

MULINIC AND ISOMULINIC ACIDS, REARRANGED DITERPENES WITH A
NEW CARBON SKELETON FROM MULINUM CRASSIFOLIUM

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Abstract. - Two new diterpenoids, mulinic and isomulinic acids, have been isolated from the aerial parts of *Mulinum crassifolium* (Umbelliferae). The structure of mulinic acid (1) was determined by a combination of spectroscopic and single-crystal X-ray diffraction analyses. The proposed structure of isomulinic acid (2) was based on spectroscopic comparison with mulinic acid and on chemical grounds. The diterpenoids 1 and 2 possess a novel carbon skeleton whose plausible biogenetic pathway from a suitable labdane derivative is briefly discussed.

INTRODUCTION

Mulinum crassifolium Phil. (Umbelliferae) is a shrub growing in the North of Chile¹ and popularly known as "chuquicán", "espinilla" or "sucurco". Bitter taste infusions of the whole plant are profusely used in folk medicine, principally in diabetes, bronchial and intestinal disorders².

In a previous communication³, some of us have reported the isolation of several phenolic compounds from the ethanolic extract of the aerial parts of this plant. We have examined the petrol ether extract of the same material, and we now wish to report the structures of mulinic and isomulinic acids (1 and 2, respectively), two rearranged diterpenoids which possess a novel carbon skeleton.

RESULTS AND DISCUSSION

Combustion analysis and low-resolution mass spectrometry indicated the molecular formula C₂₀H₃₀O₄ for mulinic acid (1), requiring six sites of unsaturation. The ¹³C NMR spectrum of mulinic acid⁴ (Table I) showed well-resolved resonances for all 20 carbons and SFORD and DEPT experiments demonstrated that 29 of the protons were attached to carbon atoms. IR absorptions at 3300-2600 br and 1695 cm⁻¹, together with a downfield carbon resonance at δ 180.8 s, revealed that the remaining proton was part of a carboxyl

functionality. Two deshielded carbon resonances at δ 124.3 (CH) and 136.9 (C) (Table I) and a deshielded proton resonance (δ 6.12 doublet of quintuplets; Table II) were assigned to a trisubstituted olefinic double bond, none of the substituents of which is the carboxyl group, since the UV spectrum of mulinic acid (1) was devoid of absorptions due to an α,β -unsaturated carboxyl chromophore.

The lack of other olefinic or carbonyl resonances in the ^{13}C NMR spectrum of compound 1 (Table I) indicated that rings had to account for the remaining four sites of unsaturation in the molecule. The ^1H and ^{13}C NMR spectra of mulinic acid (1; Tables II and I, respectively), together with ^1H COSY and one-bond ^1H - ^{13}C shift correlation spectra and ^1H double resonance experiments, revealed the presence of an isopropyl group [δ_{C} 31.5 (CH), 22.6 (CH_3), and 22.5 (CH_3); δ_{H} 1.07 d and 0.88 d ($J=5.8$ Hz in both signals), 1.53 overlapped signal], a tertiary methyl group [δ_{C} 33.8 (CH_3); δ_{H} 1.06 s], another methyl group attached to a substituted olefinic carbon [δ_{C} 20.5 (CH_3); δ_{H} 1.91 d, $J_{\text{allylic}}=1.6$ Hz], and two deshielded proton resonances at δ 4.58 ddd and 4.41 dq, which were tentatively assigned to the geminal protons of an endoperoxide bridge [methine carbons bearing oxygen atom at δ 77.4 (CH) and 80.5 (CH)], taking into account the unsaturation number of mulinic acid (1) and the easy loss of molecular oxygen from the molecular ion (ion fragment at 302 m/z , relative intensity 56%) observed in the mass spectrum of this new diterpenoid.

