

Terpenoids from *Azorella cryptantha*

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ABSTRACT

One diterpenoid with the azorellane carbon skeleton termed azorelaldehyde and two sesquiterpenoids with the guaiane carbon skeleton termed guai-1(10)-ene-11-ol and 1 α ,5 β -guaiane-4 β ,5 β ,11-triol, were isolated from the *Azorella cryptantha* and characterized by 1D and 2D NMR spectroscopy.
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KEYWORDS

Azorella cryptantha;
Apiaceae;
Azorelaldehyde;
Guai-1(10)-ene-11-ol;
1 α ,5 β -guaiane-4 β ,5 β ,11-triol.

INTRODUCTION

Continuing with our studies of *Azorella cryptantha* seven was isolated you terpenic structures. Three diterpene, yaretol and mulinol isolated previously of *Azorella madrepórica*^[4] and *A. compacta*^[3], respectively and azorelaldehyde 1, derivate of azorelolido isolated previously *Azorella cryptantha*^[2]. Four sesquiterpenos type guayanolido. Pancherione isolated of *Neocallitropsis pancheri*^[5], teucladiol isolated of *Teucrium leucocladum*^[1]. The two remaining metabolites (2, 3) they were identified as of novel structures.

RESULTS AND DISCUSSION

Azorelaldehyde 1 was isolated as oil yellow. The EIMS showed a molecular ion to m/z 304, the presence of the pick to m/z 43 made us suppose the existence of a group acetyl or group isopropyl in the molecule.

The spectrum of IR showed two important absorptions to 3400 cm^{-1} and 1790 cm^{-1} that made us suppose the existence of groups hydroxyls and aldehydes in the molecule.

The ¹H-NMR (TABLE 1) it presented two methyl doublets to δ_{H} 0.82 and 0.75, both with a coupling constant of 6.6 Hz. This system was assigned to a group isopropyl and they confirmed that observed in the EIMS.

The ¹³C-NMR and DEPT experiments (TABLE 1) indicated the presence of four primary, seven secondary, five tertiary and four quaternary carbons and suggested that 1 was a tetracyclic diterpene.

The sign to δ_{C} 207.9 ppm and their correlation with the sign sail you to δ_{H} 9.8 in the HETCORR, it was assigned to a group aldehyde in the molecule in agreement with the IR spectrum.

The sign to δ_{C} 10.2 that it was correlated with the signs to δ_{H} 0.28 and 0.95 were assigned to a group cyclopropane.

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TABLE 1: $^1\text{H-NMR}$ (200.13 MHz) and $^{13}\text{C-NMR}$ (50.4 MHz) spectroscopic data of azorelaldehyde (1)^a

Atom number	1	
	δ_{H} (mult., J , Hz)	δ_{C} (mult.)
1	0.86 <i>m</i> 1.21 <i>m</i>	20.0 <i>t</i>
2	1.16 <i>m</i> 1.77 <i>m</i>	27.5 <i>t</i>
3	1.14 <i>m</i>	58.8 <i>d</i>
4	1.40 <i>m</i>	31.0 <i>d</i>
5		42.1 <i>s</i>
6	1.78 <i>m</i>	34.1 <i>t</i>
7	1.68 <i>m</i>	32.0 <i>t</i>
8		47.2 <i>s</i>
9		20.0 <i>s</i>
10	2.34 (<i>dd</i> , 12.1, 7.3)	46.9 <i>d</i>
11	0.28 (<i>t</i> , 33) 0.95 (superpuesto)	10.2 <i>t</i>
12	0.89 <i>m</i>	20.7 <i>d</i>
13		68.9 <i>s</i>
14	1.30 <i>m</i>	31.3 <i>t</i>
15	1.39 <i>m</i>	28.0 <i>t</i>
16	1.19 <i>s</i>	29.7 <i>q</i>
17	9.80 <i>s</i>	207.9 <i>d</i>
18	0.82 (<i>d</i> , 6.6)	22.0 <i>q</i>
19	0.75 (<i>d</i> , 6.6)	16.5 <i>q</i>
20	0.74 <i>s</i>	22.2 <i>q</i>

^aIn ClCD_3 , TMS as internal standard

The sign to δ_{C} 68.9 for a quaternary carbon indicated the presence of a group hydroxyl and it was assigned to the C-13.

