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ZOAPATANOLIDE A AND B, TWO NEW HELIANGOLIDES FROM *MONTANOA TOMENTOSA**

L. QUIJANO, J. S. CALDERÓN, F. GÓMEZ and T. RÍOS

Instituto de Química de la Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, México 20, D.F., Mexico

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Key Word Index—*Montanoa tomentosa*; Compositae; Heliantheae; new heliangolides; sesquiterpene lactones; zoapatanolide A and B.

Abstract—The re-investigation of *Montanoa tomentosa* afforded, in addition to known diterpenoids of the kaurene class, two new sesquiterpene lactones, zoapatanolide A and B, of the heliangolide type.

INTRODUCTION

Montanoa tomentosa and *M. frutescens* are Mexican plants commonly known as 'zoapatles', which have been used in Mexico since ancient times in folk medicine for their putative oxytocic properties.

In a previous paper, we described the isolation and structure elucidation of montafrusin (**1a**), a germacrolide from *M. frutescens* [1]. Recently, an investigation of the active components of *M. tomentosa*, culminated in the structure elucidation of two biologically active oxepane diterpenoids, zoapatanol and montanol [2, 3]. Previous studies of the plant demonstrated the presence of several diterpenoids [4] and the sesquiterpene lactone tomentosin (**2**), the structure of which was established by ^{1}H NMR studies [5].

During our work on the structure elucidation of montafrusin (**1a**) we realized that some of the published ^{1}H NMR data of tomentosin (**2**) were not in good agreement with the proposed structure, especially the small values of $J_{6,7}$ and $J_{7,13}$ which suggested that the molecule might be a heliangolide. A similar observation was recently made by Herz, but he suggested a *trans-trans*-germacra-1(10),4-*cis*-6,12-olide-type structure [6].

RESULTS AND DISCUSSION

To verify the above hypothesis, we have undertaken a re-investigation of *M. tomentosa* Cerv. and isolated a new sesquiterpene lactone. The ^{1}H NMR and IR spectra of the new compound indicated that it was identical with the sesquiterpene lactone obtained during the isolation of the active components zoapatanol and montanol [2]. The ^{1}H NMR shifts, however, differed from those which have been published for tomentosin (**2**) [5]. This fact suggested that we were dealing with a different substance, which we named zoapatanolide A (**3a**). Its structure was established by extensive ^{1}H NMR studies and spin–spin decoupling experiments as well as chemical evidence.

Zoapatanolide A (**3a**), $C_{20}H_{26}O_6$, mp 194–196°, $[\alpha]_D - 83.5°$ was a conjugated γ-lactone which showed UV absorption at 206 nm (ϵ 21358) and the typical IR absorption at 1770 cm^{-1}. A strong absorption band at 3440 cm^{-1} indicated the presence of hydroxyl groups, which was confirmed by acetylation to give a diacetate (**3b**). The presence of an angelate (1710 cm^{-1}) was indicated by MS ion peaks at *m/z* 83 (C_5H_7O, 100) and 55 (C_4H_7, 50.5) characteristic of this type of ester, and the typical vinyl proton quartet at δ 6.14 in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum of zoapatanolide A (**3a**) run in $CDCl_3$ (Table 1) showed the typical signals of the exocyclic methylene at δ 6.29 as a broad doublet ($^4J = 2$) and 5.62 as a broad doublet which might be considered as a doublet of doublets ($^4J = 2$, $^2J = 1$). The small allylic coupling constant suggested either a *cis*-fused lactone ring according to Samek's rule [7] or a *trans*-fused heliangolide type structure [8]. The ^{1}H NMR spectrum determined in acetone-d_6 displayed a two-proton set of signals at δ 4.1–4.4 which were assigned to H-9 and the C-9-hydroxyl interaction since they collapsed to a one-proton doublet ($J = 10$) centred at δ 4.2 (H-9) upon D_2O addition, besides the disappearance of a singlet at δ 2.8. The remaining proton signals were assigned by spin–spin decoupling experiments; H-7 was located as a doublet of quartets at δ 2.95 ($J = 10$, $J = 1$) since irradiation of this signal collapsed the exocyclic methylene signals to singlets, converted a triplet at δ 4.95 ($J = 10$) to a doublet and sharpened a broad doublet at δ4.79 ($J = 10$). Irradiation at the frequency of H-9 (δ 4.2) converted the triplet at δ 4.95 into a doublet. Thus the signals at δ 4.95 and 4.79 were assigned to H-8 and H-6 respectively. Irradiation at the frequency of a vinyl methyl group doublet at δ1.75 ($J = 1$) converted a broad doublet at δ 5.08 ($J = 10$) into a thin doublet of doublets ($J = 10$, $J = 1$)

