ABSOLUTE CONFIGURATION OF 18-ACETOXY-CIS-CLERODA-3,13E-DIEN-15-OIC ACID

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ABSTRACT

In this paper we report the absolute configuration which has been determined from the refinement of the Flack parameter, x = 0.05(5), which indicate that the correct configuration had been assigned against 1353 (99%) CuKα Bijvoet pairs. On this basis the absolute configuration was assigned as C5*R*, C8*S*, C9*R* and C10*S*. The structure of 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid consists of a clerodane skeleton and the corresponding methyl groups are α-oriented (C8, C9) while C5 is β-oriented. The acidic lateral chain is β-oriented and the double bond between C13 and C14 has E isomeric. The ciclohexene, cyclohexane rings are cis fused, and in an sofa and chair conformation respectively. In the crystal the molecules are linked by one intermolecular O—H···O hydrogen bond forming 1D-dimensional chain with distance donor-acceptor of 2.060(6)Å with graph-set notation $C_1^1(15)$.

INTRODUCTION

Following our program to isolate interesting metabolites from the Atacama Desert Flora, Northern Chile[10,12-17]. Now we are dedicated to the study of *Haplopappus rigidus* Phil., one of the species known in Chile by the popular name "baylahuen", is an endemic plant found at a height of over 3000 m in the Andes mountains and used as medicinal by the local population and Aymara aboriginals since precolombian times[1]. All the related Chilean *Haplopappus* "baylahuen" species produce a resinous exudate with interesting biologically active compounds including, several clerodane and labdane diterpenoids and flavonoids with biological significance[2]. Indeed, the Mapuche medicinal plants *Haplopappus multifolius*, *H. taeda*, *H. baylahuen* and *remyanus* showed the presence of flavonoids and labdane terpenoids with known antioxidant, choleretic, anti-inflammatory and cholagogue properties[3,4,5]. In the Aymara region, the infusion of *H. rigidus* has been used to cure or prevent liver diseases, gastro-intestinal disorders, cough and has also been employed as a sexual stimulant attributed to the presence of diterpenoids and flavonoids[1,6,7]. Moreover, from the resinous exudate of *H. incinatus* the antibacterial potent clerodane diterpenoid: 18-acetoxy-cis-cleroda-3-en-15-oic acid was isolated[2,8], while from the aerial parts of *H. rigidus* Phil, the related main clerodane rigidusol (13-hydroxy-18-acetoxy-cis-cleroda-3,14-diene) exhibited moderate cytotoxic activity against human breast adenocarcinoma cell line MCF-7[9]. In this work we report the absolute configuration of the rigidusol derivative 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid by X-ray diffraction methods, which is consistent with the previously reported for this compound[2,8] (Figure 1).

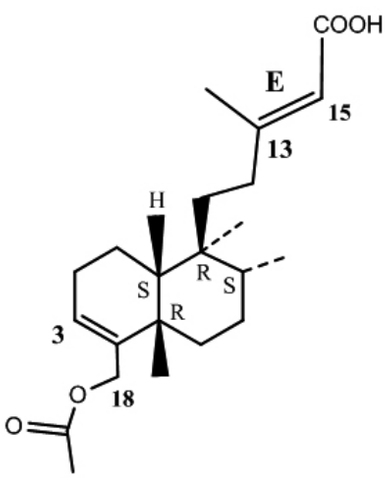

Figure 1. Absolute configuration of 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid.