The sign to δ_{H} 1.19 were assigned to the methyl 16 together to the carbon carbinolic. These assignments together with the one of the group aldehyde to C-17 it allowed us to conclude that this metabolite was derived of the azorelolido.

The full proton and carbon NMR spectral assignments were performed using a combination of COSY, HETCORR and NOESY 2D experiments.

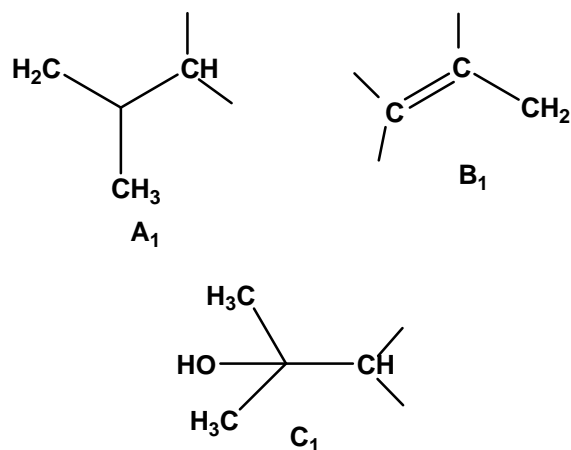
The EIMS of 2 showed a molecular ion to m/z 222 consistent with a molecular formula of $\text{C}_{15}\text{H}_{26}\text{O}$. The ^1H NMR spectrum displayed three methyl proton singlets at δ 1.09 (Me-12 and Me-13) and 1.59 (Me-15), and one methyl proton doublets at δ 0.84 ($^3J = 6.9$ Hz).

The IR spectrum showed as important absorption a band to 3400 cm^{-1} characteristic of group hydroxyls.

The $^{13}\text{C-NMR}$ and DEPT experiments (TABLE 2) indicated the presence of four primary, five secondary, three tertiary and three quaternary carbons and suggested that 2 were a bicyclic sesquiterpenoid.

The structure of 2 and its ^1H and ^{13}C NMR spectral parameters were deduced from the concerted application of homonuclear and both direct and long-range heteronuclear chemical shift correlation experiments. Firstly, the establishment of the proton connectivities was deduced from the COSY spectrum. Then, one bond proton-carbon chemical shift correlations were achieved using a proton detected C,H-correlation experiment (HECTCORR sequence), while assignments of the CH_n groups were obtained from analysis of the long-range correlation responses over two or three bonds (2J or 3J couplings) using the COLOC technique.

Beginning from the connectivities observed between the methyl proton shifts and the carbons α and β to these grupos en the COLOC diagram, the two following partial structures A_1 , B_1 and C_1 were determined:



Expansion to include other groups in structural segments A_1 , B_1 and C_1 became feasible using (i) the proton intercoupling network obtained from the COSY diagram, (ii) the long-range $^1\text{H-}^{13}\text{C}$ correlation responses observed for the ^1H resonances other than methyls, (iii) chemical shift assignments (e.g. the deshielding of ^1H signals sited in α -position of the double bond function) and (iv) the previously reported 1D NMR results. From these data (TABLE 2), the structure of 2 was deduced to be guai-1(10)-ene-11-ol

TABLE 2 : $^1\text{H-NMR}$ (200.13 MHz) and $^{13}\text{C-NMR}$ (50.4 MHz) spectroscopic data of 2^a

Atom number	2	
	δ_{H} (mult., J, Hz)	δ_{C} (mult.)
1		128.5 <i>s</i>
2	1.14 <i>m</i>	27.5 <i>t</i>
3	2.05 <i>m</i>	34.7 <i>t</i>
4	2.08 <i>m</i>	38.8 <i>d</i>
5	2.34 <i>m</i>	46.1 <i>d</i>
6	1.75 <i>m</i>	28.6 <i>t</i>
7	1.39 <i>m</i>	53.9 <i>d</i>
8	2.19 <i>m</i>	30.1 <i>t</i>
9	1.30 <i>m</i> 1.61 <i>m</i>	32.8 <i>t</i>
10		141.1 <i>s</i>
11		73.4 <i>s</i>
12	1.09 <i>s</i>	26.9 <i>q</i>
13	1.09 <i>s</i>	27.0 <i>q</i>
14	0.84 (<i>d</i> , 6.9)	15.2 <i>q</i>
15	1.59 <i>s</i>	25.1 <i>q</i>

