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CRYSTAL AND MOLECULAR STRUCTURE OF 14α-ACETOXY-13α-HYDROXYMULIN-11-EN-20-OIC ACID MONOHYDRATE

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ABSTRACT

The structure of this 14α-acetoxy-13α-hydroxymulin-11-en-20-oic acid monohydrate consists of a mulinane skeleton and the corresponding isopropyl, methyl, carboxyl and methyl groups at C3, C8, C5, C13, respectively, which are β-oriented, whereas the hydroxyl and acetoxy groups at C13 and C-14 are α-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 organic molecules are linked by the water molecules by two intermolecular O—H···O hydrogen bond forming 1D-dimensional chains with graph-set notation (9).

Keywords: mulinane, diterpenoid, *Azorella* compacta, X-ray diffraction, crystal and supramolecular structure.

INTRODUCTION

Azorella compacta Phil. (Apiaceae) is a compact resinous cushion shrub that grows in the Andes Peru, Bolivia, Argentina and Chile under extreme conditions altitude and desert-like environment. This is a plant which grows at an extremely slow growth rate of only 1 cm in 20 years[1]. Azorella compacta together with *Mulinum crassifolium* are a rich source of diterpenoids with both the mulinane and azorellane skeletons[2-6].The rare diterpenoids isolated from these genera have displayed a wide variety of interesting biological activities, including trypanomicidal, trichomonicidal, toxoplasmocidal, antiplasmodial, antibacterial, spermicidal, antihyperglycemic, antitubercular, antiinflamatory and analgesic activities[7]. Both plants are extensively used in folk medicine against diabetes, bronchitis, high-altitude sickness, pneumonia, rheumatism, and have also wound healing effects[1-8]. The goal of the preparation of the title compound is to prove activity against Mycobacterium tuberculosis strains[9].

EXPERIMENTAL

Dried aerial parts of *Azorella compacta* Phil. (Apiaceae), Fig. 2 (745 g) were defatted with *n*-hexane (3 times, 1 L each time, 1 day/extraction) and the remaining plant material was extracted with ethyl acetate (3 times, 1 L each time, 1 day/extraction) at room temperature for one day each and concentrated as stated above to yield 7.2 g of a brown gum. This extract was filtered and submitted to a medium pressure column chromatography system composed of an 4.0 cm x 39 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 ten combined fractions were obtained. Fraction 5 (450 mg) was rechromatographed and was obtained the known compounds mulinolic acid[10] (30 mg) and 13α,14α-dihydroxymulin-11-en-20-oic acid[3,11] (60 mg). The latter compound (40 mg) was acetylated with Ac_2O and pyridine in the usual manner, yielding 32 mg (71.7 %) of pure compound[3]. Recrystallization of this compound from n-hexane : EtOAc (9.5:0.5) a room temperature yielded white crystals, which were suitable for X-ray diffraction analysis.

Plant Material

Azorella compacta Phil. were collected in *El Tatio*, Antofagasta, Chile in March 2011. Voucher herbarium specimen this deposited at the Laboratory of Natural Products, University of Antofagasta with the numbers Ac-031511.

14α-acetoxy-13α-hydroxymulin-11-en-20-oic acid monohydrate: Colourless crystals, m.p. 123-125 °C. The molecular weight was determined by orbitrap HR-ESI-MS/MS with a mass spectrometer (Q-exactive Focus, Bremen, Germany). $[M-H]^-$: required: 377.23335, found: 377.23322 for $C_{22}H_{33}O_5^-$ (Fig. 1). IR cm^{-1}: 3300-2800 br, 1703 (COOH), 1735 ($OCOCH_3$).^{1}H NMR (Bruker Avance 300 MHz, $CDCl_3$) δ ppm: 0.85 (3H, d, J = 5.5 Hz, Me-18), 1.07 (3H, s, Me-17), 1.02 (3H, d, J = 5.5 Hz, Me-19), 1.15 (1H, dd, J = 2.9, 14.0 Hz, H-15β), 1.32 (3H, s, Me-16), 1.47 (2H, m), 1.56 (3H, m), 1.76 (3H, m), 1.83 (1H, dd, J = 13.5/11.8 Hz, H-15 α), 2.10 (3H, s, $COCH_3$), 2.10 (1H, dd, H-9), 2.30 (2H, m), 2.44 (1H, m, H-3), 2.60 (1H, m), 2.83 (1H, dd, J=13.5/11.8 Hz), 5.01 (1H, dd, J = 11.8/2.9 Hz, H-14), 5.54 (1H, d, J = 12.4 Hz, H-12), 5.76 (1H, d, J = 12.4/8.4 Hz, H-11). ^{13}C NMR (^{13}C NMR Bruker Avance 75 MHz, $CDCl_3$) δ ppm: 180.1 (COOH), 170.1 (O**C**OCH_3), 136.2 (C-11), 132.4 (C-12), 72.2 (C-13), 58.0 (C-5), 57.4 (C-3), 50.7 (C-10), 47.0 (C-9), 41.9 (C-7), 75.2 (C-14), 36.1 (C-8), 32.1 (C-6), 31.7 (C-4), 36.6 (C-15), 28.7 (C-2), 29.3 (Me-16), 27.3 (Me-17), 24.8 (C-1), 22.3 (Me-19), 22.7 (Me-18), 21.3 ($OCOCH_3$**)**. These data together with HR-MS are consistent with the structure of 14α-acetoxy-13α-hydroxymulin-11-en-20-oic (Fig. 1).

