Ozonolysis of cholesterol and other Δ^5-steroids in the presence of alcohols: a revised mechanism and hydroperoxide structure of the solvent-participated product, confirmed by X-ray analysis

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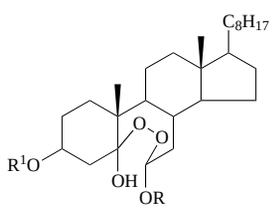
The structure of the product formed in the course of the reaction of cholesterol acetate and other Δ^5-steroids with ozone in alcohol-containing solvents has been revised. The solvent-participated products are hydroperoxides, 5α-hydroperoxy-7α-alkoxy-5α-B-homo-6-oxasteroids 3 and not the previously claimed cyclic hemiperacetals, 5α-hydroxy-7a-alkoxy-B-dihomo-6,7-dioxacholestane derivatives 1. The final evidence has been obtained from X-ray crystal structure analysis of the selected hydroperoxides 3a, 3c and 3j. It is proposed that hydrogen bonding involving the hydroperoxy and the alkoxy group is responsible for the stability of these hydroperoxides and also exerts a directive effect in the nucleophilic attack on the Criegee intermediate 12 by the alcohol.

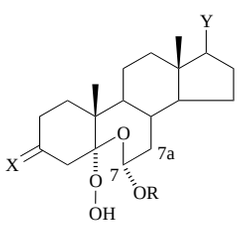
Oxysterols are compounds of biochemical and biomedical interest,[1] and are commonly encountered in human blood, other tissues and foods.[2] Their variety of biological activities and important effects on cell membranes have also been recognized.[3] The formation of oxysterols results from facile oxidation of cholesterol and related sterols by active oxygen species.[4] Ozone might be a potential oxidizing reagent. In 1949, Criegee postulated the existence of carbonyl oxides as key intermediates in the ozonolysis of alkenes.[5] Since that time investigations have been carried out, both theoretical and experimental, toward understanding the electronic nature and the structure of carbonyl oxides, as well as their chemical reactivity.[6] The known reactions of carbonyl oxides include isomerization, nucleophilic trapping, cycloadditions and oxygen transfer.[7]

The reaction of cholesterol with ozone results in formation of the expected ozonide, which could be reduced to the 5,6-secosterol.[8] The 5,6-epoxide was also found among the products of cholesterol ozonolysis in ethyl acetate and other polar solvents.[9] However, the reaction takes a different pathway in hydroxylic solvents, like water or alcohols. In 1957, the reaction of cholesterol with ozone carried out in non-polar solvents in the presence of alcohols (MeOH, EtOH or $C_5H_{11}OH$) was reported by Lettre and Jahn.[10] The major product of this reaction was assigned the structure **1** (the hydroxy-peroxide) on the basis of its chemical properties. The structure represented by the general formula **1**, with the hydroxy-peroxide moiety, was then adopted for the principal products of ozonolysis of other steroidal Δ^5-olefins reacting in similar conditions.[11].

Gumulka and Smith published an extensive study on the ozonation of cholesterol in participating[9] and non-participating[12] solvents. Their elaborate analysis of the extensive spectral data[13] was interpreted in favour of the structure **1** for the major product formed in the reaction of cholesterol with ozone carried out in hydroxylic solvents (H_2O or alcohols). In the ^1H NMR spectra of compounds with the proposed general structure **1** the presence of a low-field signal, that was originally not observed,[9] at *ca.* δ 10 constitutes a weakness in their structural assignment. The chemical evidence‡ supports either peroxyhemiacetal structure **1** or structure **3** with hydroperoxy group at C-5.

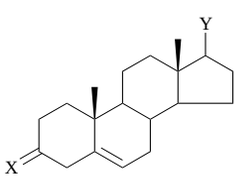


1 R^1 = H or Ac
R^1 = CH_3 or C_2H_5 or C_5H_{11}



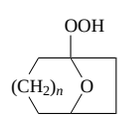
3

a	R = Me	X = β-OAc, H	Y = C_8H_{17}
b	R = Et	X = β-OAc, H	Y = C_8H_{17}
c	R = Pr^i	X = β-OAc, H	Y = C_8H_{17}
d	R = Bu^t	X = β-OAc, H	Y = C_8H_{17}
e	R = Me	X = β-Cl, H	Y = C_8H_{17}
f	R = Me	X = β-OEt, H	Y = C_8H_{17}
g	R = Me	X = O	Y = C_8H_{17}
h	R = Me	X = H_2	Y = C_8H_{17}
i	R = Me	X = β-OAc, H	Y = C_2H_5
j	R = Me	X = β-OAc, H	Y = H



2

a	X = β-OAc, H	Y = C_8H_{17}
b	X = β-Cl, H	Y = C_8H_{17}
c	X = β-OEt, H	Y = C_8H_{17}
d	X = O	Y = C_8H_{17}
e	X = H_2	Y = C_8H_{17}
f	X = β-OAc, H	Y = C_2H_5
g	X = β-OAc, H	Y = H



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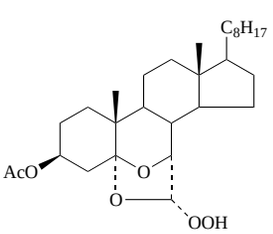

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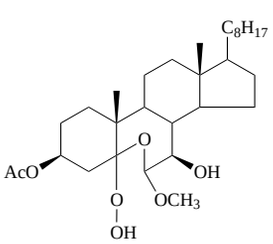

6

In general, hydroperoxides are characterized by a low-field signal in their spectra at δ_H 7–10.[14,15] In oxabicyclic peroxides of partial structure **4**, a low-field signal at δ 8.3–9.3 was assigned to a hydrogen of a hydroperoxy group.[16] Recently, we have reported[17] the steroidal hydroperoxides **5** (δ_H 9.80) and **6** (δ_H 9.95). These results together with the literature data cast some

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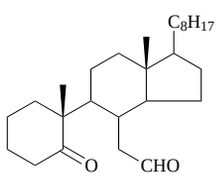
‡ The negative test for the presence of the hydroperoxide group was most probably a result of its tertiary character.[14]

doubt on the correctness of the structure **1**. In order to obtain unequivocal evidence for the structure of the solvent-participated product, we have reinvestigated the ozonolysis of cholesterol acetate[18] and other Δ^5-steroids.