EXPERIMENTAL

The title compound $C_{22}H_{34}O_4$, is a clerodane diterpenoid firstly isolated from aerial parts of *Croton chilensis* (Muell. Arg) and identified as 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid by spectral data and relative configuration by X-ray diffraction methods[10]. The later compound was also isolated and reported from *Haplopappus desertícola*[11]. Following our program to isolate interesting metabolites from the Atacama Desert Flora, Northern Chile[12-18], dried aerial parts of *Haplopappus rigidus* (1050 g) collected in april 2015 in "El Tatio", Andean mountain range of the of Atacama Desert, II Region, Northern Chile, were defatted with hexane (3 liters, 3 times in the dark, 24 hours each time) and 58.88 g were obtained after evaporation of a ethyl acetate solution. A portion of the extract (30.30 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 (226) were combined according to TLC analysis (Kieselgel F254 plates, developed with Hexane: EtOAc 7:3 v/v and spots visualized by spraying with vanillin: sulfuric acid 2 % in ethanol and heating). From fractions 24-37, the known compound: 13-hydroxy-18-acetoxy-cis-cleroda-3,14-diene[9] (2.42 g) was isolated and from fractions 94-135 the known compound **1** (18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid, 1.53 g) was isolated previously from the botanic specie Croton chilensis[10]

Recrystallization from ethyl acetate/n-hexane a room temperature yielded pure white crystals of the last compound, suitable for X-ray diffraction analysis.

The relative stereochemistry of the title compound was assigned by spectroscopic data and by chemical transformations[8]. In this paper we report the absolute configuration of the title compound which has been determined from the refinement of the Flack parameter[19], x = 0.0(5), which indicate that the correct configuration had been assigned against 1353 (99%) CuKα Bijvoet pairs. On this basis the absolute configuration was assigned as C5*R*, C8*S*, C9*R* and C10*S*.

White crystals, m.p. 100-102 °C. The molecular weight was determined by Q-orbitrap HESI-MS/MS with a mass spectrometer (Q-exactive Focus, Bremen, Germany) $[M-H]^-$: 361.23825 calcd. for $C_{22}H_{33}O_4^-$: 361.23843 (Figure 2). ^{1}H NMR (Bruker Avance 300 MHz, $CDCl_3$) δ ppm: 5.70 (2H, s, H-3/H-14), 4.59 (2H, s, H-18), 2.19 (3H, s, Me-16), 2.16 (2H, m, H-2), 2.07 (3H, s, $COCH_3$), 2.03 (1H, m, H-1), 2.01 (2H, m, H-12), 1.95 (1H, m, H-6), 1.81 (1H, m, H-1'), 1.62 (1H, m, H-11), 1.42 (2H, m, H-8/H-11'), 1.36 (2H, m, H-7/H-10), 1.29 (2H, m, H-6'/H-7'), 1.19 (3H, s, Me-19), 0.79 (3H, s, Me-20), 0.77 (3H, s, Me-17). ^{13}C NMR (^{13}C NMR Bruker Avance 75 MHz, DMSO-d6) δ ppm: 17.2 (C-1), 23.9 (C-2), 129.2 (C-3), 138.2 (C-4), 40.20 (C-5), 37.1 (C-6), 28.7 (C-7), 37.5 (C-8), 36.3 (C-9), 45.1 (C-10), 36.1 (C-11), 34.7 (C-12), 164.6 (C-13), 114.8 (C-14), 170.9 (C-15), 19.5 (C-16), 15.9 (C-17), 66.6 (C-18), 34.6 (19), 17.2 (20), 171.0 (21), 21.2 (C-22). These data, together with ESI-MS/MS and correlations observed in the HSQC and HMBC spectra, are consistent with the structure of 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid

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(Figures 1 and 3) confirmed by comparison of spectroscopic data with those reported in the literature for this structure[8-11] .

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 F^2 by full-matrix least-squares methods using SHELXL[20]. 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[20], program used to refine structure: SHELXL-2013[20], molecular graphics[21].

RESULTS AND DISCUSSION

The X-ray crystal-structure determination of the title compound was undertaken in order to establish its absolute configuration. The structure of 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid consists of a clerodane skeleton and the corresponding methyl groups are α-oriented (C8,C9) while C5 is β-oriented. The acidic lateral chain is β-oriented and the double bond between C13 and C14 has a E isomeric configuration.

