SHORT PAPER

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An unusual ozonolysis of the $\Delta^{8(14)}$-unsaturated steroids†

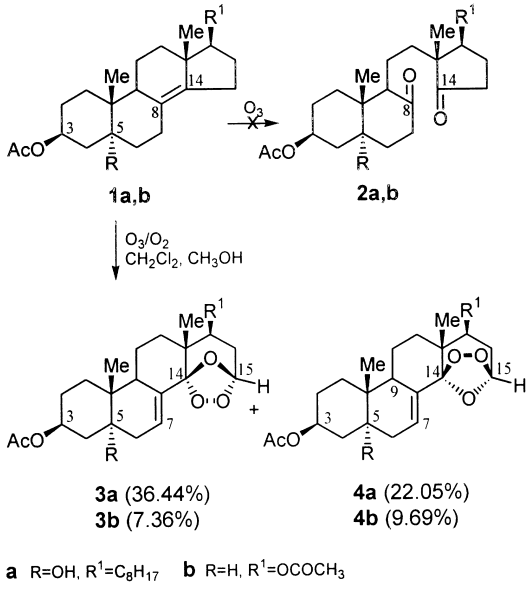
Natalija M. Krstić[a], Ljubinka B. Lorenc[b,*], Vladimir D. Pavlović[b], Jaroslav Kalvoda[c], Bernard Tinant[d] and Jean-Paul Declercq[d]

[a]Center for Chemistry, ICTM, P. O. Box 473, YU-11001 Belgrade, [b]Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, PO Box 158, YU-11001 Belgrade, [c]Leimgrubenweg 21,CH-4102 Binningen, Switzerland, [d]Laboratoire de Chimie Physique et de Cristalographie, Université Catholique de Louvain, 1 Place Louis Pasteur, B-1348 Louvain-la-Neuve, Belgium

Ozonolysis of the $\Delta^{8(14)}$-unsaturated steroids 1a,b afforded, instead of the expected 8,14-dioxo-8,14-seco derivatives 2a,b two stereoisomeric Δ^{7}-unsaturated ozonides 3a,b and 4a,b.

Keywords: ozonolysis, $\Delta^{8(14)}$-unsaturated steroids

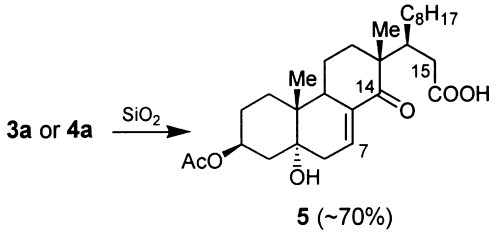
In an attempt to prepare 8,14-dioxo-8,14-secosteroids **2a,b** by ozonolysis of the 5-hydroxy-5α-cholest-8(14)-en-3β-yl acetate (**1a**)[1] and 5α-androst-8(14)-ene-3β,17β-diyl diacetate (**1b**)[2] (Scheme 1), these compounds were treated with ozone in dichloromethane at –78°C, followed by reductive work up with dimethyl sulfide. However, in both cases instead of the expected 8,14-dioxo-8,14-seco derivatives **2a**[1] and **2b**, two compounds were obtained, to which were assigned structures of the two stereoisomeric Δ^{7}-unsaturated ozonides **3a,b** and **4a,b,** respectively (Scheme 1).



Scheme 1

Microanalysis and mass spectra of the products showed that they contain three additional oxygens and two hydrogens less than the starting molecules **1a,b**, thus indicating that the reaction resulted in the introduction of an olefinic double bond and ozonide bridge into the structure of **1a,b**. The new olefinic double bond was trisubstituted as established by the ^{1}H NMR (the olefinic proton appearing at δ 6.12 and 6.30 ppm, respectively for **3a,b**, and δ 6.28 and 6.14 ppm, respectively for **4a,b**), and the ^{13}C NMR spectra (singlets for hydrogen-free carbon at 132.1 and 129.9 ppm, respectively for **3a,b** and 133.3 and 131.2 ppm, respectively for **4a,b**, and doublet for a mono-protonated carbon at 120.9 and 125.0 ppm, respectively for **3a,b** and 122.9 and 123.6 ppm, respectively for **4a,b**). Similarly, insertion of ozone into a trisubstituted olefinic double bond was suggested by the ^{1}H-NMR (a triplet at 5.87 ppm, *J*=1.8 Hz for **3a** and 5.85 ppm, *J*=3.8 Hz for **3b** and a *dd* at 5.82 ppm, *J*=5.4, 1.2 Hz for **4a** and a doublet at 5.82 ppm, *J*≈1 Hz for **4b**), and ^{13}C-NMR spectra (the oxygenated carbons appearing as singlet at 109.8 and 109.5 ppm, respectively for **3a,b** and 107.7 and 108.4 ppm, respectively for **4a,b**, and doublet at 102.9 and 101.3 ppm, respectively for **3a,b** and 101.8 and 100.8 ppm, respectively for **4a,b**).

