

ABSOLUTE CONFIGURATION OF 13-EPIMULINOLIC ACID

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ABSTRACT

The molecular structure of the title compound [iupac name: 13-epi-8-hydroxy-3-isopropyl-5a,8-dimethyl-2,3,4,5,5a,6,7,8,10a,10b-decahydrocyclohepta[e]indene-3a(1H) acid] is similar to the structure of the known compound mulinolic acid. The structure of this mulinolic acid epimer consists of a mulinane skeleton and the isopropyl, methyl group, carboxylic acid and the hydroxyl group at C3, C8, C5, C13, respectively, which are β -oriented, whereas the methyl group at C13 are α -oriented. The cyclopentane (A), cyclohexane (B) and cycloheptene (C) rings are trans (A/B) and (B/C) cis fused, and are in an envelope, chair, and twist chair conformation respectively. In the crystal the molecules are linked by two strong intermolecular O—H...O hydrogen bond forming bidimensional supramolecular structures with graph-set notation (12) and (46). The absolute configuration of the title compound which has been determined from the refinement of the Flack parameter¹⁶. On this basis the absolute configuration was assigned as C3R, C5S, C8S, C9S, C10S and C13S.

Keywords: Absolute configuration, Diterpenoid, X-ray diffraction, Crystal and Supramolecular structure.

INTRODUCTION

In the course of studies on diterpenoids from species of the tribe Mulinae (Umbelliferae), we have reported on the structures of several mulinane derivatives obtained from the petrol extracts of *Laretia acaulis* (Cav.) Gill et Hook., *Mulinum crassifolium* and *Azorella compacta* Phil¹⁻⁵. In the continuation of our phytochemical investigation of Umbelliferae we have study again *Laretia acaulis* (Cav.) Gill et Hook., known in Chile as "llareta de la zona central". This plant is a yellowish-green, compact resinous cushion shrub, which grows in the Andes of Chile. Whole plant infusions plan are widely used as diabetes treatment in folk medicine⁶. The relative stereochemistry of 13-epimulinolic acid was assigned on the careful spectroscopic comparison with mulinolic acid and by chemical transformation⁵. In this paper we report the absolute configuration of the title compound which has been determined from the refinement of the Flack parameter¹⁶, $x = -0.01(12)$, indicated that correct configuration had been assigned against 1220, CuK α Bijvoet pairs. On this basis the absolute configuration was assigned as: C3R, C5S, C8S, C9S, C10S and C13S.

EXPERIMENTAL

The title compound C₂₀H₃₂O₃ was isolated using medium pressure column chromatography (MPCC). In a continuation of our program for the chemical study of the Atacama Desert Flora⁷⁻¹⁰, dried aerial parts of *Laretia acaulis* (1130 g) collected in april 2014 in the pre-Andean mountain range of Atacama Desert, precordillera de la III Region, Northern Chile were extracted with n-hexane (3 liters, 3 times in the dark, 24 h each time) and 180 g of a yellow gum were obtained after evaporation of the solvent. A portion of the extract (20.0 g) was filtered and submitted to a medium pressure column chromatography system composed of an 2.5 cm x 48 cm medium pressure column (Aceglass inc, Vineland, NY, USA) packed with silicagel (Kieselgel 60 H, Merck, Darmstadt, Germany) using an isocratic solvent system of n-hexane-ethyl acetate (9.5:0.5 v:v) pumped with a medium pressure pump (FMI lab pump, Syosset, NY, USA) with a flow rate of 10 mL-minute. The collected fractions (75) were combined according to TLC analysis (Kieselgel F254 plates, developed with Hexane: EtOAc 8:2 v/v, and spots visualized by spraying with vanillin:sulfuric acid 2 % in ethanol and heating) and ten combined fractions were obtained. Fraction 2 (350 mg) was re-chromatographed using the same chromatographic system and the known compounds: mulin-11, 13-dien-20-oic acid (40 mg), mulinolic acid⁵ and 13-epimulinolic acid⁶ (120 mg). Recrystallization of 13-epimulinolic acid from ethyl acetate a room temperature yielded white crystals, which were suitable for X-ray diffraction analysis.

