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MULINOL, A DITERPENOID FROM *AZORELLA COMPACTA*

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Key Word Index—*Azorella compacta*; Umbelliferae; diterpenoid; mulinane derivatives; mulinic acid; mulinol.

Abstract—In addition to mulinic acid, a new diterpenoid, mulinol has been isolated from the aerial parts of *Azorella compacta*. Its structure was based on spectroscopic comparison with mulinolic acid and chemical grounds.

INTRODUCTION

In the course of our studies on the constituents of umbelliferous plants, we have already reported the isolation of several rearranged diterpenoids, isolated from the petrol extract of the aerial parts of *Mulinum crassifolium* [1–4] and of *Azorella compacta* [5], suggesting the name mulinane for their new carbon skeleton.

Further investigation of the fractions more polar chromatographic of the same extract of *Azorella compacta* led us to isolate mulinic acid (**1**) and a new diterpenoid with a mulinane skeleton, mulinol (**2**). The structure of **2** was established by spectroscopic analysis and chemical results as 13,20-dihydroxymulin-11-en.

RESULTS AND DISCUSSION

The petrol extract of *A. compacta* Phil. on chromatographic purification on a silica gel column yielded a new diterpenoid **2** and the known diterpenic acid, mulinic acid (**1**), previously isolated from *Mulinum crassifolium* Phil. (Umbelliferae) [1].

Combustion analysis and low resolution mass spectrometry indicated the molecular formula $C_{20}H_{34}O_2$, requiring four sites of unsaturation. The ^{13}C NMR spectrum of **2** (Table 1) showed well-resolved resonances for all 20 carbons. DEPT analysis using a nutation angle of 90°, indicated two sp^2 methine carbons at δ 133.7 and 136.3 and four saturated methine at δ 57.9, 50.9, 47.3 and 31.7. In addition, the DEPT 135° spectrum showed seven methylene and four methyl carbons indicating that the carbons at δ 71.2, 47.7 and 35.8 were quaternary, after comparison with the decoupled spectrum (Table 1).

The almost identical ^{13}C chemical shifts of the carbon atoms in **2** with those of mulinolic acid (**3**) [4], together with the similarities observed in their ^1H NMR spectra (Table 1), indicated the mulinane diterpenoid skeleton in **2**. The IR (3300–2990 cm^{-1}), ^1H NMR and ^{13}C NMR data of **2** showed the presence of a tertiary hydroxyl group (δ 71.2, *s*) and a primary hydroxyl group (δ 3.51, 1H, *d*, J = 11.6 Hz; δ 3.73, 1H, *d*, J = 11.6 Hz, δ 59.8, *t*). The ^1H NMR spectrum of **2** showed signals due four methyl groups at δ 0.84 (3H, *d*, J = 6.0 Hz), 0.94 (3H, *s*), 1.02 (3H, *d*, J = 6.0 Hz, and 1.28 (3H, *s*). 2D COSY experiments indicated that the signals at δ 0.84 and 1.02 were coupled with the signal at δ 1.67 (1H, *m*). The HMQC spectra showed cross-peaks between the signal at δ 0.84 with the signal at δ 23.4 and the signal at δ 1.02 with the signal at δ 23.3. The signal at δ 0.94 showed connectivities with the signal at δ 27.6. The above data revealed the presence of an isopropyl group and one tertiary methyl group. The fourth methyl group was attached to carbon bearing a hydroxyl group (δ_C 33.7, *q*; δ_H 1.28, *s*). Two deshielded carbon resonances at δ_C 133.7, *d* and 136.3, *d* and two deshielded proton resonances at δ_H 5.40 (*dd*, J = 12.4, 7.8 Hz) and 5.53 (*d*, J = 12.4 Hz) were assigned to a disubstituted olefinic double bond. All of the above data can be accommodated in the mulinane carbon skeleton, as depicted in formula **2**.