Similar spectral characteristics found for compounds of type **3** and **4** indicated that they are stereoisomers which differ by spatial arrangment at the oxygenated carbons. This was substantiated by hydrolytic cleavage of the ozonide bridge in compounds **3a** and **4a** which, after a prolonged stirring with SiO_2 in methanol solution at room temperature, were transformed to the same carboxylic acid **5** (Scheme 2). The spectroscopic data for structure **5** are given in the Experimental section.



Scheme 2

The assignment of stereochemistry at C(15) in **3a,b** and **4a,b** was based on multiplicity of the H-C(15) resonance. According to molecular models of ozonides with α-oriented peroxide bridge the H-C(15) signal, was expected to be a triplet, due to nearly equal dihedral angle between the H-C(15) and $H_2C(16)$ protons. This was found for compounds **3a** and **3b** whilst in the isomers **4a** and **4b** the corresponding H-C(15) signals appear as doublets.

However, an unequivocal confirmation of the proposed structure of **3b** was obtained by X-ray crystallografic determination (Table 1). ORTEP plots of **3b** is presented in Fig. 1.

Isolation of some stable steroidal[5,6] and non-steroidal ozonides[7] is not uncommon. However, introduction of an

* To receive any correspondence. E-mail: lorenc@helix.chem.bg.ac.yu
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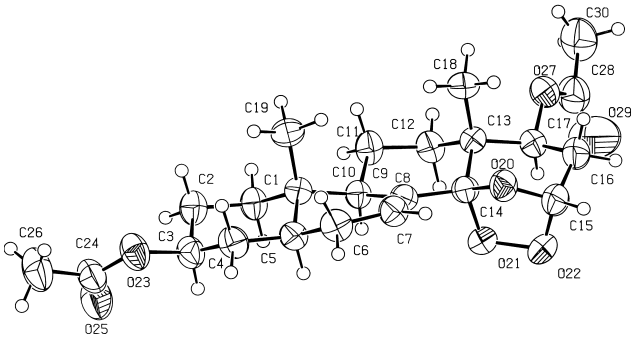

Fig. 1 Crystal structure of ozonide **3b**.

Table 1 Crystal data and structure refinement for **3b**

Empirical formula	$C_{23}H_{32}O_7$
Formula weight	420.49
Temperature	293(2) K
Wavelength	0.71069 Å
Crystal system	Monoclinic
Space group	P21
Unit cell dimensions	*a*=10.053(4) Å α=90 deg.
	b=7.447(3) Å β=109.57 deg.
	c=15.592(7) Å γ = 90 deg.
Volume	1099.9(8) Å^3
Z	2
Calculated density	1.270 Mg/m^3
Absorption coefficient	0.093 mm^{-1}
F(000)	452
Crystal size	0.25 × 0.20 × 0.08 mm
Θ range for data collection	2.13 – 25.36°
Limiting indices	$-12\le h\le 12$, $-8\le k\le 8$, $-18\le l\le 18$
Reflections collected	6193
Independent reflections	3601 [*R*(int) = 0.050]
Completeness to Θ = 25.36	95.7 %
Refinement method	Full-matrix least-squares on Fsqd
Data /restraints/ parameters	3601 / 1 / 348
Goodness-of-fit on Fsqd	1.097
Final R indices [$I>2\sigma(I)$]	*R*1 = 0.0507, w*R*2 = 0.1273
R indices (all data)	*R*1 = 0.0585, w*R*2 = 0.1405
Absolute structure parameter	2.3(12)
Extinction coefficient	0.213(17)
Largest diff. peak and hole	0.293 and –0.264 e.Å^{-3}

olefinic double bond with ozone molecule accompanied by migration of the original double bond, according to our knowledge, has not been reported as yet. The observed unique reaction is probably due to the steric characteristic of the steroidal $\Delta^{8(14)}$-double bond.