^aIn ClCD_3 , TMS as internal standard

The molecular formula for 3 was established as $\text{C}_{15}\text{H}_{28}\text{O}_3$ (EIMS: $[\text{M}]^+ = m/z$ 256). Its IR spectrum showed the presence of a hydroxyl group (3650 cm^{-1}). The $^{13}\text{C-NMR}$ and DEPT experiments (TABLE 3) indicated the presence of four primary, five secondary, three tertiary and three quaternary carbons and suggested that 3 were a bicyclic sesquiterpenoid.

The signs to δ_{C} 73.6, 75.7 and 90.7 allowed establishing that three functions hydroxyls existed in the molecule and that the same ones were located on quaternary carbons.

The $^1\text{H-NMR}$ spectrum (TABLE 3) showed three singlets at δ_{H} 1.28, 1.18 and 1.09 assigned to the methyls 12, 13 and 15, respectively. The sign doublet at δ_{H} 0.93 was assigned to H-14.

In the spectrum of correlation heteronuclear (COLOC) was observed correlation between the signs of the methyls 12 and 13 with the signs at δ_{C} 73.6 and 47.9 assigned to C-11 and C-7, respectively. Furthermore, was observed the correlation between δ_{H} 1.09 (I-15) and δ_{C} 75.7, 19.2 and 90.7.

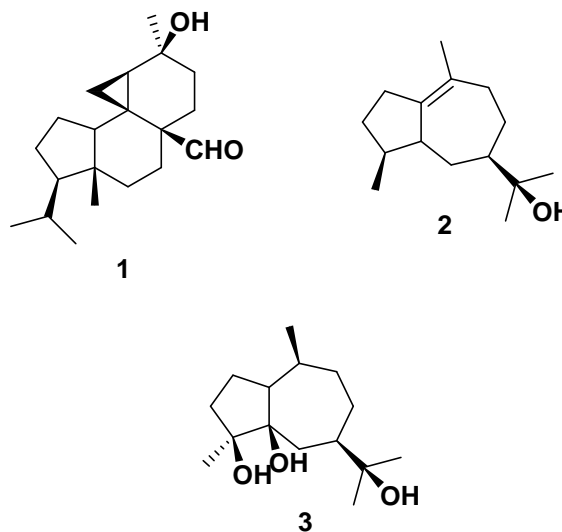
TABLE 3 : $^1\text{H-NMR}$ (200.13 MHz) and $^{13}\text{C-NMR}$ (50.4 MHz) spectroscopic data of 3^a

Atom number	3	
	δ_{H} (mult., J, Hz)	δ_{C} (mult.)
1	1.68 <i>m</i>	36.4 <i>d</i>
2	1.67 <i>m</i> 2.08 <i>m</i>	26.1 <i>t</i>
3	1.60 <i>m</i> 1.99 <i>m</i>	19.1 <i>t</i>
4	1.40 <i>m</i>	75.7 <i>s</i>
5		90.7 <i>s</i>
6	1.47 <i>m</i>	24.5 <i>t</i>
7	1.83 <i>m</i>	47.9 <i>s</i>
8	1.32 <i>m</i> 2.48 <i>m</i>	32.3 <i>t</i>
9	1.36 <i>m</i> 1.85 <i>m</i>	29.6 <i>t</i>
10	2.60 <i>m</i>	35.4 <i>d</i>
11		73.6 <i>s</i>
12	1.28 <i>s</i>	29.1 <i>q</i>
13	1.18 <i>s</i>	29.3 <i>q</i>
14	0.93 (<i>d</i> , 6.9)	15.1 <i>q</i>
15	1.09 <i>s</i>	26.7 <i>q</i>

^aIn ClCD_3 , TMS as internal standard

The correlation between δ_{H} 1.83 (H-7) and δ_{C} 24.5, 90.7 allowed to establish the correct position of the groups hydroxyls in the molecule.

