Phytochemistry, Vol. 23, No. 4, pp. 829–831, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

SESQUITERPENE AND DITERPENE LACTONES FROM *MELAMPODIUM LONGIPILUM*

LEOVIGILDO QUIJANO* and NIKOLAUS H. FISCHER†

Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, U.S.A.

(*Revised received* 12 *August* 1983)

Key Word Index—*Melampodium longipilum*; Asteraceae; Heliantheae; diterpene lactones; 17-hydroxy- and 17-acetoxyacanthoaustralide; sesquiterpene lactones; repandin E; melampolides; repandolide.

Abstract—The aerial parts of *Melampodium longipilum* afforded two new diterpene lactones, 17-hydroxy- and 17-acetoxyacanthoaustralide, and a new sesquiterpene lactone, repandin E, besides the known melampolides enhydrin and fluctuanin.

INTRODUCTION

The genera *Melampodium, Enhydra, Polymnia, Tetragonotheca* and *Acanthospermum* contain as characteristic constituents sesquiterpene lactones, mainly of the melampolide type [1]. Recent investigations of *Acanthospermum australe* [2] resulted in the isolation of novel diterpene lactones, which are most likely derived from geranylgeraniol by oxidative biomodifications. We describe in this paper the isolation and structure determination of the first two new members of this type of diterpene lactones from *Melampodium* species. Besides the two new diterpene lactones, 17-acetoxyacanthoaustralide (**1b**) and 17-hydroxyacanthoaustralide (**1a**), a new sesquiterpene lactone (**3**) of the repandolide type [3] was also isolated from *M. longipilum*. In addition, the known melampolides enhydrin (**4a**) [4] and fluctuanin (**4b**) [5] were obtained from less polar fractions. Their identity was established by ^{1}H NMR spectral comparison with authentic samples. The co-occurrence of compound **3** with the two melampolide-4,5-epoxides is a further indication that the latter compounds represent biogenetic precursors of the repandolides, as we have previously suggested [3].

RESULTS AND DISCUSSION

17-Hydroxyacanthoaustralide (**1a**), $C_{20}H_{32}O_5$, was isolated as a gum from the more polar chromatography fractions. The IR spectrum indicated the presence of hydroxyl (3450 cm^{-1}) and carbonyl groups (1730 cm^{-1}). The ^{1}H NMR spectrum of **1a** (Table 1) clearly indicated the presence of two vinyl methyl groups appearing as broad singlets at δ1.72 and 1.77. A three-proton singlet at δ1.34 suggested a tertiary methyl group on a carbon bearing an oxygen function. A doublet of doublets at δ3.45 (J = 11.0, 2.0 Hz), was assigned to a proton on a carbon bearing a secondary hydroxyl group. Two doublets of doublets centred at δ4.18 and 4.00, which are the AB part of an ABX pattern, and an overlapped singlet at δ4.07 are due to the methylene protons of primary hydroxyl groups, since these signals shifted downfield upon acetylation. Three broad triplets at δ5.23 (J = 6.5 Hz), 5.54 (J = 7.0 Hz) and 6.35 (J = 5.0 Hz) indicated the presence of three vinyl protons, the latter possibly being β to a carbonyl group (IR band at 1700 cm^{-1}). All these ^{1}H NMR spectral features closely resembled those of acanthoaustralide (**1g**), a diterpene lactone recently isolated from *A. australe* [2]. The spectral data strongly suggested that **1g** and the new compound (**1a**) differ by the presence of a hydroxyl function either at C-16 or C-17 in **1a**. This was verified by the following chemical transformations.

Acetylation of **1a** afforded a mixture of the tricetate **1c** and the diacetate **1d** which were separated by prep. TLC. The ^{1}H NMR spectra of **1c** and **1d** showed minor but distinct differences. While the spectrum of **1c** clearly indicated the presence of three sharp acetate peaks and a downfield shift of the H-1, H-6 and H-16 (or H-17) signals, the spectrum of **1d** only exhibited two acetate signals and the downfield shift of the H-1 and H-16 (or H-17) signals. The presence of a secondary hydroxyl group at C-6 in **1d** was indicated by a nearly unchanged doublet of doublets at δ 3.49 as well as the IR band at 3460 cm^{-1}.

