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CHEMOSELECTIVE OXIDATION OF OLEANOLIC ACID DERIVATIVES WITH OZONE

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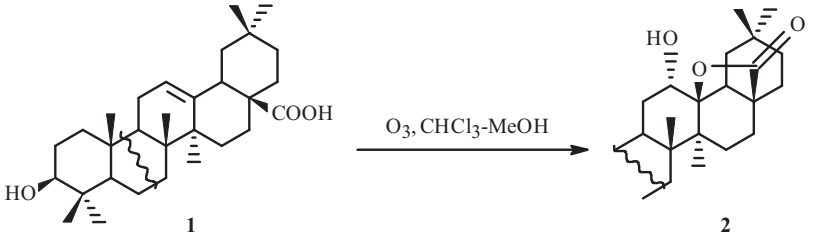
A method for preparing methyl esters of 12-oxoolean-28-oic and 3,12-dioxoolean-28-oic acids via ozonolysis of oleanolic acid methyl ester in CH_2Cl_2 at –60°C was proposed. It was found that oxidation of 2-cyano-3,4-seco-4(23)-oleanenoic acid was chemoselective depending on the amount of ozone used.

Keywords: oleanolic acid, chemoselectivity, oxidation, ozonolysis.

Oleanolic acid (**1**) belongs to a class of pentacyclic triterpenoids that is observed in >120 plants of various species (e.g., ginseng, apple and olive skin, calendulum and silphium flowerheads, white mistletoe, etc. [1]). It is responsible for several valuable medicinal properties of their extracts. Oleanolic acid and its derivatives exhibit a broad spectrum of pharmacological activity including hepatoprotective, anti-inflammatory, antimicrobial, antiviral, antitumor, etc. [2, 3]. Oleanolic acid is approved for use in China to treat liver diseases including hepatitis [4]. 2-Cyano-3,12-dioxoolea-1,9(11)-dien-28-oic acid (CDDO) inhibits proliferation of a large number of human tumor cells and is undergoing preclinical trials [5]. The nitrile of 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid showed very strong inhibition of NO production in murine macrophages (IC_{50} 1 pM) [6]. The oleanolic acid ozonolysis product 3β,12α,13β-trihydroxy-28$\beta\rightarrow$13-olide was observed to inhibit α-glucosidase, an enzyme controlling the glucose level in blood [7]. Thus, development of new synthetic approaches to transformations of oleanolic acid and its derivatives is a timely problem.

Ozonolysis of polycyclic derivatives with a sterically hindered double bond occurs most often without destroying it [8, 9]. According to the literature [7], ozonolysis of oleanolic acid (**1**) occurs with formation of a C(12)-C(13) epoxide that reacts with the C(28)OOH carboxylic acid to form lactone **2**.



1 **2**

We showed that oleanolic acid methyl ester **(3)** reacts otherwise with ozone. Both the composition and structure of the products depend considerably on the amount of ozone passed through the solution. Passing two equivalents of ozone through a solution of **3** at –60°C forms 3β-hydroxy-12-oxoolean-28-oic acid methyl ester (**4**) (Scheme 1). Increasing the amount of ozone to four molar equivalents per mole of **3** introduces a ketone at the site of the double bond and simultaneously oxidizes the C(3)-OH group to a ketone to form 3,12-dioxoolean-28-oic acid methyl ester (**5**) via oxidation by oxygen that is

