A new route to polyoxygenate C and D rings of steroids by oxidation of a $\Delta^{8,14}$-diene steroid with the methyltrioxorhenium–H_2O_2–urea system

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In order to find new ways of introducing oxygenated functions in the 15-, 9- and 11-position on steroid rings and at the same time test the reactivity of a conjugated diene steroid toward methyltrioxorhenium (MTO)-catalyzed oxidation with the urea–hydrogen peroxide adduct (UHP), the reactions of 5α-cholesta-8,14-dien-3β-yl acetate **1** with the MTO–UHP system are performed in aprotic solvents. These oxidations are performed both at 0 °C and 25 °C in $CHCl_3$ or diethyl ether as solvent and in the presence of pyridine ligand. From the reaction of diene **1** in $CHCl_3$ we isolate two new sterols, 9β-hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl acetate **3** and 9α,11α,15α-trihydroxy-5α-cholest-8(14)-en-3β-yl acetate **7**, while oxidation in Et_2O in the presence of pyridine ligand allows us to isolate the new epoxysteroid 9α,11α-epoxy-15α-hydroxy-5α-cholest-8(14)-en-3β-yl acetate **13**. The structure of all new steroids is secured on the basis of chemical evidence and interpretation of spectral data, which include H–H COSY, HMBC and NOESY experiments. These results represent a new and mild method for the functionalization of C and D rings from an 8,14-diene steroid, to give 15-oxygenated sterols, a class of compounds remarkable for their inhibitory action on sterol synthesis in animal cell culture systems.

Introduction

In recent years our research line has regarded the oxidations of unsaturated organic compounds with transition metal oxides. Specifically, we have studied the oxidative cyclization of 1,5-dienes with RuO_4, which gave stereospecifically *cis*-tetrahydrofuran products.[1] Previously, we have also reported that oxidation of a number of monoene steroids,[2] conjugated diene steroids[3] and alkenes[4] with RuO_4, used in equimolar amounts and in the absence of a co-oxidant, gave almost exclusively 1,2-diols and/or α-hydroxy ketones without giving rise to scission products. Interestingly, some conjugated diene steroids by treatment with RuO_4 furnished mainly epoxy diol and epoxy ketol products when the double bonds were located in a hindered position.[3,5] Furthermore, we have demonstrated that these reactions proceeded through ruthenium(VI) diesters.[6–8] Recently, we have also carried out a preliminary study on the oxidation of some conjugated diene steroids with aq. hydrogen peroxide in the presence of catalytic amounts of methyltrioxorhenium (CH_3ReO_3, MTO).[9] This very stable and easily accessible catalyst has attracted much attention in the last ten years due to (i) its ability to activate oxidants such as molecular oxygen or hydrogen peroxide and to (ii) its highly selective behavior in the oxidation of a wide variety of organic compounds.[10] Particularly interesting and applicable in synthesis is MTO-catalyzed oxidation, with hydrogen peroxide, of C–C double bonds. This reaction was first described by Herrmann's group[11] and successively explored by other research groups.[12–15] Active species involved in oxygen transfer to an olefinic double bond are the two peroxorhenium complexes shown in Chart 1.[16] Although the oxidation of olefins in many cases leads efficiently and selectively to the corresponding epoxides, a typical side reaction is the conversion, catalyzed by the electropositive Re^{VII} centre, of the hydrolytically sensitive epoxides to 1,2-diols.[11] To a certain degree this problem can be overcome by employing the urea–hydrogen peroxide complex (UHP) as stoichiometric oxidant[17] or by using catalytic amounts of pyridine ligand in combination with aq. hydrogen peroxide in aprotic solvents.[18]

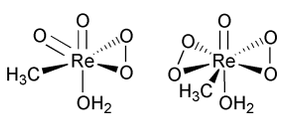


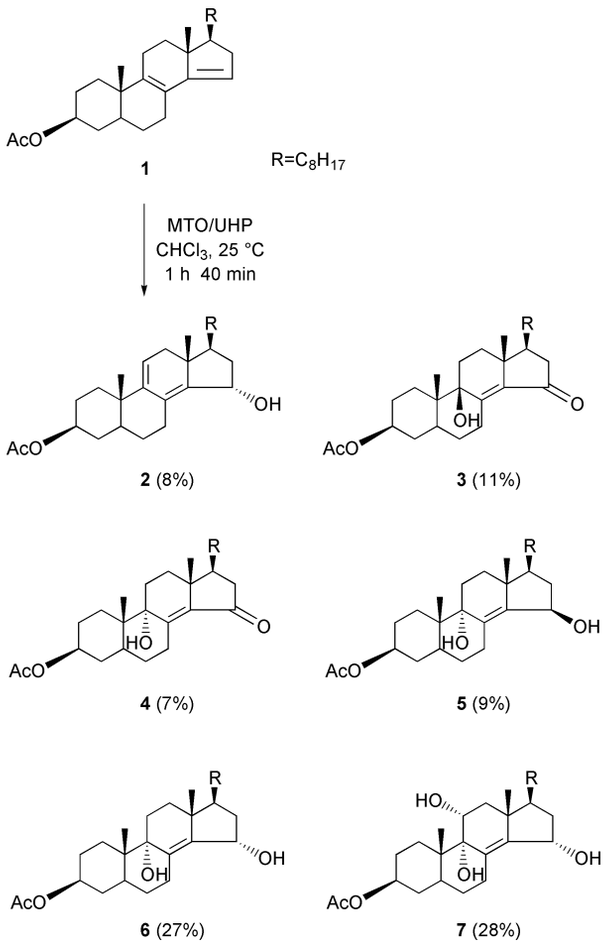
Chart 1

In continuing our study concerning the functionalization of suitable positions on the steroid nucleus[2–5] through oxidative methods, in the present paper we report MTO-catalyzed oxidations, with UHP, of 5α-cholesta-8,14-dien-3β-yl acetate **1** in various solvents and under various temperature conditions and in the presence of pyridine ligand. From these reactions we have isolated and well characterized some new steroids (**3**, **7** and **13**) on which bioassay is in progress to evaluate their possible biological activity. Furthermore, we present a new and mild method for the functionalization of C and D steroid rings obtaining 15-oxygenated sterols, a class of compounds remarkable for their inhibitory action on sterol synthesis in animal cell culture systems.[19]

Results and discussion

MTO-catalyzed oxidation, with UHP in $CHCl_3$, of 5α-cholesta-8,14-dien-3β-yl acetate **1**, prepared from reaction of cholesta-5,7-dien-3β-ol with Ac_2O and HCl in AcOH at reflux,[20] gave, both at 0 °C and at 25 °C in 1 h and 40 min, 15α-hydroxy-5α-cholesta-8(14),9(11)-dien-3β-yl acetate **2** (8%

yield), 9α-hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl acetate **4** (7%), 9α,15β-dihydroxy-5α-cholest-8(14)-en-3β-yl acetate **5** (9%) and 9α,15α-dihydroxy-5α-cholest-8(14)-en-3β-yl acetate **6** (27%) besides new sterols 9β-hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl acetate **3** (11%) and 9α,11α,15α-trihydroxy-5α-cholest-8(14)-en-3β-yl acetate **7** (28%) (Scheme 1).



