Yaretol, a Norditerpenoid from *Azorella madreporica*

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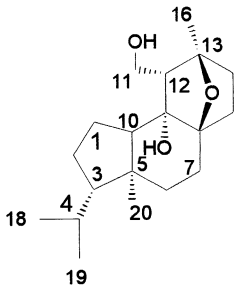
A new norditerpenoid, yaretol (**1**), was isolated from the whole plant of *Azorella madreporica*. The structure of **1** was established by one- and two-dimensional NMR techniques and confirmed by X-ray diffraction analysis.

Azorella madreporica Clos (Apiaceae), known locally as "llareta", is a yellow-green, compact resinous cushion shrub that grows in the high Andes of Central Chile. This species is distributed from the mountains of Coquimbo to the northern part of O'Higgins Province. Bitter-tasting infusions of the whole plant are used in folk medicine, principally as a gastric stimulant.[1] In previous phytochemical studies on samples of this species collected in Chile at Laguna Dolores, Departamento de Colina, Región Metropolitana, the isolation of an azorellane diterpenoid, 13α-hydroxyazorellane, was reported from a petroleum ether extract.[1] As a part of our continuing search for diterpenoids from plants belonging to the Apiaceae, we have collected *A. madreporica* from Vallenar, in the III Region (Chile). Investigation of its constituents resulted in the characterization of mulinolic acid[2] and a new norditerpenoid (**1**).



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The molecular formula of yaretol (**1**), $C_{19}H_{32}O_3$, was deduced from its HREIMS data (*m*/*z* 308.2339 $[M]^+$, $C_{19}H_{32}O_3$ requires 308.2351). Thus, compound **1** could be assigned with four degrees of unsaturation. The analysis of the IR, ^{1}H NMR, and ^{13}C NMR spectra (Table 1) did not show features assignable to multiple bonds, indicating that structure **1** is tetracyclic. The IR and ^{1}H NMR spectra suggested the presence of two hydroxyl groups (3538 and 3431 cm^{-1}; δ_H 5.05 and 4.53 s). The ^{13}C NMR spectrum showed signals assignable to four oxygenated carbons, three quaternary carbons at δ_C 86.2, 85.7, and 79.7, and one methylene carbon at δ_C 60.3. The ^{1}H-decoupled ^{13}C NMR and HMBC spectra of **1** showed resonances for 19 carbons. DEPT analysis, using a 90° angle, indicated four saturated methines at δ 58.3, 57.4, 58.2, and 30.4. The 135° DEPT spectrum showed seven methylene and four methyl carbons, indicating that the carbons at δ 86.2, 85.7, 79.7, and 42.0, after comparison with the decoupled spectrum (Table 1), were not bonded to hydrogen atoms.

The ^{1}H and ^{13}C NMR spectra of **1** (Table 1), together with data from ^{1}H COSY, HMQC, and HMBC experiments, revealed the presence of an isopropyl group [δ_C 30.4 (CH), 22.3 (CH_3), and 22.9 (CH_3); δ_H 1.46, 0.81 (3H, d, J = 6.6 Hz), and 0.90 (3H, d, J = 6.6 Hz)], a tertiary methyl group [δ_C 12.4 (CH_3), δ_H 1.03 s], and another tertiary methyl group [δ_C 22.4 (CH_3), δ_H 1.55] bonded to an oxygenated carbon. After a detailed analysis of ^{1}H COSY and HMBC experiments, it could be concluded that the structure **1** shows the structural features of the A and B rings from mulinane and azorellane diterpenoids isolated from this plant and other species of the genus *Azorella*.[3]

The ^{1}H−^{1}H COSY NMR spectrum of **1** indicated connectivities between the methyl groups (C-19 and C-20), showing a correlation with H-3. The long-range ^{1}H−^{13}C correlations (Table 1) of the H-18 and H-19 protons with C-3 and C-4 confirmed the position of the isopropyl group in ring A. The HMBC spectrum showed correlations between the H-3 proton with C-20, C-2, C-5, C-6, and C-10 and connectivities of the H-10 signal with C-1, C-3, C-6, C-9, and C-20. In turn, H-7 showed correlations with the C-9, C-8, C-6, and C-5 carbons. These data confirmed the partial structure of yaretol (**1**) (Figure 1).

The ^{1}H−^{1}H COSY spectrum showed connectivities between the protons of the CH_2OH group at δ 4.06 (1H, t, J = 9.9 Hz) and 3.89 (1H, m), with the signal at H-12. In a similar manner, in the HMBC spectrum, connectivities were observed between the protons from the CH_2−O group at C-9 and with the signals of C-12 and C-13. In the HMQC spectrum, the signal at δ 58.3 (C-12) correlated with the proton at δ 2.01. This proton, in the HMBC spectrum, correlated with the C-8, C-9, C-10, C-11, C-13, C-14, and C-16 signals. In the HMBC spectrum, the H-16 protons correlated with carbons C-12 and C-14. The correlation of Me-16 with C-8 was indicative of an ether bridge between C-13 and C-8 and suggested a loss of the corresponding methyl group at the C-8 carbon of the azorellane-type diterpenoids.

