# Synthesis of 19β,28-epoxy-23,24-dinor-*A*-*neo*-18α-olean-4-en-3-one from betulin

*N. I. Medvedeva,[a] O. B. Flekhter,[a]* ★ *O. S. Kukovinets,[a] F. Z. Galin,[a] G. A. Tolstikov,[b] I. Baglin,[c] and C. Cavé[d]*

[a] *Institute of Organic Chemistry, Ufa Research Center of the Russian Academy of Sciences, 71 prosp. Oktyabrya, 450054 Ufa, Russian Federation. Fax: +7 (347 2) 356066. E-mail: obf@anrb.ru*
[b] *N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Division of the Russian Academy of Sciences, 9 prosp. Akad. Lavrent´eva, 630090 Novosibirsk, Russian Federation.*
[c] *EA 3660, School of Pharmacy, Department of Molecular Biology, BP 87900, 21079 Dijon Cedex, France**
[d] *BioCis Laboratory 8076, Department of Pharmacy, 92296 Châtenay-Malabry Cedex, France***

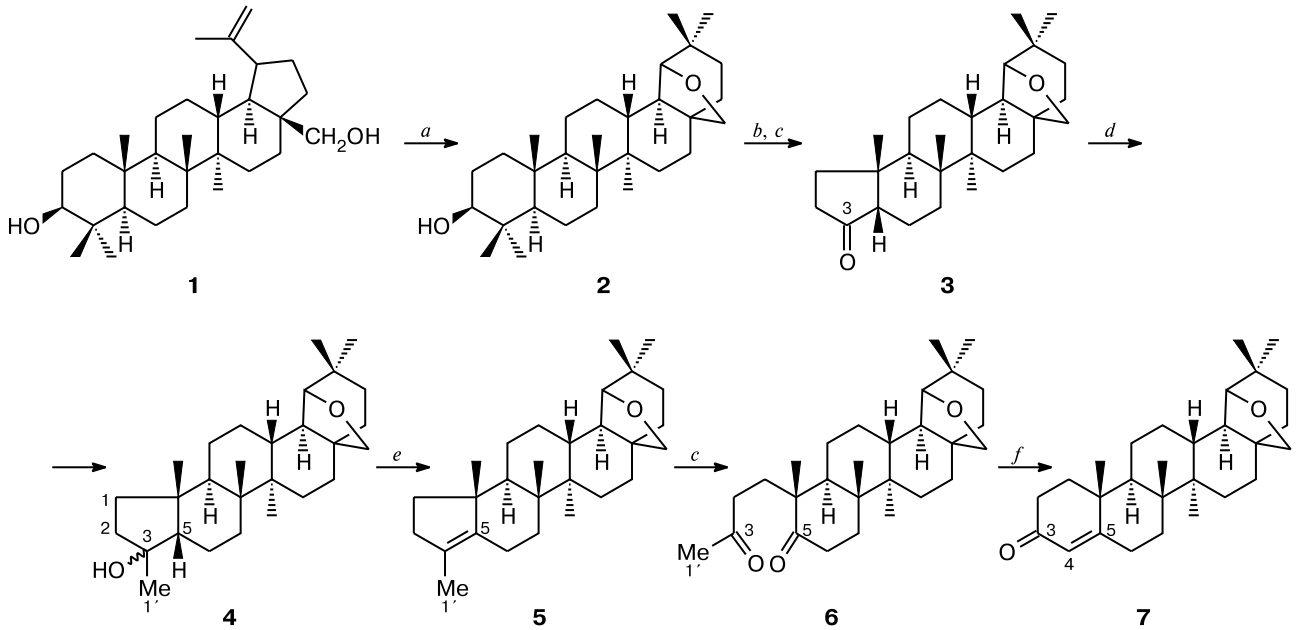
19β,28-Epoxy-23,24-dinor-*A*-*neo*-18α-olean-4-en-3-one was obtained from betulin in a total yield of 18%.

