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Azorellanol : A Diterpenoid with a New Carbon Skeleton from *Azorella compacta*

Luis Alberto Loyola*, Jorge Bórquez, Glauco Morales

Laboratorio de Productos Naturales, Facultad de Ciencias Básicas, Universidad de Antofagasta, Camino a Coloso S/N, Antofagasta, Chile

Aurelio San-Martín, Víctor Manríquez

Departamento de Química, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Santiago, Chile.

Oscar Wittke

Departamento de Física, Facultad de Ciencias Físicas y Matemáticas, Universidad de Chile, Beaucheff 850 Santiago, Chile.

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Abstract: A new diterpenoid, azorellanol (**5**), was isolated from the whole plant of *Azorella compacta* (Umbelliferae) .The structure was established by using mainly one- and two-dimensional NMR techniques, whereas the absolute stereochemistry was determined by X-ray diffraction analysis. The diterpenoid (**5**) possesses a novel hydrocarbon skeleton for which we suggest the name azorellane

Keywords: *Azorella compacta;* Umbelliferae; azorellane; diterpenoid; azorellanol; X-ray analysis.

Azorella compacta Phil. (Umbelliferae), llareta, is a yellow-green, compact resinous cushion shrub, which grows in the high Andes of southern Perú Bolivia, northeastern Chile and northwestern Argentina.[1] Bitter taste infusions of the whole plant are used in folk medicine, principally in the treatment of diabetes as well as for asthma, colds, bronchitis, kidney and womb complaints.[1]

In previous communications,[2,3] we reported the structures of the mulinane derivatives, mulinolic acid (**1**), mulinic acid (**2**), mulin-11,13-dien-20-oico acid (**3**) and mulinol (**4**), which are obtained from the petrol ether extract from *Azorella compacta* Phil.[2,3] We now wish to report the structure of azorellanol (**5**), a diterpenoid that possesses a novel carbon skeleton.

RESULTS AND DISCUSSION

Azorellanol (**5**) has the molecular formula $C_{22}H_{36}O_3$, as deduced by a combination of ^{13}C-NMR data and High-resolution mass spectrometry on [M-H_2O]$^+$ (obtained 330.2406 ; required 330.2559). The ^{1}H-decoupled ^{13}C-NMR spectrum of **5** showed resonances of 22 carbons; **DEPT** analysis using an angle

*E-mail address: aloyola@uantof.cl

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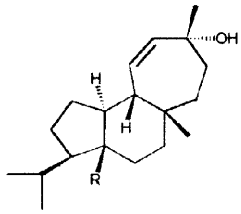
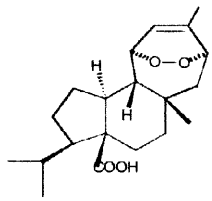
of 90°, indicated five saturated methines at δ 78.5, 59.2, 47.4, 31.7 and 25.9. The **DEPT** 135° spectrum showed six methylenes and six methyl carbons indicating, after comparison with a decoupled spectrum (Table 1), that the carbons at δ 170.9, 69.6, 42.4, 34.4, and 26.6 were not attached to hydrogens. The IR, ^{1}H-NMR and ^{13}C-NMR data of **5** suggested the presence of an acetoxyl group (1.745 cm^{-1}, δ 2.02 s and δ 170.9 s) and revealed that the remaining proton was part of a tertiary hydroxyl group (3.465 cm^{-1} br, δ 69.6 s).



1 R=COOH
4 R=CH_2OH



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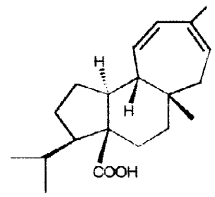

