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OXEPANE DITERPENOIDS AND SESQUITERPENE LACTONES FROM 'ZOAPATLE' (*MONTANOA TOMENTOSA*), A MEXICAN PLANT WITH OXYTOCIC ACTIVITY*

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Key Word Index—*Montanoa tomentosa*; Compositae; sesquiterpene lactones; biologically active oxepane diterpenoids.

Abstract—Investigation of the 'tea' prepared from the leaves of 'zoapatle' (*Montanoa tomentosa*), resulted in the isolation of the known biologically active oxepane diterpenoids zoapatanol and montanol, the known sesquiterpene lactones zoapatanolide A, C and D as well as two new biologically active oxepane diterpenoids and a new sesquiterpene lactone. The structures of the new compounds were established by spectroscopic methods.

INTRODUCTION

In previous papers we have described the isolation and structure elucidation of sesquiterpene lactones from *Montanoa tomentosa* and *M. frutescens*, two Mexican species commonly known as 'zoapatle' [1–3]. Other authors have published on the isolation and identification of the biologically active components. The putative oxytocic material was isolated as a complex mixture called 'triplet mixture' from which zoapatanol (**1a**) and montanol (**1b**) were separated and identified. The third component could not be separated and remained unidentified [4].

In a new investigation of the 'tea', we have isolated and identified other components of the 'triplet mixture'. Besides the known sesquiterpene lactones zoapatanolide A [2], C and D [3], the oxepane diterpenoids zoapatanol (**1a**), montanol (**1b**) [4] and tomexanthin (**3c**) [5], we have also isolated a new guaianolide, zoapatanolide E (**4a**), and two new oxepane diterpenoids which we have named tomentol (**2a**) and tomexanthol (**3a**). Tomentol (**2a**) was shown to be as active as the 'triplet mixture' and zoapatanol.† The details and results of the biological test utilized to prove the uterotonic activity will be published elsewhere.

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† The biological activity was tested at the Division de Biología Molecular del Centro Médico Nacional del Instituto Mexicano del Seguro Social.

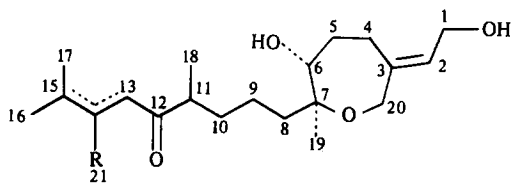
Table 1. ^{1}H NMR data of tomentol (**2a**), tomenxanthol (**3a**) and their acetates **2b** and **3b** (80 MHz, $CDCl_3$, TMS as int. standard)

H	2a	3a	2b	3b
1	4.18 *d* (*br*) (7)	4.17 *d* (*br*) (7)	4.57 *d* (*br*) (7)	4.56 *d* (7)
2	5.45 *t*(*br*) (7)	5.44 *t*(*br*) (7)	5.39 *t*(*br*) (7)	5.39 *t*(*br*) (7)
6	3.54 *dd* (6)	3.53 *dd* (6)	4.70*	~4.70*
13	3.18 *s* (*br*)	6.28 *d* (16)	3.16 *s* (*br*)	6.26 *d* (16)
14	—	6.87 *d* (16)	—	6.85 *d* (16)
16	1.10 *d* (7)	1.40 *s*	1.07 *d* (7)	1.45 *s*
17	1.05 *d* (7)	1.40 *s*	1.03 *d* (7)	1.45 *s*
18	1.05 *d* (7)	1.10 *d* (7)	1.03 *d* (7)	1.08 *d* (7)
19	1.17 *s*	1.15 *s*	1.13 *s*	1.13 *s*
20	4.10 *s* (*br*)	4.10 *s* (*br*)	4.09 *s* (*br*)	4.08 *s* (*br*)
21a	4.78 *s* (*br*)	—	4.76 *s* (*br*)	—
21b	4.97 *s* (*br*)	—	4.95 *s* (*br*)	—
AcO	—	—	2.05 *s*	2.04 *s*

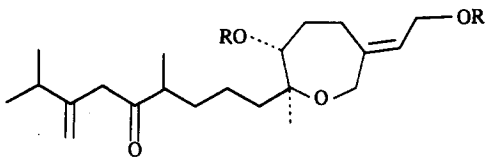
Figures in parentheses are coupling constants or line separations in Hz.
*Obscured by other signals.

