Pergamon

PII: S0031–9422(97)01062–5

Phytochemistry, Vol. 49, No. 4, pp. 1091–1093, 1998

Printed in Great Britain
0031–9422/98/$—see front matter

11,12-EPOXY-MULIN-13-EN-20-OIC ACID, A DITERPENOID FROM *AZORELLA COMPACTA*

LUIS A. LOYOLA,* JORGE BÓRQUEZ, GLAUCO MORALES and AURELIO SAN-MARTÍN†

Laboratorio de Productos Naturales, Departamento de Química, Facultad de Ciencias Básicas, Universidad de Antofagasta, Antofagasta, Chile; † Departamento de Química, Facultad de Ciencias, Universidad de Chile, Chile

(*Received in revised form* 27 *October* 1997)

Key Word Index—*Azorella compacta* Phil.; Umbelliferae; diterpenoid; mulinane derivatives; 11,12-epoxy-mulin-13-en-20-oic acid.

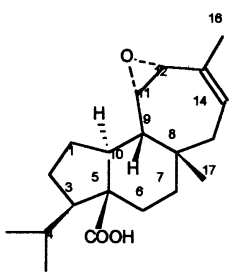
Abstract—A new diterpenoid, 11,12-epoxy-mulin-13-en-20-oic acid, was isolated from the aerial parts of *Azorella compacta* Phil. (Umbelliferae). Its structure determination was based on spectroscopic comparison with isomulinic acid.

INTRODUCTION

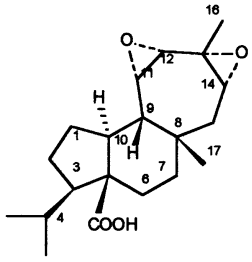
In previous communications [1, 2], we reported the structures of four mulinane diterpenoids isolated from the petroleum ether extract *Azorella compacta* Phil. (Umbelliferae). This plant, commonly known as ''llareta'', is a yellowish-green, compact, resinous cushion shrub growing in the north of Chile and used in folk medicine against diabetes and bronchial and intestinal disorders [3]. A study of the less polar chromatographic fractions of the same extract has now allowed the isolation of a new diterpenoid acid with a mulinane skeleton, the structure of which was established by spectroscopic analysis as 11,12-epoxy-mulin-13-en-20-oic acid (**1**).

RESULTS AND DISCUSION

Mass spectrometry indicated the molecular formula $C_{20}H_{30}O_3$ (six sites of unsaturation) for **1**, and its IR spectrum revealed the presence of a carboxyl group. (3500–2500 br, 1720 cm^{-1}) The ^{13}C NMR spectrum of compound **1** (Table 1) showed resonances for twenty carbons atoms, and DEPT experiments demonstrated that 29 protons were attached to carbon atoms. A downfield carbon resonance at δ 181.5 (C) revealed that the remaining proton was involved in a carboxylic functionality. Two deshielded carbon resonances at 132.5 (CH) and 149.3 (C) correlated in HMQC experiments with one deshielded proton resonance at δ 5.70 *br s* (Table 2) were assigned to a trisubstituted olefinic double bond.



1



2

The ^{13}C NMR and ^{1}H NMR spectra of compound **1** (Tables 1 and 2), together with ^{1}H COSY, revealed the presence of an isopropyl group [δ_C 31.5 (CH), 22.6 (Me) and 22.5 (Me); δ_H 1.48, 1H, overlapped signal, 0.82, 3H, *d* and 1.01, 3H, *d*, ($J = 6.5$ Hz in both signals)], a tertiary methyl group [δ_C 32.8 (Me); δ_H 1.08, 3H, *s*] and another methyl attached to a substituted olefinic carbon [δ_C14.9 (Me); δ_H 1.60, 3H, *s*], and two oximethine [δ_C 48.8, (CH) and 51.1 (CH); δ_H 3.0, 1H, *dd*, ($J = 8.4$ and 1.3 Hz) and 2.96, 1H, *br s*] indicating the presence of a epoxide ring in **1**.

The lack of other olefinic or carbonyl resonances in the ^{13}C NMR of compound **1** (Table 1) indicated that rings had to account for the remaining three sites of unsaturation in the molecule.

All the above data can be accommodated in the mulinane carbon skeleton, as depicted in formula **1**. The almost identical ^{13}C chemical shift (Table 1) of the carbon atoms in **1** and isomulinic acid [4] **2**, together with the similarities observed in ^{1}H NMR spectra also supported this assumption. In fact, the observed differences between the NMR spectroscopy

* Author to whom correspondence should be addressed.