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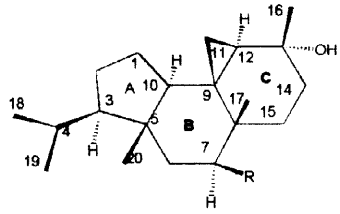
The lack of olefinic or other carbonyl resonances in the ^{13}C NMR spectrum of compound **5** (Table 1) indicated that rings had to account for remaining four sites of unsaturation in the molecule. The ^{1}H and ^{13}C-NMR spectra of azorellanol (**5**) (Table 1), together with ^{1}H COSY and HMQC and HMBC experiments, revealed the presence of an isopropyl group [δ_C 31.7 (CH), 22.7 (CH_3) and 22.5 (CH_3); δ_H 1.50 overlapped signal, 0.84 d and 0.93 d (J = 6.0 Hz in both signals)], two tertiary methyl groups [δ_C 19.0 (CH_3), 17.7 (CH_3) ; δ_H 1.10 s, 0.91 s], another tertiary methyl group attached to hydroxyl carbon [δ_C 30.2 (CH_3), 69.6 (C) ; δ_H 1.27 s], a secondary acetate group [δ_C 21.5 (CH_3), δ_H 2.02 s, δ_C 78.5 (CH), and 5.28 dd (J = 11.2 and 7.0 Hz)], and three shielded proton resonances at δ_H 0.13 t (J = 4.6 Hz), 0.69 dd (J = 10.0 and 4.6 Hz) and 0.76 dd (J = 10.0 and 4.6 Hz), which were assigned to the protons of a cyclopropane ring (methylene carbon at δ_C 10.7, methine carbon at δ_C 25.9 and quaternary carbon at δ_C 26.6).

All the above data could be accommodated in a mulinane carbon skeleton with C-9, C-11 and C-12 forming part of a cyclopropane ring, as depicted in formula **5**.



5 R=OAc
6 R=OH
7 R=O-p-Br-Bz

Table 1 . ^{1}H and ^{13}C-NMR Data[a,b] for Azorellanol (5).

Position	δ ^{1}H	m	J (Hz)	δ ^{13}C	m
1	α 0.91	+		20.9	t
	β 1.21	+			
2	α 1.21	+		27.7	t
	β 1.80	m			
3	1.24	+		59.2	d
4	1.50	+		31.7	d
5				42.4	s
6	α 2.20	dd	13.8 and 7.0	39.5	t
	β 1.50	dd	13.8 and 11.2		
7	α 5.28	dd	11.2 and 7.0	78.5	d
8	-			34.4	s
9	-			26.6	s
10	2.41	dd	12.0 and 7.1	47.4	d
11	β 0.13	t	4.6	10.7	t
	α 0.76	dd	10.0 and 4.6		
12	α 0.69	dd	10.0 and 4.6	25.9	d
13				69.6	s
14	β 1.21	+		30.9	t
	α 1.33	+			
15	β 1.16	+		31.9	t
	α 1.50	+			
16	1.27	s		30.2	q
17	1.10	s		19.0	q
18	0.84	d	6.0	22.7	q
19	0.93	d	6.0	22.5	q
20	0.91	s		17.7	q
21				170.9	s
22	2.02	s		21.5	q

a) Bruker AMX 200 instrument operating at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR, using TMS as internal standard.
b) Assignments aided by ^{1}H- ^{1}H-COSY, HMQC, HMBC, ROESY.
+) Overlapped signal.

Most the structural fragments of azorellanol were identified by ^{1}H COSY, HMQC and HMBC spectrum (Fig. 1).

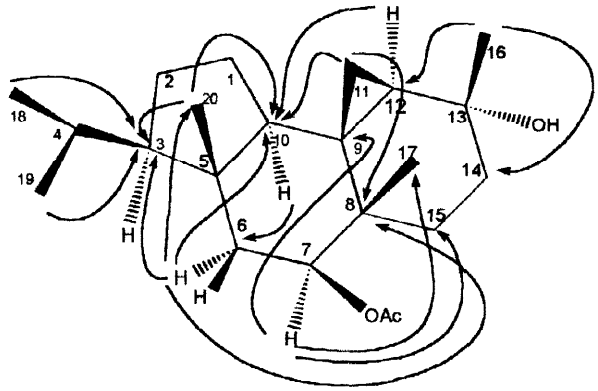
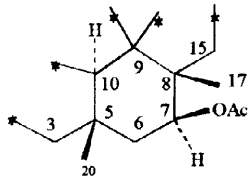


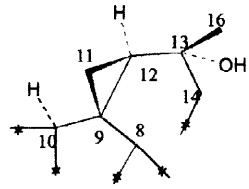
Fig.1. Main ^{1}H-^{13}C long-range correlations for azorellanol (5) detected by HMBC spectrum.

The signal at δ 5.28 was assigned to H-7, while those at δ 2.20 and 1.50 were attributed to C-6 protons. The long range ^{1}H-^{13}C correlations (Fig. 1) of the proton H-7 with C-6, C- 9, C-15, C-17 and C-21 and H-6 (δ 2.20) with C-3, C-5, C-7, C-8, C-10 and C-20 supported the partial structure A in **(5)**.