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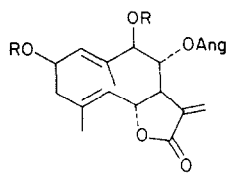
Table 1. ^{1}H NMR data of compounds **3a** and **6** and their derivatives [200-MHz (**3a** and **6**) or 80-MHz, $CDCl_3$, TMS as int. standard]

	3a	**6**	**3b**	**4b**	**5a**	**5b**
H-1	5.33 *t(br)*	5.45 *t(br)*	5.42 *t(br)*	5.42 *t(br)*	*	*
H-3	4.74 *dd*	4.74 *dd*	5.57 *dd*	5.68 *dd*	5.77 *dd*	5.75 *dd*
H-5	5.10 *d(br)*	5.16 *d(br)*	5.15 *d(br)*	5.35 *br*	5.1–5.4	5.0–5.4
H-6	4.77 *d(br)*	4.72 *d(br)*	4.80 *d(br)*	5.35 *br*	4.62	4.65 *d(br)*
H-7	2.78 *dq*	2.78 *dq*	2.85 *d(br)*	*	*	*
H-8	4.99 *t*	3.74 *t*	5.1–5.4	4.50 *t*	5.1–5.4	5.0–5.4
H-9	4.13 *d*	5.13 *d*	5.1–5.4	5.06 *d*	5.1–5.4	5.0–5.4
H-13a	5.62 *d*	5.95 *br*	5.63 *d*	1.36 *d*	5.62 *br*	5.75 *br*
H-13b	6.29 *d*	6.50 *br*	6.30 *d*		6.27 *br*	6.37 *br*
H-14	1.89 *br*	1.89 *br*	1.80 *br*	1.76 *br*	1.64 *s*	1.65 *s*
H-15	1.79 *d*	1.79 *d*	1.85 *br*	1.90 *br*	1.77 *br*	1.84 *br*
H-3′	6.14 *qq*	6.16 *qq*	6.1 *dq*	6.1 *qq*	6.08 *qq*	*
H-4′	1.97 *dq*	2.01 *dq*	1.9–2.0 *m*	1.9–2.0 *m*	1.85–1.95 *m*	1.42 *s*
H-5′	1.90 *quint*	1.94 *quint*	1.9–2.0 *m*	1.9–2.0 *m*	1.85–1.95 *m*	1.25,1.28 *d*†
AcO	—	—	2.04,2.10 *s*	2.05 *s*	2.02,2.05 *s*	2,07,2.10 *s*

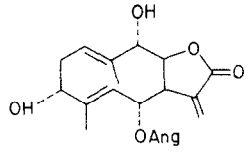
*Signal obscured.

†Diastereomeric mixture.

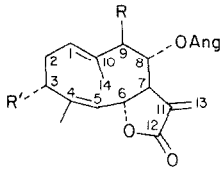
J(Hz): 1, 2 = 8; 2, 3 = 12 and 5; 5, 6 = 11; 6, 7 = 1.5; 7, 8 = 10; 8, 9 = 10; 7, 13a = 1.5; 7, 13b = 2; 5, 15 = 1.2; 3′, 4′ = 7; 3′, 5′ = 1.5; 4′, 5′ = 1.5.



