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Mulinane-type diterpenoids from *Mulinum spinosum*☆

Alejandra I. Chiaramello, Carlos E. Ardanaz, Eduardo E. García, Pedro C. Rossomando*

Química Orgánica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco 917, D5700 BWS, San Luis, Argentina

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Abstract

In addition to the known mulinolic and mulinenic acids, two diterpenoids, mulin-11-ene-13-α,14-α-dihydroxy-20-oic and mulin-12-ene-14-one-20-oic acids, were isolated from the aerial parts of *Mulinum spinosum* (Cav.) Pers. (Apiaceae). Their structures were determined based on spectroscopic studies.

Keywords: *Mulinum spinosum*; Apiaceae; Diterpenoid; Mulinane derivatives; Mulin-11-ene-13-α,14-α-dihydroxy-20-oic acid; Mulin-12-ene-14-one-20-oic acid

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1. Introduction

Mulinum (Apiaceae) is a genus of 15–20 species confined to Argentina, Bolivia and Chile (Constance, 1988). *M. spinosum* (Cav.) Pers. is a shrub of the central Andes in Argentina and Chile and Patagonia steppe in Argentina, where it is known as 'neneo' or 'yerba negra' and is used as a medicinal plant to treat dental neuralgias, hepatic diseases and altitude sickness (Ruiz Leal, 1972; San Martín, 1983). Previous studies on this plant reported the presence of resinic acids, α- and β-pinene and limonene (Riveros et al., 1982) as well as two mulinane diterpenoid acids, mulin-11,13-dien-20-oic acid and mulin-11,13-dien-17-hydroxy-20-oic acid (Nicoletti et al., 1996).

This paper describes the isolation and structural elucidation of two new mulinane derivatives, mulin-11-ene-13-α,14-α-dihydroxy-20-oic acid (**1**) studied as its acetoxy derivative **1a**, and mulin-12-ene-14-one-20-oic acid (**2**). The structure of **1** was established by spectroscopic analysis and on chemical grounds while that of **2** was determined by spectroscopic analysis only. Along with the new compounds **1** and **2**, we isolated the known diterpenoids mulinolic (**3**) (Loyola et al., 1996) and mulinenic (**4**) (Loyola et al., 1991) acids, both previously isolated from *M. crassifolium*.

2. Results and discussion

HREIMS of **1a** showed, a peak consistent with the [M–H_2O] ion, ($C_{22}H_{32}O_4$, required *m*/*z* 360.23006, found *m*/*z* 360.231997). The ^{13}C NMR spectrum of **1a** (Table 2) showed well-resolved resonances for all 22 carbons. DEPT analysis using a 90° angle, indicated two sp^2 methine carbons at δ_C 136.2 and 132.4, and five saturated methines at δ_C 75.2, 57.4, 50.7, 47.0 and 31.7. In addition, the DEPT 135° spectrum showed five methylene and five methyl carbons indicating that the carbons at δ_C 180.1, 170.1, 72.2, 58.0 and 36.1 were quaternary, after comparison with the decoupled spectrum. The ^{1}H NMR (Table 1) and ^{13}C NMR spectral data of **1a** showed the presence of a tertiary hydroxyl group (δ_C 72.2, *s*) and a secondary acetoxyl group (δ_H 5.01, 1H, *dd*, $J = 11.8$, 2.9 Hz; δ_C 75.2, *d*). The presence of the acetoxyl group was also evidenced by signals at 1736 cm^{-1} (IR); δ_H 2.09 (3H, *s*) (^{1}H NMR); δ_C 21.3, *q*; and δ_C 170.1, *s* (^{13}C NMR). The ^{1}H NMR spectrum of **1a** showed signals due to five methyl groups at δ_H 0.85 and 1.02 (both 3H, *d*, $J = 5.5$ Hz), δ_H 1.07, 1.31 and 2.09 (all of them 3H, *s*). Phase sensitive 2D COSY experiments indicated that the signals at δ_H 0.85 and 1.02 were

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* Corresponding author. Fax: +54-2652-426711.
E-mail address: prosso@unsl.edu.ar (P.C. Rossomando).

