having hydroxy functions in the alkyl side chain, which were characterized as 17α,25- and 20*S*,25-dihydroxylated derivatives (**27**, 8% yield and **28**, respectively), together with the expected 25-monohydroxylated one (**26**, 45% yield) as the main product.[14] A small amount of 14α,25-dihydroxylated product (**29**) was also formed. However, 17α- and 20*S*-monohydroxylated 5α-cholastan-3β-yl acetates could not be isolated.

Meanwhile, when 5α-ergostan-3β-yl acetate **7** was treated with DMDO for 24 h, the corresponding 24*R*- and 25-hydroxylated compounds (**30**, 31% and **31**, 40% yield, respectively) were isolated as the major products, accompanied by a small amount (total yield, 17%) of four dihydroxylated mixtures (by GC-MS). Exposure of 5α-stigmastan-3β-yl acetate **8** with DMDO for 24 h similarly produced the corresponding 24*S*- and 25-hydroxylated compounds (**34**, 18% and **35**, 32% yield, respectively), but in this case at least three other compounds were also isolated concurrently in addition to a small amount of three unidentified components; those were identified as the 17α- and 20*S*-hydroxylated (**32**, 5% and **33**, 8% yield respectively) and 17α,25-dihydroxylated (**36**, 10% yield) derivatives. The above results suggest that the reactivity and regioselectivity of the *O*-insertion reaction are also strongly influenced by an alkyl substituent attached directly to methine carbons in the substrates.

The structures of the individual isolated products (**9**–**36**), which include a number of novel compounds, were characterized by their IR, ^{1}H- and ^{13}C-NMR (Table S1), † and MS data. The validity of the structural assignments was further confirmed by comparing the previously reported literature data for ^{1}H-NMR,[3,7,15–21] ^{13}C-NMR,[14–23] and MS[17,20,24,25] of analogous steroids. For instance, identification of 16-oxo steroids **16**, **21**, **22**, **24**, and **25** was made as follows: in the ^{1}H-NMR spectrum, the 18-methyl proton signal is shifted downfield by *ca.* 0.15–0.16 ppm, compared with that of the respective parent compounds **3**–**5**,[7b] while the C-20 carbon signal in the ^{13}C-NMR spectrum is shifted to up field by 4.5–4.9 ppm due to the γ-effect of the ketogenesis, and the resonance position of the C-16 carbon is in good agreement with that (δ_C 217.4 ppm) of methyl 3,7,16-trioxo-5β-cholan-24-oate [unpublished]; diagnostic fragment ions occurring in the MS of the 16-oxo steroids,[24] which are usually absent in those of 15-oxo analogs,[25] supported the structures proposed. Furthermore, the presence of the cyclic γ-lactone ring at the C-17 position in compound **18** was elucidated by a characteristic absorption peak at 1771 cm^{-1} in the IR spectrum, a considerable down field shift of the C-21 methyl protons (relative to that of **3**) at δ 1.45 as a singlet,[26] and the disappearance of the methyl ester protons in the ^{1}H-NMR spectrum as well as those of the C-20 (δ_C 88.4) and C-24 (δ_C 177.4) signals and the absence of a C-25 methyl ester signal ($\delta_C \approx 51.5$) in the ^{13}C-NMR spectrum, and intense fragment ions at *m*/*z* 414 (14%) and 354 (85%) forming by the loss of one and two acetic acid molecules from the molecular ion, respectively.[17]

With the aid of suitably chosen substrates, the reactivity and site selectivity of *O*-insertion into an unactivated tertiary methine C–H induced by DMDO were found to be dependent significantly not only on the structural and steric environments, but also on the degree of electron density of the methine carbon under consideration. According to a previous study,[12b] DMDO inserts an oxygen atom into methine C–H bonds in such a way that the electrophilic attack of a DMDO molecule is directed along the O–O bond axis toward the relevant methine carbon atoms of a substrate and proceeds *via* the transition state of a bridge structure. The stereochemical configuration of the resulting C–OH is, therefore, retained completely.



Assuming that a DMDO molecule approaches from the same side as that of the methine C–H bond, the position of *O*-insertion can be rationally deduced from the structural and/or steric environments of the substrates. Thus, access of a DMDO molecule to C-5α C–H in compounds **6**–**8** from the α-face on ring A is prevented by steric hindrance, whereas that to C-5β C–H in compounds **1**–**5** from the β-face on ring A is much less hindered; convenient 5β-hydroxylation in 5β-steroids is, therefore, due to the favored steric factor at this position. A recent study showed that *O*-insertion into 5β-bile acid methyl ester–peracetate derivatives occurs preferentially at C-5β C–H, and that the subsequent competitive *O*-insertion at other tertiary C–H positions is dependent on a combined effect of the presence of deactivating groups (see below) and/or the steric environment of methine carbons under consideration.[17] In a similar manner, attack of a DMDO molecule on C-17α C–H in compound **2** having an axial 7α-acetoxy group is not hindered by a steric interaction to give compounds **11**–**13**, and C-14α C–H and C-16 CH_2 in compound **3** having an equatorial 7β-acetoxy group shows no significant steric hindrance to yield compounds **15**, **16** and **18**, together with the concurrent formation of compounds **14**, **17** and **19**. In compounds **1**–**3**, 14α- and/or 17α-hydroxylation takes place, but they do not occur at all in analogous compounds **4** and **5**, in which an axially oriented 12α-acetoxy group effectively masks both the 14α- and 17α-position.