**Key words:** triterpenoids, betulin, allobetulin.

Achievements of the last few years in the development of novel triterpenoid drugs stimulate interest in new synthetic transformations of compounds of this class.[1—4] For instance, derivatives of glycyrrhizic and glycyrrhetic acids have been used to create highly effective anti-HIV, anti-inflammatory, and antiulcer drugs;[5] oleanolic acid is successfully employed in China as a hepatoprotector.[6] Betulinic acid is subjected to preclinical tests as a means

* Unité de Molécules d´Intérêt Biologique, EA 3660, UFR de Pharmacie, BP 87900, 21079 Dijon Cedex, France.
** UMR CNRS BioCis 8076, Faculté de Pharmacie, 5, Rue J.-B. Clément, 92296 Châtenay-Malabry Cedex, France.

**Scheme 1**




**Reagents and conditions:** *a*. $CF_3COOH$, $CH_2Cl_2$, 8 min, 22 °C; *b*. $PCl_5$, $C_6H_6/C_7H_8$, 30 min, 0 °C; *c*. $O_3$, $CH_2Cl_2$, −60 °C, Zn/AcOH; *d*. MeMgI, $THF/Et_2O$, 3 h, 22 °C; *e*. $POCl_3$, 24 h, 22 °C; *f*. KOH/MeOH, 30 min, 64 °C.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 4, pp. 804—806, April, 2007.

for curing metastatic melanomas and 2-cyano-3,12-dioxooleane-1,9(11)-dien-28-oic acid has been recommended for clinical tests as an inhibitor of NO synthase and a drug for curing inflammations and malignant tumors.[7] It is expected that Bevirimat based on 3′,3′-dimethylsuccinylbetulinic acid will be a highly effective anti-HIV drug.[8] Allobetulin and its derivatives are also interesting and promising objects of investigations because they exhibit an antibacterial[9] and antiviral effects on influenza A (see Ref. 10) and B viruses (see Ref. 11).

Here we describe the synthesis of an allobetulin derivative containing the 23,24-dinorolean-4-en-3-one fragment in ring A, which is characteristic of steroids (Scheme 1). We were the first to perform isomerization of betulin **1** into allobetulin **2** in the presence of $CF_3COOH$. The reaction completion within a few minutes and the 97% yield of allobetulin make this method promising for preparative purposes. The synthesis of 19β,28-epoxy-4,23,24-trinor-5β-*A*-*neo*-18α-olean-3-one (**3**) from allobetulin has been described earlier.[12,13]

The action of MeMgI on a solution of ketone **3** produced a mixture of diastereomeric alcohols **4** with close $R_f$ values (ratio 1 : 4, types of the isomers not specified). Dehydration of this mixture of alcohols **4** with $POCl_3$ in pyridine at room temperature gave individual 19β,28-epoxy-3-methyl-4,23,24-trinor-18α-olean-3-ene (**5**). After purification and crystallization from MeOH—$CHCl_3$, the yield was 80%. Ozonization of olefin **5** in $CH_2Cl_2$ at −60 °C followed by reduction of the peroxide products with Zn/AcOH afforded 3-methyl 3,5-seco-3,5-diketone (**6**). Compound **6** was purified by column chromatography; the yield was 60%.

Aldol condensation of diketone **6** in 5% KOH in methanol gave allobetulin enone **7**. The total yield of compound **7** from betulin was 18%.

## Experimental

NMR spectra were recorded on a Bruker AM-300 spectrometer (300.13 and 75.47 MHz, respectively) in $CDCl_3$ with $Me_4Si$ as the internal standard. Melting points were measured on a Boetius hot-stage microscope. Specific rotation was determined on a Perkin—Elmer 241 MC polarimeter in a 1-dm tube. Thin-layer chromatography was carried out on Silufol plates (Chemapol, Czechia) in chloroform—methanol (25 : 1). Spots were visualized with 5% EPTA and subsequent heating at 100—120 °C for 2—3 min. Column chromatography was carried out on neutral $Al_2O_3$ (Reakhim). After isolation and purification, the reaction products were crystallized from $CHCl_3$—MeOH. An Ozon-2K instrument was used as an ozone source. Betulin was isolated as described earlier.[14] Compound **3** was synthesized according to a known procedure.[13]

**19β,28-Epoxy-18α-olean-3β-ol (allobetulin, 2).** Trifluoroacetic acid (2 mL) was added dropwise to a stirred solution of betulin **1** (0.80 g, 2 mmol) in $CHCl_3$ (10 mL). The reaction was completed in 8 min (monitoring by TLC). The organic layer was washed with a saturated solution of $Na_2CO_3$ (3×20 mL) and water (3×20 mL), dried over $Na_2SO_4$, and concentrated *in vacuo*. The yield was 0.86 g (97%), m.p. 264—266 °C (*cf.* Ref. 15: m.p. 265—266 °C).

