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ABSOLUTE CONFIGURATION OF 3-ACETYLBETULINIC ACID

© **E. M. Suleimen,[1] K. Van Hecke,[2] L. Van Meervelt,[2] Wim Deborggraeve,[2] and Wim Dehaen[2]**

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The absolute configuration of 3-acetoxybetulinic acid is determined by single crystal X-ray diffraction.

Keywords: betulinic acid, 3-acetoxybetulinic acid, single crystal X-ray diffraction analysis.

Betulinic acid **1** derived from betulin has a complex of valuable pharmacological properties: antiinflammatory, wound-healing, hepatoprotecting antiviral, and antitumor [1-3]. Some derivatives of betulin were found to be effective as anti-HIV agents [4].

In this paper we present the absolute configuration of 3-acetoxybetulinic acid **2**, which was obtained by the interaction of acetic anhydride in pyridine [5]. The spectral data of **2** proved to be identical to those described in the literature [6].

Compound **2** was characterized in the chiral space group $P2_12_12_1$ (No. 19) with one molecule in the asymmetric center and one molecule of chloroform solvent (Fig. 1).

The cyclopentane ring adopts a twisted *envelope* conformation at C22-C23, while all cyclohexane rings adopt a *chair* conformation.

The acetoxy group is in the equatorial position relative to the C3 atom. The C2-O2-C3-C4 torsion angle is 118.9(3)°.

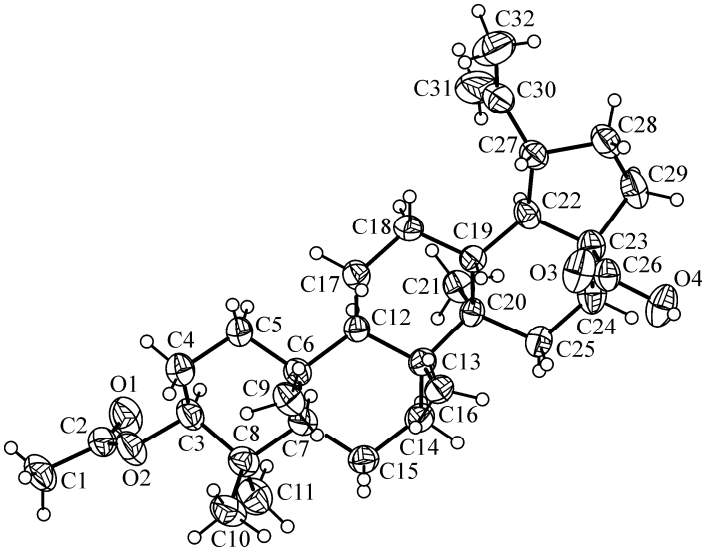

Fig. 1. Molecular and crystal structure of **2** according to X-ray crystallography. Thermal displacement ellipsoids are drawn at the 40% probability level. A chloroform molecule is omitted.

[1]L. N. Gumilev Eurasian National University, Astana, Kazakhstan; syerlan75@yandex.ru. [2]Katholieke Universiteit Leuven, Belgium; Kristof.VanHecke@chem.kuleuven.be. Translated from *Zhurnal Strukturnoi Khimii*, Vol. 54, No. 1, pp. 195-197, January-February, 2013. Original article submitted April 16, 2012.

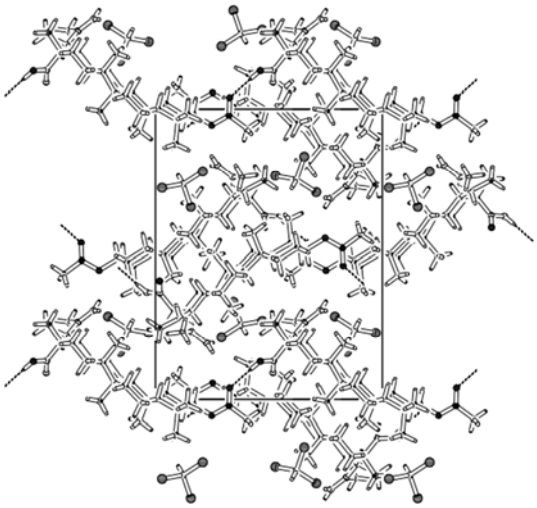

Fig. 2. Crystal packing of the structure of compound **2** in the (100) plane. The molecules are hydrogen bonded along the [010] direction.

The isopropyl group is also equatorially positioned on the C27 atom of the cyclopentane ring. The C32-C30-C27-C28 torsion angle is 78.1(5)°. Other similar structures of betulinic acid derivatives, containing an acetoxy group at the same position, have been reported in [7, 8].