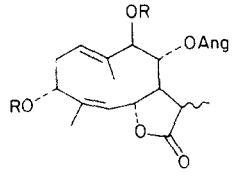
1a R = H
1b R = Ac



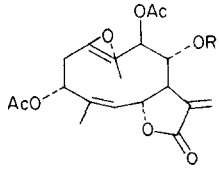
2



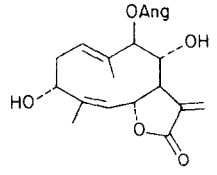
3a R = R′ = OH
3b R = R′ = OAc
3c R = H, R′ = OH



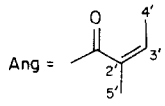
4a R = H
4b R = Ac



5a R = Ang
5b R = Epoang



6



assigning these signals to the C-4-Me group and H-5 which still conserved a residual coupling probably with H-3. Further irradiation at the frequency of the remaining vinyl methyl signals at δ 1.9 sharpened a triplet at δ 5.4 ($J = 8$) assigning this signal to H-1 and collapsed the vinyl proton quartet of the angelic moiety into a singlet.

According to the above data, the structure of zoapatanolide can be represented by a heliangolide type structure. Furthermore, comparison of the published ^{1}H NMR spectral parameters of 3-epi-nobilin (**3c**) [9] with those of zoapatanolide A (**3a**) indicated close similarities.

In contrast to montafrusin (**1a**), which showed a typical CD curve of substances of the costunolide type, the CD curve of zoapatanolide (**3a**) had a strong negative Cotton effect at 218 nm corresponding to the π, π^* transition of the *trans*-annular-cross conjugated double bonds, besides a strong positive band at 235 nm due to the n, π^* transition of the unsaturated γ-lactone, similar to 3-epi-nobiline (**3c**) and other heliangolides [9].

In spite of the fact that the CD profile of zoapatanolide as well as most of the ^{1}H NMR signals are also in accord with a *trans-trans*-germacra-1(10), 4-*cis*-6,12-olide, we propose a heliangolide type structure based on the fact that pyrolysis of zoapatanolide diacetate (**3b**) carried out under the same conditions as used for montafrusin diacetate (**1b**) did not afford the expected Cope rearrangement product. On the other hand, substances of *trans-trans*-germacra-1(10),4-*cis*-6,12-olide type as well as costunolide type afford Cope rearrangement products [1, 10].

Examination of Dreiding models showed that the magnitudes of $J_{5,6}$, $J_{6,7}$, $J_{7,8}$ and $J_{8,9}$ (11, ~1, 10 and 10 Hz) were keeping with the stereochemistry depicted in the formulas. The small value $J_{6,7} = 1$ in zoapatanolide was also in accord with a heliangolide rather than a germacra-1 (10), 4-diene-*cis*-6, 12-olide, which shows a larger value of $J_{6,7} = 6$–7 [6, 10].

Reduction of zoapatanolide (**3a**) with sodium borohydride gave the dihydro derivative (**4a**), mp 203–204°, $[M]^+$ 364. Its ^{1}H NMR spectrum showed a new secondary methyl group at δ 1.25 ($J = 7.0$) and lacked the exocyclic methylene signals. Acetylation of **4a** afforded the corresponding diacetate **4b**, whose ^{1}H NMR clearly showed the H-9 and H-8 signals as a doublet and a triplet at δ 5.06 ($J = 9.5$) and δ 4.51 ($J = 9.5$) respectively.

Treatment of zoapatanolide A diacetate (**3b**) with *meta*-chloro perbenzoic acid furnished a mixture of epoxides which were identified spectroscopically as the 1 (10) monoepoxizoapatanolide (**5a**) and the diepoxi derivative **5b** in which the ester side-chain double bond was also oxidized giving a diasteroisomeric mixture of 2′, 3′-epoxides as was shown by the ^{1}H NMR which displayed two secondary methyl group doublets at δ 1.26 ($J = 6$ Hz) and 1.31 ($J = 6.0$ Hz).

Based on all these facts we propose **3a** as the more likely structure for zoapatanolide A.

Finally, the 200 MHz ^{1}H NMR (Table 1) of one of the chromatography fractions obtained during the isolation, showed it contained a mixture of zoapatanolide A and another sesquiterpene lactone which has to be the corresponding isomer **6** in which the relative position of the hydroxyl group and the side chain ester at C-9 and C-8 were interchanged, as was clearly shown by the presence of an extra triplet at δ 3.74 ($J = 10$ Hz) and a doublet at δ 5.13 ($J = 10$ Hz) due to H-8 and H-9 respectively. We have named this new heliangolide zoapatanolide B (**6**).

