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TWO OXEPANE-TYPE DITERPENE LACTONES FROM *MELAMPODIUM DIFFUSUM*

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Key Word Index—*Melampodium diffusum*; Asteraceae; Heliantheae; oxepane diterpene lactones; melfusanolide derivatives.

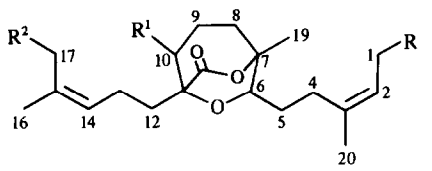
Abstract—The isolation and structure elucidation of two new oxepane diterpene lactones, 1,10,17-trihydroxymelfusanolide and 1,10-dihydroxy-17-acetoxymelfusanolide, from *Melampodium diffusum* are reported. The structure determination involved chemical and spectral methods.

INTRODUCTION

In our previous papers we have described the isolation and structure elucidation of sesquiterpene lactones from *Melampodium diffusum* [1] and *M. longipilum* [2] and more recently the isolation of diterpene lactones from *M. longipilum* [3]. We wish to report our results on the isolation and structural elucidation of two novel oxepane-type diterpene lactones from *M. diffusum* which appear to be derived biogenetically from the the known 17-acetoxy acanthoaustralide type percursors **3** [3].

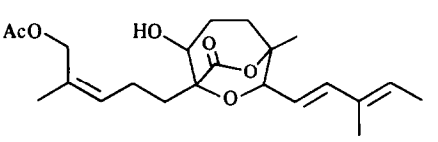
RESULTS AND DISCUSSION

1,10-Dihydroxy-17-acetoxymelfusanolide‡ (**1a**), $C_{22}H_{34}O_7$, was an oil with UV end absorption at 203 nm and IR bands at 3450 and 1730 cm^{-1}, which were assigned to hydroxyl and carbonyl groups, respectively. The signals of the ^1H NMR spectrum (Table 1) closely resembled those of 17-acetoxyacanthoaustralide (**3**) isolated from *M. longipilum* [3]. It exhibited two overlapping vinyl methyl signals appearing as a broad singlet at δ 1.74 and a tertiary methyl signal at δ 1.28 which must be on a carbon bearing an oxygen function. A sharp singlet at δ 2.06 also indicated the presence of an acetoxy group in the melfusanolide **1a**. As in the known lactone **3**, two broadened triplets at δ 5.48 and 5.45 were assigned to the vinyl protons H-2 and H-14. The chemical shift of a two-proton AB-pattern centred at δ 4.6 (J = 12 Hz) suggested that the acetoxy group was attached to C-17. The broad doublet at δ 4.12 (J = 7.0 Hz) was in agreement with a methylene group containing a hydroxyl group (2 H-1). The doublets of doublets at δ 3.69 (H-10, J = 7.5 Hz, J = 5.0 Hz) and 3.52 (H-6, J = 10.0 Hz, J = 3.0 Hz) were assigned to the methine protons (H-10 and H-6) bearing a secondary hydroxyl group and an ether function, respectively. The chemical shifts of H-6 in **1a** and derivatives indicated that the ether oxygen must be attached to C-6 rather than C-7 [3]. The ^{13}C NMR spectrum of **1a** clearly indicated the presence of



	R	R¹	R²
1a	OH	OH	OAc
1b	OH	OH	OH
1c	OAc	OAc	OAc
1d	OTAC	OTAC	OTAC
1e	=O	OH	OAc
1f	=O	=O	OAc

TAC = —CO—NH—CO—CCl_3



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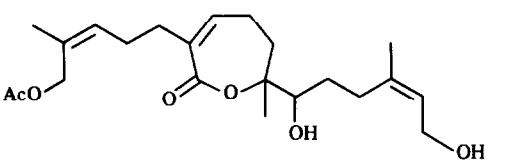

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‡The name melfusanolide has been reserved for the unsubstituted diterpene.

