Pergamon

PII: S0031-9422(96)00603-6

Phytochemistry, Vol. 44, No. 4, pp. 649–651, 1997

0031-9422/97 $17.00+0.00

DITERPENOIDS FROM *AZORELLA COMPACTA*

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

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* 29 *July* 1996)

Key Word Index—*Azorella compacta*; Umbelliferae; diterpenoid; mulinane derivatives; mulinolic acid; mulin-11,13-dien-20-oic acid.

Abstract—In addition to mulinolic acid, a new diterpenoid, mulin-11,13-dien-20-oic acid, has been isolated from the aerial parts of *Azorella compacta*. Its structure was based on a spectroscopic comparison with mulinolic acid and on chemical grounds. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

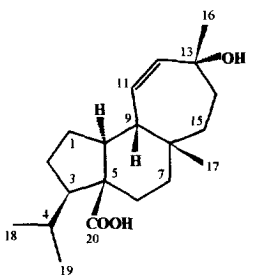
In the course of our studies on Umbelliferae diterpenoids, we have reported on the structures of the mulinane derivatives, mulinic acid [1], isomulinic acid [1], mulinenic acid [2] and 17-acetoximulinic acid [3], and recently mulinolic acid [4], obtained from the petrol extract from *Mulinum crassifolium* Phil. The unique skeleton of the diterpenoid acid was of interest from a biogenetic point of view and the search for its homologues in *Azorella compacta* Phil. was undertaken. *A. compacta*, Llareta, is a yellow-green, compact resinous cushion shrub, which grows in the high Andes of southern Peru, Bolivia, northeastern Chile and northwestern Argentina [5]. Bitter taste infusions of the whole plant are used in folk medicine, principally in the treatment of diabetes as well for asthma, colds, bronchitis, kidney and womb complaints [5].

This paper describes the isolation and structural elucidation of a new diterpenoid acid, the structure of which was established by spectroscopic analysis and on chemical grounds as mulin-11,13-dien-20-oic acid (**2**), and mulinolic acid (**1**) from *A. compacta*.

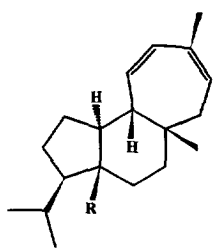
RESULTS AND DISCUSSION

The petrol extract of *A. compacta* on chromatographic purification on a silica gel column yielded a new diterpenoid acid (**2**) and the known diterpenoid acid, mulinolic acid (**1**) [4].

Combustion analysis and low-resolution, mass spectrometry indicated the molecular formula



1



2 R = COOH
3 R = COOMe

$C_{20}H_{30}O_2$ (six sites of unsaturation) for **2**. The ^{13}C NMR decoupled spectrum of **2** (Table 2) showed well-resolved resonances for all 20 carbons. DEPT analysis using a rotation angle of 90°, indicated three sp^2 methine carbons at δ 133.2, 128.4, and 125.9 and four saturated methines at δ 58.2, 55.6, 51.0 and 32.3. The DEPT 135° spectrum showed five methylene and four methyl carbons indicating, after comparison with the decoupled spectrum (Table 2), that the carbons at δ 182.1, 132.3, 59.1 and 35.4 were non-hydrogenated.

The almost identical ^{13}C chemical shifts (Table 2) of the carbon atoms in **2** and mulinolic acid (**1**), together with the similarities observed in the ^1H NMR spectra (Table 1), were indicative of the presence of a mulinane diterpenoid moiety in **2**. The IR, ^1H NMR and ^{13}C NMR data of **2** showed the presence of a carboxyl group (3300–2500 *br*, 1690 cm^{-1}, δ 11.52 *br s* and δ 182.1 *s*). Treatment of **2** with diazomethane afforded the corresponding methyl ester (**3**) (1730 cm^{-1}; δ 3.67, 3H, *s*, and δ 174.7).

The ^1H NMR spectra of **2** showed absorptions due to four methyl groups at δ 0.94 (6H, *br s*), 1.11 (3H, *d*) and 1.87 (3H, *br s*). 2D COSY experiments indicated that the signals at δ 0.94 and 1.11 were coupled

* Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR (300 MHz) spectral data of compounds **1** and **2**

	1	2
1	1.55*	1.73
	1.93*	2.13 *dd* (2.5, 11.3)
2	1.46*	1.57*
	1.91*	2.0 *m*
3	1.51*	1.55*
4	1.47*	1.55*
6	1.47*d* (9.4)	1.42*
	2.42 *dd* (3.0, 9.4)	2.50 *br d* (13.1)
7	1.38*	2.77 *br d* (17.1)
	1.47*	1.73*
9	2.08 *dd* (6.0, 10.8)	2.37 *dd* (5.8, 9.4)
10	2.16 *br d* (10.8)	1.77 *m*
11	5.58*	5.62 *dd* (5.8, 12.6)
12	5.58*	5.71 *d* (12.6)
14	1.84 *dd* (4.6, 12.5)	5.53 *d* (7.4)
15	1.10 *dd* (4.6, 15.0)	1.46 *br d* (14.0)
	2.61 *d* (15.0)	1.69 *dd* (7.4, 14.0)
16	1.32 *s*	1.87 *s*
17	0.94 *s*	0.94 *s*
18	0.86 *d* (5.8)	0.94 *d* (5.6)
19	1.04 *d* (5.8)	1.11 *d* (5.6)
20	11.35 *br s*	11.52 *br s*

At 300 MHz in $CDCl_3$. Chemical shifts are relative to TMS.

