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# ALLYLIC OXIDATION OF 19β,28-EPOXY-A-*NEO*-5β-METHYL-25-NOR-18α-OLEAN-9-ENE

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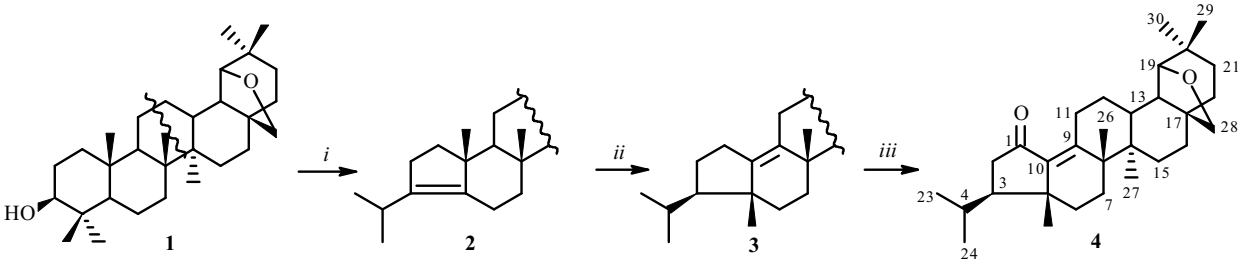
*Allylic oxidation of 19β,28-epoxy-A-*neo*-5β-methyl-25-nor-18α-olean-9-ene by ozone produced 19β,28-epoxy-A-*neo*-5β-methyl-25-nor-18α-olean-9-en-1-one.*

**Keywords:** betulin, allobetulin, ozonolysis, oxidation, antitumor activity.

The oleanane triterpenoid allobetulin (**1**) is an available betulin derivative [1]. Allobetulin is known to exhibit moderate inhibiting activity against type B flu virus [2] whereas 28-oxoallobetulone is an effective inhibitor of type A flu virus replication [3]. Derivatives of allobetulin have manifested antifeedant activity against larvae of *Heliothis zea* [4] and *Leptinotarsa decemlineata* [5]. The antitumor activity of allobetulin derivatives is practically unstudied [6].

Herein we report the synthesis of a new allobetulin derivative that is of interest for modifications on rings A and B. Allobetulin (**1**) was dehydrated to 19*β*,28-epoxy-A-*neo*-18*α*-olean-3(5)-ene (**2**), which was then isomerized into 19*β*,28-epoxy-A-*neo*-5*β*-methyl-25-nor-18*α*-olean-9-ene (**3**) in 75% yield by the action of acetylchloride upon refluxing in $CH_3CN$. Reaction with ozone caused allyl oxidation of **3** to form 19*β*,28-epoxy-A-*neo*-5*β*-methyl-25-nor-18*α*-olean-9-en-1-one (**4**), the structure of which was confirmed by NMR spectroscopy. Thus, the $^{13}C$ NMR spectra exhibited 30 resonances that were assigned using DEPT spectra [7] to quaternary, methine, methylene, and methyl groups. Resonances of 8 quaternary C atoms, 5 methines, 10 methylenes, and 7 methyls were found. Resonances of carbonyl C-1 ($\delta_C$ 208.13 ppm), methine C-19 (87.93), and methylene C-28 (71.21) C atoms, which were oxygenated, had characteristic chemical shifts.




*i*. $PCl_5$, $CHCl_3$, 20°C; *ii*. AcCl, $CH_3CN$, 82°C; *iii.* $O_3$, $CH_2Cl_2$, –40°C

COSY and HETCOR spectra were analyzed in order to establish the positions of resonances for protonated C atoms and their protons in **4**. According to COSY spectra, the methylene protons in the *α*-position relative to the carbonyl ($H_{ax}$-2, $\delta_H$ 2.06 ppm and $H_{eq}$-2, $\delta_H$ 2.41 ppm) formed an ABX-system with the H-3 proton ($\delta_H$ 1.39). The spin–spin coupling constant was 12.3 Hz, indicating a *trans*-diaxial coupling and an axial position for H-3. Therefore, the isopropyl group occupied the equatorial position. Correlation peaks in the HETCOR spectrum $H_{ax}$-2($H_{eq}$-2)/C-2 and H-3/C-3 and cross peaks of H-3 with the methine proton of the isopropyl group H-4 ($\delta_H$ 1.69) in the COSY spectrum enabled the structure and substitution stereochemistry of five-membered ring A to be found. The chemical shifts of quaternary C atoms on the C-9 ($\delta_C$ 156.10 ppm) and C-10 ($\delta_C$ 139.76) double bond corresponded with its position in ring B [8].

