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# SYNTHESIS AND ANTIVIRAL ACTIVITY OF 2,3-*seco*-DERIVATIVES OF BETULONIC ACID

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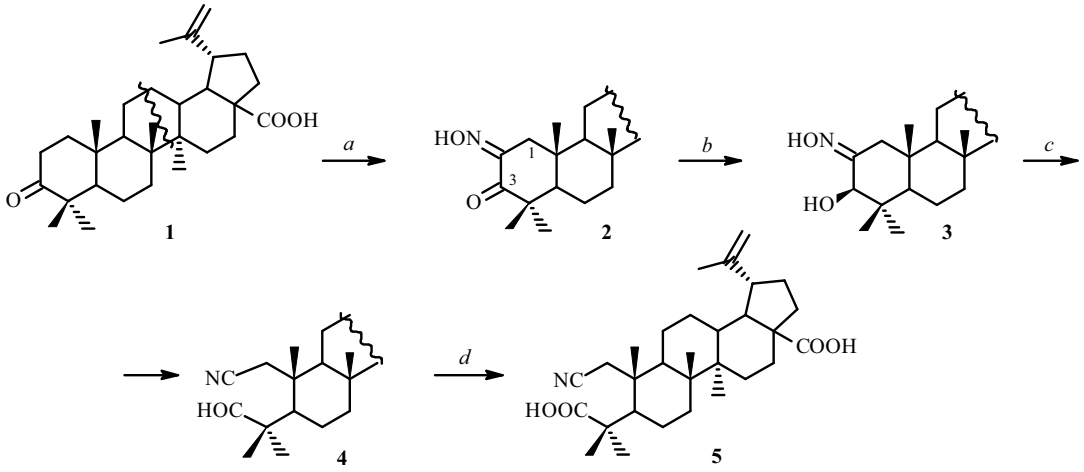
*A lupane 2,3-*seco*-aldehydoacid that was oxidized further to the 2,3-*seco*-diacid was synthesized by Beckmann fragmentation of the α-hydroxyoxime of betulonic acid. It was found that both 2,3-*seco*-derivatives were capable of suppresing reproduction of Herpes type 1 and flu A viruses ($EC_{50}$ from 1.9 to 21.3 μM).*

**Key words:** 2,3-*seco*-triterpenoids, betulonic acid, Beckmann fragmentation, antiviral activity.

Lupane triterpenoids of plant origin such as betulin and betulinic and betulonic acids exhibit a variety of biological activity and are interesting as starting materials for chemical and biocatalytic transformations [1, 2]. Semi-synthetic lupane derivatives include compounds with high antitumor and antiviral activity [1–3]. We have previously proposed a method for preparing cytotoxic 2,3-*seco*-derivatives of the lupane and oleanane type that included Beckmann cleavage of α-hydroxyoximes based on the methyl ester of betulonic acid and allobetulone [4, 5]. Herein we present an analogous approach that was used to synthesize lupane derivatives with an opened ring A and a free C-28 carboxylic acid.

The starting material for the synthesis of the 2,3-*seco*-derivatives was betulonic acid (**1**). During the process (Scheme 1) the corresponding hydroxyiminoketone **2** and hydroxyiminoalcohol **3** were prepared. The hydroxyimine substituent on C-2 in **2** and **3** was indicated by absorption bands in the IR spectra at 1644 $cm^{-1}$ and 3248–3400. PMR spectra of **2** and **3** recorded in DMSO-$d_6$ solutions had a resonance for the hydroxyimino proton at 12.17 ppm (**2**) or 10.59 (**3**).




**1** **2** **3** **4** **5**

*a*. *i*-$C_6H_{11}NO_2$/*t*-$C_4H_9OH$/*t*-$C_4H_9OK$; *b*. $NaBH_4/CH_3OH$; *c*. $TsCl/C_5H_5N$; *d*. $Cr_2O_3/H_2SO_4/(CH_3)_2CO$

Scheme 1

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TABLE 1. Antiviral Properties of Compounds **4** and **5**

| Compound No. | MTC (RD/CEF), μM | Herpes virus | | Flu virus | |
|---|---|---|---|---|---|
| | | $EC_{50}$ ($I_{95}$)* $EC_{90}$($I_{95}$), μM | ratio MTC/$EC_{50}$ MTC/$EC_{90}$ | $EC_{50}$ ($I_{95}$) $EC_{90}$($I_{95}$), μM | ratio MTC/$EC_{50}$ MTC/$EC_{90}$ |
| **4** | 26.7/26.7 | 1.9 (2911.5÷0.001)<br>17.9 (28269÷0.01) | 14.0<br>1.5 | 7.7 (8.8÷6.6)<br>12.8 (15.0÷11.1) | 3.5<br>2.1 |
| **5** | 206.6/103.3 | 21.3 (186.8÷2.5)<br>81.8 (716.9÷9.3) | 9.7<br>2.5 | 63.8 (72.1÷56.4)<br>101.0 (114.3÷89.3) | 1.6<br>1.0 |

*$I_{95}$ is the confidence interval.

