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MULINENIC ACID, A REARRANGED DITERPENOID FROM *MULINUM CRASSIFOLIUM*

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ABSTRACT.—From the petroleum ether extract of the aerial parts of *Mulinum crassifolium*, a new diterpenoid, mulinenic acid, has been isolated. Its structure [4] was established by spectroscopic means and by a single-crystal X-ray diffraction analysis. Mulinenic acid [4] possesses the rearranged mulinane carbon skeleton, which has recently been found in three other diterpenoids 1, 2, and 3 isolated from the same plant.

In previous communications (1, 2), we reported the structures of three rearranged diterpenoids, mulinic [1], isomulinic [2] (1), and 17-acetoxymulinic [3] (2) acids, isolated from the petroleum ether extract of the aerial parts of *Mulinum crassifolium* Phil. (Umbelliferae), a shrub growing in the north of Chile and used in folk medicine against diabetes and bronchial and intestinal disorders (3). A study of the less polar chromatographic fractions of the same extract has now allowed the isolation of another minor constituent, mulinenic acid, the structure of which was established by spectroscopic and X-ray crystallographic analyses as 13β,17-epoxy-mulin-11-en-20-oic acid [4]. The numbering system in the mulinane skeleton is based on that in labdane diterpenes,

since it could be biogenetically derived from a labdane derivative (1).

Combustion analysis and low-resolution ms indicated the molecular formula $C_{20}H_{30}O_3$ for mulinenic acid [4], and its ir spectrum revealed the presence of a carboxyl group (3500–2500 br, 1720 cm^{-1}). The ^{13}C-nmr spectrum of compound 4 (Table 1) showed resonances for all of the 20 carbon atoms, and DEPT experiments demonstrated that 29 of the protons were attached to carbon atoms. A downfield carbon resonance at δ 179.4 (C) revealed that the remaining proton was involved in a carboxylic functionality. Two deshielded carbon resonances at δ 131.9 (CH) and 136.6 (CH), correlated with two deshielded proton resonances at δ 5.46 dd ($J = 10.9, 2.1$ Hz) and δ 5.61 dd ($J = 10.9, 2.5$ Hz), re-
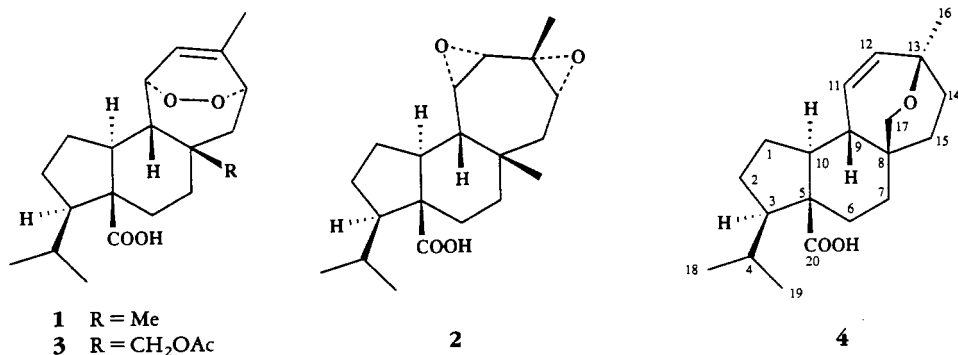

1 R = Me
3 R = CH₂OAc

2

4

TABLE 1. ¹³C-nmr Chemical Shift
Assignments for Compound **4**.ᵃ

Carbon	**4**	Carbon	**4**
C-1 . . .	25.0(2)ᵇ	C-11 . .	131.9(1)
C-2 . . .	28.5(2)	C-12 . .	136.6(1)
C-3 . . .	57.7(1)	C-13 . .	71.1(0)
C-4 . . .	31.6(1)	C-14 . .	33.2(2)
C-5 . . .	57.0(0)	C-15 . .	25.9(2)
C-6 . . .	32.0(2)	C-16 . .	29.1(3)
C-7 . . .	35.8(2)	C-17 . .	74.8(2)
C-8 . . .	36.9(0)	C-18ᶜ . .	22.7(3)
C-9 . . .	50.2(1)	C-19ᶜ . .	22.4(3)
C-10 . . .	50.1(1)	C-20 . .	179.4(0)

ᵃChemical shifts are reported in ppm downfield
from internal TMS. Assignments are based on
¹H-¹H and ¹H-¹³C chemical shift correlated 2D
nmr spectroscopy.

ᵇThe number in parentheses indicates the
number of hydrogens attached to the correspond-
ing carbon and was determined from DEPT ex-
periments.

ᶜThese carbon numbers are interchangeable.

