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Mulinane-type diterpenoids from *Laretia acaulis*

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Abstract

In addition to the known mulinolic acid and mulin-11, 13-dien-20-oic acid, 13-epimulinolic acid has been isolated from the aerial parts of *Laretia acaulis* (Cav.) Gill et Hook (Umbelliferae). Its structure was based on spectroscopic comparison with mulinolic acid and by chemical characterization. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Laretia acaulis*; Umbelliferae; Diterpenoid; Mulinane derivatives; Mulinolic acid; Mulin-11, 13-dien -20-oic acid; 13-epimulinolic acid

1. Introduction

In the course of studies on diterpenoids from species of the tribe Mulinae (Umbelliferae), we have reported on the structures of several mulinane derivatives obtained from the petrol extracts of Mulinum *crassifolium* Phil. and *Azorella compacta* Phil. (Loyola, Bórquez, Morales & San-Martín, 1996, 1997a, 1997b; Loyola et al., 1990, 1991). In the continuation of our phytochemical investigation of Umbelliferae we have studied *Laretia acaulis* (Cav.) Gill et Hook., known in Chile as 'llareta de la zona central'. This plant is a yellowish-green, compact resinous cushion shrub, which grows in the high Andes of Chile. Whole plant infusions are widely used as diabetes treatment in folk medicine.

This paper describes the isolation of the two known diterpenoids: mulinolic acid (**1**) (Loyola et al., 1997a) and mulin-11, 13-dien-20-oic acid (**2**) (Loyola et al., 1997b) and a new diterpenic acid from petrol extract of *L. Acaulis*. The structure of compound **3** was established by spectroscopic analysis and chemical grounds as 13-epimulinolic acid.

2. Results and discussion

After chromatographic purification on silica gel the petrol extract of *L. acaulis* yielded a new diterpenic acid (**3**) and two known diterpenic acids, mulinolic acid (**1**), and mulin-11-13-dien-20-oic acid (**2**), recently isolated from *Mulinum crassifolium* Phil. (Loyola et al., 1997b) and *Azorella compacta* Phil. (Umbelliferae) (Loyola et al., 1997a), respectively.

13-Epimulinolic acid (**3**), $[\alpha]_D^{24} = -49.20$ ($CHCl_3$; c 0.26), had the molecular formula $C_{20}H_{32}O_3$ determined by HR-EIMS (found 320.2370, calculated 320.2351). Its ^1H- and ^{13}C-NMR spectral data (see Table 1) were almost identical with these of mulinolic acid (**1**). In fact, the main differences between the ^1H-NMR spectra of **3** and **1** were in the chemical shifts corresponding to the CH_3-16 ($\Delta\delta - 0.10$), H-12 ($\Delta\delta - 0.10$), H-10 ($\Delta\delta - 0.33$), H-14α ($\Delta\delta + 0.16$), H-14β ($\Delta\delta - 0.12$) and H-15α ($\Delta\delta - 0.44$). Moreover, a comparison between the ^{13}C-NMR spectra of **3** and **1** (Table 1) showed a significant difference in the C-11 ($\Delta\delta + 4.3$), C-12 ($\Delta\delta - 9.5$), C-13 ($\Delta\delta + 2.7$) and C-16 ($\Delta\delta - 5.3$) chemical shifts. These data could be attributed to an opposite stereochemistry at C-13 of **3** in comparison with the mulinolic acid (**1**), in which the hydroxyl group at C-13 was α-orientated (Loyola et al., 1996).

Additional proof of the relative stereochemistry of

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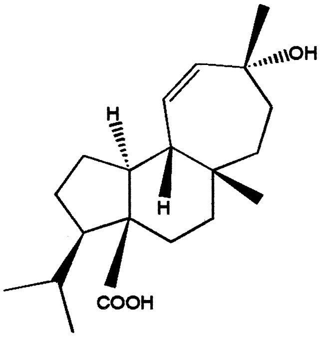
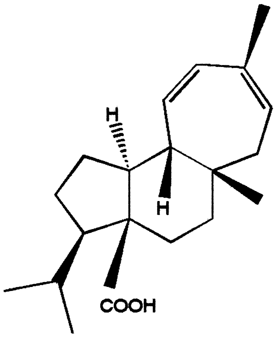


1



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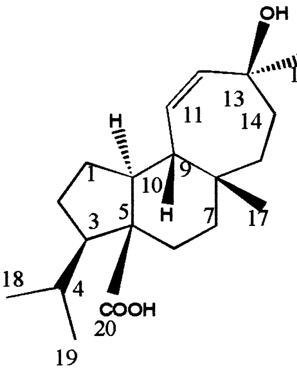

3

3 was obtained from a ROESY NMR experiment. This experiment showed a correlation between the signal at δ 2.04 for the H-9β and the signals at δ 1.45 (H-1β), δ 2.35 (H-6β) and δ 0.93 (Me-17) which should be in the same face of the molecule. In addition, H-6β showed correlation with the signal at δ 1.28 (H-7) and δ 1.51 (H-14) indicating that they are in the same configuration. Moreover, the signal at δ 2.17 (H-15α) showed correlation with the signal at δ 1.23 (Me-16) and both signals also showed correlations with the signal at δ 1.83 for the H-10α proton which should be in α configuration. Thus, we concluded that **3** has S-configuration at C-13.

Both mulinolic acid (**1**) and compound **3** with acid gave mulin-11, 13-dien-20-oic acid (**2**) by *p*-toluensulfonic treatment, as was confirmed by TLC, IR, NMR and $[\alpha]^{24}_{\mathrm{D}}$. Therefore, the structure of 13-epimulinolic acid was determined as **3**.