The structure of 2,3-*seco*-aldehydonitrile **4** was confirmed by spectral data. The IR spectrum of **4** contained a band at 2248 $cm^{-1}$ that corresponded to nitrile vibrations. The PMR spectrum showed the C-3 aldehyde proton as a singlet with chemical shift 9.67 ppm. The $^{13}C$ NMR spectrum had characteristic resonances for nitrile (117.99 ppm) and aldehyde (206.10 ppm) C atoms. Oxidation of **4** produced diacid **5**, the structure of which was confirmed by IR and NMR spectroscopy. The nature of the C-1 methylene resonances in **4** and **5**, as described earlier for 2,3-*seco*-derivatives [4], depended on the nature of the C-3 substituent. For the aldehyde (**4**), the C-1 protons resonated as two doublets at 2.23 and 2.59 ppm; for the carboxylic acid (**5**), as a singlet at 2.55 ppm.

The antiviral activity of **4** and **5** was studied against flu A virus and Herpes Simplex virus type 1 (Table 1). It was found that **4** and **5** were capable of suppressing reproduction of Herpes virus type 1 ($EC_{50}$ = 1.9 and 21.3 μM; ratio MTC/$EC_{50}$ = 14.0 and 9.7, respectively). Also, **5** was slightly active against flu A virus (MTC/$EC_{50}$ = 1.6) whereas **4** exhibited moderate antiviral activity ($EC_{50}$ = 7.7 μM, MTC/$EC_{50}$ = 3.5).

## EXPERIMENTAL

PMR and $^{13}C$ NMR spectra were recorded in $CDCl_3$ or DMSO-$d_6$ solutions on a Varian Mercury+ spectrometer (USA) at operating frequency 300 or 75.5 MHz with HMDS internal standard. IR spectra were recorded in mineral oil on a Specord M80 spectrophotometer (Germany). Melting points were measured on a PTP instrument for determining melting points (Russia). Specific optical rotation was determined for $CHCl_3$ or $CHCl_3$:EtOH (4:1) solutions on a Perkin–Elmer Model 341 polarimeter (USA) at 589 nm. Elemental analyses (C, H, N) were perfomed using a Leco CHNS-9321P elemental analyzer (Netherlands) and agreed with those calculated.

TLC was carried out on Sorbfil plates (Russia) using solvents selected individually for each compound. Compounds were detected using phosphomolybdic acid in EtOH (20%) and heating at 100–120°C for 2–3 min. Column chromatography was performed over silica gel (Merck, 60–200 μm) with a compound:sorbent ratio of about 1:50. The eluent was selected individually for each compound. Solvents were purified and dried by literature methods [6]. Betulonic acid [7] was prepared by oxidation of betulin using Jones reagent [8].

**2-Hydroxyimino-3-oxolup-20(29)-en-28-oic Acid (2).** The literature synthesis was used [4]. Purification by column chromatography (eluent $CHCl_3$:EtOAc, 10:1) afforded **2** (2.1 g, 70%), $R_f$ 0.42 ($CHCl_3$:MeOH, 20:1), mp 169–171°C (EtOH), $[\alpha]_D^{21}$ +95.4° (*c* 0.4, $CHCl_3$), $C_{30}H_{47}NO_4$.

IR spectrum (ν, $cm^{-1}$): 3400 (OH), 1710 (COOH), 1698 (C=O), 1644 (C=N).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.82, 0.96, 0.99, 1.10, 1.15 (5 × 3H, 5s, $5CH_3$), 1.69 (3H, s, $CH_3$-30), 2.09, 3.00 (2H, 2d, $J_{AB}$ = 18.8, 2H-1, AB-system), 3.01 (1H, m, H-19), 4.61, 4.74 (2H, 2s, 2H-29). PMR spectrum (300 MHz, DMSO-$d_6$, δ, ppm): 12.17 (1H, s, NOH).

$^{13}C$ NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.53, 15.30, 16.91, 19.32, 20.21, 21.64, 25.49, 29.03, 29.67, 30.49, 32.04, 32.84, 35.63, 37.03, 38.37, 38.83, 40.40, 41.81, 42.49, 45.86, 46.80, 48.31, 49.09, 52.31, 56.30, 109.84 (C-29), 150.27 (C-20), 153.47 (C-2), 181.76 (C-28), 203.21 (C-3).

**3$\beta$-Hydroxy-2-hydroxyiminolup-20(29)-en-28-oic Acid (3).** The literature synthesis was used [4]. Purification using column chromatography (eluent $CHCl_3$:EtOAc, 10:1) afforded **3** (1.16 g, 64%), $R_f$ 0.3 ($CHCl_3$:MeOH, 20:1), mp 240–242°C ($CHCl_3$:EtOAc), $[\alpha]_D^{21}$ +23.9° (*c* 0.4, $CHCl_3$:EtOH, 4:1), $C_{30}H_{47}NO_4$.

