

## ABSOLUTE CONFIGURATION OF MULINOLIC ACID

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## ABSTRACT

The structure of this mulinolic acid consists of a mulinane skeleton and the corresponding isopropyl, methyl, carboxyl and methyl groups at C3, C8, C5, C13, respectively, which are  $\beta$ -oriented, whereas the hydroxyl group at C13 is  $\alpha$ -oriented. The cyclopentane (A), cyclohexane (B) and cycloheptene (C) rings are trans (A/B) and (B/C) cis fused, and in an envelope, chair, and twist conformation respectively.

In the crystal the molecules are linked by two intermolecular O—H...O hydrogen bond forming bidimensional supramolecular structures with graph-set notation R<sub>4</sub>(12), R<sub>4</sub>(40), R<sub>6</sub>(46) and C<sub>4</sub>(46). The absolute configuration of the title compound has been determined from the refinement of the Flack parameter<sup>16</sup>. On this basis the absolute configuration was assigned as C3R, C5S, C8S, C9S, C10S and C13R.

**Keywords:** absolute configuration, diterpenoid, X-ray diffraction, crystal and supramolecular structure.

## INTRODUCTION

*Mulinum crassifolium* is small cushion shrub growing up to 15 cm commonly known as “chuquicán”, “susurco” o “espinilla” which grows in the north of Chile at altitudes above 3500m. Bitter tasting infusions of the whole plant are used widely in folk medicine, principally in the treatment of diabetes, and bronchial and intestinal disorders<sup>1</sup>. This plant is well recognized as an important source of diterpenes bearing the mulinane skeleton with interesting biological activities<sup>2</sup>. The relative stereochemistry of the title compound was assigned by spectroscopic data and by chemical transformations<sup>3</sup>. In this paper we report the absolute configuration of the title compound which has been determined from the refinement of the Flack parameter<sup>15</sup>,  $x = 0.05(11)$ , which indicate that the correct configuration had been assigned against 925, CuK $\alpha$  Bijvoet pairs. On this basis the absolute configuration was assigned as C3R, C5S, C8S, C9S, C10S and C13R.

## EXPERIMENTAL

The title compound C<sub>20</sub>H<sub>32</sub>O<sub>3</sub> was isolated using medium pressure column chromatography (MPCC). In a continuation of our program for the chemical study of the Atacama Desert Flora<sup>2-6</sup>, dried aerial parts of *Mulinum crassifolium* (750 g) collected in April 2014 in the pre-Andean mountain range of Atacama Desert, precordillera de la II Region, Northern Chile were extracted with n-hexane (1 liter, 3 times in the dark, 24 h each time) and 22 g of a yellow gum were obtained after evaporation of the solvent. A portion of the extract (10.0 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 (9.0:1.0 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 (80) 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 fifteen combined fractions were obtained. Fraction 5 (350 mg) was re-chromatographed using the same chromatographic system and the known compounds: mulin-11,13-dien-20-oic acid<sup>6</sup> (120 mg) and mulinolic acid<sup>7</sup> (60 mg). Recrystallization de mulinolic acid from ethyl acetate a room temperature yielded white crystals, which were suitable for X-ray diffraction analysis. The spectroscopic data showed resemblance with those similar mulinane structures reported in the literature<sup>4-11</sup>. The relative stereochemistry of mulinolic acid was assigned based on careful analysis of the ROESY experiment<sup>6</sup>, and in this work we have assigned the absolute configuration of this diterpene using X-ray crystal analysis.

Mulinolic acid: white crystals, m.p. 178-180 °C. The molecular weight was determined by orbitrap HR-ESI-MS/MS with a mass spectrometer. [M-H]<sup>-</sup>: required: 319.22752, found: 319.22766 for C<sub>20</sub>H<sub>32</sub>O<sub>3</sub> (Fig. 1). <sup>1</sup>H NMR (Bruker Avance 300 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 0.86 (3H, d, J = 5.8 Hz, Me-18), 0.94 (3H, s, Me-17), 1.04 (3H, d, J = 5.8 Hz, Me-19), 1.10 (1H, dd, J = 4.6,