Analysis of the chemical shifts in the ^{13}C NMR spectrum, together with the presence of four rings in the yaretol **1**, the partial structure (Figure 1), and its comparison with

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Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data of Compound **1**[a]

position	δ_C[b]	δ_H[c]	NOESY	HMBC
1α	18.8 t	1.79 m ax	HO-9	2, 3, 5
β		1.75 m eq	H-12	
2α	27.6 t	1.25 dd eq		1, 5, 10
β		1.81 m ax		
3*β*	58.3 d	0.90 dd (9.5, 9.5)	H-10	18, 19, 20, 2, 4, 6, 5, 10
4	30.4 d	1.46 m	Me-20	18, 19, 5
5	42.0 s			
6α	37.0 t	1.83 dd eq	H-3 and H-10	20, 7, 5, 10
β		1.63 ddd ax	Me-18, Me-19 and Me-20	
7α	27.1 t	2.12 ddd ax (4.8, 14.2, 16)		15, 6, 5, 8, 9
β		1.93 m eq	Me-20	
8	86.2 s			
9	85.7 s			
10α	57.4 d	1.89 m	H-12	20, 1, 6, 5, 3, 12, 9
11α	60.3 t	4.06 t eq (9.9)	OH-9	12, 13, 9
β		3.89 m		
12*β*	58.3 d	2.01 t (9.9)	H-10	8, 16,14,10, 11, 13, 9
13	79.7 s			
14α	31.0 t	2.65 ddd eq (6.3, 9.6, 15.7)		8,15, 12, 13,
β		1.48 m ax	15α	
15α	32.5 t	1.96 m eq	OH-11	14, 15, 13, 8
β		1.47 m ax		
16	22.5 q	1.55 s	H-11	14, 12, 8
18	22.3 q[c]	0.90 d (6.6)	H-4	20, 19, 4, 3
19	22.9 q[c]	0.81 d (6.6)	H-4	20, 19, 4, 3
20	12.3 q	1.03 s	H-4, H-7*β*, H-6*β*	6, 5, 10, 3
OH-11		5.05 s	H-15α	
OH-9		4.53 s	H-11	13, 8

[a] Spectra taken in pyridine-d_5. [b] Multiplicity determined from DEPT. [c] Coupling constants (J in Hz) in parentheses. [d] Interchangeable.

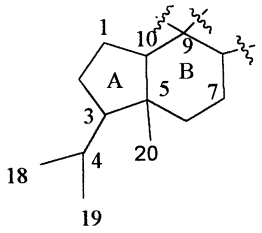

Figure 1. Partial structure of yaretol.

the carbon skeleton of the azorellane-type diterpenoids allow us to suggest that the structure of **1** is related to these diterpenoids by means of a bond cleavage at C-9−C-11, eliminating the cyclopropane ring and loss of the C-17 methyl group. Structure **1** was consistent with a detailed analysis of the ^{1}H and ^{13}C NMR spectral data (Table 1). These NMR spectra, plus the ^{1}H COSY, HMQC, and HMBC experiments, allowed us to assign, besides the structural correlations, the chemical shift of the carbons and protons unequivocally. Thus, long-range $^{1}H-^{13}C$ correlations were observed between the signal at H-7 and C-8 and C-15 and between H-15 and C-8 and C-13. In turn, C-8 showed connectivities with H-12*β*, H-12α, and H-16. Additionally, the hydroxyl group resonance at δ 4.53 was correlated with the signals at C-8 and C-13, indicative of the position of this group at C-9. Therefore, we propose structure **1** for yaretol. The trivial name proposed is based on the English transliteration of the local name of the plant origin.

The stereochemistry of **1** was assigned according to the NOESY spectrum (Table 1) and its correlation to the azorellane skeleton. The *trans*-fusion of rings A and B was suggested by the absence of any correlation between the protons from Me-20 (α) and H-10 (*β*). The signal at δ 1.89 (H-10*β*) was correlated with the signal at δ 0.90 (H-3*β*), indicating that the isopropyl group has an α-configuration. The signal at δ 1.03 (Me-20) showed a correlation with the signals at H-4, H-6, and H-7, indicating that they have the same configuration. Moreover, the signal at δ 2.01 (H-12) showed a correlation with the signal at H-10, so it was inferred that the hydroxymethylene group (C-11) also has a α-configuration. The lack of any connectivity between H-12 and Me-16 suggested an α-configuration for the latter. All this evidence allowed us to propose the relative stereochemistry for yaretol shown in structure **1**.