Most of the structural fragments of mulinol (**2**) were identified by the heteronuclear multiple bond connectivity (HMBC) spectrum (Fig. 1). Correlations were observed between the signal at δ_H 1.28 (3H, *s*, assigned to the C-16 protons) with the signal at δ_C

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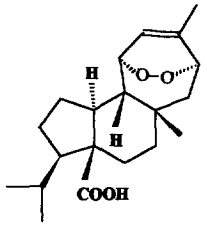
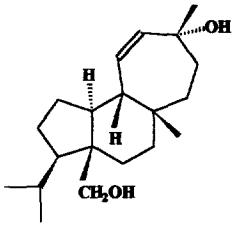
Table 1. NMR signals of compounds **2** and **3**

	Mulinol (**2**)		Mulinolic acid (**3**)	
	δ_C	δ_H	δ_C	δ_H
1	24.3 (*t*)	β1.46*	25.2 (*t*)	α1.55*
		α1.05*		β1.93*
2	28.4 (*t*)	α1.36*	28.9 (*t*)	β1.46*
		β1.84*		α1.91*
3	57.9 (*d*)	1.14 *m*	57.7 (*d*)	1.51*
4	31.7 (*d*)	1.67 *m*	32.0 (*d*)	1.47*
5	47.7 (*s*)		58.4 (*s*)	
6	29.6 (*t*)	α1.36*	32.4 (*t*)	α1.47 *d* (9.4)
		β2.13 *dt* (9.6, 3.0)		β2.42 *dd* (3.0; 9.4)
7	39.9 (*t*)	β1.23*	42.2 (*t*)	β1.38*
		α1.46*		α1.47*
8	35.8 (*s*)		35.9 (*s*)	
9	47.3 (*d*)	1.97 *dd* (12.8, 7.8)	48.7 (*d*)	2.08 *m*
10	50.9 (*d*)	1.84 *m*	51.6 (*d*)	2.16 *m*
11	133.7 (*d*)	5.40 *dd* (12.4, 7.8)	133.8 (*d*)	5.58 *d*
12	136.3 (*d*)	5.53 *d* (12.4)	136.5 (*d*)	5.58 *d*
13	71.2 (*s*)		71.4 (*s*)	
14	36.1 (*t*)	β1.60*	36.2 (*t*)	β1.63 *dd* (9.3; 12.5)
		α1.80*		α1.84 *dd* (4.6; 12.5)
15	30.2 (*t*)	β0.94	30.5 (*t*)	β1.10 *dd* (4.6; 15.0)
		α2.61 *ddd* (13.0, 1.8)		α2.61 *dt* (15)
16	33.4 (*c*)	1.28 *s*	33.7 (*c*)	1.32 *s*
17	27.6 (*c*)	0.94 *m*	27.5 (*c*)	0.94 *s*
18	23.4 (*c*)	0.84 *d* (6.0)	22.9 (*c*)	0.86 *d* (5.8)
19	23.3 (*c*)	1.02 *d* (6.0)	22.6 (*c*)	1.04 *d* (5.8)
20	59.8 (*t*)	3.73 *d* (11.6)	180.2 (*s*)	
		3.51 *d* (11.6)		

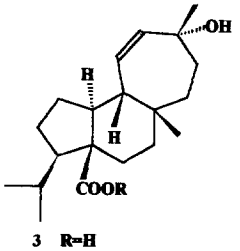
At 75/300 MHz in $CDCl_3$. Chemical shift are relative to TMS.
* Overlapped signal the chemical shift of these protons was measured on the HMQC spectra.



1



2



3 R=H
4 R=Me

136.3 *d* (olefinic C-12), δ_C 71.2 *s* (C-13) and δ_C 36.1 *t* (C-14). The signals at δ_H 5.40 (*dd*, J = 12.4, 7.8 Hz) and 5.53 (*d*, J = 12.4 Hz) assigned to the H-11 and H-12 correlated with the signal at δ_C 71.2 *s* (C-13). These facts confirmed the location of a hydroxyl group in C-13 and an olefinic functionality at C-11/C-12. The C-17 protons (δ 0.94, *s*) showed correlation with C-15, C-9 and C-7 carbons (at δ_C 30.2 *t*, δ 47.3 *d* and δ 39.9 *t*, respectively) and H-9 proton C-12 (δ_H 1.97, *dd*, J = 12.8, 7.8 Hz) with C-8, C-10, C-11 and C-15 carbons (at δ_C 35.8 *s*, δ_C 50.9 *d*, δ_C 133.7 *d*, δ_C 136.3 *d* and δ_C 30.2 *t*, respectively). Similarly, the H-6 (δ_H 2.13 *dd*, J = 9.6, 3.0 Hz) showed correlation with the C-8 and C-7 carbons (at δ_C 35.8 *s* and δ_C 39.9 *t*, respectively). The C-18 and C-19 protons (δ_H 0.84 *d*, J = 6.0 Hz) and δ_H 1.02 *d*, J = 6.0 Hz) show connectivities with C-2, C-3 and C-4 carbons (at δ_C 28.4 *t*, δ_C 57.9 *d* and δ_C 31.7 *d*, respectively). The location of the CH_2OH substituent in mulinol (**1**) is confirmed by the connectivities between H-20 (δ_H 3.51, *d*, J = 11.6 Hz) and 3.73 *d*, J = 11.6 Hz with C-3 (δ_C 57.9 *d*), C-5 (δ_C 47.7 *s*), C-6 (δ_C 29.6 *t*) and C-10 (δ_C 50.9 *d*).