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

Table 2. ^{13}C NMR chemical shifts of compounds **1**, **2**, and **3** (75 MHz, $CDCl_3$, TMS int. standard)

C	DEPT	Mulinolic acid (**1**)	2	3
1	*t*	25.2	25.2	24.9
2	*t*	28.9	29.4	29.0
3	*d*	57.7	58.2	57.7
4	*d*	32.0	32.3	32.0
5	*s*	58.5	59.1	58.6
6	*t*	32.4	33.2	32.9
7	*t*	42.2	36.9	36.5
8	*s*	35.9	35.4	34.6
9	*d*	48.7	51.0	50.5
10	*d*	51.6	55.6	55.3
11	*d*	133.8	133.2	132.6
12	*d*	136.6	128.4	127.7
13	*s*	71.4	132.3	131.5
14	*t*/*d*/*d*	36.2	125.9	125.2
15	*t*	30.5	41.7	41.4
16	*q*	33.7	26.2	25.8
17	*q*	27.5	27.7	27.3
18	*q*	22.9	23.3*	22.9*
19	*q*	22.6	22.9*	22.5*
20	*s*	180.2	182.1	174.7
OMe	*q*			50.7

* May be interchanged.

with signal at δ 1.55 (1H, *m*). The HMQC spectra showed cross-peaks between the signal at δ 0.94 and the signals at δ 22.9 and 27.7. While the signals at δ 1.11 showed connectivities with the signal at δ 22.7. The above data revealed the presence of an isopropyl group and one tertiary methyl group. The fourth methyl group had to be attached to an olefinic carbon.

The doublet of doublet at δ 5.62 (J = 5.8 and 12.6 Hz), the doublet at δ 5.71 (J = 12.6 Hz) and the doublet at δ 5.53 (J = 7.4) showed the presence of two olefinic double bonds in **2**. 1H-1H COSY experiments and the UV spectra [λ_{max} 248 nm (ε 9.995)] indicated the presence of a homoanular diene systems. The proton at δ 5.62 is coupled with the proton at δ 5.71, while the HMQC spectrum showed that the signal at δ 5.62 is connected with the signal at δ 133.2, that at δ 5.71 with the signal at δ 128.4 and that at δ 5.53 with the signal at δ 125.9. ^{1}H–^{1}H COSY experiments showed cross-peaks between the signal at δ 5.53 and the signal of the methyl group at δ 1.87. The above data revealed the presence of two olefinic double bonds, one being *cis* disubstituted and the other trisubstituted in a homoanular diene system.

Treatment of mulinolic acid (**1**) with *p*-toluensulphonic acid gave, in high yield, a product identical in all respects to **2**. Thus, in the molecule of **2** the isopropyl group is located at C-3, the COOH group is connected at C-5 and the homoanular diene system involves C-11 to C-14 of the mulinane carbon skeleton. Consequently, **2** was characterized as mulin-11,13-dien-20-oic acid. The stereochemistry shown by **2** is in agreement with the relative stereochemistry of all mulinane derivatives previously isolated from *M. crassifolium* [4] and with the transformation of mulinolic acid (**1**) to **2**. This finding is supported by analysis of all the spectral data in particular the 2D NMR (DQF–COSY), DEPT and HMQC allowed us to assign all the chemical shifts in the ^{1}H and ^{13}C NMR spectra. For example, the signals at δ 2.37 and 51.0, 1.77 and 55.6 are assigned to the resonances of sp^3 methines in C-9 and C-10, respectively. The signal at δ 2.37 (*dd*) is coupled with the signal at δ 1.77 (*m*) and the signal δ 5.62 (*dd*) of the olefinic proton attached to C-11. The signal δ 5.53 assigned to the olefinic proton at C-14 shows connectivity with the protons of the C-16 methyl at δ 1.87.

This is the first report of the isolation of mulinane diterpenes from a plant other than *M. crassifolium*. Both the genus *Azorella* and *Mulinum* are placed in the Tribe Mulineae and the co-occurrence of these diterpenoid acids indicates a close affinity between them. It is important to mention that the *M. crassifolium* Phil. (chuquican) and the *A. compacta* Phil. (Llareta) are widely used in folk medicine, principally in the treatment of diabetes [5].