The full proton and carbon NMR spectral assignments were performed using a combination of COSY, HETCORR and COLOC 2D experiments.



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EXPERIMENTAL

General experimental procedures

The 1D and 2D NMR experiments were recorded on Bruker AC-200 spectrometer using CDCl_3 as solvent and TMS as an internal standard. Chemical shifts are given in ppm downfield from TMS and coupling constants are measured in Hz. COSY, DEPT, HETCOR, COLOC and NOESY experiments were obtained using standard Bruker software. Optical rotation were determined on a Jasco P-1010 polarimeter. IR spectra were recorded in a Nicolet 5-SXC-FTIR spectrophotometer. EIMS were collected on a Finnigan 3300 F-100 at 70 eV by direct inlet. CC was performed on silica gel (70-230 mesh ASTM) (Merck) and silica gel 60 (230-400 mesh ASTM) (Merck). TLC was performed on silica gel 60 GF₂₅₄ (Merck).

Plant material

Azorella cryptantha was collected near Bauchazeta, Department of Iglesia, Province of San Juan, Argentina, during December 2003 and was identified by Dr. Luis Ariza Espinar. A voucher specimens is deposited in Museo Botánico Córdoba (CORD 506), Córdoba, Argentina.

Extraction and isolation

The leaves and stem (1250 g) of *A. cryptantha* were air-dried and exhaustively extracted with EtOH. The residue obtained after evaporation of the solvent (246 g) was suspended in EtOH/H₂O and extracted sequentially with hexane and Cl_3CH . The organic layers were evaporated under reduced pressure, yielding gummy residues of 39 g and 59 g, respectively.

The Cl_3CH extract was purified by CC eluting with Hexane, Hexane-EtOAc, EtOAc and the combined according to their TLC profiles. The fractions 1-2 were further purified by repeated CC to give 15 mg of azorelaldehyde (1). The fractions 6-7 were further purified by repeated CC to give 22 mg of yaretol. The fractions 9-11 were further purified by repeated CC to give 24 mg of teucladiol. The fractions 25-34 were further purified by repeated CC to give 235 mg of 1 α ,5 β -guaiane-4 β ,5 β ,11-triol (3). The fractions 40-48 were further purified by repeated CC to give 136 mg of guai-1(10)-ene-11-ol (2). The fractions 50-57 were fur-

ther purified by repeated CC to give 35 mg of Pancherione.

Azorelaldehyde (1)

$\text{C}_{20}\text{H}_{32}\text{O}_2$; oil, $[\alpha]_{\text{D}}^{25.1} -0.67$ (CH_3Cl *c* 0.75) IR (AgCl) ν_{max} 3402, 1720 cm^{-1} . EIMS 70 eV *m/z* 304 $[\text{M}]^+$, 286 $[\text{M}-18]^+$. ^1H and ^{13}C NMR data, see TABLE 1;

guai-1(10)-ene-11-ol (2)

$\text{C}_{15}\text{H}_{26}\text{O}$; oil, $[\alpha]_{\text{D}}^{25.9} +9.49$ (CH_3Cl *c* 1.17) IR (AgCl) ν_{max} 3421; 2926 cm^{-1} . EIMS 70 eV *m/z* 222 $[\text{M}]^+$, 204 $[\text{M}-18]^+$. ^1H and ^{13}C NMR data, see TABLE 2;

1 α ,5 β -guaiane-4 β ,5 β ,11-triol (3)

$\text{C}_{15}\text{H}_{28}\text{O}_3$; oil, $[\alpha]_{\text{D}}^{25.9} -48.21$ (CH_3Cl *c* 0.68) IR (AgCl) ν_{max} 3441 cm^{-1} . EIMS 70 eV *m/z* 256 $[\text{M}]^+$, 238 $[\text{M}-18]^+$, 220 $[\text{M}-(2 \times 18)]^+$, 202 $[\text{M}-(3 \times 18)]^+$. ^1H and ^{13}C NMR data, see TABLE 3;

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