From the less polar fractions, another diterpene lactone, 17-acetoxyacanthoaustralide (**1b**) was isolated. The IR and ^{1}H NMR spectra of **1b** were similar to those of **1a**, indicating that the difference resided in the presence of an additional acetyl group in **1b** (IR band at 1730 cm^{-1}) instead of a hydroxyl group in **1a**. The acetate must be at C-17 since in the ^{1}H NMR spectrum of **1b** the C-17 two-proton methylene singlet was shifted downfield to δ4.58. This was in accord with the finding that acetylation of **1b** gave the triacetate **1c** which was identical with that obtained from **1a**.

The stereochemistry at Δ^2 was assigned to have a Z-configuration based on the chemical shift of the C-3 methyl group (H-20) in **1a** and **1b**, which were in good agreement with the chemical shift of the C-3 methyl group of the Δ^2 (Z)-farnesols rather than the Δ^2 (E)-farnesols [6]. Oxidation of **1b** with manganese dioxide confirmed that the 2,3-double bond must have the Z-configuration, since the C-3 methyl group signal appeared at δ1.99 in the

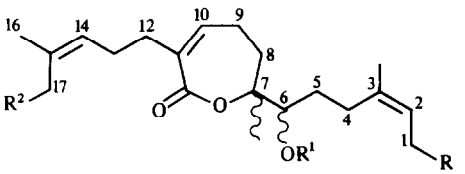
*Permanent address: Instituto de Quimica, UNAM, Mexico D.F., Mexico.

†To whom correspondence should be addressed.

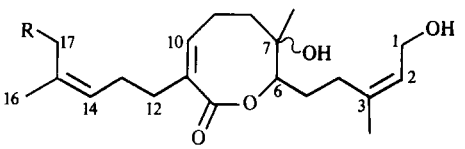
Table 1. ^{1}H NMR data for the diterpene lactones and derivatives (200 MHz, $CDCl_3$, TMS as int. standard, 27°)

Signals	1a	1b	1c	1d	2a	2b
H-1	4.0 *dd* (12, 7)*	4.01 *dd* (12, 7)	4.52 *br d* (7)	4.62 *br d* (7)	4.04 *br d* (7, 5)	4.05 (obs)
H-1′	4.18 *dd* (12, 8)	4.20 *dd* (12, 8)	—	—	—	—
H-2	5.54 *br t* (7)	5.57 *br t* (7)	5.36 *br t* (7)	5.37 *br t* (7)	5.45 *br t* (7)	5.42 *br t* (7)
H-6	3.45 *dd* (11, 2)	3.46 *dd* (11, 2)	4.97 *dd* (11, 2)	3.49 *dd* (11, 2)	4.49 *dd* (9.5, 2.5)	4.5 *dd* (10, 2)
H-10	6.35 *br t* (5)	6.33 *br t* (5)	6.30 *br t* (5)	6.32 *br t* (5)	5.99 *br t* (4.5)	5.99 *br t* (4.5)
H-14	5.23 *br t* (6, 5)	5.37 *br t* (6, 5)	5.36 *br t* (9)	5.37 *br t* (7)	5.37 *br t* (6.5)	5.23 *br t* (6.5)
H-17	4.07 *br s*	4.58 *br s*	4.57 *br s*	4.57 *br s*	4.55 *br s*	4.05 *br s*
C-15-Me	1.77 *br s*	1.73 *br s*	1.73 *br s*	1.73 *br s*	1.73	1.73 *br s*
C-7-Me	1.34 *s*	1.34 *s*	1.35 *s*	1.35 *s*	1.26 *s*	1.24 *s*
C-3-Me	1.72 *br s*	1.73 *br s*	1.77 *br s*	1.76 *br s*	1.73	1.77
AcO	—	2.07 *s*	2.13, 2.07, 2.05 *s*	2.05, 2.06 *s*	2.07	—

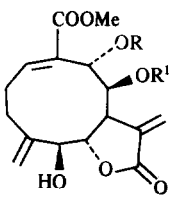
*Figures in parentheses are coupling constants or line separations in Hz.