RESULTS AND DISCUSSION

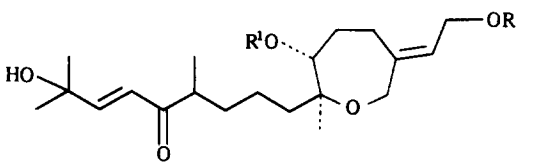
Tomentol (**2a**), $C_{21}H_{36}O_4$, was isolated as a gum, by extended TLC purification of the 'triplet mixture'. The IR spectrum indicated hydroxyl functions (3420 cm^{-1}) and possibly a saturated ketone (1720 cm^{-1}). The mass spectrum did not show a molecular ion, but an $[M-H_2O]^+$ ion was present at m/z 334. Acetylation of tomentol (**2a**) yielded a diacetate (**2b**) whose MS showed the molecular ion at m/z 436 ($C_{25}H_{40}O_6$) confirming the M_r of 352 ($C_{21}H_{36}O_4$) and the presence of two hydroxyl groups in **2a**. The structure of tomentol (**2a**) could be deduced from the ^1H NMR spectrum (Table 1) since it was very similar to that of the known montanol (**1b**) [4]. Tomentol (**2a**) differs from **1b** by the presence of a terminal methylene group instead of a vinyl methyl group at C-14. The ^1H NMR spectrum of **2a** showed the two vinyl protons of the terminal methylene as two broad singlets at δ4.78 and 4.97 instead of the vinyl methyl group signal. The vinyl proton signal at C-13 was replaced by a two-proton broad singlet at δ3.18. All the ^1H NMR spectral data of tomentol and its acetate are in good agreement with the structure **2a**. The assignments are supported by the mass spectral fragmentation pattern (Fig. 1).



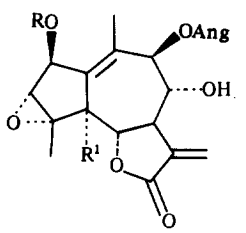
1a Δ^{14}, R = H
1b Δ^{13}, R = Me



2a R = H
2b R = Ac



3a R = R¹ = H
3b R = R¹ = Ac
3c R = Ac, R¹ = H



4a R = H, R¹ = OH
4b R = Ac, R¹ = OH
4c R = Ac, R¹ = H

Tomexanthol (**3a**), $C_{20}H_{34}O_5$, was a second new oxepane diterpene isolated from the 'triplet mixture'. The ^1H NMR spectrum of **3a** (Table 1) indicated that this compound was the desacetyl derivative of tomexanthin (**3c**). Accordingly, the H-1 doublet was shifted upfield from δ4.60 to 4.17 and the acetate methyl singlet was missing. The MS showed a weak molecular ion at m/z 354 in agreement with the molecular formula $C_{20}H_{34}O_5$. Acetylation of **3a** afforded the diacetate **3b**. The ^1H NMR spectrum of **3b** showed the acetate methyl signals at δ2.04 and the downfield shift of H-1 and H-6 from δ4.17 and 3.53 in tomexanthol (**3a**) to 4.56 and 4.70 respectively in the diacetate **3b**. All the ^1H NMR and mass spectral data were in full agreement with the structure **3a** for tomexanthol.

Zoapatanolide E (**4a**), $C_{20}H_{24}O_8$, was isolated as a gum which exhibited a strong IR absorption at 1775 cm^{-1} indicating an α,β-unsaturated γ-lactone. Further IR absorptions at 3490 and 1720 cm^{-1} indicated the presence of hydroxyl groups and an unsaturated ester. The ^1H NMR spectrum of **4a** was very similar to that of zoapatanolide C (**4b**), but the acetate methyl singlet was missing and the H-2 signal shifted upfield from δ5.62 to 4.53. These differences between the ^1H NMR spectra of **4a** and **4b** indicated that zoapatanolide E must be the desacetyl derivative of zoapatanolide C.