Most of the structural fragments of mulinic acid (1) were identified by 2D COSY and RELAY-COSY experiments. The signal at δ 6.12 was assigned to H-12, while those at δ 4.58 and 4.41 were attributed to the C-11 and C-14 protons respectively, because the first proton showed a large coupling value with H-12 ($J=7.4$ Hz) whereas the other one was coupled with two different protons (J values of 4.7 and 1.6 Hz) resonating in the aliphatic region (C-15 methylene protons, δ 2.31 and 1.56). The C-11 proton showed correlation with another methine proton at δ 1.90 (H-9), which in turn was coupled with H-10 (signal at δ 2.15). Moreover, the RELAY-COSY spectrum clearly revealed the correlation between the C-11 and C-10 methine protons via H-9. The COSY spectrum also showed connectivities between H-12 and the methyl group at δ 1.91 (C-16) and between the two most upfield methyl groups (C-18 and C-19) and an overlapped proton at δ 1.53 (H-4), which in turn did not show correlation with H-3. However, the absence of additional cross peaks in the RELAY-COSY spectrum clearly established that the C-3 and C-4 methine protons resonated at the same field (δ 1.53). The COSY spectrum also revealed the existence of a methyl group (C-17) attached to a non protonated carbon. An unambiguous assignment for all the remaining C-1, C-2, C-6, and C-7 methylene protons was not possible from the above experiments due to the overlap in the aliphatic part of the spectrum. However, assignments for the two protons attached to the same carbon and for those corresponding to every one of the fragments (C-1, C-2) and (C-6, C-7) were made from a one-bond ^1H - ^{13}C shift correlation spectrum with F1-decoupling (see Table II).

It was not possible to accommodate all the identified fragments on any known diterpene carbon skeleton so the structure of mulinic acid (1) was solved by single-crystal X-ray diffraction analysis.

Table I. ^{13}C NMR data of compounds 1 and 2^a

C	1	2	C	1	2
1	25.5 t ^{b,c}	24.2 t ^c	11	77.4 d	59.1 d
2	28.4 t ^c	28.4 t ^c	12	124.3 d	60.2 d
3	57.4 d	57.3 d	13	136.9 s	56.0 s ^e
4	31.5 d	31.7 d	14	80.5 d	60.5 d
5	57.0 s	57.5 s ^e	15	42.5 t	32.5 t
6	41.6 t ^d	43.1 t ^d	16	20.5 q	22.5 q
7	32.7 t ^d	32.5 t ^d	17	33.8 q	27.7 q
8	33.6 s	33.7 s	18 ^f	22.6 q	22.6 q
9	49.4 d	45.6 d	19 ^f	22.5 q	22.4 q
10	49.1 d	48.8 d	20	180.8 s	179.9 s

^aChemical shifts are reported in parts per million downfield from internal TMS. Assignments are based on ^1H - ^{13}C COSY experiments. ^bProton attachments were determined by SFORD experiments and DEPT pulse sequences. ^{c,d,e}Ambiguous assignments, but those given here are considered to be most likely. ^fThese carbon numbers are interchangeable.

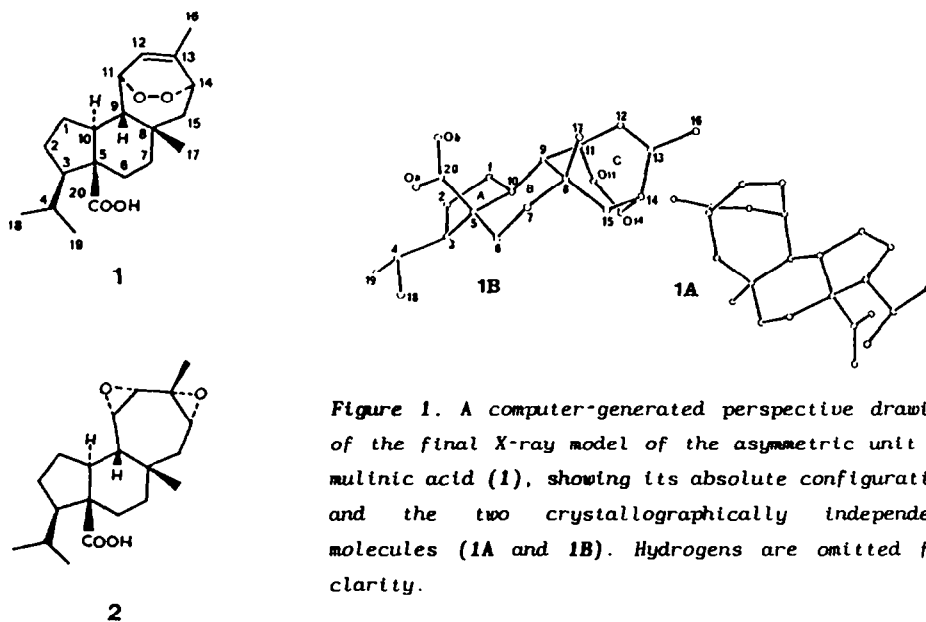


Figure 1. A computer-generated perspective drawing of the final X-ray model of the asymmetric unit of mulinic acid (1), showing its absolute configuration and the two crystallographically independent molecules (1A and 1B). Hydrogens are omitted for clarity.