A comparison of the oxidation products of compounds **6**–**8** further clarified that the presence of electron-donating alkyl substituents such as methyl, ethyl, isopropyl or dimethyl attached directly to a target methine carbon is favorable to the reaction and accelerates the reactivity of *O*-insertion site selectively, as compared with the other tertiary C–H positions, verifying the electrophilicity of DMDO.[12] For example, compound **6** having a dimethyl group at C-25 was converted exclusively into the 25-hydroxylated derivatives **26**–**29** in 95% yield after 36 h, indicating that DMDO inserts relatively rapidly into the C-25 C–H bond. A similar trend was also observed in 24-alkylated sterol acetates **7** and **8** having both methyl (or ethyl) and isopropyl groups to give **24**- and **25**-hydroxylated compounds (**30**, **31**, **34**, **35**, and **36**) concurrently as the major oxidation products.

In contrast, *O*-insertion reaction with DMDO was deactivated by the presence of electron-withdrawing groups such as an acetoxy function and a methyl ester situated in the vicinity of a target methine carbon. In fact, the total conversion of compounds **1**–**5** into the corresponding oxyfunctionalization products decreased according to an increasing number of acetoxy groups: **1**, 91%; **2**–**4**, 54–77%; **5**, 42% for 36 h reaction. Furthermore, so long as the methyl ester **1** is situated at C-24, no 20*S*-oxygenation took place, but analogous **6** with a C_8 alkyl side chain resulted in the formation of 20*S*-hydroxylated product (**28**). The observation indicates that a methyl ester at C-24 in **1** is also a deactivating group against DMDO reaction. In particular, for compound **5** possessing four deactivating groups (three acetoxy groups and one methyl ester), total conversion into the oxyfunctionalized products (**23**–**25**) was only 54%, even though the DMDO reaction was carried out over a period of 60 h.

In conclusion, the oxygen-transfer reaction induced by DMDO into unactivated methine carbons in 5α- and 5β-steroids was effectively achieved under simple overall procedures and mild conditions to give the corresponding mono- and/or dioxygenated derivatives stereoselectively in reasonable isolated yields. The reactivity and site selectivity of this remote oxyfunctionalization depended on a combined effect of structural and steric environments of the substrates as well as the degree of electron density on the target methine carbon atoms. Since the remote oxyfunctionalization in ring D and side chain in bile acids and sterols, which are abundantly available from natural sources, is a key transformation into bioactive steroids such a brassinolides, ecdysonic compounds, 25-hydroxyvitamin D derivatives, and/or cardiotonic steroids, the method described herein may be usefully utilized for promising non-enzymatic syntheses.

Experimental

Materials and methods

Mps were determined on an electric micro hot stage and are uncorrected. Infrared (IR) spectra were obtained on a Bio Rad FTS-7 FT-IR spectrometer (Philadelphia, USA) for samples in KBr tablets. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were obtained on a JEOL JNM-EX 270 FT NMR instrument (Tokyo, Japan) at 270 and 67.80 MHz, respectively, with $CDCl_3$ containing 0.1% Me_4Si as the solvent; chemical shifts are expressed as δ/ppm relative to Me_4Si. ^{13}C-NMR signals corresponding to the methyl (CH_3), methylene (CH_2), methine (CH), and quaternary (C) carbons were differentiated by means of DEPT experiments. Low-resolution-electron impact mass spectra (EI-MS) were recorded on a JEOL JMC-Automass 150 gas chromatography (GC)–mass spectrometry at 70 eV. High-resolution EI-MS were

recorded on a JEOL DX-303 mass spectrometer at 70 eV. High-resolution MS were also performed using a JEOL JMS-LCmate double-focusing magnetic mass spectrometer equipped with an electrospray ionization (ESI) probe under the positive-ion detection mode: the resolution of the mass spectrometer was set at 3 000, and the voltages for electrospray, orifice, and ring lens were 2.5 kV, 30 V and 100 V, respectively; the temperatures of orifice and desolvating plate were 150 and 250 °C, respectively. A Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector was used isothermally at 300 °C; it was fitted with a chemically bonded fused silica capillary column (25QC3/HT5; 25 m × 0.32 mm i.d.; film thickness, 0.1 μm; SGE). The apparatus used for medium pressure liquid chromatography consisted of a Shimamura YRD-880 RI-detector (Tokyo, Japan) and a uf-3040S chromatographic pump using silica gel 60 (230–400 mesh, Nacalai Tesque) as adsorbent and Et_2O–benzene–acetone (1.5 : 8 : 2, v/v/v) or $CHCl_3$–methanol (99.5 : 0.5–99:1, v/v) mixtures as eluent.

Compound **7** was prepared in five steps from (22*E*)-ergosta-5,7,22-trien-3β-ol, *via* $\Delta^{7,22}$-, $\Delta^{8(14)}$-, and Δ^{14}-unsaturated derivatives.[27] A concentrated DMDO solution (0.33–0.35 mol) in $CHCl_3$ was prepared as described in detail in the literature.[17]

General procedure for the oxidation by DMDO

To a solution of steroidal acetate (2 mmol) in CH_2Cl_2 (10 ml) was added a freshly prepared solution of DMDO (4 mmol; 12 ml) in $CHCl_3$. The mixture was left at room temperature for 12 h, and excess of the reagent and solvent were evaporated off under reduced pressure. The above procedure was repeated for two (24 h) to four runs (48 h). The reaction products were purified by passage through a column of silica gel (70–230 mesh) and elution with benzene–EtOAc (4 : 1–1 : 4, v/v) or diethyl ether–benzene–acetone (1.5 : 8 : 0.5–1.5 : 8 : 2, v/v/v) mixtures and then by medium-pressure liquid chromatography on silica gel (230–400 mesh) and elution with $CHCl_3$–methanol (99.5 : 0.5–99 : 1, v/v) or benzene–methanol (99 : 1, v/v) mixtures.