Experimental

General: Removal of solvents was carried out under reduced pressure. Prep. column chromatography: sillica gel 0.04–0.063 mm. TLC: control of reaction and separation of products on sillica gel (Stahl) with benzene/AcOEt 9:1, 8:2 and 7:3, detection with 50% aq. H_2SO_4 soln. M.p.s uncorrected. IR spectra: Perkin-Elmer-337 spectrophotometer; ν in cm^{-1}. NMR spectra: Varian Gemini 200 (^{1}H at 200 MHz, ^{13}C at 50.28 MHz); $CDCl_3$ soln at r.t., TMS as internal standard; chemical shifts in ppm as δ values, *J* in Hz. Mass spectra: Finnigan-MAT 8230. UV spectra: Beckman DU-50 spectrophotometer.

Ozonolysis of 5α–hydroxy-5α-cholest-8(14)-en-3β-yl acetate (**1a**): A solution of 5α-hydroxy-5α-cholest-8(14)-en-3β-yl acetate (**1a**) (1.2 g) in dichloromethane (72 ml) and methanol (0.2 ml) was treated with ozone at –78°C until the solution turned a pale blue colour (about 1.5 hour) and then flushed with nitrogen. The mixture was treated with $(CH_3)_2S$ (2 ml), stirred at –40°C for 2 hours, and left in the refrigerator overnight. The mixture was washed with water, saturated aq. $NaHCO_3$ and water, dried over $CaSO_4$ and evaporated to dryness. The residue (1.07 g, 81%) was chromatographed on SiO_2 (100 g) (toluene–EtOAc, 90:10) to give a mixture of **3a** and **4a** (700 mg). It was rechromatographed on SiO_2 (80 g). Elution with toluene–EtOAc (92.5:7.5) gave ozonide **3a** (481.4 mg, 36.44%) as a white solid, m.p. 108–112°C (from acetone–MeOH). IR (KBr): 3454, 2954, 1734, 1468, 1366, 1246, 1104, 1029, 981. ^{1}H-NMR: 0.85 (*s*, 3HCH$_3$(19)), 0.88 (*s*, 3HCH$_3$(18)), 0.90 (*d, J*=6.4, 6H, CH$_3$(26), CH$_3$(27)), 0.95 (*d*, 3H, *J*=6.6, CH$_3$(21)), 2.02 (*s*, 3H, AcO-C(3)), 5.10 (*m*, 1H, H-C(3)), 5.87 (*t*, 1H, *J*=1.8, H-C(15)), 6.12 (*br.d*, 1H, *J*=5.8, H-C(7)). ^{13}C-NMR: 170.4 (*s*, OCOCH$_3$), 132.1 (*s*, C(8)), 120.9 (*d*, C(7)), 109.8 (*s*, C(14)), 102.9 (*d*, C(15)), 73.1 (*s*, C(5)), 70.5 (*d*, C(3)), 42.7 (*d*, C(17)), 42.4 (*s*, C(13)), 41.9 (*d*, C(9)), 39.3 (*t*, C(24)), 38.8 (*s* C(10)), 37.9 (*t*, C(12)), 36.6 (*t*, C(4)), 33.5 (*t*, C(22)), 32.3 (*t*, C(6)), 30.6 (*t*, C(1)), 30.5 (*d*, C(20)), 28.3 (*t*, C(16)), 26.9 (*t*, C(2)), 27.9 (*d*, C(25)), 25.8 (*t*, C(23)), 22.6 (*q*, C(27)), 22.5 (*q*, C(26)), 21.4 (*q*, C(21)), 21.3 (*q*, OCOCH3), 18.7 (*t*, C(11)), 18.0 (*q*, C(19)), 15.3 (*q*, C(18)). MS: *m*/*z* = 490 (M$^+$). Anal. calc. for $C_{29}H_{46}O_6 \times 1/2$ CH$_3$OH (506.708): C 69.92, H 9.54; found: C 69.64, H 9.83.