EXPERIMENTAL

M. tomentosa Cerv. was collected on the U.N.A.M. campus, Mexico City, on 15 July 1980. A voucher is on deposit at Herbarium of Instituto de Biología (U.N.A.M.), México. Dried leaves (2.9 kg) were extracted with $CHCl_3$ at room temp. and the resultant extract (215 g) was percolated on a column packed with 1 kg of Tonsil optimum extra (supplied by Tonsil Mexicana) and eluted with $CHCl_3$ and mixtures of $CHCl_3$–EtOAc. Fractions (300-ml), were collected and all were monitored by TLC. Similar fractions were combined and chromatographed on Si gel.

From the less polar fractions eluted with $CHCl_3$, a mixture of β-sitosterol and stigmasterol was isolated, besides kaurenoic acid and 16α-hydroxy kaurenoic acid, mp 280° (lit. 281–283° [11]).

Zoapatanolide A (**3a**). From fractions eluted with $CHCl_3$–EtOAc (1:1) (7.5 g) chromatography over 120 g Si gel gave a dark brown syrup which was crystallized from Et_2O. Zoapatanolide was recrystallized from $CHCl_3$–Et_2O, mp 194–196°, $[\alpha]_D - 83.5°$ ($CHCl_3$); UV λ_{max}^{MeOH} nm (ϵ): 206 (21 358); IR ν_{max}^{KBr} cm^{-1}: 3440, 1770, 1650. CD (MeOH); 235 nm ($[\theta] + 11\,700$), 218 nm ($[\theta] - 20\,700$); MS *m/z* (rel. int.): 362 $[M]^+$ (0.12) 344 $[M - H_2O]^+$ (0.42), 326 $[M - 2H_2O]^+$ (0.13), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$(23.6).

Zoapatanolide A acetate (**3b**). 160 mg **3a**, 2 ml Ac_2O and 0.5 ml pyridine were combined and left 4 hr at room temp. Usual work-up gave 145 mg of **3b**, mp. 190–191° (Me_2CO); $[\alpha]_D - 13.7°$ ($CHCl_3$); UV λ_{max}^{MeOH} nm (ϵ): 203 (26837); IR ν_{max}^{film} cm^{-1}: 1775, 1735, 1650; MS *m/z* (rel. int.): 387 $[M - 59]^+$ (3.8), 344 $[M - 59 - 43]^+$ (1.2), 226 $[M - 120]^+$ (3.1), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (24.2), 43 $(C_2H_3O)^+$ (29.2).

Reduction of zoapatanolide A (**3a**). To a soln of 100 mg **3a** in 10 ml MeOH was added 100 mg $NaBH_4$ at 5° and the mixture was allowed to react for 15 min. The mixture was then diluted with H_2O, acidified with 5% aq. HCl and extracted with EtOAc. The washed and dried extracts were evaporated and the residue purified by TLC to give 65 mg **4a**, mp 203–205°; IR ν_{max}^{KBr} cm^{-1}: 3420, 1766, 1690, 1650; MS *m/z* (rel. int.): 364 $[M]^+$ (0.5), 264 $[M - 100]^+$ (0.4), 100 $[C_5H_8O_2]^+$ (58.1), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (36.1).

Epoxidation of acetate **3b**. To a soln of the acetate **3b** (115 mg) in $CHCl_3$, *m*-chloro-perbenzoic acid (100 mg) was added and the reaction monitored by TLC. The $CHCl_3$ soln was washed with 5% aq. $NaHCO_3$, dried (Na_2SO_4), evaporated and the residue purified by TLC.

The less polar compound was identified as the monoepoxide **5a**, mp 225–227°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1750, 1740, 1645; MS *m/z* (rel. int.): 403 $[M - 59]^+$ (0.7), 402 $[M - 60]^+$ (0.6), 363 $[M - 99]^+$ (1.6), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (39.0), 43 $[C_2H_3O]^+$ (45.3).

The more polar compound was the diepoxi-derivative **5b**, mp 236–238°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1772, 1750, 1665, 1235; MS *m/z* (rel. int.): 478 $[M]^+$ (0.4), 419 $(M - 59]^+$ (2.5), 363 $[M - 115]^+$ (1.6), 83 $[C_5H_7O]^+$ (43.3), 55 $[C_4H_7]^+$ (21.7), 43 $[C_2H_3O]^+$ (100).

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