Table 1. ^{1}H NMR data for melfusanolides **1a** and **1b** and derivatives (200 MHz, $CDCl_3$, TMS as int. standard, 27°)

	1a	**1a** (C_6D_6)	**1b**	**1c**	**1d**	**1e**†	**1f**†	**2** (C_6D_6)
H-1	4.12 (*br*) *d* (7)*	4.18 (*br*) *d* (7)	4.22 (*br*) *d* (7)	4.60 (*br*) *d* (7)	4.78 *dd* (8; 2.5)	9.95*d* (8)	10.02 *d* (8)	1.55 (*br*) *d* (7)
H-2	5.48 (*br*) *t* (7)	5.44 (*br*) *t* (7)	4.49 (*br*) *d* (7)	5.43 (*br*) *t* (7)	5.50 (*br*) *t* (7)	5.90 (*br*) *d* (8)	5.92 (*br*) *d* (8)	5.52 *m*
H-4	—	—	—	2.2–2.4	—	—	—	6.31 (*br*) *dd* (16)
H-5	1.5–1.7	1.29 *m*	1.5–1.7	1.45–1.75	1.5–1.7	—	—	5.25 *dd* (16; 6.5)
H-6	3.52 *dd* 10; 3)	3.44 *dd* (6; 6)	3.50 *dd* (10; 3)	3.62 *dd* (7; 5)	3.63 *dd* (8; 4.5)	3.62 *dd* (8; 4)	3.88 (*br*) *t* (6)	3.97 (*br*) *d* (6.5)
H-10	3.69 *dd* (8; 5)	3.60 *dd* (6; 6)	3.68 (8; 5)	4.92 *dd* (7.5; 5)	4.95 *dd* (8; 5.5)	3.74 *dd* (7.5; 5)	—	3.62 *dd* (10; 5)
H-14	5.45 (*br*) *t* (7)	5.41 *t* (*br*) (7)	5.35 (*br*) *t* (7)	5.43 (*br*) *t* (7)	5.40 (*br*) *t* (7)	5.47 (*br*) *t* (7)	5.38 (*br*) *t* (7)	5.45 *m*
H-17a	4.52 *d* (12)	4.60 *d* (12)	4.17 *d* (12)	4.60 (*br*) *s*	4.52*d* (12)	4.51 *d* (12)	4.58 (*br*) *s*	4.54 *d* (12)
H-17b	4.68 *d* (12)	4.76 *d* (12)	4.25 *d* (12)		4.63 *d* (12)	4.72 *d* (12)		4.76 *d* (12)
H-16	1.75 (*br*) *s*	1.73 *s* (*br*)	1.78 *(br) s*	1.75 (*br*) *s*	1.73 *d* (~1)	1.76 (*br*) *s*	1.73 (*br*) *s*	1.57 (*br*) *s*
H-19	1.30 *s*	0.98 *s*	1.30 *s*	1.35 *s*	1.37 *s*	1.33 *s*	1.40 *s*	1.00 *s*
H-20	1.75 (*br*) *s*	1.65 (*br*) *s*	1.74 (*br*) *s*	1.80 (*br*) *s*	1.82 *d* (~1)	2.02 *d* (~1)	2.24 (*br*) *s*	1.69 (*br*) *s*
AcO	2.06 *s*	—	—	2.04, 2.06, 2.07 *s*	2.06 *s*	*2.06 s*	2.06	1.70 *s*

* Figures in parentheses are coupling constants or line separations in Hz.
† Obtained at 100 Hz.

two carbonyl groups in the molecule and the remaining signals (Experimental) were in good agreement with structure **1a**. Acetylation of **1a** confirmed the presence of primary and secondary hydroxyls at C-1 and C-10, since both the H-1 and H-10 signals underwent the typical downfield ^{1}H NMR shifts (compare data of **1a**, **1b** and **1c** in Table 1). *In situ* acylation with trichloroacetyl isocyanate (TAI) gave **1d** which also supported the above findings. Oxidation of **1a** with manganese dioxide confirmed the presence of the primary allylic hydroxyl group, giving the corresponding α,β-unsaturated aldehyde **1e** with a diagnostic aldehyde signal at δ 9.95 (*d*, J = 8.0 Hz). The chemical shift of the C-3 methyl group at δ 2.02 in **1e** indicated that the sterochemistry at Δ^2 must be *Z* as in the acanthoaustralide derivatives isolated from *M. longipilum* [3]. Aldehyde **1d** slowly isomerized at room temperature giving a mixture of *Z*- and *E*-isomers, as indicated by the ^{1}H NMR spectrum which showed new signals at δ 2.2 (C-3-Me) and 10.0 (H-1) assigned to the *E*-isomer; this was in full agreement with the analogous proton signals in the ^{1}H NMR spectrum of citral [4]. Furthermore, oxidation of **1a** with PCC gave the ketoaldehyde derivative **1f**. The ^{1}H NMR spectrum of **1f** indicated isomerization of the Δ^2 double bond during oxidation since the C-3 methyl group signal appeared at δ 2.24 caused by the deshielding effect of the carbonyl group upon the C-3-Me. The mass spectrum of **1e** showed a molecular ion at *m/z* 408 and prominent peaks at *m/z* 348 $[M-AcOH]^+$ and 330 $[M-AcOH-H_2O]^+$.