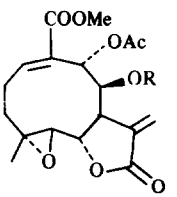
	R	R^1	R^2
1a	OH	H	OH
1b	OH	H	OAc
1c	OAc	Ac	OAc
1d	OAc	H	OAc
1e	=O	H	OAc
1f	OH	H	=O
1g	OH	H	H
1h	=O	H	OH



	R
2a	OH
2b	OAc



3 R = Ac, R^1 = angelate, or vice versa



4a R = angelate epoxide
4b R = angelate

^{1}H NMR spectrum of the aldehyde **1e** as in that of the *Z*-isomer in the spectrum of a citral mixture [7]. Although the ^{1}H NMR parameters for H-1, H-2 and the C-3-Me of compounds **1a** and **1b** were very similar to the chemical shifts of the reported acanthoaustralides [2], our assignments based on chemical shift data of aldehyde **1e** are opposite to the configurational assignments reported in the literature [2].

The Δ^{14}-double bond configuration was assigned to be *Z* on the basis of the chemical shift of the aldehydic proton in the ^{1}H NMR spectrum of the aldehyde **1f** obtained from **1a** by oxidation with manganese dioxide, the chemical shift δ10.08 being in agreement with a *cis*-aldehydic proton [8]. The stereochemistry at C-6 and C-7 remains open.

The ^{1}H NMR spectra of **1a** and **1b** indicated the presence of small amounts of another compound which must represent the isomeric eight-membered ring lactones 17-hydroxyisoacanthoaustralide (**2a**) and 17-acetoxy isoacanthoaustralide (**2b**). The assignments were based on the comparison of the ^{1}H NMR parameters with their analogues described in the literature [2]. Separation of compounds **1a** and **2a** by semi-preparative reverse phase HPLC on a 30 cm C-18 bondapak column (H_2O–MeOH, 1:1) gave baseline separations of the two lactones. However, after removal of the solvent mixture *in vacuo*, both fractions provided the same ratio of compounds **1a** and **2a**, as indicated by 200 MHz ^{1}H NMR. This strongly suggests that compounds **1a** and **2a** interconvert under the given conditions, most likely via intramolecular acyltransfer.

The structure of repandin E (**3**), $C_{23}H_{28}O_9$, mp 172–174°, was deduced mainly from the ^{1}H NMR and MS spectral data by comparison with the spectral parameters of the known repandins A–D [3]. The mass spectrum of repandin E showed the molecular ion at *m/z* 448. Further peaks at 388 $[M-60]^+$, 349 $[M-99]^+$, 83 $[C_5H_7O]^+$ and 55 $[C_4H_7]^+$ and diagnostic ^{1}H NMR signals indicated the presence of acetyl and angelyl ester side chains. The ^{1}H NMR spectrum also suggested that the two ester groups must be attached to C-8 and C-9, since the chemical shifts and couplings of the doublet of doublets at δ 6.21 (J = 10.3 Hz) and the doublet at 6.06 (J = 10 Hz), assigned to H-8 and H-9, respectively, were nearly identical with those of the known repandins [3]. These above ^{1}H NMR assignments and those of all other

protons were confirmed by spin-spin decoupling experiments of **3** (see Experimental). On the basis of the great similarity of the ^{1}H NMR and CD parameters of repandin E with the repandins A–D [3], it appears that the new compound has the configurations and conformation of the medium ring skeleton as previously established for the other repandolides [3]. The attachments of the two ester groups as shown in structure **3** with the acetate at C-9 and the angelate group at C-8 is solely based on the co-occurence of the known melampolide fluctuanin (**4b**), which appears to represent the biogenetic precursor for **3** [3]. Therefore, the sites of attachments of the two ester groups in **3** remain tentative.

EXPERIMENTAL

Melampodium longipilum Robins. was collected on 1 Sept. 1976 in Mexico: Puebla *ca* 30 miles north-west of Huajuapan de Leon on Highway 190. (Hartman & Funk No. 4152; voucher deposited at O.S., U.S.A.) Leaves and stems (49.0 g) were extracted with $CHCl_3$. Standard work-up [9] provided 2.0 g of crude syrup which was chromatographed on silica gel (80 g) using petrol–$CHCl_3$ (1:1), $CHCl_3$ and mixtures of $CHCl_3$–Me_2CO (5, 10, 20, 50%) as eluant; 100 ml fractions were taken and monitored by TLC. Earlier fractions eluted with petrol–$CHCl_3$ provided the sesquiterpene lactones enhydrin (**4a**) [4] and fluctuanin (**4b**) (longipin acetate) [5]. Their identity was established by ^{1}H NMR spectral comparison with authentic samples. Diterpene lactones **1a** (200 mg) and **1b** (160 mg) were obtained as gums from fractions eluted with $CHCl_3$–Me_2CO (19:1) and $CHCl_3$–Me_2CO (1:1).

