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MONTAFRUSIN, A NEW GERMACROLIDE FROM *MONTANOA FRUTESCENS**

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Key Word Index—*Montanoa frutescens*; Compositae; Heliantheae; a new germacrolide type sesquiterpene lactone; montafrusin.

Abstract—The investigation of *Montanoa frutescens* afforded a new sesquiterpene lactone of the germacrolide type, montafrusin, besides the known diterpenes kaurenic acid and its 15α-isovalerate.

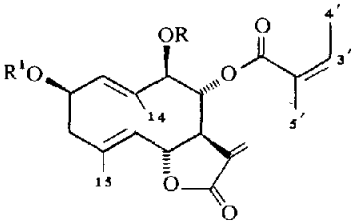
INTRODUCTION

Montanoa frutescens and *M. tomentosa* (Compositae, Heliantheae) are Mexican plants commonly known as 'zoapatles'. Previous studies of *Montanoa tomentosa* have shown the presence of several diterpenoid compounds [1] and a sesquiterpene lactone [2].

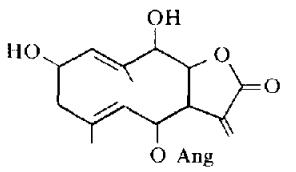
RESULTS AND DISCUSSION

Recently we have undertaken the study of *Montanoa frutescens* and have isolated kaurenic acid and its corresponding 15α-isovalerate, and a new sesquiterpene lactone of the germacrolide type which we named montafrusin (**1a**). The proposed structure and stereochemistry of **1a** were established by spectroscopic methods. Montafrusin (**1a**) $C_{20}H_{26}O_6$, mp 184–6° showed IR absorptions at 3540 and 3450 cm^{-1} indicating the presence of OH groups. An absorption at 1765 cm^{-1} was typical of α,β-unsaturated γ-lactones, a band at 1710 cm^{-1} corresponded to an α,β-unsaturated ester and one at 1650 cm^{-1} to double bonds. The MS showed a molecular ion at *m/e* 362 ($C_{20}H_{26}O_6$) and other spectral peaks at 344 ($M^+ - 18$), 262 ($M^+ - 100$), 244 ($M^+ - 118$) as well as the strongest peaks at *m/e* 83 (100%) and 55 which suggested the presence of a five-carbon ester side chain, which must be an angelate group on the basis of the vinyl proton signal appearing at 6.1 ppm in the ^1H NMR spectrum [3, 4].

The ^1H NMR spectrum (Table 1) of **1a** exhibited doublets of doublets typical of the lactonic exocyclic methylene with absorptions at δ 6.09 ($^4J = 3.8$, $^2J = 1$) and 5.64 ($^4J = 3.5$, $^2J = 1$), the large allylic coupling constant suggesting a *trans*-fused lactone ring [5, 6]. A doublet of doublets at 4.19 ($J = 10$, $J = 4$) which was assigned to H-9, collapsed to a doublet ($J = 10$) upon D_2O addition. All other proton assignments were determined by spin-spin decoupling experiments. The doublet of doublets at 4.54 ($J = 10$, $J = 3$) was assigned to H-8 since irradiation of this signal affected H-9. The overlapping signals at 4.8–5.05 were assigned to H-2 and H-5, the signal being affected by irradiations of the absorptions centred at 5.6 (H-1) and the doublet of doublets at 5.33 (H-6). The latter resonances were assigned to H-1 and H-6, respectively, on the basis that irradiation at the centre of δ 5.6 sharpened the C-10



1a R = R′ = H
1b R = R′ = Ac



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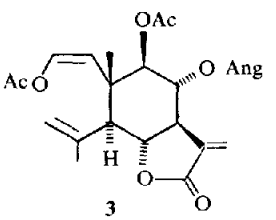

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Table 1. ^{1}H NMR data* of montafrusin (**1a**), montafrusin diacetate (**1b**) and Cope rearrangement product (**3**)