The signal at δ 0.69 (1H, dd, J = 10.0 and 4.6) was assigned to H-12 and the signals at δ 0.13 (1H, t, J = 4.6) and 0.76 (1H, dd J = 10.0 and 4.6) were attributed to C-11 protons, and the signal at δ 1.27 (3H, s) was assigned to the protons of Me-16. The long range ^{1}H-^{13}C correlations of the proton H-11 at δ 0.13 with C-8, C-9, C-10, C-12 and C-13, and the protons H-16 with C-12, C-13, and C-14 supported the presence of partial structure B in **5**.

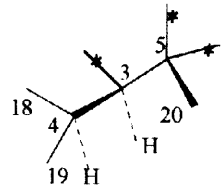


A



B

The ^{1}H COSY spectrum showed connectivities between the methyl groups (C-18 and C-19) at δ 0.84 and 0.93 (3H, d, J = 6.0 Hz) and an overlapped proton at δ 1.50 (H-4) which correlates with H-3 (δ 1.24, 1H, overlapped signal). The long range ^{1}H-^{13}C correlations (fig 1) of the protons H-18 and H-19 with C-3 and C-4 confirmed the position of the isopropyl group in **(5)**.



The principal results from ROESY NMR experiments (Fig. 2) suggested that azorellanol had the stereochemistry **5**, in agreement with the relative stereochemistry of all mulinane diterpenoids previously isolated from *Mulinum crassifolium*[4,5,6] and *Azorella compacta*.[2,3] The ROESY NMR experiment showed a correlation between the signal at δ 2.41 (1H, dd, J =12 and 7.1 Hz) for the α H-10 and the signals at δ 1.24 (H-3) and δ 5.28 (H-7), which should be in α configuration and that the isopropyl and acetate groups are in the β configuration. In addition α H-7 showed correlation with the signal at δ 2.20 (H-6) and 1.50 (H-15) indicating that they are in the same configuration. Moreover, the signal at δ 1.10 (3H, s) for β H-

17 showed correlation with the signals at δ 0.91 (Me-20) and δ 0.13 (H-11) which should also be β. A correlation between H-11 and H-14 (δ 1.21) and Me-16 (δ 1.27, s) indicated that the hydroxyl must be α.

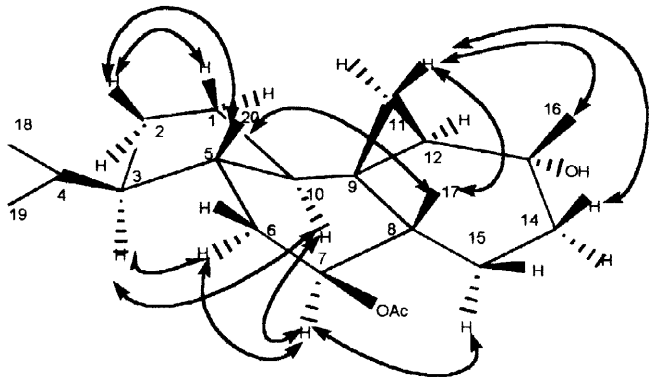


Fig. 2. Roesy results for azorellanol (**5**).

In order to confirm the stereochemistry and to establish the absolute configuration of **5**, a single crystal X-ray analysis was performed on the 7-p-bromobenzoyl derivative of 7-deacetylazorellanol (**6**). Fig. 3 shows a perspective drawing of the molecule of 7- p-Bromobenzoyl-7-deacetylazorellanol (**7**).

