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Madreporanone, a unique diterpene with a novel skeleton from *Azorella madreporica*

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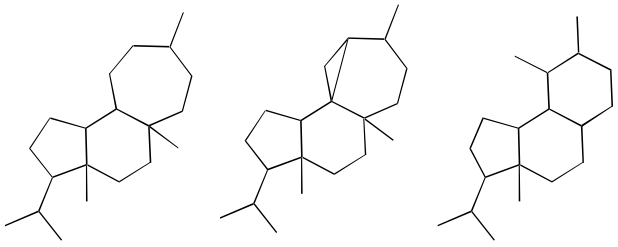
Abstract—A unique diterpene **1**, was isolated from the altiplane Andean plant *Azorella madreporica* Clos (Apiaceae). The structure and stereochemistry were established by spectral methods and molecular mechanics calculations. The compound possesses a novel hydrocarbon skeleton for which we suggest the name madreporane. © 2002 Elsevier Science Ltd. All rights reserved.

In Chile, 'llareta de Coquimbo' is a popular name[1] of several species of the genera *Azorella* and *Laretia* (Apiaceae), and "chuquicán" is used[2] for *Mulinum crassifolium* (Apiaceae). Today, 15[3–19] naturally occurring diterpenes derived from three skeletal classes have been isolated from species of these genera, and it is noteworthy that, although belonging to different genera, these species have a common feature: they are all biosynthesized diterpenes with a common mulinane skeleton, which was found first[3] in *M. crassifolium*. The other skeletons azorellane, isolated from *Azorella compacta*[13] as well as from *Azorella madreporica*,[12] and yaretane, from *Azorella madreporica*,[19] appear to be derived from mulinane by rearrangement and degradation.

In continuing our investigation of medicinal plants from Chile we have studied *Azorella madreporica* Clos, a compact resinous cushion shrub with a widespread distribution along the Andes from Argentina, Chile, and Bolivia to Perú. This species, distributed in Chile from the mountains of Coquimbo to the northern part of the O'Higgins Province, was collected from Vallenar, in the III Region. In this paper we describe a unique nor-diterpene madreporanone **1** with a novel carbon skeleton for which we propose the trivial name of madreporane **2**.

Madreporanone **1** was obtained[20] as a colourless oil from the hexane/ethyl acetate (60:40) of the vacuum flash chromatography fraction of the crude petroleum ether extract followed by gel filtration and purification by recycling-HPLC. NMR data coupled with a $[M-H_2O]^+$ peak at m/z 308.2302 in the HREIMS of **1** suggested a molecular formula of $C_{19}H_{32}O_3$ (calcd for $C_{19}H_{32}O_3$, 308.2351).



Mulinane Azorellane Yaretane

The ^{13}C NMR spectrum of **1** (Table 1) showed signals for 19 carbons whose multiplicities were determined from the DEPT spectrum: five methyls, six methylenes, three methines, and five nonprotonated carbons (two bearing oxygen and one carbonyl). One of the oxygens of the molecular formula should be in the form of a methyl carbinol (H_3-16: δ 1.0; C-13: δ 72.7) and although it should be expected that the signal at δ_C 74.4 would correspond to a carbon bearing oxygen, it was a surprise to find that the MS spectrum of **1** and some HMBC correlations demonstrate that it was really due to a quaternary carbon (C-10) vicinal to a carbonyl

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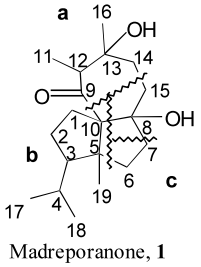
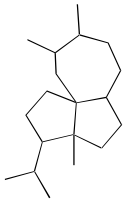
group. The IR absorption at 3432 and 1686 cm^{-1} were consistent with both oxygenated functionalities: two hydroxyls and one carbonyl. The hydroxylic functions do not react with acetic anhydride and pyridine, suggesting they are tertiary. Since the IR spectrum revealed no absorption for additional unsaturations, the molecule is tricyclic.