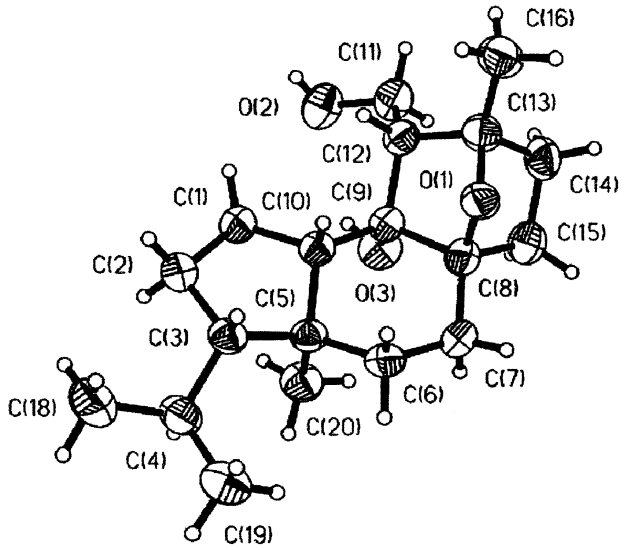

Figure 2. X-ray structure of compound **1**.

To confirm the proposed structure, based on spectroscopic data, a single-crystal X-ray analysis of yaretol was performed. The relative stereochemistry was solved by direct phase determination. A perspective view of the molecule, with displacement ellipsoids and the atom labeling, is given in Figure 2. The molecular structure of the title compound consists of three fused rings: a five-membered ring (A), in an envelope conformation, fused to a six-membered ring (B), in near chair conformation, and a six-membered ring (C) in boat conformation with an

epoxide group at C(8)−O(1)−C(13). Torsional angles of ring A and B about the common C(5)−C(10) bond are −167.3(2)° and −179.2(2)°, respectively, whereas those of rings B and C about C(8)−C(9) are 156.7(2)° and 172.0(2)°. As substituents, the molecule possesses a methyl and hydroxyl groups axially disposed on one side of the molecule. The methyl at C(13), the hydroxymethylene, and the isopropyl groups are equatorially bonded to the molecule.

Experimental Section

General Experimental Procedures. These are as previously reported.

Plant Material. *Azorella madreporica* Clos was collected in March 1999, in the III Region of northern Chile. A voucher specimen was deposited at the Herbarium of the Universidad de Concepción, Concepción, Chile.

Extraction and Isolation. Dried and finely powdered whole plant material of *A. madreporica* (5.1 kg) was extracted with petroleum ether at room temperature to give a gum (311 g). This extract was chromatographed by flash chromatography on silica gel.[5,6] A fraction that eluted with petroleum ether−EtOAc (95:5) (8.7 g) was further separated by silica gel chromatography and eluted with petroleum ether−EtOAc (5%) to yield 13α-hydroxyazorellane (530 mg).[1]

The fraction that eluted with petroleum ether−EtOAc (20%) (45.3 g) was chromatographed on silica gel (350 g), eluted with 15% petroleum ether−EtOAc, to yield mulinolic acid[2] (1.8 g) and yaretol (**1**, 360 mg).

Yaretol (1): white crystals, from petroleum ether−EtOAc; mp 165 °C; $[\alpha]^{24}_D$ −23.3 (*c*, 0.24 $CHCl_3$); IR (KBr) ν_{max} 3538, 3431, 1510, 1390, 1220, 990, and 600 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 308 $[M]^+$, 290 $[M - H_2O]^+$, 272 $[M - 2H_2O]^+$, 137 [base peak]; HREIMS *m*/*z* 308.2339 (calcd for $C_{19}H_{32}O_3$ 308.2351); 137.1035 (calcd for $C_9H_{13}O$ 137.0966).

X-ray Diffraction of 2.[6] The title compound is orthorhombic, space group $P2_12_12_1$, with unit cell parameters $a = 11.810(2)$ Å, $b = 12.166(2)$ Å, $c = 12.281(2)$ Å, $V = 1764.5(5)$ Å^3, and $Z = 4$. A colorless crystal of dimensions 0.90 × 0.28 × 0.26 mm was used on a Siemens *R*3*m* diffractometer, graphite-monochromated Mo Kα radiation. Intensity data for 2495 total reflections, $\theta_{max} = 27.56°$, were collected in the $\theta/2\theta$ scan mode. The structure was solved by direct phase determination. Positional and anisotropic displacement parameters for all non-H atoms were refined on F^2, with 2315 independent reflections, by full-matrix least-squares calculations. A riding model was applied to H atoms, which were calculated geometrically, with C−H = 0.96 Å, and an isotropic displacement parameter equal to 1.2 times the equivalent isotropic displacement parameter of their corresponding parent atom. Exception was made with the positional parameters of the H atoms of the hydroxyl groups which were also refined. Refinement converged to a conventional index $R = 0.0396$, for 1752 observed reflections, and 206 variable parameters, with $\Delta\rho_{max} = 0.18$ and $\Delta\rho_{min} = -0.13$ e Å^{-3} on a final difference Fourier map. The absolute configuration could not be determined reliably.

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References and Notes

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(6) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 185486). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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