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SYNTHESIS OF 2,3-*seco*-TRITERPENE HYDRAZONOHYDRAZIDES OF THE LUPANE AND 19β,28-EPOXY-18α-OLEANANE TYPES

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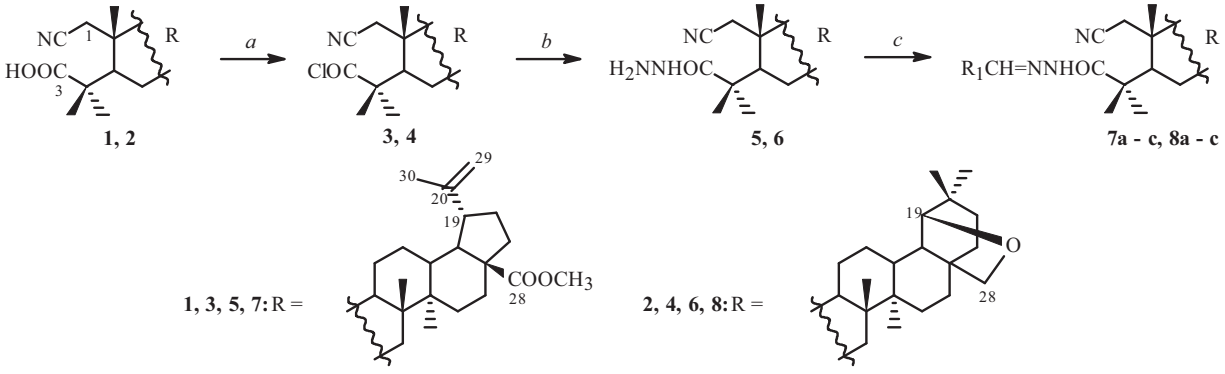
*Hydrazonohydrazides were synthesized by acid-catalyzed condensation of 2,3-*seco*-triterpene 1-cyano-3-hydrazides with aldehydes. The antiviral action of the hydrazonohydrazides was studied against vesicular stomatitis Indiana virus.*

Keywords: A-*seco*-triterpenoids, betulin, betulinic acid, allobetulone, hydrazides, hydrazones, vesicular stomatitis virus.

Betulin is a polycyclic triterpenoid that is readily available and exhibits a broad spectrum of biological activity. Its derivatives betulonic acid and allobetulone are promising starting materials for chemical transformations [1, 2]. The transformation pathways of these triterpenoids into *N*-containing biologically active derivatives include the formation of C-3 and C-28 amides or imines in the triterpene backbone. It was shown that additional modification of the triterpenoid carbon framework by cleavage of the C2–C3 bond of ring A helps in particular to increase the antiviral activity of the basic triterpenoids [3, 4]. We recently synthesized 2,3-*seco*-triterpene hydrazones with high levels of therapeutic and preventative activity against enveloped vesicular stomatitis Indiana virus (VSIV) by the reaction of lupane and 19β,28-epoxy-18α-oleanane 2,3-*seco*-aldehydonitriles with substituted hydrazines [5–7]. The search for new anti-VSIV derivatives and reports of the antiviral properties of triterpene hydrazides of betulinic and betulonic acids against enveloped Group A and Herpes Simplex Type I viruses [8] prompted us to prepare 2,3-*seco*-hydrazonohydrazides of the lupane and 19β,28-epoxy-18α-oleanane types.

A-*seco*-Triterpene hydrazonohydrazides **7a**–**c** and **8a**–**c** were prepared from C3-carboxy derivatives **1** and **2** [9] through the corresponding acid chlorides **3** and **4** that were used directly without purification in the hydrazinolysis reaction with subsequent acid-catalyzed condensation of the resulting 1-cyano-3-hydrazides **5** and **6** with aldehydes.