Scheme 1

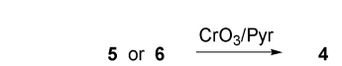
Compounds **2** and **6** were previously obtained in 23 and 26% yield, respectively, by MCPBA epoxidation of diene **1** using a two-phase buffered system.[21a] Compounds **2**, **4**, **5** and **6**, although previously reported in the literature,[21] have not been completely characterized. 2D-NMR spectra allowed us to obtain more complete ^{1}H and ^{13}C NMR assignments of these products. In particular, comparative data (^{1}H, ^{13}C, HMBC) of compounds **3** and **4** are presented in Table 1, while ^{1}H and ^{13}C NMR assignments of compounds **5** and **6**, achieved by ^{1}H–^{1}H COSY, DEPT and HMBC experiments, are reported in Table 2.

New steroid **3** has molecular formula $C_{29}H_{46}O_4$, as revealed HREIMS spectrum (M^+, *m/z* 458.3358. calc. *M*, 458.3396), and contains an unsaturated carbonyl group, a tetrasubstituted double bond and a tertiary alcohol function, as denoted by ^{13}C and DEPT NMR spectra which included resonances at δ_C 208.1, 145.7, 144.3 and 84.5. The UV maximum at 255 nm [$\varepsilon(CHCl_3)$ = 8544], the strong downfield shift of the 7-H$^\beta$ proton at δ 3.97 (dm, *J* 13.4 Hz) and the signal for 16-H$^\alpha$ at δ 2.43 (dd, *J* 18.8, 7.6 Hz), typical of a proton next to a carbonyl group, provided evidence for the presence of an α,β-unsaturated ketone group in the molecule. The β stereochemistry of the allylic 9-OH group was deduced from chemical-shift values of H_3-18 and -19 and from pyridine-induced shifts of some proton signals, relative to the spectrum recorded in $CDCl_3$.[22]

The molecular formula of new sterol **7** was determined as $C_{29}H_{48}O_5$ on the basis of the molecular-ion peak at *m/z* 476.3485 in the HREIMS spectrum. Complete ^{1}H and ^{13}C NMR assignments of **7**, achieved by the combination of ^{1}H–^{1}H COSY, HMBC and NOESY experiments, are reported in Table 3. Specifically, the ^{13}C NMR spectrum, showing resonances at δ_C 134.8 and 150.1, revealed the presence of a tetrasubstituted double bond in the molecule, positioned between carbons 8 and 14 on the basis of the DEPT NMR spectrum and HMBC correlations (see Table 3). Furthermore, the ^{13}C NMR spectrum included signals relative to carbons bonding oxygen, at δ_C 75.1 (C-9), 69.5 (C-15) and 66.6 (C-11). The ^{1}H NMR spectrum of **7** recorded in $CDCl_3$ showed two resonances of hydroxymethine protons at δ 4.63 (d, *J* 4.8 Hz), relative to 15-H, and δ 4.11 (dd, *J* 12.2, 4.4 Hz), attributed to 11-H$^\beta$ for its correlations with methylene proton signals of a CH_2 group next to a blocked position [δ 2.12 (dd, *J* 12.4, 4.4 Hz) and 1.36 (dd, *J* 12.4, 12.2 Hz)] as revealed by an H–H COSY experiment. The stereochemistry at the oxygen-bearing centres C-9, C-11 and C-15 was determined by combined proton–proton coupling constants and NOESY analysis. In particular, α stereochemistry for 9-OH and 11-OH was assigned on the basis of NOESY correlations of the 11-H$^\beta$ signal at δ 4.11 with those for H_3-18 (δ 0.88), H_3-19 (δ 0.89) and 12-H$^\beta$ (δ 2.12), apart from NOESY correlations between the exchangeable proton at δ 3.71 (br s), relative to 9-OH, and signals at δ 2.67 (br s, 11-OH), 1.36 (12-H$^\alpha$) and 2.21 (m, 5-H). Similarly, NOESY correlations of the 15-H signal at δ 4.63 with signals at δ 2.48 (7-H$^\beta$), 1.82 (16-H$^\beta$) and 0.88 (H_3-18) revealed an α orientation for the hydroxy group at C-15. Support for the assigned stereochemistry at the chiral centres C-9, C-11 and C-15 came also from the ^{1}H NMR spectrum of **7**, recorded in [$^2H_5$]pyridine solution, that showed the expected downfield pyridine-induced shifts,[22] relative to the spectrum recorded in $CDCl_3$, for resonances of 5-H ($\Delta\delta$ −0.30), 7-H$_{ax}$ ($\Delta\delta$ −0.47) and 12-H^{ax} ($\Delta\delta$ −0.38) (see Table 3).

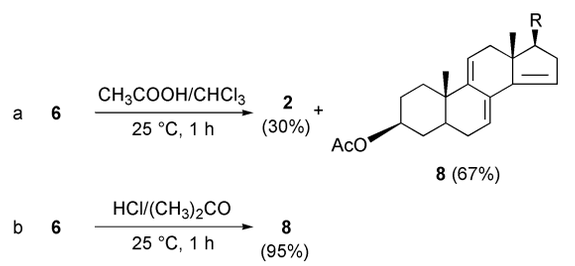
The ^{1}H NMR spectrum of **2**, recorded in [$^2H_5$]pyridine, included a resonance of an olefinic proton at δ 5.44 (dd, *J* 6.4, 2.0 Hz, 11-H) which, being vicinal to a methylene group next to a blocked position [δ 2.35 (dd, *J* 16.1, 6.4 Hz, 12-H$^\beta$) and 2.15 (dd, *J* 16.1, 2.0 Hz, 12-H$^\alpha$)], as revealed by selective H–H decoupling experiments, was attributed to 11-H. An hydroxymethine signal at δ 4.92 (br t, *J* 5.0 Hz), showing a correlation with a signal at δ 2.25 (dd, *J* 12.8, 5.0 Hz, 16-H$^\beta$) and with the exchangeable proton signal at δ 5.71 (br d, *J* 5.0 Hz, 15-OH), was assigned to 15-H with β stereochemistry according to proton–proton coupling-constant values and to pyridine-induced shifts of some proton signals, relative to the spectrum recorded in $CDCl_3$.[22]

Finally, structures of compounds **2**, **4**, **5** and **6** were also confirmed through chemical evidence. In fact, oxidation of **5** and **6** with CrO_3 in pyridine gave, in both cases, the expected product **4** (Scheme 2), while dehydration of diol **6** with AcOH in



Scheme 2

$CHCl_3$ at 25 °C furnished, after 1 h, the expected compound **2** (30% yield) and a triene steroid, namely **8** (67%, Scheme 3a), obtained from **6** after loss of two water molecules.