Dehydration of **1a** with *p*-toluenesulfonic acid gave the diene **2** (UV, λ_{max} = 220 nm, $[M]^+$ = 392). The ^{1}H NMR of **2** clearly indicated the presence of an extra vinyl methyl doublet at δ 1.55 (J = 7.0 Hz). A *trans*-double bond was suggested by signals at δ 6.31 appearing as a broad doublet (H-4, J = 16 Hz) and doublet of doublets at δ 5.25 (J = 16.0 Hz, J = 6.5 Hz) due to H-5. Since in **2** the olefinic signal due to H-5 was vicinally coupled to H-6, the sequence of carbons from C-1 to C-6 in **2** and therefore in **1a** was established.

All the chemical and spectral data of the new melfusanolide are in good agreement with a skeletal arrangement as given in structure **1a** except the stereochemical centers C-6, C-7, C-10 and C-11, which could not be established from the ^{1}H NMR data.

A second oxepane diterpene lactone, 1,10,17-trihydroxymelfusanolide (**1b**), was isolated from the more polar chromatography fractions. As in **1a**, the IR showed absorption bands typical for hydroxyl and carbonyl groups. The exhibited molecular ion at *m/z* 368 and further peaks at *m/z* 350, 332 and 314 due to the successive losses of one, two and three molecules of water, suggested the presence of three hydroxyl groups in the molecule. The ^{1}H NMR clearly indicated that 1,10,17-trihydroxymelfusanolide (**1b**) corresponds to 17-desacetyl-1,10-dihydroxymelfusanolide, since it lacked the methyl acetate signal and the H-17 signal was shifted upfield. Alkaline hydrolysis of the melfusanolide **1a** confirmed the above assumption, since the saponification product of **1a** was identical with the melfusanolide **1b**.

The stereochemistry at the Δ^{14}-double bond in **1a** and **1b** was assigned a *Z*-configuration based on the chemical shift of the C-15 methyl group which was nearly identical with the analogous parameters of the acanthoaustralide derivative **3** [3], which had been established by ^{1}H NMR spectral comparisons with model compounds [5].

*Tentative assignments based on chemical shifts only.

EXPERIMENTAL

Melampodium diffusum Cass. was collected on 1 Sept. 1976, in Mexico: Guerrero, road to Ayatla, *ca* 1 mile south of Tierra Colorada (Hartman & Funk No. 4211, voucher deposited at O.S., U.S.A.). Aerial parts (280 g) were extracted with $CHCl_3$ and worked up under standard conditions [6]. The crude terpenoid syrup (3.37 g) was chromatographed over 70 g silica gel as previously described [1]. From fraction 9, which was eluted with $CHCl_3$–Me_2CO (1:4) and TLC purification (Et_2O, × 7), 50 mg of **1b** were obtained as a gum. Fraction 7 provided 200 mg of **1a**.

1,10,17-*Trihydroxymelfusanolide* (**1b**). $C_{20}H_{32}O_6$, gum: IR ν_{max}^{film} cm^{-1}: 3400 (OH), 1730 (C=O), 1650 (double bond); UV λ_{max}^{MeOH} nm: end absorption at 203 (ε 7924); EIMS (probe) *m/z* (rel. int.): 368 $[M]^+$ (0.9), 350 $[M-H_2O]^+$ (8.8), 332 $[M-2H_2O]^+$ (2.9), 314 $[M-3H_2O]^+$ (1.3), 138 (18.0), 121 (19.6), 109 (35.1), 93 (62.3), 81 (100.0), 69 (54.2), 55 (37.1).

