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OXIDATION OF URSOLIC ACID BY OZONE

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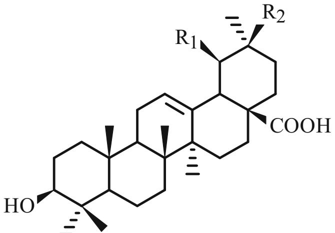
Oxidation of methyl esters of ursolic and 3-acetoxyursolic acids by ozone formed products with a 12-oxo-11S,13R-oxetane moiety on ring C.

Keywords: ursolic acid, oxidation, ozonolysis, oxetanes.

Oleanolic **(1)** and ursolic **(2)** acids are classified as pentacyclic triterpenoids and have been detected in more than 100 plants of various species (e.g., apple and olive skins, ginseng root, calendula and silphium inflorescences, white mistletoe, etc. [1]). Their extracts exhibit several valuable medicinal properties.



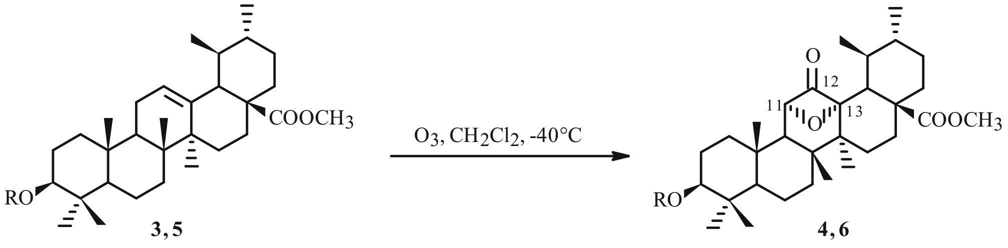
1, 2

1: $R_1 = H, R_2 = CH_3$
2: $R_1 = CH_3, R_2 = H$

An effective method for functionalizing triterpenoids is oxidation by ozone [2–5]. For example, $3\beta,12\alpha,13\beta$-trihydroxy-28→13-olide, the ozonolysis product of oleanolic acid, was discovered to inhibit α-glucosidase, the enzyme responsible for the blood glucose level [6].

We recently showed that oxidation of oleanolic acid methyl ester with an excess of ozone produced 3,12-dioxoolean-28-oic acid [7]. Oxidation of 11-deoxoglycyrrhetic acid occurred analogously to form the 3,12-diketone [5]. Ozonolysis of ursolic acid derivatives has not been reported.

We established that the product from reaction of acetylursolic acid methyl ester (**3**) with an excess of ozone was 12-oxo-11,13-epoxide (oxetane) **4** (Scheme 1). Moreover, the C-3 hydroxyl of ursolic acid methyl ester (**5**) was stable to the action of ozone, in contrast with oleanolic and 11-deoxoglycyrrhetic acids [5, 7]. The reaction produced 3β-hydroxyoxetane **6**.



3, 4: R = Ac; **5, 6:** R = H

Scheme 1

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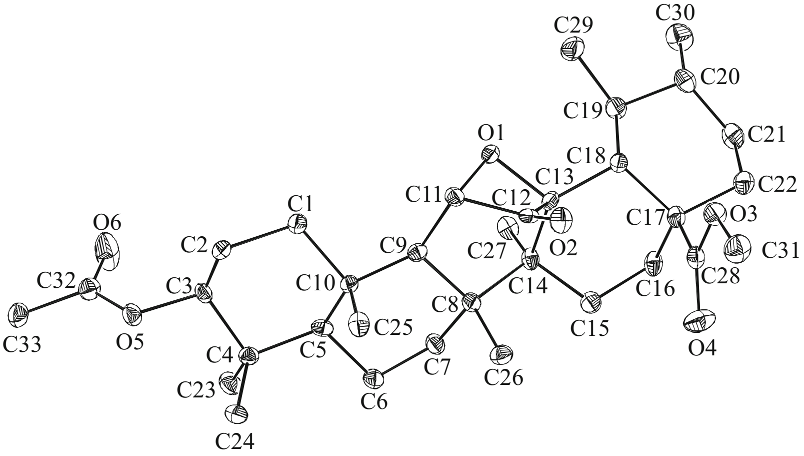

Fig. 1. Molecular structure of 3*β*-acetoxy-12-oxo-11*S*,13*R*-epoxyurs-28-oic acid (**4**) with 50% probability of thermal ellipsoids.