17-*Acetoxyacanthoaustralide* (**1b**). $C_{22}H_{34}O_6$, gum; UV λ_{max}^{MeOH} nm(ε): 202 (12 944), 220sh (7643); IR ν_{max}^{film} cm^{-1}: 3450, 1730, 1680, 1230; EIMS (probe) *m/z* (rel. int.): 350 $[M-44]^+$ (0.6), 349 $[M-45]^+$ (1.8), 348 $[M-46]^+$ (1.6), 334 $[M-AcOH]^+$ (1.5), 316 $[M-AcOH-H_2O]^+$ (1.2), 265 $[M-C_7H_{13}O_2]^+$ (2.2), 205 $[265-AcOH]^+$ (43.9), 167 (15.0), 151 (56.9), 149 (26.1), 147 (53.4), 133 (38.1), 121 (35.7), 119 (40.7), 93 (53.6), 91 (42.0), 83 (66.3), 81 (62.1), 43 (100.0).

17-*Hydroxyacanthoaustralide* (**1a**). $C_{20}H_{32}O_5$, gum; UV λ_{max}^{MeOH} nm(ε): 203 (11 776), 220 sh (4972); IR ν_{max}^{film} cm^{-1}: 3450, 1690, 1620; EIMS (probe) *m/z* (rel. int.): 362 $[M]^+$ (not present), 279 (10.0), 205 (11.4), 167 (31.3), 151 (28.1), 149 (100.0), 133 (20.7), 121 (24.1), 119 (23.6), 109 (25.5), 107 (21.9), 105 (26.1), 95 (27.8), 93 (35.3), 91 (24.4), 83 (31.3), 81 (45.3), 79 (33.1), 71 (31.6), 69 (29.4), 67 (28.1), 57 (26.1), 55 (37.6), 53 (17.0), 43 (67.6), 41 (32.4).

Triacetate (**1c**) *and diacetate* (**1d**). Acetylation of **1b** (50 mg) with Ac_2O (0.5 ml) and pyridine (0.2 ml) under standard work-up conditions gave after prep. TLC (Et_2O–petrol, 1:1, × 4) 14 mg of **1c** from the less polar band and 11 mg **1d**.

Triacetate (**1c**). $C_{26}H_{38}O_8$, gum; UV λ_{max}^{MeOH} nm(ε): 203 (9924), 220 (5873); IR ν_{max}^{film} cm^{-1}: 1740 (esters), 1695 (conj. double bond), 1235 (acetate); EIMS (probe) *m/z* (rel. int.): 418 $[M-AcOH]^+$ (2.1), 358 $[M-2AcOH]^+$ (1.6), 298 $[M-3AcOH]^+$ (2.9), 279 (10.8), 205 (20.3), 164 (21.0), 167 (29.4), 151 (16.9), 149 (100.0), 133 (23.7), 121 (19.5), 119 (28.5), 109 (10.9), 107 (15.8), 105 (25.0), 95 (17.7), 93 (35.0), 91 (21.4), 83 (12.5), 81 (30.4), 79 (22.1), 71 (16.9), 69 (14.2), 67 (18.4), 57 (14.7), 55 (16.3), 43 (81.4).

Diacetate (**1d**). $C_{24}H_{36}O_7$, gum; UV λ_{max}^{MeOH} nm(ε): 202 (8909), 220 sh (4739); IR ν_{max}^{film} cm^{-1}: 3460 (OH), 1740 (esters), 1700 sh, 1240 (acetate); EIMS (probe) *m/z* (rel. int.): 376 $[M-AcOH]^+$ (1.4), 316 $[M-2AcOH]^+$ (2.8), 298 $[M-2AcOH-H_2O]^+$ (1.9), 205 (49.4), 164 (24.4), 151 (60.8), 149 (19.3), 147 (45.9), 133 (29.1), 121 (27.0), 119 (34.2), 109 (24.4), 107 (22.7), 105 (31.7), 95 (31.4), 93 (50.6), 91 (33.1), 83 (21.4), 81 (50.0), 79 (33.8), 71 (9.5), 69 (22.9), 67 (32.8), 55 (24.7), 43 (100.0).