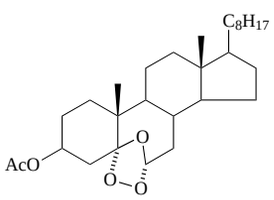
Results and discussion

In the reaction of cholesterol acetate **2a** with ozone carried out in chloroform–alcohol (MeOH, EtOH Pr^{i}OH or Bu^{t}OH) 1:1 mixtures at −70 °C we obtained a series of crystalline compounds **3a**–**3d**. Their physical and spectral properties were similar to those previously described.[9,10,13] The evidence presented below shows that these and other peroxidic products are in fact hydroperoxides and have the structure depicted in the general formula **3**.

In the ^{1}H NMR spectrum of compounds **3a**–**3d** an important signal at δ *ca.* 10 should be assigned to the proton of the OOH group. Also, the strong band in the range 3250–3315 cm^{-1} in the IR spectra of these compounds (taken as chloroform solutions) is consistent with the presence of a hydroperoxy group.[19] In order to confirm this assumption, the chemical evidence for the presence of a hydroperoxy group in **3** was required.



7a 3β-OAc
b 3β-OH
c Δ^3



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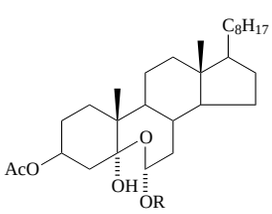
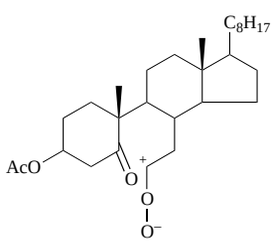


9a R = Me
b R = Et



13

The reduction of **3a** with lithium aluminium hydride in diethyl ether at −60 °C resulted in formation of the known B-seco steroids **7a**–**7c**,[8,9,12,20] while the reaction of **3a** with potassium iodide in acetic acid gave **7a** quantitatively. Treatment of **3a** with trifluoroacetic acid in a benzene–hexane mixture at 0 °C resulted in formation of a mixture, from which the ozonide[12] **8** was isolated by chromatography (15% yield). The ozonide **8** was quite stable when pure and its spectral properties were in accordance with those published.[12] The formation of **8** suggested the 5α-configuration of the hydroperoxy group in **3a**. It was expected that mild reduction of the hydroperoxides **3** would enable the isolation of the respective hydroxy derivatives **9** without cleavage of the ring B. Indeed, brief treatment of the methoxy-hydroperoxide **3a** with dimethyl sulfide without solvent gave, after evaporation of the excess of Me_2S, a product of higher polarity [$R_F = 0.25$ as compared with $R_F = 0.51$ of **3a** (in benzene–ethyl acetate, 5:1)], which decomposed to the seco compound (**7a**) on contact with silica gel. Also, addition of hydroxylic solvents (H_2O, ROH, AcOH) transformed it immediately to **7a**. However, when the crude reduction product was dissolved in ethanol-free [$^2H_3$]chloroform, it gave a ^{1}H NMR spectrum perfectly consistent with structure **9a**. It showed the following characteristic signals: a singlet for the methoxy (δ 3.39), a singlet for the hydroxy (δ 3.00, the signal disappears after the addition of ^2H_2O), and a triplet of one proton (δ 4.58, J 7.6 Hz) arising from the C*H*(OR)$_2$ group. The IR spectrum of **9a** showed absorption for both a free and an associated hydroxy group at 3555 and 3420 cm^{-1}, respectively. Similarly, the ethoxy-hydroperoxide **3b** was reduced to the bisacetal **9b**.

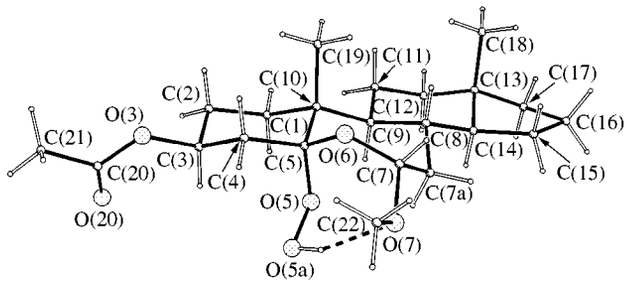


Fig. 1 Perspective view of the hydroperoxide **3j**

In the ^{1}H NMR spectra, the similar positions for the 7-H signal (δ 4.60 in **3a**, δ 4.58 in **9a**; δ 4.72 in **3b**, δ 4.69 in **9b**) as well as a similar splitting pattern for this signal (for example: both triplets, J 7.8 and 7.6 Hz for **3a** and **9a**, respectively) are indicative of the same size and conformation of ring B in compounds **3** and **9**.

Moreover, the formation of 5α-hydroperoxy compounds in the course of ozonolysis of Δ^5-steroids was found to be independent of their substitution at position 3. This was proved when cholest-5-ene derivatives **2b**–**2e** were treated with ozone in chloroform–methanol solution. As a result, the respective hydroperoxides **3e**–**3g** were isolated. Similarly, cholest-5-ene **2e** gave the crystalline hydroperoxide **3h** in 64% yield. All the compounds gave IR and ^{1}H NMR spectra characteristic of 5-hydroperoxy-7α-alkoxy-6-oxa B-homosteroids (see Experimental).