TABLE 2. ¹H-nmr Data of Compound **4**.ᵃ

Proton	**4**	*J* values (Hz)	
H-1 . . .	1.68,ᵇ 1.90	4,18(19)	5.9
H-2 . . .	1.42, 1.90	9β,10α	10.8
H-3 . . .	1.43	9β,11	2.1
H-4 . . .	1.43	9β,12	2.5
H-6 . . .	1.16, 2.37 m	11,12	10.9
H-7 . . .	1.14, 1.18	17A,17Bᶜ	10.0
H-9 . . .	2.52 br d	17A,15Bᶜ	1.5
H-10 . . .	1.73		
H-11 . . .	5.46 dd		
H-12	5.61 dd		
H-14 . . .	1.68, 1.86		
H-15 . . .	1.24, 2.04 m		
H-16 . . .	1.14 s		
H-17ᶜ . .	3.42 dd, 3.74 d		
H-18ᵈ . .	0.80 d		
H-19ᵈ . .	0.96 d		

ᵃChemical shifts are reported in ppm downfield
from internal TMS. Assignments are based on
¹H-¹H and ¹H-¹³C chemical shift correlated 2D
nmr spectroscopy and double resonance experi-
ments.

ᵇSignals without indication of their multiplic-
ity appeared overlapped.

ᶜThe H_A-17 proton (δ 3.42 dd) is the pro-*S* hy-
drogen, close to the H-9β hydrogen. This was
clearly established from the NOESY spectrum
(see Discussion).

ᵈThese carbon numbers are interchangeable.

spectively, were assigned to a cis-disub-
stituted olefinic double bond. The lack
of other olefinic or carbonyl resonances
in the ¹³C-nmr spectrum of compound **4**
(Table 1) indicated that rings had to ac-
count for the remaining four sites of un-
saturation in the molecule.

The ¹H- and ¹³C-nmr spectra of
mulinenic acid {**4**} (Tables 2 and 1, re-
spectively), together with ¹H COSY,
one-bond ¹H-¹³C shift correlation, and
¹H double resonance experiments, re-
vealed the presence of an isopropyl group
[δ_C 31.6 (CH), 22.7 (Me), and 22.4
(Me); δ_H 1.43, 1H, overlapped signal,
0.80, 3H, d, and 0.96, 3H, d (*J* = 5.9
Hz in both signals)], a tertiary methyl
group [δ_C 29.1 (Me); δ_H 1.14, 3H, s],
and an ether bridge between a fully sub-
stituted sp³ carbon atom [δ_C 71.1 (C)]
and a methylene group [δ_C 74.8 (CH₂);
δ_H 3.74 d (*J* = 10.0 Hz) and 3.42 dd
(*J* = 10.0, 1.5 Hz)].

Most of the structural features of
mulinenic acid {**4**} were identified by
using 2D COSY, RELAY-COSY, and
NOESY experiments, which also al-
lowed the unequivocal assignment of
two isolated CH₂-CH₂ fragments.

All the above data of mulinenic acid
can be accommodated in the mulinane
carbon skeleton just as is depicted in for-
mula **4**. The almost identical ¹³C chemi-
cal shift of the C-1 to C-10 and C-18 to
C-20 carbon atoms in compounds **1-4**,
together with the similarities observed
in their ¹H-nmr spectra (1, 2) (Tables 1
and 2) also supported this assumption.

In order to confirm structure **4** and es-
tablish its absolute configuration, a
single-crystal X-ray diffraction analysis
of mulinenic acid was undertaken. The
X-ray molecular model of this new diter-
penoid (Figure 1) confirmed all the
above deductions on its structure **4** and
established the absolute configuration
shown in Figure 1 and formula **4**, identi-
cal with that found (1) in mulinic acid
{**1**} (see also Experimental). Fractional
coordinates of the carbon and oxygen
atoms are provided in Table 3. The crys-
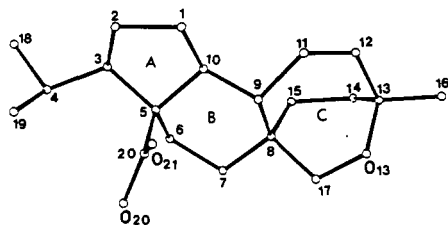tal structure of compound **4** shows that

FIGURE 1. A computer-generated perspective drawing of the final X-ray model of mulinenic acid [4] (hydrogens are omitted for clarity).

the angles between rings A/B and B/C are 3° and 23°, respectively. The conformation of the rings has been analyzed in the crystalline state through the torsion angles; ring A presents an envelope conformation with the flap at C-5, ring B shows a distorted chair conformation, and ring C (C-8, C-9, C-11, C-12, C-13, O-13, C-17) possesses a twist conformation, which is also shown for the other seven-membered (C-8, C-9, C-11,