7a, 8a: $R_1 = (CH_3)_2CH$-; **7b, 8b:** $R_1 = (CH_3)_3C$-; **7c, 8c:** $R_1 = C_6H_5$-
a. $(COCl)_2/CH_2Cl_2$; *b*. $NH_2NH_2 \cdot H_2O/CH_2Cl_2$; *c*. $R_1CHO/C_2H_5OH/CH_3COOH$

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The formation of substituted hydrazides **7a**–**c** and **8a**–**c** was confirmed by IR and NMR spectroscopy. IR spectra of **7a**–**c** and **8a**–**c** showed absorption bands in the range 1652–1658 (C=N), 2237–2241 (C≡N), and 3230–3250 (NH) cm^{-1}. PMR spectra of **7a**–**c** and **8a**–**c** contained resonances for protons characteristic of the triterpene moiety and for imine and amide protons at 7.27–8.12 and 8.36–8.86 ppm, respectively. ^{13}C NMR spectra had a resonance characteristic of C-3 (174.12–175.07 ppm).

The synthesized compounds (**7a**–**c** and **8a**–**c**) did not exhibit inhibition against VSIV upon addition to a cell suspension before introducing the virus [6]. This indicated that the 2,3-*seco*-triterpene hydrazonohydrazides did not exhibit preventative VSIV-inhibiting activity.

EXPERIMENTAL

IR spectra (ν, cm^{-1}) were taken in mineral oil mulls on an IFS 66/S IR-Fourier spectrometer (Bruker). PMR and ^{13}C NMR spectra (δ, ppm, J/Hz) were recorded in $CDCl_3$ solution on a Mercury+ spectrometer (Varian, USA) at operating frequency 300 or 75.5 MHz. Melting points were determined on an OptiMelt MPA100 instrument (USA). TLC was performed on Sorbfil plates (Russia) using hexane:EtOAc (7:3). Compounds were detected by treatment with H_2SO_4 (5%) with subsequent heating of the plate at 95–100°C. Column chromatography used Merck silica gel (60–200 μm) with an ~1:20 compound:sorbent ratio and elution by hexane:EtOAc (7:1).

General Method for Preparing *N′*-Substituted Hydrazides (7a–c, 8a–c). A solution of acid (**1** or **2**, 0.54 mmol) in anhydrous CH_2Cl_2 (12 mL) under Ar was treated with oxalylchloride (1.1 mmol) and stirred at room temperature for 6 h. The solvent was distilled in vacuo (water aspirator) to dryness on a water bath (30°C). The solid was treated with anhydrous CH_2Cl_2 (10 mL). The solvent was evaporated. The procedure was repeated three times. The resulting acid chloride in CH_2Cl_2 was stirred, cooled to 0 ± 5°C, and treated dropwise with hydrazine hydrate (36 mmol, 98%). The solvent was evaporated. The hydrazide-containing solid was dissolved in EtOH (20 mL), treated with aldehyde (0.54 mmol) and glacial acetic acid (2 drops), and left at room temperature for 7 h. The reaction was monitored by TLC. The solvent was evaporated. The solid was purified by column chromatography.

28-Methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic Acid *N′*-(2-Methylpropylidene)hydrazide (7a). Yield 0.07 g (23%), R_f 0.2 (hexane:EtOAc, 7:3), mp 113.5°C (hexane:EtOAc, 5:1), $[\alpha]_D^{20}$ –6.4° (*c* 0.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1653 (C=N), 1724 ($COOCH_3$), 2241 (C≡N), 3243 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.93, 0.98, 1.30 (9H, 3s, $3CH_3$), 1.00 (6H, s, $2CH_3$), 1.11 and 1.23 [6H, 2d, J = 6.9, $CH(C\underline{H}_3)_2$], 1.66 (3H, s, 3H-30), 2.41 and 2.50 (2H, 2d, J_{AB} = 18.7, AB-system, 2H-1), 2.67 [1H, sept, J = 6.9, $C\underline{H}(CH_3)_2$], 2.91–3.02 (1H, m, H-19), 3.65 (3H, s, $COOC\underline{H}_3$), 4.59 and 4.71 (2H, 2s, 2H-29), 7.27 (1H, d, J = 7.8, CH=N), 8.46 (1H, s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.55, 15.68, 17.56, 19.17, 19.83 (2C), 20.12, 21.18, 21.75, 25.45, 28.23, 29.24, 29.56, 30.40, 31.53, 31.87, 33.24, 36.80, 38.18, 40.57, 42.59, 42.79, 44.76, 44.86, 46.82, 49.14, 51.27, 52.58, 56.45, 109.84 (C-29), 119.35 (C-2), 150.14 (C-20), 157.04, 174.12 (C-3), 176.54 (C-28).