The final evidence for the structure of ozonation products **3** was obtained from X-ray analysis of the selected compounds: **3a**, **3c** and **3j** (Table 1). Since crystals of **3a** were poorly diffracting and crystals of **3c** showed some signs of disorder (see Experimental), it was hoped that steroidal hydroperoxides with the shorter side chain would give crystals suitable for satisfactory measurements. Therefore, ozonolyses of 3β-acetoxypregn-5-ene **2f** and of 3β-acetoxyandrost-5-ene **2g** were carried out to give hydroperoxides **3i**, and **3j**, respectively. Of these two compounds, hydroperoxide **3j** gave crystals suitable for X-ray studies. Thus, two crystal structure analyses were fully completed, *i.e.* for compounds **3c** and **3j**.

Both compounds **3c** and **3j** crystallize in the orthorhombic system, the former with one, and the latter with two molecules in the asymmetric unit. The skeleton geometry of the three independent molecules does not differ significantly. Their close conformational similarity can be seen from Table 2 which lists endocyclic torsion angles. The perspective view of one of the two crystallographically independent molecules of compound **3j** is shown in Fig. 1. The stereochemistry of the four-ring skeleton is *trans–transoid–trans–transoid–trans*. The six-membered A and C rings have chair conformations slightly flattened at the junction with the seven-membered B ring. In both rings mirror symmetry dominates; in the A rings, about the planes through C(3) and C(10), and in the C rings about the planes through C(9) and C(13). Independent of the presence or lack of substituent at C(17), the five-membered D rings have a conformation intermediate between a 13β-envelope and 13β,14α half-chair. The side chain in **3c** has an extended conformation and shows some signs of disorder at its end. In fact C(24) has been refined in two alternative positions, labelled as C(24) and C(24B), with the ratio of site-occupation factors 0.70:0.30, respectively.

Of special interest in the two structures (three molecules) are the seven-membered B rings. Their conformation can be described as a twist chair with a twofold symmetry axis passing

Table 1 Crystal data and details of X-ray measurements

	3a	**3c**	**3j**
Empirical formula	$C_{30}H_{52}O_6$	$C_{32}H_{56}O_6$	$C_{22}H_{36}O_6$
Formula mass	508.1	536.2	396.5
Temperature/K	293	293	293
Wavelength/Å	Cu-Kα (1.541 78)	Cu-Kα (1.541 78)	Cu-Kα (1.541 78)
Crystal system	Monoclinic	Orthorhombic	Orthorhombic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions/Å	a = 18.976(4)	a = 10.131(2)	a = 6.490(1)
	b = 6.398(1)	b = 10.494(3)	b = 21.126(4)
	c = 25.519(5)	c = 30.036(8)	c = 31.319(6)
	β = 102.47(2)°	—	
Volume/Å^3	3025(2)	3193(2)	4294(2)
Z	4	4	8
D_c/g cm^{-3}	1.117	1.115	1.227
Absorption coefficient/mm^{-1}	0.57	0.59	0.68
Crystal size/mm	0.62 × 0.05 × 0.01	0.32 × 0.24 × 0.12	0.62 × 0.22 × 0.17
$2\theta_{max}$ for data collection	120°	120°	127°
Index ranges	$-21 \leqslant h \leqslant 21$	$-11 \leqslant h \leqslant 11$	$-7 \leqslant h \leqslant 7$
	$0 \leqslant k \leqslant 7$	$0 \leqslant k \leqslant 11$	$0 \leqslant k \leqslant 24$
	$0 \leqslant l \leqslant 28$	$0 \leqslant l \leqslant 33$	$0 \leqslant l \leqslant 36$
Reflections collected	4940	4607	7013
Observed reflections [$F > 4\sigma(F)$]	866	1657	3959
Extinction parameter		not applied	2.6(2) × 10^{-3}
Weighting scheme		$w = 1/[\sigma^2(F_o)^2 + (0.1151P)^2]$	$w = 1/[\sigma^2(F_o)^2 + (0.0858P)^2]$
		$P = (\max F_o^2 + 2F_c^2)/3$	$P = (\max F_o^2 + 2F_c^2)/3$
Goodness-of-fit		0.805	0.988
Final R indices		R_1 = 0.067	R_1 = 0.048
		wR_2 = 0.160	wR_2 = 0.125
Largest diff. peak		−0.16	−0.16
and hole/e Å^{-3}		0.20	0.20

Table 2 Endocyclic torsion angles (°)