Scheme 3

Table 1 δ_H and δ_C assignments and HMBC correlations of ketols **3** and **4**

	3			**4**		
C	δ_H[a]	δ_C[b]	HMBC[c]	δ_H[a]	δ_C[b]	HMBC[c]
1		29.6		1.78, 1.48	29.5	
2		26.8		1.90, 1.44	26.9	
3	4.67 m	72.8	CH_3COO	4.73	72.9	CH_3COO
4_{eq}	1.76	33.6	3, 5	1.75	33.8	2, 3, 5, 10
4_{ax}				1.31		3, 5, 6
5	2.18 m	35.7	4, 6, 10	2.20 m	34.5	4, 6, 10, 19
6		26.4		1.43, 1.28	27.8	
7_{eq}	3.97 dm (13.4)	23.2	6, 8, 14	3.93 dm (13.4)	22.6	5, 6, 8, 9, 14
7_{ax}				2.01		6, 8, 9
8		145.7			148.0	
9		84.5			74.3	
10		42.6			41.3	
11		29.1		1.63	28.3	12, 13
12		34.4		2.02	33.6	9, 11, 13, 14
13		43.4			43.1	
14		144.3			141.6	
15		208.1			208.4	
16	2.43 dd (18.8, 7.6)	42.4	13, 14, 15, 17	2.41	42.5	13, 14, 15, 17
				2.05		15, 17, 20
17	1.55	50.7		1.53	50.4	
18	0.95 s	17.4	12, 13, 14, 17	0.97 s	17.3	12, 13, 14, 17
19	0.84 s	15.8	1, 5, 9, 10	0.82 s	15.5	1, 5, 9, 10
20	1.57	35.2		1.57	35.1	
21	0.99 d (6.0)	19.0	17, 20, 22	1.02 d (6.0)	19.2	17, 20, 22
22	1.38, 1.00	35.7		1.33, 1.07	35.7	
23	1.31, 1.17	23.5		1.32, 1.17	23.5	
24	1.14, 1.09	39.3	22, 25	1.13	39.3	
25	1.53	27.9	24, 26, 27	1.52	27.9	24, 26, 27
26	0.86 d (6.2)	22.5[d]	24, 25, 27	0.86 d (6.2)	22.5[d]	24, 25, 27
27	0.86 d (6.2)	22.7[d]	24, 25, 26	0.86 d (6.2)	22.7[d]	24, 25, 26
CH_3COO	2.01 s	21.3	CH_3COO	2.03	21.4	CH_3COO, 3
CH_3COO		170.5			170.6	

[a] δ-Values are in ppm from the residual $CHCl_3$ signal (δ 7.26); coupling constants (in Hz) are given in parentheses; δ assignments aided by ^1H–^1H COSY experiment. [b] δ-Values are in ppm from $CHCl_3$ signal (δ_C 77.0); ^{13}C assignments were assisted by HMBC experiment. [c] HMBC correlations from H to C. [d] Assignments may be interchanged within a column.

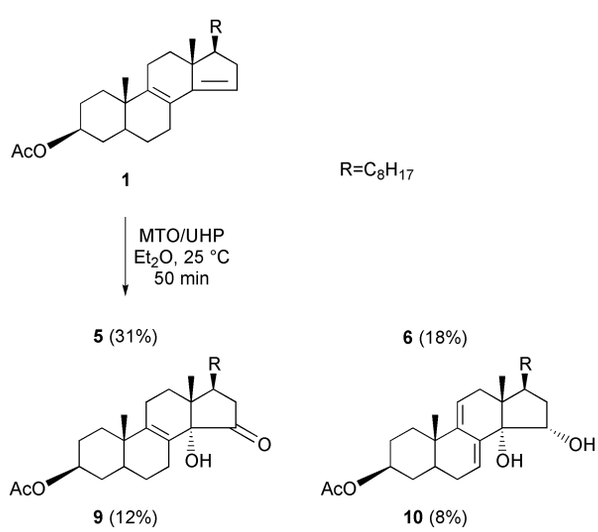
Since steroid **8** is a good starting substrate to obtain polyoxygenated products, we tried to optimize the yield of reaction that affords this steroid. Best results were achieved by treatment of diol **6** with HCl (4%, aq.) in acetone at 25 °C: the reaction finished after *ca.* 1 h, giving triene **8** with 95% yield (Scheme 3b).

Compound **8**, although previously reported in the literature,[23] appears to have never been fully characterized. Then, with support of H–H COSY, HMBC and DEPT NMR experiments, we were able to obtain complete ^1H and ^{13}C NMR assignments for this compound (see Table 4).

When the reaction of diene **1** with the MTO–UHP oxidizing system was performed in diethyl ether at 25 °C the unsaturated diols **5** (31%) and **6** (18%), 14α-hydroxy-15-oxo-5α-cholest-8-en-3β-yl acetate **9** (12%) and the new sterol 14α,15α-dihydroxy-5α-cholesta-7,9(11)-dien-3β-yl acetate **10** (8%) were obtained after 50 min (Scheme 4). In this case an inversion in the yields of compounds **5** and **6** relative to the reaction performed in $CHCl_3$ was observed. The same reaction carried out at 0 °C gave solubility problems.

The structure of **9** was determined by comparing its NMR spectral data with those of an authentic sample obtained recently in our laboratory from the reaction of $\Delta^{8,14}$-diene steroid **1** with RuO_4.[5]

The ^1H NMR spectrum of **10** denoted the presence in the molecule of two trisubstituted double bonds, showing resonances at δ 5.43 (dd, *J* 6.6, 2.5 Hz) and 5.07 (d, *J* 5.2 Hz), and of a CH(OH) group, including a signal at δ 4.91 (br t, *J* 2.3 Hz). Selective H–H decoupling experiments allowed us to assign the signal at δ 5.43 to 11-H, due to its correlations with a methylene group next to a blocked position [δ 2.38 (dd, *J* 17.4, 6.6 Hz, 12-H^{β}) and δ 2.22 (dd, *J* 17.4, 2.5 Hz, 12-H^{α})], the signal at



Scheme 4

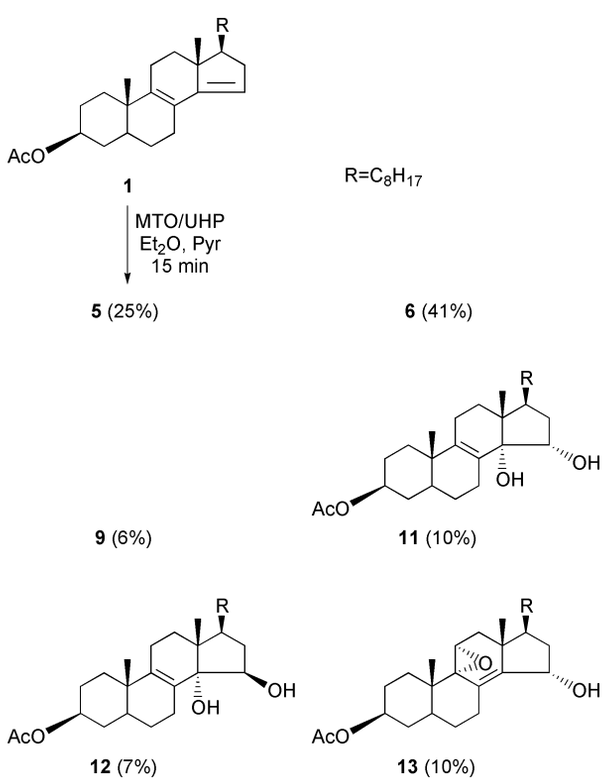
δ 5.07 to 7-H and that at δ 4.91 to 15-H. Furthermore, the ^1H NMR spectrum contained two resonances of exchangeable protons, at δ 6.48 (s) and 5.89 (br s), relative to two alcohol functions, the tertiary of which was positioned at C-14 on the basis of chemical-shift values of H_3-18 that resulted in agreement with the calculated value.[24]