28-Methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic Acid *N′*-(2,2-Dimethylpropylidene)hydrazide (7b). Yield 0.02 g (7%), R_f 0.3 (hexane:EtOAc, 7:3), mp 109.5°C (hexane:EtOAc, 5:1), $[\alpha]_D^{20}$ 0° (*c* 0.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1653 (C=N), 1723 ($COOCH_3$), 2241 (C≡N), 3247 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.93, 0.98, 1.01, 1.22, 1.30 (15H, 5s, $5CH_3$), 1.14 (9H, s, $3CH_3$), 1.66 (3H, s, 3H-30), 2.42 and 2.50 (2H, J_{AB} = 18.8, AB-system, 2H-1), 2.93–3.03 (1H, m, CH-19), 3.66 (3H, s, $COOCH_3$), 4.59 and 4.71 (2H, 2s, 2H-29), 7.34 (1H, s, CH=N), 8.36 (1H, s, NH).

28-Methoxy-28-oxo-1-cyano-2,3-*seco*-lup-20(29)-en-3-oic Acid *N′*-(Benzylidene)hydrazide (7c). Yield 0.1 g (33%), R_f 0.3 ($CHCl_3$:EtOAc, 10:1), mp 119°C (hexane:EtOAc, 7:1), $[\alpha]_D^{20}$ 0° (*c* 0.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1658 (C=N), 1724 ($COOCH_3$), 2239 (C≡N), 3250 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.94, 1.28, 1.37 (9H, 3s, $3CH_3$), 1.01 (6H, s, $2CH_3$), 1.66 (3H, s, 3H-30), 2.44 and 2.53 (2H, 2d, J_{AB} = 18.6, AB-system, 2H-1), 2.96 (1H, td, J = 10.2, 6.0, H-19), 3.66 (3H, s, $COOC\underline{H}_3$), 4.59 and 4.71 (2H, 2s, 2H-29), 7.37 (3H, t, J = 2.9, arom.), 7.73 (2H, t, J = 3.5, arom.), 8.11 (1H, s, CH=N), 8.86 (1H, s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 14.59, 15.72, 17.60, 19.21, 20.33, 21.70, 21.82, 22.91, 25.48, 28.52, 28.94, 29.59, 30.44, 31.88, 33.23, 36.81, 38.19, 40.60, 42.66, 42.80, 44.77, 45.31, 46.84, 49.16, 51.29, 52.42, 56.48, 109.86 (C-29), 119.40 (C-2), 127.81 (2C), 128.57 (2C), 130.37, 147.97, 150.17 (C-20), 175.07 (C-3), 176.55 (C-28).