C-12, C-13, C-14, C-15) and six-membered (C-8, C-15, C-14, C-13, O-13, C-17) rings. Furthermore, the carboxylic group participates in an intermolecular hydrogen bond with the ethereal oxygen atom (O-13) [$(-x, y - \frac{1}{2}, \frac{1}{2} - z)$ O-20 . . . . O-13 = 2.674 Å, O-20 - - - H = 0.893 Å, H . . . . O-13 = 1.899 Å; O-20 - - - H . . . . O-13 = 144.6°]. Any other contact between molecules smaller than 3.2 Å has not been observed in the crystal.

Mulinenic acid [4], mulinic acid [1], isomulinic acid [2] (1), and 17-acetoxymulinic acid [3] (2) are up to now the only mulinane derivatives found in nature.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.— Mp was determined in a Kofler apparatus and is uncorrected. Optical rotation was measured with a Perkin-Elmer 241 MC polarimeter, with a 1 dm cell. Elemental analysis was carried out with the help of a Heraeus CHN-O-Rapid analyzer.

TABLE 3. Fractional Atomic Coordinates and Equivalent Isotropic Thermal Parameters[a] for the Non-hydrogen Atoms of Compound 4, With Estimated Standard Deviations in Parentheses.

Atom	x	y	z	U_{eq}
C-1	0.2061(2)	0.8039(4)	0.3629(10)	44(2)
C-2	0.2104(2)	0.7012(4)	0.4815(10)	49(2)
C-3	0.1672(2)	0.7091(4)	0.6761(8)	40(2)
C-4	0.1460(2)	0.6073(4)	0.7796(10)	50(2)
C-5	0.1167(2)	0.7869(4)	0.6049(7)	36(1)
C-6	0.0816(2)	0.8422(4)	0.7823(9)	43(2)
C-7	0.0425(2)	0.9298(4)	0.6830(9)	42(2)
C-8	0.0786(2)	1.0062(4)	0.5480(8)	38(1)
C-9	0.1233(2)	0.9535(4)	0.3831(9)	40(2)
C-10	0.1592(2)	0.8673(4)	0.4926(8)	37(1)
C-11	0.1700(2)	1.0256(4)	0.2801(9)	45(2)
C-12	0.1668(2)	1.1256(4)	0.2690(11)	51(2)
C-13	0.1129(2)	1.1884(4)	0.3576(10)	47(2)
C-14	0.1149(3)	1.1901(4)	0.5982(10)	52(2)
C-15	0.1148(3)	1.0834(4)	0.6918(9)	50(2)
C-16	0.1225(3)	1.2953(4)	0.2564(13)	68(2)
C-17	0.0294(4)	1.0680(4)	0.4296(10)	50(2)
C-18	0.1985(4)	0.5646(5)	0.9191(13)	81(3)
C-19	0.1238(3)	0.5250(4)	0.6210(14)	68(2)
C-20	0.0730(2)	0.7374(4)	0.4420(9)	39(2)
O-13	0.0555(2)	1.1418(3)	0.2855(7)	55(1)
O-20	0.0188(1)	0.7077(3)	0.5291(7)	53(1)
O-21	0.0842(2)	0.7233(3)	0.2589(6)	58(1)

[a]The thermal parameters as $U_{eq} = (\frac{1}{3})\Sigma[UIJ \cdot AI^* \cdot AJ^* \cdot AI \cdot AJ \cdot \cos(AI \cdot AJ)] \cdot 10^3$.

The ir spectrum was determined on a Perkin-Elmer 681 spectrophotometer. The low-resolution mass spectrum was obtained on a VG 12-250 spectrometer (mode ei, 70 eV, solid probe). ^{1}H-nmr (300 MHz) experiments were performed at 30° on a Varian XL-300 spectrometer. COSY and RELAY-COSY experiments were performed in magnitude mode. 512*1K data matrices were obtained, which were zero-filled to 1K*1K prior to Fourier transformation and symmetrization. 2D-NOESY experiments were performed in the phase sensitive mode, with a mixing time of 2 sec. A similar data matrix was obtained. ^{13}C-nmr (50.3 MHz) experiments were performed at 30° on a Bruker AM-200 apparatus equipped with a dual probe. DEPT experiments were obtained using proton pulses of 90° and 135°. ^{1}H-^{13}C shift-correlation experiments were performed with and without F1-decoupling, using standard Bruker software. A 64*8K data matrix was obtained in both cases, which was zero-filled to 128*8K prior to Fourier transformation.

For general details on the collection of *M. crassifolium* and extraction of the diterpenoids see Loyola *et al.* (1).