		Compound **2**	Compound **3**	
			Molecule I	Molecule II
Ring A	C(10)–C(1)–C(2)–C(3)	−54.7(9)	−54.3(5)	−55.9(5)
	C(1)–C(2)–C(3)–C(4)	54.3(8)	54.5(4)	54.9(5)
	C(2)–C(3)–C(4)–C(5)	−55.4(8)	−56.3(5)	−55.0(4)
	C(3)–C(4)–C(5)–C(10)	56.1(8)	56.2(4)	54.9(4)
	C(4)–C(5)–C(10)–C(1)	−50.2(8)	−51.1(4)	−50.8(4)
	C(5)–C(10)–C(1)–C(2)	50.4(8)	50.3(4)	51.6(4)
Ring B	C(10)–C(5)–O(6)–C(7)	−89.9(7)	−89.1(4)	−89.2(4)
	C(5)–O(6)–C(7)–C(7A)	37.8(8)	40.0(4)	41.2(4)
	O(6)–C(7)–C(7A)–C(8)	40.6(9)	40.4(4)	39.1(4)
	C(7)–C(7A)–C(8)–C(9)	−79.7(7)	−82.1(4)	−82.4(4)
	C(7A)–C(8)–C(9)–C(10)	67.8(7)	68.5(4)	69.7(4)
	C(8)–C(9)–C(10)–C(5)	−58.6(7)	−54.4(4)	−54.4(4)
	C(9)–C(10)–C(5)–O(6)	76.8(7)	71.2(3)	69.7(4)
Ring C	C(9)–C(8)–C(14)–C(13)	53.2(8)	55.0(4)	52.6(4)
	C(8)–C(14)–C(13)–C(12)	−59.7(8)	−62.0(4)	−61.9(4)
	C(14)–C(13)–C(12)–C(11)	58.1(8)	57.4(4)	59.3(5)
	C(13)–C(12)–C(11)–C(9)	−56.5(9)	−53.1(5)	−52.9(5)
	C(12)–C(11)–C(9)–C(8)	48.3(8)	46.4(4)	42.6(5)
	C(11)–C(9)–C(8)–C(14)	−43.7(7)	−44.3(4)	−41.0(4)
Ring D	C(13)–C(14)–C(15)–C(16)	−32.2(7)	−30.3(4)	−32.6(4)
	C(14)–C(15)–C(16)–C(17)	6.4(7)	5.6(5)	7.6(4)
	C(15)–C(16)–C(17)–C(13)	21.0(7)	20.9(4)	20.1(5)
	C(16)–C(17)–C(13)–C(14)	−40.1(7)	−39.4(4)	−39.7(4)
	C(17)–C(13)–C(14)–C(15)	45.1(7)	43.1(4)	44.7(4)

through C(7) and the midpoint of the C(10)–C(9) bond. Such a conformation reduces to its lowest value the strain energy associated with the *trans* fusion of the 6-membered rings at C(5)–C(10) and C(8)–C(9), and the presence of the substituent at C(7).[21] It is additionally stabilised by an intramolecular hydrogen bond between the hydroperoxy hydrogen and the oxygen atom of the alkoxy substituent at C(7). This internal hydrogen bond closes another seven-membered ring whose conformation approximates to a twist-boat form, with an approximate twofold axis passing through C(5) and the midpoint of the hydrogen bridge H(5)···O(7). Intramolecular hydrogen-bond parameters are listed in Table 3, and the conformation of the two bridged seven-membered rings is illustrated in Fig. 2. The internal hydrogen bond must have a stabilizing effect on molecular conformation, since it is maintained in the solid state, with no intermolecular hydrogen bonds being present. Presumably, it might also be responsible for the relative stability of this group of hydroperoxides.

Table 3 Geometry of the intramolecular O5A–H5···O7 hydrogen bonds[a]

Compound		d(D···A)/Å	d(H···A)/Å	∠D–H···A/°
2		2.780(6)	1.98	138
3	Molecule I	2.717(3)	1.94	158
3	Molecule II	2.679(4)	1.83	150

[a] D–H bond lengths have been standardized to a value 0.97 Å.

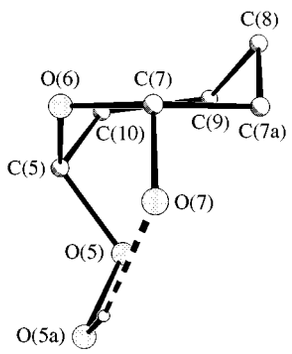


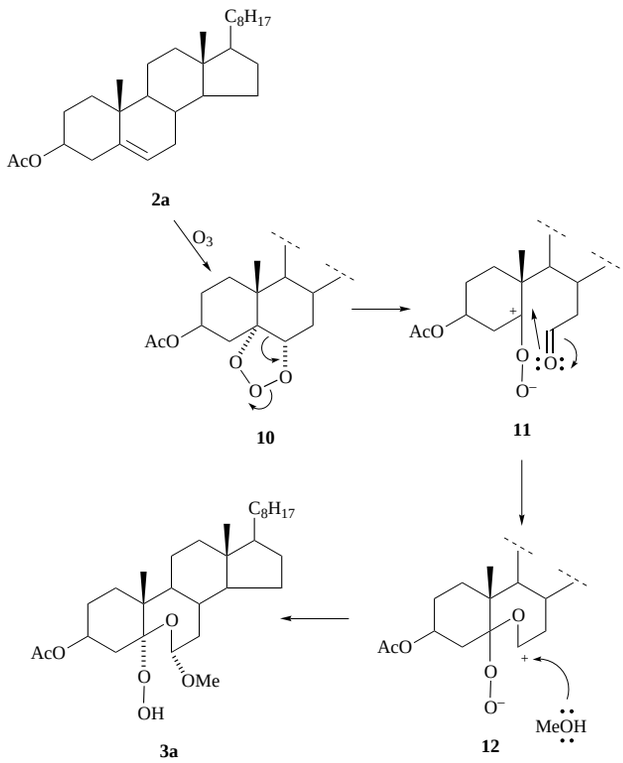
Fig. 2 Perspective view of the ring B of the hydroperoxide **3j** with the hydrogen-bonded 5α-hydroperoxy group

Seven-membered carbocycles occur in many classes of natural products, and most commonly they are fused to at least one additional ring. Seven-membered ε-lactone rings are present in brassinosteroids, the recently characterised plant growth-promoting steroids[22] and in kaurane type diterpenoids.[23] In the few crystal structures reported, the atomic coordinates of which are available through the Cambridge Structural Database,[24] the seven-membered ε-lactone ring invariably adopts the chair conformation, with an approximate mirror plane passing either through C(10)[23a] or C(9)[22c,23d] carbon atoms.