Oxidation products of methyl 3α-acetoxy-5β-cholan-24-oate 1. *Methyl 3α-acetoxy-5β-hydroxycholan-24-oate* ***9***. Isolated from the reaction product of **1** as colorless prisms (Fr.1) crystallized from aq. acetone; mp 165–167 °C (lit.,[14] 167–168 °C); IR ν_{max}/cm^{-1} 1707, 1732 (C=O), 3444 (OH); ^{1}H-NMR δ 0.65 (3H, s, 18-H_3), 0.91 (3H, s, 19-H_3), 0.92 (3H, d, *J* 7.6 Hz, 21-H_3), 2.03 (3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$), 5.10 (1H, br m, 3β-H); MS *m*/*z* 370 (M − AcOH − H_2O, 32%), 334 (68), 273 (M − AcOH − S.C., 22), 255 (M − AcOH − H_2O − S.C., 69), 213 (M − AcOH − H_2O − S.C. − ring D, 100).

Methyl 3α-acetoxy-5β,17α-dihydroxycholan-24-oate ***10***. Isolated from the reaction product of **1** as colorless needles (Fr. 2) crystallized from aq. acetone; mp 189–191 °C. IR ν_{max}/cm^{-1} 1712, 1731 (C=O), 3470 (OH); ^{1}H-NMR δ 0.73 (3H, s, 18-H_3), 0.90 (3H, d, *J* 6.8 Hz, 21-H_3), 0.91 (3H, s, 19-H_3), 2.02 (3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$), 5.07 (1H, br m, 3β-H); EI-MS *m*/*z* 368 (M − AcOH − $2H_2O$, 15%), 313 (M − $2H_2O$ − S.C., 18), 281 (36), 271 (M − AcOH − H_2O − S.C., 15), 253 (M − AcOH − $2H_2O$ − S.C., 69), 207 (62), 171 (100); ESI-MS Calc. for $C_{27}H_{44}O_6$·Na (*M* + Na$^+$): 487.3036. Found: *m*/*z*, 487.3033.

Oxidation products of methyl 3α,7α-diacetoxy-5β-cholan-24-oate 2. *Methyl 3α,7α-diacetoxy-17α-hydroxy-5β-cholan-24-oate* ***12***. Isolated from the reaction product of **2** as a colorless amorphous solid (Fr. 1) crystallized from EtOAc–hexane; mp 157–160 °C (lit.,[17] viscous oil); IR ν_{max}/cm^{-1} 1728 (C=O), 3545 (OH); ^{1}H-NMR δ 0.74 (3H, s, 18-H_3), 0.91 (3H, d, *J* 7.0 Hz, 21-H_3), 0.94 (3H, s, 19-H_3), 2.02, 2.06 (each 3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$), 4.59 (1H, br m, 3β-H), 4.91 (1H, m, 7β-H); EI-MS *m*/*z* 446 (M − AcOH, 4%), 428 (M − AcOH − H_2O, 19), 386 (M − 2AcOH, 100), 368 (M − 2AcOH − H_2O, 82), 353 (M − 2AcOH − H_2O − CH_3, 18), 332 (91), 313 (M − AcOH − H_2O − S.C., 35), 286 (M − AcOH − H_2O − S.C. − ring D, 12), 253 (M − 2AcOH − H_2O − S.C., 37), 226 (M − 2AcOH − H_2O − S.C. − part of ring D, 40), 211 (M − 2AcOH − H_2O − S.C. − ring D, 30).

Methyl 3α,7α-diacetoxy-5β-hydroxycholan-24-oate ***11***. Isolated from the reaction product of **2** as a colorless amorphous solid (Fr. 2) crystallized from EtOAc–hexane; mp 155–157 °C (lit.,[17] 158–159 °C); IR ν_{max}/cm^{-1} 1735 (C=O), 3475 (OH); ^{1}H-NMR δ 0.65 (3H, s, 18-H_3), 0.91 (3H, s, 19-H_3), 0.92 (3H, d, *J* 7.3 Hz, 21-H_3), 2.03, 2.07 (each 3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$) 4.92 (1H, m, 7β-H), 5.02 (1H, br m, 3β-H); EI-MS *m*/*z* 428 (M − AcOH − H_2O, 19%), 386 (M − 2AcOH, 100), 368 (M − 2AcOH − H_2O, 81), 353 (M − AcOH − H_2O − CH_3, 17), 332 (91), 313 (M − AcOH − H_2O − S.C., 35), 286 (M − AcOH − H_2O − S.C. − part of ring D, 13), 271 (M − 2AcOH − S.C., 92), 253 (M − 2AcOH − H_2O − S.C., 37), 226 (M − 2AcOH − H_2O − S.C. − part of ring D, 40), 211 (M − 2AcOH − H_2O − S.C. − ring D, 30).

Methyl 3α,7α-diacetoxy-5β,17α-dihydroxycholan-24-oate ***13***. Isolated from the reaction product of **2** as a colorless amorphous solid (Fr. 3) crystallized from aq. methanol; mp 172–174 °C; IR ν_{max}/cm^{-1} 1713, 1731 (C=O), 3473 (OH); ^{1}H-NMR δ 0.73 (3H, s, 18-H_3), 0.91 (3H, d, *J* 5.9 Hz, 21-H_3), 0.92 (3H, s, 19-H_3), 2.03, 2.08 (each 3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$), 4.94 (1H, m, 7β-H), 5.01 (1H, br m, 3β-H); EI-MS *m*/*z* 384 (M − Ac − H_2O, 2%), 366 (M − 2AcOH − $2H_2O$, 19), 351 (M − 2AcOH − H_2O − CH_3, 3), 311 (M − 2AcOH − $2H_2O$ − S.C., 5), 269 (M − 2AcOH − H_2O − S.C., 8), 251 (M − 2AcOH − $2H_2O$ − S.C., 100), 226 (M − 2AcOH − H_2O − S.C. − part of ring D, 16); ESI-MS Calc. for $C_{29}H_{46}O_8$·Na (*M* + Na$^+$): 545.3090. Found: *m*/*z*, 545.3097.