13-epimulinolic acid, white crystals, m.p. 240-243 °C. The molecular weight was determined by orbitrap ESI-MS/MS with a mass spectrometer (Q-exactive Focus, Bremen, Germany) [M-H]⁻: 319. 22752 calcd. for C₂₀H₃₁O₃: 319.22787. ¹H NMR (Bruker Avance 300 MHz, CDCl₃) δ ppm: 0.80 (3H, d, J = 5.8 Hz, Me-18), 0.93 (3H, s, Me-17), 0.97 (3H, d, J = 5.8 Hz, Me-19), 1.07

(1H, dd, J = 4.8, 14.6 Hz, H-15 β), 1.23 (3H, s) 1.315 (3H, m, H-7 β , H-6 α , H-2 α), 1.36 (2H, m, H-3, H-4), 1.43 (2H, d, H-1 β , H-7 α), 1.87 (3H, m, H-1 α , H-2 β , H-10 α), 1.51 (1H, dd, J = 5.9, 13.5 Hz, H-14 β), 2.00 (1H, dd, J = 13.5, 14.6 Hz, H-14 α), 2.05 (1H, dd, J = 8.2, 11.0 Hz, H-9 β), 2.17 (1H, t, J = 2.17 Hz), 2.35 (1H, m, H-6 β), 5.26 (1H, dd, J = 8.2, 12.9 Hz, H-11), 5.48 (1H, d, H-12). ¹³C NMR (¹³C NMR Bruker Avance 75 MHz, CDCl₃) δ ppm: 177.0 (COOH), 138.1 (C-11), 127.0 (C-12), 74.1 (C-13), 57.8 (C-5), 57.4 (C-3), 50.9 (C-10), 48.0 (C-9), 41.9 (C-7), 36.8 (C-14), 35.6 (C-8), 32.5 (C-6), 31.7 (C-4), 30.7 (C-15), 28.7 (C-2), 28.4 (Me-16), 27.5 (Me-17), 24.7 (C-1), 22.5 (Me-19), 22.1 (Me-18). These data, together with HR-MS data and correlations observed in the HSQC and HMBC spectra, are consistent with the structure of 13-epimulinolic acid (Fig. 1). The spectroscopic data showed resemblance with those similar mulinane structures reported in the literature¹⁰⁻¹².

Data collection, structural determination and refinement was performed with a Bruker AXS D8-Venture, Triumph- μ -Cu with graphite-monochromated CuK α radiation (1.54178 Å). The structure was solved by direct method, and was refined against F² by full-matrix least-squares methods using SHELXL¹³. All of the non-hydrogen atoms were refined anisotropically. The hydrogen atoms was located from a difference Fourier map and allowed to ride on their parent C atoms, with isotropic displacement parameters related to the refined values of the corresponding parent atoms. H atoms bonded to O atoms were freely refined with isotropic displacement parameters. The final Fourier maps, the electron-density residuals were not significant. Crystallographic data, details of data collection and structure refinement parameters for the title compound is summarized in Table 1. Program used to solve structure: SHELXS-201313, program used to refine structure: SHELXL-201313, molecular graphics¹⁴. The absolute configuration of the title compound which has been determined from the refinement of the Flack parameter¹⁶, $x = -0.01(12)$, indicated that correct configuration had been assigned, against 1220, CuK α Bijvoet pairs.