The isolation of both types of products, a hydroperoxide and a hydroxyperoxide, from ozonation of cyclobutene[19b] and indene[25] derivatives has been reported. The double bond in these substrates is incorporated into four- or five-membered rings and the resulting hydroperoxides are tetrahydrofuran and isochroman derivatives, respectively. The present work also shows that seven-membered, solvent-participated products are effectively formed in the course of ozonolysis of rigid cyclohexene derivatives.

The formation of the hydroperoxides **3** is rationalized in terms of the Criegee mechanism.[5] Furthermore, the unequivocal evidence for the structure **3** accounts for the revision of the mechanism of ozonolysis of cholesterol and other Δ^5-steroids in alcohol-containing solvents (Scheme 1). The primary ozonide **10** which, for steric reasons, has most probably the 5α stereochemistry, is the precursor of the more stable, tertiary carbonyl oxide intermediate **11**. The intramolecular partial capture of **11** by the 6-carbonyl oxygen gives the dipolar intermediate **12**. The reaction with methanol then follows, resulting in **3a**. Thus, the previously claimed secondary carbonyl oxide **13**[9] is not an intermediate in the ozonolysis of cholesterol. The formation of the 7α-alkoxy derivatives **3a**–**3d**, irrespective of the bulkiness of the nucleophilic alcohol, suggests that directed[25] α-axial incorporation of the alkoxy group takes place. Otherwise, the isomer of **3** with the equatorial 7β-alkoxy group would be expected to arise, possibly as a major product. It was also shown, that hydroperoxides **3** are not formed from 1,2,4-trioxolane **8**, as this compound is stable in methanol solution at room temperature.

In view of the results presented, all structures **1** assigned to the principal products obtained in the ozonolyses of cholesterol in water or alcohols reported previously[9,10,11,13] should be revised. Hence, isomeric structures **3** with hydroperoxy group are correct for these compounds. It also appears, that the dimeric and oligomeric structures proposed for products obtained during ozonation of cholesterol in non-participating solvents[12] should be revised accordingly, since all these compounds show, in their ^{1}H NMR spectra, a lowfield proton signal characteristic of a hydroperoxy group.



Scheme 1

Experimental

Mp values were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were determined with a Perkin-Elmer 580 grating spectrophotometer for solutions in chloroform. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL FX90Q or Varian Gemini 300 VT spectrophotometers operating in the Fourier transform mode using solutions in deuteriochloroform. Coupling constants J are given in Hz, and the chemical shifts (δ) are expressed in ppm relative to tetramethylsilane. Electron impact mass spectra were recorded with a JEOL LMS D-100 spectrometer. Column chromatography was performed by using silica gel 60 (Merck 70–230 mesh, no. 7734). The progress of reactions was monitored by TLC using a precoated aluminium-backed silica plates (Merck, no. 5554).

X-Ray analysis and structure refinement

Single crystals of **3a** were obtained from the isopropyl alcohol–diisopropyl ether solution, and **3c** and **3j** were grown from methanol. In general, it was difficult to obtain crystals of a quality suitable for X-ray measurement. Crystals of **3a** were poorly diffracting and although we were able to solve the structure, it was apparent that the number of observed reflections was too low to successfully refine it. Therefore, further refinement of compound **3a** was not undertaken. X-Ray data for compound **3c** were of higher quality, but it soon became apparent that some of the atoms of the side chain at C(17) showed large displacement parameters, suggesting positional disorder. Consequently, C(24) was refined in two alternative positions labelled as C(24) and C(24B) with a partial occupancy ratio 70:30, respectively. The crystal structure of hydroperoxide **3j**, which lacks the side chain, was obtained with the highest precision. Crystal data and details of the X-ray measurements are summarized in Table 1. For all three compounds the reflection

intensities were measured on a four-circle KM-4 (KUMA Diffraction)[26] diffractometer equipped with a graphite monochromator. The cell constants and the orientation matrix were obtained from a least-squares fit of at least 25 centred reflections. The intensities were measured using the ω–2θ scan technique with variable scan rate, and a scan range ω 0.75–1.45°. Background measurements were estimated from a 68-step profile. The intensities were corrected for Lorentz and polarisation effects; absorption corrections were not applied. The structures were solved by direct methods with SHELXS-86[27] and refined with SHELXL-93.[28] Refinement was completed only for structures **3c** and **3j**. Heavy atoms (C, O, N) were refined anisotropically. The positions of the H-atoms attached to the C-atoms were calculated, with the exception of the OH H-atoms which were located from difference Fourier maps. All H atoms were refined using a riding model with an isotropic temperature factor 1.2 times U_{eq} of the atom to which they are bonded. A Siemens Stereochemical Workstation was used to prepare the drawings.[29] Details of the present X-ray analyses and crystal data are collected in Table 1.§

The general procedure for ozonolysis of steroidal olefins in an alcohol–chloroform solution

The steroid olefin (**2a**–**2g**) was dissolved in a mixture of chloroform–alcohol (ROH) (in most cases 1:1) to get an approximately 1% solution, that was cooled to −78 °C. Ozone was passed through this solution until the substrate disappeared (TLC test). The excess ozone was removed with a stream of oxygen. Solvents were evaporated under reduced pressure and the residue was recrystallized. The following hydroperoxides were obtained [the yield of the chromatographically pure (TLC) product is given].

3β-Acetoxy-5α-hydroperoxy-7α-methoxy-5α-B-homo-6-oxacholestane 3a. (56% yield) mp 150–153 °C (from MeOH) (lit.,[9] mp 143–145 °C); the IR, ^{1}H NMR and ^{13}C NMR spectra were in accordance with the data given in ref. 9.