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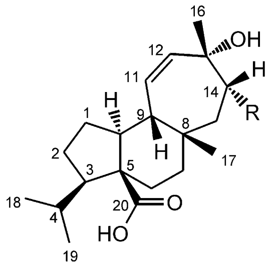
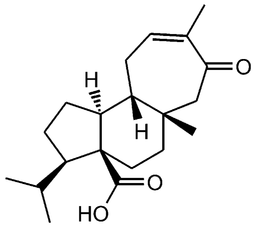


	R
1	OH
1a	OAc
3	H



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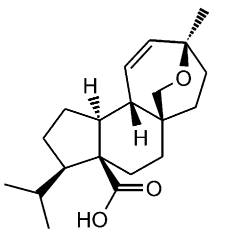

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coupled with the signal at δ_H 1.48 (1H, *m*). The heteronuclear correlation (HETCORR) spectrum showed cross-peaks between δ_H 0.85 and δ_C 22.7 and the signal at δ_H 1.02 and δ_C 22.3. The signal at δ_H 1.07 showed connectivities with δ_C 27.3. The above data revealed the presence of an isopropyl group and a tertiary methyl group. The fourth methyl group was attached to carbon bearing a hydroxyl group (δ_H 1.31; δ_C 29.3, *q*). Two deshielded carbon resonances at δ_C 132.4, *d* and 136.2, *d* and two deshielded proton resonances at δ_H 5.54 (*d*, $J = 12.4$ Hz) and 5.76 (*dd*, $J = 12.4$, 8.4 Hz) were assigned to a disubstituted olefinic double bond with a *cis* configuration. All the above data can be accomodated in the mulinane carbon skeleton as depicted in formula **1a**.

Most of the relevant structural fragments of **1a** were identified by the heteronuclear long range correlations COLOC, aided by the phase sensitive COSY experiment. Correlations were observed between the signal at δ_H 1.31 (3H, *s*, H-16) and the signals at δ_C 132.4, *d* (olefinic C-12), δ_C 72.2, *s* (C-13) and δ_C 75.2, *d* (C-14). The signal at δ_H 5.76 (H-11) correlated with the resonance at δ_C 72.2 (C-13). The resonance at δ_C 170.1 assigned to the carbonyl group of the acetoxyl segment showed cross-peaks with δ_H 2.83 (1H, *dd*, $J = 13.5$, 11.8 Hz, H-15α). In addition HH COSY spectrum revealed δ correlations between H-14 and the α and β protons on C-15. A cross-peak between H-11 and H-9 at δ_H 2.09 (1H, *dd*, $J = 11.6$, 8.4 Hz) was also observed. The C-17 protons (δ_H 1.07, *s*) showed correlations with C-15, C-9 and C-8 carbons (δ_C 36.6, *t*; 47.0, *d* and 36.1, *s*, respectively). Similarly, the H-18 (δ_H 0.85, *d*) and H-19 (δ_H 1.02, *d*) protons showed connectivities with C-3 (δ_C 57.4, *d*) and C-4 (δ_C 31.7, *d*) carbons.

Table 1
^1H NMR[a] spectral data for compounds **1a**–**3**

	1a	**2**	**3**
Position	δ_H	δ_H	δ_H
3	2.44[b] *m*		
4	1.48[b] *m*		
6			β 2.42 *dd* (9.9, 2.9)
9	2.09[b] *dd* (11.6, 8.4)		
10	2.3[b] *m*		
11	5.76 *dd* (12.4, 8.4)		5.58 *m*
12	5.54 *d* (12.4)	6.17 *brd*	5.58 *m*
14	5.01 *dd* (11.8, 2.9)		
15	α 2.83 *dd* (13.5, 11.8)	α 2.77 *d* (12.4)	α 2.61 *dd* (14.8, 2.5)
	β 1.15 *dd* (13.5, 2.9)	β 2.33 *d* (12.4)	
16	1.31 *s*	1.82 *d* (2.3)	1.32 *s*
17	1.07 *s*	1.04 *s*	0.94 *s*
18	0.85 *d* (5.5)	0.85 *d* (5.5)	0.86 *d* (5.6)
19	1.02 *d* (5.5)	1.01 *d* (5.5)	1.03 *d* (5.6)
$OCO\underline{C}H_3$	2.09 *s*		

[a] At 200.13 MHz in $CDCl_3$. Chemical shifts are relative to TMS. (*J* in Hz).