Resonances in spectra of **4** were assigned completely using 2D ^{1}H–^{1}H COSY and HETCOR correlation spectroscopy. A total of 33 resonances was observed according to DEPT spectra. These corresponded to nine quaternary, seven methine, eight methylene, and nine methyl C atoms. The formation of the 12-oxo-11,13-epoxy derivative was confirmed by resonances of quaternary C atoms C-12 (δ_C 191.48) and C-13 (δ_C 112.88) and that of methine C-11 (δ_C 95.35, δ_H 4.82). The oxetane had the 11*S*,13*R* configuration according to an x-ray structure analysis (XSA) (Fig. 1).

Thus, oxidation of the methyl esters of ursolic and 3-acetoxyursolic acids by ozone occurred stereospecifically and formed 12-oxo-(11*S*,13*R*)-epoxyurs-28-oic acid derivatives. The structure of **4** was proven by an XSA.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS internal standard on a Bruker AM-300 spectrometer (300 and 75.5 MHz, respectively). Melting points were determined on a Boetius microstage. Optical density was measured in a 1-dm tube on a Perkin–Elmer 241 MC polarimeter. An Ozon-2K ozonator was used. TLC analysis was performed on Sorbfil plates (ZAO Sorbpolimer, Russia) using $CHCl_3$:EtOAc (40:1). Compounds were detected using H_2SO_4 (10%) with subsequent heating at 100–120°C for 2–3 min. Ursolic acid was isolated as before [8]. Its methyl ester **5** and acetate **3** were prepared using standard methods.

Ozonolysis of 3 and 5. A solution of **3** or **5** (1 mmol) in CH_2Cl_2 (50 mL) was purged with an excess of ozone at –40°C and held for 3 h at room temperature. The solvent was evaporated using a water aspirator. The solid was chromatographed over a column of Al_2O_3 with elution by benzene.

3*β*-Acetoxy-12-oxo-11*S*,13*R*-epoxyurs-28-oic Acid Methyl Ester (4). Yield 0.42 g (77%), R_f 0.73, mp 252°C, $[\alpha]_D^{20}$ +95° (*c* 0.33, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.84 (3H, s, 3H-24), 0.85 (3H, s, 3H-23), 0.91 (1H, dd, $^3J_{5\text{-}6ax} = 11.8$, $^3J_{5\text{-}6eq} = 2.3$, H-5), 0.96 (3H, d, $^3J = 6.2$, 3H-29), 0.96 (1H, m, H-20), 1.00 (3H, s, 3H-25), 1.15 (3H, s, 3H-26), 1.15 (3H, d, $^3J = 6.2$, 3H-30), 1.23 (1H, m, Heq-22), 1.25 (1H, m, Hax-21), 1.30 (1H, m, Heq-15), 1.32 (3H, s, 3H-27), 1.33 (1H, m, Heq-7), 1.40 (1H, m, Hax-6), 1.43 (1H, m, Hax-7), 1.54 (1H, m, Heq-21), 1.56 (1H, ddd, $^2J = 11.6$, $^3J_{1ax\text{-}2ax} = 14.8$, $^3J_{1ax\text{-}2eq} = 2.1$, Hax-1), 1.58 (1H, m, H-19), 1.59 (1H, m, Heq-6), 1.60 (1H, dtd, $^2J = 11.2$, $^3J_{16eq\text{-}15eq} = 2.2$, $^3J_{16eq\text{-}15ax} = 2.2$, $^4J_{16eq\text{-}22} = 1.1$, Heq-16), 1.70 (1H, m, Hax-2), 1.72 (1H, m, Heq-2), 1.74 (1H, m, Hax-22), 1.84 (1H, ddd, $^2J = 11.2$, $^3J_{16ax\text{-}15ax} = 13.7$, $^3J_{16ax\text{-}15eq} = 3.6$, Hax-16), 1.87 (1H, ddd, $^2J = 11.6$, $^3J_{1eq\text{-}2ax} = 2.3$, $^3J_{1eq\text{-}2eq} = 2.3$, Heq-1), 2.05 (3H, s, OAc), 2.17 (1H, s, H-9), 2.51 (1H, td, $^2J = 13.7$, $^3J_{15ax\text{-}16ax} = 13.7$, $^3J_{15ax\text{-}16eq} = 2.2$, Hax-15), 2.55 (1H, dd, $^3J_{18\text{-}19} = 11.0$, $^4J_{18\text{-}16eq} = 1.2$, H-18), 3.77 (3H, s, OCH_3), 4.50 (1H, dd, $^3J_{3\text{-}2ax} = 11.1$, $^3J_{3\text{-}2eq} = 5.1$, H-3), 4.82 (1H, s, H-11).