Table II ^1H NMR data of compounds 1 and 2

Proton on Carbon n ^o	1	2	J(Hz)	1	2
1	1.95 ^b , 1.76 ^{b(+)}	2.02 ^{b,c}	4,18(19)	5.8	5.8
2	2.00 ^b , 1.42 ^{b(+)}	2.02 ^{b,c}	7 α ,7 β	b	12.9
3	1.53 ^b	1.46 ^b	7 α ,6 α	b	3.5
4	1.53 ^b	1.48 ^b	7 α ,6 β	b	3.5
6	1.48 ^b , 1.33 ^{b(s)}	1.56 ^b , 1.55 ^{b(+)}	11 β ,9 β	5.0	3.8
7	2.42 ^b , 1.39 ^{b(s)}	2.40 dt (α proton) 1.08 ^b (β proton) ⁽⁺⁾	11 β ,12	7.4	3.8
9	1.90 ^b	2.05 ^b	11 β ,14 β	1.6	0.0
10	2.15 ^b	2.03 ^b	12,14 β	1.6	0.8
11	4.85 ddd	3.15 t	12,16	1.6	0.0
12	6.12 d of quintuplets	3.24 dd	14 β ,15 α	1.6	7.3
14	4.41 dq	2.89 td	14 β ,15 β	4.7	7.3
15	2.31 ^b , 1.56 ^b	2.11 ^b , 1.56 ^b			
16	1.91 d	1.51 s			
17	1.06 s	1.05 s			
18 ^d	1.07 d	1.04 d			
19 ^d	0.88 d	0.85 d			

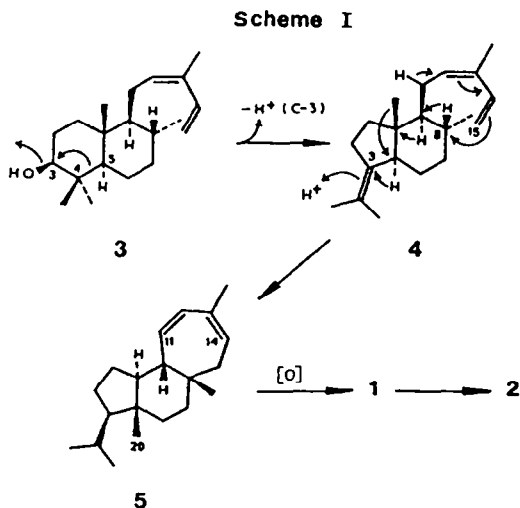
^aChemical shifts are listed in parts per million downfield from internal TMS. Assignments are based on ^1H - ^1H and ^1H - ^{13}C COSY spectra and double resonance experiments. ^bOverlapped signal. ^cThe other methylene proton was not determined. ^dThese carbon numbers are interchangeable. (+, s, τ) Assignments bearing the same sing may be interchanged.

A computer-generated perspective drawing of the final X-ray model is given in Figure 1, showing the structure, the absolute configuration, and the two crystallographically independent molecules of compound 1 in the asymmetric unit. Both molecules (1A and 1B) are clearly similar as regards conformation, but they present some differences in bond lengths and angles. The conformation of the rings has been analyzed in the crystalline state through the torsion angles. The seven-membered carbocyclic ring presents a boat conformation, whereas the 1,2-dioxepan ring is a chair. The six-membered carbocyclic ring possesses a chair conformation ($^6\text{C}_0$), but ring C-11, C-12, C-13, C-14, O-14, O-11 is a boat ($\text{B}_{11,14}$). Finally, the five-membered ring is an envelope with the flap at C-5. In the

crystal, molecules 1A and 1B are held by two hydrogen bonds between the carboxylic OH of molecule 1A and the carboxylic C=O of molecule 1B, and vice versa. Furthermore, there is an intermolecular contact less than the van der Waals radii [O-11 (1A)...C-11 (1B) = 3.13(1) Å (1-X, 1/2+Y, 1-Z)].