3β-Acetoxy-5α-hydroperoxy-7α-ethoxy-5α-B-homo-6-oxacholestane 3b. (70% yield) mp 132–136 °C (from Et_2O–MeOH) (lit.,[9] mp 138-140 °C); the IR, ^{1}H NMR and ^{13}C NMR spectra were in accordance with the data given in ref. 9.

3β-Acetoxy-5α-hydroperoxy-7α-isopropoxy-5α-B-homo-6-oxacholestane 3c. (55% yield) mp 133–135 °C (from ethanol); ν_{max}/cm^{-1} 3250, 1725; δ_H 10.32 (1 H, s, 5α-OOH), 4.83 (3 H, m, 3α-H), 4.79 (1 H, m, 7β-H), 4.05 (1 H, septet, O-*CH*Me$_2$), 2.62 (1 H, m, 4α-H), 2.03 (3H, s, OAc), 1.06 (10-Me), 0.65 (13-Me); δ_C 69.8 (C-3), 98.6 (C-7), 111.4 (C-5), 170.1 (CH_3COO); *m*/*z* 536, 518, 459, 399, 291 (Found: C, 71.5; H, 10.6. $C_{32}H_{56}O_6$ requires C, 71.60; H. 10.52%).

3β-Acetoxy-5α-hydroperoxy-7α-*tert*-butoxy-5α-B-homo-6-oxacholestane 3d. (60% yield) mp 185–189 °C (from benzene–hexane); ν_{max}/cm^{-1} 3280, 1720; δ_H 10.73 (1 H, s, 5α-OOH), 5.00 (1 H, m, 7β-H), 4.91 (1 H, m, 3α-H), 2.63 (1 H, m, 4α-H), 2.02 (3 H, s, Ac), 1.26 (9 H, s, Bu^t), 1.05 (3 H, s, 10-Me), 0.65 (3 H, s, 13-Me); δ_C 69.9 (C-3), 94.6 (C-7) and 111.3 (C-5) (Found: C, 72.1; H, 10.4. $C_{33}H_{58}O_6$ requires C, 71.96; H, 10.61%).

3β-Chloro-5α-hydroperoxy-7α-methoxy-5α-B-homo-6-oxacholestane 3e. (58% yield) mp 111–114 °C (from Et_2O–MeOH); ν_{max}/cm^{-1} 3270, 1190 and 1030; δ_H 10.05 (1 H, s, 5α-OOH), 4.61 (1 H, t, *J* 7.9, 7β-H), 4.03 (1 H, m, 3α-H), 3.48 (3 H, s, 7-OCH_3), 2.85 (1 H, m, 4α-H), 1.07 (3 H, s, 10-Me) and 0.65 (3 H, s, 13-Me); *m*/*z* 467, 452, 434, 398 and 249 (Found: C, 69.5; H, 10.0. $C_{28}H_{49}ClO_4$ requires C, 69.32; H, 10.18).

3β-Ethoxy-5α-hydroperoxy-7α-methoxy-5α-B-homo-6-oxacholestane 3f. (66% yield) mp 137–139 °C (from Et_2O–MeOH); ν_{max}/cm^{-1} 3280, 1100, 1050, 1035 and 1010; δ_H 10.00 (1 H, s, 5α-OOH), 4.61 (1 H, t, *J* 7.8, 7β-H), 3.49 (3 H, s, 7-OCH_3), 3.38 (1 H, m, 3α-H), 2.70 (1 H, m, 4α-H), 1.20 (3 H, t, OCH_2CH_3), 1.05 (3 H, s, 10-Me) and 0.65 (3 H, s, 13-Me); *m*/*z* 494, 476, 445, 315 and 263 (Found: C, 73.0; H, 10.9. $C_{30}H_{54}O_5$ requires C, 72.83; H, 11.00).

5α-Hydroperoxy-7α-methoxy-5α-B-homo-6-oxacholestane-3-one 3g. (57% yield) mp 124–127 °C (from EtOH); ν_{max}/cm^{-1} 3260, 1720, 1048 and 1030; δ_H 10.13 (1 H, s, 5α-OOH), 4.55 (1 H, m, 7β-H), 3.48 (3 H, s, OMe), 3.29 (1 H, dd, *J* 15.6, *J* 2.1, 4α-H), 2.61 (1 H, d, *J* 15.6, 4β-H), 1.24 (3 H, s, 10-Me) and 0.69 (3 H, s, 13-Me); *m*/*z* 464, 446, 363, 263 and 135 (Found: C, 72.3; H, 10.2. $C_{28}H_{48}O_5$ requires C, 72.36; H, 10.42).

5α-Hydroperoxy-7α-methoxy-5α-B-homo-6-oxacholestane 3h. (65% yield) mp 127–129 °C (from MeOH); ν_{max}/cm^{-1} 3220, 1140, 1030 and 1010; δ_H 9.59 (1 H, s, 5α-OOH), 4.59 (1 H, m, 7β-H), 3.48 (3 H, s, OMe), 2.30 (1 H, m, 4α-H), 1.04 (3 H, s, 10-Me) and 0.66 (3 H, s, 13-Me); *m*/*z* 450, 432, 401 and 263 (Found: C, 74.4; H, 11.3. $C_{28}H_{50}O_4$ requires C, 74.62; H, 11.18).