Data collection, structural determination and refinement was performed with a Bruker AXS D8-Venture, Triumph-μI-Cu with graphite-monochromated CuKα radiation (1.54178 Å). The structure was solved by direct method, and was refined against F² by full-matrix least-squares methods using SHELXL[12.] Disordered solvent molecules were not modelled and the disordered density was taken into account using the SQUEEZE/PLATON[13] procedure. 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-2013[12], program used to refine structure: SHELXL-2013[12], molecular graphics[14].

RESULTS AND DISCUSSION

HREIMS of 1 showed an ion consistent with a molecular formula $C_{22}H_{33}O_5^-$ (requires m/z 377.23335, found 377.23322) and its IR spectrum revealed the presence of a carboxyl group (3500-2500 br) and ester group. The total of 22 carbons suggested the presence of an acetilated diterpene. The ^{1}H NMR and ^{13}C NMR spectral data were easily assigned by comparison with NMR data of related compounds and in particular with the parent compounds[2,5,6,9]. These spectral data together with ^{1}H COSY, show the presence of an isopropyl group [δ_{C-4} 31.7 (CH), 22.7 (Me-18) and 22.3 (Me-19); δ_{H-4} 1.47, overlapped signal, 0.85 (Me-18) and 1.02 (Me-19), a tertiary methyl group [δ_C 27.3; δ_H 1.07 s, Me-17), the methyl group at δ_H 1.32 (Me-16), a shift typical for a methyl group germinal to a hydroxyl group, showed cross-peaks to carbons at δ_{C-12} 132.4 and δ_{C-13} 72.2, a secondary acetoxy group [δ_{H-14} 5.01 (1H, dd, J=11.8/2.9 Hz; δ_{C-14} 75.2, d). All lack above data can be accommodated in the mulinane skeleton.

The molecular structure and the relative configuration of the title compound was confirmed by x-ray diffraction analysis of suitable single

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crystals (Fig. 3). Colorless block crystals of title compound with approximate dimensions 0.140 x 0.100 x 0.110 mm was obtained by slow evaporation of a ethyl acetate solution. The structure of this 14α-acetoxy-13α-hydroxymulin-11-en-20-oic acid monohydrate consists of a mulinane skeleton and the corresponding isopropyl, methyl, carboxyl and methyl groups at C3, C8, C5, C13, respectively, which are β-oriented, whereas the hydroxyl and acetoxy groups at C13 and C-14 are α-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. The Cremer and Pople parameters[15] for the cyclopentane (A), cyclohexane (B) and cycloheptene (C) rings are: $Q_2 = 0.419(7)$ Å, $\varphi_2 = 116.6(10)°$; $Q_T = 0.582(6)$ Å, $\theta = 172.8(7)°$, $\varphi = 130(5)°$ and $Q_2 = 0.251(7)$ Å, $\varphi_2 = 123.0(5)°$, $Q_3 = 0.613(6)$ Å, $\varphi_3 = 331.0(6)°$, 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 main differences between the title compound and the similar compounds[17,24] are in the conformation of the C ring, (chair in both compounds). In other closely related compounds, the conformation of the rings A, B and C are retained respect to of the title compound[22,23].

All stereochemistry and geometric parameters are similar to the parent compounds (mulinane diterpenoid) and all bond distances and angles are comparable and normal.

In the crystal the organic molecules are linked by the water molecules by two intermolecular O—H···O hydrogen bond, with the average H···O distance of 2.046(7) Å and O–H···O angles of 164.7(5)° forming 1D-dimensional chains with graph-set notation[16] (9), along [010] direction, Fig. 4. One weak intramolecular hydrogen bond interaction is observed between O6–H···O3.