^{13}C NMR spectrum (δ, ppm): 16.20 (C-24), 18.32 (C-6), 19.64 (C-27), 20.04 (C-30), 20.38 (C-25), 21.26 (C-29), 21.27 (C-33), 21.68 (C-26), 23.25 (C-2), 24.46 (C-16), 26.87 (C-15), 27.72 (C-23), 29.17 (C-21), 35.73 (C-7), 36.23 (C-10), 36.66 (C-1), 37.95 (C-4), 38.55 (C-22), 39.09 (C-20), 39.16 (C-19), 42.61 (C-8), 46.34 (C-18), 46.78 (C-14), 47.74 (C-17), 51.78 (C-31), 55.21 (C-5), 60.48 (C-9), 80.31 (C-3), 95.35 (C-11), 112.88 (C-13), 170.87 ($\underline{C}OCH_3$), 177.34 (C-28), 191.48 (C-21). $C_{33}H_{50}O_6$.

X-ray Structure Analysis of 3*β*-Acetoxy-12-oxo-11*S*,13*R*-epoxyurs-28-oic Acid Methyl Ester (4). Colorless needle-like crystals, $C_{33}H_{50}O_6$ (MW = 542.73), orthorhombic at 100 K, $a = 8.0611(8)$ Å, $b = 14.4044(14)$, $c = 24.906(3)$, $V = 2892.0(5)$ Å^3, space group $P2_12_12_1$, $Z = 4$, $d_{calcd} = 1.247$ mg/mm^3. Intensities of 29,741 reflections were measured on a SMART APEX2 CCD diffractometer (λ Mo-Kα = 0.71073 Å, graphite monochromator, ω-scanning in steps of 0.5°, $2\theta < 54°$) for a single crystal of size 0.20 × 0.18 × 0.14 mm^3. The initial dataset of measured intensities was processed using the programs SAINT [9] and SADABS [10]. The structure was solved by direct methods and refined by anisotropic full-matrix least-squares methods for nonhydrogen atoms over F^2_{hkl}. Hydrogen atoms were located at the geometrically calculated positions and refined using a rider model [$U_{iso}(H) = nU_{eq}(C)$, where $n = 1.5$ for methyl C atoms and 1.2 for other C atoms]. Refinement used 3555 independent reflections ($R_{int} = 0.1085$). The agreement factors over all independent reflections were $wR_2 = 0.0983$ [$R_1 = 0.0457$ over 2763 reflections with $I > 2\sigma(I)$]. All calculations were conducted on an IBM PC using the SHELXTL program set [11]. Atomic coordinates and thermal factors were deposited in the Cambridge Crystallographic Data Centre (CCDC) No. 773222; http://www.ccdc.cam.ac.uk/products/csd/request/.

3*β*-Hydroxy-12-oxo-11*S*,13*R*-epoxyurs-28-oic Acid Methyl Ester (6). Yield 0.37 g (73%), R_f 0.33, mp 210–212°C, $[\alpha]_D^{20}$ +62° (*c* 0.1, $CHCl_3$).

PMR spectrum (δ, ppm, J/Hz): 0.75, 0.79, 0.91, 0.93, 1.11, 1.13, 1.25 (21H, 7s, $7CH_3$), 1.29–2.41 (21H, m, CH_2, CH), 2.51 (1H, d, J = 10.6, H-18), 3.15–3.27 (1H, m, H-3), 3.78 (3H, s, OCH_3), 4.82 (1H, s, H-11).

^{13}C NMR spectrum (δ, ppm): 15.0, 18.3, 19.6, 19.9, 20.3, 21.2, 21.6, 24.4, 26.8, 26.9, 27.7, 28.1, 29.1, 35.7, 36.2, 36.6, 38.8, 38.9, 39.0, 42.5, 46.2, 46.7, 47.6, 51.7, 55.0, 60.6, 78.5 (C-3), 95.3 (C-11), 112.8 (C-13), 177.3 (C-28), 191.5 (C-12). $C_{31}H_{48}O_5$ (MW 500.0).

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