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MULINOLIC ACID, A DITERPENOID FROM *MULINUM CRASSIFOLIUM*

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Key Word Index—*Mulinum crassifolium*; Umbelliferae; diterpenoid; mulinane derivative; mulinolic acid.

Abstract—Mulinolic acid, a new diterpenoid, has been isolated from the aerial parts of *Mulinum crassifolium*. Its structure was established by spectroscopic means. Mulinolic acid has a rearranged mulinane carbon skeleton of the type which has been found in four other diterpenoids from the same plant. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

In previous communications [1–3], we have reported on the structures of four rearranged diterpenoids, mulinic (**1**), 17-acetoxymulinic (**2**), isomulinic (**3**) and mulinenic (**4**) acid, isolated from the petrol extract of the aerial parts of *Mulinum crassifolium* Phil. This plant, commonly known as 'chuquicán', 'susurco' or 'espinilla', is a shrub which grows in the north of Chile at altitudes above 3000 m. Bitter tasting infusions of the whole plant are used widely in folk medicine, principally in the treatment of diabetes, and bronchial and intestinal disorders [4].

In our continuing studies of the petrol extract, we isolated a new diterpenoid acid with a mulinane skeleton, mulinolic acid, the structure of which was established by spectroscopic analysis as 13-hydroxymulin-11-en-20-oic acid (**5**).

RESULTS AND DISCUSSION

Combustion analysis and low-resolution mass spectrometry indicated the molecular formula $C_{20}H_{32}O_3$ for mulinolic acid (**5**), requiring five sites of unsaturation. The ^{13}C NMR spectrum (Table 1) showed well-resolved resonances for all 20 carbons and SFORD and DEPT experiments demonstrated that 30 of the protons were attached to carbon atoms. The IR and ^{13}C NMR data showed the presence of a tertiary hydroxyl group (3390 cm^{-1} and δ_C 71.4 *s*) and a carboxyl group (3300–2500 *br*, 1690 cm^{-1} and δ_C 180.2 *s*). The methyl ester was easily obtained by reaction with ethereal diazomethane. Two deshielded carbon resonances at δ_C 133.8 (*d*) and 136.5 (*d*) and two deshielded proton resonances (δ_H 5.58 overlapped signal) were assigned to a disubstituted olefinic double bond. The lack of other olefinic or carbonyl resonances in the ^{13}C NMR spectrum, suggested a tricyclic molecule.

The ^1H and ^{13}C NMR spectra of mulinolic acid (**5**) (Table 1), together with ^1H COSY and the heteronuclear multiple quantum coherence (HMQC) spectrum, revealed the presence of an isopropyl group (δ_C 32.0, *d*, 22.9, *q*, 22.6, *q*; δ_H 0.86, 3H, *d*, 1.04, 3H, *d* (J = 5.8 Hz in both signals) and 1.47, 1H, overlapped signal), a tertiary methyl group (δ_C 27.5, *q*; δ_H 0.94, 3H, *s*), and another tertiary methyl group attached to carbon bearing a hydroxyl group (δ_C 33.7, *q*; δ_H 1.32, 3H, *s*).

All of the above data can be accommodated in the mulinane carbon skeleton, as depicted in formula **5**. The almost identical ^{13}C chemical shift (Table 2) of the C-1 to C-13 and C-18 to C-20 carbon atoms in mulinenic acid (**4**) (the structure **4** was confirmed by X-ray analysis) and **5**, together with the similarities observed in ^1H NMR spectra also supported this assumption. The resonance of C-13, carrying the hydroxyl and methyl group (C-16), is assigned to the signal at δ_C 71.4 (*s*) (δ_C 71.1, *s* in **4**). The isopropyl group is located at C-3 as in **4**, as shown by an identical ^{13}C chemical shift (δ_C 57.7, *d*). Similarly, the carboxyl group (δ_C 180.2 *s*) has been located at C-5 (δ_C 58.5 *s* in **5** and 57.0 *s* in **4**) and the olefinic system at C11–C12 (δ_C 133.8 *d* and 136.5 *d* in **5** and 131.9 *d* and 136.6 *d* in **4**).

The differences are consistent with the presence in mulinolic acid (**5**) of a C-17 methyl group attached to a fully substituted sp^3 carbon atom (C-8) and a tertiary hydroxyl group attached to C-13, instead of the ether bridge between C-13 and C-17 found in **4**; thus suggesting that the new diterpenoid (**5**) is 13-hydroxymulin-11-en-20-oic acid.

