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Studies Towards the Synthesis of Polyoxygenated Steroids[1]. Reaction of 7,9(11)-Diene Steroids with RuO_4.

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Abstract. In order to find an entry to C-ring oxygenated steroids, the reaction of ruthenium tetroxide with some $\Delta^{7,9(11)}$-sterols has been explored. The reaction has been performed both at -70°C and room temperature using an equimolecular amount of the oxidant and acetone-water as solvent system in the absence of a cooxidant. The change in the temperature conditions moderately affects the yields of the reaction products. When 3β,6α- and 3β,6β-diacetoxy-$\Delta^{7,9(11)}$-sterols were used the diene system was predominantly attacked at the 9(11)-double bond from the α-face of the molecule giving Δ^7-9α-hydroxy-11-keto- and Δ^7-9α,11α-dihydroxy-sterols. RuO_4 oxidation of cholesta-7,9(11)-dien-3β-yl acetate, yielded, in addition to the above oxidation products, minor amounts of three related C-7,C-11 oxigenated 8α,9α-epoxysterols, arising from the oxidation of both double bonds of the diene system. These results, that in part parallel those we recently found on RuO_4 oxidation of trisubstituted [Δ^4, Δ^5 and Δ^7] steroidal alkenes, shed further light on the reactivity of this oxidizing agent which, neverthless, seems far from being completely understood. In addition, our procedure furnishes a reliable route to Δ^7-9α,11β-dihydroxysterols *via* $LiAlH_4$ reduction of the 11-keto group.

INTRODUCTION

Ruthenium tetroxide along with a cooxidant such as sodium metaperiodate or sodium hypochloride is a powerful oxidizing reagent[2-6]. It has been usually employed in catalytic amounts to oxidize a variety of compounds and for the oxidative cleavage of aromatics rings[7-10] and carbon-carbon double bonds[11-18]. On the contrary, ruthenium tetroxide oxidation of alkenes in absence of a secondary oxidant has attracted limited attention[19-21] possibly due to low yields.

Recently, we have tested the reactivity of a number of tri- and tetra- substituted nuclear monoene steroids using equimolecular amounts of ruthenium tetroxide in the absence of a cooxidant[22]. In contrast with previously reported results on RuO_4 oxidation of carbon-carbon double bonds[2,3,6] we found that trisubstituted double bonds gave α-hydroxyketones and/or 1,2-diols rather than the expected products deriving from the scission of the double bond.

This unexpected outcome prompted us to extend our oxidation procedure to some steroidal dienes. In this paper we report the results of our studies concerning the 7,9(11)-diene system.

RESULTS AND DISCUSSION

Our attention was primarily addressed to the 7,9(11)-diene system because we were above all interested in developing oxidative methods which would have allowed to synthesize C-9 and/or C-11 oxygenated steroids, recently isolated metabolites from the sponges genus *Dysidea*[23-27].

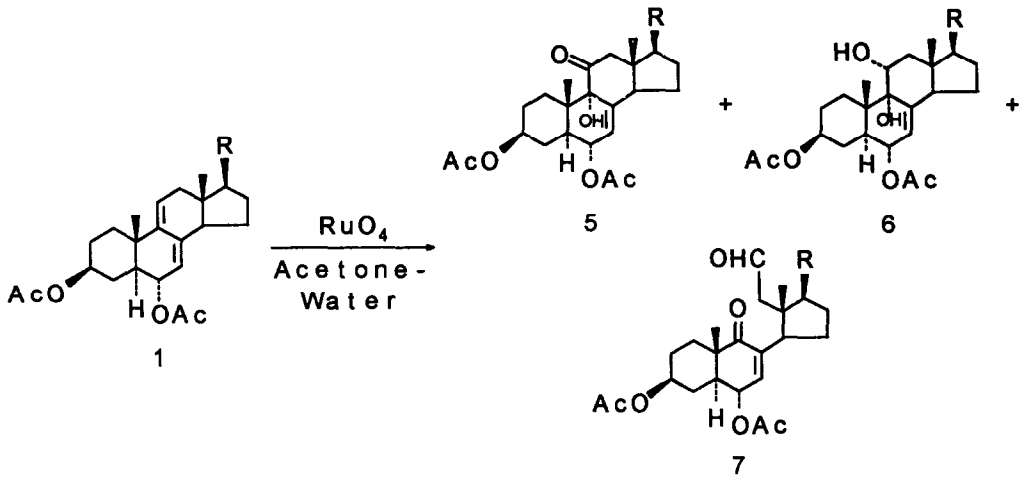
As first substrate we choose 5α-cholesta-7,9(11)-diene-3β,6α-diol diacetate (**1**) (Table 1, entry 1), a steroid recently synthesized in our laboratory starting from 7-dehydrocholesterol[28], which on oxidation with ruthenium tetroxide at room temperature afforded 3β,6α-diacetoxy-9α-hydroxy-5α-cholest-7-en-11-one (**5**) and the corresponding 9α,11α-dihydroxysterol, 5α-cholest-7-ene-3β,6α,9α,11α-tetrol 3,6-diacetate (**6**), in 42% and 22% yields, respectively. Minor amounts (8% yield) of the 9,11-secosterol **7**, 3β,6α-diacetoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al, deriving from the scission of the $\Delta^{9(11)}$-double bond, was also found (Scheme 1).

Spectral data for compounds **5-7** were in good agreement with the proposed structures. In particular, the presence in **5** of a 9α-hydroxy-11-keto grouping, in place of the $\Delta^{9(11)}$-double bond, was deduced as follows. The presence of a carbonyl and a tertiary hydroxyl group in the molecule was inferred from IR (ν_{max} 1715 and 3457 cm^{-1}), ^{13}C-NMR [δ 208.74 (s) and 76.97 (s)] and ^{1}H-NMR (δ 2.48, s, exchangeable proton) data. (Table 2). The ^{1}H-NMR spectrum of **5** recorded in $CDCl_3$ also contained an olefinic signal at δ 5.48 (1H, bdd, J=1.8 and 1.8 Hz, H-7) and two mutually coupled doublets at δ 2.84 and 2.54 (1H each, J=15.9 Hz,) that indicated the presence of an isolated methylene (H_2-12) next to the carbonyl group. The pyridine induced-shifts[29] for protons H_{ax}-1, H-5 and H-14, in the spectrum recorded in pyridine-d_5 (Table 2) , were in agreement with the presence in the molecule of a 9α-OH group (these protons were not assigned in the spectrum recorded in $CDCl_3$ since they fell in the crowded region below 2.15 ppm). Likewise, the H_{eq}-1 proton experienced a strong downfield shift in the same solvent due to the vicinity in the space with the C-11 carbonyl group. Final proof for structure **5** was obtained by $LiAlH_4$ reduction that afforded a 1:5 mixture of 5α-cholest-7-ene-3β,6α,9α,11α-tetrol, a steroid recently synthesized in our laboratory[28], and its 11β-epimer. The ^{1}H-NMR spectrum of the mixture contained, *inter alia*, the characteristic sharp triplet (J= 1.8 and 1.8 Hz) attributable to the Hα-11 proton[30] of the 11β-epimer.

Compound **6** was easily recognized as the 9α,11α-diol corresponding to the α-ketol **5**. ^{1}H- (Table 3) and ^{13}C-NMR spectra of **6** contained the resonances pertinent to the C-11 hydroxymethine group[26,28] [^{1}H-NMR: δ 4.12 (1H, ddd, J= 11.7, 5.9 Hz, H_β-11); ^{13}C-NMR: δ 69.34 (d)] which replaces the C-11 keto group present in **5**. The α-orientation of the 11-OH group followed from the value of the coupling constants (11.7 and 5.9 Hz) of its geminal proton (H-11) with H-12. Finally, deacetylation of **6** with $LiAlH_4$ gave a product indistinguishable from 5α-cholest-7-ene-3β,6α,9α,11α-tetrol[28].

The spectral data of compound 7 were consistent with the presence of the Δ^7-9,11-secosterol structure. The IR spectrum showed a carbonyl absorption at 1678 cm^{-1}, typical of a conjugated ketone, and an absorption at 1718 cm^{-1} attributed to an aldehyde on the basis of ^1H and ^{13}C-NMR data [^1H-NMR: δ 9.90 (d,J=3.4 Hz, H-11);^{13}C-NMR: δ 203.57 (d,C-11)].