Table 1. ^{13}C NMR chemical shifts of compounds **1** and **2** ($CDCl_3$, TMS as internal reference)

	1	**2**
1	25.6 (*t*)	24.2 (*t*)
2	28.2 (*t*)	28.4 (*t*)
3	58.0 (*d*)	57.3 (*d*)
4	31.5 (*d*)	31.7 (*d*)
5	56.2 (*s*)	57.5 (*s*)
6	37.5 (*t*)	43.1 (*t*)
7	33.2 (*t*)	32.5 (*t*)
8	43.6 (*s*)	33.7 (*s*)
9	46.8 (*d*)	45.6 (*d*)
10	51.0 (*d*)	48.8 (*d*)
11	48.7 (*d*)	59.1 (*d*)
12	51.1 (*d*)	60.2 (*d*)
13	149.3 (*s*)	56.0 (*s*)
14	132.5 (*d*)	60.5 (*d*)
15	34.4 (*t*)	32.5 (*t*)
16	14.9 (*q*)	22.5 (*q*)
17	32.8 (*q*)	27.7 (*q*)
18	22.6 (*q*)	22.6 (*q*)
19	22.5 (*q*)	22.4 (*q*)
20	181.50 (*s*)	179.9 (*s*)

Table 2. ^{1}H NMR (500 MHz) spectral data of compounds **1** and **2**

	1	**2**
1	α 1.75 *m*	2.02
	β 1.01†	
2	α 1.90 *m*	2.02
	β 1.40†	
3	1.38	1.46
4	1.48†	1.48
6	α 1.44†	1.56
	β 1.22	1.55
7	α 2.37 *dt* (12.9, 3.4)	2.40 *dt*
	β 1.20†	1.08
9	1.70 *m*	2.05
10	1.33 *m*	2.03
11	3.00 *d* (8.4)	3.15 *t*
12	2.96 *br s*	3.25 *dd*
14	5.70 *br s*	2.89 *td*
15	β 1.23†	2.11
	α 1.60†	1.56
16	1.60 *s*	1.51 *s*
17	1.08 *s*	1.05 *s*
18	1.01 *d* (6.5)	1.04 *d*
19	0.82 *d* (6.5)	0.85 *d*

At 500 MHz in $CDCl_3$. Chemical shifts are relative to TMS. All these assignments were in agreement with the HMQC and HMBC.

† Overlapped signal. The chemical shift of these protons was measured on the HMQC spectra.

data of isomulinic acid and **1** were consistent, in the latter compound, with the presence of an oxirane ring at C-11, C-12 [δ_C 48.8 (C-11) and 51.1 (C-12); δ_H 3.0 (H-11), *dd*, ($J = 8.4$ and 1.3 Hz), 2.96 (H-12), *br s*] and a trisubstituted olefinic double bond in C-13, C-14 [δ_C 149.3 (C-13) and 132.5 (C-14); δ_{CH} 5.70 (H-14), *br s*], instead of two oxirane rings at the C-11, C-12 and C-13, C-14 positions of the former, thus suggesting that the new diterpenoid **1** was the 11,12-epoxy-mulin-13-en-20-oic acid.

Most of the structural fragments of **1** were identified by HMBC spectrum. Correlations were observed between the signal at δ_H 5.70 (1H, *br s*, assigned to the C-14 proton) with the signal at δ_C 14.9 *q* (Me C-16), δ_C 51.1 (C-12) and δ_C 149.3 (C-13). The signals at δ_H 3.0 (*dd*, $J = 8.4$ and 1.3 Hz) and 2.96 (*br s*) assigned to the H-11 and H-12 correlated with the signal at δ_C 149.3 *s*. (C-13). These facts confirmed the location of one oxirane ring at C-11/ C-12 and olefinic functionality at C-13/C-14. The H-12 and H-11 correlated with C-9 (δ_C 46.8 *d*) and H-11 correlated with C-10 (δ_C 51.0 *d*) and C-8 (δ_C 43.6 *s*). The correlations observed in the experiment are shown in Fig. 1.

The absolute configuration of compound **1** was not ascertained. However, on biogenetic grounds, it is reasonable to assume that it possesses the same configuration as isomulinic acid (**2**), whose absolute configuration has been established from an X-ray diffraction analysis [4]. The principal results from ROESY NMR experiments suggested that 11,12-epoxy-mulin-13-en-20-oic acid had the relative stereochemistry shown in Fig. 2. The ROESY NMR experiment showed a correlation between the signal at δ 1.70 (H-9) and the signals at δ 3.00 (H-11); δ 1.08 (Me-17) and δ 1.20 (H-7) which should be in a β-configuration. In addition Hβ-7 showed correlation with the signal at δ 1.44. (H-6) and Me-17 protons showed correlation with the signal at δ 1.23. (H-15) indicating that they were in the same configuration. Moreover, the signal at δ 1.33 for the α-orientated H-10 showed correlation with the signals at δ 1.22 (H-6) and δ 1.38 (H-3) which should be in the α-configuration. The signal at δ 1.22