RESULTS AND DISCUSSION

The molecular structure and the absolute configuration of the title compound was confirmed by x-ray diffraction analysis of suitable single crystals (Fig.2). Colorless block crystals of title compound with approximate dimensions 0.250 x 0.200 x 0.200 mm was obtained by slow evaporation of a ethyl acetate solution. The molecular structure [iupac name: 8-hydroxy-3-isopropyl-5a,8-dimethyl-2,3,4,5,5a,6,7,8,10a,10b-decahydrocyclohepta[e]indene-3a(1H) acid] consists of a mulinic acid skeleton and the isopropyl, methyl group, carboxylic acid and hydroxyl group at C3, C8, C5, C13 respectively, which are β -oriented, whereas the methyl group at C13 is α -oriented, Fig.2 The Cremer and Pople parameters¹⁵ for the cyclopentane (A), cyclohexane (B) and cycloheptene (C) rings are: Q₂= 0.425(3)Å, $\varphi_2=112.2(4)^\circ$; Q₁=0.578(3)Å, $\theta=173.8(3)^\circ$, $\varphi=164(2)^\circ$ and Q₂=0.104(3)Å, $\varphi_2=106.7(15)^\circ$, Q₃=0.577(3), $\varphi_3=333.9(3)^\circ$, so the conformation for A, B and C rings are: envelope, chair, and twist chair respectively. The A/B and B/C rings are trans and cis fused respectively. The

OH groups are involved in hydrogen bonding interactions with the average H...O distance of 1.895 Å and O—H...O angles of 171.5°, Table 2, so in the crystal the molecules are linked by two strong intermolecular O—H...O hydrogen bond forming bidimensional supramolecular structures with graph-set notation (12) and (46)¹⁷, Fig.3 and Fig.4. All bond distances and angles are normal.

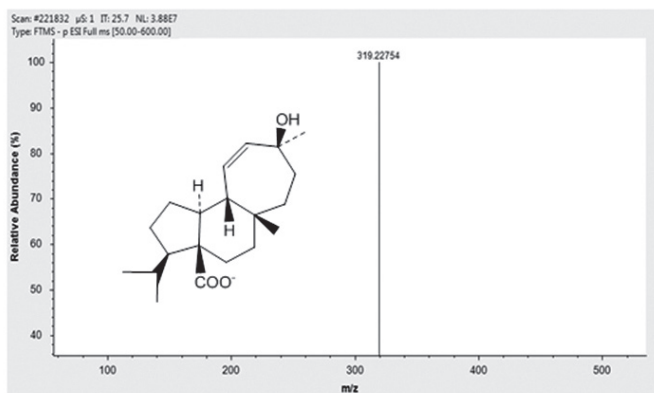


Fig. 1: Full Orbitrap HESI (-) spectra of compound 13-epimulinolic acid.

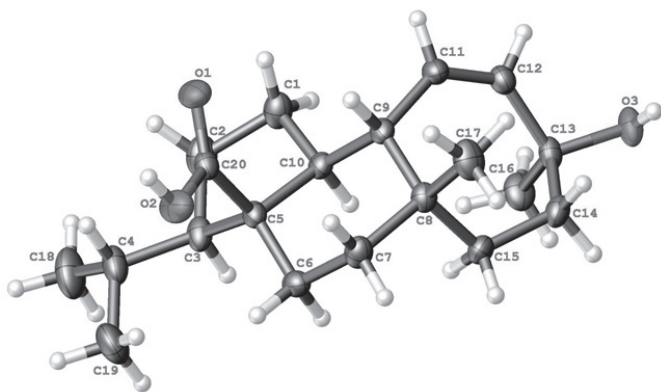


Fig.2: ORTEP Diagram of 13-epimulinolic acid. The ellipsoids are shown at the 30% probability level.

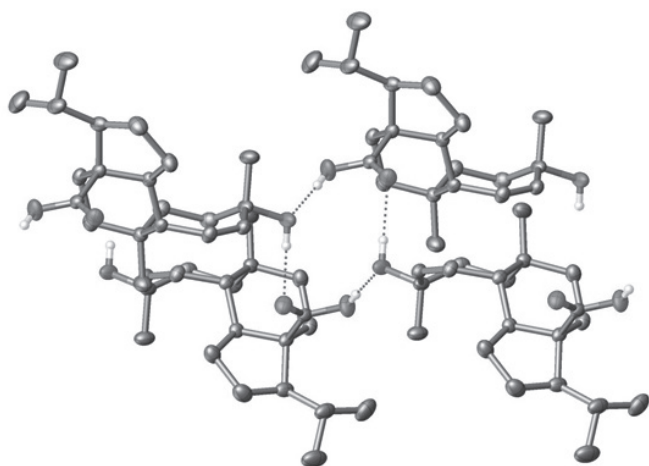


Fig.3 A view of the bi-dimensional supramolecular aggregate, showing the formation of ring with set-graph motif (12).