3β-Acetoxy-5α-hydroperoxy-7α-methoxy-5α-B-homo-6-oxapregnane 3i. (56% yield) mp 127–130 °C (from MeOH); ν_{max}/cm^{-1} 3250 and 1720; δ_H 10.04 (1 H, s, 5α-OOH), 4.90 (3 H, m, 3α-H), 4.62 (1 H, t, *J* 7.9, 7β-H), 3.48 (3 H, s, OMe), 2.68 (1 H, m, 4α-H), 2.03 (3 H, s, Ac), 1.08 (3 H, s, 10-Me) and 0.55 (3 H, s, 13-Me) (Found: C, 67.8; H, 9.2. $C_{24}H_{40}O_6$ requires C, 67.89; H, 9.50).

3β-Acetoxy-5α-hydroperoxy-7α-methoxy-5α-B-homo-6-oxaandrostane 3j. (65% yield) mp 138–142 °C (from MeOH-$CHCl_3$); ν_{max}/cm^{-1} 3250, 1720, 1250, 1140, 1040 and 1020; δ_H 10.03 (1 H, s, 5α-OOH), 4.90 (1 H, m, 3α-H), 4.62 (1 H, t, *J* 7.5, 7β-H), 3.8 (3 H, s, OMe), 2.67 (1 H, m, 4α-H), 2.03 (3 H, s, OAc), 1.08 (3 H, s, 10-Me) and 0.70 (3 H, s, 13-Me); δ_C 69.7 (C-3), 102.3 (C-7), 111.5 (C-5) and 170.0 (CH_3COO); *m*/*z* 396, 378, 347, 287, 243 and 151 (Found: C, 66.4; H, 9.4. $C_{22}H_{36}O_6$ requires C, 66.64; H, 9.15).

Reduction of 3a with lithium aluminium hydride

To a stirred solution of **3a** (187 mg) in anhydrous diethyl ether (100 ml) under argon, lithium aluminium hydride (18 mg) was added at −60 °C. After 2 h, methanol (30 ml) was added. The usual workup gave the residue (169 mg), which showed three spots on TLC. The ^{1}H NMR spectrum indicated the presence of compounds **7a**,[20] **7b**[8,12] and **7c**[20b] in the ratio 5:2:3, as estimated from the integration of characteristic low-field signals at δ 9.61, 6.77, 5.82, 5.38 and 4.51.

Reaction of 3a with potassium iodide

To a solution of **3a** (85 mg) in acetic acid (5 ml) a solution of potassium iodide (30 mg) in AcOH (1 ml) was added and the deep colour of iodine developed quickly. After 0.5 h diethyl ether–benzene mixture (1:1, 10 ml) was added and the solution was washed with aq. $NaHSO_3$ (5%), aq. Na_2CO_3 (5%) and brine. The organic layer was dried ($MgSO_4$) and evaporated to give chromatographically pure **7a** (68 mg). The ^{1}H NMR and IR (in CCl_4) spectra were in accordance with the data given in ref. 20.

Reaction of 3a with trifluoroacetic acid

To a solution of the hydroperoxide **3a** (830 mg) in hexane–benzene 1:12 mixture (6 ml), trifluoroacetic acid (1 ml) was added and the reaction mixture was kept at 0 °C for 12 h. The solution was washed with cold aq. NaOH (5%), water, dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel (40 g) with chloroform as eluent to give 116 mg of the semicrystalline ozonide **8**. Its ^{1}H NMR, IR and mass spectra were well in accordance with the literature.[12]

§ Atomic coordinates, anisotropic displacement parameters and tables of bond distances and angles for compounds **3c** and **3j** have been deposited at the Cambridge Crystallographic Data Centre. For details, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 2*, 1996, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number, 188/93.

Reduction of hydroperoxide 3a with dimethyl sulfide to the cyclic hemiacetal 9a

Into an NMR tube compound **3a** (20 mg) and Me_2S (1 ml) were added. After 0.5 h the solvent was removed under reduced pressure, dry $CDCl_3$ or C_6D_6 (0.5 ml) was added and the ^1H NMR spectrum recorded. It showed the following signals of **9a**: $\delta_H(CDCl_3)$ 4.90 (1 H, m, 3α-H), 4.58 (1 H, t, *J* 7.7, 7β-H), 3.39 (3 H, s, OCH_3), 3.00 (1 H, s, 5-OH, disappears on addition of D_2O), 0.99 (3 H, s, 10-CH_3), 0.66 (3 H, s, 13-CH_3); $\delta_H(C_6H_6)$ 5.21 (1 H, m, 3α-H), 4.55 (1 H, dd, *J* 10, *J* 8.1, 7β-H), 3.62 (1 H, s, OH), 3.26 (3 H, s, OMe), 1.76 (3 H, s, AcO), 1.02 (s, 10-Me), 0.67 (s, 13-Me); ν_{max}/cm^{-1} (in CCl_4) 3555, 3420, 1732 and 1237; *m*/*z* 460 ($M^+ - MeOH$), 354, 318, 247 and 110.

Reduction of hydroperoxide 3b with dimethyl sulfide to the cyclic hemiacetal 9b

Compound **9b** was obtained according to the procedure described in the preceding experiment: ν_{max}/cm^{-1} (in CCl_4) 3555, 3400, 1735 and 1242; δ_H 4.96 (1 H, m, 3α-H), 4.69 (1 H, t, *J* 8.1, 7α-H), 3.86 and 3.46 (2 H, two q, *J* 7.2, 7-OCH_2CH_3), 2.98 (1 H, s, 5-OH, disappears on addition of D_2O), 1.20 (3 H, t, *J* 7.2, 7-OCH_2CH_3), 0.99 (3 H, s, 10-Me), 0.66 (3 H, s, 13-Me); *m*/*z* 460 ($M^+ - EtOH$), 354, 318 and 110.