The Cremer and Pople parameters[22] for the ciclohexene and ciclohexane rings are:

Q_T = 0.455(10) Å, θ = 134.4(13)°, φ = 115.2(17)° and Q_T = 0.560(9) Å, θ = 12.7(9)°, φ = 179.4(4)° so the onformation is sofa and chair respectively. The ciclohexene, ciclohexane rings are cis fused. In the crystal the molecules are linked by one intermolecular O—H···O hydrogen bond forming 1D-dimensional chain with graph-set notation (15)[23] (O2—H2···O4^i 149°; O2···O4^i 2.797(9) Å; H2···O4^i 2.06 Å (symmetry code (i) x,-1+y,1+z), (Figure 4). The absolute configuration of the title compound which has been determined from the refinement of the Flack parameter[19], x = 0.0(5), which indicate that the correct configuration had been assigned against 1353 (99%) CuKα Bijvoet pairs. On this basis the absolute configuration was assigned as C5*R*, C8*S*, C9*R* and C10*S*. All distances and angles are normal.

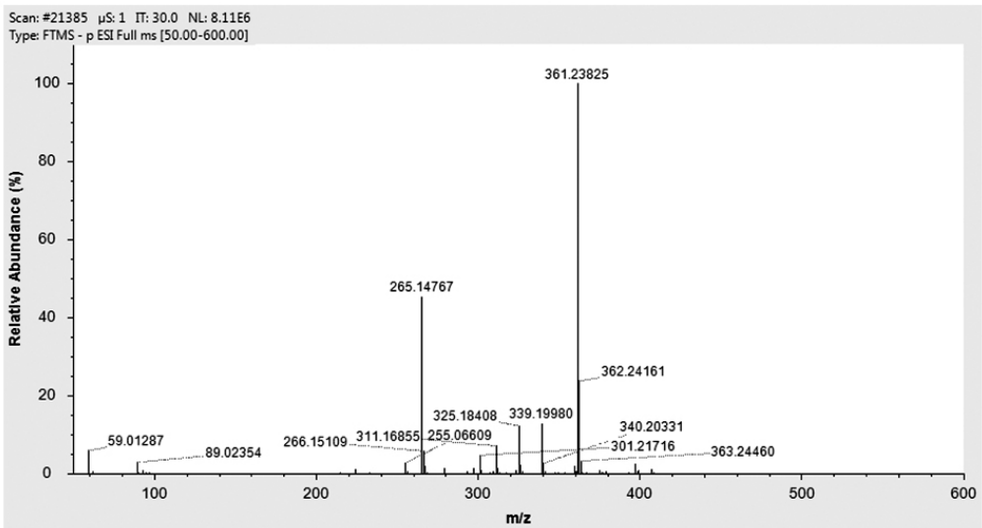

Figure 2. Full Q-Orbitrap HESI (-) spectra of compound 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid.

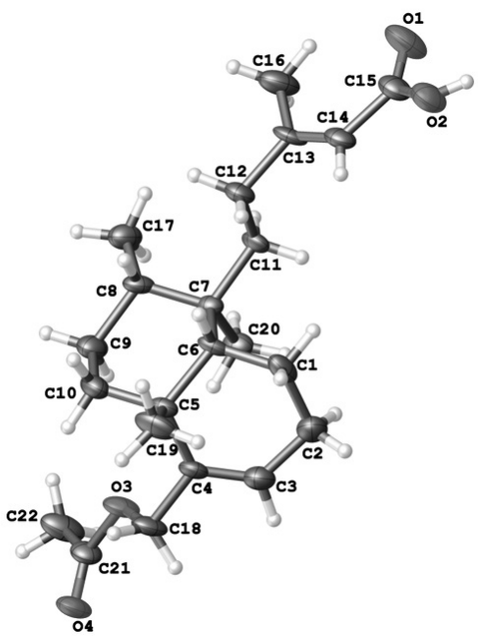

Figure 3. ORTEP Diagram of 18-acetoxy-cis-cleroda-3,13E-dien-15-oic acid. The ellipsoids are shown at the 30% probability level.