Oxidation products of methyl 3α,7β-diacetoxy-5β-cholan-24-oate 3. *Methyl 3α,7β-diacetoxy-16-oxo-5β-cholan-24-oate* ***16***. Isolated from the reaction product of **3** as a noncrystalline substance (Fr. 1). IR ν_{max}/cm^{-1} 1732 (C=O); ^{1}H-NMR δ 0.85 (3H, s, 18-H_3), 1.00 (3H, d, *J* 7.8 Hz, 21-H_3), 1.01 (3H, s, 19-H_3), 2.00, 2.03 (each 3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$), 4.65 (1H, br m, 3β-H), 4.88 (1H, br m, 7α-H); MS *m*/*z* 504 (M, 2%), 489 (M − CH_3, 39), 429 (M − AcOH − CH_3, 39), 375 (M − S.C. − CH_3 + 1, 35), 337 (M − 2AcOH − CH_3 − OCH_3, 100), 255 (M − 2AcOH − S.C. − CH_3 + 1, 54), 213 (M − 2AcOH − S.C. − ring D, 25); EI-MS Calc. for $C_{29}H_{44}O_7$ (*M*): 504.3087. Found: M$^+$, 504.3081.

(20S)-3α,7β-Diacetoxy-5β-cholane O-24,20-lactone ***18***. Isolated from the reaction product of **3** as a colorless amorphous solid (Fr. 2) crystallized from aq. methanol; mp 202–204 °C; IR ν_{max}/cm^{-1} 1743, 1771 (C=O); ^{1}H-NMR δ 0.84 (3H, s, 18-H_3), 0.98 (3H, s, 19-H_3), 1.45 (3H, s, 21-H_3), 2.03, 2.05 (each 3H, s, $COCH_3$), 4.68 (1H, br m, 3β-H), 4.76 (1H, br m, 7α-H); EI-MS *m*/*z* 414 (M − AcOH, 14%), 354 (M − 2AcOH, 85), 339 (M − 2AcOH − CH_3, 41), 313 (10), 300 (M − AcOH − S.C. − CH_3, 10), 288 (M − AcOH − S.C. − part of ring D, 8), 281 (7), 253 (56), 241 (19), 228 (M − 2AcOH − S.C. − part of ring D, 94), 227 (100), 213 (M − 2AcOH − S.C. − ring D, 75); ESI-MS Calc. for $C_{28}H_{42}O_6$·Na (*M* + Na$^+$): 497.2879. Found: *m*/*z*, 497.2890.

Methyl 3α,7β-diacetoxy-14α-hydroxy-5β-cholan-24-oate ***15***. Isolated from the reaction product of **3** as a noncrystalline substance (Fr. 3) (lit.,[17] viscous oil); IR ν_{max}/cm^{-1} 1737 (C=O), 3518 (OH); ^{1}H-NMR δ 0.79 (3H, s, 18-H_3), 0.90 (3H, d, *J* 6.2 Hz, 21-H_3), 0.98 (3H, s, 19-H_3), 2.00, 2.02 (each 3H, s, $COCH_3$), 3.67 (3H, s, $COOCH_3$), 4.66 (1H, br m, 3β-H), 5.15 (1H, br m, 7α-H); EI-MS *m*/*z* 428 (M − AcOH − H_2O, 8%), 368 (M − 2AcOH − H_2O, 24), 353 (M − 2AcOH − H_2O − CH_3, 9), 314 (9), 281 (5), 253 (M − 2AcOH − H_2O − S.C., 100), 239 (10), 212 (25).

Methyl 3α,7β-diacetoxy-17α-hydroxy-5β-cholan-24-oate ***14***. Isolated from the reaction product of **3** as a noncrystalline substance (Fr. 4) (lit.,[17] viscous oil); IR ν_{max}/cm^{-1} 1735 (C=O), 3545 (OH); ^{1}H-NMR δ 0.76 (3H, s, 18-H$_3$), 0.90 (3H, d, *J* 6.8 Hz, 21-H$_3$), 0.98 (3H, s, 19-H$_3$), 1.98, 2.03 (each 3H, s, COCH$_3$), 3.67 (3H, s, COOCH$_3$), 4.63 (1H, br m, 3β-H), 4.80 (1H, br m, 7α-H); EI-MS *m*/*z* 428 (M − AcOH − H$_2$O, 1%), 386 (M − 2AcOH, 19), 368 (M − 2AcOH − H$_2$O, 35), 353 (M − 2AcOH − H$_2$O − CH$_3$, 13), 332 (57), 271 (M − 2AcOH − S.C., 69), 253 (M − 2AcOH − H$_2$O − S.C., 100).

Methyl 3α,7β-diacetoxy-5β-hydroxycholan-24-oate ***17***. Isolated from the reaction product of **3** as colorless thin plates (Fr. 5) crystallized from aq. acetone; mp 152–154 °C (lit.,[17] 148–149 °C); IR ν_{max}/cm^{-1} 1712, 1736 (C=O), 3487 (OH); ^{1}H-NMR δ 0.68 (3H, s, 18-H$_3$), 0.92 (3H, d, *J* 6.2 Hz, 21-H$_3$), 0.93 (3H, s, 19-H$_3$), 1.99, 2.02 (each 3H, s, COCH$_3$), 3.67 (3H, s, COOCH$_3$), 4.65 (1H, br m, 7α-H), 5.10 (1H, br m, 3β-H); EI-MS *m*/*z* 386 (M − 2AcOH, 16%), 368 (M − 2AcOH − H$_2$O, 36), 332 (51), 271 (M − 2AcOH − S.C., 64), 253 (M − 2AcOH − H$_2$O − S.C., 78), 110 (100).