Oxidation of **1** in diethyl ether at 25 °C in the presence of pyridine ligand furnished, after only 15 min, unsaturated diols **5** (25%) and **6** (41%), ketol **9** (6%), 14α,15α-dihydroxy-5α-cholest-8-en-3β-yl acetate **11** (10%), 14α,15β-dihydroxy-5α-cholest-8-en-3β-yl acetate **12** (7%), and the new steroid 9α,11α-epoxy-

Table 2 δ_H and δ_C assignments and HMBC correlations of diols **5** and **6**

	5			**6**		
C	δ_H[a]	δ_C[b]	HMBC[c]	δ_H[a]	δ_C[b]	HMBC[c]
1_{ax}	2.13 td (13.6, 6.4)	29.9	2, 10	2.16	30.0	2, 9, 10, 19
1_{eq}	1.51			1.55		2, 3, 5, 10
2	1.91, 1.53	27.6			27.7	
3	4.86 m	73.4	CH_3COO	5.13 m	73.7	CH_3COO
4_{eq}	1.66 br dt (12.0, 3.8)	34.0	3, 5		33.9	
4_{ax}	1.32			1.38		2, 3, 5, 10
5	2.37 m	35.8		2.55 m	35.5	10
6_{eq}	1.24	29.3	7, 8	1.29	28.9	4, 5, 8, 10
6_{ax}	1.29					
7_{eq}	3.08 dt (13.7, 2.5)	27.3	6, 8, 14	2.88 m	27.9	5, 6, 8, 9, 14
7_{ax}	2.92 td (13.7, 7.2)			2.92 m		5, 6, 8, 9, 14
8		132.7			135.6	
9		84.0			73.9	
10		42.0			40.1	
11	2.23	27.6	8, 9, 12, 13	1.78	29.2	8, 9, 12, 13
12_{eq}	2.35	36.9	18	1.90	34.5	9, 11, 13, 14, 18
12_{ax}	1.99		9, 13, 14, 18			
13		43.8			44.1	
14		152.5			149.3	
15	5.01 br d (6.0)	69.7	8, 13, 14, 17	4.95 br d (5.4)	69.3	8, 13, 14, 17
16_{β}	2.18	40.1	17	2.11	40.7	8, 13, 14, 15, 17
16_{α}	1.90		13, 14, 15, 17			
17	1.86	54.0			53.7	
18	0.95 s	18.3	12, 13, 14, 17	0.95 s	18.3	12, 13, 14, 17
19	0.94 s	16.5	1, 5, 9, 10	0.92 s	16.1	1, 5, 9, 10
20	1.53	34.5		1.52	34.7	17, 21, 22
21	0.96 d (6.6)	19.3	17, 20, 22	0.96 d (6.4)	19.3	17, 20, 22
22	1.38, 1.00	36.4		1.43, 1.02	36.4	21, 23, 24
23	1.31, 1.17	23.9		1.29, 1.12	23.9	
24	1.14, 1.09	39.7	22, 25	1.16, 1.11	39.7	
25	1.43	28.1	24, 26, 27	1.46	28.2	24, 26, 27
26	0.82 d (6.8)	22.6[d]	24, 25, 27	0.87 d (6.4)	22.6[d]	24, 25, 27
27	0.82 d (6.8)	22.9[d]	24, 25, 26	0.87 d (6.4)	22.9[d]	24, 25, 26
CH_3COO	2.03 s	21.3	CH_3COO	2.03 s	21.3	CH_3COO, 3
CH_3COO		170.2			170.2	

[a] δ-Values are in ppm from the residual C_5D_4HN signals (δ 8.71, 7.55, 7.19); coupling constants (in Hz) are given in parentheses; δ assignments aided by ^1H–^1H COSY experiment. [b] δ-Values are in ppm from C_5D_5N signals (δ_C 149.9, 135.5, 123.5); ^{13}C assignments assisted by HMBC experiment. [c] HMBC correlations from H to C. [d] Assignments may be interchanged within a column.



Scheme 5

15α-hydroxy-5α-cholest-8(14)-en-3β-yl acetate **13** (10%) (Scheme 5). An increase in the overall yield in oxidation products (99%) and a higher yield (41%) of the remarkable sterol **6**, in comparison with both reactions performed in $CHCl_3$ and diethyl ether, were observed.

Compounds **11** and **12** were identified by comparing their spectral data with those of authentic materials obtained by $NaBH_4$ reduction of **9**.

The high-resolution EIMS spectrum of the new steroid **13** showed an M^+ ion peak at *m/z* 458.3347 (calc. *M*, 458.3396) corresponding to the molecular formula $C_{29}H_{46}O_4$. lts ^1H NMR spectrum, recorded in [$^2H_5$]pyridine, included the doublet at δ 4.85 (br d, *J* 4.4 Hz), assigned to the hydroxymethine proton 15-H^{β} and the double doublet at δ 3.36 (*J* 7.2, 1.4 Hz), due to a proton of a trisubstituted epoxide ring (see Table 4). The epoxide signal at δ 3.36 was attributed to 11-H, showing correlation with the double doublet at δ 2.30 (*J* 14.2, 7.2 Hz, 12-H^{β}) relative to a proton that is part of a methylene group next to a blocked position, as revealed from selective H–H decoupling experiments. Furthermore, the epoxide signal showed the same doublet appearance as reported in the literature from Tori *et al.*[25] for the 11β proton of the 9α,11α-epoxy steroids. The ^{13}C NMR spectrum of **13** included an hydroxymethine carbon signal at δ_C 68.4 (C-15), two epoxide signals, at δ_C 60.3 (C-9) and 53.1 (C-11), and resonances at δ_C 151.5 and 131.6, relative to a tetrasubstituted double bond positioned between C-8 and C-14 on the basis of HMBC correlations. Stereochemistry at chiral centres C-9, C-11 and C-15 was confirmed by evaluation of 11-H and 15-H proton signal coupling constants and by the