**19β,28-Epoxy-3-methyl-4,23,24-trinor-5β-*A*-*neo*-18α-olean-3-ol (4).** A 0.6 *M* solution of MeMgI (5 mL, 3 mmol) in anhydrous $Et_2O$ was added dropwise at 5—10 °C to a solution of compound **3** (0.80 g, 2 mmol) in anhydrous THF (10 mL). The mixture was stirred at ~20 °C for 3 h, treated with a saturated solution of $NH_4Cl$ (10 mL), and filtered. The organic layer was washed with water (3×20 mL). The product was extracted from the aqueous layer with $CHCl_3$ (3×20 mL) and the combined extracts were washed with water (3×20 mL), dried over $Na_2SO_4$, and concentrated *in vacuo*. The yield of a 1 : 4 mixture of two isomers (NMR data) was 0.68 g (83%). $^1$H NMR ($CDCl_3$), δ: 0.80 (s, 3 H, Me); 0.87 (s, 2.4 H, Me) and 0.90 (s, 0.6 H, Me); 0.95, 0.98 (both s, 3 H each, Me); 1.01 (s, 2.4 H, Me) and 1.04 (s, 0.6 H, Me); 1.09—1.80 (m, 24 H, $CH_2$, CH); 1.27 (s, 0.6 H, Me (H(1′)) and 1.29 (s, 2.4 H, Me (H(1′))); 3.55 (br.s, 1 H, H(19)); 3.70—3.80 (m, 2 H, H(28)). $^{13}$C NMR, δ: 13.1 (0.2 C); 13.4 (0.8 C); 15.0 (0.2 C); 15.1 (0.8 C); 16.9 (0.2 C); 18.2 (0.8 C); 23.1 (0.2 C); 23.5 (0.8 C); 23.6 (0.2 C(1′)); 24.1 (0.8 C(1′)); 26.3; 26.6 (0.2 C); 26.7 (0.8 C); 26.8; 27.4 (0.2 C); 27.9 (0.8 C); 28.4 (0.2 C); 28.9 (0.8 C); 29.0 (0.8 C); 29.5 (0.2 C); 32.8; 34.5 (0.2 C); 35.0 (0.8 C); 35.1 (0.8 C); 36.3(0.2 C); 36.4; 36.8; 38.9 (0.2 C); 39.1 (0.8 C); 39.5; 40.0 (0.8 C); 40.3 (0.2 C); 40.7 (0.8 C); 41.0 (0.2 C); 41.6 (0.8 C); 42.5 (0.2 C); 43.5; 45.6 (0.8 C); 46.8 (0.2 C); 46.9 (0.8 C); 47.9 (0.2 C); 53.8 (0.2 C); 53.9 (0.8 C); 71.4 C(28); 83.2 C(3); 88.0 C(19).

**19β,28-Epoxy-3-methyl-4,23,24-trinor-*A*-*neo*-18α-olean-3(5)-ene (5).** Phosphoryl chloride (4.5 mL) was added dropwise at 0—5 °C to a solution of compound **4** (1 mmol, 0.39 g) in anhydrous pyridine (10 mL). The reaction mixture was stirred at ~20 °C for 24 h and poured onto ice (10 g). The product was extracted with $CHCl_3$ (3×20 mL) and the combined extracts were washed with water (3×20 mL), dried over $Na_2SO_4$, and concentrated *in vacuo*. The product was chromatographed on $Al_2O_3$ with benzene as an eluent. The yield was 0.31 g (80%), $R_f$ 0.80, m.p. 199—200 °C, $[\alpha]^{20}_D$ +118.1° (*c* 2.60, $CHCl_3$). Found (%): C, 84.90; H, 11.90. $C_{28}H_{44}O$. Calculated (%): C, 84.79; H, 11.18. $^1$H NMR ($CDCl_3$), δ: 0.78, 0.87, 0.94, 1.05 (all s, 15 H, 5 Me); 1.28 (s, 3 H, H(1′)); 1.10—1.75 (m, 22 H, $CH_2$, CH); 1.92 (d, 1 H, H(2), $J$ = 14.0 Hz); 2.01 (dd, 1 H, H(6), $J$ = 15.8 Hz, $J$ = 9.2 Hz); 2.22 (dt, 1 H, H(6), $J$ = 15.8 Hz, $J$ = 3.9 Hz); 2.31 (dd, 1 H, H(2), $J$ = 14.0 Hz, $J$ = 2.0 Hz); 3.40 and 3.80 (both d, 1 H each, H(28), $J$ = 7.7 Hz); 3.55 (s, 1 H, H(19)). $^{13}$C NMR, δ: 13.4, 13.6, 14.2, 19.2, 19.8, 23.6, 24.5, 24.5, 26.2, 26.4, 26.7, 28.8, 32.2, 32.7, 34.6, 34.8, 36.2, 36.7, 40.5, 41.4, 42.4, 46.8, 49.9, 50.0, 71.3 C(28), 87.9 C(19), 126.1 C(3), 141.6 C(5).