The treatment of mulinolic acid (**3**) with ethereal diazomethane gave, in high yield the methyl ester **4**. Lithium aluminium hydride reduction of **4** yielded a product identical in all respects to **2** (mp. [α], IR, NMR). Consequently, mulinol (**2**) was characterized as 13,20-dihydroxymulin-11-en.

The principal results from ROESY NMR experiments suggested that mulinol had the stereochemistry shown in **2**, in agreement with the relative stereochemistry of mulinolic acid (**3**), previously isolated

from *Mulinum crassifolium* [4], and *Azorella compacta* [5]. The ROESY NMR experiment showed a correlation between the signal at δ 1.84 for the Hα-10 and the signals at δ 1.14 (H-3) and δ 1.36 (H-6) which should be in an α-configuration. In addition, Hα-6 showed correlation with the signal at δ 2.61 (H-15) and δ 1.80 (H-14) showed correlation with the signal at δ 2.61 (H-15) indicating that they were in the same configuration. Moreover, the signal at δ 1.97 for the Hβ-9 showed correlation with the signals at δ 0.94 (Me-17), δ 3.51 and 3.73 (H-20), δ 1.84 (H-2) and δ 5.40 (H-11) which should be the β-configuration. A correlation between H_3-17 and H-14 (δ 1.63) and H_3-16 (δ 1.32) with H-14 (δ 1.63) indicated that the C-13 hydroxy function occupied the α-configuration. The signal at δ 2.13 for the Hβ-6 showed correlation with the signal at δ 1.23 (Hβ-7) and δ 1.04 (H_3-19), indicating that the isopropyl group was also in the β-configuration. Mulinol (**2**) is the first mulinane diterpenoid isolated with a hydroxymethylene group at C-5, compared with the other known mulinane diterpenoids with a carboxyl group in C-5.

EXPERIMENTAL

Mp are uncorr. Plant materials were collected in November 1994 in Tatio in northern Chile, voucher specimens 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 extract 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 petrol–EtOAc gradient. The fraction eluted with petrol–EtOAc (5%) (6.2 g) was rechromatographed on silica gel (140 g) eluted with 5% petrol–EtOAc to yield mulinic acid (**1**) (140 mg) and **2** (90 mg).

Mulinol (**2**). Amorphous powder, mp 112–114°; $[\alpha]_D^{24}$ −85° ($CHCl_3$; *c* 0.155). IR ν_{max}^{KBr} cm^{-1}: 3200–2990, 1450, 1390, 1050. ^1H NMR (see Table 1). ^{13}C NMR (see Table 1). EIMS (70 eV, direct inlet) *m/z* (rel. int.): 306 $[M]^+$ (2), 288 (22), 258 (29), 257 (58), 227 (19), 189 (21), 163 (37), 149 (30), 121 (43), 119 (52), 108 (81), 91 (77), 79 (80), 69 (65), 55 (63), 43 (100), 41 (61). Found: C, 77.89; H, 10.93%; Calcd for $C_{20}H_{34}O_2$: C, 78.40; H, 11.20%.

Methylation of **3**. Compound **3** (300 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 **4** (270 mg) as an amorphous powder, IR ν_{max}^{KBr} cm^{-1}: 3500, 1750 (COOMe), 1450, 1390, 1050.

Reduction of compound **4** *with* $LiAlH_4$. Compound **4** (200 mg) in Et_2O (15 ml) was treated with $LiAlH_4$ (50 mg) at reflux during 6 hr. The usual work-up yielded **2** (70 mg).

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