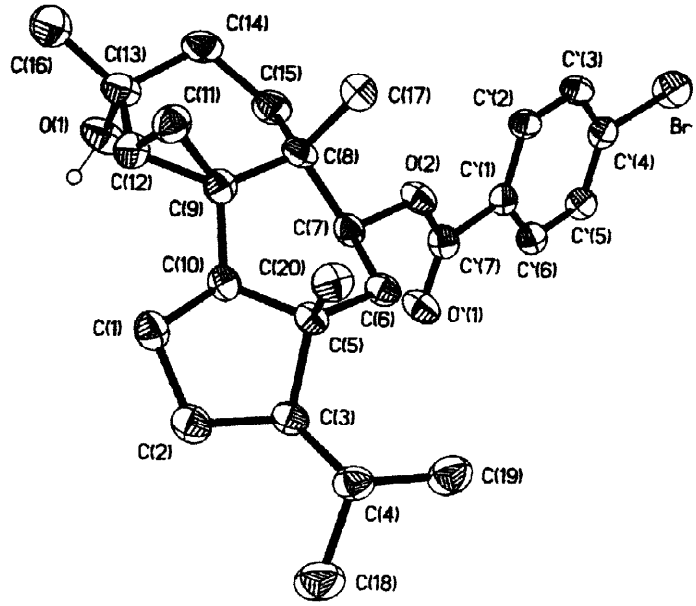


Fig. 3. Perspective drawing of 7 with atoms label.

7- p-Bromobenzoyl-7-deacetylazorellanol possesses a novel tricyclic carbon skeleton, with a six-membered ring with a methylene bridge between C-9 and C-12 that forms a 1,2-methylencyclohexane ring (C). It also has a six -membered ring (B) fused to a five membered ring (A) with methyl, hydroxyl, isopropyl and bromobenzoate substituents. The methyl substituents at C-5 and C-8 are axially disposed on one side of molecule, and the α-tertiary hydroxyl substituent is axially disposed on the other side. The methyl at C-13, bromobenzoate and isopropyl substituents are equatorially bonded to the molecule.

The 1,2-methylencyclohexane ring C is in a distorted half-chair conformation with C-9 as the flap -0.17 Å out of the mean plane defined by C-8, C-12, C-13 and C-15 as compared to 0.71 and 0.99 Å for C-14 and C-11. This flattening of ring C is essentially determined by the presence of C-11 bonded to C-9 and C-12 to form a cyclopropane ring that tends to constrain half of the ring to a planar arrangement. In the cylopropane ring C-9, C-11 and C-12 the geometry is almost a regular triangle with the C-9 and C-11 distance being slightly longer. This increased distance probably due to the different substitution between C-9 and C-12 atoms. On the other hand, the cyclohexane ring B adopts a twist boat conformation with C-7 and C-10 lying 0.69 and 0.56 above the best plane through the C-5, C-6, C-8 and C-9 atoms. Finally, the five-membered ring A is an envelope; C-1, C-2, C-3 and C-5 have a maximum deviation of 0.11 Å from their mean plane, whereas C-10 deviate from this plane by 0.64 Å.

The torsional angles of ring C and ring B about the common C-8 and C-9 bond are 131 and -127°, respectively, whereas those of ring B and A about C-5 and C-10 are 178 and -169°. The absolute configuration of **7** deduced by the X-ray experiment is that shown in Fig. 3.

EXPERIMENTAL

General Methods : All types of spectral and physical measurements as chromatographic systems are as previously reported. [4,5]

Extraction and Isolation. : *Azorella compacta* (llareta) was collected in November 1994 in Tatio in northern Chile.[1] A voucher specimen was deposited in the Herbarium of Universidad de Concepción, Concepción, Chile. Dried and finely powdered whole plant of *Azorella compacta* Phil. (750 g) was extracted with petroleum ether at room temperature to give a gum (28.5 g). This extract was chromatographed by flash chromatography on Si gel.[1] The fraction eluted with petroleum ether-EtOAc (80:20) (6.2 g) was submitted to a further Si gel column eluted with petroleum ether-EtOAc (10%) to yield azorellanol (**5**) (1.2 g).

Azorellanol (**5**) : Obtained as white crystal from petroleum ether-EtOAc m.p 149 ^{0} C . $_{589}[\alpha]^{20}$ - -10.23 ($CHCl_3$; c 1.036). IR ν^{KBr} cm^{-1}: 3.465 br. , 1730, 1250 . ^{1}H-NMR (see Table 1). ^{13}C-NMR (see Table 1).

HREIMS found 330.2406 (calculated 330.2559 for $C_{22}H_{34}O_2$) ; EIMS (70 ev, direct inlet) m/z (rel. int.): 330 [M - H_2O]$^+$, (16), 270 (27), 255 (36), 228 (28), 227 (31), 201 (19), 189 (24), 185 (42), 173 (18), 145 (24), 133 (23), 119 (42), 109 (23), 107 (25), 93 (22), 84 (100), 82 (87), 78 (23), 69 (18), 55 (32).