Scheme 1



The UV absorption at 237 nm (ε = 5013) and ^{13}C-NMR data [202.82 (s, C-9), 143.03 (d, C-7) and 137.68 (s, C-8)] confirmed the presence of an α,β-unsaturated ketone group in the molecule. The ^1H-NMR spectrum also included an olefinic proton signal at δ 6.42 (d, J= 1.9 Hz, H-7).

Δ^7-9,11-Secosterols are natural substances recently isolated in our laboratory from the marine sponge *Spongia officinalis*[28,31]. In particular, steroid 7 is the 3,6-diacetylderivative of the natural 3β,6α-dihydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al[28]. Indeed, acetylation of the natural ketoaldehyde with Ac_2O-pyridine gave a product identical in all respects to compound 7.

When the above reaction was performed at -70°C an inversion in the yields of compounds 5 and 6 [5 (20%), 6 (34%)] and an increasing of the yield of aldehyde 7 (23%) was observed.

It is to be noted that RuO_4 shows in this reaction a behaviour similar to that shown by other oxidizing agents such as osmium tetroxide and *m*-chloroperbenzoic acid which react, with the above substrate, forming the osmate ester and the epoxyderivative through the preferential attack at the $\Delta^{9(11)}$-unsaturation while leaving, as expected, the more hindered Δ^7-double bond unaffected[28].

Table 1. RuO_4 oxidation of some $\Delta^{7,9(11)}$-diene steroids.

Entry	*Substrate*	*Products*	*Yield (%)[a] T=25 °C*	*Yield (%)[a] T= -70 °C*
1	5α-cholesta-7,9(11)-diene-3β,6α-diol diacetate	**5**	42	20
		6	22	34
		7	8	23
2	5α–cholesta-7,9(11)-diene-3β,5,6α-triol	**8**	62	68
	3,6-diacetate	**9**	13	9
3	5α-cholesta-7,9(11)-diene-3β,5,6β-triol	**10**	71	81
	3,6-diacetate	**11**	16	10
		12	traces	traces
4	5α-cholesta-7,9(11)-dien-3β-yl acetate	**13**	6	30
		14	8	6
		15	3	7
		16	16	30
		17	12	14
		18	6	2

a. Yields are for isolated (HPLC) products.

When the reaction was performed on 5α-cholesta-7,9(11)-diene-3β,5,6α-triol 3,6-diacetate (**2**) at room temperature (Table 1, entry 2), 3β,6α-diacetoxy-5,9α-dihydroxy-5α-cholest-7-en-11-one (**8**) (62% yield) and 5α-cholest-7-ene-3β,5,6α,9α,11α-pentol 3,6-diacetate (**9**) (13% yield) were obtained (Scheme 2).

A reasoning similar to that used for the structure elucidation of compound **5** applies in the case of **8**. IR and ^{13}C-NMR data indicated the presence of a ketonic function and one more tertiary hydroxyl group in the molecule showing, respectively, absorptions at 3422 and 1717 cm^{-1} and resonances at δ 206.90 (s, C-11) and 78.57 (or 78.03; s, C-9). The ^{1}H-NMR spectrum of compound **8** ($CDCl_3$, Table 2) included the resonances pertinent to the isolated C-12 methylene group next to the C-11 carbonyl function, at δ 2.82 and 2.58 (1H each, d's, J=13.7 Hz), the H-7 olefinic proton at δ 5.28 and two one-proton singlets for exchangeable hydrogens at δ 4.60 and 2.56. The proton spectrum recorded in pyridine-d_5 (Table 2) showed the expected pyridine-induced shifts[29] for H_{ax}-1, H_{eq}-1 and H-14, in agreement with the presence of a 9α-OH group. Further support for the C-9 stereochemistry came from a nOe experiment; irradiation on the signal of the C-9 hydroxyl group resulted in the enhancement of the H_{ax}-1 and H-14 signals.

Compound **9** had ^{1}H- and ^{13}C-NMR spectra very similar to those of **6**. The proton spectrum (Table 3) included the resonances for the H-7 and Hβ-11 protons at δ 5.23 (bs) and 3.98 (ddd, J=11.0, 11.0 and 5.5 Hz), respectively, while the ^{13}C-NMR spectrum showed signals for C-9 and C-11 carbon atoms at δ 75.35 (s) and 73.42 (d), respectively. Further evidence for structure **9** came from the acetylation of **9** with Ac_2O-pyridine which gave a product having nuclear proton resonances in good agreement with those exhibited by 24-

Table 2. Selected ^{1}H-NMR data for ketols **5,8,10** and **13**[a].

Proton	*5*		*8*		*10*		*13*	
	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N	$CDCl_3$	C_5D_5N
1ax		2.31,ddd (14.2,14.2,3.4)		2.75,ddd (14.0,14.0,2.9)				2.30,ddd (14.2,3.4,3.4)
1eq		2.81,ddd (14.2,3.4,3.4)		2.66,ddd (14.0,4.4,4.4)		2.76,bdd (9.8, 2.9)	2.08,ddd (13.4,3.7,3.7)	2.80,ddd (14.2,3.9,3.9)
3	4.67, m	4.89, m	5.04, m	5.56, m	5.12, m	5.67, m	4.69, m	4.88, m
4ax				1.93,dd (12.5,12.5)		2.32,dd (11.7,11.7)		
4eq		2.29,dd (14.2, 3.4)		2.45,dd (12.5,4.4)		2.23 ddd (11.7,4.9,1.5)		
5		2.55,ddd (10.2,3.4,3.4)						
6	5.05,bddd (10.2,1.8,1.8)	5.44,bd (10.2)	5.11,bs ($W_{1/2}$=4.4)	5.50,bs ($W_{1/2}$=4.4)	5.00, dd (5.3, 2.2)	5.66,dd (5.4,2.4)		
7	5.48, bdd (1.8,1.8)	5.69,bs ($W_{1/2}$=4.9)	5.28,bs ($W_{1/2}$=4.9)	5.74,bs ($W_{1/2}$=5.1)	5.49,dd (5.3, 1.8)	5.81,dd (5.4,2.0)	5.57,bs ($W_{1/2}$=10.0)	5.53,bs ($W_{1/2}$=10.2)
12α	2.84, d (15.9)	3.13, d (13.7)	2.82, d (13.7)	3.05, d (13.2)	2.84, d (15.0)	3.07, d (13.7)	2.82,d (15.9)	3.14, d (13.7)
12β	2.54, d (15.9)	2.74, d (13.7)	2.58, d (13.7)	2.73, d (13.2)	2.58, d (15.0)	2.79,d (13.7)	2.50, d (15.9)	2.74, d (13.7)
14	2.61,bdd (8.8,8.8)	3.11,bdd (8.8,8.8)	2.98,bdd (9.7,9.7)	3.18,bdd (9.6,9.6)	2.86,bdd (10.6,10.6)	3.16,bdd (9.3,9.3)	2.57,bdd (9.2,9.2)	3.06,bdd (8.3,8.3)
18	0.66,s	0.62,s	0.55,s	0.61,s	0.62,s	0.68,s	0.66,s	0.67,s
19	1.04,s	1.28,s	1.20,s	1.42,s	1.17,s	1.55,s	0.94,s	1.19,s
21	0.89, d (5.7)	0.82, d (5.9)	0.90, d (6.2)	0.83, d (6.6)	0.90, d (6.2)	0.83, d (6.3)	0.90, d (6.1)	0.84, d,(5.4)
26,27	0.86, d (6.6)	0.85, d (6.8)	0.86, d (6.6)	0.87, d (6.6)	0.86, d (6.6)	0.86, d (6.8)	0.86, d (6.7)	0.86, d (6.6)
OH's	2.48, bs	7.85, s	4.60, 2.56, s's	7.76, 6.47, s's	4.38, 3.63, s's	7.69, 7.01, s's	2.36,s	7.28,s
Acetates	2.07, 2.03, s's	2.03, 2.01, s's	2.15, 2.03, s's	2.01, 1.85, s's	2.08, 2.04, s's	2.04, 1.98, s's	2.02, s	2.02, s

a. δ Values are in ppm from the residual $CHCl_3$ and pyridine signals (7.26 and 8.71, respectively). Coupling constants (hertz) are given in parentheses.