15.0 Hz, H-15 $\beta$ ), 1.32 (3H, s, Me-16), 1.38 (1H, m, H7 $\beta$ ), 1.47 (4H, m, H-6 $\alpha$ , H-4 $\alpha$ , H-7 $\alpha$ , H-2 $\beta$ ), 1.51 (1H, m, H-3 $\alpha$ ), 1.55 (1H, m, H-1 $\alpha$ ), 1.63 (1H, dd, J = 9.3/12.5 Hz, H-14 $\beta$ ), 1.84 (1H, dd, J=4.6/12.5 Hz, H-14 $\alpha$ ), 1.91 (1H, m, H-2 $\alpha$ ), 1.93 (1H, m, H-1 $\beta$ ), 2.08 (1H, m, dd, J = 6.0/10.8, H-9 $\beta$ ), 2.16 (1H, br d = 10.8 Hz, H-10 $\alpha$ ), 2.42 (1H, dd, J = 3.0/9.4 Hz, H-6 $\beta$ ), 2.61 (1H, dt, J = 15.0 Hz, H-15 $\alpha$ ), 5.58 (1H, d, J = 12.8 Hz, H-11), 5.58 (1H, d, J=12.9 Hz, H-12). <sup>13</sup>C NMR (<sup>13</sup>C NMR Bruker Avance 75 MHz, CDCl<sub>3</sub>)  $\delta$  ppm: 180.2 (COOH), 133.8 (C-11), 136.5 (C-12), 71.4 (C-13), 58.4 (C-5), 57.7 (C-3), 51.6 (C-10), 48.7 (C-9), 42.2 (C-7), 36.2 (C-14), 35.9 (C-8), 32.4 (C-6), 32.0 (C-4), 30.5 (C-15), 28.9 (C-2), 33.7 (Me-16), 27.5 (Me-17), 25.2 (C-1), 22.6 (Me-19), 22.9 (Me-18). These data, together with HR-MS data and correlations observed in the HSQC and HMBC spectra, are consistent with the structure of mulinolic acid (Figure 1).

Data collection, structural determination and refinement was performed with a Bruker AXS D8-Venture, Triumph- $\mu$ -Cu with graphite-monochromated CuK $\alpha$  radiation (1.54178 Å). The structure was solved by direct method, and was refined against F<sup>2</sup> by full-matrix least-squares methods using SHELXL<sup>12</sup>. 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 and O atoms, with isotropic displacement parameters related to the refined values of the corresponding parent atoms. 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-201312, program used to refine structure: SHELXL-201312, molecular graphics<sup>13</sup>. The absolute configuration of the title compound which has been determined from the refinement of the Flack parameter<sup>15</sup>,  $x = 0.05(11)$ , indicated that correct configuration had been assigned, against 925, CuK $\alpha$  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 0.200 x 0.150 x 0.150 mm was obtained by slow evaporation of a ethyl acetate solution. The molecular structure consists of a mulinolic acid skeleton with the required isopropyl, methyl, carboxyl and methyl group at C3, C8, C5, C13 respectively, which are  $\beta$ -oriented, as in mulinolic acid, whereas the hydroxyl group at C13 is  $\alpha$ -oriented, (Fig.1). The Cremer and Pople parameters<sup>15</sup> for the cyclopentane (A), cyclohexane (B) and cycloheptene (C) rings are: Q<sub>5</sub> = 0.412(6) Å,  $\varphi_5$  = 115.6(9)°; Q<sub>1</sub> = 0.595(6) Å,  $\theta$  = 174.3(6)°,  $\varphi$  = 107(6)° and Q<sub>2</sub> = 0.207(6) Å,  $\varphi_2$  = 104.4(18)°, Q<sub>3</sub> = 0.592(6) Å,  $\varphi_3$  = 334.9(6)°, 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 1.955 Å and O—H...O angles of 149° (Table 2), so in the crystal the molecules are linked by two intermolecular O—H...O hydrogen

bond forming bidimensional supramolecular structures with graph-set notation  $R_4^4(12)$ ,  $R_4^4(40)$ ,  $R_6^6(46)$  and  $C_4^4(46)$ .<sup>16</sup>, (Fig. 3 and Fig. 4). All bond distances and angles are normal.