IR spectrum (ν, $cm^{-1}$): 3380, br. 3248 (OH), 1690 (COOH), 1644 (C=N).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.70, 0.74, 0.90, 0.98, 1.09 (5 × 3H, 5s, $5CH_3$), 1.68 (3H, s, $CH_3$-30), 2.20, 3.40 (2H, 2d, $J_{AB}$ = 12.9, 2H-1, AB-system), 3.00 (1H, m, H-19), 3.79 (1H, s, H-3), 4.59, 4.73 (2H, 2s, 2H-29).

PMR spectrum (300 MHz, DMSO-$d_6$, δ, ppm): 10.59 (1H, s, NOH).

$^{13}C$ NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.69, 15.57, 15.61, 16.55, 18.22, 19.35, 21.23, 25.36, 28.35, 29.70, 30.56, 32.12, 34.00, 36.93, 38.01, 38.21, 40.99, 41.18, 42.45, 42.94, 46.90, 49.46, 49.87, 54.92, 56.55, 78.53 (C-3), 109.68 (C-29), 150.43 (C-20), 158.43 (C-2), 176.71 (C-28).

**2,3-*seco*-1-Cyanolup-20(29)-en-3-al-28-oic Acid (4).** The literature synthesis was used [4]. Purification using column chromatography (eluent hexane:EtOAc, 5:1) afforded **4** (0.82 g, 57%), $R_f$ 0.46 (hexane:EtOAc, 7:3), mp 169–171°C (hexane:EtOAc), $[\alpha]_D^{21}$ +30.6° (*c* 0.5, $CHCl_3$), $C_{30}H_{45}NO_3$. IR spectrum (ν, $cm^{-1}$): 2248 (C≡N), 1722 (COOH), 1688 (CHO).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.89, 0.94, 1.04, 1.08, 1.14 (5 × 3H, 5s, $5CH_3$), 1.68 (3H, s, $CH_3$-30), 2.23, 2.59 (2H, 2d, $J_{AB}$ = 18.3, 2H-1, AB-system), 3.00 (1H, td, J = 10.5, 5.7, H-19), 4.61, 4.72 (2H, 2s, 2H-29), 9.67 (1H, s, H-3).

$^{13}C$ NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.59, 15.72, 18.74, 19.17, 19.50, 20.08, 21.78, 22.78, 23.50, 25.37, 29.63, 30.40, 31.87, 33.19, 36.93, 38.37, 40.57, 42.21, 42.83, 44.52, 46.83, 48.93, 49.05, 50.75, 56.33, 110.00 (C-29), 117.99 (C-2), 150.00 (C-20), 182.08 (C-28), 206.10 (C-3).

**2,3-*seco*-1-Cyanolup-20(29)-en-3,28-dioic Acid (5).** The literature synthesis was used [4]. Purification using column chromatography (eluent $CHCl_3$:EtOAc, 10:1) afforded **5** (0.43 g, 62%), $R_f$ 0.2 ($CHCl_3$:EtOAc, 10:1), mp 72–74°C (hexane:EtOAc), $[\alpha]_D^{21}$ +18.1° (*c* 0.2, $CHCl_3$), $C_{30}H_{45}NO_4$.

IR spectrum (ν, $cm^{-1}$): 2244 (C≡N), 1710, 1690 (COOH), .

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.92, 0.98, 1.03, 1.27, 1.35 (5 × 3H, 5s, $5CH_3$), 1.68 (3H, s, $CH_3$-30), 2.55 (2H, s, 2H-1), 3.00 (1H, m, H-19), 4.61, 4.72 (2H, 2s, $CH_2$-29).

$^{13}C$ NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.59, 15.84, 18.35, 19.22, 21.01, 21.68, 25.18, 25.31, 26.95, 29.15, 29.73, 30.40, 31.93, 33.24, 36.80, 38.32, 40.51, 42.33, 42.73, 44.89, 46.45, 46.87, 48.95, 50.94, 56.46, 109.94 (C-29), 118.29 (C-2), 150.06 (C-20), 182.84 (C-28), 184.49 (C-3).

**Antiviral Properties of 4 and 5.** Antiviral activity was determined using cell cultures with Herpes Simplex virus type 1 (HSV, strain 1C) and flu A/FPV/Rostock/34 (H7N1). Compounds were dissolved beforehand in EtOH (10%, stock solution concentration 5 mg/mL) and then in medium to the required concentrations. Antiviral activity was studied by estimating the cytopathic effect on a transfer culture of human rhabdomyosarcoma (RD) cells with HSV-1 and by the reduction of plaque in cell culture of primary chicken embryo fibroblasts (CEF) with FPV [9]. The criterion for antiviral activity was a decrease of virus titre in the presence of the tested compounds compared with a control. The concentrations of compounds suppressing virus multiplication by 50 and 90% (mean-effective concentration, $EC_{50}$, and concentration for 90% reduction of virus titre, $EC_{90}$) were determined using the Fung computer program [10] based on probit analysis and weighted linear regression. The ratios of maximum tolerated concentration (MTC) of the compounds to $EC_{50}$ and $EC_{90}$ were also calculated. MTC of the compounds were determined from the maximum concentration of a compound that did not affect the morphology of uninfected cell culture after incubation for 72 h.


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