EXPERIMENTAL

Montanoa tomentosa Cerv (zoapatle) was collected at UNAM campus, México City in October 1983. Fresh leaves of zoapatle (3.2 kg) were extracted with hot water (10 l.) for 2 hr and filtered to afford a dark tea. The aq. extract was extracted with EtOAc (about 6 l.) and the solvent evaporated *in vacuo* giving 7.6 g of a dark crude extract, which was chromatographed over 80 g of silica gel (Merck 70–230 mesh) using petrol and mixtures of petrol–EtOAc as eluant; 160 fractions (200 ml) were taken and monitored by TLC.

Percolation of fractions 21–41 (290 mg) over silica gel with CH_2Cl_2 and Me_2CO yielded 29 mg kaurenoic acid. TLC purification (CH_2Cl_2–Me_2CO, 19:1) of fractions 42–46 (95 mg) yielded 15 mg zoapatanolide D (**4c**) [3] and 5 mg tomexanthin (**3c**) [5]. From fractions 61–80 (350 mg), after TLC purification on silica gel G (CH_2Cl_2–Me_2CO, 97:3), 40 mg of zoapatanolide A [2] were obtained. Fractions 81–106 (1.2 g) were rechromatographed over 50 g of silica gel (Merck 70–230 mesh) using heptane–EtOAc mixtures of increasing polarity, to yield 211 fractions of 200 ml. Fractions 42–54 provided 170 mg zoapatanolide C (**4b**). Crystallization of fractions 81–90 have 105 mg zoapatanolide A. Further preparative TLC (CH_2Cl_2–Me_2CO, 7:3) of fractions 91–169 gave 180 mg of an oily mixture ('triplet mixture' [4]) whose ^1H NMR spectrum showed the presence of the oxepane diterpenoids zoapatanol (**1a**) and montanol (**1b**) and other compounds.