With the structure of mulinic acid securely established as 1, we turned our attention to isomulinic acid (2). This compound also possessed the molecular formula $C_{20}H_{30}O_4$ and its 1H and ^{13}C NMR spectra (Tables II and I, respectively) were very similar to those of mulinic acid (1). In fact, the observed differences between the NMR spectroscopic data of compounds 1 and 2 were consistent with the presence in the latter of two oxirane rings at the C-11, C-12 and C-13, C-14 positions [δ_{H-11} 3.15 t, $J_{11,9}=J_{11,12}=3.8$ Hz; δ_{H-12} 3.24 dd, $J_{12,14}=0.8$ Hz; δ_{H-14} 2.89 td, $J_{14,15}=J_{14,15}=7.3$ Hz; epoxide carbons at δ 59.1 d (C-11), 60.2 d, (C-12), 56.0 s, (C-13), and 60.5 d, (C-14)] instead of the allylic endoperoxide fragment of the former.

When mulinic acid (1, mp 185-187°C) was heated at 190°C for 5 minutes without any solvent, isomulinic acid (2) was obtained (56% yield), besides minor quantities of the starting material (1, 23%), and traces of other decomposition products. This transformation, which takes place via a thermal rearrangement with retention of the configuration⁵ at C-11 and C-14, established that isomulinic acid possessed the structure and absolute configuration depicted in 2.



Mulinic and isomulinic acids (1 and 2, respectively) are rearranged diterpenes with a new hydrocarbon skeleton for which we suggest the name of mulinane. Biogenesis of these new diterpenoids may proceed as is outlined in Scheme I. A labdane derivative such as 3 can be biogenetically transformed⁶ into its 5(4+3)-abeo derivative 4, which in turn, via formation of a new carbon-carbon bond (C-8 - C-15) in connection with a 8,3-friedo rearrangement, may produce the mulinane derivative 5 (Scheme I). Oxidation at C-20 and a 1,4 addition of singlet oxygen to the 11,13-diene from

the α face of compound 5 would generate mulinic acid (1), from which isomulinic acid (2) is formed (see above).

Endoperoxide and 1,3-diepoxy functionalities as those of mulinic and isomulinic acids (1 and 2) have been found in other natural products such as rugosal A⁷, crotepoxide^{5b,8}, and stemolide^{5c,9}.

EXPERIMENTAL

Melting points were determined in a Kofler apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter, with a 1-dm cell. Elemental analyses were carried out with the help of a Heraeus CHN-O-Rapid analyzer. IR spectra were determined on a Perkin-Elmer 681 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained at 200 MHz and 50.3 MHz, respectively, on a Bruker AM-200 spectrometer, in CDCl_3 solution with TMS as an internal standard. Low-resolution mass spectra were obtained on a VG 12-250 spectrometer (mode EI, 70 eV, solid probe)

Extraction and isolation of the diterpenoids. Dried and finely powdered aerial parts of *Mulinum crassifolium* Phil.¹⁰ (560 g) were extracted with petroleum ether (5 L) for three days at room temperature. The solvent was removed in vacuo to yield 166 g of a gummy extract, which was partitioned between petroleum ether and aqueous methanol (90%). The hydroalcoholic fraction was concentrated in vacuo, water was added, and the resulting suspension was exhaustively extracted with CHCl_3 . Evaporation of the combined organic extracts in vacuo gave a residue (11.8 g), which was fractionated via silica gel (Merck, No. 7734, deactivated with 15% water, 400g) column chromatography (step gradient: petroleum ether to EtOAc) to give crude mulinic acid (1, 230 mg, eluted with petroleum ether-EtOAc 9:2) and isomulinic acid (2, 70 mg, eluted with petroleum ether-EtOAc 3:1).

Mulinic acid (1). Mp 185–187°C dec. (EtOAc - n-hexane, colourless needles); $[\alpha]_{\text{D}}^{20}$ -133.2° (c 0.307, CHCl_3); IR (KBr) ν_{max} (cm^{-1}) 3300–2600 br, 1695 (carboxyl group), 2960, 2920, 2870, 1445, 1385, 1255, 1200, 1015, 960, 750, 700; ^1H NMR: see Table II; ^{13}C NMR: see Table I; MS, m/z (relative intensity) 334 (M^+ , 5), 319 (1.5), 305 (3), 302 (56), 287 (11), 271 (6), 205 (16), 175 (31), 147 (32), 133 (34), 119 (34), 109 (37), 107 (37), 105 (46), 95 (48), 91 (51), 81 (41), 79 (48), 69 (59), 43 (100, base peak), 41 (99). Anal. Calcd. for $\text{C}_{20}\text{H}_{30}\text{O}_4$: C, 71.82; H, 9.04. Found: C, 71.96; H, 9.11.