Methyl 3α,7β-diacetoxy-5β,17α-dihydroxycholan-24-oate ***19***. Isolated from the reaction product of **3** as a noncrystalline substance (Fr. 6); IR ν_{max}/cm^{-1} 1732 (C=O), 3519 (OH); ^{1}H-NMR δ 0.76 (3H, s, 18-H$_3$), 0.91 (3H, d, *J* 5.4 Hz, 21-H$_3$), 0.95 (3H, s, 19-H$_3$), 1.99, 2.03 (each 3H, s, COCH$_3$), 3.67 (3H, s, COOCH$_3$), 4.67 (1H, br m, *J* 5.4 Hz, 7α-H), 5.10 (1H, br m, *J* 5.1 Hz, 3β-H); EI-MS *m*/*z* 430 (M − AcOH − H$_2$O − CH$_3$ + 1, 4%), 412 (M − AcOH − 2H$_2$O − CH$_3$ + 1, 12), 370 (M − 2AcOH − H$_2$O − CH$_3$ + 1, 32), 352 (M − 2AcOH − 2H$_2$O − CH$_3$ + 1, 93), 335 (M − 2AcOH − 2H$_2$O − 2CH$_3$ + 1, 21), 316 (24), 269 (M − 2AcOH − H$_2$O − S.C., 27), 251 (M − 2AcOH − 2H$_2$O − S.C., 100); ESI-MS Calc. for C$_{29}$H$_{46}$O$_8$·Na: (*M* + Na$^+$), 545.3090. Found: *m*/*z*, 545.3070.

Oxidation products of methyl 3α,12α-diacetoxy-5β-cholan-24-oate 4. *Methyl 3α,12α-diacetoxy-16-oxo-5β-cholan-24-oate* ***21***. Isolated from the reaction product of **4** as a noncrystalline substance (Fr. 1); IR ν_{max}/cm^{-1} 1738 (C=O); ^{1}H-NMR δ 0.89 (3H, s, 18-H$_3$), 0.93 (3H, d, *J* 6.2 Hz, 21-H$_3$), 0.94 (3H, s, 19-H$_3$), 2.02, 2.04 (each 3H, s, COCH$_3$), 3.66 (3H, s, COOCH$_3$), 4.72 (1H, br m, 3β-H), 5.10 (1H, m, 12β-H); EI-MS *m*/*z* 444 (M − AcOH, 5%), 429 (M − AcOH − CH$_3$, 10), 390 (M − S.C. + 1, 9), 384 (M − 2AcOH, 10), 375 (M − S.C. − CH$_3$ + 1, 15), 371 (57), 352 (25), 330 (M − AcOH − S.C. + 1, 16), 315 (M − AcOH − S.C. − CH$_3$ + 1, 28), 311 (54), 270 (M − 2AcOH − S.C. + 1, 27), 255 (M − 2AcOH − S.C. − CH$_3$ + 1, 66), 170 (100); EI-MS Calc. for C$_{29}$H$_{44}$O$_7$ (*M*): 504.3087. Found: M$^+$, 504.3099.

Methyl 3α,12α-diacetoxy-5β-hydroxycholan-24-oate ***20***. Isolated from the reaction product of **4** as colorless needles (Fr. 2) crystallized from Et$_2$O–hexane; mp 129–130 °C (lit.,[14] 127–128 °C); IR ν_{max}/cm^{-1} 1730 (C=O), 3475 (OH); ^{1}H-NMR δ 0.73 (3H, s, 18-H$_3$), 0.81 (3H, d, *J* 6.2 Hz, 21-H$_3$), 0.88 (3H, s, 19-H$_3$), 2.03, 2.10 (each 3H, s, COCH$_3$), 3.66 (3H, s, COOCH$_3$), 5.05 (1H, br m, 3β-H), 5.10 (1H, m, 12β-H); EI-MS *m*/*z* 428 (M − AcOH − H$_2$O, 7%), 386 (M − 2AcOH, 3), 368 (M − 2AcOH − H$_2$O, 31), 332 (M − 2CH$_3$ − S.C. − part of ring D, 22), 331 (M − AcOH − S.C., 17), 313 (M − AcOH − H$_2$O − S.C., 18), 271 (M − 2AcOH − S.C., 23), 253 (M − 2AcOH − H$_2$O − S.C.,100), 211 (M − 2AcOH − H$_2$O − S.C. − ring D, 18).

Methyl 3α,12α-diacetoxy-5β-hydroxy-16-oxocholan-24-oate ***22***. Isolated from the reaction product of **4** as a noncrystalline substance (Fr. 3); IR ν_{max}/cm^{-1} 1735 (C=O), 3475 (OH); ^{1}H-NMR δ 0.82 (3H, s, 18-H$_3$), 0.88 (3H, s, 19-H$_3$), 0.90 (3H, d, *J* 7.0 Hz, 21-H$_3$), 2.03, 2.17 (each 3H, s, COCH$_3$), 3.67 (3H, s, COOCH$_3$), 5.06 (1H, br m, 3β-H), 5.22 (1H, m, 12β-H); EI-MS *m*/*z* 460 (M − AcOH, 3%), 442 (M − AcOH − H$_2$O, 13), 400 (M − 2AcOH, 12), 382 (M − 2AcOH − H$_2$O, 100), 367 (M − 2AcOH − H$_2$O − CH$_3$, 25), 349 (M − S.C. − ring D, 37), 317 (18), 277 (22), 267 (M − 2AcOH − H$_2$O − S.C., 71), 249 (47), 211 (M − 2AcOH − H$_2$O − S.C. − ring D, 39); EI-MS Calc. for C$_{31}$H$_{44}$O$_8$ (*M*): 520.3036. Found: M$^+$, 520.3040.