Madreporanone, **1**

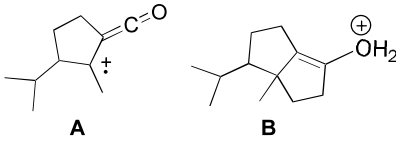

Madreporane, **2**

In addition to the methyl carbinol, the ^1H NMR spectrum showed high field signals for three secondary methyl groups (δ 0.85, δ 0.90 and δ 1.03) and one angular methyl group (δ 1.14) corresponding to a diterpene skeleton. At this point we anticipated that although madreporanone has five methyl groups, as expected in a regular diterpene skeleton, the compound is a degraded diterpene metabolite by loss of one isoprenic methyl group. Thus, the fifth, non-isoprenic, methyl comes from rearrangement of the cyclopropane ring containing a possible precursor compound **4**, as shown in the proposed biogenetic sequence of **1** in Fig. 2.

Chemical shift arguments, ^1H–^1H COSY and HMBC correlations allowed the assignment of fragments **a–c** as shown in **1**. From the ^1H–^1H COSY NMR spectrum it was clearly possible to differentiate two discrete spin systems (H_3-11–H-12 and H_2-6–H_2-7) and only a part of another spin system (H-4–H-3, H_2-1–H_2-2) due to the complex overlapping signals of methines and methylene protons. The coupling of very downfield methine H-12 at (δ 3.55) with a secondary H_3-11 methyl group (δ 1.03) together with the long-range HMBC correlations: H_3-11/C-9, C-13; methyl carbinol H_3-16/C-12, C-14 as well as the correlation H-12/C-9, C-11, C-13 established the connectivity of the H_3-11–H-12 in fragment **a**. On the other hand, an isopropyl unit was revealed by a clear coupling of the remaining two secondary methyl groups (H_3-17, H_3-18) with H-4 (δ 1.67), leading us to suspect that the methyl and isopropyl substituted cyclopentane ring fragment **b**, characteristic of naturally occurring diterpenes from this species,[3,13] could be a part of **1**. This was confirmed by: (a) the HMBC correlation of the three C-17, C-18 and C-19 methyl groups with H-3, (b) the C_2/C_3 linkage established by the HMBC correlation H_2-2/C-4, C-5, C-10; H_2-1/C-2, C-3 and the COSY correlated H_2-1–H_2-2 fragment, and (c) the HMBC correlation H_3-19/C-3, C-5, C-10.

The coupling of the downfield signal of a proton of the H_2-7 (δ 2.0) with H_2-6 (δ 1.32 and δ 1.58) established the connectivity of H-6–H-7 fragment **c**.

HMQC and HMBC data and biogenetic considerations were used to establish the connectivity between fragments **a**/**b**/**c**. The linkage C-9/C-10 was secured by the correlation H_2-1/C-9. This was supported by MS which showed a base peak fragment at m/z 151 for $C_{10}H_{15}O$



Scheme 1. Selected MS fragments of **1**.

Table 1. ^1H, ^{13}C and HMBC data of compound **1** [500 MHz, δ ppm, (J) Hz, chloroform-d]