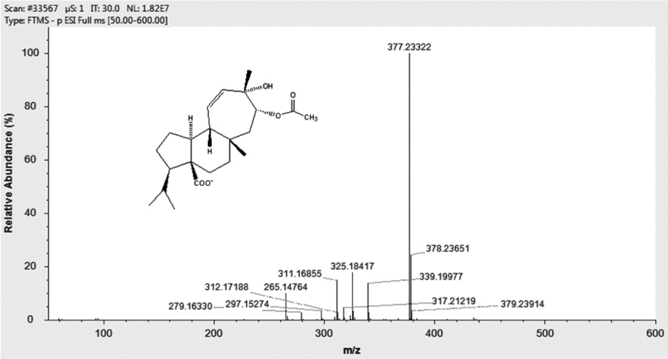

Fig. 1: Full Orbitrap HESI (-) spectra of compound 14α-acetoxy-13α-hydroxymulin-11-en-20-oic acid monohydrate

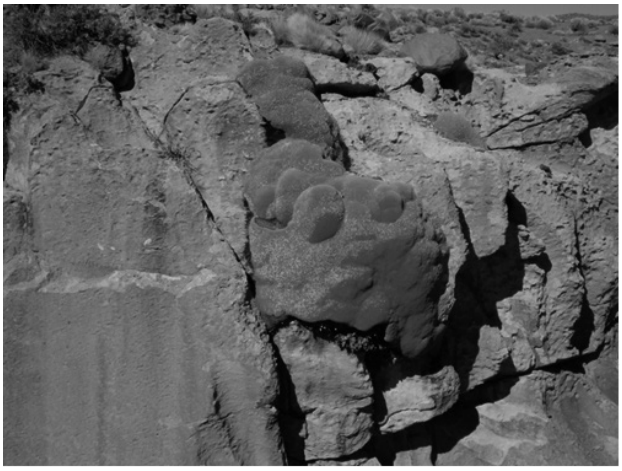

Fig. 2 *Azorella compacta* from Northern Chile. (Taken by Jorge Bórquez, on March 2011).

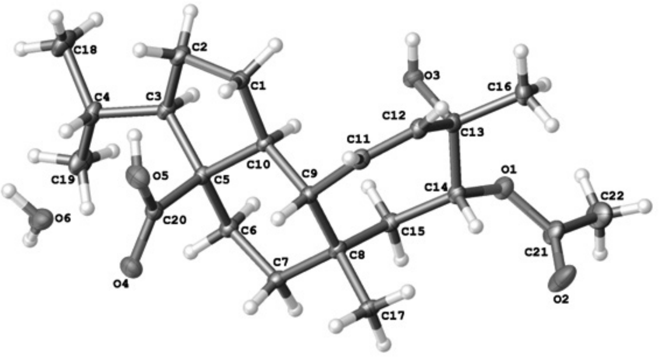

Fig. 3. ORTEP Diagram of the title compound. The ellipsoids are shown at the 20% probability level.

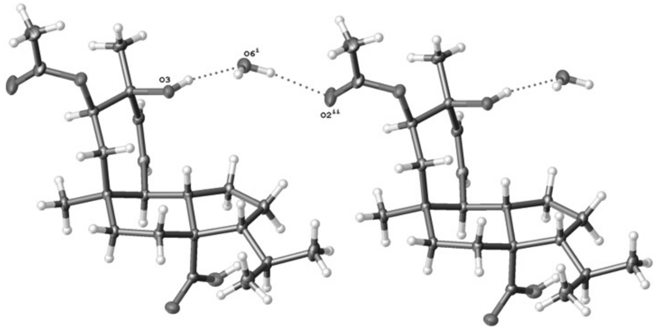

Fig. 4 A view of the one-dimensional supramolecular aggregate, showing the formation of chains with set-graph motif (9) as representative example. [Symmetry code: (i) -1+x,y,z, (ii) x,-1+y,z]

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

Crystal data

$C_{22}H_{34}O_5{\cdot}H_2O$ V = 1336.7(5) Å^3
Mr = 396.51 Z = 2
Monoclinic, $P2_1$ CuKα (λ = 1.54178 Å)
a = 9.089(2) Å α = 90° μ = 0.57 mm^{-1}
b = 10.055(2) Å β = 104.068(14)° T = 296(2) K
c = 15.079(4) Å γ = 90° Colourless, Block, 0.140 x 0.100 x 0.110 mm
Density (calculated)/Mg/m^3 0.985 F(000) = 432

Data Collection

Diffractometer Bruker AXS D8-Venture, Triumph-Iμ-Cu
2660 reflections with I>2σ(I)
17057 measured reflections $R_{int} = 0.099$
3796 independent reflections
2θ range for data collection 10.032 to 118.314°
Index ranges -9 ≤ h ≤ 10, -10 ≤ k ≤ 11, -16 ≤ l ≤ 16

Refinement

$R[F^2>2\sigma(F^2)] = 0.066$ 259 parameters
$wR[F^2] = 0.202$ S = 1.06
Flack parameter[23] 0.4(2) $\Delta\rho_{max} = 0.22$ e·Å^{-3}
$\Delta\rho_{min} = -0.21$ e·Å^{-3}

CONCLUSIONS

All stereochemistry and geometric parameters are similar to the parent compounds (mulinane diterpenoid). The main observed difference with same related compounds is the cycloheptene ring conformation. All bond distances and angles are normal.

Supplementary material

CCDC- 1572802 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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