Most of the structural fragments of mulinolic acid (**5**)

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Table 1. DEPT multiplicities, ^{13}C NMR, one-bond ^{1}H–NMR connectivities (HMQC) and J_{HH} (Hz) of mulinolic acid (**5**)*

C	DEPT multiplicity	^{13}C NMR (δ)	^{1}H NMR (δ)	J_{HH} (Hz)
1	CH_2	25.2	1α 1.55	†
			1β 1.93	†
2	CH_2	28.9	2β 1.46	†
			2α 1.91	†
3	CH	57.7	1.51	†
4	CH	32.0	1.47	†
5	-C-	58.4		
6	CH_2	32.4	6α 1.47	*d* 9.4
			6β 2.42	*dd* 3.0; 9.4
7	CH_2	42.2	7β 1.38	†
			7α 1.47	†
8	-C-	35.9		
9	CH	48.7	2.08	*dd* 6.0; 10.8
10	CH	51.6	2.16	*br d* 10.8
11	CH	133.8	5.58	†
12	CH	136.5	5.58	†
13	-C-	71.4		
14	CH_2	36.2	14β 1.63	*dd* 9.3; 12.5
			14α 1.84	*dd* 4.6; 12.5
15	CH_2	30.5	15β 1.10	*dd* 4.6; 15.0
			15α 2.61	*dt* 15.0
16	CH_3	33.7	1.32	*s*
17	CH_3	27.5	0.94	*s*
18	CH_3	22.9	0.86	*d*, 5.8
19	CH_3	22.6	1.04	*d*, 5.8
20	-C-	180.2		

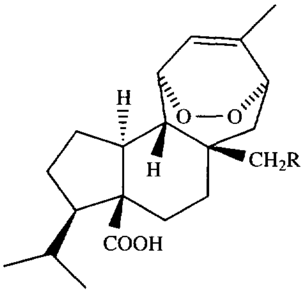
*At 75/300 MHz in $CDCl_3$. Chemical shifts are relative to TMS. All these assignments were in agreement with the HMQC and HMBC.

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

Table 2. ^{13}C NMR chemical shifts of compounds **4** and **5** ($CDCl_3$, TMS as internal standard)

		5	**4**
C	DEPT	(δ_C)	(δ_C)
1	*t*	25.2	25.0
2	*t*	28.9	28.5
3	*d*	57.7	57.7
4	*d*	32.0	31.6
5	*s*	58.5	57.0
6	*t*	32.4	32.0
7	*t*	42.2	35.8
8	*s*	35.9	36.9
9	*d*	48.7	50.2
10	*d*	51.6	50.1
11	*d*	133.8	131.9
12	*d*	136.6	136.6
13	*s*	71.4	71.1
14	*t*	36.2	33.2
15	*t*	30.5	25.9
16	*q*	33.7	29.1
17	*q*/*t*	27.5	74.8
18	*q*	22.9	22.7
19	*q*	22.6	22.4
20	*s*	180.2	179.4

were identified by means of the HMBC spectrum (Fig. 1). Correlations were observed between the signal at δ_H 1.32 (3H, *s*) assigned to Me-16 protons with the signals at δ_C 136.5 (*d*, olefinic C-12), δ_C 71.4 (*s*, C-13) and δ_C 36.2 (*t*, C-14). The signal at δ_H 5.58 (overlapped signals) assigned to H-11 and H-12 correlated with the signal at δ_C 71.4 (*s*, C-13). These facts confirmed the location of the hydroxyl group in C-13 and the olefinic functionality at C-11–C-12. The Me-17 protons (δ 0.94, *s*) showed correlation with C-15, C-9 and C-7 (δ_C 30.5, *t*, 48.7, *d* and 42.2, *t*, respectively) and H-9 (δ_H 2.08, *dd*) with C-8, C-11 and C-12 (δ_C 35.9, *s*, 133.8, *d* and 136.6, *d*, respectively). Similarly, H-6 (δ_H 2.42, *dd*) showed correlation with C-8, C-10 and C-5 (δ_C 35.9, *s*, 51.6, *d* and 58.5, *s*, respectively). The Me-18 and Me-19 protons (δ_H 0.86, *d*, and 1.04, *d*) showed connectivities with C-3 and C-4 (δ_C 57.7, *d*, and 32.0, *d*, respectively). The location of the carboxyl substituent in mulinolic acid (**5**) was confirmed by the connectivities between H-10 (δ_H 2.16, *br d*), H-3 and H-6 (δ_H 1.51 and 1.47 overlapped signal) with C-20 (δ_C 180.2, *s*). The correlations observed in the experiment are shown in Fig. 1.



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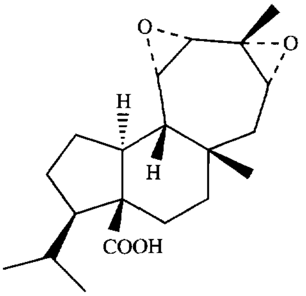
2 R = OAc



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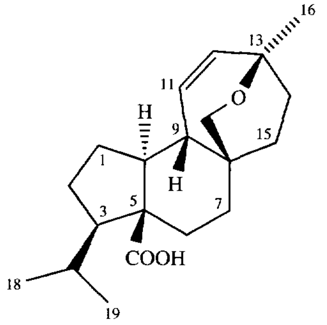