Further elution with same eluent gave ozonide **4a** (291.5 mg, 22.05%) as an oil. IR (KBr): 3490, 2954, 1733, 1468, 1367, 1249, 1087, 1027, 906. ^{1}H-NMR: 0.86 (*d*, 6H, *J*=6.6, CH$_3$(26), CH$_3$(27)), 0.88 (*s*, 6H, CH$_3$(18), CH$_3$(19)), 0.93 (*d*, 3H, *J*=7.0, CH$_3$(21)), 2.02 (*s*, 3H, AcO-C(3)), 5.12 (*m*, 1H, H-C(3)), 5.82 (*dd*, 1H, *J*=5.4, 1.2, H-C(15)), 6.28 (*br.d*, 1H, *J*=5.4, H-C(7)). ^{13}C-NMR: 170.5 (*s*, OCOCH$_3$), 133.3 (*s*, C(8)), 122.9 (*d*, C(7)), 107.7 (*s*, C(14)), 101.8 (*d*, C(15)), 73.0 (*s*, C(5)), 70.5 (*d*, C(3)), 44.7 (*d*, C(17)), 42.9 (*d*, C(9)), 42.9 (*s*, C(13)), 39.2 (*t*, C(24)), 38.8 (*t*, C(4)), 38.1 (*t*, C(12)), 37.9 (*s*, C(10)), 37.2 (*t*, C(22)), 33.3 (*t*, C(6)), 31.1 (*d*, C(20)), 30.6 (*t*, C(1)), 28.5 (*t*, C(16)), 27.8 (*d*, C(25)), 26.9 (*t*, C(2)), 25.7 (*t*, C(23)), 22.6 (*q*, C(27)), 22.5 (*q*, C(26)), 21.3 (*q*, OCOCH$_3$), 20.9 (*q*, C(21)), 18.5 (*t*, C(11)), 17.6 (*q*, C(19)), 14.8 (*q*, C(18)). MS: *m*/*z* = (M$^+$) 490. Anal. calc. for $C_{29}H_{46}O_6 \times 1/2$ CH$_3$OH (506.708): C 69.92, H 9,54; found: C 69.76, H 9.25.

Ozonolysis of 5α-androst-8(14)-ene-3β,17β-diyl diacetate (**1b**): A solution of 5α-androst-8(14)-ene-3β,17β-diyl diacetate (**1b**) (500 mg) in dichloromethane (32 ml) and methanol (0.1 ml) was treated with ozone at –78°C as previously described (about 1 hour). Work up with $(CH_3)_2S$ as above gave residue (312 mg, 62%) which was chromatographed on SiO_2 (50 g). Elution with toluene–EtOAc (92:8) afforded ozonide **3b** (41.3 mg, 7.36%) as a white solid, m.p. 150–152°C (from acetone–MeOH). IR (KBr): 2943, 1731, 1446, 1366, 1242. ^{1}H-NMR: 0.81 (*s*, 3H, CH$_3$(19)), 0.98 (*s*, 3H, CH$_3$(18)), 2.02 (*s*, 3H, AcO-C(3)), 2.05 (*s*, 1H, AcO-C(17)), 4.68 (*m*, 1H, H-C(3)), 5.30 (*q*, 1H, H-C(17)), 5.85 (*t*, 1H, *J*=3.8, H-C(15)), 6.30 (*br.d*, 1H, *J*=5.2, H-C(7)). ^{13}C-NMR: 170.5 and 170.4 (*2s*, 2OCOCH$_3$), 129.9 (*s*, C(8)), 125.0 (*d*, C(7)),109.5 (*s*, C(14)), 101.3 (*d*, C(15)), 72.9 (*d*, C(17)), 72.5 (*d*, C(3)), 47.9 (*d*, C(9)), 42,2 (*s*, C(13)), 38.6 (*d*, C(5)), 36.1 (*t*, C(12)), 34.1 (*s*, C(10)), 33.3 (*t*, C(6)), 33.0 (*t*, C(16)), 31.8 (*t*, C(1)), 19.2 (*t*, C(4)), 27.2 (*t*, C(2)), 21.3 and 21.0 (2*q*, 2OCOCH$_3$), 18.5 (*t*, C(11)), 15.0 (*q*, C(19)), 12.7 (*q*, C(18)). CI-MS: *m*/*z* = 421 (M$^+$ + 1). Anal. calc. for $C_{23}H_{32}O_7$ (420.502): C 65.70, H 7.67; found: C 65.55, H 7.46.