**19β,28-Epoxy-3-methyl-3,5-seco-4,23,24-trinor-*A*-*neo*-18α-oleane-3,5-dione (6).** Ozone was passed at −60 °C through a solution of compound **5** (0.79 g, 2 mmol) in $CH_2Cl_2$ (50 mL) until the starting reagent disappeared (monitoring by TLC). The temperature was elevated to 0 °C and glacial AcOH (10 mL) and zinc dust (1 g) were added. The mixture was stirred for 1 h and filtered. The filtrate was washed with a saturated solution of $Na_2CO_3$ (3×20 mL) and water (3×20 mL), dried over $Na_2SO_4$, and concentrated *in vacuo*. The product was chromatographed on $Al_2O_3$ with chloroform as an eluent. The yield was 0.47 g (60%), $R_f$ 0.65, m.p. 134—136 °C, $[\alpha]^{20}_D$ +138.5° (*c* 1.00,

$CHCl_3$). Found (%): C, 78.68; H, 10.50. $C_{28}H_{44}O_3$. Calculated (%): C, 78.46; H, 10.35. $^1$H NMR ($CDCl_3$), δ: 0.80, 0.95, 1.01, 1.02 (all s, 15 H, 5 Me); 1.10—1.80 (m, 19 H, $CH_2$, CH); 2.12 (s, 3 H, H(1′)); 2.25—2.35 (m, 2 H, H(6)); 2.36—2.45 (m, 2 H, H(2)); 3.45 and 3.75 (both d, 1 H each, H(28), $J$ = 7.8 Hz); 3.55 (s, 1 H, H(19)). $^{13}$C NMR, δ: 13.3, 15.5, 20.6, 23.4, 24.5, 25.9, 26.8, 28.7, 28.7, 29.9, 30.1, 32.6, 33.2, 34.6, 35.1, 36.2, 36.6, 39.1, 39.5, 40.7, 41.5, 44.7, 46.5, 49.8, 71.4 (C(28)), 87.9 (C(19)), 208.6 (C(3)), 217.1 (C(5)).

**19β,28-Epoxy-23,24-dinor-*A-neo*-18α-olean-4-en-3-one (7).** A 5% solution of KOH in MeOH (5 mL) was added to a solution of compound **6** (0.86 g, 2 mmol) in MeOH (50 mL). The reaction mixture was refluxed under argon for 30 min and then poured into 5% HCl (50 mL). The resulting precipitate was filtered off, washed with water, and dried. The yield was 0.77 g (90%), $R_f$ 0.38, m.p. 109—112 °C, $[\alpha]^{20}_D$ +95.0° (*c* 3.00, $CHCl_3$). Found (%): C, 81.60; H,10.45. $C_{28}H_{42}O_2$. Calculated (%): C, 81.90; H, 10.31. $^1$H NMR ($CDCl_3$), δ: 0.80, 0.93, 0.98, 1.15 (all s, 15 H, 5 Me); 1.20—1.70 (m, 19 H, $CH_2$, CH); 2.05—2.20 (m, 2 H, H(6)); 2.45—2.55 (m, 2 H, H(2)); 3.45 and 3.85 (both d, 1 H each, H(28), $J$ = 7.4 Hz); 3.55 (s, 1 H, H(19)); 5.75 (s, 1 H, H(4)). $^{13}$C NMR, δ: 13.2, 15.0, 18.3, 21.8, 24.5, 24.5, 26.1, 26.5, 28.8, 28.8, 30.0, 32.7, 33.5, 34.3, 36.2, 36.7, 37.6, 38.8, 40.6, 41.0, 41.5, 46.7, 49.4, 71.2 (C(28)), 87.9 (C(19)), 123.9 (C(4)), 171.9 (C(5)), 199.5 (C(3)).