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TABLE 1. Antitumor Activity *in vitro* of **3** at Concentration $10^{-5}$ M against 60 Lines of Human Cancer Cells

| Cell line | Percent of growth | Cell line | Percent of growth |
|---|---|---|---|
| Lung cancer | | Leukemia | |
| A549/ATCC | 88.82 | CCRF-CEM | 104.55 |
| EKVX | 107.53 | HL-60(TB) | 105.21 |
| HOP-62 | 119.79 | K-562 | 106.61 |
| NCI-H226 | 102.49 | MOLT-4 | 94.22 |
| NCI-H322M | 116.36 | RPMI-8226 | 106.72 |
| NCI-H23 | 102.55 | SR | – |
| NCI-H460 | 107.00 | Kidney cancer | |
| NCI-H522 | 100.45 | 786-0 | 105.25 |
| HOP-92 | 92.75 | A498 | 110.63 |
| Colon cancer | | ACHN | 107.87 |
| COLO 205 | 118.66 | CAKI-1 | 90.91 |
| HCC-2998 | 106.96 | RXF 393 | 112.90 |
| HCT-116 | 114.16 | SN12C | 99.48 |
| HCT-15 | 111.78 | TK-10 | 101.91 |
| HT29 | 100.61 | UO-31 | 106.19 |
| KM12 | 102.23 | Melanoma | |
| SW-620 | 105.86 | LOX IMVI | 89.06 |
| Breast cancer | | MALME-3M | 92.22 |
| MCF7 | 94.96 | M14 | 96.02 |
| MDA-MB-231/ATCC | 99.06 | MDA-MB-435 | 95.77 |
| HS 578T | – | SK-MEL-2 | 99.07 |
| BT-549 | – | SK-MEL-28 | 106.01 |
| T-47D | 115.86 | SK-MEL-5 | 103.27 |
| MDA-MB-468 | 105.73 | UACC-257 | 97.17 |
| Ovary cancer | | UACC-62 | 96.90 |
| IGROV1 | 111.50 | Prostate cancer | |
| OVCAR-3 | 116.90 | PC-3 | 86.16 |
| OVCAR-4 | 109.24 | DU-145 | 101.55 |
| OVCAR-5 | 102.38 | CNS cancer | |
| OVCAR-8 | 101.38 | SF-268 | 95.04 |
| NCI/ADR-RES | 107.78 | SF-295 | 104.80 |
| SK-OV-3 | 111.52 | SF-539 | 105.46 |
| | | SNB-19 | 101.53 |
| | | SNB-75 | – |
| | | U251 | 100.83 |

The *in vitro* antitumor activity of **3** was studied. The cytotoxicity was determined against 60 cell lines of nine different human tumors (lung, colon, CNS, ovary, kidney, prostate, breast, leukemia, and melanoma) at the National Cancer Institute (USA) using the published method [9–12]. Preliminary testing of the activity was carried out in cell culture medium at a final concentration of $10^{-5}$ M for 48 h, after which the growth of the treated cells was evaluated against untreated control cells. Table 1 presents results for the growth of treated cells against control cells (negative values correspond to cell death). According to Table 1, **3** did not exhibit antitumor activity.

## EXPERIMENTAL

PMR and $^{13}C$ NMR spectra were recorded in $CDCl_3$ on Bruker AM-300 and AMX-300 pulsed spectrometers at operating frequency 300 ($^1H$) and 75 ($^{13}C$) MHz with 5-mm QNP probes at constant sample temperature 298 K. Chemical shifts in $^{13}C$ NMR and PMR spectra are given in ppm vs. the resonance of TMS internal standard. The delay between pulse sequences was set to achieve full relaxation. Additional zeroes and multiplication of the Fourier-transform of the spectrum by an exponential function (lb = 0.1 Hz for $^1H$ and 1 Hz for $^{13}C$) were applied in order to increase the digital resolution. $^{13}C$ NMR spectra with proton-decoupling (WALTZ-16) were recorded with spectral window 18 kHz, 64k points, exciting pulse (30°)

length 2.2 μs, relaxation delay 1.5 s, and 1500-5000 scans. $^{13}C$ NMR spectra were edited based on DEPT-90 and DEPT-135 experiments [7]. The pulse length and regenerating transverse magnetization were set at 6 μs (DEPT-90) and 9 μs (DEPT-135); the refocusing delay 1/2J = 3.6 ms. A total of 64k points were accumulated during 1024 scans with spectral window 18 kHz and exponential line broadening 1 Hz.