[b] Overlapped signals. The chemical shifts of these protons were measured on the phase sensitive HH COSY and on HETCOR spectra.

The main results from phase sensitive NOESY spectra suggested that **1a** had the stereochemistry shown, in agreement with the relative stereochemistry of mulinolic

Table 2
^{13}C NMR[a] spectral data for compounds **1a**–**3**

	1a	**2**	**3**
Position	δ_C	δ_C	δ_C
1	24.8 *t*	24.8 *t*	25.0 *t*
2	28.7 *t*	28.6 *t*	28.7 *t*
3	57.4 *d*	58.1 *d*	57.4 *d*
4	31.7 *d*	31.8 *d*	31.8 *d*
5	58.0 *s*	57.2 *s*	58.1 *s*
6	32.1 *t*	33.1 *t*	32.1 *t*
7	41.9 *t*	40.3 *t*	41.9 *t*
8	36.1 *s*	37.4 *s*	35.6 *s*
9	47.0 *d*	44.3 *d*	48.4 *d*
10	50.7 *d*	48.7 *d*	51.3 *d*
11	136.2 *d*	28.2 *t*	133.5 *d*
12	132.4 *d*	135.2 *d*	136.3 *d*
13	72.2 *s*	140.6 *s*	71.1 *s*
14	75.2 *d*	206.1 *s*	35.9 *t*
15	36.6 *t*	54.7 *t*	30.2 *t*
16	29.3 *q*	19.5 *q*	33.5 *q*
17	27.3 *q*	30.1 *q*	27.3 *q*
18	22.7 *q*	22.6 *q*	22.7 *q*
19	22.3 *q*	22.2 *q*	22.3 *q*
20	180.1 *s*	180.3 *s*	179.9 *s*
$OCO\underline{C}H_3$	21.3 *q*		
$O\underline{C}OCH_3$	170.1 *s*		

[a] At 50.3 MHz in $CDCl_3$. The assignments were confirmed by HETCOR and COLOC experiments.

acid (**3**) (Loyola et al., 1996). H-14 showed dipolar correlations with H-16 and H-17 protons, both with β configuration, that confirms the α-orientation of the acetate group on C-14. Other selected NOESY correlations were H-9/H-11, H-9/H-17, H-12/H-16 and H-3/H-19. Based on these findings we assigned **1a** the structure of mulin-11-ene-13-α-hydroxy-14-α-acetoxy-20-oic acid.

HREIMS of **2** showed an ion consistent with the molecular formula $C_{20}H_{30}O_3$ (required m/z 318.219495, found m/z 318.217643). The ^{13}C NMR spectral data of **2** (Table 2) showed signals at δ_C 180.3, *s* (C-20) and δ_C 206.1, *s* (C-14) typical for a carboxylic acid and a ketone, respectively. Two carbons at δ_C 135.2, *d* (C-12) and 140.6, *s* (C-13) together with the corresponding signal of one vinylic proton H-12 (δ_H 6.17, *brd*) in the ^{1}H NMR spectrum (Table 1) indicated the presence of a trisubstituted double bond. The proton shift of the methyl signal at δ_H 1.82 (3H, *d*, $J=2.3$ Hz, H-16) and its COLOC correlations with C-12, C-13 and C-14 as well as the C-14/H-12 cross-peak suggested the presence of an α,β-unsaturated ketone as depicted in **2**. A connectivity between H-12 and H-16 in the phase sensitive H–H COSY spectrum reinforced the allylic relationship ($J_{12,16}=2.3$ Hz). The location of the keto carbonyl group (C-14) was also evidenced by the strong deshielding of the C-15β proton compared with the same proton in **1a**, as well as for its coupling pattern simplification [δ_α 2.77 and δ_β 2.33 (both *d*, $J=12.4$ Hz)]. In addition the H-15α showed cross-peaks with H-17 (δ_H 1.04, 3H, *brs*) with a $J_{15\alpha,17}=1.3$ Hz. On the other hand the H-17 showed COLOC correlations with C-7, C-8, C-9 and C-15 carbons at δ_C 40.3, *t*; 37.4, *s*; 44.3, *d* and 54.7, *t*, respectively. A dipolar correlation between the signals at δ_H 2.33 (H-15β) and δ_H 1.04 (H-17) confirms the proposed configuration of C-15 protons. Based on these findings we assigned **2** the structure of mulin-12-ene-14-one-20-oic acid.