No.	1		
	H	C	HMBC
1	α: 1.40 dd (4.8, 13.5); β: 1.72 m	33.5	C_2, C_3, C_9
2	α: 1.64 m; β: 1.25 m	28.5	C_4, C_5, C_{10}
3	1.24 m	59.2	C_2, C_4, C_5, C_6, C_{10}
4	1.67 m	28.2	C_2, C_3, C_5
5		53.2	
6	α: 1.32 ddd (6.5, 13.0, 13.0); β: 1.58 m	37.9	C_3, C_5, C_7, C_8, C_{10}, C_{19}
7	α: 2.00 ddd (7.3, 12.8, 12.8); β: 1.54 dd (6.5, 12.2)	40.4	C_6, C_8, C_{15}
8		79.1	C_{10}
9		212.0	
10		74.4	
11	1.03 d (6.8)	12.2	C_9, C_{12}, C_{13}
12	3.55 q (6.8)	52.4	C_9, C_{11}, C_{13}, C_{14},C_{16}
13		72.7	
14	α: 2.27 td (2.4, 13.3); β: 1.63 m	39.0	C_8, C_{13}, C_{15}, C_{16}
15	α: 1.65 m; β: 1.75 ddd (2.8, 5.6, 14.9)	35.3	C_8, C_{10}, C_{13}, C_{14}
16	1.00 s	21.7	C_{12}, C_{13}, C_{14}
17	0.90 d (7.2)	23.4	C_3, C_4
18	0.85 d (7.2)	22.7	C_3, C_4
19	1.14 s	22.7	C_3, C_5, C_6, C_{10}

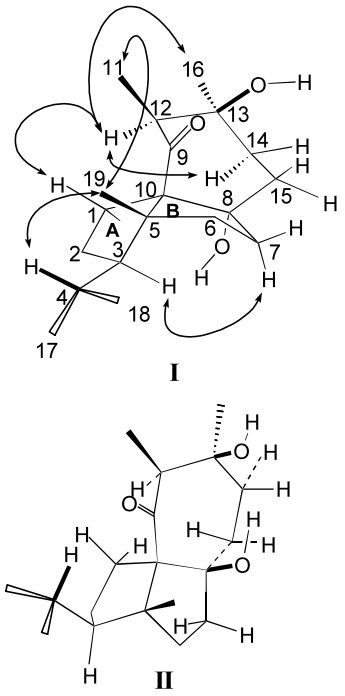


Figure 1. Selected NOE and configurations of **1**.

(HRMS) consistent with a fragment **A**. The C-5/C-6 linkage was confirmed by the correlation of H_2-6 with C-5, C-19, C-3 and C-10. At this time, the location of the remaining two carbons, C-15 and the quaternary oxygenated C-8, followed from the correlation H_2-15/C-7, C-8. C-13 and C-10 and the correlation H_2-14/C-15 and C-8. This was also reinforced by the MS fragment at m/z 181 for $C_{12}H_{21}O$ (HRMS) consistent with a fragment **B** (Scheme 1). The above correlations also allowed us to establish the connectivity of the fragment **a**/**c** through the linkage C-7/C-8 completing thus the overall planar structure of madreporanone **1** with the required four degrees of insaturation.

The relative configurations for C-3, C-5, C-8, C-10, C-12 and C-13 chiral centres of **1** were assigned by NOESY experiments and molecular mechanics calculations. A NOE effect observed between H-4 with Me-19 and Me-19 with Me-11 suggested the same configuration for these methyl groups and the isopropyl group as depicted in **I** (Fig. 1), which is coincident with the stereochemistry shown for the related methyl and isopropyl groups of all the naturally occurring diterpenes found in species of these genera.

The NOE observed between H-12 and H_3-16 suggested an opposite stereochemistry of this methyl group relative to the others. On the other hand, the NOE observed between H-3α and H-7α indicated that the rings A/B must be *cis*-fused with C-10 bearing a β alkyl substituent. Thus, only the stereochemistry at C-8 remains to be defined. The above-mentioned NOE as well as other observed NOEs between H-12α and H-1β; H-12α and H-14α are compatible with configurations **I** and **II** for C-8.

Molecular mechanics calculation allowed us to discriminate between them. After minimization the calculated ^3J coupling constants between H-14α and the vicinal pair of methylene protons were: 0.2, 12.7 Hz in **I** and 1.45, 6.1 Hz in **II**. The measured value of 2.4, 13.3 Hz agree with the proposed configuration for C-8 as depicted in **I** (Fig. 1).