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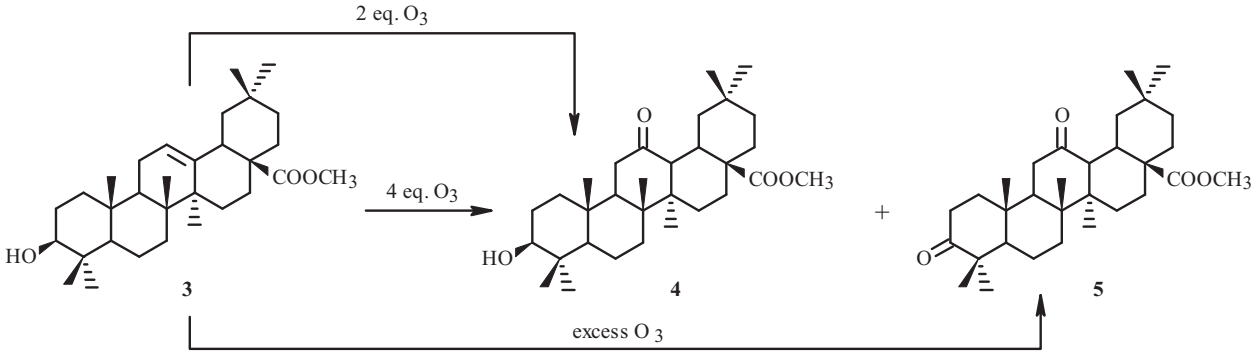
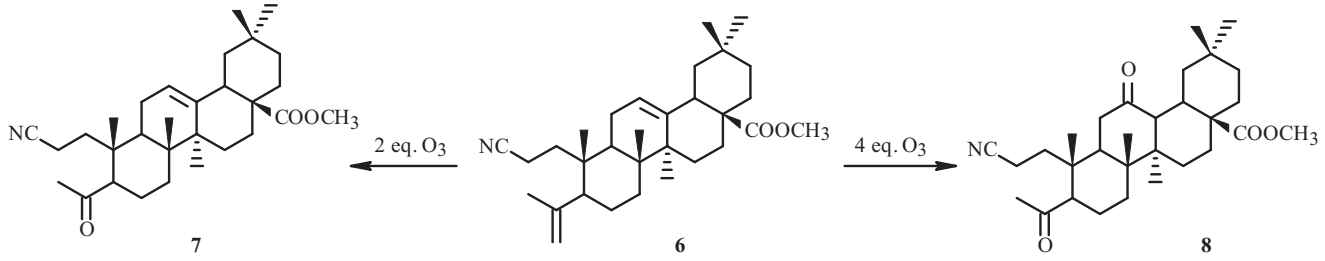
initiated by ozone. The oxidation is complete if the reaction is carried out in CH_2Cl_2 with an excess of ozone. Apparently the capability of C(3)-OH for facile oxidation depends on the type of triterpene framework. Analogous transformations were noted for glycyrrhetic acid derivatives and their mechanism was proposed [10]. Nevertheless, we have not observed oxidation by ozone of the alcohol groups of betulin derivatives.



Scheme 1

The formation of **5** was confirmed by NMR spectral data. Resonances of carbonyl C3 and C12 were found in the ^{13}C NMR spectrum at δ 210.8 and 216.5 ppm. The one-step method proposed by us for preparing 3,12-dioxo-acid methyl ester **5** in 84% yield via ozonolysis has a definite preparative advantage over the four-step method reported previously (50% yield) [11].

Reaction of 2-cyano-3,4-*seco*-4(23)-oleanenoic acid (**6**) with two equivalents of ozone at –60°C gave the 4-oxo derivative **7** in 75% yield (Scheme 2). Passing a four-fold excess of ozone through a solution of **6** in CH_2Cl_2 produced the 4,12-dioxo derivative **8** (71% yield). Resonances of the ketone were observed in the ^{13}C NMR spectrum at δ 209.4 and 210.7 ppm. Thus, chemoselective oxidation of 2-cyano-3,4-*seco*-4(23)-oleanenoic acid (**6**) was possible depending on the amount of ozone. Selective transformations of rings A and C could be carried out.



Scheme 2

EXPERIMENTAL

PMR and ^{13}C NMR spectra in $CDCl_3$ were recorded on a Bruker AM-300 spectrometer (300 and 75.5 MHz, respectively) with TMS internal standard. 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 for ozonation. TLC was performed on Sorbfil plates (ZAO Sorbpolimer, Russia) using $CHCl_3$:EtOAc (40:1). Compounds were detected using H_2SO_4 solution (10%) with subsequent heating at 100–120°C for 2–3 min. Methyl esters of oleanolic acid (**3**) and 2-cyano-3,4-*seco*-4(23)-oleanenoic acid (**6**) were prepared as before [12].

General Method for Ozonolysis of 3 and 6. A solution of **3** or **6** (1 mmol) in CH_2Cl_2 (50–70 mL) at –60°C was purged with ozone and left at room temperature for 3 h. Solvent was vacuum distilled (water aspirator). The solid was chromatographed over a column of Al_2O_3 with elution by $CHCl_3$.