Prep. TLC (CH_2Cl_2–Me_2CO, 4:1) of fractions 107–117 of the

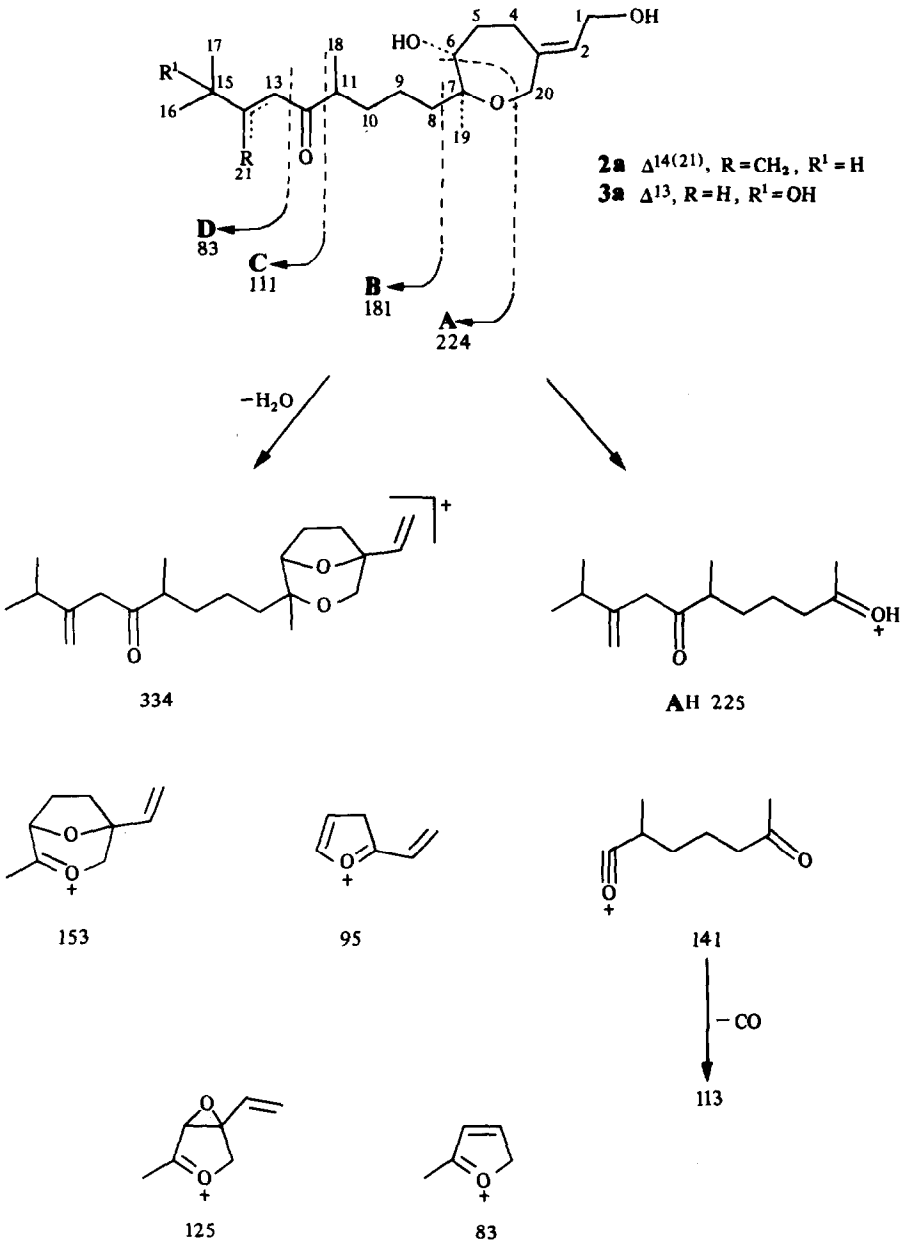


Fig. 1. Mass spectral fragmentation of **2a**.

first chromatography afforded 53 mg zoapatanolide C (**4b**) and 80 mg of the 'triplet mixture'.

Isolation of tomentol (**2a**). TLC of a 25 mg sample of the 'triplet mixture' on silica gel G (0.25 mm × 10 × 20 cm) impregnated with a 10% soln of $AgNO_3$ (CH_2Cl_2–Me_2CO, 4:1, × 3) gave three bands. The less polar fraction (band 1) gave an oil which was identified spectroscopically as montanol (**1b**). The next fraction (band 2) yielded zoapatanol (**1a**) which was identified by NMR and comparison with an authentic sample. The most polar fraction (band 3) contained tomentol (**2a**). IR ν_{max}^{film} cm^{-1}: 3420, 1710, 1680, 890; UV λ_{max}^{MeOH} nm (ε): 204 (4065); EIMS (probe) 70 eV *m/z* (rel. int.): 334 $[M-H_2O]^+$ (1.0), 316 $[M-2H_2O]^+$ (0.3), 241 $[M-C]^+$ (2.5), 225 $[A+H]^+$ (16), 171 $[M-B]^+$ (5.3), 153 $[M-B-H_2O]^+$ (6.8), 141 $[A-D]^+$ (50), 113 $[A-C]^+$ (85), 111 $[C]^+$ (30), 95 $[C_6H_7O]^+$ (56), 83 $[D]^+$ (20), 81 (15), 71 (20), 69 (21), 67 (41), 55 (70), 43 (100), 41 (48).

Isolation of zoapatanolide E (**4a**) *and tomexanthol* (**3a**). TLC of the rest of the 'triplet mixture' (235 mg) on silica gel G (2 mm × 10 × 20 cm) impregnated with a 10% soln of $AgNO_3$ (CH_2Cl_2–Me_2CO, 4:1, × 3) gave four bands. Further TLC purification (CH_2Cl_2–Me_2CO, 4:1) of band 1 afforded 12 mg zoapatanolide E (**4a**). Band 2 was fractionated over silica gel using CH_2Cl_2 and CH_2Cl_2–Me_2CO (3:2). Further preparative TLC (CH_2Cl_2–Me_2CO, 4:1, × 3) of the polar fraction yielded 7 mg tomexanthol (**3a**). The least polar fraction contained the dehydration product of montanol (18 mg) [4]. Purification of

band 3 using the same conditions gave further quantities of the 'triplet mixture' and tomenxanthol (**3a**) (10 mg). Band 4 contained 9 mg tomentol (**2a**).

Tomexanthol (**3a**). Colourless gum. IR ν_{max}^{film} cm^{-1}: 3370, 1690, 1630; UV λ_{max}^{MeOH} nm (ε): 204 (10,450), 223 (8200); EIMS (probe) 70 eV *m/z* (rel. int.): 354 $[M]^+$ (0.2), 336 $[M-H_2O]^+$ (0.5), 318 $[M-2H_2O]^+$ (0.3), 227 $[AH]^+$ (2.1), 209 $[AH-H_2O]^+$ (10), 149 (15), 141 $[A-D]^+$ (27), 113 $[C]^+$ (46), 111 (32), 95 $[C_6H_7O]^+$ (37), 85 $[D]^+$ (24), 83 (28), 81 (26), 71 (47), 69 (37), 67 (32), 55 (39), 43 (100).