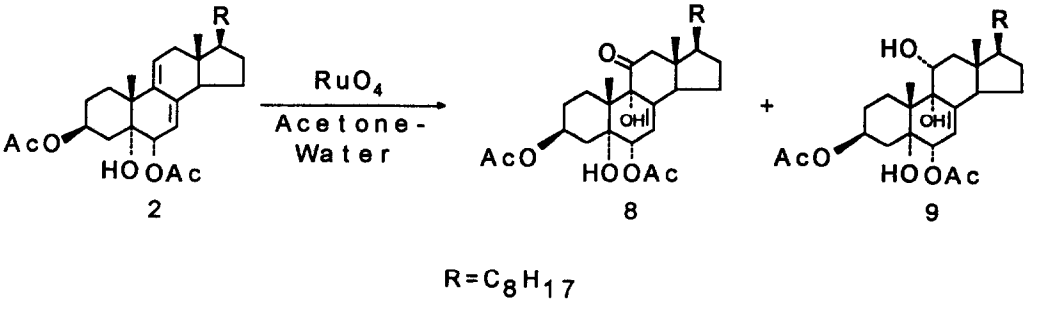
methylene-5α-cholest-7-ene-3β,5,6α,9α,11α,-pentol 3,6,11-triacetate, the triacetate derivative of a natural pentahydroxysterol recently isolated by Kashman *et al.* from the marine sponge *Dysidea herbacea*[26].

Table 3. Selected ^{1}H-NMR data ($CDCl_3$) for compounds **6, 9, 11** and **14**[a].

Proton	*6*	*9*	*11*	*14*
3	4.66,m	5.04,m	5.12,m	4.67,m
6	4.98,bd (10.2)	4.98,bs ($W_{1/2}$=4.9)	4.92,bdd (5.5,1.8)	
7	5.28, bd ($W_{1/2}$=4.4)	5.23,bs ($W_{1/2}$=4.9)	5.40,bdd (5.5,1.8)	5.40,ddd (5.4,2.4,2.4)
11	4.12, ddd (11.7, 5.9,5.9)	3.98,ddd (11.0,11.0,5.5)	4.12, ddd (11.0,5.5,5.5)	4.11,ddd (11.7,5.4,5.4)
14	2.33,bdd (9.3,9.3)	2.52,bdd (11.0,11.0)	2.48,bdd (9.7,9.7)	2.29,m
18	0.58,s	0.58,s	0.61,s	0.57,s
19	1.08,s	1.20,s	1.25,s	0.98,s
21	0.93,d (5.4)	0.94,d (5.5)	0.94,d (5.5)	0.94,d (5.9)
26,27	0.86,d (6.8)	0.82,d (6.7)	0.86, d (6.7)	0.86,d (6.3)
OH's	2.37,s	4.33, 2.56, s's	4.02, 3.44, s's	
Acetates	2.07, 2.03, s's	2.14, 2.03, s's	2.07, 2.03, s's	2.02,s

a. δ Values are in ppm from the residual $CHCl_3$ signal (7.26). Coupling constants (hertz) are given in parentheses.

Scheme 2

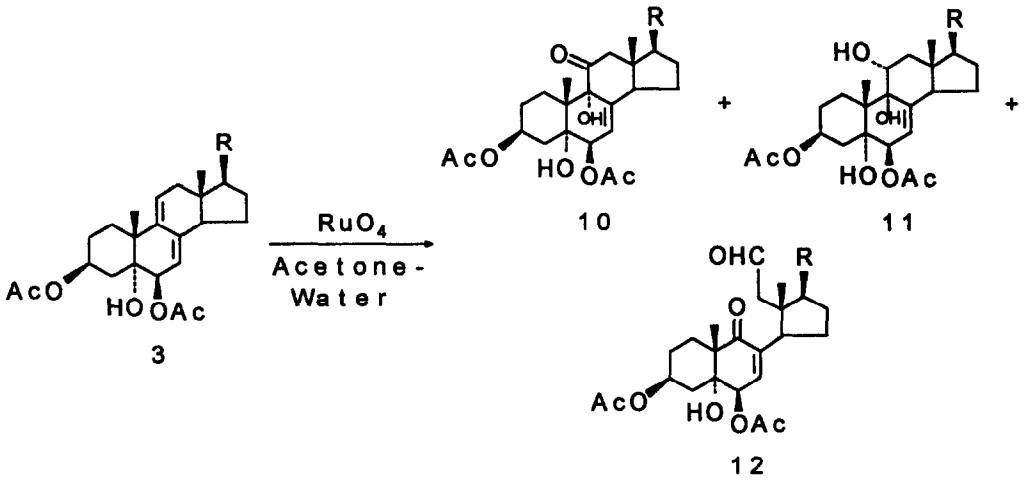


When the reaction was performed at -70 °C componds **8** and **9** were obtained in 68% and 9% yields, respectively.

RuO_4 oxidation at room temperature of the C-6 epimer of **2**, 5α-cholesta-7,9(11)-diene-3β,5,6β-triol 3,6-diacetate (**3**) (Table 1, entry 3), gave 3β,6β-diacetoxy-5,9α-dihydroxy-5α-cholest-7-en-11-one (**10**, 71% yield), 5α-cholest-7-ene-3β,5,6β,9α,11α-pentol 3,6-diacetate (**11**, 16% yield) and 3β,6β-diacetoxy-5-hydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**12**, traces) corresponding to an increasing in the overall yield in oxidation products, in comparison with the reaction performed on the corresponding 6α-epimer **2**, of 12% (Scheme 3) .

The structure elucidation of compounds **10**, **11** and **12** was secured by spectral studies (for ^{1}H-NMR spectra of **10** and **11** see Tables 2 and 3, respectively) and comparison with compounds **5-7** and **8,9**. Secoaldehyde **12** has not been isolated but traces of it were detected by ^{1}H-NMR in the 95% pure HPLC fraction containing the trioldiacetate **11**.

Scheme 3



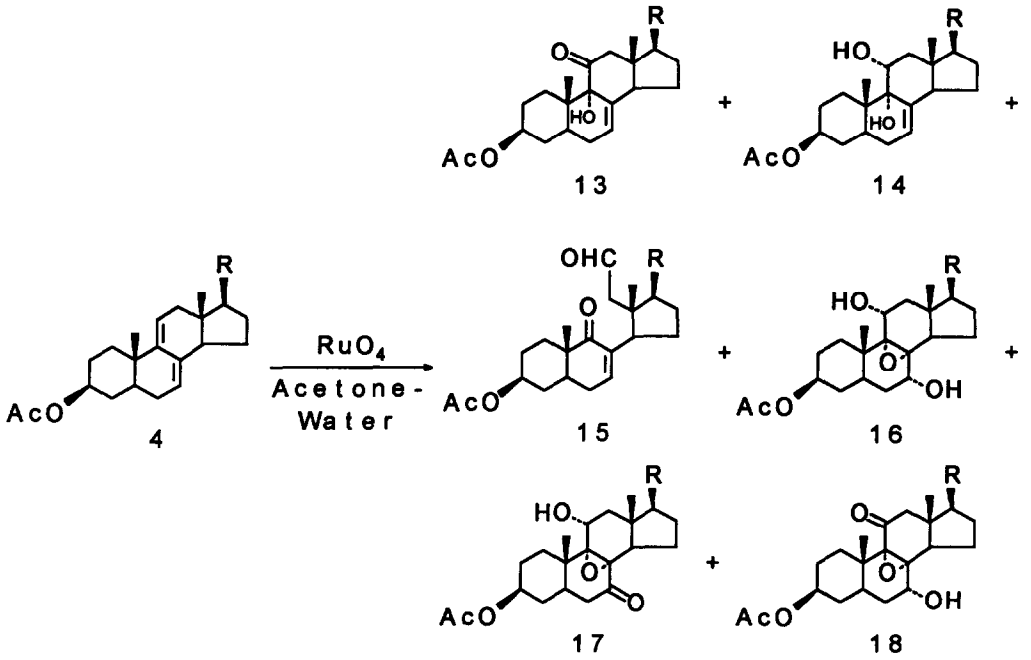
The 7-deacetylderivative of secoaldehyde **12** has recently been isolated in our laboratory from the sponge *Spongia officinalis* and its structure secured by synthesis[32]. Indeed, the ^{1}H-NMR spectrum of the compound obtained from the natural product by acetylation, contained signals superimposable with those observable (H-11, H-7, H-6, H-14 and H_3-18) in the proton spectrum of the mixture of **11** and **12** and pertinent to **12**.

When the reaction was performed at -70°C, compounds **10-12** were obtained in the following yields: **10** (81 %), **11** (10 %), **12** (traces).

