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MONTAFRUSIN B, A GERMACROLIDE FROM *MONTANOA FRUTESCENS* AND THE MOLECULAR STRUCTURE OF MONTAFRUSIN A*

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Key Word Index—*Montanoa frutescens*; Asteraceae; Heliantheae; germacrolides; sesquiterpene lactones; montafrusins A and B; crystal structure.

Abstract—Chemical analysis of *Montanoa frutescens*, provided a new germacrolide, montafrusin B. The structure of the new compound was established by spectral methods, mainly ^{1}H NMR correlations with montafrusin A, the structure of which was confirmed by single crystal X-ray diffraction. Montafrusin A was found to have the unique [$^{15}D_5$, $^1D_{14}$] conformation, in which the methyl group at C-4 is β-oriented, and the methyl group at C-10 is α-oriented.

INTRODUCTION

In our chemical studies of the genus *Montanoa* we have previously analysed the sesquiterpene lactones of the Mexican species *M. frutescenes* [1], *M. tomentosa* [2, 3] and *M. grandiflora* [4]. To date eight species have been chemically studied. Most of them, *M. pteropoda* [5], *M. hibiscifolia* [6], *M. atriplicifolia* [7], *M. revealii* and *M. mollissima* [8], have been shown to contain 6,12-*cis*-germacranolides. Two species, *M. frutescens* [1, 9] and *M. tomentosa* [2, 3, 10] afforded 6,12-*trans*-lactones. One species contained 8,12-*trans*-lactones [4]. Now we have further investigated *M. frutescens* and isolated besides the already described montafrusin A (**1a**) [1] a new germacrolide which we named montafrusin B (**1b**). The molecular structure of montafrusin A (**1a**) was determined by single crystal X-ray diffraction. The X-ray data demonstrate that the ten membered ring exists in the crystal in a unique conformation [$^{15}D_5$, $^1D_{14}$], contrary to that of 6-epidesacetyllaurenobiolide (**3**) isolated from *M. grandiflora* [4].

RESULTS AND DISCUSSION

Montafrusin B (**1b**), $C_{15}H_{26}O_6$, mp 175–176°, $[\alpha]_D$ +23.2°, was characterized as an α,β-unsaturated-γ-lactone, containing hydroxyl group(s), and an α,β-unsaturated ester (IR absorption at 1765, 3420 and 1710 cm^{-1}). The ^{1}H NMR spectrum exhibited absorptions very similar to those of montafrusin A (**1a**), except for the signals that indicated the difference in the side chain ester. Diagnostic ^{1}H NMR absorptions at δ5.69 (1H), 1.93 (3H) and 2.15 (3H), together with strong mass spectral peaks at *m/z* 83 and 55 indicated the presence of a senecioate moiety in montafrusin B (**1b**). The other signals of the basic skeletal arrangement of montafrusin B were very similar in their chemical shifts and multiplicities to those of montafrusin A (**1a**).

Acetylation of **1b** gave the diacetate **1c**, which lacked the hydroxyl absorptions, but instead gave an additional carbonyl band at 1740 cm^{-1}. The ^{1}H NMR spectrum of **1c** showed two three-proton singlets at δ2.02 and 2.07 indicating the presence of two acetate groups in the molecule. As in the case of montafrusin A (**1a**), confirmation of the structure **1b** was achieved by pyrrolysis of the diacetate **1c**, which afforded the Cope rearrangement product **2**.

Since both compounds **1a** and **1b** exhibited very similar spectral parameters (^{1}H NMR, IR, MS) montafrusin A (**1a**) was chosen for X-ray diffraction studies. Torsion angles demonstrated that the ten membered ring exists in the crystal in a unique boat–boat type conformation [$^{15}D_5$, $^1D_{14}$] [11], in which the methyl group at C-4 is β-oriented and the methyl group at C-10 is α-oriented, instead of the double chair type [$^{15}D_5$, $_1D^{14}$] typical of the C(6)-*trans*-germacrolides. The double bonds are approximately parallel, and their centres are separated by 3.003 Å. This is the first case of such a conformation in *trans*-6α,12-germacrolides. A similar conformation is found in pertilide [12], in which C-14 and C-15 are forced to be *anti* by lactonization. Since the ^{1}H NMR spectral data for the medium ring portion of montafrusin B (**1b**) and A (**1a**) were nearly identical, this strongly suggests that the conformation in both compounds must be the same.