Oxidation products of methyl 3α,7α,12α-triacetoxy-5β-cholan-24-oate 5. *Methyl 3α,7α,12α-triacetoxy-16-oxo-5β-cholan-24-oate* ***24***. Isolated from the reaction product of **5** as a noncrystalline substance (Fr. 1); IR ν_{max}/cm^{-1} 1732 (C=O); ^{1}H-NMR δ 0.91 (3H, s, 18-H$_3$), 0.92 (3H, d, *J* 5.1 Hz, 21-H$_3$), 0.95 (3H, s, 19-H$_3$), 2.05, 2.07, 2.10 (each 3H, s, COCH$_3$), 3.67 (3H, s, COOCH$_3$), 4.60 (1H, br m, 3β-H), 4.88 (1H, m, 7β-H), 5.11 (1H, m, 12β-H); EI-MS *m*/*z* 487 (M − AcOH − CH$_3$, 78%), 442 (M − 2AcOH, 9), 433 (M − S.C. − CH$_3$ + 1, 9), 382 (M − 3AcOH, 9), 351 (M − 3AcOH − OCH$_3$, 28), 333 (28), 309 (66), 295 (26), 268 (M − 3AcOH − S.C. + 1, 31), 267 (M − 3AcOH − S.C., 34), 253 (M − 3AcOH − S.C. − CH$_3$ + 1, 72), 170 (100); EI-MS Calc. for C$_{31}$H$_{46}$O$_9$ (*M*): 562.3142. Found: M$^+$, 562.3138.

Methyl 3α,7α,12α-triacetoxy-5β-hydroxycholan-24-oate ***23***. Isolated from the reaction product of **5** as a noncrystalline substance (Fr. 2) (lit.,[17] 87–89 °C); IR ν_{max}/cm^{-1} 1736 (C=O), 3489 (OH); ^{1}H-NMR δ 0.73 (3H, s, 18-H$_3$), 0.82 (3H, d, *J* 6.2 Hz, 21-H$_3$), 0.89 (3H, s, 19-H$_3$), 2.04, 2.08, 2.10 (each 3H, s, COCH$_3$), 3.66 (3H, s, COOCH$_3$), 4.95 (1H, m, 7β-H), 5.00 (1H, br m, 3β-H), 5.10 (1H, m, 12β-H); EI-MS *m*/*z* 444 (M − 2AcOH, 6%), 426 (M − 2AcOH − H$_2$O, 18), 384 (M − 3AcOH, 48), 366 (M − 3AcOH − H$_2$O, 71), 351 (M − 3AcOH − H$_2$O − CH$_3$, 36), 330 (M − AcOH − H$_2$O − S.C. − ring D, 29), 329 (M − 2AcOH − S.C., 72), 311 (M − 2AcOH − H$_2$O − S.C., 29), 269 (M − 3AcOH − S.C., 72), 251 (M − 3AcOH − H$_2$O − S.C., 100).

Methyl 3α,7α,12α-triacetoxy-5β-hydroxy-16-oxocholan-24-oate ***25***. Isolated from the reaction product of **5** as a noncrystalline substance (Fr. 3); IR ν_{max}/cm^{-1} 1738 (C=O), 3538 (OH); ^{1}H-NMR δ 0.91 (3H, s, 18-H$_3$), 0.92 (3H, d, *J* 6.5 Hz, 21-H$_3$), 0.93 (3H, s, 19-H$_3$), 2.05, 2.11, 2.18 (each 3H, s, COCH$_3$), 3.66 (3H, s, COOCH$_3$), 4.92 (1H, m, 7β-H), 5.02 (1H, br m, 3β-H), 5.12 (1H, m, 12β-H); EI-MS *m*/*z* 578 (M, 5%), 503 (M − AcOH − CH$_3$, 14), 464 (M − S.C. + 1, 9), 449 (M − S.C. − CH$_3$ + 1, 9), 401 (9), 380 (M − 3AcOH − H$_2$O, 14), 365 (M − 3AcOH − H$_2$O − CH$_3$, 22), 349 (33), 325 (M − 2AcOH − H$_2$O − S.C., 58), 311 (M − 2AcOH − H$_2$O − S.C. − CH$_3$ + 1, 36), 283 (M − 3AcOH − S.C., 25), 269 (M − 2AcOH − H$_2$O − S.C. − ring D − CH$_3$, 49), 251 (M − 3AcOH − H$_2$O − S.C. − CH$_3$ + 1, 100), 207 (85); ESI-MS Calc. for C$_{31}$H$_{46}$O$_{10}$·Na (*M* + Na$^+$): 601.2988. Found: *m*/*z*, 601.2967.

Oxidation products of 5α-cholestan-3β-yl acetate 6. *25-Hydroxy-5α-cholestan-3β-yl acetate* ***26***. Isolated from the reaction product of **6** as a colorless amorphous solid (Fr. 1) crystallized from aq. methanol; mp 124–126 °C (lit.,[26] 119–121 °C); IR ν_{max}/cm^{-1} 1728 (C=O), 3392 (OH); ^{1}H-NMR δ 0.65 (3H, s, 18-H$_3$), 0.82 (3H, s, 19-H$_3$), 0.91 (3H, d, *J* 6.8 Hz, 21-H$_3$), 1.21 (6H, s, 26- and 27-H$_3$), 2.02 (3H, s, COCH$_3$), 4.68 (1H, br m, *J* 5.4 Hz, 3α-H); EI-MS 428 (M − H$_2$O, 14%), 413 (M − H$_2$O − CH$_3$, 8), 353 (M − AcOH − H$_2$O − CH$_3$, 15), 315 (44), 255 (44), 215 (M − AcOH − S.C. − D, 43), 95 (100).