The reaction performed on 5α-cholesta-7,9(11)-dien-3β-yl acetate (**4**) at room temperature (Table 1, entry 4) gave, falling into line with the previously shown results, 3β-

acetoxy-9α-hydroxy-5α-cholest-7-en-11-one (**13**, 6 % yield), 5α-cholest-7-ene-3β,9α,11α-triol 3-acetate (**14**, 8 % yield) and 3β-acetoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (**15**, 3 % yield). Moreover, three related C-7,C-11 oxygenated 8α,9α-epoxysteroids were obtained namely 8α,9α-epoxy-5α-cholesta-3β,7α,11α-triol 3-acetate (**16**, 16 % yield), 3β-acetoxy-8α,9α-epoxy-11α-hydroxy-5α-cholestan-7-one (**17**, 12 % yield) and 3β-acetoxy-8α,9α-epoxy-7α-hydroxy-5α-cholestan-11-one (**18**, 6 % yield) (Scheme 4).

Scheme 4



Ketol **13** had spectral features which clearly indicated the presence of the C-9,C-11 α-ketol function next to the Δ^7-unsaturation [^{1}H-NMR (Table 2): δ 2.82 and 2.50 (AB system, J=15.9 Hz, H_2-12), 5.57 (bs, $W_{1/2}$=10.0 Hz, H-7); ^{13}C-NMR: δ 211.89 (s, C-11), 136.69 (s, C-8), 125.07 (d, C-7), 77.80 (s, C-9)].

Compounds **14** and **15** were easily identified as 5α-cholest-7-ene-3β,9α,11α-triol 3-acetate and 3β-acetoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al, respectively, on the basis of spectral data (see Table 3 and the experimental section). Compound **14** has previously been synthesized by osmium tetroxide oxidation of 5α-cholesta-7,9(11)-dien-3β-yl acetate (**4**)[33]

while the 9,11-secosterol **15** has previously been obtained by periodic acid scission of diol **14**[33].

The structures of the strictly related 8α,9α-epoxysterols **16-18** were deduced from spectral analyses. In particular, the stereochemistry at the C-7 and/or C-11 chiral centres in all these compounds was established on the basis of nOe experiments and evaluation of coupling constants of the H-7 and H-11 proton signals in their ^{1}H-NMR spectra. The 8α,9α-stereochemistry of the epoxide ring, though strongly suggested by the steric requirement of diene **4**, which forces the oxidant to attack the diene system from the less hindered α-face of the molecule, was further supported by chromium trioxide-pyridine oxidation of all three compounds **16-18**. In fact, oxidation of compound **16** gave the expected 3β-acetoxy-8α,9α-epoxy-5α-cholestan-7,11-dione along with unreacted **16**, and **17** and **18** in the approximate ratio of 1:1:2:2, as estimated by ^{1}H-NMR analysis. The chemical shift value of the Me-18 and Me-19 resonances in the ^{1}H-NMR spectrum of 3β-acetoxy-8α,9α-epoxy-5α-cholestan-7,11-dione are in good agreement with calculated values (Me-18, found: 0.73 ppm, calculated: 0.76 ppm; Me-19, found: 1.24 ppm, calculated: 1.25 ppm)[34]. The obtainment of the intermediate oxidation products **17** and **18** also correlated compounds **16-18** at all common chiral centres. Oxidation of **17** gave 3β-acetoxy-8α,9α-epoxy-5α-cholestan-7,11-dione and unreacted **17** in the ratio of 1:1.5 while oxidation of **18** afforded the same diketone and unreacted **18** in a 1:1 ratio.

Compounds **5-13** and **17** and **18** have not been synthesized before. Although a literature search had revealed that a 3β-acetoxy-8,9-oxido-7,11-dihydroxycholestane had previously been synthesized, the matter is covered by patent[35].

CONCLUSION

The above results show that ruthenium tetroxide when used in equimolecular amounts in the absence of a secondary oxidant exhibits a chemical reactivity similar to that previously shown with steroidal alkenes , that is, prevalently it is able to oxidize carbon-carbon double bonds without giving rise to their scission[22].

RuO_4 oxidation of $\Delta^{7,9(11)}$-sterols provides a viable route to Δ^7-9α-hydroxy-11-ketosteroids which, in turn, seem to be good precursors of Δ^7-9α,11β-dihydroxysteroids through stereoselective $LiAlH_4$ reduction of the 11-keto group[36], as seen in the case of 3β,6α-diacetoxy-9α-hydroxy-5α-cholest-7-en-11-one (**5**). On the other hand, RuO_4 oxidation also gives Δ^7-9α,11α-dihydroxysteroids but the yields are low (Table 1).

Finally, although our RuO_4 oxidation procedure has been applied only to some tri- and tetra-substituted steroidal alkenes[22] and now to the 7,9(11)-diene steroidal system, the method appears to be promising because it furnishes α-hydroxyketones, if the substrates are alkenes, and unsaturated α-hydroxyketones, when conjugated dienes are oxidized, in a simple and fast way and in mild conditions. As far as we know, the direct conversion of alkenes into α-ketols was previously carried out by potassium permanganate under conditions of carefully controlled pH[37-39] or supported on copper sulfate pentahydrate[40], while zinc permanganate supported on silica gel has been tested only in one case and the yield of the conversion is low[41,42]. Thus, the set up of more efficient and general procedures able to perform this transformation would be desirable. On the other hand, no direct methods seem to be available for the

conversion of conjugated dienes into unsaturated α-hydroxyketones. These cosiderations prompt us to explore the possibility of extending our procedure to other steroidal and non-steroidal, cyclic and acyclic, alkenes and conjugated dienes. The ability of RuO_4 of forming epoxydes is also currently under investigation.

EXPERIMENTAL SECTION

^1H- and ^{13}C-NMR spectra were recorded on Bruker WM 270 and 400 spectrometers in $CDCl_3$ or pyridine-d_5 solutions. Proton chemical shifts were referenced to the residual $CHCl_3$ and pyridine signals (7.26 and 8.71 ppm, respectively). ^{13}C-NMR chemical shifts were referenced to the solvents ($CDCl_3$: 77.0 ppm; C_5D_5N: 149.9). The multiplicity of ^{13}C-NMR resonances was determined by DEPT experiments. Electron impact mass spectra (EIMS) were recorded on a TRIO 2000 mass spectrometer. High resolution electron impact mass spectra (HREIMS) were recorded on a KRATOS AEI-MS 902 mass spectrometer. Fourier transform IR (FTIR) spectra were obtained with a Perkin-Elmer 1760-X FT-IR spectrophotometer. Ultraviolet (UV) spectra were recorded with a Perkin-Elmer Model 550S spectrophotometer. High performance liquid chromatographies (HPLC) were performed using a Varian 2510 pump equipped with a Waters dual cell refractometer using Hibar LiChrosorb Si-60 (250 x 10 mm, and 250 x 4 mm) columns. Melting points were determined on a Reichert Termovar type 300429 Kofler hot stage melting apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter in $CHCl_3$ solutions. Column chromatography was carried out on Merck silica gel 40 (70-230 mesh) and 60 (230-400 mesh). Thin-layer chromatography (TLC) analyses were performed on precoated silica gel F_{254} plates (0.25 thick, Merck).

Synthesis of the starting products: 5α-cholesta-7,9(11)-diene-3β,6α-diol diacetate (1), 5α-cholesta-7,9(11)-diene-3β,5,6α-triol 3,6-diacetate (2), 5α-cholesta-7,9(11)-diene-3β,5,6β-triol 3,6-diacetate (3) and 5α-cholesta-7,9(11)-dien-3β-yl acetate (4).

Dienes **1** and **2** were synthesized according to previously described procedures[28,43] starting from commercially available 7-dehydrocholesterol.

Compound **3** was obtained from 7-dehydrocholesterol as follows. Borohydride reduction of 3β-acetoxy-5-hydroxy-5α-cholest-7-en-6-one, synthesized as described for its Δ^{22} C-24 methyl homolog by Fieser *et al.*[44], gave 5α-cholest-7-ene-3β,5,6β-triol 3-acetate whose acetylation with Ac_2O-pyridine, followed by mercuric acetate dehydrogenation[45], afforded the desired diene **3**.

Compound **4** was obtained from commercially available 5α-cholest-7-en-3β-ol by acetylation with Ac_2O-pyridine followed by conjugation with mercuric acetate[45].

3β,6α-Diacetoxy-9α-hydroxy-5α-cholest-7-en-11-one (5), 5α-cholest-7-ene-3β,6α,9α,11α-tetrol 3,6-diacetate (6) and 3β,6α-diacetoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (7) (entry 1).