Two-dimensional $^1H$–$^1H$ COSY spectra [13, 14] were recorded using standard modes of multi-pulse sequences in the instrument software. The matrix size was 2k for 1024 experiments with spectral window 3 kHz. A sinusoidal bell-shaped weighting function for F1 and F2 projections (ssb = 0) was used for the spectrum magnitude. HETCOR heteronuclear correlation spectra [15] (matrix size 1k for 256 experiments, 18 kHz for F2-projection and 3 kHz for F1) had a refocusing delay that was optimized for observation of $J_{CH}$ = 145 Hz.

Melting points were determined on a Boetius microstage. Optical rotation was measured on a Perkin–Elmer 241 MS polarimeter (Germany) in a 1-dm tube. 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. Allobetulin (**1**) was obtained as before [16].

**19β,28-Epoxy-A-*neo*-18α-olean-3(5)-ene (2).** A solution of **1** (0.84 g, 2 mmol) in anhydrous $CHCl_3$ (40 mL) was treated with $PCl_5$ (1.04 g, 5 mmol) and stirred at room temperature for 2 h (TLC monitoring). The organic layer was separated, washed with $H_2O$, dried over $CaCl_2$, and evaporated in vacuo. The solid was crystallized from EtOH, $R_f$ 0.80, yield 0.67 g (82%), mp 211–213°C, $[\alpha]_D^{20}$ +58° (*c* 1.00, $CHCl_3$) {lit. [17] mp 216-218°C, $[\alpha]_D^{22}$ +48.2° (*c* 1.31, $CHCl_3$)}, $C_{30}H_{48}O$ (MW 424.7).

PMR spectrum (δ, ppm, J/Hz): 0.79, 0.85, 0.86, 1.00, 1.18 (15H, 5s, $5CH_3$), 0.93 (3H, d, J = 6.8, 24-$CH_3$), 0.97 (3H, d, J = 6.8, 23-$CH_3$), 1.00-2.20 (23H, m, CH, $CH_2$), 2.65 (1H, septet, J = 6.8, H-4), 3.46 and 3.81 (2H, both d, J = 7.8, H-28), 3.55 (1H, s, H-19).

$^{13}C$ NMR spectrum (δ, ppm): 13.5, 15.1, 15.7, 19.4, 22.8, 23.3, 23.5, 24.6, 26.3, 26.4, 26.7, 28.3, 28.9, 32.8, 33.7, 34.3, 36.3, 36.9, 39.8, 40.4, 40.7, 41.6, 44.5, 42.2, 46.8, 50.1, 71.3 (C-28), 87.9 (C-19), 136.1 (C-5), 139.8 (C-3).

**19β,28-Epoxy-A-*neo*-5β-methyl-25-nor-18α-olean-9-ene (3).** A solution of **2** (0.43 g, 1 mmol) in anhydrous $CH_3CN$ (15 mL) was treated dropwise with freshly distilled AcCl (1 mL), refluxed for 5 h, and treated with cold water (100 mL). The resulting precipitate was filtered off, washed with $H_2O$ until neutral, dried in air, and crystallized from hexane. Yield 0.32 g (75%), mp 148–150°C, $[\alpha]_D$ +45° (*c* 1, $CHCl_3$) (lit. [18] mp 147–149°C), $C_{30}H_{48}O$ (MW 424.7).

PMR spectrum (δ, ppm, J/Hz): 0.79, 0.81, 0.82, 0.94, 1.12 (15H, 5s, $5CH_3$), 0.88 (3H, d, J = 6.9, 24-$CH_3$), 0.95 (3H, d, J = 6.9, 23-$CH_3$), 1.13–2.31 (24H, m, CH, $CH_2$), 3.45 and 3.80 (2H, both d, J = 7.5, H-28), 3.55 (1H, s, H-19).