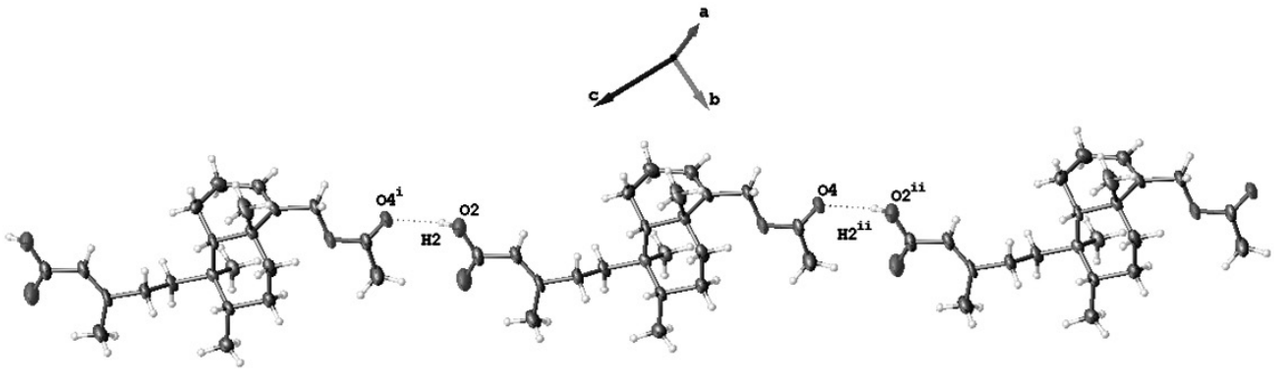

Figure 4. A view of the one-dimensional supramolecular aggregate, showing the formation of chain with set-graph motif (15)[23]. [Symmetry codes: (i) x, -1+y, 1+z; (ii) x, 1+y, -1+z].

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

Crystal data

$C_{22}H_{34}O_4$		V = 1038.7(2) Å^3
Mr = 362.49		Z = 2
Monoclinic, $P2_1$		CuKα (λ = 1.54178 Å)
a = 10.0779(11) Å	α = 90°	μ = 0.62 mm^{-1}
b = 8.0098(9)Å	β = 109.788(7)°	T = 296(2) K
c = 13.6747(13)Å	γ = 90°	Colourless, Block, 0.180 x 0.170 x 0.09 mm
Density (calculated)/Mg/m^3 1.159		F(000) = 396

Data Collection

Diffractometer Bruker AXS D8-Venture, Triumph-Iμ-Cu	
1485 reflections with I>2σ(I)	
15972 measured reflections	
2972 independent reflections	
2θ range for data collection	6.870 to 118.01°
Index ranges	$-11 \leq h \leq 11, -8 \leq k \leq 8, -15 \leq l \leq 15$

Refinement

$R[F^2>2\sigma(F^2)] = 0.069$	241 parameters
$wR[F^2] = 0.183$	Flack parameter[19] 0.0(5)
$\Delta\rho_{max} = 0.22$ e·Å^{-3}	Number Bijvoet pairs: 1353(99%)
$\Delta\rho_{min} = -0.20$ e·Å^{-3}	S = 0.94

CONCLUSIONS

In the title compound the molecular structure and its relative configuration were established by spectroscopic and crystallographic methods twenty three year ago[10], but not of the absolute configuration, due to the experiment condition used (Mo, radiation). In order to establish its absolute configuration, a single-crystal X-ray diffraction analysis of was undertaken, using Cu radiation. The X-ray molecular model of the title compound (Figure 3), confirmed all the above deductions on its structure and established the absolute configuration as C5*R*, C8*S*, C9*R* and C10*S*.

Supplementary material

CCDC-1812263 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.as.uk/data_request/cif.

ACKNOWLEDGEMENTS

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