A solution of 5α-cholesta-7,9(11)-diene-3β,6α-diol diacetate (**1**, 13.0 mg, 0.027 mmol) in 10 mL of acetone was treated at room temperature with a solution of ruthenium tetroxide in 5:1 (v/v) acetone-water.

The oxidant was prepared by stirring at room temperature $RuO_2 \cdot 2H_2O$ (4.6 mg, 0.027 mmol) with a suspension of sodium metaperiodate (60 mg, 0.28 mmol) in 5:1 (v/v) acetone-water (12 mL) until the rection mixture became yellow (1h).

The progress of the reaction was followed by TLC. When the reaction was complete (10 min.), 2-propanol was added to reduce RuO_4 excess and the black precipitate formed was centrifuged and washed with methanol. The residue was concentrated under reduced pressure and fractionated by HPLC on a Hibar LiChrosorb Si-60 (250 x 4 mm) column using hexane-ethyl acetate (85:15) as eluent to give **5** (5.7 mg, 42 % yield), **6** (2.9 mg, 22 % yield) and **7** (1.1 mg, 8 % yield).

The same reaction was also performed at -70 °C using 28.0 mg (0.060 mmol) of diene **1** dissolved in 10 mL of acetone. After 10 min the reaction was quenched by adding 2-propanol and worked-up as above. HPLC separation of the resulting mixture in the above conditions gave **5** (6 mg, 20 % yield), **6** (10.6 mg, 34 % yield) and **7** (7 mg, 23 % yield).

5: m.p. 198-200 °C (light petroleum-$CHCl_3$, 5:1); $[\alpha]_D$= +32.0 (c=0.3, $CHCl_3$); FTIR (film) ν_{max} 3457, 1734, 1715 and 1245 cm^{-1}; ^{1}H-NMR (see Table 2); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) δ 210.35 (s), 171.21 (s), 170.51 (s), 140.47 (s), 124.15 (d), 72.30 (d), 72.00 (d), 55.93 (d), 54.59 (t), 50.91 (d), 44.15 (s), 39.66 (s), 39.40 (t), 38.67 (d), 35.96 (d), 35.73 (t), 29.61 (t), 29.32 (t), 27.97 (t), 26.69 (t), 23.77 (t), 22.76 (q), 22.51 (q), 22.25 (t), 21.34 (q), 21.20 (q), 18.54 (q), 14.55 (q), 13.99 (q); ^{13}C-NMR (py-d_5, 100.1 MHz) δ 208.74 (s), 171.08 (s), 170.22 (s), 142.74 (s), 124.29 (d), 76.97 (s), 72.89 (d), 72.78 (d), 55.34 (d), 54.81 (t), 50.78 (d), 45.06 (s), 40.00 (s), 39.67 (t), 39.60 (d), 36.07 (d), 36.07 (t), 30.31 (t), 30.17 (t), 28.21 (d), 28.08 (t), 27.49 (t), 24.03 (t), 22.91 (t), 22.91 (q), 22.65 (q), 21.24 (q), 21.03 (q), 18.62 (q), 14.66 (q), 13.14 (q); EIMS *m/z* 456 (M^+-CH_3COOH), 414 (M^+-CH_3COOH-CH_2CO), 396 (M^+-$2CH_3COOH$), 381 (M^+-$2CH_3COOH$-CH_3, base peak), 378 (M^+-$2CH_3COOH$-H_2O); 283 (M^+-$2CH_3COOH$-side chain), 265 (M^+-$2CH_3COOH$-side chain-H_2O); HREIMS found *m/z* 456.3251 (M^+-CH_3COOH) $C_{29}H_{44}O_4$ requires 456.3240.

6: colourless oil ; $[\alpha]_D$= +32.6 (c=0.6, $CHCl_3$); FTIR (film) ν_{max} 3456, 1736 and 1245 cm^{-1}; ^{1}H-NMR (see Table 3); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) δ 171.25 (s), 170.57 (s), 141.54 (s), 123.82 (d), 74.17 (s), 72.58 (d), 72.17 (d), 69.34 (d), 55.82 (d), 50.76 (d), 46.79 (t), 42.98 (s), 40.61 (s), 39.42 (t), 38.75 (d), 35.99 (d), 35.91 (t), 31.66 (t), 30.07 (t), 27.98 (d), 27.81 (t), 27.09 (t), 23.62 (t), 22.84 (t), 22.77 (q), 22.52 (q), 21.38 (q), 21.25 (q), 18.74 (q), 15.62 (q), 12.27 (q); EIMS *m/z* 458 (M^+-CH_3COOH), 440 (M^+-CH_3COOH-H_2O), 398 (M^+-CH_3COOH-H_2O-CH_2CO), 380 (M^+-$2CH_3COOH$-H_2O), 365 (M^+-$2CH_3COOH$-H_2O-CH_3, base peak), 362 (M^+-$2CH_3COOH$-$2H_2O$), 347 (M^+-$2CH_3COOH$-$2H_2O$-CH_3), 285 (M^+-$2CH_3COOH$-side chain), 267 (M^+-$2CH_3COOH$-side chain-H_2O), 249 (M^+-$2CH_3COOH$-side chain-$2H_2O$); HREIMS found *m/z* 458.3380 (M^+-CH_3COOH) $C_{29}H_{46}O_4$ requires 458.3396.

7: amorphus powder; $[\alpha]_D$= +23.2 (c=0.3, CH_3OH); FTIR (film) ν_{max} 1737, 1718, 1678 and 1243 cm^{-1}; UV (CH_3OH) λ_{max} 237 nm (ε= 5013); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 9.90 (1H, d, J=3.4 Hz, H-11), 6.42 (1H, d, J=1.9 Hz, H-7), 5.52 (1H, dd, J=10.2 and 1.9 Hz, Hβ-6), 4.64 (1H, m, 3α- H), 3.59 (1H, dd, J=10.7 and 8.3 Hz, H-14), 2.27 (1H, dd, J=16.6

and 3.9 Hz, Ha-12), 2.14, 2.04 (3H each, s's, acetates), 1.95 (1H, d, J=16.6 Hz, Hb-12), 1.17 (3H, s, H_3-19), 0.91 (3H, d, J=6.8 Hz, H_3-21), 0.86 (6H, d, J=6.3 Hz, H_3-26 and H_3-27), 0.70 (3H, s, H_3-18); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) δ 203.57 (d), 202.82 (s), 170.84 (s), 170.41 (s), 143.03 (d), 137.68 (s), 71.58 (d), 71.06 (d), 52.13 (d), 50.92 (t), 46.12 (s), 44.98 (d), 44.75 (s), 43.48 (d), 39.41 (t), 35.37 (t), 34.79 (d), 31.43 (t), 28.98 (t), 27.93 (d), 26.48 (t), 26.44 (t), 26.12 (t), 24. 28 (t), 22.75 (q), 22.53 (q), 21.28 (q), 21.03 (q), 19.32 (q), 16.49 (q), 15.89 (q); EIMS *m/z* 516 (M^+), 474 (M^+-CH_2CO) , 473 (M^+-CH_2CHO), 456 (M^+-CH_3COOH), 414 (M^+-CH_3COOH-CH_2CO) , 413 (M^+-CH_3COOH- CH_2CHO), 396 (M^+-$2CH_3COOH$), 381 (M^+- $2CH_3COOH$-CH_3), 343 (M^+-CH_3COOH-side chain), 283 (M^+-$2CH_3COOH$-side chain), 69 (base peak); HREIMS found *m/z* 456.3235 (M^+-CH_3COOH) $C_{29}H_{44}O_4$ requires 456.3240.

$LiAlH_4$ reduction of compound 5.

Ketol **5** (3.0 mg) dissolved in ether (1 mL) was treated with excess lithium aluminum hydride and kept under stirring for 20 min. Excess reagent was destroyed by addition of a few drops of water, the ether layer was decanted and the white precipitate was washed with ether. The combined ether phases were dried ($MgSO_4$) and taken to dryness to give an oily product which was subjected to HPLC purification on a Hibar LiChrosorb Si-60 (250 x 4 mm) column using $CHCl_3$-CH_3OH (9:1) as eluent; 2.5 mg of a 1:5 mixture of 5α-cholest-7-ene-3β,6α,9α,11α-tetrol[28] and its 11β-epimer (^{1}H-NMR analysis) were obtained .