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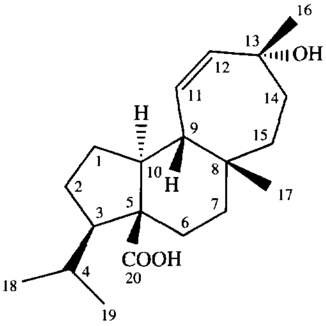

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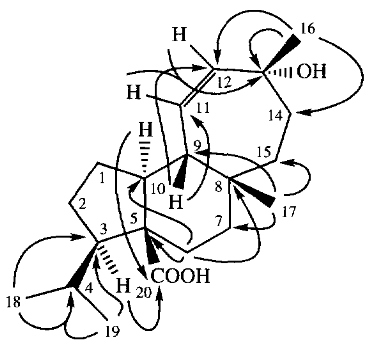

Fig. 1. $^{1}H-^{13}C$ long-range correlations for mulinolic acid (**5**) detected by HMBC spectrum.

From all above data, it was evident that mulinolic acid had a planar structure depicted in **5**. The principal results from ROESY NMR experiments suggested that mulinolic acid (**5**) has the stereochemistry shown in Fig. 2, in agreement with the relative stereochemistry of all mulinane derivatives (1–4) previously isolated from *M. crassifolium*. The ROESY NMR experiment showed a correlation between the signal at δ 2.16 for H-10α and the signals at δ 1.55 (H-1), 1.47 (H-6) and 2.61 (H-15) which should be in an α-configuration. In addition H-15α showed a correlation with the signals at δ 1.47 (H-6) and δ 1.84 (H-14) indicating that they are in the same configuration. Moreover, the signal at δ 2.08 for H-9β showed correlation with the signals at δ 0.94 (Me-17), 1.38 (H-7), 1.63 (H-14) and 5.58

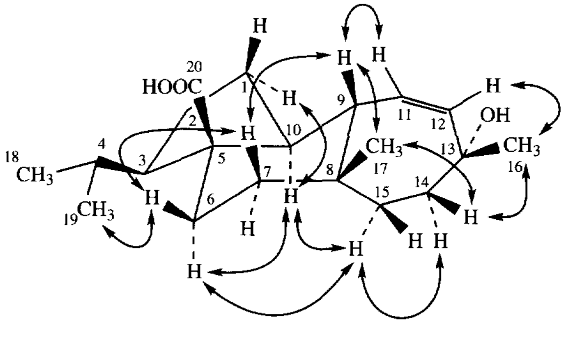

Fig. 2. ROESY results for compound **5**.

(H-11). A correlation between H-14β (δ 1.63) and Me-16 (δ 1.32) indicated that the C-13 hydroxy function has an α configuration. The signal at δ 1.38 for H-7β showed correlation with the signal at δ 2.42 (H-6β) and this signal correlated with the signal at δ 1.04 (Me-19), indicating that the isopropyl group is also in the β configuration. It is proposed that the configuration of the carboxyl group (C-20) β, as in the case of the analogous compounds **1–4**.

EXPERIMENTAL

General. Mp: uncorr. Plant materials were collected in November 1994 in Tatio in northern Chile, voucher specimens are deposited in the Herbarium of Universidad de Concepción, Concepción, Chile.

Extraction and isolation of mulinolic acid (**5**). Dried and finely powdered aerial parts of *M. crassifolium.* (870 g) were extracted with petrol at room temp. After filtration, the solvent was evapd. to dryness under red. pres. and low temp. yielding a gum (32.3 g). The concd. petrol extract was absorbed on silica gel (100 g) and slurried onto the top of a column containing silica gel (500 g) in petrol and eluted with a petrol–EtOAc gradient with increasing amounts of EtOAc.

The fr. eluted with petrol–EtOAc (1 : 9) (12.9 g) was rechromatographed on silica gel (150 g) eluted with petrol–EtOAc (1 : 19) to yield 350 mg of mulinolic acid (**5**) which was recrystallized from Et_2O: mp 178–180°; $[\alpha]_D^{24}$ +85.0 ($CHCl_3$; *c* 0.20). IR ν_{max}^{KBr} cm^{-1}: 3.390 (OH), 3.300–2.500 *br*, 1690 (COOH). ^1H NMR: (Table 1); ^{13} C NMR: Table 1; EI-MS (70 eV, direct inlet) *m/z* (rel. int.): 320 $[M]^+$ (9), 303 (23), 302 (67), 287 (15), 275 (22), 274 (38), 259 (30), 257 (93), 249 (15), 241 (21), 232 (14), 231 (55), 229 (19), 221 (17), 213 (31), 203 (17), 191 (47), 189 (35), 187 (30), 177 (29), 175 (38), 153 (40), 149 (28), 147 (30), 145 (23), 95 (87), 94 (42), 93 (79), 92 (34), 91 (64), 84 (62), 83 (37), 81 (83), 78 (43), 69 (97), 67 (81), 57 (25), 55 (100), 53 (39). Found: C, 74.43; H, 10.15%; calcd for $C_{20}H_{32}O_3$: C, 75.00; H, 10.10%.

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