Table 3 δ_H and δ_C assignments and HMBC correlations of compound **7**

	7			
C	$\delta_H(CDCl_3)$[a]	$\delta_H(C_5D_5N)$[a]	$\delta_C(CDCl_3)$[b]	HMBC[c]
1	1.71		31.5	
2	1.86, 1.44		27.3	
3	4.69 m	4.90 m	73.3	
4	1.72, 1.30		33.6	
5	2.21 m	2.51 m	35.8	
6	1.37, 1.23		28.6	
7_{eq}	2.48	3.13 dt (14.0, 2.5)	27.8	5, 8, 9
7_{ax}	2.46	2.93 td (14.0, 7.5)		5, 6, 8, 14
8			134.8	
9			75.1	
10			40.9	
11_{β}	4.11 dd (12.2, 4.4)	4.39 br ddd (11.7, 5.0, 5.0)	66.6	
12_{eq}	2.12 dd (12.4, 4.4)	2.32 dd (11.7, 4.3)	38.7	9, 11, 13, 14
12_{ax}	1.36 dd (12.4, 12.2)	1.74 t (11.7)		9, 11, 13, 18
13			44.5	
14			150.1	
15	4.63 d (4.8)	4.88 br d (5.0)	69.5	8, 13, 14, 17
16_{β}	1.82		44.3	13, 14, 15
16_{α}	1.61			
17	1.68		53.4	
18	0.88 s	0.98[d] s	18.88	12, 13, 14, 17
19	0.89 s	1.00[d] s	15.8	1, 5, 9, 10
20			34.1	
21	0.97 d (6.2)	0.90 d (6.4)	18.95	17, 20, 22
22	1.32, 0.99		36.0	
23	1.33, 1.13		24.0	
24	1.12, 1.07		39.3	
25	1.52		27.9	23, 24, 26, 27
26	0.86 d (6.6)	0.83 d (6.4)	22.8[d]	24, 25, 27
27	0.86 d (6.6)	0.83 d (6.4)	22.5[d]	24, 25, 26
CH_3CO	2.01 s	2.04 s	21.4	
CH_3CO			170.8	
15-OH	4.20 br s	5.81 br s		
9-OH	3.71 br s	5.53 br s		
11-OH	2.67 br s	5.55 br d (5.0)		

[a] δ-Values are in ppm from the residual solvent signals ($CHCl_3$: δ 7.26; C_5D_4HN: δ 8.71, 7.55, 7.19); coupling constants (in Hz) are given in parentheses; δ assignments aided by ^1H–^1H COSY experiment, in $CDCl_3$ solution, and selective H–H decoupling experiments, in C_5D_5N solution. [b] δ-Values are in ppm from $CDCl_3$ signal (δ_C 77.0); ^{13}C assignments assisted by HMBC experiment. [c] HMBC correlations in $CDCl_3$ solution from H to C. [d] Assignments may be interchanged within a column.

chemical-shift values of H_3-18 and H_3-19 which were in agreement with the calculated values.[24] Final proof for structure **13** came from epoxidation of **2** to **13** with *m*-chloroperbenzoic acid.

Conclusions

In this paper we report MTO-catalyzed oxidations, with UHP, of the conjugated diene steroid 5α-cholesta-8,14-dien-3β-yl acetate **1** in various solvents and under various temperature conditions and in the presence of pyridine ligand. From analysis of the various oxidation products it should be clear that unsaturated epoxides are initial products of the oxidative process and that these intermediates undergo ring opening, even in the presence of pyridine, due to their high instability towards the strong Lewis acid MTO. Nevertheless, pyridine has an accelerating effect on the reaction rate, as denoted by reaction times (see Experimental section), according to data previously reported in the literature[18] and allows an increase in the overall yield in oxidation products (99%) and a higher yield (41%) of the noteworthy sterol **6**, in comparison with both reactions performed in $CHCl_3$ and in diethyl ether. Furthermore, carrying out oxidation of **1** with MTO–UHP in diethyl ether in the presence of pyridine, we were able to isolate an epoxide product (**13**, Scheme 5), probably derived by epoxidation of compound **2**. In all cases it is reasonable that epoxide-ring opening occurs prevalently for 1,4-water attack to the unsaturated oxiranes to give unsaturated 1,4-diols, as can be seen by reaction-product analysis. However, these reactions permit polyoxygenation of steroid rings in suitable positions and are an attractive alternative to direct C–C double-bond steroid dihydroxylation with RuO_4.[2,3] Specifically, application of this oxidative procedure to $\Delta^{8,14}$-diene steroids allowed us to oxygenate C and D steroid rings and obtain various 15-oxygenated sterols, a class of sterols noteworthy because most of them act as inhibitors of sterol synthesis in animal cell-culture systems.[19]

Furthermore, dehydration of diol **6** with a solution of HCl (4% aq.) in acetone at 25 °C led to an interesting triene substrate (**8**, 95% yield, Scheme 3b), which we hope will be successively utilized to polyoxygenate simultaneously B, C and D rings of the steroid nucleus.

Finally, from these reactions we have isolated and well characterized the new steroids **3**, **7** and **13**, the biological activity of which is under investigation.

Experimental

UV spectra were recorded with a Perkin-Elmer Model 550S spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AMX-500 and Varian 200 spectrometers for samples in $CDCl_3$ and [$^2H_5$]pyridine solutions. Proton chemical shifts were referenced to residual $CHCl_3$ (δ 7.26) and C_5D_4HN (δ 8.71, 7.55, 7.19) signals. ^{13}C NMR chemical shifts were referenced to the solvent ($CDCl_3$: δ_C 77.0; C_5D_5N: δ_C 149.9, 135.5, 123.5). *J*-Values are given in Hz. 2D-NMR spectra were recorded at 500 MHz on a Bruker AMX-500 spectrometer. In particular, ^1H–^1H COSY experiments were

Table 4 δ_H and δ_C assignments and HMBC correlations of compounds **8** and **13**

