

## Betulin 3,28-Bis-*O*-trifluoroacetate: Synthesis and Molecular Structure

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Received January 22, 2010

**Abstract**—The reaction of betulin with excess trifluoroacetic anhydride gave the corresponding 3,28-bis-*O*-trifluoroacetate whose molecular structure as a slightly bent “stairway” was determined by X-ray analysis.

**DOI:** 10.1134/S1070428010100088

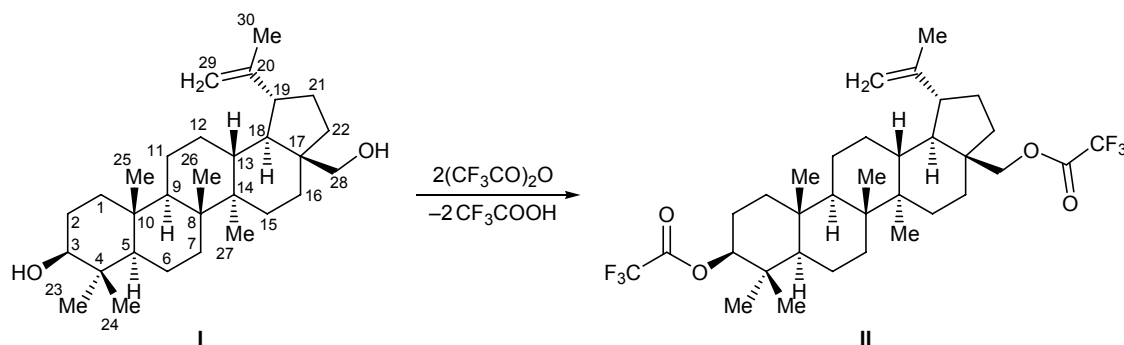
Betulin (**I**) is a lupane triterpenoid possessing high biological activity (antiphlogistic, choleric, antiviral, antitumor, immune modulating, etc.) [1]. The main natural source of betulin is birch bark, where its concentration ranges from 10 to 35%. The ease of isolation and purification of betulin determines sharply increased interest in the synthesis of various its derivatives. Esterification of betulin whose molecule possesses two hydroxy groups gave a number of *O*-acyl derivatives, some of which were found to be promising as anti-HIV agents [1].

In the present work we synthesized betulin 3,28-bis-*O*-trifluoroacetate (**II**), taking into account that perfluorocarboxylic acid esters are known to exhibit stronger and more diverse biological activity than their fluorine-free analogs. Compound **II** was obtained in 89% yield by reaction of betulin with excess trifluoroacetic anhydride (1 : 16) in carbon tetrachloride at 0 to 10°C (Scheme 1). Bis-trifluoroacetate **II** was

isolated previously [2] as a mixture with 28-*O*-trifluoroacetate in the oxidation of betulin with dimethyl sulfoxide activated by trifluoroacetic anhydride.

Diester **II** is an almost colorless crystalline substance, which is readily soluble in chloroform, methylene chloride, and acetone, poorly soluble in ethyl acetate, and even more poorly soluble in alcohol. The structure of compound **II** was confirmed by the IR and <sup>1</sup>H and <sup>19</sup>F NMR spectra and X-ray analysis. The IR spectrum of **II** lacked absorption at about 3446 cm<sup>-1</sup>, which is typical of initial diol **I**, but contained a strong ester carbonyl absorption band (1792 cm<sup>-1</sup>); also, absorption bands due to stretching vibrations of C=C (1640 cm<sup>-1</sup>) and C–F bonds (1220 cm<sup>-1</sup>) were observed. Compound **II** displayed in the <sup>19</sup>F NMR spectrum two signals with equal intensities at δ<sub>F</sub> –76.08 and –76.49 ppm, which confirmed the presence of two trifluoroacetoxy groups on C<sup>3</sup> and C<sup>28</sup>. In the <sup>1</sup>H NMR spectrum of **II** protons in the CH<sub>2</sub>O group resonated as

Scheme 1.



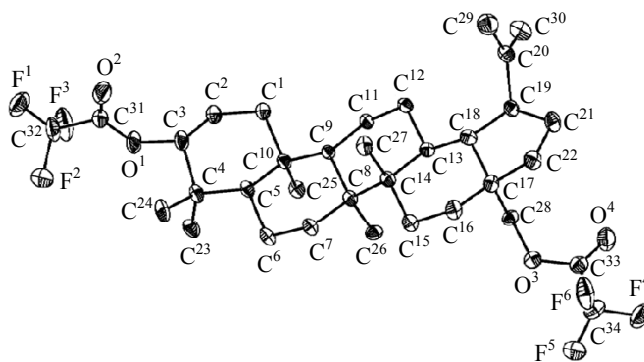
two doublets at  $\delta$  4.55 and 4.13 ppm ( $^2J = 11.0$  Hz), which appeared in a weaker field relative to the corresponding signals of initial betulin (**I**) ( $\delta\Delta = 0.74$  and 0.79 ppm, respectively). The 3-H proton signal was displaced downfield even to a stronger extent, by 1.50 ppm; it was a doublet of doublets located at  $\delta$  4.67 ppm ( $J = 11.0, 4.4$  Hz). Signals due to olefinic protons in the isopropenyl group almost did not change their position ( $\delta$  4.70, 4.62 ppm), indicating that addition of trifluoroacetic acid (which is formed during the process) at the double C=C bond did not occur.