Isomulinic acid (2). Mp 179–182°C (EtOAc - n-hexane, colourless plates); $[\alpha]_{\text{D}}^{20}$ -75.7° (c 0.103, CHCl_3); IR (KBr) ν_{max} (cm^{-1}) 3140 br, 1718 (carboxyl group), 2960, 2875, 1460, 1385, 1240, 1185, 1165, 950, 900, 800, 745; ^1H NMR: see Table II; ^{13}C NMR: see Table I; MS, m/z (relative intensity) 334 (M^+ , 6), 319 (2), 305 (4), 271 (11), 245 (11), 205 (29), 187 (29), 147 (28), 119 (31), 109 (48), 91 (39), 79 (38), 69 (39), 55 (46), 43 (100, base peak), 41 (70). Anal. Calcd. for $\text{C}_{20}\text{H}_{30}\text{O}_4$: C, 71.82; H, 9.04. Found: C, 71.68; H, 9.17.

Transformation of mulinic acid (1) into isomulinic acid (2). Mulinic acid (1, 30 mg), in a round-bottom flask and without any solvent, was heated under argon in a silicone oil bath at 190°C for 5 minutes. The reaction mixture was subjected to column chromatography (silica gel, n-hexane - EtOAc 3:1 as eluent) yielding 7 mg of the starting material (1, less polar constituent) and 17 mg of a compound identical (mp, mixed mp, $[\alpha]_{\text{D}}$, TLC, ^1H NMR, MS) with natural isomulinic acid (2).

X-ray structure determination of mulinic acid (1). A rather poor colourless crystal of compound 1 with approximate dimensions of 0.20 x 0.18 x 0.15 mm was used for the crystal diffraction analysis. The cell dimensions [$a=14.554(2)$, $b=11.374(1)$, $c=12.837(1)$ Å, $\beta=114.238(3)^\circ$] were determined from least-squares analysis of 21 high-angle reflections, range of 2θ from 10° to 50° . The crystal of compound 1, $C_{20}H_{30}O_4$, is monoclinic, space group $2P_1$, $Z=4$, $D_c=1.147$ g cm $^{-3}$ and $\mu=5.935$ cm $^{-1}$. The lattice parameters and the intensities were measured on a Philips PW 1100 four-circle diffractometer, with graphite monochromated $CuK\alpha$ radiation (1.5418 Å) and the $\omega/2\theta$ scan technique, with a scan width of 1.60 and a scan speed of 0.060 s $^{-1}$. Two reference reflections were monitored every 90 reflections in order to check crystal alignment and/or decomposition: their intensities showed no variation and no significant crystal decay. A total of 3613 independent reflections with $2^\circ < \theta < 65^\circ$ were scanned, from these 2561 with $I > 2\sigma(I)$ were considered as observed and used for the structure determination and refinement. The intensities were corrected for Lorentz and polarization effects, but no correction for absorption or extinction were applied. The structure was solved by direct methods (*MULTAN*¹¹ and *DIRDIF*¹²). Least-squares refinement with isotropic, then with anisotropic temperature factors for non-hydrogen atoms was applied. The hydrogen atoms were located in successive Fourier maps, but those corresponding to the methyl group were added in theoretical calculated positions and their isotropic thermal factors included in the subsequent refinement, but they were not refined. The weighting scheme was chosen to give no trends in $\langle w\Delta^2 F \rangle$ over ranges of $\langle F_o \rangle$ and $\langle \sin \theta / \lambda \rangle$. The final values of unweighted and weighted discrepancy indices are $R=8.8$ and $R_w=10.5$. The relatively high R value of 8.8 is attributable to the preponderance of weak diffraction maxima resulting from the poor crystal and considerable thermal motion in some regions of the molecule. In the determination of the absolute configuration the anomalous dispersion effects of O and C were used¹³. On consideration of reflections with $F_o > 10\sigma(F_o)$ there are 50 Bijvoet pairs with $\Delta F_c > 0.09$, showing an averaged Bijvoet difference (Δ^+) of 1.011 for the correct enantiomer versus 1.060 for the wrong one (Δ^-). All the calculations were performed on a VAX 11/750 computer using the X-RAY 76 package¹⁴. Atomic scattering factors were taken from the literature¹⁵ and molecular plots were obtained by *ORTEP*¹⁶. Lists of atom parameters, anisotropic thermal parameters, structure factors, and H-atom parameters corresponding to the two crystallographically independent molecules (1A and 1B) of mulinic acid (1) have been deposited at the Cambridge Crystallographic Data Centre.

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