EXPERIMENTAL

Mp uncorr. Plant material was collected in November 1994 in Tatio in northern Chile, voucher speci-

mens were deposited in the Herbarium of Universidad de Concepción, Concepción, Chile.

Extraction and isolation of diterpenoids. Dried and finely powdered whole plant of *Azorella compacta* Phil. (750 g) was extracted with petrol at room temp. After filtration, the solvent was evapd to dryness under red. pres. and low temp. yielding a gum (28.5 g). The concd petrol ext. was absorbed on silica gel (60 g) and slurried onto the top of a column containing silica gel (450 g) in petrol and eluted with a petrol-EtOAc gradient with increasing amounts of EtOAc.

The fraction eluted with petrol-EtOAc (10%) (12.9 g) was rechromatographed on silica gel (150 g) eluted with 5% petrol-EtOAc to yielded mulinolic acid (**1**) (1.2 g) and **2** (420 mg).

Mulin-11,13-dien-20-oic acid (**2**). Needles, mp 82–85° (petrol-EtOAc); $[\alpha]_D^{24} - 178.62$ ($CHCl_3$; c 0.153). IR ν_{max}^{KBr} cm^{-1}: 3300–2500 *br*, 1690 (COOH), 1450, 1250; UV λ_{max} 248 nm (ε 9995), ^1H NMR: Table 1; ^{13}C NMR: Table 2; EIMS (70 eV, direct inlet) *m/z* (rel. int.): 302 $[M]^+$ (41), 287 (33), 279 (7), 259 (6), 257 (14), 241 (17), 229 (12), 213 (24), 209 (15), 192 (13), 175 (10), 163 (16), 159 (13), 157 (14), 149 (28), 147 (15), 145 (20), 135 (23), 134 (23), 133 (24), 132 (27), 123 (17), 121 (78), 120 (89), 119 (75), 117 (22), 115 (22), 109 (33), 108 (86), 107 (40), 106 (32), 105 (42), 95 (44), 94 (96), 93 (100), 92 (59), 91 (88), 84 (11), 83 (18), 81 (47), 80 (39), 79 (59), 77 (31), 69 (21), 67 (81), 55 (24), 53 (16), 43 (70), 41 (40). Found: C, 78.66; H, 9.84%; Calcd for $C_{20}H_{30}O_2$: C, 79.40; H, 10.00%.

Methylation of **2**. Compound **2** (50 mg) was dissolved in Et_2O (5 ml) and treated with ethereal CH_2N_2. After usual work-up, recrystallization of the product from Et_2O gave the methyl ester **3**. Amorphous powder; IR ν_{max}^{KBr} cm^{-1}. 1730 (COOMe), 1440, 1200; ^1H NMR. δ 0.83 (*br. s*, 6H), 1.00 (*d*, 3H, $J = 6.2$ Hz), 1.37 (*m*, 4H), 1.65 (*m*, 4H), 1.78 (*s*, 3H), 1.80–2.20 (*m*, 4H), 2.43 (*dd*, 1H, $J = 2.5$ and 12 Hz), 2.53 (*br. d*, 1H), 3.66 (*s*, 3H), 5.45 (*d*, 1H, $J = 6.5$), 5.50 (*dd*, 1H, $J = 5.6$ and 12.6), 5.62 (*dd*, 1H, $J = 0.9$ and 12.6); ^{13}C NMR: Table 2.

Dehydration of mulinolic acid (**1**). Compound **1** (92.8 mg) was dissolved in C_6H_6 (6 ml) and treated with pTSOH for 1 hr at room temp. The reaction mixt. was evaporated *in vacuo* and partitioned between EtOAc and H_2O. The EtOAc layer was concd to give a residue, which was further purified by Sephadex LH-20 chromatography (MeOH) to afford **2** (79.7 mg).

Acknowledgements—We thank Prof. C. Marticorena, University de Concepción (Chile), for the botanical classification of the plant material and Prof. B. Rodríguez, Instituto de Química Orgánica-CSIC (Mardrid-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. Q., Morales, G., Rodríguez, B., Jiménez-Barbero, J., de la Torre, M. C., Perales, A. and Torres, M. R., *Tetrahedron*, 1990, **46**, 5413.
2. Loyola, L. A., Morales, G., de la Torre, M. C., Pedreros, S. and Rodriguez, B., *Phytochemistry*, 1990, **29**, 3950.
3. Loyola, L. A., Morales, G., Rodríguez, B., Jiménez-Barbero, J., Pedreros, S., de la Torre, M. C. and Perales, A., *Journal of Natural Products*, 1991, **54**, 1404.
4. Loyola, L. A., Bórquez, J., Morales, G. and San Martin, A., *Phytochemistry*, in press.
5. Wickens, G. E., *Economic Botany*, 1995, **49**(2), 207.