14α,25-Dihydroxy-5α-cholestan-3β-yl acetate ***29***. Isolated from the reaction product of **6** as colorless thin plates (Fr. 2) crystallized from Et$_2$O–hexane; mp 138–140 °C; IR ν_{max}/cm^{-1} 1733 (C=O), 3438 (OH); ^{1}H-NMR δ 0.80 (3H, s, 18-H$_3$), 0.84 (3H, s, 19-H$_3$), 0.89 (3H, d, *J* 6.5 Hz, 21-H$_3$), 1.21 (6H, s, 26 and 27-H$_3$), 2.02 (3H, s, COCH$_3$), 4.68 (1H, br m, *J* 5.1 Hz, 3α-H); EI-MS *m*/*z* 426 (M − 2H$_2$O, 12%), 411 (M − 2H$_2$O − CH$_3$, 7), 351 (M − AcOH − 2H$_2$O − CH$_3$, 6), 341 (54), 315 (46), 281 (21), 255 (M − AcOH − H$_2$O − S.C., 74), 204 (100); ESI-MS Calc. for C$_{29}$H$_{50}$O$_4$·Na (*M* + Na$^+$): 485.3607. Found: *m*/*z*, 485.3627.

17α,25-Dihydroxy-5α-cholestan-3β-yl acetate ***27***. Isolated from the reaction product of **6** as colorless thin plates (Fr. 3) crystallized from acetone–hexane; mp 152–154 °C; IR ν_{max}/cm^{-1}

1731 (C=O), 3481 (OH); ^{1}H-NMR δ 0.74 (3H, s, 18-H_3), 0.83 (3H, s, 19-H_3), 0.90 (3H, d, J 6.8 Hz, 21-H_3), 1.22 (6H, s, 26- and 27-H_3), 2.02 (3H, s, $COCH_3$), 4.68 (1H, br m, J 4.9 Hz, 3α-H); EI-MS m/z 411 (M − H_2O − CH_3, 5%), 333 (M − S.C., 66), 315 (M − H_2O − S.C., 60), 255 (M − AcOH − H_2O − S.C., 97), 215 (M − AcOH − S.C. − ring D, 27), 95 (100); ESI-MS Calc. for $C_{29}H_{50}O_4$·Na (M + Na^+): 485.3607. Found: m/z, 485.3613.

(20S)-20,25-Dihydroxy-5α-cholestan-3β-yl acetate ***28***. Isolated from the reaction product of **6** as a colorless amorphous solid (Fr. 4) crystallized from aq. methanol; mp 106–108 °C; IR ν_{max}/cm^{-1} 1728 (C=O), 3260 (OH); ^{1}H-NMR δ 0.82 (3H, s, 18-H_3), 0.84 (3H, s, 19-H_3), 1.22 (6H, s, 26- and 27-H_3), 1.28 (3H, s, 21-H_3), 2.02 (3H, s, $COCH_3$), 4.68 (1H, br m, J 5.2 Hz, 3α-H); EI-MS m/z 426 (M − $2H_2O$, 14%), 411 (M − $2H_2O$ − CH_3, 10), 361 (17), 351 (M − AcOH − $2H_2O$ − CH_3, 18), 343 (29), 315 (82), 301 (23), 283 (26), 255 (100), 215 (M − AcOH − S.C. − ring D, 59); ESI-MS Calc. for $C_{29}H_{50}O_5$·Na (M + Na^+): 485.3607. Found: m/z, 485.3584.

Oxidation products of 5α-ergostan-3β-yl acetate 7. *(24R)-24-Hydroxy-5α-ergastan-3β-yl acetate* ***30***. Isolated from the reaction product of **7** as a colorless amorphous solid (Fr. 1) crystallized from aq. methanol; mp 169–172 °C; IR ν_{max}/cm^{-1} 1712 (C=O), 3506 (OH); ^{1}H-NMR δ 0.65 (3H, s, 18-H_3), 0.81 (3H, s, 19-H_3), 0.88 (3H, d, J 7.0 Hz, 21-H_3), 0.91 (6H, d, J 7.0 Hz, 26- and 27-CH_3), 1.07 (3H, s, 28-H_3), 2.02 (3H, s, $COCH_3$), 4.68 (1H, br m, J 5.1 Hz, 3α-H); EI-MS m/z 442 (M − H_2O, 16%), 427 (M − H_2O − CH_3, 7), 417 (22), 399 (18), 358 (100), 339 (36), 315 (44), 257 (M − AcOH − S.C., 68), 215 (M − AcOH − S.C. − ring D, 40); EI-MS Calc. for $C_{30}H_{50}O_2$ (M − H_2O): 442.3805. Found: m/z, 442.3811.

25-Hydroxy-5α-ergostan-3β-yl acetate ***31***. Isolated from the reaction product of **7** as colorless thin plates (Fr. 2) crystallized from aq. methanol; mp 160–163 °C; IR ν_{max}/cm^{-1} 1717 (C=O), 3526 (OH); ^{1}H-NMR δ 0.65 (3H, s, 18-H_3), 0.82 (3H, s, 19-H_3), 0.89 (3H, d, J 6.5 Hz, 21-H_3), 0.91 (6H, s, 26- and 27-H_3), 0.92 (3H, d, J 6.2 Hz, 28-H_3), 2.02 (3H, s, $COCH_3$), 4.68 (1H, br m, J 5.1 Hz, 3α-H); EI-MS m/z 442 (M − H_2O, 17%), 427 (M − H_2O − CH_3, 6), 358 (19), 343 (21), 327 (11), 315 (36), 283 (34), 257 (M − AcOH − S.C., 81), 215 (M − AcOH − S.C. − ring D, 100); EI-MS Calc. for $C_{30}H_{50}O_2$ (M − H_2O): 442.3805. Found: m/z, 442.3827.