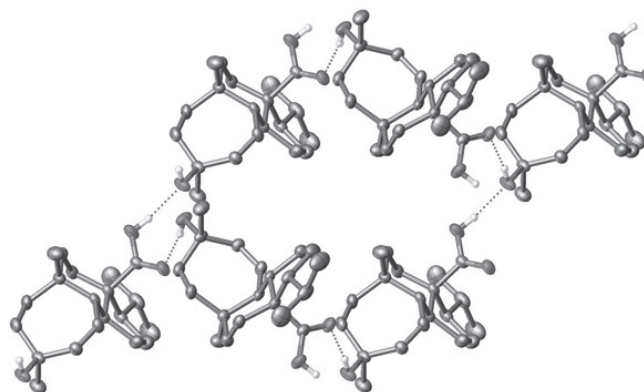


Fig.4 A view of the bi-dimensional supramolecular aggregate, showing the formation of ring with set-graph motif (46).

Table 1. Crystallographic data, details of data collection and structure refinement parameters for title compound

Crystal data

$C_{20}H_{32}O_3$	$V = 3874.8(2) \text{ \AA}^3$
$M_r = 320.45$	$Z = 8$
Orthorhombic, $C222_1$	$CuK\alpha (\lambda = 1.54178 \text{ \AA})$
$a = 12.3345(4) \text{ \AA}$	$\alpha = 90^\circ$
$b = 17.2102(5) \text{ \AA}$	$\beta = 90^\circ$
$c = 18.2531(5) \text{ \AA}$	$\gamma = 90^\circ$
	$\mu = 0.564 \text{ mm}^{-1}$
	$T = 296(2) \text{ K}$
	Colourless, Block, 0.25 x 0.20 x 0.20 mm

Data Collection

Diffractometer Bruker AXS D8-Venture, Triumph-I μ -Cu	
2793 reflections with $I > 2\sigma(I)$	
14145 measured reflections	$R_{int} = 0.0568$
2793 independent reflections	
2 θ range for data collection	8.82 to 118.09°
Index ranges	$-13 \leq h \leq 13, -17 \leq k \leq 19, -19 \leq l \leq 20$

Refinement

$R[F^2 > 2\sigma(F^2)] = 0.0335$	215 parameters
$wR[F^2] = 0.0818$	$S = 1.052$
	$\Delta\rho_{max} = 0.14 \text{ e-\AA}^{-3}$
	$\Delta\rho_{min} = -0.12 \text{ e-\AA}^{-3}$
Flack parameter ¹⁶ -0.01(12)	Goodness-of-fit on F^2 1.052

Table 2: Hydrogen-bonding geometry (Å, °)

D—H...A	D—H	H...A	D...A	D—H...A
O3—H1...O1 ⁱ	0.82	1.98	2.794(2)	171
O2—H2...O3 ⁱⁱ	0.82	1.81	2.624(3)	172

symmetry code: (i) $-1/2+x, 3/2-y, 1-z$; (ii) $1/2+x, -1/2+y, z$

CONCLUSIONS

In order to establish its absolute configuration, a single-crystal X-ray diffraction analysis of 13-epimulinolic acid was undertaken. The X-ray molecular model of the title compound (Fig.2), confirmed all the above deductions on its structure and established the absolute configuration as C3R, C5S, C8S, C9S, C10S and C13S.

Supplementary material

CCDC- 1518397 contains the supplementary crystallographic data for

this article. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

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