3β-Hydroxy-12-oxoolean-28-oic acid methyl ester (4) was prepared from **3** using two equivalents of ozone. Yield 0.40 g (82%), R_f 0.27, mp 132–133°C, $[\alpha]_D^{20}$ –13° (*c* 0.4, CH_2Cl_2), $C_{31}H_{50}O_4$ (MW 486.73).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.69, 0.75, 0.80, 0.84, 0.87, 0.89 (21H, 6s, $7CH_3$), 1.15–2.20 (22H, m, CH_2, CH), 2.25 (2H, d, J = 4, H-11), 2.65 (1H, d, J = 14, H-13), 3.08 (1H, m, H-3), 3.58 (3H, s, OCH_3).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 14.9, 15.2, 15.8, 18.0, 20.3, 22.5, 22.9, 26.7, 27.3, 27.7, 30.3, 31.6, 31.7, 32.7, 33.1, 34.2, 35.9, 36.6, 36.6, 37.7, 38.2, 38.5, 40.9, 41.6, 47.0, 49.5, 51.5, 54.8, 78.1 (C3), 178.2, 211.5 (C12).

3,12-Dioxoolean-28-oic acid methyl ester (5) was prepared from **3** using four equivalents of ozone. Yields of **4** and **5** after chromatographic separation were 0.08 g (21%) and 0.35 g (73%), respectively. The yield of **5** after passing an excess of ozone through a solution of **3** was 0.41 g (84%), R_f 0.45, mp 117–118°C, $[\alpha]_D^{20}$ +5.1° (*c* 0.1, CH_2Cl_2), $C_{31}H_{48}O_4$ (MW 484.71).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.87, 0.92, 0.94, 0.95, 0.98, 1.01, 1.05 (21H, 7s, $7CH_3$), 1.10–2.00 (19H, m, CH_2, CH), 2.12 (2H, m, H-2), 2.62 (2H, d, J = 4, H-11), 2.75 (1H, d, J = 10.2, H-13), 3.65 (3H, s, OCH_3).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 12.0, 14.7, 15.7, 19.4, 20.3, 21.0, 22.6, 23.0, 26.1, 27.4, 30.4, 31.0, 31.8, 32.7, 33.2, 33.7, 34.3, 36.1, 36.5, 36.5, 38.4, 41.1, 41.9, 47.1, 47.2, 49.0, 51.7, 54.8, 178.2, 210.8 (C12), 216.5 (C3).

2-Cyano-3,4-*seco*-4-oxo-23-nor-olean-12-en-28-oic acid methyl ester (7) was prepared from **6** using two equivalents of ozone. Yield 0.35 g (75%), R_f 0.30, mp 128–131°C, $[\alpha]_D^{20}$ +13.5° (*c* 0.2, CH_2Cl_2), $C_{30}H_{45}NO_3$ (MW 467.69).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.73, 0.81, 0.84, 0.94, 1.06 (15H, 5s, $5CH_3$), 1.15–2.00 (19H, m, CH_2, CH), 2.05 (3H, s, CH_3C=O), 2.40–2.50 (2H, m, H-2), 2.52 (1H, d, J = 4, H-5), 2.79 (1H, dd, J = 3, 13, H-18), 3.54 (3H, s, OCH_3), 5.21 (1H, d, J = 3.4, H-12).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 11.6, 15.2, 16.8, 21.6, 22.8, 23.0, 23.5, 25.6, 27.6, 30.3, 30.4, 30.6, 32.2, 32.9, 33.7, 34.4, 37.8, 38.9, 39.0, 41.3, 42.1, 45.6, 46.6, 51.5, 56.4, 119.9, 121.4 (C12), 143.9 (C13), 178.0, 211.6 (C4).

2-Cyano-3,4-*seco*-4,12-dioxo-23-nor-olean-28-oic acid methyl ester (8) was prepared from **6** using four equivalents of ozone. Yield 0.34 g (71%), R_f 0.25, mp 201–203°C, $[\alpha]_D^{20}$ +2.6° (*c* 0.2, CH_2Cl_2), $C_{30}H_{45}NO_4$ (MW 483.69).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 0.87, 0.94, 0.95, 1.00 (15H, 4s, $5CH_3$), 1.00–1.90 (20H, m, CH_2, CH), 2.13 (3H, s, CH_3C=O), 2.40–2.50 (2H, m, H-2), 2.53 (1H, d, J = 4, H-5), 2.71 (1H, d, J = 9.7, H-13), 3.64 (3H, s, OCH_3).

^{13}C NMR spectrum ($CDCl_3$, δ, ppm): 11.4, 15.9, 18.3, 20.3, 21.6, 22.6, 23.0, 27.4, 29.6, 30.5, 30.5, 31.8, 32.7, 33.2, 33.8, 34.3, 36.1, 37.9, 38.6, 40.2, 40.8, 42.3, 47.1, 51.7, 51.8, 55.8 119.9, 178.4, 209.4 (C12), 210.7 (C4).

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