$^{13}C$ NMR spectrum (δ, ppm): 15.5, 17.9, 22.9, 23.4, 24.6, 25.7, 26.0, 26.1, 26.5, 27.1, 27.6, 28.3, 28.8, 29.4, 29.8, 32.7, 35.7, 36.3, 36.7, 37.3, 40.6, 41.1, 41.7, 42.8, 46.8, 57.9, 71.2 (C-28), 87.5 (C-19), 131.2 (C-10), 141.8 (C-9).

**19β,28-Epoxy-A-*neo*-5β-methyl-25-nor-18α-olean-9-en-1-one (4).** Ozone was passed through a solution of **3** (0.44 g, 1 mmol) in $CH_2Cl_2$ (25 mL) until the starting material disappeared (TLC monitoring). The solvent was evaporated in vacuo. The product was chromatographed over a column of $Al_2O_3$ with elution by $CHCl_3$. Yield 0.27 g (63%), mp 202–204°C, $[\alpha]_D$ +93° (*c* 0.26, $CHCl_3$), $C_{30}H_{46}O_2$ (MW 438.7).

PMR spectrum (δ, ppm, J/Hz): 0.79 (3H, s, 29-$CH_3$), 0.82 (3H, s, 27-$CH_3$), 0.91 (3H, d, $^3J$ = 6.5, 23-$CH_3$), 0.93 (1H, m, $H_{ax}$-12), 0.94 (3H, s, 25-$CH_3$), 0.94 (3H, s, 30-$CH_3$), 1.01 (3H, d, $^3J$ = 6.5, 24-$CH_3$), 1.17 (3H, s, 26-$CH_3$), 1.24 (1H, m, $H_{eq}$-21), 1.26 (1H, m, $H_{ax}$-6), 1.32 (1H, m, $H_{eq}$-15), 1.39 (1H, m, H-3), 1.41 (1H, m, $H_{ax}$-16), 1.42 (1H, m, $H_{ax}$-21), 1.44 (1H, m $H_{ax}$-22), 1.45 (1H, m, $H_{eq}$-7), 1.49 (1H, m, H-18), 1.50 (1H, m, H-13), 1.55 (1H, m, $H_{eq}$-6), 1.56 (1H, m, $H_{eq}$-16), 1.63 (1H, m, $H_{ax}$-7), 1.67 (1H, m, $H_{eq}$-12), 1.69 (1H, m, H-4), 1.95 (1H, m, $H_{eq}$-22), 1.98 (1H, m, $H_{ax}$-15), 1.99 (1H, ddd, $^2J$ = 15.0, $^3J_{11ax\text{-}12ax}$ = 11.5, $^3J_{11ax\text{-}12eq}$ = 3.3, $H_{ax}$-11), 2.06 (1H, dd, $^2J$ = 18.6, $^3J_{2ax\text{-}3ax}$ = 12.3, $H_{ax}$-2), 2.41 (1H, dd, $^2J$ = 18.6, $^3J_{2ex\text{-}3eq}$ = 8.0, $H_{eq}$-2), 3.49 (1H, d, $^2J$ = 7.8, $H_{anti}$-28), 3.56 (1H, d, $^4J_{19\text{-}21eq}$ = 0.8, H-19), 3.82 (1H, dd, $^2J$ = 7.8, $^4J_{28syn\text{-}22ax}$ = 1.4, $H_{syn}$-28), 4.06 (1H, ddd, $^2J$ = 15.0, $^3J_{11eq\text{-}12ax}$ = 2.1, $^3J_{11eq\text{-}12eq}$ = 2.1, $H_{eq}$-11).

$^{13}C$ NMR spectrum (δ, ppm): 15.9 (C-27), 18.5 (C-25), 22.8 (C-24), 22.9 (C-23), 23.5 (C-11), 24.5 (C-29), 26.1 (C-26), 26.2 (C-12), 26.4 (C-16), 27.1 (C-7), 28.7 (C-30), 28.9 (C-15), 30.1 (C-4), 32.7 (C-6), 35.2 (C-13), 36.3 (C-20), 36.6 (C-21), 37.1 (C-22), 41.6 (C-14), 42.1 (C-17), 42.4 (C-8), 42.9 (C-2), 42.9 (C-5), 46.6 (C-18), 52.9 (C-3), 71.2 (C-28), 87.9 (C-19), 139.8 (C-10), 156.1 (C-9), 208.1 (1).

The test procedure for *in vitro* antitumor activity of **3** is given at the website www.dtp.nci.nih.gov.

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