	8			**13**	
C	$\delta_H(CDCl_3)$[a]	$\delta_C(CDCl_3)$[b]	HMBC[c]	$\delta_H(C_5D_5N)$[a]	$\delta_C(C_5D_5N)$[b]
1		34.1			29.2
2	1.96, 1.60	27.6	3, 10		27.3
3	4.67 m	73.1	CH_3CO, 2	4.93 m	73.1
4	1.80, 1.38	33.8			33.8
5	1.56	38.3			36.2
6	1.98	30.3	4, 5, 7, 8		29.0
7	6.05 br t (3.3)	120.3	5, 6, 8, 9	$_{eq}$ 3.03, dt (15.0, 2.5) $_{ax}$ 2.50 m	28.5
8		128.3			131.6
9		142.0			60.3
10		35.2			39.9
11	5.51 br d (6.4)	118.2	8, 10, 12, 13	3.36 dd (7.2, 1.4)	53.1
12_{eq} 12_{ax}	2.43 dd (17.1, 6.8) 2.13 br d (17.1)	41.8	9, 11, 13, 14, 18	2.30 dd (14.2, 7.2)	41.7
13		45.3			44.1
14		148.0			151.5
15	5.80 br s	118.2	8, 13, 14, 16, 17	4.85 br d (4.4)	68.4
16_β 16_α	2.41 br dd (17.8, 6.7) 2.00	36.0	13, 14, 15, 17 14, 15, 17, 20	1.82 dd (13.3, 6.8) 1.58 br dd (13.3, 2.6)	40.1
17	1.62	58.3	12, 13, 18	2.19 br d (6.8)	53.4
18	0.81 s	17.4	12, 13, 14, 17	1.02 s	19.1
19	0.94 s	18.7	1, 5, 9, 10	1.02 s	16.8
20	1.61	34.4			34.1
21	0.93 d (6.0)	18.2	17, 20, 22	0.87 d (6.8)	19.1
22	1.35, 1.12	35.5			36.3
23	1.33, 1.10	23.8			23.9
24	1.15, 1.09	39.5			39.7
25	1.54	28.0	23, 24	1.53	28.2
26	0.86 d (6.4)	22.7[d]	24, 25, 27	0.84 d (6.6)	22.9[d]
27	0.86 d (6.4)	22.5[d]	24, 25, 26	0.84 d (6.6)	22.7[d]
CH_3CO	2.03 s	21.3		2.04 s	21.3
CH_3CO		170.6			170.4

[a] δ-Values are in ppm from the residual solvent signals ($CHCl_3$: δ 7.26; C_5D_4HN: δ 8.71, 7.55, 7.19); coupling constants (in Hz) are given in parentheses; δ assignments aided by ^1H–^1H COSY experiment for **8** and by selective H–H decoupling experiments for **13**. [b] δ-Values are in ppm from solvent signals ($CDCl_3$: δ_C 77.0; C_5D_5N: δ_C 149.9, 135.5, 123.5); ^{13}C assignments assisted by HMBC experiment. [c] HMBC correlations from H to C. [d] Assignments may be interchanged within a column.

recorded by employing the conventional pulse sequence[26] while HMBC and NOESY experiments were performed according to the method of Bax and co-workers.[27] Positive-ion FAB mass spectra were obtained on a VG Autospec mass spectrometer. Electron-impact mass spectra (EIMS) were recorded on a Trio 2000 mass spectrometer. High-resolution electron-impact mass spectra (HREIMS) were obtained by electron impact at 70 eV on a Kratos AEI-MS 902 mass spectrometer. Mps were determined with a Reichert microscopic hot-stage apparatus and are uncorrected. High-performance liquid chromatography (HPLC) separations were performed on a Varian 2510 apparatus equipped with a Waters R403 dual-cell refractometer, using semipreparative Hibar Lichrosorb Si-60 (250 × 10 mm) column. TLC analyses were performed on precoated silica gel F_{254} plates (0.25 mm thick, Merck).

Oxidation of 1 with MTO–UHP in $CHCl_3$ (Scheme 1)

Methyltrioxorhenium (MTO, 6.0 mg, 0.024 mmol) was mixed with the urea–hydrogen peroxide adduct (UHP, 88.3 mg, 0.939 eq.) in $CHCl_3$ (2 cm^3) and the mixture was stirred at 25 °C for 10 min. A solution of **1** (100 mg, 0.235 mmol) in 2 cm^3 of $CHCl_3$ was then added dropwise to the stirred yellow suspension. The progress of the reaction was followed by TLC. When the reaction was complete (1 h and 40 min) 10 cm^3 of water were added. The mixture was extracted with $CHCl_3$ (3 × 15 cm^3) and the combined organic phases were dried with $MgSO_4$ and then evaporated under reduced pressure to give 110 mg of crude products. Separation of the reaction mixture by HPLC using hexane–AcOEt (75 : 25 v/v, φ 3.5 cm^3 min^{-1}) as eluent gave pure samples of **2** (8.3 mg, 8%, t_R 3.9 min), **3** (11.8 mg, 11%, t_R 4.8 min), **4** (7.5 mg, 7%, t_R 5.4 min), **5** (9.7 mg, 9%, t_R 12.4 min), **6** (29.1 mg, 27%, t_R 16.7 min) and **7** (31.3 mg, 28%, t_R 24.8 min) (Scheme 1), as solids.

15α-Hydroxy-5α-cholesta-8(14),9(11)-dien-3β-yl acetate 2. TLC R_f 0.43 (hexane–AcOEt, 8 : 2); δ_H(200 MHz; $CDCl_3$) 5.45 (1 H, dd, *J* 6.4, 2.0, 11-H), 4.71 (1 H, m, 3-H^α), 4.67 (1 H, br d, *J* 5.2, 15-H^β), 2.66 (1 H, dt, *J* 15.2, 4.0, 7-H^β), 2.36 (1 H, dd, *J* 16.3, 6.4, 12-H^β), 2.06 (1 H, dd, *J* 16.3, 2.0, 12-H^α), 2.03 (3 H, s, CH_3COO), 0.94 (3 H, s, H_3-19), 0.91 (3 H, d, *J* 6.4, H_3-21), 0.85 (6 H, d, *J* 6.4, H_3-26 and -27), 0.73 (3 H, s, H_3-18); δ_H(200 MHz; C_5D_5N) 5.71 (1 H, br d, *J* 5.0, 15-OH), 5.44 (1 H, dd, *J* 6.4, 2.0, 11-H), 4.92 (1 H, br t, *J* 5.0, 15-H^β), 4.83 (1 H, m, 3-H^α), 2.91 (1 H, dd, *J* 16.8, 4.0, 7-H^β), 2.62 (1 H, td, *J* 16.8, 6.2, 7-H^α), 2.35 (1 H, dd, *J* 16.1, 6.4, 12-H^β), 2.25 (1 H, dd, *J* 12.8, 5.0, 16-H^β), 2.15 (1 H, dd, *J* 16.1, 2.0, 12-H^α), 2.04 (3 H, s, CH_3COO), 0.97 (3 H, s, H_3-19), 0.94 (3 H, d, *J* 6.4, H_3-21), 0.90 (3 H, s, H_3-18), 0.84 (6 H, d, *J* 6.4, H_3-26 and -27); *m*/*z* (EI) 442.3478 (M^+. $C_{29}H_{46}O_3$ requires *M*, 442.3447).

9β-Hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl acetate 3. TLC R_f 0.34 (hexane–AcOEt, 8 : 2); $\lambda_{max}(CHCl_3)$/nm 255 (ε/dm^3 mol^{-1} cm^{-1} 8544); δ_H(200 MHz; $CDCl_3$) see Table 1; δ_C(50.1 MHz; $CDCl_3$) see Table 1; *m*/*z* (EI) 458 (M^+, 6%), 440 ($M^+ - H_2O$, 6), 410 ($M^+ - H_2O - 2CH_3$, 6), 398 (M^+ − AcOH, 17), 380 (M^+ − AcOH − H_2O, 9), 290 [$M^+ - C_8H_{17}$-(side chain) − C_3H_3O(D ring), 44], 237 ($M^+ - C_8H_{17}$ − AcOH − H_2O − $2CH_3$, 13), 197 ($M^+ - C_8H_{17} - C_3H_3O$ − AcOH − H_2O − CH_3, 22), 177 [$M^+ - C_8H_{17} - C_3H_3O$ − (A ring), 100]; *m*/*z* (EI) 458.3358 (M^+. $C_{29}H_{46}O_4$ requires *M*, 458.3396).