This work was financially supported by the Russian Foundation for Basic Research (Project No. 05-03-32832).

## References

1. I. Baglin, A.-C. Mitaine-Offer, M. Nour, K. Tan, C. Cavé, and M.-A. Lacaille-Dubois, *Mini Rev. Med. Chem.*, 2003, **3**, 525.
2. G. A. Tolstikov, O. B. Flekhter, E. E. Shul´ts, L. A. Baltina, and A. G. Tolstikov, *Khim. Interesakh Ustoich. Razvit.*, 2005, **13**, 1 [*Chem. Sustain. Dev.*, 2005, **13**, 1 (Engl. Transl.)].
3. P. Dzubak, M. Hajduch, D. Vydra, A. Hustova, M. Kvasnica, D. Biedermann, L. Markova, M. Urban, and J. Sarek, *Nat. Prod. Rep.*, 2006, **23**, 394.
4. T. G. Tolstikova, I. V. Sorokina, G. A. Tolstikov, A. G. Tolstikov, and O. B. Flekhter, *Bioorg. Khim.*, 2006, **32**, 291 [*Russ. J. Bioorg. Chem.*, 2006, **32**, 261 (Engl. Transl.)].
5. G. A. Tolstikov, E. E. Shul´ts, L. A. Baltina, and T. G. Tolstikova, *Khim. Interesakh Ustoich. Razvit.*, 1997, **5**, 57 [*Chem. Sustain. Dev.*, 1997, **5** (Engl. Transl.)].
6. J. Liu, *J. Ethnopharmacol.*, 2005, 92.
7. P. A. Krasutsky, *Nat. Prod. Rep.*, 2006, **23**, 919.
8. Z. Temesgen and J. E. Feinberg, *Curr. Opin. Invest. Drugs*, 2006, **7**, 75.
9. P. A. Krasutsky, R. M. Carlson, and R. A. J. Karim, US Pat. 2004 097 436, 2004.
10. O. B. Flekhter, L. R. Nigmatullina, L. A. Baltina, L. T. Karachurina, F. Z. Galin, F. S. Zarudii, G. A. Tolstikov, E. I. Boreko, N. I. Pavlova, S. N. Nikolaeva, and O. V. Savinova, *Khim.-Farm. Zh.*, 2002, **36**, 9, 26 [*Russ. Chem. Pharm. J.*, 2002, **36**, 484 (Engl. Transl.)].
11. V. G. Platonov, A. D. Zorina, M. A. Gordon, N. P. Chizhov, L. V. Balykina, Yu. D. Mikhailov, D. R. Ivanen, Tran Kim Tvi, and A. G. Shavva, *Khim.-Farm. Zh.*, 1995, **29**, 42 [*Russ. Chem. Pharm. J.*, 1995, **29**, 38 (Engl. Transl.)].
12. G. R. Pettit, B. Green, and W. J. Bowyer, *J. Org. Chem.*, 1961, **26**, 2879.
13. N. I. Medvedeva, O. B. Flekhter, E. V. Tret´yakova, F. Z. Galin, L. A. Baltina, L. V. Spirikhin, and G. A. Tolstikov, *Zh. Org. Khim.*, 2004, **40**, 1140 [*Russ. J. Org. Chem.*, 2004, **40**, 1092 (Engl. Transl.)].
14. O. B. Flekhter, L. T. Karachurina, L. R. Nigmatullina, T. A. Sapozhnikova, L. A. Baltina, F. S. Zarudii, F. Z. Galin, L. V. Spirikhin, G. A. Tolstikov, O. A. Plyasunova, and A. G. Pokrovskii, *Bioorg. Khim.*, 2002, **28**, 543 [*Russ. J. Bioorg. Chem.*, 2002, **28**, 494 (Engl. Transl.)].
15. T. S. Li, J.-X. Wang, and X.-J. Zheng, *J. Chem. Soc., Perkin Trans. 1*, 1998, 3957.

*Received November 2, 2006;*
*in revised form April 24, 2007*