Bond distances (esds 0.009–0.02 Å) and angles (esds 0.6–1.1°) are given in supplementary material and are normal. The lactone ring is in the envelope conformation with the sum of its five endocyclic torsion angle magnitudes 77°. The angelate substituent is nonplanar, with a twist of 21.5° about the bond between C-16 and C-17. Molecules are linked in the solid state by a network of weak hydrogen bonds. Hydroxyl group O-4 serves as acceptor in a hydrogen bond from hydroxyl group O-3 of a neighboring molecule (O . . . . O 2.905(7) Å), and donates to a weaker interaction with lactone oxygen O-1 (3.066(8) Å).

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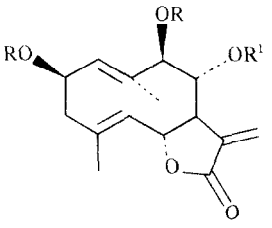
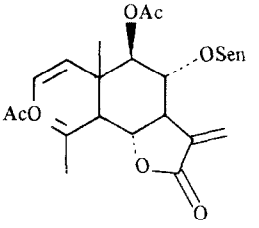


1a R = H, R^1 = Ang
1b R = H, R^1 = Sen
1c R = Ac, R^1 = Sen



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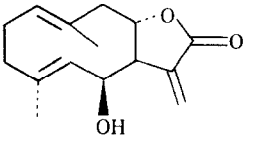

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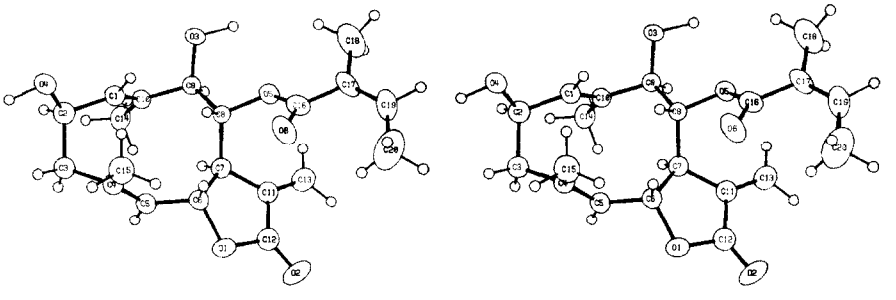

Fig. 1. Stereoscopic representation of the structure of montafrusin A.

EXPERIMENTAL

Montanoa frutescens (Mairet) Hemsl. (1 kg), collected in Morelos, Mexico, *ca* 60 km S. of Mexico City in October 1980, was extracted as described before [1]. The crude syrup (65 g) was chromatographed on a Tonsil optimum extra (supplied by Tonsil Mexicana) column (500 g) with $CHCl_3$–Me_2CO mixtures of increasing polarity, 65 fractions of 250 ml each, were collected. From fractions eluted with $CHCl_3$–Me_2CO (95:5) was obtained montafrusin A (**1a**; 215 mg), mp 185–187° (lit. 184–186° [1]).

Fractions eluted with $CHCl_3$–Me_2CO (9:1 and 4:1) were combined and rechromatographed on a silica gel (100 g) column with $CHCl_3$–Me_2CO mixtures, fractions of 200 ml each being collected. From these, fractions eluted with $CHCl_3$–Me_2CO (9:1) yielded montafrusin B (**1b**; 125 mg).

Montafrusin B (**1b**). $C_{20}H_{26}O_3$; mp 175–177° (Et_2O); $[\alpha]_D$ +23.3°; UV λ_{max}^{MeOH} nm (ε): 211 (22200); IR ν_{max}^{KBr} cm^{-1}: 3420, 1765, 1710, 1650; EIMS (probe) *m/z* (rel. int.): 362 $[M]^+$ (0.1), 344 $[M-H_2O]^+$ (0.3), 262 $[M-C_5H_8O_2]^+$ (3), 244 $[M-C_5H_8O_2-H_2O]^+$ (4), 83 $[C_5H_7O]^+$ (100), 55 $[C_5H_7]^+$ (20). (Found: C, 65.81; H, 7.25; O, 26.8. Calc. for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23; O, 26.49%.)

Montafrusin B acetate (**1c**). Acetylation of 60 mg of **1b**, with Ac_2O–pyridine gave after usual work up, the diacetate **1c** (30 mg) as a gum after TLC purification (petrol–EtOAc, 1:1) (30 mg). $[\alpha]_D$ +28.1°; UV λ_{max}^{MeOH} nm (ε): 212 (26800); IR ν_{max}^{film} cm^{-1}: 1770, 1740, 1720, 1650. EIMS (probe) *m/z* (rel. int.): 446 $[M]^+$ (0.1), 387 $[M-AcO]^+$ (1.2), 326 $[M-2AcOH]^+$ (0.3), 226 $[M-2AcOH-C_5H_8O_2]^+$ (15), 83 $[C_5H_7O]^+$ (100), 55 $[C_5H_7]^+$ (42), 43 $[Ac]^+$ (52).