Zoapatanolide E (**4a**). Colourless gum. IR ν_{max}^{film} cm^{-1}: 3490, 1775, 1720, 1645; UV λ_{max}^{MeOH} nm (ε): 207 (12,880); EIMS (probe) 70 eV *m/z* (rel. int.): 374 $[M-H_2O]^+$ (0.2), 292 $[M-AngOH]^+$ (0.3), 274 $[M-H_2O-AngOH]^+$ (0.6), 167 (4), 149 (11), 83 (100), 55 (30), 43 (10); ^{1}H NMR (80 MHz, $CDCl_3$) δ1.66 (3H, *s*, H-15), 1.78 (3H, *s* (*br*), H-14), 1.97 (3H, *m*, H-5′), 2.01 (3H, *d* (*br*), H-4′), 3.15 (*br*, OH), 3.66 (*d*, J = 2.5 Hz, H-3) 3.84 (H-6, H-7, H-8), 4.53 (*s* (*br*), H-2), 6.1 (H-9, H-3′), 6.20 (*s* (*br*), H-13a, H-13b).

Tomentol diacetate (**2b**). Acetylation of 11 mg **2a** in Ac_2O–C_5H_5N, followed by usual work-up, gave the diacetate **3b**. IR ν_{max}^{film} cm^{-1}: 1745, 1712, 1643, 900; EIMS (probe) 70 eV *m/z* (rel. int.): 436 $[M]^+$ (0.8), 376 $[M-AcOH]^+$ (1.4), 333 $[M-AcOH-Ac]^+$ (0.3), 233 $[M-2AcOH-D]^+$ (20), 225 $[AH]^+$ (3.5), 153 $[B-C_2H_4]^+$ (8), 141 $[A-D]^+$ (18), 113 $[A-C]^+$ (29), 111 $[C]^+$ (14), 92 $[M-A-2AcOH]^+$ (37), 95 $[C_6H_7O]^+$ (26), 83 $[D]^+$ (11), 81 (13), 71 (5), 69 (15), 67 (24), 55 (33), 43 (100).

Tomexanthol diacetate (**3b**). Acetylation of 17 mg **3a** with Ac_2O–C_5H_5N gave, after TLC purification (CH_2Cl_2–Me_2CO, 19:1), the diacetate **3b**. IR ν_{max}^{film} cm^{-1}: 3695, 1730, 1605; EIMS (probe) 70 eV *m/z* (rel. int.): 378 $[M-AcOH]^+$ (0.2), 209 $[AH-H_2O]^+$ (2.5), 153 $[M-B-AcO-Ac]^+$ (6), 149 (8), 141 $[A-D]^+$ (6), 113 $[C]^+$ (10), 111 (14), 95 $[C_6H_7O]^+$ (13), 85 $[D]^+$ (9), 83 (14), 81 (11), 71 (13), 69 (10), 67 (9), 55 (18), 43 (100).

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REFERENCES

1. Quijano, L., Calderón, J. S., Gómez, G. F. and Ríos, T. (1979) *Phytochemistry* **18**, 843.
2. Quijano, L., Calderón, J. S., Gómez, G. F. and Ríos, T. (1982) *Phytochemistry* **21**, 2041.
3. Quijano, L., Gómez, G. F., Calderón, J. S., López, P. J. and Ríos, T. (1984) *Phytochemistry* **23**, 125.
4. Kanojia, R. M., Wachter, M. P., Levine, S. D., Adams, R. E., Chen, R., Chin, E., Cotter, M. L., Hirsch, A. F., Huettemann, R., Kane, V. V., Ostrowski, P., Shaw, C. J., Mateos, J. L., Noriega, L., Guzman, A., Mijarez, A., Tovar, L. and Shefter, E. (1982) *J. Org. Chem.* **47**, 1310.
5. Seaman, F. C., Malcolm, A. J. and Fischer, N. H. (1984) *Phytochemistry* **23**, 464.