	1a	1b	3
H-1	5.6†	5.77†	4.42 *d*(7.5)
H-2	4.92 *brt* (8)	†	6.99 *d* (7.5)
H-3a	2.72 *dd* (13, 8)	2.78 *dd* (14, 7)	5.11 *t* (1.5)
H-3b	2.36 *brd* (13)	2.33 *dd* (14, 2)	4.82 *br*
H-5	4.95 *brd* (11)	4.9 *brd* (10)	2.95 *d* (11.5)
H-6	5.33 *dd* (10, 8)	5.33 *dd* (10, 8.5)	4.26 *dd* (11.5)
H-7	2.75 *m*	2.69 *m*	3.02 *m*
H-8	4.54 *dd* (10.3)	4.78 *dd* (10.3)	5.51 *dd* (7.6)§
H-9	4.19 *dd* (10.4)‡	5.14 *d* (10)	5.61 *d* (7)
H-13	6.09 *d* (3.8)	6.23 *d* (3.8)	6.11 *d* (3)
H-13′	5.64 *d* (3.5)	5.61 *d* (3.5)	5.43 *d* (3)
H-14	1.9 *s*	1.87 *s*	1.36 *s*
H-15	1.8 *br*	1.87 *s*	1.86 *br*
H-3′	6.1 *brq*	6.1 *brq*	6.18 *brq*
H-4′	1.9 2.0 *m*	1.9 2.1 *m*	2.01 *m*
H-5′	1.9–2.0 *m*	1.9–2.1 *m*	1.86 *m*
Ac	– –	2.03, 2.11 *s*	1.94, 2.2 *s*

*Run at 100 MHz in $CDCl_3$ with TMS as internal standard. **1a** was run in acetone-d_6. Values are in ppm (δ). Values in parentheses are coupling constants in Hz.

†Signal obscured.

‡Changes to a sharp doublet (J = 10) on D_2O exchange.

§No first order pattern.

vinylic methyl absorption at 1.8 and irradiation at 5.33 affected the H-7 signal at 2.8. Conversely, irradiation at 2.8 (which affects one of H-3 signals) collapsed the exocyclic methylene proton signals to singlets and the H-6 and H-8 signals at 5.33 and 4.54, respectively, to broad doublets (J = 10), the overlapping H-2 signals at 4.92 also being affected by this irradiation. It is interesting to point out that irradiation at 4.91 (H-2 and H-5) not only affected the H-3, H-1 and H-6 signals but also the C-10 methyl absorption which appeared as a doublet (J = 1.5) indicating a long range coupling between H-2 and the C-10 methyl group.

According to the above data, montafrusin could be represented by either **1a** or **2** exclusive of stereochemistry, which would be the 2-OH isomer of the structure reported for tomentosin [2]. Acetylation of montafrusin (**1a**) afforded the diacetate **1b** with IR absorptions at 1775, 1735, 1720, 1650 and 1600 cm^{-1}. The ^{1}H NMR spectrum displayed two sharp acetate methyl signals at δ2.03 and 2.11 and a downfield absorption at 5.14.

Montafrusin showed a CD curve typical of a C-6 *trans*-fused γ-lactone in accord with the Stöcklin–Waddell–Geissman rule [7]. A negative Cotton effect was observed at 262 nm corresponding to the $n \rightarrow \pi^*$ transition of the unsaturated γ-lactone, besides a strong positive band at 214 nm due to the $\pi \rightarrow \pi^*$ transition of the *trans* annular-cross conjugated double bonds.

Final confirmation of the structure of **1a** was achieved by obtaining the Cope rearrangement product **3** by pyrolysis of the diacetate **1b**. The ^{1}H NMR spectrum of the enol-acetate **3**, exhibited the H-5 signal as a doublet at δ 2.95 (J = 11.5) and the H-6 signal as a doublet of doublets at 4.26 (J = 11.5, J = 11.5) indicating the *trans* diaxial relationship between H-5 and H-6 which indicates a *trans*-fused lactone ring, since H-7 and H-5 are generally α in germacrolide-derived elemanolides. These chemical shift values and coupling constants for H-5 and H-6 are similar to those reported for the Cope rearrangement products of chihuahuin [8] and eupaserrin acetates [9]. The ^{1}H NMR spectrum also exhibited a three-proton singlet at δ 1.36 and two vinyl proton signals at 5.11 and 4.82. The H-1 and H-2 signals appeared as an AX pattern at 6.99 and 4.42 (J = 7.5) indicating a *cis* relationship of the enol-acetate. This result placed the second hydroxyl group of the molecule at C-2 and assigned the β-configuration of this OH group in **1a** [10]. The H-8 and H-9 proton signals represented the AB part of an ABX pattern with the H-9 signals appearing as a doublet centred at 5.61 (J = 7) and H-8 at 5.51 as a doublet of doublets (J = 7, J = 6).

Concerning the stereochemistry at C-8 and C-9, the large coupling constant (J = 10), observed between H-8 and H-9 in **1a** suggested a diaxial relationship between these protons placing H-8 β-axial and H-9 α-axial. Furthermore, the assigned H-8 stereochemistry is in accord with the observed splitting ($\sim$1 Hz) of the exocyclic methylene doublet signals, due to the geminal coupling in α,β-unsaturated C-6 γ-lactones with either a C-8 α-OH or ester side chain [11].