Pyrolysis of **1c**. A 30 mg sample of **1c** was heated for 10 min under high vacuum in a sublimation tube, to give the Cope rearrangement product **2** (15 mg) as a gum after TLC purification (petrol–EtOAc, 3:2). IR ν_{max}^{film} cm^{-1}: 1770, 1750, 1711, 1635. EIMS (probe) *m/z* (rel. int.): 446 $[M]^+$ (0.1), 386 $[M-AcOH]^+$ (0.3), 326 $[M-2AcOH]$ (0.3), 244 $[M-AcOH-C_5H_8O_2-CH_2CO]^+$ (1.3), 83 $[C_5H_7O]^+$ (100), 55 $[C_5H_7]^+$ (9), 43 $[Ac]^+$ (10).

X-ray data. A crystal of dimensions 0.32 × 0.44 × 0.56 mm was used for data collection on an Enraf–Nonius CAD4 diffractometer equipped with MoKα radiation and a graphite monochromator. Crystal data are: $C_{20}H_{26}O_6$, M_r = 362.4, orthorhombic space group $P2_12_12_1$, a = 7.803(3), b = 12.766(6), c = 19.551(8) Å, V = 1947(2) $Å^3$, Z = 4, d_c = 1.236 $g.cm^{-3}$, λ

Table 1. ^{1}H NMR* signals for compounds **1b**, **1c** and **2** (**1b** at 200 MHz, **1c** and **2** at 80 MHz)

H	1b	1c	2
1	5.63 †	5.4–5.6†	4.40 *d* (8)
2	4.95 *t* (8)	5.79 *t* (8)	6.97 *d* (8)
3a	2.67 *dd* (13,8)	2.75 *dd* (13,8)	5.10 *br s*
3b	2.41 *d* (13)	2.34 *dd* (13)	4.79 *br s*
5	4.83 *br d* (10)	4.86 *br d* (10)	2.92 *d* (12)
6	5.32 *br t* (10)	5.20 *dd* (10,8)	4.20 *t* (12)
7	2.54 *m*	2.6 *m*	2.9 *m*
8	4.49 *dd* (10,3)	4.72 *dd* (10,3)	5.3–5.6‡
9	4.53 *d* (10)	5.05 *d* (10)	5.3–5.6‡
13a	5.58 *d* (3)	5.66 *d* (3)	5.49 *d* (13)
13b	6.20 *d* (3.5)	6.22 *d* (3.5)	6.12 *d* (3)
14	1.93 *br s*	1.88 *br*	1.34 *s*
15	1.78 *br s*	1.88 *br*	1.85 *br s*
2′	5.69 *m*	5.62 *m*	5.65 *m*
4′	1.93 *br s*	1.88 *br*	1.92 *d* (1)
5′	2.15 *d* (1)	2.14 *d* (1)	2.19 *d* (1)
AcO	—	2.02, 2.07 *s*	1.95, 2.21 *s*

*$CDCl_3$, TMS as internal standard Numbers in parentheses are coupling constants or line separations in Hz.

†Obscured by other signals.

‡No first order pattern.

= 0.71073 Å, μ(MoKα) = 0.85 cm^{-1}. Data were collected by ω-2θ scans of variable speed, designed to yield $I \simeq 25\sigma(I)$ for all significant reflections. One octant of data having $1° < \theta < 25°$ was measured, yielding 1976 unique data, of which, owing to the rather low quality of the crystals, only 955 had $I > 3\sigma(I)$, and were used in the refinement. Data reduction included corrections for background, Lorentz, and polarization effects; no absorption correction was necessary.

The structure was solved by direct methods, using MULTAN 78 [13], and refined by full matrix least squares, using the Enraf–Nonius SDP programs [14]. Due to the lack of sufficient observed data, carbon atoms C-1 through C-12 were treated isotropically, while only substituent carbon and oxygen atoms were refined anisotropically. Hydrogen atoms were located from difference maps and included as fixed contributions to the structure factors. Convergence was achieved with $R = 0.068$, $R_w = 0.069$ based on observed reflections, and the maximum residual electron density was 0.26 eÅ^{-3}. Coordinates, H atom coordinates, anisotropic thermal parameters, distances, angles and structure factors amplitudes have been deposited at the Cambridge Crystallographic Data Centre.

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