Bis-trifluoroacetate **II** readily undergoes hydrolysis with alkali to give betulin (**I**) in almost quantitative yield, and the hydrolysis product was purer than a sample of **I** used for the synthesis of compound **II**. Thus the synthesis of betulin 3,28-bis-*O*-trifluoroacetate (**II**) and its subsequent hydrolysis may be regarded as a method for purification of betulin.

The molecular structure of compound **II** (Fig. 1) was determined by X-ray analysis; it was similar to the structure of analogous 3,28-bis-*O*-acetate [3]. The cyclopentane ring in molecule **II** in crystal adopts a flattened *twist* conformation with the C<sup>19</sup> and C<sup>21</sup> atoms deviating by 1.08(1) and 1.00(1) Å from the plane formed by the other carbon atoms in that ring. All cyclohexane rings have a *chair* conformation, and every two neighboring cyclohexane rings are linked to each other as *chair-chair-chair*, so that the molecule looks like a slightly bent stairway. Replacement of acetyl groups in betulin 3,28-bis-*O*-acetate by trifluoroacetyl groups radically changes supramolecular organization of compound **II** in crystal (Fig. 2). Molecules **II** in crystal are held together mostly through C–H···F and F···F interactions [the shortest C···F and F···F distances are 3.16(1) and 2.67(1) Å, respectively]. Weaker H···H contacts, which predominate in the crystalline structure of betulin 3,28-bis-*O*-acetate and are responsible for the formation of zigzag chains therein [3], ensure only additional stabilization of the three-dimensional network in the crystalline structure of compound **II**.

## EXPERIMENTAL

The IR spectra were recorded on a Shimadzu FTIR-8400S instrument from samples prepared as KBr pellets. The <sup>1</sup>H NMR spectra were measured from solutions in CDCl<sub>3</sub> on a Bruker NW-400 spectrometer at 400.1 MHz (internal stabilization by the <sup>2</sup>H resonance). The <sup>19</sup>F NMR spectra were obtained on a Bruker AS-200 instrument (188.3 MHz) using CFC<sub>3</sub> as refer-

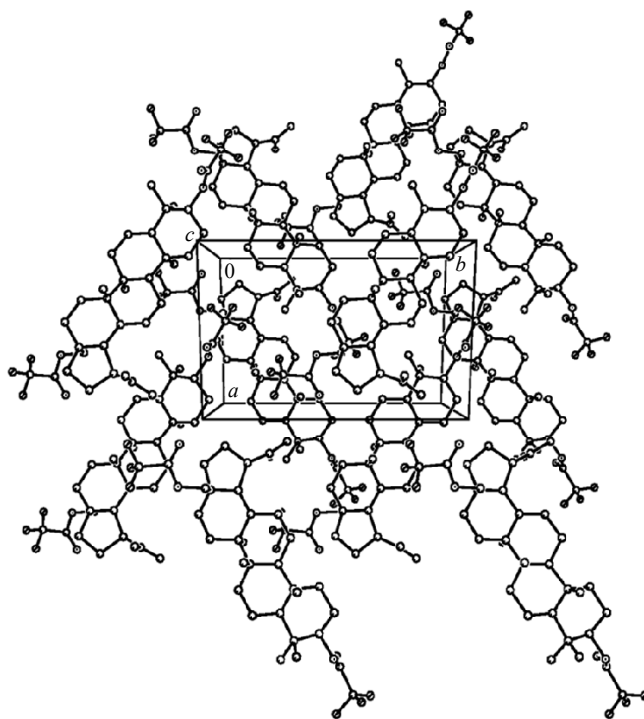


**Fig. 1.** Structure of the molecule of betulin 3,28-bis-*O*-trifluoroacetate (**II**) according to the X-ray diffraction data. Non-hydrogen atoms are shown as thermal vibration ellipsoids with a probability of 50%.

ence and CDCl<sub>3</sub> as solvent. The optical rotation  $[\alpha]_D^{20}$  was determined in chloroform on a Perkin–Elmer 241MC polarimeter.

Trifluoroacetic anhydride was commercial product (R&M) containing 99% of the main substance. Carbon tetrachloride, toluene, and petroleum ether were dried according to standard procedures.