Intermolecular hydrogen bonds between the carboxyl O-H group and the acetoxy C=O group of a symmetry-equivalent molecule (O(H)…O distance of 2.682(4) Å) are observed, which link the molecules together in the [010] direction (Fig. 2).

The absolute configuration of the structure was determined and the following atoms were established as C3(*S*), C6(*R*), C7(*R*), C12(*S*), C13(*R*), C19(*R*), C20(*R*), C23(*S*), C27(*R*).

Experimental. 3-Acetoxybetulinic acid. 500 mg (1.1 mmol) of betulinic acid was dissolved in 0.350 ml of pyridine (4.38 mmol) and 4.76 ml (50.4 mmol) of acetic anhydride were added. The mixture was kept for 24 h at room temperature and afterwards mixed with 20 ml of ice water. The mixture was stirred for 20 min at 0°C. The product was filtered and the residue was chromatographed on Silicagel using a mixture of heptane and dichloromethane solvents (2/8). After recrystallization white crystals (437 mg, 80%) were obtained.

Yield: 80%.

ESI: *m*/*z* 499 (M), *m*/*z* 500 (M+H).

HRMS: $C_{32}H_{50}O_4$. Calculated: 498.37091 Found: 498.37278.

IR: 2948 (CH sp^3), 1722 (C=O), 1687 (C=C), 1246 (C–O).

^{1}H NMR: (300 MHz, $CDCl_3$, ppm): δ 4.75 (s, 1H, $H^{29\alpha}$, 4.61 (s, 1H, H^{29b}), 4.54-4.53 (m, 1H, H^3), 3.08-2.94 (m, 1H, H^{19}), 2.33-1.91 (m, 7H), 2.04 (s, 3H, H^{32}), 1.80-1.14 (m, 21H), 0.99-0.76 (m, 18H), 0.97 (s, 3H, CH_3), 0.84 (s, 3H, CH_3), 0.84 (s, 3H, CH_3).

^{13}C NMR: (100 MHz, $CDCl_3$, ppm): δ 181.49 (C28); 171.09 (C31); 150.39 (C20); 109.76 (C29); 80.95 (C3); 56.36 (C17); 55.40 (C5); 55,38 (C9); 49.24 (C18); 46.93 (C19); 42.41 (C14); 40.68 (C8); 38.39 (C1); 38.36 (CH_2); 37.79 (CH_2); 37.10 (CH_2); 36.80 (CH_2); 34.22 (C7); 30.54 (CH_2); 29.67 (CH_2); 28.79 (CH_2); 27.94 (CH_2); 25.43 (C2); 23.70 (CH_2); 21.34 (C32); 20.83 (C11); 19.34 (CH_2); 18.15 (C6); 16.47; 16.18; 16.03 (C23, C26, C25); 14.65 (C27).

Single crystal X-ray diffraction analysis. Compound **2** was crystaliized from a mixture of heptane/ dichloromethane solvents (2/8) at room temperature.

The X-ray crystallographic data were collected on a SMART 6000 diffractometer equipped with a CCD detector using CuK_α radiation (λ = 1.54178 Å), ϕ and ω scans. The data were interpreted and integrated with the program SAINT (Bruker) [9]. Both structures were solved by a direct method and refined by full-matrix least-squares on F^2 using the

SHELXTL program package [2]. Non-hydrogen atoms were refined anisotropically and the hydrogen atoms were refined in the riding mode and isotropic temperature factors fixed at 1.2 times *U*(eq) of the parent atoms (1.5 times for methyl and hydroxyl groups). CCDC-876596 contains the supplementary crystallographic data for this paper and can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

Crystal data for compound 2. $C_{33}H_{51}Cl_3O_4$, $M = 618.09$, orthorhombic, $P2_12_12_1$ (No. 19), $a = 11.651(1)$ Å, $b = 14.835(2)$ Å, $c = 19.131(2)$ Å, $V = 3306.5(6)$ Å^3, $T = 293(2)$ K, $Z = 4$, $\rho_{calc} = 1.242$ g/cm^3, $\mu(CuK_\alpha) = 2.775$ mm^{-1}, $F(000) = 1328$, crystal dimensions 0.4×0.4×0.3 mm, 6249 independent reflections ($R_{int} = 0.0877$). Final $R = 0.0569$ for 4863 reflections with $I > 2\sigma(I)$ and $wR2 = 0.1650$ for all data. The absolute configuration of the structure was determined with a final Flack parameter of 0.01(3) [11].

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