5α-cholest-7-ene-3β,6α,9α,11β-tetrol: ^{1}H-NMR (CD_3OD, 270 MHz) δ 5.31 (1H, bdd, J=1.8 and 1.8 Hz, H-7), 3.99 (1H, bdd, J=1.8 and 1.8 Hz, Hα-11), 3.75 (1H, bddd, J=9.8, 1.8 and 1.8 Hz, Hβ-6), 3.45 (1H, m, Hα-3), 2.35 (1H, bdd, J=9.8 and 9.8 Hz, H-14), 2.21 (1H, bd, J=13.4 Hz, Heq-1), 2.12 (1H, bdd, J=14.0 and 1.8 Hz, Hα-12), 1.88 (1H, d, J=14.0 Hz, Hβ-12), 1.19 (3H, s, H_3-19), 0.99 (3H, d, J=6.7 Hz, H_3-21), 0.89 (6H, d, J=6.1 Hz, H_3-26 and H_3-27), 0.81 (3H, s, H_3-18); ^{1}H-NMR (py-d_5, 270 MHz) δ 6.12 (1H, d, J=5.6 Hz, OH-6), 5.92 (1H, d, J=3.9 Hz, OH-3), 5.89 (1H, bs, H-7), 5.57 (1H, s, OH-9), 5.46 (1H, d, J=3.9 Hz, OH-11), 4.76 (1H, bs, $W_{1/2}$ =9.3 Hz, Hα-11), 4.42 (1H, bdd, J=7.3 and 7.3 Hz, Hβ-6), 4.02 (1H, m, Hα-3), 3.07 (1H, bddd, J=12.7, 2.9 and 2.9 Hz, Heq-1), 2.50 (1H, bd, J=12.7 Hz, Ha-12), 2.35 (1H, dd, J=12.7 and 2.9 Hz, Hb-12), 1.79 (3H, s, H_3-19), 1.22 (3H, s, H_3-18), 0.99 (3H, d, J=6.3 Hz, H_3-21), 0.85 (6H, d, J=6.8 Hz, H_3-26 and H_3-27).

$LiAlH_4$ reduction of compound 6.

2.0 Mg of diol **6** were reduced with $LiAlH_4$ as described above for compound **5**. HPLC purification in the above conditions afforded 1.5 mg of pure 5α-cholest-7-ene-3β,6α,9α,11α-tetrol identical in all respects to a reference sample[28].

3β,6α-Diacetoxy-5,9α-dihydroxy-5α-cholest-7-en-11-one (8) and 5α-cholest-7-ene-3β,5,6 α, 9α,11α-pentol 3,6-diacetate (9) (entry 2).

A solution of 5α-cholesta-7,9(11)-diene-3β,5,6α-triol 3,6-diacetate (**2**, 28.0 mg, 0.056 mmol) in 10 mL of acetone was treated at room temperature as described above for compound **1** with 12 mL of acetone-water (5:1, v/v) solution of ruthenium tetroxide prepared from 9.5 mg (0.056 mmol) of hydrated form of ruthenium dioxide. The reaction was complete after 5 min. HPLC separation of the resulting mixture on a Hibar LiChrosorb Si-60 (250 x 4 mm) column eluted with hexane/ethyl acetate (78:22) gave pure compounds **8** (18.6 mg, 62%) and **9** (3.9 mg, 13 %).

The reaction performed at -70 °C on 18.0 mg (0.036 mmol) of diene **2** dissolved in 10 mL of acetone afforded 13 mg of **8** (68%) and 2.0 mg of **9** (9%).

8: m.p. 233-234°C (CH_3OH); $[\alpha]_D$= +30.3 (c=0.7, $CHCl_3$); FTIR (film) ν_{max} 3422, 1742, 1717 and 1232 cm^{-1}; ^{1}H-NMR (see Table 2); ^{13}C-NMR (py-d_5, 100.1 MHz) δ 206.90 (s), 170.90 (s), 170.36 (s), 143.09 (s), 120.77 (d), 78.57 (s), 78.03 (s), 73.93 (d), 70.34 (d), 55.22 (d), 54.50 (t), 50.23 (d), 44.96 (s), 42.00 (s), 39.68 (t), 36.56 (t), 36.09 (t), 36.09 (t), 28.21 (d), 28.16 (t), 27.19 (t), 26.31 (t), 24.06 (t), 22.92 (q), 22.70 (q), 22.67 (t), 21.26 (q), 20.76 (q), 18.64 (q), 18.48 (q), 13.21 (q); EIMS *m/z* 472 (M^+-CH_3COOH), 454 (M^+-CH_3COOH-H_2O), 412 (M^+-2CH_3COOH, base peak), 394 (M^+-2CH_3COOH-H_2O), 379 (M^+-2CH_3COOH-H_2O-CH_3), 281 (M^+-2CH_3COOH-H_2O-side chain); HREIMS found *m/z* 472.3201 (M^+-CH_3COOH) $C_{29}H_{44}O_5$ requires 472.3189.

9: amorphus powder; $[\alpha]_D$= +15.3 (c=0.4, $CHCl_3$); FTIR (film) ν_{max} 3425, 1737, and 1244 cm^{-1}; ^{1}H-NMR (see Table 3); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) 170.65 (s), 170.22 (s), 144.22 (s), 117.18 (d), 77.80 (s), 75.35 (s), 73.42 (d), 70.01 (d), 69.74 (d), 55.78 (d), 50.17 (d), 46.35 (t), 42.77 (s), 42.36 (s), 39.46 (t), 36.88 (t), 36.01 (t), 35.94 (d), 27.99 (t), 27.89 (t), 27.70 (d), 26.65 (t), 23.81 (t), 22.78 (t), 22.67 (q), 22.53 (q), 21.37 (q), 21.17 (q), 19.43 (q), 18.71 (q), 12.37 (q); EIMS *m/z* 474 (M^+-CH_3COOH), 456 (M^+-CH_3COOH-H_2O), 414 (M^+-2CH_3COOH), 396 (M^+-2CH_3COOH-H_2O), 283 (M^+-2CH_3COOH-H_2O-side chain), 265 (M^+-2CH_3COOH-2H_2O-side chain), 239 (M^+-2CH_3COOH-side chain-44, base peak); HREIMS found *m/z* 474.3348 (M^+-CH_3COOH) $C_{29}H_{46}O_5$ requires 474.3345.

3β,6β-Diacetoxy-5,9α-dihydroxy-5α-cholest-7-en-11-one (10), 5α-cholest-7-ene-3β,5,6β,9α, 11α-pentol 3,6-diacetate (11) and 3β,6β-diacetoxy-5-hydroxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (12) (entry 3).

A solution of 5α-cholesta-7,9(11)-diene-3β,5,6β-triol 3,6-diacetate (**3**, 12.0 mg, 0.024 mmol) in 10 mL of acetone was treated at room temperature as described above with 12 mL of acetone-water (5:1, v/v) solution of ruthenium tetroxide prepared from 4.0 mg (0.024 mmol) of hydrated form of ruthenium dioxide. After 5 min. the reaction was quenced by adding 2-propanol and worked-up as described above. HPLC separation of the resulting mixture on a Hibar LiChrosorb Si-60 (250 x 4 mm) column eluted with hexane/ethyl acetate (80:20) gave 9 mg of pure compound **10** (71%) and 2.2 mg of a mixture of compounds **11** and **12** in the approximate ratio of 95:5, as deduced from ^{1}H-NMR analysis. Further purification of compound **11** was achieved by HPLC using hexane-ethyl acetate (82:18) as eluent. 2.0 Mg (16%) of pure **11** were obtained from this separation.

The reaction performed at -70 °C on 3.0 mg (0.006 mmol) of diene **3** dissolved in 5 mL of acetone afforded 2.6 mg of **10** (81%) and 0.5 mg of the mixture of **11** (estimated yield by ^{1}H-NMR 10%) and **12**.