The upfield chemical shift of H-8 requires a comment. The alternative possibility of a C-8 lactone could explain the upfield chemical shift of H-8. The negative Cotton effect observed in the CD spectrum at 262 nm is predicted either for a C-6 *trans*-fused γ-lactone or a C-8 *cis*-fused γ-lactone [7], but the later case would not be in agreement with Samek's rule [5, 6], since the ^4J values observed for the exocyclic methylene signals in montafrusin are 3.8 and 3.5 Hz. According to these facts and the Cope rearrangement product **3**, montafrusin must be a C-6 *trans*-fused γ-lactone. In an attempt to interpret the upfield chemical shift of H-8, we have observed that in C-6 *trans*-fused germacrolides with α C-8 ester attachments, tulipinolide, tulipinolide diepoxi [12], chihuahuin [8], lanuginolide 11,13 dehydro [13], the β H-8 (axial) signal has a higher chemical shift (δ4.5–5.2) than the αH-8 (equatorial) signal in germacrolides with β C-8 ester attachments, epitulipinolide [12], eupatoriopicrin [14], eupasserrin [9], lipiferolide [15], epitulipinolide-diepoxi [15], costunolide 8β angeloxy, 3β-9-α-dihydroxy [16], which are always further downfield (δ 5.7–5.9) due to the equatorial position and the deshielding effect of the $\Delta^{11,13}$ bond. On the other hand, C-6 *trans*-fused γ-lactones with a C-8 β side chain ester normally have a small $J_{7,8}$ ($\leq$1 Hz) and C-6 *trans*-fused γ-lactones with a C-8 α side chain ester have a large $J_{7,8}$ (7–9 Hz). However, the $J_{7,8}$ (3 Hz) observed for montafrusin lies between both values, indicating that a certain torsion of the dihedral angle H-C_7-C_8-H allowing a value near 120° might exist, and could be due to the combined influence of the $\Delta^{11,13}$ bond and the C-8 α angeloxy function. Based on all these facts we propose **1a** as the more likely structure for montafrusin.

EXPERIMENTAL

Isolation of montafrusin (**1a**). *Montanoa frutescens* (Mairet) Hemsl. was collected in Morelos, México, 60 km S of México City on 1 November 1976. A voucher is deposited at the Instituto de Biologia (UNAM), México.

A 3 kg sample of the leaves, flowers and stems was extracted first with petrol, then with $CHCl_3$, and the resultant extracts chromatographed on a Si gel column. From the chromatography of the petrol extract caryophyllene, taraxasterol acetate, kaurenic acid and its corresponding 15α-isovalerate were isolated.

From the chromatography of the $CHCl_3$ extract in the fractions eluted with EtOAc, a dark brown syrup was obtained which crystallized upon addition of $CHCl_3$. Montafrusin was recrystallized from EtOAc–$CHCl_3$, mp 184–6°. UV λ_{max}^{EtOH}: 213 nm (ε = 23650); IR ν_{max}^{KBr} cm^{-1}: 3540, 3450, 1765, 1710, 1650; CD (MeOH): 262 nm ([θ] − 2093), 214 nm ([θ] + 14953); MS *m/e*: 362 (M^+), 344 ($M^+ - H_2O$), 262 ($M^+ - C_4H_7COOH$), 244 ($M^+ - H_2OC_4H_7COOH$), 83 (C_5H_7O), 55 (C_4H_7).

Montafrusin acetate (**1b**). A 30 mg sample of **1a**, 2 ml Ac_2O and 0.5 ml Py were combined and left overnight at room temp. The resultant residue, after removing the excess of Ac_2O and Py under high vacuum, was purified by TLC ($CHCl_3$–Me_2CO, 9:1) yielding the oily diacetate. UV λ_{max}^{EtOH} 213 nm (ε = 14200); IR ν_{max}^{film} cm^{-1}: 1775, 1735, 1720, 1650, 1600.

Pyrolysis of **1b**. Montafrusin diacetate (**1b**) (25 mg) was heated for 10 min under high vacuum at 200° in a sublimation tube, to give a colourless oil. The ^1H NMR spectrum indicated the presence of one major component which was in accord with structure **3** (Table 1). IR ν_{max}^{film} cm^{-1}: 1775, 1755, 1720, 1675, 1650.

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