Oxidation products of 5α-stigmastan-3β-yl acetate 8. *(20S)-20-Hydroxy-5α-stigmastan-3β-yl acetate* ***33***. Isolated from the reaction product of **8** as a colorless amorphous solid (Fr. 1) crystallized from aq. methanol; mp 103–104 °C; IR ν_{max}/cm^{-1} 1728 (C=O); ^{1}H-NMR δ 0.82 (3H, s, 18-H_3), 0.83 (6H, d, J 5.4 Hz, 26- and 27-H_3), 0.84 (3H, s, 19-H_3), 0.85 (3H, t, J 7.8 Hz, 29-H_3), 1.29 (3H, s, 21-H_3), 2.02 (3H, s, $COCH_3$), 4.67 (1H, br m, 3α-H); EI-MS m/z 456 (M − H_2O, 55%), 441 (M − H_2O − CH_3, 9), 381 (M − AcOH − H_2O − CH_3, 15), 358 (47), 343 (100), 315 (47), 301 (51), 283 (86), 255 (96), 243 (51), 229 (57), 251 (M − AcOH − S.C. − ring D, 72); EI-MS Calc. for $C_{31}H_{54}O_3$ (M); 474.4073. Found: M^+, 474.4054.

17α-Hydroxy-5α-stigmastan-3β-yl acetate ***32***. Isolated from the reaction product of **8** as colorless thin plates (Fr. 2) crystallized from aq. methanol; mp 178–180 °C; IR ν_{max}/cm^{-1} 1732 (C=O), 3595 (OH); ^{1}H-NMR δ 0.77 (3H, s, 18-H_3), 0.83 (3H, s, 19-H_3), 0.81 and 0.84 (each 3H, d, J 7.0 Hz, 26- and 27-H_3), 0.83 (3H, t, J 7.0 Hz, 29-H_3), 0.89 (3H, d, J 7.0 Hz, 21-H_3), 2.05 (3H, s, $COCH_3$), 4.68 (1H, br m, 3α-H); EI-MS m/z 381 (M − AcOH − H_2O − CH_3, 7%), 333 (M − S.C., 75), 315 (M − H_2O − S.C., 44), 255 (M − AcOH − H_2O − S.C., 93), 215 (M − AcOH − S.C. − ring D, 17), 95 (100); EI-MS Calc. for $C_{31}H_{54}O_3$ (M): 474.4073. Found: M^+, 474.4074.

(24S)-24-Hydroxy-5α-stigmastan-3β-yl acetate ***34***. Isolated from the reaction product of **8** as colorless needles (Fr. 3) crystallized from aq. methanol; mp 153–155 °C; IR ν_{max}/cm^{-1} 1716 (C=O), 3538 (OH); ^{1}H-NMR δ 0.65 (3H, s, 18-H_3), 0.82 (3H, s, 19-H_3), 0.88 (3H, t, J 7.3 Hz, 29-H_3), 0.89 (6H, d, J 6.2 Hz, 26- and 27-H_3), 0.92 (3H, d, J 6.8 Hz, 21-H_3), 2.02 (3H, s, $COCH_3$), 4.68 (1H, br m, 3α-H); EI-MS m/z 456 (M − H_2O, 8%), 443 (7), 358 (100), 343 (28), 315 (50), 298 (22), 285 (33), 275 (M − S.C. − ring D, 19), 257 (M − AcOH − S.C., 59), 230 (M − AcOH − S.C. − part of ring D, 18), 229 (38), 251 (M − AcOH − S.C. − ring D, 66); EI-MS Calc. for $C_{31}H_{52}O_2$: (M − H_2O): 456.3967. Found: m/z, 456.3990.

25-Hydroxy-5α-stigmastan-3β-yl acetate ***35***. Isolated from the reaction product of **8** as colorless needles (Fr. 4) crystallized from aq. methanol; mp 197–199 °C; IR ν_{max}/cm^{-1} 1728 (C=O), 3376 (OH); ^{1}H-NMR δ 0.65 (3H, s, 18-H_3), 0.82 (3H, s, 19-H_3), 0.92 (3H, d, J 6.5 Hz, 21-H_3), 0.95 (3H, t, J 7.3 Hz, 29-H_3), 1.17 (6H, s, 26- and 27-H_3), 2.02 (3H, s, $COCH_3$), 4.68 (1H, br m, 3α-H); EI-MS m/z 456 (M − H_2O, 21%), 441 (M − H_2O − CH_3, 3), 396 (M − AcOH − H_2O, 9), 381 (M − AcOH − H_2O − CH_3, 9), 358 (25), 343 (20), 315 (29), 298 (14), 283 (24), 257 (M − AcOH − S.C., 59), 230 (M − AcOH − S.C. − part of ring D, 17) 229 (26), 215 (M − AcOH − S.C. − ring D, 100); EI-MS Calc. for $C_{31}H_{52}O_2$: (M − H_2O): 456.3967. Found: m/z: 456.3959.

17α,25-Dihydroxy-5α-stigmastan-3β-yl acetate ***36***. Isolated from the reaction product of **8** as colorless needles (Fr. 5) crystallized from aq. acetone; mp 174–176 °C; IR ν_{max}/cm^{-1} 1728 (C=O), 3495 (OH); ^{1}H-NMR δ 0.74 (3H, s, 18-H_3), 0.83 (3H, s, 19-H_3), 0.91 (3H, d, J 6.8 Hz, 21-H_3), 0.96 (3H, t, J 7.8 Hz, 29-H_3), 1.18 (6H, s, 26- and 27-H_3), 2.02 (3H, s, $COCH_3$), 4.68 (1H, br m, 3α-H); EI-MS m/z 439 (M − $2H_2O$ − CH_3, 5%), 401 (3), 370 (8), 355 (8), 341 (18), 333 (M − S.C., 10), 315 (M − H_2O − S.C., 59), 281 (17), 255 (M − AcOH − H_2O − S.C., 100), 215 (M − AcOH − S.C. − ring D, 25); ESI-MS Calc. for $C_{31}H_{54}O_4$·Na (M + Na^+): 513.3920. Found: m/z, 513.3939.

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