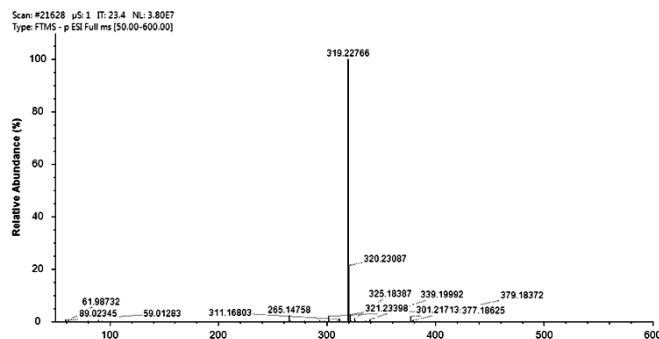


Fig. 1: Full Orbitrap HESI (-) spectra of compound mulinolic acid.

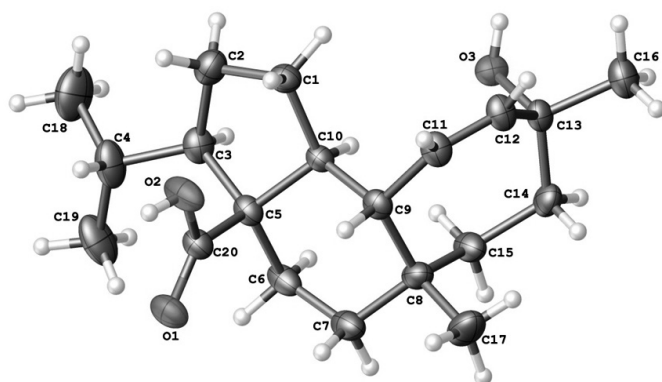


Fig.2: ORTEP Diagram of mulinolic acid. The ellipsoids are shown at the 30% probability level.

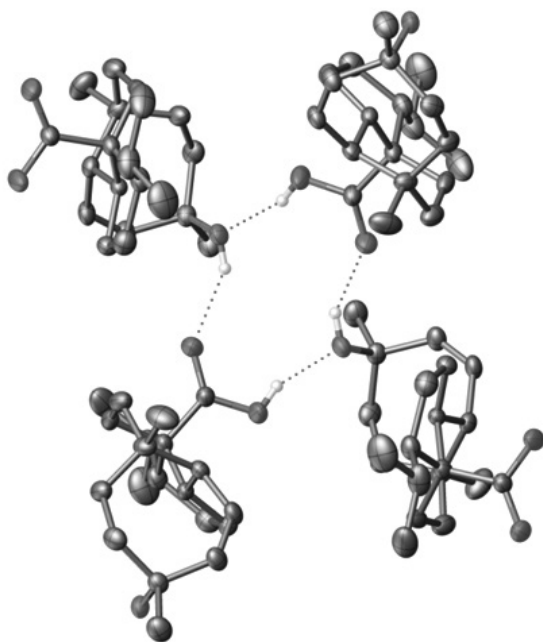


Fig.3 A view of the bi-dimensional supramolecular aggregate, showing the formation of ring with set-graph motif  $R_4^4(12)$  as representative example. For the sake of clarity, H-atoms bonded to C atoms have been omitted.

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

#### Crystal data

$C_{20}H_{32}O_3$	$V = 4151.3(9) \text{ \AA}^3$
$M_r = 320.45$	$Z = 8$
Orthorhombic, $C222_1$	$CuK\alpha (\lambda = 1.54178 \text{ \AA})$
$a = 14.7039(19) \text{ \AA}$	$\mu = 0.526 \text{ mm}^{-1}$
$b = 22.772(3) \text{ \AA}$	$T = 296(2) \text{ K}$
$c = 12.3979(15) \text{ \AA}$	$\alpha = 90^\circ$
	$\beta = 90^\circ$
	$\gamma = 90^\circ$
Density (calculated)/ $Mg/m^3$	1.025
	$F(000) = 1408.0$