3. Experimental

3.1. General

M.p. uncorr. Plant material was collected in Uspallata, Las Heras, Mendoza, Argentina, in March 2000 and voucher specimens (Del Vitto et al., N° 9092, UNSL) were deposited in the Herbario of Universidad Nacional de San Luis, San Luis, Argentina. CC: silica gel 60 (Merck), 0.063–0.200 mm; TLC: Kieselgel mit Fluoreszenz-Indicator UV_{254} (Macherey-Nagel), 0.25 mm; IR: in the 4000–225 cm^{-1} range with 32 scans using the KBr pellet technique. Spectral resolution: 4 cm^{-1}. (Nicolet Protégé 460 spectrometrer). NMR spectra was obtained using a Bruker AC-200 spectrometer. HRMS was performed on a Micromass, VG AutoSpec. EIMS: at 70 eV on GCQ Plus instrument with MS/MS.

3.2. Extraction and isolation of diterpenoids

Dried and finely powdered tissues from the aerial parts of *M. spinosum* (3.65 g) were extracted with acetone at room temp. (3 × 48 h). After filtration, the solvent was evapd. to dryness under red. pres. and low temp. yielding 330 g of dark syrup. The conc. acetone extract was fractionated on silica gel column (1 kg) with hexane and hexane–EtOAc mixtures of increasing polarity as elution solvents. The fraction hexane–EtOAc 30% (47.5 g) was separated on silica gel using the same elution solvents yielding 31.5 mg of **1** (hexane–EtOAc 20%). The fraction hexane–EtOAc 10% (24.5 g) was rechromatographed using the above conditions giving 25.7 mg of **2** (hexane–EtOAc 10%). Compound **1** (30 mg) was acetylated with Ac_2O and 4-dimethylamine-pyridine in the usual manner, yielding 24 mg (72%) of pure **1a**.

3.3. Mulin-11-ene-13-α-hydroxy-14-α-acetoxy-20-oic acid (1a)

Amber oil; $[\alpha]_D^{20}$ −82.4 (Me_2CO; *c* 0.26); IR ν_{max}^{KBr}: cm^{-1}: 3300–2800 *br*, 1717 (COOH), 1736 ($OCOCH_3$), 1159, 1122, 876; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; EIMS m/z (rel. int.): 360 [M^+-H_2O] (3), 318 [M^+-HOAc] (20), 300 [318-H_2O] and [360-HOAc] (28), 285 [300-15] (9), 272 (66), 255 (34), 229 (100), 211 (23), 175 (29), 145 (26), 105 (42), 91 (36). HREIMS: found 360.231997 (calc. 360.23006 for $C_{22}H_{32}O_4$).

3.4. Mulin-12-ene-14-one-20-oic acid (2)

White solid; m.p.: 218–220 °C; $[\alpha]_D^{20}$ −46.1 (Me_2CO; *c* 0.36); IR ν_{max}^{KBr}: cm^{-1}: 3300–2500 *br*, 1686 (COOH), 1671 (C=O), 1446, 1385, 954; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2; EIMS m/z (rel. int.): 318 [M^+] (36), 300 [M^+-H_2O] (7), 273 [M^+-CO_2H] (40), 272 [M^+–HCOOH] (80), 257 [M^+–HCOOH–CH_3] (7), 229 [M^+–HCOOH–C_3H_7] (75), 175 (100), 147 (27), 133 (40), 119 (34), 105 (65), 91 (47). HREIMS: found 318.217643 (calc. 318.219495 for $C_{20}H_{30}O_3$), found 272.213884 (calc. 272.214016 for $C_{19}H_{28}O$).

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