9α-Hydroxy-15-oxo-5α-cholest-8(14)-en-3β-yl acetate 4. TLC R_f 0.34 (hexane–AcOEt, 8 : 2); δ_H(500 MHz; $CDCl_3$) see Table 1; δ_C(125 MHz; $CDCl_3$) see Table 1; *m*/*z* (EI) 458.3366 (M^+. $C_{29}H_{46}O_4$ requires *M*, 458.3396).

9α,15β-Dihydroxy-5α-cholest-8(14)-en-3β-yl acetate 5. TLC R_f 0.15 (hexane–AcOEt, 75 : 25); δ_H(500 MHz; C_5D_5N) see Table 2; δ_H(200 MHz; $CDCl_3$) 4.75 (1 H, br d, *J* 5.6, 15-H$^{\alpha}$), 4.68 (1 H, m, 3-H$^{\alpha}$), 2.18 (1 H, dt, *J* 12.2, 4.4, 7-H$^{\beta}$), 2.00 (3 H, s, CH_3COO), 1.74 (1 H, br dd, *J* 12.4, 5.6, 16-H$^{\alpha}$), 0.94 (3 H, d, *J* 6.4, H_3-21), 0.89 (3 H, s, H_3-18), 0.86 (6 H, d, *J* 6.6, H_3-26 and -27), 0.80 (3 H, s, H_3-19); δ_C(125 MHz; C_5D_5N) see Table 2; *m*/*z* (EI) 460.3511 (M^+. $C_{29}H_{48}O_4$ requires *M*, 460.3553).

9α,15α-Dihydroxy-5α-cholest-8(14)-en-3β-yl acetate 6. TLC R_f 0.17 (hexane–AcOEt, 7 : 3); δ_H(500 MHz; C_5D_5N) see Table 2; δ_H(200 MHz; $CDCl_3$) 4.71 (1 H, m, 3-H$^{\alpha}$), 4.64 (1 H, br d, *J* 5.1, 15-H$^{\beta}$), 2.01 (3 H, s, CH_3COO), 0.96 (3 H, d, *J* 6.4, H_3-21), 0.86 (6 H, d, *J* 6.4, H_3-26 and -27), 0.84 (3 H, s, H_3-18), 0.81 (3 H, s, H_3-19); δ_C(125 MHz; C_5D_5N) see Table 2; *m*/*z* (EI) 460 (M^+, 5%), 442 ($M^+ - H_2O$, 16), 427 ($M^+ - H_2O - CH_3$, 8), 409 ($M^+ - 2H_2O - CH_3$, 3), 400 (M^+ − AcOH, 32), 382 (M^+ − AcOH − H_2O, 33), 367 (M^+ − AcOH − H_2O − CH_3, 57), 349 (M^+ − AcOH − $2H_2O$ − CH_3, 16), 311 [$M^+ - C_8H_{17}$-(side chain) − $2H_2O$, 100]; *m*/*z* (EI) 460.3525 (M^+. $C_{29}H_{48}O_4$ requires *M*, 460.3553).

Acetylation of 6. Compound **6** (10 mg, 0.022 mmol) was acetylated with pyridine–Ac_2O (2 : 1) overnight at room temperature. After the usual work-up the crude residue was purified on a TLC plate (hexane–AcOEt, 8 : 2 v/v) to give the pure acetyl derivative (10.4 mg, 95%); δ_H(200 MHz; C_5D_5N) 5.98 (1 H, br d, *J* 6.1, 15-H$^{\beta}$), 5.90 (1 H, br s, 9-OH), 4.94 (1 H, m, 3-H$^{\alpha}$), 2.68 (1 H, td *J* 13.4, 7.6, 7-H^{ax}), 2.56 (1 H, m, 5-H), 2.19 (1 H, dt *J* 13.4, 2.5, 7-H^{eq}), 2.05 (3 H, s, CH_3COO), 1.96 (3 H, s, CH_3COO), 0.90 (3 H, s, H_3-19), 0.89 (3 H, d, *J* 6.4, H_3-21), 0.84 (6 H, d, *J* 6.4, H_3-26 and -27), 0.84 (3 H, s, H_3-18).

9α,11α,15α-Trihydroxy-5α-cholest-8(14)-en-3β-yl acetate 7. TLC R_f 0.09 (hexane–AcOEt, 7 : 3); δ_H(500 MHz; $CDCl_3$) see Table 3; δ_H(200 MHz; C_5D_5N) see Table 3; δ_C(500 MHz; $CDCl_3$) see Table 3; *m*/*z* (EI) 476 (M^+, 7%), 458 ($M^+ - H_2O$, 58), 440 ($M^+ - 2H_2O$, 38), 422 ($M^+ - 3H_2O$, 19), 416 (M^+ − AcOH, 32), 398 (M^+ − AcOH − H_2O, 31), 380 (M^+ − AcOH − $2H_2O$, 69), 365 (M^+ − AcOH − $2H_2O$ − CH_3, 27), 345 [$M^+ - C_8H_{17}$-(side chain) − H_2O, 13], 327 ($M^+ - C_8H_{17} - 2H_2O$, 100), 309 ($M^+ - C_8H_{17} - 3H_2O$, 26); *m*/*z* (EI) 476.3485 (M^+. $C_{29}H_{48}O_5$ requires *M*, 476.3502).

Chromium trioxide–pyridine oxidation of compounds 5 and 6 (Scheme 2)

To a solution of **5** (10 mg, 0.022 mmol) or **6** in pyridine (1.5 cm^3) was added an excess of chromium trioxide–pyridine complex.[28] The mixture was stirred at room temperature for 16 h, then diluted with water (4 cm^3) and extracted with diethyl ether (3 × 6 cm^3). The ethereal solution, taken to dryness, was chromatographed by HPLC using hexane–AcOEt (7 : 3 v/v) as eluent to give 8 mg (79%) of α,β-unsaturated ketone **4**.

Dehydration of compound 6 (Scheme 3)

Treatment of diol **6** (20 mg, 0.043 mmol) with CH_3COOH (1 cm^3) in $CHCl_3$ (1 cm^3) at 25 °C for 1 h furnished, after HPLC separation [eluent: hexane–AcOEt (8 : 2 v/v)], 5.8 mg of compound **2** (30%) and 12.3 mg of triene **8** (67%, Scheme 3a), as solids. Dehydration of diol **6** (20 mg, 0.043 mmol) with HCl (1 cm^3; 4% aq.) in acetone (1 cm^3) at 25 °C for 1 h gave triene **8** in 95% yield (17.5 mg, Scheme 3b).