#### Data Collection

Diffractometer Bruker AXS D8-Venture, Triumph-I $\mu$ -Cu	
2621 reflections with $I > 2\sigma(I)$	
22363 measured reflections	$R_{int} = 0.069$
2298 independent reflections	
2 $\theta$ range for data collection	7.156 to 111.436 $^\circ$
Index ranges	$-15 \leq h \leq 15, -23 \leq k \leq 24, -13 \leq l \leq 13$

#### Refinement

$R[F^2 > 2\sigma(F^2)] = 0.072$	215 parameters
$wR[F^2] = 0.201$	$S = 1.14$
	$\Delta\rho_{max} = 0.53 \text{ e}\cdot\text{\AA}^{-3}$
	$\Delta\rho_{min} = -0.23 \text{ e}\cdot\text{\AA}^{-3}$
Flack parameter <sup>15</sup> 0.05(11)	Goodness-of-fit on $F^2$ 1.139

**Table 2:** Hydrogen-bonding geometry ( $\text{\AA}$ ,  $^\circ$ ).

D—H...A	D—H	H...A	D...A	D—H...A
O2—H2...O3 <sup>i</sup>	0.82	1.83	2.600(6)	156
O3—H3A...O1 <sup>ii</sup>	0.82	2.08	2.776(6)	142

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

## CONCLUSIONS

In order to establish its absolute configuration, a single-crystal X-ray diffraction analysis of mulinolic 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  $C13R$ .

#### Supplementary material

CCDC- 1548215 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](http://www.ccdc.cam.ac.uk/data_request/cif).

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## REFERENCES

- 1.- C. Munizaga, H. Gunkel, Notas etnobotánicas del pueblo atacameño de Socaire. Publicación No. 5. Universidad de Chile, 1958.
- 2.- J.Bórquez, A. Ardiles, L. A. Loyola, L.M.Peña-Rodriguez, G.M. Molina-Salinas, J. Vallejos, I.G.Collado, M.J. Simirgiotis. *Molecules*, **19**, 3898, (2014)
- 3.- I.Brito, M. J. Simirgiotis, A. Brito, Werner, M. R., Bórquez, J., Winterhalter, P., A. Cárdenas. *Journal of the Chilean Chemical Society* **60**, (1), 2864, (2015)
- 4.- I.Brito, J. Bórquez, M.Simirgiotis, M. Neves-Vieira, G.Jerz, P.Winterhalter, M. Bolte, A. Cárdenas. *Zeitschrift für Kristallographie-New Crystal Structures* **229**, 399, (2014)

- 5.- J. Bórquez, N.L. Bartolucci, C. Echiburú-Chau, P. Winterhalter, J. Vallejos, G. Jerz, M.J. Simirgiotis. *Journal of the Science of Food and Agriculture* **96**, (8), 2832, (2016)
- 6.- L. A. Loyola, J. Bórquez, G. Morales, A. San Martín. *Phytochemistry*, **44**, 649, (1997)
- 7.- L. A. Loyola, J. Bórquez, G. Morales, A. San Martín. *Phytochemistry*, **4**, (1), 165, (1996)
- 8.- L.A. Loyola, J. Bórquez, G. Morales, A. San Martín., *Phytochemistry*, **53**, 96, (2000)
- 9.- L.A. Loyola, J. Bórquez, G. Morales, A. San Martín. *Phytochemistry*, **45** (7), 1465, (1997)
- 10.- I. Brito, J. Bórquez, L. A. Loyola, A. Cárdenas, M. López-Rodríguez. *Acta Cryst. E* **64**, o1209, (2008)
- 11.- I. Brito, J. Bórquez, J. Albanez, M. Bolte, L. M. Peña-Rodríguez. *Acta Cryst. E* **66**, 02452, (2010)
- 12.- G. M. Sheldrick, *Acta Cryst.* **112**, A64, (2008)
- 13.- O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, *J. Appl. Cryst.*, **42**, 339, (2009)
- 14.- D. Cremer, J.A. Pople. *J. Am. Chem. Soc.* **97**, 1354, (1975)
- 15.- H.D. Flack. *Acta Cryst.* **A39**, 876, (1983)
- 16.- J. Bernstein, R.E. Davis, L. Shimoni, N.-L. Chang. *Angew. Chem. Int. Ed. Engl.* **34**, 1555, (1995)