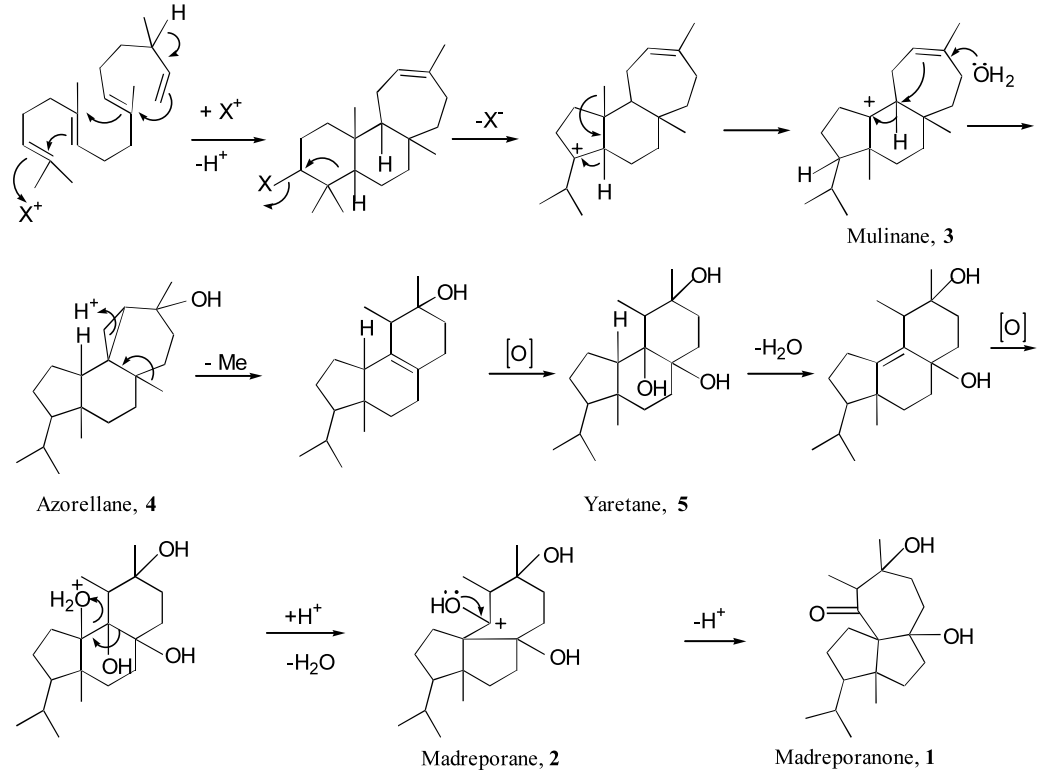


Figure 2. Possible biogenesis of madreporane **1**.

Biogenetically, compound **1** appears to be directly derived from the yaretane skeleton, **5**, which is a rearranged and degraded diterpene form of a parent precursor mulinane, **3**, following the sequence: mulinane, **3**→azorellane, **4**→yaretane, **5**→madreporane, **2**→madreporanone, **1**, shown in Fig. 2. An array of possible new metabolites generated from this novel metabolic pathway should be expected.

Acknowledgements

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20. **Madreporanone 1**. Colourless oil; $[\alpha]_D^{25}$ +63.5 (*c* 1.7, $CHCl_3$); IR ν_{max} 3432, 1686, 1455, 1380, 1095, 1065, 898 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; EI MS *m*/*z* (%), 308 (M^+; 0.5), 290 (M^+–H_2O; 19), 272 (M^+–$2H_2O$; 21), 181 (80), 151 (100). EIHRMS calcd for $C_{19}H_{32}O_3$ (M^+), 308.2351 found 308.2302; calcd for $C_{19}H_{30}O_2$ (M^+–H_2O), 290.2245 found 290.2253; calcd for $C_{19}H_{28}O$ (M^+–$2H_2O$), 272.2140 found 272.2122; calcd for $C_{12}H_{21}O$, 181.1592 found 151.1568; calcd for $C_{10}H_{15}O$ (base peak), 151.1122 found 151.1083.