10: m.p. 189-190 °C (CH_3OH); $[\alpha]_D$= -124.0 (c=0.9, $CHCl_3$); FTIR (film) ν_{max} 3420, 1734, 1718 and 1239 cm^{-1}; ^{1}H-NMR (see Table 2);^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ 207.66 (s), 170.84 (s), 170.33 (s), 143.48 (s), 118.55 (d), 78.00 (s), 77.01 (s), 72.54 (d), 70.24 (d), 55.72 (d), 54.29 (t), 50.38 (d), 43.77 (s), 40.29 (s), 39.45 (t), 35.97 (t), 35.97 (t), 35.79 (t), 28.00 (d), 27.84 (t), 26.68 (t), 25.62 (t), 23.80 (t), 22.79 (q), 22.52 (q), 22.52 (d), 21.42 (q), 21.30 (q), 18.79 (q), 18.60 (q), 13.55 (q); EIMS *m/z* 514 (M^+-H_2O), 472 (M^+-CH_3COOH, base peak), 454 (M^+-CH_3COOH-H_2O), 412 (M^+-2CH_3COOH), 394 (M^+-2CH_3COOH-H_2O), 379 (M^+-2CH_3COOH-H_2O-CH_3), 281 (M^+-2CH_3COOH-H_2O-side chain); HREIMS found *m/z* 514.3283 (M^+-H_2O) $C_{31}H_{46}O_6$ requires 514.3294.

11: m.p 195-197 °C (CH_3OH); $[\alpha]_D$= -62.3 (c=0.2, $CHCl_3$); FTIR (film) ν_{max} 3424,1735 and 1241cm^{-1}; ^{1}H-NMR (see Table 3); EIMS *m/z* 474 (M^+-CH_3COOH), 456 (M^+-CH_3COOH-H_2O), 414 (M^+-2CH_3COOH), 396 (M^+-2CH_3COOH-H_2O), 265 (M^+-2CH_3COOH-2H_2O-side chain), 239 (M^+-2CH_3COOH-side chain-44), 221 (M^+-2CH_3COOH-side chain-H_2O-44), 123 (base peak); HREIMS found *m/z* 474.3355 (M^+-CH_3COOH) $C_{29}H_{46}O_5$ requires 474.3345.

12: ^{1}H-NMR (data from the mixture of **11** and **12**) ($CDCl_3$, 400 MHz) δ 9.90 (1H, d, J=3.7 Hz, H-11), 6.42 (1H, d, J=5.1 Hz, H-7), 5.21 (1H, d, J=5.1 Hz, H-6), 3.64 (1H, dd, J=10.2 and 10.2 Hz, H-14), 0.72 (3H, s, H_3-18);

3β-Acetoxy-9α-hydroxy-5α-cholest-7-en-11-one (13), 5α-cholest-7-ene-3β,9α,11α-triol 3-acetate (14), 3β-acetoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al (15), 8α,9α-epoxy-5α-cholesta-3β,7α,11α-triol 3-acetate (16), 3β-acetoxy-8α,9α-epoxy-11α-hydroxy-5α-cholestan-7-one (17) and 3β-acetoxy-8α,9α-epoxy-7α-hydroxy-5α-cholestan-11-one (18) (entry 4).

A solution of 5α-cholesta-7,9(11)-diene-3β-yl acetate (**4**, 20.0 mg, 0.047 mmol) in 10 mL of acetone was treated at room temperature, as described above, with 12 mL of acetone-water (5:1, v/v) solution of ruthenium tetroxide prepared from 8.0 mg (0.047 mmol) of hydrated form of ruthenium dioxide. After 5 min. the reaction was quenced and worked-up as above. HPLC separation of the resulting mixture on a Hibar LiChrosorb Si-60 (250 x 10 mm) column eluted with hexane/ethyl acetate (7: 3) gave pure compound **16** (2 mg, 12%) and 12 mg of a mixture of compounds **13-15, 17** and **18** which was further separated on a Hibar LiChrosorb Si-60 (250 x 4 mm) column using hexane/ethyl acetate (87:13) as eluent. This separation afforded pure samples of **13** (1.0 mg, 6%), **14** (1.3 mg, 8%), **15** (0.4 mg, 3%), **17** (2.7 mg, 16%) and **18** (1.0 mg, 6%).

The reaction performed at -70 °C on 16.7 mg (0.039 mmol) of diene **4** dissolved in 6 mL of acetone afforded, after HPLC separation as above, 5.2 mg of **13** (30%), 1.0 mg of **14** (6%), 1.2 mg of **15** (7%), 5.6 mg of **16** (30 %), 2.6 mg of **17** (14%), and 0.5 mg of **18** (2%).

13: m.p. 170-171 °C (CH_3OH); $[\alpha]_D$= -35.9 (c=0.4, $CHCl_3$); FTIR (film) ν_{max} 3502, 1735, 1708 and 1248 cm^{-1}; ^{1}H-NMR (see Table 2); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) δ

211.89 (s), 170.67 (s), 136.69 (s), 125.07 (d), 77.80 (s), 72.73 (d), 55.76 (d), 54.68 (t), 51.12 (d), 44.23 (s), 39.48 (t), 38.41 (s), 36.13 (d), 35.81 (t), 33.61 (t), 33.56 (d), 29.86 (t), 28.86 (t), 28.08 (t), 28.05 (d), 27.34 (t), 23.85 (t), 22.84 (q), 22.58 (q), 22.46 (t), 21.47 (q), 18.61 (q), 13.95 (q), 13.43 (q); EIMS *m/z* 458(M^+, base peak), 430 (M^+-CO), 416 (M^+-CH_2CO), 398 (M^+-CH_3COOH), 380 (M^+CH_3COOH-H_2O), 345 (M^+-side chain); HREIMS found *m/z* 458.3376 (M^+) $C_{29}H_{46}O_4$ requires 458.3396.

14: m.p. 158-160°C (hexane) [lit.[33]: 158-160 °C]; $[\alpha]_D$= -34.8 (c=0.3, $CHCl_3$) [lit.[33]: -43.0]; FTIR (film) ν_{max} 3452, 1735 and 1245 cm^{-1}; ^{1}H-NMR (see Table 3); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) δ 170.64 (s), 138.45 (s), 124.41 (d), 74.70 (s), 72.85 (d), 69.63 (d), 55.81 (d), 50.66 (d), 47.01 (t), 42.78 (s), 39.87 (s), 39.45 (t), 36.09 (d), 35.95 (t), 34.20 (t), 33.90 (d), 31.25 (t), 30.11 (t), 27.99 (d), 27.86 (t), 27.66 (t), 23.84 (t), 23.07 (t), 22.79 (q), 22.53 (q), 21.44 (q), 18.76 (q), 14.52 (q), 12.07 (q); EIMS *m/z* 460 (M^+), 442 (M^+-H_2O), 424 (M^+-$2H_2O$), 400 (M^+-CH_3COOH), 382 (M^+-H_2O-CH_3COOH), 329 (M^+-H_2O-side chain, base peak).

15: m.p. 101-103 °C (CH_3OH) [lit.[33]: 102-105 °C]; $[\alpha]_D$= -37.0 (c=0.1, $CHCl_3$) [lit.[33]: -33.6]; FTIR (film) ν_{max} 1734, 1717 and 1244 cm^{-1}; UV (EtOH) λ_{max} 240 nm (ε= 4730); ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 9.92 (1H, d, J= 3.4 Hz, H-11), 6.66 (1H, dd, J=4.9 and 3.4 Hz, H-7), 4.67 (1H, m, Hα-3), 3.60 (1H, dd, J=10.2 and 10.2 Hz, H-14), 2.03 (3H, s, acetate), 1.07 (3H, s, H_3-19), 0.91 (3H, d, J=6.8 Hz, H_3-21), 0.86 (6H, d, J=6.4 Hz, H_3-26 and H_3-27), 0.69 (3H, s, H_3-18); ^{13}C-NMR ($CDCl_3$, 67.9 MHz) δ 204.25, 204.23, 170.52, 144.59, 136.20, 72.27, 52.04, 50.85, 46.05, 43.72, 43.53, 39.42, 39.20, 35.39, 34.95, 32.99, 30.92, 30.10, 29.69, 27.94, 26.91, 26.61, 26.22, 24.28, 22.77, 22.54, 21.34, 19.26, 16.33, 14.43; EIMS *m/z* 458 (M^+), 416 (M^+-CH_2CO), 398 (M^+-CH_3COOH), 388 (M^+-CH_2CO-CO), 301 (base peak), 275 (M^+-CH_2CO-CO-side chain).