1-Cyano-19β,28-epoxy-2,3-*seco*-18α-olean-3-oic Acid *N*′-(2-Methylpropylidene)hydrazide (8a). Yield 0.1 g (40%), R_f 0.3 ($CHCl_3$:MeOH, 20:1), mp 143.2°C (hexane:EtOAc, 5:1), $[\alpha]_D^{20}$ –17.2° (*c* 0.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1653 (C=N), 2240 (C≡N), 3238 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.78, 0.92, 0.96, 0.99, 1.01, 1.23, 1.32 (21H, 7s, $7CH_3$), 1.11 and 1.12 [6H, 2d, J = 6.6, $CH(C\underline{H}_3)_2$], 2.45 and 2.53 (2H, 2d, J_{AB} = 18.6, AB-system, 2H-1), 2.66 [1H, sept, J = 6.6, $C\underline{H}(CH_3)_2$], 3.44 and 3.75 (2H, 2d, J_{AB} = 7.7, AB-system, 2H-28), 3.51 (1H, s, H-19), 7.29 (1H, d, J = 6.6, CH=N), 8.45 (1H, s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 13.41, 15.59, 17.82, 19.83, 19.85, 20.08, 21.16, 21.83, 24.48, 26.10, 26.31, 26.37, 28.37, 28.72, 29.30, 31.53, 32.64, 32.77, 34.24, 36.22, 36.61, 40.48, 41.12, 41.42, 42.66, 44.85, 45.23, 46.54, 52.71, 71.21 (C-28), 87.78 (C-19), 119.31 (C-2), 157.02, 174.86 (C-3).

1-Cyano-19β,28-epoxy-2,3-*seco*-18α-olean-3-oic Acid *N*′-(2,2-Dimethylpropylidene)hydrazide (8b). Yield 0.1 g (43%), R_f 0.3 (hexane:EtOAc, 7:3), mp 112.9°C (hexane:EtOAc, 7:1), $[\alpha]_D^{20}$ –24.0° (*c* 0.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1652 (C=N), 2241 (C≡N), 3230 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.78, 0.92, 0.96, 0.99, 1.01, 1.23, 1.32 (21H, 7s, $7CH_3$), 1.15 (9H, s, $3CH_3$), 2.45 and 2.53 (2H, 2d, J_{AB} = 17.4, AB-system, 2H-1), 3.44 and 3.75 (2H, 2d, J_{AB} = 7.7, AB-system, 2H-28), 3.52 (1H, s, CH-19), 7.36 (1H, s, CH=N), 8.42 (1H, s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 13.42, 15.61, 17.85, 20.09, 21.12, 21.84, 24.48, 26.12, 26.32, 26.39, 27.28 (2C), 28.31, 28.74, 29.32, 29.67, 32.65, 32.79, 34.25, 34.99, 36.24, 36.61, 40.49, 41.14, 41.44, 42.67, 44.89, 45.24, 46.55, 52.72, 71.22 (C-28), 87.80 (C-19), 119.33 (C-2), 159.60, 174.78 (C-3).

1-Cyano-19β,28-epoxy-2,3-*seco*-18α-olean-3-oic Acid *N*′-(Benzylidene)hydrazide (8c). Yield 0.2 g (34%), R_f 0.3 ($CHCl_3$:EtOAc, 10:1), mp 203.4°C (hexane:EtOAc, 7:1), $[\alpha]_D^{20}$ 0° (*c* 0.5, $CHCl_3$). IR spectrum (ν, cm^{-1}): 1655 (C=N), 2237 (C≡N), 3249 (NH).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.78, 0.92, 0.97, 1.00, 1.03, 1.29, 1.39 (21H, 7s, $7CH_3$), 2.47 and 2.57 (2H, 2d, J_{AB} = 18.6, AB-system, 2H-1), 3.44 and 3.75 (2H, 2d, J_{AB} = 8.0, AB-system, 2H-28), 3.51 (1H, s, H-19), 7.37 (3H, t, J = 3.0, arom.), 7.73 (2H, t, J = 3.8, arom.), 8.12 (1H, s, CH=N), 8.86 (1H, s, NH).

^{13}C NMR spectrum (75.5 MHz, $CDCl_3$, δ, ppm): 13.46, 15.62, 17.86, 20.27, 21.60, 21.87, 24.50, 26.12, 26.33, 26.40, 28.74, 29.02, 32.65, 32.78, 34.26, 36.24 (2C), 36.61, 40.51, 41.13, 41.44 (2C), 42.72, 45.26, 46.56, 52.58, 71.22 (C-28), 87.80 (C-19), 119.39 (C-2), 127.81 (2C), 128.57 (2C), 130.38, 133.55, 147.99, 175.04 (C-3).

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