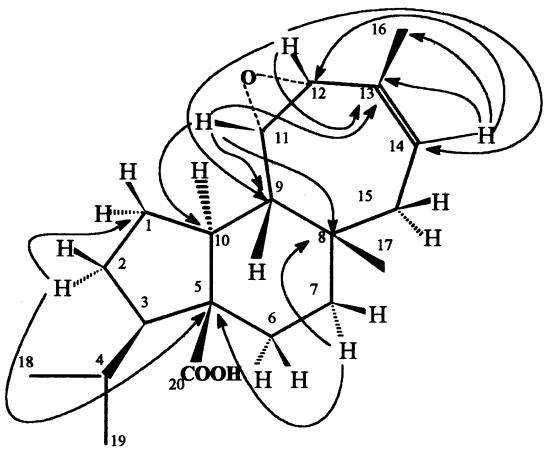


Fig. 1. ^{1}H-^{1}C long-range correlations for compounds **1** detected by the HMBC spectrum.

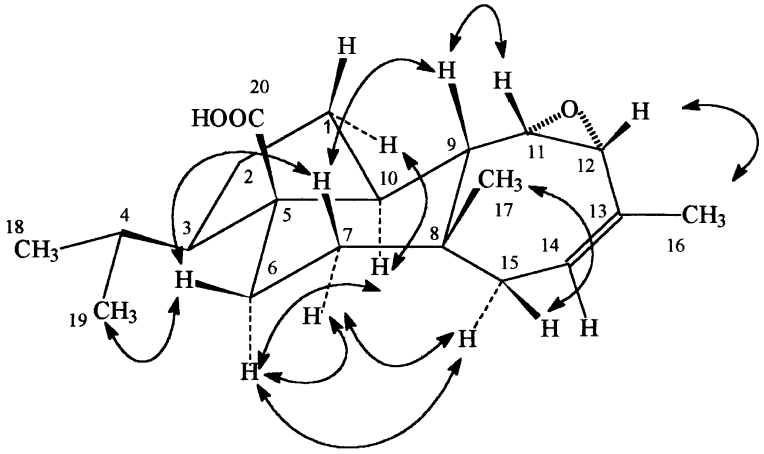


Fig. 2. Results of a ROESY experiment of compound **1**.

for the α-orientated H-6 showed correlation with the signals at δ 2.35 (H-7) and δ 1.60. (H-15) indicating that they were in the same configuration. The correlations observed in the ROESY NMR experiment are shown in Fig 2.

Results from this work, in combination with our previous communications [1, 2], show that the principal chemical components of *Azorella compacta* are mulinane-type diterpenoids.

EXPERIMENTAL

General

Mps: uncorr. Plant material was collected in November 1994 in Tatio in northern Chile. The plant was identified by Prof. Clodomiro Marticorena, Universidad de Concepción and a voucher specimens (CONC.133002) were deposited in the Herbarium of Universidad de Concepción, Concepción, Chile.

Extraction and isolation of 11,12-epoxy-mulin-13-en-20-oic acid (**1**)

The extraction and subsequent fractionation (by silica gel CC) of the extract from dry whole plant (750 g) of *Azorella compacta* Phil. is described in previous papers [1, 2]. The chromatographic fraction obtained before elution of mulinolic acid [1] was evaporated to dryness and the residue (1.2 g) chromatographed on a Silica gel column eluting with petrol–EtOAc (3%) to yield compound **1** (220 mg).

11,12-epoxy-mulin-13-en-20-oic acid (**1**). Amorphous powder, mp 116–118°C; IR_{max}^{KBr} cm^{-1}: 3500–2500 br, 1720, 1230. ^1H NMR (see Table 2). ^{13}C NMR (see Table 1). EIMS (70 eV, direct inlet) *m/z* (rel. int.): 318 $[M]^+$ (4), 303 (8), 302 (12), 282 (7), 257 (10), 241 (6), 189 (11), 163 (10), 149 (7), 147 (14), 133(15), 132 (12), 121 (13), 120 (21), 119 (25), 108 (16), 107 (17), 105 (29), 93 (25), 91 (37), 79 (37), 69 (17), 55 (33), 43 (100), 41 (94).

Acknowledgements—We thank Prof. C. Marticorena, Universidad de Concepción (Chile), for the botanical classification of the plant material and Prof. B. Rodríguez, Instituto de Química Orgánica-CSIC (Madrid-España) for the mass spectral measurements. This work was subsidized by the FONDECYT (Chile, Grant No 1951001) and DICYT- (Universidad de Antofagasta).

REFERENCES

1. Loyola, L. A., Bórquez, J., Morales G. and San-Martín A., *Phytochemistry*, 1997, **44**, 649.
2. Loyola, L. A., Bórquez, J., Morales G. and San-Martín A., *Phytochemistry*, 1997, **45**, 1465.
3. Wickens, G. E., *Economic Botany*, 1995, **54**(2), 207
4. Loyola, L. A., Morales, G., Rodríguez, B., Jiménez-Barbero, J., de la Torre, M. C., Perales, A. and Torres M. R., *Tetrahedron*, 1990, **46**, 5413.