References

1 E. P. C. Kilsdonk, D. W. Morel, W. J. Johnson and G. H. Rothblat, *J. Lipid Res.*, 1995, **36**, 505 and references cited therein.
2 M. F. Grey, T. D. V. Lawrie and C. J. W. Brooks, *Lipids*, 1971, **6**, 836; N. B. Javitt, E. Kok, S. Burstein, B. Cohen and J. Kutscher, *J. Biol. Chem.*, 1981, **256**, 12 644; N. Kumar and O. P. Singhal, *J. Sci. Food Agric.*, 1991, **55**, 497; L. L. Smith and B. H. Johnson, *Free Rad. Biol. Med.*, 1989, **7**, 285.
3 X. Pannecoucke, J.-P. Starck, A. Milon, G. Ourisson and B. Luu, *Bull. Soc. Chim. Fr.*, 1994, **131**, 693 and references cited therein.
4 L. L. Smith, *J. Liquid Chromatogr.*, 1993, **16**, 1731 and references cited therein.
5 R. Criegee and G. Wenner, *Chem. Ber.*, 1949, **564**, 9; R. Criegee, *Angew. Chem., Int. Ed. Engl.*, 1975, **11**, 745.
6 P. S. Bailey in *Ozonation in Organic Chemistry*, Academic Press, New York, 1978, vol. 1; 1982, vol. 2.
7 W. H. Bunnelle, *Chem. Rev.*, 1991, **91**, 335 and references cited therein.
8 J. W. Cornforth, G. D. Hunter and G. Popjak, *Biochem. J.*, 1953, **54**, 590.
9 J. Gumulka and L. L. Smith, *J. Am. Chem. Soc.*, 1983, **105**, 1972.
10 H. Lettre and A. Jahn, *Angew Chem.*, 1957, **69**, 266.
11 K. Tanabe and Y. Morisawa, *Bull. Chem. Soc. Jpn.*, 1963, **11**, 536; L. Brown, W. J. S. Lyall, C. J. Suckling and K. E. Suckling, *J. Chem. Soc., Perkin Trans. 1*, 1987, 595.
12 K. Jaworski and L. L. Smith, *J. Org. Chem.*, 1988, **53**, 545.
13 K. Jaworski and L. L. Smith, *Magn. Reson. Chem.*, 1988, **26**, 104.
14 H. Kropf and H. von Wallis, *Synthesis*, 1981, 237.
15 D. Swern, A. H. Clements and T. M. Luong, *Anal. Chem.*, 1969, **41**, 412.
16 T. S. Lillie and R. C. Ronald, *J. Org. Chem.*, 1985, **50**, 5084.
17 Z. Paryzek, J. Martynow and W. Swoboda, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1220.
18 Z. Paryzek, J. Martynow and W. Swoboda, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1222.
19 (*a*) R. E. Keay and G. A. Hamilton, *J. Org. Chem.*, 1973, **39**, 3604; (*b*) K. Griesbaum and G. Kiesel, *Chem. Ber.*, 1989, **122**, 145.
20 (*a*) K. Tanabe and Y. Morisawa, *Chem. Pharm. Bull.*, 1963, **11**, 536; P. Morand and M. Kaufman, *J. Org. Chem.*, 1969, **34**, 2175; (*b*) P. Yates and S. Stiver, *Can. J. Chem.*, 1988, **66**, 1209.
21 J. B. Hendrickson, *Tetrahedron*, 1963, **19**, 1387.
22 (*a*) M. J. Thompson, N. Mandava, J. L. Flippen-Anderson, J. F. Worley, S. R. Dutky, W. E. Robbins and W. Lusby, *J. Org. Chem.*, 1979, **44**, 5002; (*b*) M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. Mandava, J. F. Worley, J. D. Warthen, Jr., G. L. Steffens, J. L. Flippen-Anderson and J. C. Cook, Jr., *Nature* (*London*), 1979, **281**, 216; (*c*) L. Kutschabsky, G. Adam and H.-M. Vorbrodt, *Z. Chem.*, 1990, **30**, 136.
23 (*a*) M. Shaiq Ali, M. K. Baynham, J. R. Hanson and P. B. Hitchcock, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2679; (*b*) M. K. Baynham, J. M. Dickinson, J. R. Hanson and P. B. Hitchcock, *J. Chem. Soc., Perkin Trans. 1*, 1987, 1987; (*c*) F. Nagashima, H. Tanaka, S. Takaoka and Y. Asakawa, *Chem. Pharm. Bull.*, 1994, **42**, 2656; (*d*) A. R. H. Kehrli, D. A. H. Taylor and M. Niven, *J. Chem. Soc., Perkin Trans. 1*, 1990, 2057.
24 3D Search and Research using the Cambridge Structural Database, F. H. Allen; O. Kennard, *Chemical Design Automation News*, 1993, **8**, 131.
25 K. J. McCullough, M. Nojima, M. Miura, T. Fujisaka and S. Kusabayashi, *J. Chem. Soc., Chem. Commun.*, 1984, 35; K. J. McCullough, T. Fujisaka, M. Nojima and S. Kusabayashi, *Tetrahedron Lett.*, 1988, **29**, 3375; N. Nakamura, T. Fujisaka, M. Nojima, S. Kusabayashi and K. J. McCullough, *J. Am. Chem. Soc.*, 1989, **111**, 1799.
26 *DATARED*, *KUMA Diffraction*, Wrocław, Poland, 1989.
27 G. M. Sheldrick, SHELXS-86, University of Göttingen,1986.
28 G. M. Sheldrick, SHELXL-93, University of Göttingen, 1993.
29 *Stereochemical Workstation Operational Manual*, Release 3.4 Siemens Analytical X-Ray Instruments, Inc., Medison, Wisconsin, USA, 1989.

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