ISOLATION OF MULINENIC ACID [**4**].—The chromatographic fraction obtained before elution of mulinic acid [**1**] (1) was evaporated to dryness and the residue (31 mg) chromatographed on a Si gel column (Merck, No. 7734, deactivated with 10% H_2O, 40 g) eluting with *n*-hexane–EtOAc (4:1) to yield compound **4** (11 mg).

Mulinenic acid [**4**] was crystallized from EtOAc/*n*-hexane as needles; mp 207–209°; $[\alpha]^{22}D -124.3°$ ($c = 0.111$, CHCl$_3$); ir (KBr) (cm^{-1}) ν max 3500–2500 br, 1720 (COOH), 2970, 2880, 1450, 1380, 1265, 1245, 1170, 1090, 1060, 880, 860, 797, 740, 710, 665; ^{1}H nmr (CDCl$_3$) see Table 2; ^{13}C nmr (CDCl$_3$) see Table 1; eims *m/z* (rel. int.) [M]$^+$ 318 (3.5), 300 (7), 288 (14), 287 (7), 272 (37), 260 (12), 243 (10), 241 (10), 229 (23), 171 (27), 105 (32), 91 (42), 79 (35), 67 (25), 55 (35), 43 (100), 41 (82); calcd for C$_{20}$H$_{30}$O$_3$, C 75.43, H 9.50, found C 75.19, H 9.62.

X-RAY CRYSTAL STRUCTURE ANALYSIS OF MULINENIC ACID [**4**][1].—Crystal data: C$_{20}$H$_{30}$O$_3$, MW = 318.45, orthorhombic, space group $P2_12_12_1$, $a = 21.457(4)$, $b = 13.096(1)$, $c = 6.252(1)$ Å (from least-squares refinement based on 17 reflections within θ range from 9° to 36°), Z = 4, V = 1756.8(6) Å^3, D$_c$ = 1.204 g·cm^{-3}, F(000) = 696, μ (CuKα radiation, λ = 1.5418 Å) = 5.878 cm^{-1}. Crystal dimensions: 0.08 × 0.15 × 0.07 mm. Three-dimensional data were collected on a Philips PW 1100 four-circle diffractometer with graphite monochromated CuKα radiation by the ω–2θ scan technique (scan width 1.6, scan speed 0.05 sec^{-1}) in the range 2°<θ<65°. The intensities of two reference reflections were monitored every 90 reflections in order to check crystal alignment and/or decomposition; their intensities showed no variation and no crystal decay. A total of 1751 unique reflections was collected, 1232 of which were considered greater than 2σ(I) and used in the structure solution and refinement. The data were corrected for Lorentz and polarization effects.

The structure was solved by MULTAN (4) and DIRDIF (5). Positions of all non-hydrogen atoms were refined by full-matrix-least-squares procedure with anisotropic temperature factors. The H-atoms were located by difference Fourier methods and included in the refinement as fixed contributors. Weighting scheme: empirical to prevent trends in $<w\Delta^2F>$ vs. $<F_0>$ and $<\sin \theta/\lambda>$. The refinement ended with R = 0.082 and R$_w$ = 0.085. The highest peak in the final difference Fourier was 0.47 eÅ^{-3}.

The absolute configuration was determined with Bijvoet differences in two experiments using the anomalous dispersion of C and O atoms with ΔF_c>0.085 and with the least experimental error, F_0>10σ(F$_1$). Thirty-one more relevant Bijvoet pairs from the raw data (*hkl* and *h̄k̄l̄* alternately measured intensities, with no extra care on recentering, scan speed, etc.) gave the following results: $R_1 = \Sigma[\Delta F_0 - \Delta F_C]/N = 1.336$ and $R_2 = \Sigma[\Delta I_0 - \Delta I_c]/\Sigma[\Delta I_0] = 0.992$ for the right enantiomer model; the R$_1$ and R$_2$ values for the wrong enantiomer are 1.352 and 1.010, respectively. This result was a poor indication of the correct absolute configuration. The next step was to select 21 strong *hkl* and *h̄k̄l̄* reflections which are more sensitive to the anomalous scattering effects and also comparing [ΔF_c]; they were carefully remeasured at very low scan-speed. The new values R$_1$ and R$_2$ are 0.360 and 0.981 for the correct enantiomer, and 0.396 and 1.085 for the wrong one. Thus, an improvement of the enantiomeric indication can be observed.

Atomic scattering factors and anomalous dispersion factors were taken from *International Tables for X-Ray Crystallography* (6). All the calculations were performed on VAX 11/750 computer using the X-RAY 76 package (7) and several local programs.

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University of Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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