Hydrolysis of azorellanol (5) : A solution of 300 mg of **5** in 15 mL of MeOH was mixed with 16 mL of MeOH saturated with K_2CO_3 and stirred at room temperature overnight, poured into H_2O and extracted repeatedly with EtOAc. The extract was dried and chromatographed over silica gel using petrol ether-EtOAc (90:10), to yield 220 mg of 7-deacetylazorellanol **6**.

Compound **6** : Prisms (from Et_2O). mp 168-170 °C . ^{1}H-NMR ($CDCl_3$): δ 4.05 (1H, dd, J = 11.3 and 6.9 Hz, H-7), 2.32 (1H, dd, J = 12.5 and 7.2 Hz, H-10), 2.20 (1H, dd, J = 13.9 and 6.9 Hz, H-6′), 1.80 (1H, m, H-2), 1.47 (2H, m, H-6, H-4), 1.28 (3H, s, Me-16), 1.21 (3H, m, H-3, H-2′, H-1), 0.96 (3H, s, Me-17), 0.96 (3H, d, J = 6.5 Hz, Me-19), 0.90 (1H,m, H-1′), 0.87 (3H, s, Me-20), 0.84 (3H,d, J = 6.5 Hz, Me-18), 0.77 (1H, dd, J = 4.6 and 10.0, H-11′), 0.66 (1H, dd, J = 4.6 and 10.0, H-12), 0,13 (1H, t, J = 4.6 Hz, H-11).). ^{13}C-NMR ($CDCl_3$): 76.5 (d, C-7), 70.0 (s, C-13), 59.5 (d, C-3), 47.3 (d, C-10), 43.4 (t, C-6), 42.4 (s, C-5), 35.2 (s, C-8), 32.4 (t, C-15), 31.7 (d, C-4), 31.1 (t, C-14), 30.4 (q, C-16), 27.7 (t, C-2), 25.8 (d, C-12), 26.4 (s, C-.9), 22.8(q, C-18), 22.7 (q, C-19), 20.9 (t, C-1), 17.7 (q, C-17), 17.6 (q, C-20), 10.8 (t, C-11). HREIMS found 306.2604 (calculated 306.2555 for $C_{20}H_{34}O_2$) ; EIMS (70 ev, direct inlet) m/z (rel. int.): 306 (1), 288 [M-H_2O]$^+$ (87), 273 (18), 255 (10), 245 (26), 227 (33), 216 (26), 203 (32), 191 (38),191 (18), 177 (22), 122 (60), 95 (100), 81 (65).

p-Bromobenzoylation of 7-deacetylazorellanol (**6**) : To a soln of **6** (200 mg) in THF (15 mL) p-bromobenzoyl chloride (213 mg) and pyridine (1 mL) were added, and the mixture was stirred at room temperature for 1 day. The reaction mixt was worked-up as usual and purified by silica gel chromatography to afford 160 mg of **7**.

Compound **7** : Prisms (from petroleum ether), mp 83-85 ° C. ^{1}H-NMR ($CDCl_3$): δ 7.86, 7.55 (each d, J =8.5 Hz, aromatic H), 5.50 (1H, dd, J = 7.0 and 11.2 Hz, H-7), 2.46 (1H, dd, J = 12.3 and 7.2 Hz, H-10), 2.31 (1H, dd, J = 7.0 and 14.0 Hz, H-6′), 1.83 (1H, m, H-2), 1.40-1.72 (3H, m, H-4, H-6, H-15), 1.10-1.40 (5H, m, H-1, H-3, H-14, H-14′, H-15′), 1.26 (3H, s, Me-16), 1.14 (3H, s, Me-17), 0.92 (3H, s, Me-20), 0.90 (1H, m, H-1′), 0.91 (3H, d, J = 6.3 Hz, Me-19), 0.83 (3H, d, J = 6.3 Hz, Me-18), 0.80 (1H, m, H-11′), 0.70 (1H, dd, J = 5.6 and 9.2 Hz, H-12), 0.15 (1H, t, J = 5.6, H-11). ^{13}C-NMR ($CDCl_3$). EIMS (70 ev, direct inlet) m/z (rel. int.): 472 [M-H_2O]$^+$ (1.9), 470 [M-H_2O]$^+$ (1.8), 391 (4.2), 270 (34.5), 255 (32), 228 (54), 201 (16), 185 (100), 157 (61), 133 (44), 121 (26), 95 (35), 81 (49) 119 (67).

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