16: m.p . 165-166°C (diisopropyl ether); $[\alpha]_D$= +15.3 (c=0.3, $CHCl_3$); FTIR (film) ν_{max} 3446, 1734 and 1246 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.68 (1H, m, Hα-3), 4.27 (1H, ddd, J= 7.9, 7.9 and 7.9 Hz, Hβ-11), 3.98 (1H, dd, J= 9.2 and 6.1 Hz, Hβ-7), 2.37 (1H, dd, J= 13.4 and 7.3 Hz, Hα-12), 2.24 (1H, d, J= 9.8 Hz, OH), 2.01 (3H, s, acetate), 1.08 (3H, s, H_3-19), 0.92 (3H, d, J= 6.7 Hz, H_3-21), 0.86 (6H, d J= 6.7 Hz, H_3-26 and H_3-27), 0.65 (3H, s, H_3-18); ^{1}H-NMR (py-d_5, 400 MHz) δ 6.00 (1H, d, J= 7.9 Hz, OH), 5.85 (1H, d, J= 9.7 Hz, OH), 4.73 (1H, m, Hα-3), 4.59 (1H, bddd, J= 8.5, 8.5 and 8.5 Hz, Hβ-11), 4.27 (1H, bdd, J= 4.9 and 4.9 Hz, Hβ–7), 2.71 (1H, ddd, J= 14.6, 4.2 and 4.2 Hz, Heq-1), 2.58 (1H, dd, J= 12.8 and 7.3 Hz, Hα-12), 2.34 (1H, dddd, J= 13.4, 13.4, 3.0 and 3.0 Hz, H-5), 2.26 (1H, m, H-15), 2.05 (1H, dd, J= 13.4 and 7.3 Hz, H-14), 1.99 (3H, s, acetate), 1.66 (1H, ddd, J= 14.0, 14.0 and 6.7 Hz, Hβ-6), 1.51 (1H, dd, J= 14.0 and 3.0 Hz, Heq-6), 1.45 (1H, dd, J=12.8 and 8.5 Hz, Hβ-12), 1.15 (3H, s, H_3-19), 0.92 (3H, d, J= 6.7 Hz, H_3-21), 0.84 (6H, d, J= 6.7 Hz, H_3-26 e H_3-27), 0.75 (3H, s, H_3-18); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) δ 170.47 (s), 72.72 (s), 72.54 (d), 69.14 (s), 65.86 (d), 64.56 (d), 53.86 (d), 52.69 (d), 46.63 (t), 42.57 (s), 39.40 (t), 36.57 (s), 36.08 (d), 35.80 (t), 34.37 (t), 33.33 (t), 33.33 (t), 30.69 (d), 28.05 (t), 27.96 (d), 27.06 (t), 23.82 (t), 23.20 (t), 22.77 (q), 22.51 (q), 21.35 (q), 18.75 (q), 15.48 (q), 13.01 (q); EIMS *m/z* 458 (M^+-H_2O), 440 (M^+-$2H_2O$), 398 (M^+-H_2O-CH_3COOH), 380 (M^+-$2H_2O$-CH_3COOH), 327 (M^+-$2H_2O$-side chain), 291 (base peak); HREIMS found *m/z* 458.3409 (M^+-H_2O) $C_{29}H_{46}O_4$ requires 458.3396.

17: m.p. 167-168 °C (acetone); $[\alpha]_D$= -24.6 (c=0.4, $CHCl_3$); FTIR (film) ν_{max} 3430, 1734, 1713 and 1246 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 4.68 (1H, m, 3α-H), 4.33 (1H, ddd, J=9.7, 9.7, and 9.7 Hz, Hβ-11), 2.48 (1H, dd, J=12.8 and 6.7 Hz, Hβ-12), 2.36 (1H, dd, J=17.7 and 6.1 Hz, Ha-6), 2.22 (1H, m, overlapped to other signals, H-5), 2.02 (3H, s, acetate), 1.95 (1H, m, overlapped to other signals, Hb-6), 1.38 (1H, d, J=9.7 Hz, OH), 1.15 (3H, s, H_3-19), 0.93 (3H, d, J=6.1 Hz, H_3-21), 0.86 (6H, d, J=6.7 Hz, H_3-26 and H_3-27), 0.68 (3H, s, H_3-18); ^{1}H-NMR (py-d_5, 400 MHz) δ 6.22 (1H, d, J=8.8 Hz, OH), 4.80 (1H, m, 3α-H), 4.62 (1H, ddd, J=8.8, 8.8 and 8.8 Hz, Hβ-11), 2.62 (1H, dd, J=12.6 and 7.1 Hz, Hβ-12), 2.46 (1H, ddd, J=14.3, 3.8 and 3.8 Hz, Heq-1), 2.23 (1H, ddd, J=14.3, 14.3 and 4.4 Hz, Hax-1), 2.02 (3H, s, acetate), 1.19 (3H, s, H_3-19), 0.93 (3H, d, J=6.6 Hz, H_3-21), 0.85 (6H, d, J=6.6 Hz, H_3-26 and H_3-27), 0.76 (3H ,s, H_3-18); ^{13}C-NMR ($CDCl_3$, 100.1 MHz) δ 203.64 (s), 170.44 (s), 72.69 (s), 71.95 (d), 67.00 (s), 66.24 (d), 52.43 (d), 50.72 (d), 46.02 (t), 43.36 (s), 41.76 (t), 39.39 (t), 37.36 (s), 36.11 (d), 35.86 (t), 33.41 (t), 33.17 (t), 32.70 (d), 28.58 (t), 27.97 (d), 27.09 (t), 23.82 (t), 23.71 (t), 22.77 (q), 22.51 (q), 21.31 (q), 18.89 (q), 15.64 (q), 12.62 (q); EIMS *m/z* 474 (M^+), 456 (M^+-H_2O), 396 (M^+-H_2O-CH_3COOH), 343 (M^+-H_2O-side chain), 162 (base peak); HREIMS found *m/z* 474.3360 (M^+) $C_{29}H_{46}O_5$ requires 474.3345.

18: $[\alpha]_D$=+44 (c=0.1, $CHCl_3$); FTIR (film) ν_{max} 3301, 1737, 1704 and 1256 cm^{-1};^{1}H-NMR ($CDCl_3$, 270 MHz) δ 4.68 (1H, m, 3α-H), 4.05 (1H, bdd, J=9.2. and 5.9 Hz, Hβ-7), 2.64 (1H, ddd, J=13.2, 3.4 and 3.4 Hz, Heq-1), 2.50 (2H, AB system, J=14.2 Hz, H_2-12), 2.01 (3H, s, acetate), 1.12 (3H, s, H_3-19), 0.88 (3H, d, J=5.4 Hz, H_3-21), 0.86 (3H, d, J=6.3 Hz, H_3-26 and H_3-27), 0.74 (3H, s, H_3-18); EIMS *m/z* 474 (M^+), 456 (M^+-H_2O), 396 (M^+-H_2O-CH_3COOH); HREIMS found *m/z* 474.3358 (M^+) $C_{29}H_{46}O_5$ requires 474.3345.

Chromium trioxide-pyridine oxidation of compounds 16-18.

To a solution of **17** (2.9 mg) in pyridine (1 mL), excess chromium trioxide-pyridine complex[46] was added. The mixture was kept under stirring at room temperature for 16 h, then diluted with water (1 mL) and extracted with ethyl ether (3 x 3 mL). The ether solution was washed with water, dried over magnesium sufate and taken to dryness. The oily residue was chromatographed on a Hibar LiChrosorb Si-60 (250 x 4 mm) column (eluent: hexane/ethyl acetate, 87:13) to give 1.1 mg of 3β-acetoxy-8α,9α-epoxy-5α-cholestan-7,11-dione and 1.5 mg of unreacted **17**.

3β-acetoxy-8α,9α-epoxy-5α-cholestan-7,11-dione: FTIR (film) ν_{max} 1734, 1713 and 1240 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 270 MHz) δ 4.68 (1H, m, Hα-3), 2.83 (1H, ddd, J=13.2, 3.4 and 3.4 Hz, Heq-1), 2.53 (2H, AB system, J=13.2 Hz, H_2-12), 2.02 (3H, s, acetate), 1.24 (3H, s, H_3-19), 0.88 (3H, d, J=5.8 Hz, H_3-21), 0.86 (6H, d, J=6.3 Hz, H_3-26 and H_3-27), 0.73 (3H, s, H_3-18).

Following the same procedure described above, oxidation of compound **16** (2.0 mg) gave a mixture of 3β-acetoxy-8α,9α-epoxy-5α-cholestan-7,11-dione, unreacted **16** and **17** and **18** in the approximate ratio of 1:1:2:2 (^{1}H-NMR analysis).

When the oxidation was performed on compound **18** (1.0 mg), 3β-acetoxy-8α,9α-epoxy-5α-cholestan-7,11-dione and unreacted **18** were obtained in the approximate ratio of 1:1 (^{1}H-NMR analysis).

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