1,10-*Dihydroxy*-17-*acetoxymelfusanolide* (**1a**). $C_{22}H_{34}O_7$, gum; IR ν_{max}^{film} cm^{-1}: 3450 (OH), 1730 (C=O), 1240; UV λ^{MeOH} nm: 203 (ε 9851); EIMS (probe) *m/z* (rel. int.): 350 $[M-AcOH]^+$ (30.0), 332 $[M-AcOH-H_2O]^+$ (16.5), 314 $[M-AcOH-2H_2O]^+$ (3.8), 138 (31.4) 121 (23.0), 109 (44.6), 93 (77.2), 81 (100.0), 69 (37.2), 55 (27.3), 43 (28.5); CIMS (isobutane): 411.10 $[MH]^+$ (2.6), 393.18 $[MH-H_2O]^+$ (39.5), 367.04 $[MH-CO_2]^+$ (15.4), 351.08 $[MH-AcOH]^+$ (78.0), 349.10 $[MH-CO_2-H_2O]^+$ (27.5), 333.16 $[MH-AcOH-H_2O]^+$ (100.0), 315.12 $[MH-AcOH-2H_2O]^+$ (4.8); ^{13}C NMR ($CDCl_3$)*: δ 171.3 and 170.1 (*s*, C=O groups), 137.9 (*s*, C-15), 130.1 (*d*, C-2), 129.6 (*s*, C-3), 125.1 (*d*, C-14), 83.6 and 82.6 (*s*, C-7 and C-11), 72.9 (*d*, C-10), 72.9 (*d*, C-6), 63.0 (*t*, C-17), 58.3 (*t*, C-1), triplets at 34.2, 29.3, 28.7, 28.5 and 27.7 (–CH_2 groups), quartets at 24.7, 22.9, 21.1 and 20.6 (Me groups).

Acetate **1c**. A mixture of 30 mg **1b**, 0.4 ml Ac_2O and 0.1 ml pyridine were allowed to react for 4 hr at 25°. Usual work-up and TLC purification (Et_2O–petrol, 1:1, × 3) gave 20 mg **1c**, $C_{26}H_{38}O_9$, gum; IR ν_{max}^{film} cm^{-1}: 1740, 1230; EIMS (probe) *m/z* (rel. int.): 434 $[M-AcOH]^+$ (7.2) 392 $[M-AcOH-C_2H_2O]^+$ (24.7), 374 $[M-2AcOH]^+$ (5.9), 332 $[M-2AcOH-C_2H_2O]^+$ (45.3), 314 $[M-3AcOH]^+$ (10.1), 138 (18.2), 151 (30.9), 121 (23.5), 109 (37.0), 93 (55.5), 81 (100.0), 69 (36.2), 55 (26.6), 43 (87.1).

Aldehyde **1e**. A soln of 50 mg **1a** in $CHCl_3$ and 150 mg of MnO_2 was stirred for 3 hr. Filtration and evaporation of the solvent provided 30 mg **1e**. $C_{22}H_{32}O_7$, gum; EIMS (probe) *m/z* (rel. int.): 408 $[M]^+$ (1.5), 366 $[M-42]^+$ (3.6), 364 $[M-44]^+$, 348 $[M-AcOH]^+$ (75.1), 330 $[M-AcOH-H_2O]^+$ (2.7), 138 (35.9), 121 (28.5), 109 (67.2), 93 (79.2), 81 (100.0), 69 (43.3), 55 (28.9), 43 (50.4).

Saponification of **1a**. To a soln of **1a** (35 mg) in 5 ml EtOH, five drops of a 50% aq. soln of KOH were added and allowed to react for 72 hr at 20°. Usual work-up provided a gum (5 mg) which was shown to be identical with **1b** by ^{1}H NMR and EIMS.

Dehydration of **1a** *with TsOH*. A soln of **1a** (30 mg) and 30 mg TsOH in dry C_6H_6 was refluxed for 3 hr, the reaction being monitored by TLC. UV λ_{max}^{MeOH} nm: 220 (ε 7260); IR ν_{max}^{film} cm^{-1}: 3450, 1735; EIMS (probe) *m/z* (rel. int.): 392 $[M]^+$ (4.5), 332 $[M-AcOH]^+$ (5.5), 121 (20.0), 109 (26.4), 93 (19.1) 91 (25.5), 81 (34.5), 69 (22.7), 67 (26.4), 55 (34.5), 43 (100.0).

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