Further elution with same eluent gave ozonide **4b** (54.2 mg, 9.69%) as an oil. ^{1}H-NMR: 0.84 (*s*, 3H, CH3(19)), 0.93 (*s*, 3H, CH3(18)), 2.03 (*s*, 3H, AcO-C(3)), 2.12 (*s*, 3H, AcO-C(17)), 4.69 (*m*, 1H, H-C(3)), 4.47 (*fd*, 1H, *J*=5.6, H-C(17)), 5.82 (*d*, 1H, *J*~1 Hz, H-C(15)), 6.14 (*t*, 1H, *J*=5.4, H-C(7)). ^{13}C-NMR: 170.9 and 170.6 (2s, 2OCOCH$_3$), 131.2 (*s*, C(8)), 123.6 (*d*, C(7)), 108.4 (*s*, C(14)), 100.8 (*d*, C(15)), 72.9 (*d*, C(17)), 71.6 (*d*, C(3)), 47.2 (*d*, C(9)), 41.5 (*s*, C(13)), 38.9 (d, C(5)), 36.3 (t, C(12)), 34.2 (s, C(10)), 33.4 (t, C(6)), 33.3 (*t*, C(16)), 32.3 (*t*, C(1)), 29.0 (*t*, C(4)), 27.2 (*t*, C(2)), 21.3 and 21.2 (2*q*, OCOCH$_3$), 19.0 (*t*, C(11)), 15.5 (*q*, C(19)), 12.7 (*q*, C(18)). CI-MS: *m*/*z* = 421 (M$^+$ + 1).

Hydrolytic cleavage of the ozonide bridge in compounds **3a** *and* **4a**: Ozonide **3a** (50 mg) was stirred with silica gel in methanol (5 ml) for 7 days at room temperature. After removal of SiO_2 and solvent the residue was chromatographed on SiO_2 (2 g). Elution with toluene–EtOAc (1: 1) gave 14-oxo-cholest-7-en-15-oic acid **5** (36 mg, 72%) as a white solid, m.p. 133–135°C (from acetone–MeOH). IR (KBr): 3426, 2955, 1713, 1696, 1668, 1266, 1217. ^{1}H-NMR: 0.83 (*s*, 3H, CH$_3$(19)), 0.86 (*d*, 6H, CH$_3$(26), CH$_3$(27)), 0.89 (*d*, 3H, CH$_3$(21)), 1.02 (*s*, 3H, CH$_3$(18)), 2.03 (*s*, 3H, AcO-C(3)), 2.29 (*d*, 2H, *J*=5, H$_2$C(16)), 5.11 (*m*, 1H, H-C(3)), 6.81 (*fs*, 1H, H-C(7)). ^{13}C-NMR: 203.2 (*s*,C(14)), 179.6 (*s*, C(15)), 170.7 (*s*, OCOCH$_3$), 136.5 (*d*, C(7)), 133.8 (*s*, C(8)), 73.0 (*s*, C(5)), 70.4 (*d*, C(3)), 50.3 (*s*, C(13)), 45.4 (*d*, C(17)), 43.2 (*d*, C(9)),39.2 (*t*, C(24)), 38.6 (*t*, C(12)), 38.0 (*s*, C(10)), 35.8 (*d*, C(20)), 34.1 (*t*, C(22)), 31.3 (*t*, C(6)), 30.4 (*t*, C(1)), 29.4 (*t*, C(4)), 29.4 (*t*, C(16)), 27.9 (*d*, C(25)), 27.1 (*t*, C(2)), 25.6 (*t*, C(23)), 23.9 (*q*, C(18)), 22.7 (*q*, C(27)), 22.5 (*q*, C(26)), 21.4 (*q*, OCOCH$_3$), 21.0 (*q*, C(21)), 18.3 (*t*, C(11)), 16.7 (*q*, C(19)). CI-

MS: m/z = 491 (M^+ + 1). Anal. calc. for $C_{29}H_{46}O_6 \times H_2O$ (508.627): C 68.50, H 9.45; found: C 68.40, H 9.66. UV: λ_{max}(EtOH): 247 nm (7100).

Ozonide **4a** (50 mg) was treated with silica gel in CH_3OH (5 ml) as above to give **5** (32 mg, 64%).

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