3. Experimental

3.1. General

Mp. uncorr. plant material, collected in Los Morteros, Vallenar, Chile, in November 1997, was identified by Prof. C. Marticorena and voucher specimens were deposited in the Herbarium of Universidad de Concepción, Concepción, Chile.

Table 1
NMR[a] spectral data for 2 and 3

	13-Epimulinolic δ_C	Acid **3** δ_H	Mulinolic δ_C	Acid **1** δ_H
1	24.7 (*t*)	β 1.45[b], α 1.91[b]	25.2 (*t*)	α 1.55[b], β 1.93[b]
2	28.7 (*t*)	α 1.34[b], β 1.83[b]	28.9 (*t*)	β 1.46[b], α 1.91[b]
3	57.4 (*d*)	1.36[b]	57.7 (*d*)	1.51[b]
4	31.7 (*d*)	1.36[b]	32.0 (*d*)	1.47[b]
5	57.8 (*s*)		58.4 (*s*)	
6	32.5 (*t*)	α 1.31[b], β 2.35 *m*	32.4 (*t*)	α 1.47 *d* (9.4), β 2.42 *dd* (3.0; 9.4)
7	41.9 (*t*)	β 1.28[b], α 1.40[b]	42.2 (*t*)	β 1.38[b], α 1.47[b]
8	35.6 (*s*)		35.9 (*s*)	
9	48.0 (*d*)	2.04 *dd* (8.2, 11.0)	48.7 (*d*)	2.08 *m*
10	50.9 (*d*)	1.83[b]	51.6 (*d*)	2.16 *m*
11	138.1 (*d*)	5.26 *dd* (8.2, 12.9)	133.8 (*d*)	5.58 *d*
12	127.0 (*d*)	5.48 *d* (12.9)	136.5 (*d*)	5.58 *d*
13	74.1 (*s*)		71.4 (*s*)	
14	36.8 (*t*)	β 1.51 *dd* (5.9, 13.5), α 2.00 *dd* (13.5, 14.6)	36.2 (*t*)	β 1.63 *dd* (9.3; 12.5), α 1.84 *dd* (4.6; 12.5)
15	30.7 (*t*)	β 1.07 *dd* (4.8, 14.6), α 2.17 *t* (14.3)	30.5 (*t*)	β 1.10 *dd* (4.6; 15.0), α 2.61 *dt* (15)
16	28.4 (*q*)	1.23 *s*	33.7 (*q*)	1.32 *s*
17	27.5 (*q*)	0.93 *s*	27.5 (*q*)	0.94 *s*
18	22.1 (*q*)	0.80 *d* (5.8)	22.9 (*q*)	0.86 *d* (5.8)
19	22.5 (*q*)	0.97 *d* (5.8)	22.6 (*q*)	1.04 *d* (5.8)
20	177.0 (*s*)		180.2 (*s*)	

[a] At 75/300 MHz in $CDCl_3$. Chemical shifts are relative to TMS.

[b] Overlaping signals; the chemical shifts of these protons was measured using HMQC spectra.

3.2. Extraction and isolation of diterpenoid

Dried and finely powdered tissues from the whole plant of *L. acaulis* (1200 g) were extracted with petrol at room temperature. After filtration, the solvent was evaporated under reduced pressure and low temperature yielding a gum (187 g). The concentrated petrol extract was adsorbed on silica gel (220 g) and slurried onto the top of a column containing silica gel (800 g) in petrol and eluted with petrol–EtOAc gradient with increasing amounts of EtOAc.

The fraction eluted with petrol–EtOAc (10%) (37.4 g) was rechromatographed on silica gel (350 g), eluted with 5% petrol–EtOAc, to yield mulinolic acid **1** (4.6 g), mulin-11, 13-dien-20-oic acid **2** (6.8 g) and 13-epimulinolic acid **3** (360 mg).

3.3. 13-Epimulinolic acid (**3**)

Needles, mp 240°C (petrol–EtOAc); $[\alpha]_D^{24} = -49.20$ ($CHCl_3$; c 0.26). IR ν_{max}^{KBr} cm^{-1}: 3355 (OH), 3150–2500 (*br*), 1690 (COOH), 1449, 1218; ^1H- and ^{13}C-NMR spectral data (see Table 1); HR-EIMS: found 320.2370, (calc. 320.2351 for $C_{20}H_{32}O_3$), 259.1667, (calc. 259.1698 for $C_{17}H_{23}O_2$); EIMS (70 eV, direct inlet) *m/z* (relative intentsity): 320 $[M]^+$ (20), 305 $[M\text{-}Me]^+$ (56), 303 $[M\text{-}OH]^+$ (23), 302 $[M\text{-}H_2O]^+$ (98), 287 $[M\text{-}Me\text{-}H_2O]^+$ (12), 260 (22), 259 $[M\text{-}H_2O\text{-}iso\text{-}Pr]^+$, (100), 257 (49), 249 (60), 241 (34), 223 (21), 191 (16), 187 (40), 177 (38), 107 (26), 105 (34), 105 (34), 95 (26), 193 (35), 81 (36), 79 (33), 55 (37).

3.4. Dehydration of 13-epimulinolic acid (**3**)

Compound (**3**) (65 mg) was dissolved in C_6H_6 (5 ml) and treated with *p*-TsOH for 1 h at room temperature. The reaction mixture was evaporated in vacuo and partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue, which was further purified by Sephadex LH-20 chromatography (MeOH) to afford (**2**) (53 mg).

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