Repandin E (**3**). $C_{23}H_{28}O_9$, was obtained from fractions eluted with $CHCl_3$–Me_2CO (19:1), mp 172–174° ($CHCl_3$–Et_2O); UV λ_{max}^{MeOH} nm(ε): 216 (17309); CD (*c* 4.7 × 10^{-5}, MeOH): $[\theta]_{214}$ − 141 072, $[\theta]_{250}$ + 7 625; IR ν_{max}^{film} cm^{-1}: 3550, 1770, 1740; EIMS (probe) *m/z* (rel. int.): 448 $[M]^+$ (0.2), 388 $[M-AcOH]^+$ (3.9), 349 $[M-AngO]^+$ (4.6), 348 $[M-AngOH]^+$ (1.4), 288 $[M-AcOH-AngOH]^+$ (1.8), 83 $[C_5H_7O]^+$ (100.0), 55 $[C_4H_7]^+$ (26.1); ^{1}H NMR ($CDCl_3$): δ6.75 (*dd*, *J* = 2 Hz, H-13b), 6.21 (*dd*, *J* = 10, 3 Hz, H-8), 6.06 (*d*, *J* = 10 Hz, H-9) 6.05 (*qq*, *J* = 7, 1.5 Hz, H-3'), 5.87 (*d*, *J* = 2 Hz, H-13a), 4.99, 5.01 (*br s*, H-15a, H-15b), 4.83 (*br d*, *J* = 9.5 Hz, H-6), 3.92 (*d*, *J* = 9.5, H-5), 3.94 (*s*, CO_2Me), 3.01 (*m*, H-7), 1.95 (*s*, OAc), 1.92 (*dq*, *J* = 7, 1.5 Hz, H-4'), 1.80 (*quint*, *J* = 1.5, H-5').

Aldehyde **1e**. A 20 mg sample of **1b** in 3 ml of CH_2Cl_2 was oxidized with MnO_2 the reaction being monitored by TLC. Usual work-up gave the gummy aldehyde **1e** (14 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1735, 1680; EIMS (probe) *m/z* (rel. int.): 392 $[M]^+$ (8.1), 332 $[M-AcOH]^+$ (30.0), 314 $[M-AcOH-H_2O]^+$ (4.6), 265 $[M-C_7H_{12}O_2]^+$ (8.7), 205 $[265-AcOH]^+$ (47.1) 167 (100.0), 149 (29.9), 147 (28.1), 133 (30.8), 121 (28.8), 119 (19.7), 109 (78.8), 105 (25.3), 95 (30.9), 93 (35.0), 91 (21.7), 83 (26.9), 81 (93.0), 79 (27.2), 71 (18.7), 69 (62.0), 67 (29.7), 55 (23.3), 43 (69.7); ^{1}H NMR ($CDCl_3$): δ9.95 (*s*, H-1), 6.34 (*br t*, *J* = 4.5 Hz, H-10) 5.92 (*br d*, *J* = 7.0 Hz, H-2), 5.37 (*br t*, *J* = 6.5 Hz, H-14) 4.57 (*br s*, H-17), 3.53 (*dd*, *J* = 11.0 Hz, *J* = 2.0 Hz, H-6) 2.07 (*s*, OAc), 1.99 (*d*, *J* = 1.0 Hz, H-20), 1.74 (*br s*, H-16), 1.35 (*s*, H-19).

Aldehyde **1f**. MnO_2 oxidation of 18 mg of **1a** gave after prep. TLC, the aldehyde **1f** (8 mg) and a small amount of the C-1 monoaldehyde derivative (**1h**) which could not be separated due to the lack of material. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1680; ^{1}H NMR ($CDCl_3$): δ10.08 (*s*, H-17), 6.48 (*br t*, *J* = 7.0 Hz, H-14), 6.36 (*br t*, *J* = 5.0 Hz, H-10), 5.58 (*br t*, *J* = 7.0 Hz, H-2), 4.22 (*dd*, *J* = 12.0 Hz; *J* = 8.0 Hz, H-1'), 4.02 (*dd*, *J* = 12.0 Hz; *J* = 7.0 Hz, H-1), 3.47 (*dd*, *J* = 11.0 Hz, *J* = 2.0 Hz, H-6), 1.78 (*br s*, H-16), 1.74 (*br s*, H-20), 1.34 (*s*, H-19).

Acknowledgements—The authors wish to thank Drs. R. Hartman and V. Funk for plant collections and Helga D. Fischer for technical assistance. This research was supported, in part, by USDA-SEA cooperative agreement No. 58-7B30-2-399.

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