5α-Cholesta-7,9(11),14-trien-3β-yl acetate 8. TLC R_f 0.90 (hexane–AcOEt, 7 : 3); δ_H(500 MHz; $CDCl_3$) see Table 4; δ_C(125 MHz; $CDCl_3$) see Table 4; *m*/*z* (EI) 424 (M^+, 14%), 364 (M^+ − AcOH, 82), 349 (M^+ − AcOH − CH_3, 27), 311 [$M^+ - C_8H_{17}$(side chain), 100], 251 ($M^+ - C_8H_{17}$ − AcOH, 82); *m*/*z* (EI) 424.3358 (M^+. $C_{29}H_{44}O_2$ requires *M*, 424.3341).

Oxidation of 1 with MTO–UHP in Et_2O (Scheme 4)

Oxidation of **1** (100 mg, 0.235 mmol) with MTO (6.0 mg, 0.024 mmol) and UHP (88.3 mg, 0.939) was also performed in diethyl ether at 25 °C. After 50 min the reaction was quenched by addition of 10 cm^3 of water and worked up as above. HPLC separation of the resulting mixture [eluent: hexane–AcOEt (75 : 25 v/v)] gave as solids, in order of elution, 12.9 mg of **9** (12%), 8.6 mg of **10** (8%), 33.4 mg of **5** (31%) and 19.4 mg of **6** (18%) (Scheme 4).

14α,15α-Dihydroxy-5α-cholesta-7,9(11)-dien-3β-yl acetate 10. TLC R_f 0.16 (hexane–AcOEt, 75 : 25); δ_H(200 MHz; C_5D_5N) 6.48 (1 H, s, OH), 5.89 (1 H, br s, OH), 5.43 (1 H, dd, *J* 6.6, 2.5, 11-H), 5.07 (1 H, d, *J* 5.2, 7-H), 4.91 (1 H, br t, *J* 2.3, 15-H$^{\beta}$), 4.79 (1 H, m, 3-H$^{\alpha}$), 2.38 (1 H, dd, *J* 17.4, 6.6, 12-H^{eq}), 2.22 (1 H, dd, *J* 17.4, 2.5, 12-H^{ax}), 2.02 (3 H, s, CH_3COO), 0.93 (3 H, s, H_3-19), 0.92 (3 H, d, *J* 6.4, H_3-21), 0.87 (3-H, s, H_3-18), 0.81 (6 H, d, *J* 6.3, H_3-26 and -27); *m*/*z* (EI) 458.3377 (M^+. $C_{29}H_{46}O_4$ requires *M*, 458.3396).

Oxidation of 1 with MTO–UHP in Et_2O in the presence of pyridine ligand (Scheme 5)

The reaction of 100 mg (0.235 mmol) of the diene **1**, 6.0 mg (0.024 mmol) of MTO, 88.3 mg (0.939 mmol) of UHP and 190 mm^3 (2.350 mmol) of pyridine at 25 °C in diethyl ether was completed after 15 min. The resulting mixture was separated by HPLC using hexane–AcOEt (75 : 25 v/v, φ 2.2 cm^3 min^{-1}) as eluent to give, in order of elution, **9** (6.4 mg, 6%), **11** (10.8 mg, 10%), **12** (7.5 mg, 7%), **13** (10.7 mg, 10%), **5** (27.0, 25%) and **6** (44.3 mg, 41%) (Scheme 5), as solids.

9α,11α-Epoxy-15α-hydroxy-5α-cholest-8(14)-en-3β-yl acetate 13. TLC R_f 0.38 (hexane–AcOEt, 7 : 3); δ_H(500 MHz; C_5D_5N) see Table 4; δ_H(200 MHz; $CDCl_3$) 4.69 (1 H, m, 3-H$^{\alpha}$), 4.61 (1 H, br d, *J* 4.4, 15-H$^{\beta}$), 3.34 (1 H, dd, *J* 7.2, 1.4, 11-H$^{\beta}$), 2.65 (1 H, dt, *J* 15.0, 2.5, 7-H$^{\beta}$), 2.36 (1 H, dd, *J* 14.1, 7.2, 12-H$^{\beta}$), 2.02 (3 H, s, CH_3COO), 1.03 (3 H, s, H_3-19), 0.93 (3 H, s, H_3-18), 0.89 (3 H, d, *J* 6.8, H_3-21), 0.86 (6 H, d, *J* 6.8, H_3-26 and -27); δ_C(125 MHz; C_5D_5N) see Table 4; *m*/*z* (FAB) 459 (MH^+, 18%), 441 ($MH^+ - H_2O$, 100), 425 ($MH^+ - O - H_2O$, 84), 423 ($MH^+ - 2H_2O$, 57), 399 (MH^+ − AcOH, 17), 381 ($MH^+ - H_2O$ − AcOH, 32), 365 ($MH^+ - H_2O - O$ − AcOH, 21), 363 ($MH^+ - 2H_2O$ − AcOH, 20); *m*/*z* (EI) 458 (M^+, 32%), 442 ($M^+ - H_2O$, 9), 440 ($M^+ - H_2O$, 100), 424 ($M^+ - H_2O - O$, 19), 422 ($M^+ - 2H_2O$, 28), 407 ($M^+ - 2H_2O - CH_3$, 7), 398 (M^+ − AcOH, 14), 380 ($M^+ - H_2O$ − AcOH, 31), 365 ($M^+ - H_2O$ − AcOH − CH_3, 28), 362 ($M^+ - 2H_2O$ − AcOH, 16), 345 ($M^+ - C_8H_{17}$, 17); *m*/*z* (EI) 458.3347 (M^+. $C_{29}H_{46}O_4$ requires *M*, 458.3396, 32%), 440.3295 ($M^+ - H_2O$. $C_{29}H_{44}O_3$ requires *m*/*z*, 440.3290, 100).

Epoxidation of compound 2

To diene **2** (10 mg, 0.023 mmol), dissolved in 1 cm^3 of diethyl ether, was added a solution of *m*-chloroperbenzoic acid (4.7 mg, 0.028 mmol) in diethyl ether (1.5 cm^3) at 25 °C. The mixture was stirred for 2 h, after which it was washed successively with aq. Na_2SO_3 and aq. $NaHCO_3$. The ethereal solution was evaporated and the residue was purified by HPLC (eluent: hexane–AcOEt, 7 : 3 v/v) to give epoxide steroid **13** (8 mg) in 77% yield.

$NaBH_4$ reduction of compound 9

Unsaturated ketol **9** (6 mg, 0.013 mmol), dissolved in 2 cm^3 of isopropyl alcohol and 1 cm^3 of methanol, was treated with

$NaBH_4$ (6.5 mg dissolved in 1 cm^3 of methanol and 0.1 cm^3 of water) at 0 °C to afford a mixture of 15α-alcohol **11** (0.4 mg, 7%) and its 15β-epimer **12** (4.2 mg, 70%), which were isolated and purified by HPLC using hexane–AcOEt (75 : 25 v/v) as eluent.

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