Betulin was obtained by extraction of upper birch bark (*Betula Pendula Roth*) [4] which was preliminarily cut into 20×5×0.1-mm pieces with a moisture content of 5% and subjected to Soxhlet extraction with



**Fig. 2.** A fragment of crystal packing of betulin 3,28-bis-*O*-trifluoroacetate (**II**); a view along the crystallographic *c* axis.

a 1:1 mixture of petroleum ether (bp 70–100°C) and toluene. The extract was cooled to 10°C, and the precipitate was filtered off and dried in air. The crude product was a yellowish powder with mp 230–257°C. It was recrystallized twice from ethanol to obtain a loose white powder with mp 251–252°C; published data [5]: mp 256–258°C. According to the <sup>1</sup>H NMR data, the product contained ≥95% of the main substance.

**Betulin 3,28-bis-*O*-trifluoroacetate (II).** Trifluoroacetic anhydride, 20.2 ml (0.145 mol), was added dropwise under stirring to a suspension of 4.0 g (9 mmol) of betulin (I) in 35 ml of carbon tetrachloride, maintaining the temperature of the reaction mixture in the range from 0 to 10°C. When the addition was complete, the mixture was stirred for 15 min at room temperature, and the solvent, excess trifluoroacetic anhydride, and trifluoroacetic acid formed during the process were distilled off under reduced pressure (12 mm) at a bath temperature not exceeding 40°C. To ensure complete removal of trifluoroacetic anhydride and trifluoroacetic acid, 2×5 ml of carbon tetrachloride was added to the distillation flask. The residue was compound II as a light brown substance. Recrystallization from ethyl acetate gave colorless crystals. Yield 5.1 g (89%), mp 183–185°C,  $[\alpha]_D^{20} = +4.3^\circ$  ( $c = 0.015$ , CHCl<sub>3</sub>). IR spectrum,  $\nu$ , cm<sup>-1</sup>: 1792 (C=O), 1640 (C=C), 1220 (C–F). <sup>1</sup>H NMR spectrum,  $\delta$ , ppm: 0.80 s, 0.88 s, 0.89 s, 0.98 s, and 1.05 s (15H, CH<sub>3</sub>), 1.20–2.40 m (26H, CH<sub>2</sub>, CH), 1.69 s (3H, 30-H), 2.43 d (1H, 19-H, <sup>3</sup> $J = 6.6$  Hz), 4.67 d.d (1H, 3-H,  $J = 11.0$ , 4.4 Hz), 4.13 d and 4.56 d (1H each, 28-H, <sup>2</sup> $J = 11.0$  Hz), 4.70 s and 4.62 s (1H each, 29-H). <sup>19</sup>F NMR spectrum,  $\delta_F$ , ppm: –76.08 s and –76.49 s (CF<sub>3</sub>). Found, %: C 64.17; H 7.52. C<sub>34</sub>H<sub>48</sub>F<sub>6</sub>O<sub>4</sub>. Calculated, %: C 64.34; H 7.62.

Crystallographic data for betulin 3,28-bis-*O*-trifluoroacetate (II): C<sub>34</sub>H<sub>48</sub>F<sub>6</sub>O<sub>4</sub>,  $M$  634.75; 0.30×0.25×0.20 mm; monoclinic crystal system, space group  $P2_1$ . Unit cell parameters (at 100 K):  $a = 9.5242(4)$ ,  $b = 14.6638(8)$ ,  $c = 11.7096(8)$  Å;  $\beta = 105.314(5)^\circ$ ;  $V = 1577.31(16)$  Å<sup>3</sup>;  $Z = 2$  ( $Z' = 1$ );  $F(000) = 678$ ;  $d_{\text{calc}} = 1.339$  g/cm<sup>3</sup>;  $\mu = 0.109$  mm<sup>-1</sup>. The unit cell parameters and intensities of 19104 reflections were measured on a Smart Apex2 CCD diffractometer at 100 K (MoK $\alpha$

irradiation, graphite monochromator,  $\omega$ -scanning,  $2\theta_{\text{max}} = 58^\circ$ ). The structure was solved by the direct method and was refined by the least-squares procedure in full-matrix anisotropic approximation with respect to  $F_{hkl}^2$ . The positions of hydrogen atoms were calculated on the basis of geometry considerations and were refined in isotropic approximation according to the riding model. The final divergence factors were  $R_1 = 0.0577$  for 4087 independent reflections with  $I > 2\sigma(I)$  and  $wR_2 = 0.1558$  for all 4309 independent reflections. All calculations were performed using SHELXTL PLUS software package [6]. The complete set of crystallographic data for compound II (coordinates of atoms, bond lengths, bond angles, and anisotropic temperature parameters) was deposited to the Cambridge Crystallographic Data Centre (entry no. CCDC 755395).

**Hydrolysis of betulin 3,28-bis-*O*-trifluoroacetate (II).** A solution of 0.101 g (2.5 mmol) of sodium hydroxide in 0.3 ml of ethanol was added to a solution of 0.503 g (0.78 mmol) of betulin 3,28-bis-*O*-trifluoroacetate (II) in 5 ml of toluene, and the mixture was heated for 3 h at 60°C. Water, 10 ml, was then added, and the colorless precipitate was filtered off, washed with water, and dried. Yield of betulin (I) 0.33 g (94%), mp 254–256°C.

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