Sesquiterpene Lactones from *Anthemis melanolepis*

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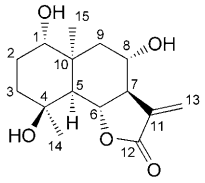
From the aerial parts of *A. melanolepis*, two new sesquiterpene lactones, melanolepin B (=1α,4β,8α-trihydroxyeudesm-11(13)-en-12,6α-olide; **1**) and melanolepin C (=1α,10β-epoxy-8β-hydroxygermacra-4(15),11(13)-dien-12,6α-olide; **2**), were isolated, together with four known compounds, desacetyllaurenobiolide, dentatin A, taraxasterol, and (3*S*,5*R*)-loliolide. Compound **1** was found to have the rare *cis*-fused eudesmane skeleton. The structures of the isolated compounds were established by means of NMR (^{1}H, ^{1}H-COSY, ^{1}H,^{13}C-HSQC, HMBC, NOESY) and MS analyses.

Introduction. – The genus *Anthemis* (Anthemideae-Asteraceae) is widely distributed in Europe, especially around the Mediterranean, in West, Southwest, and Central Asia, as well as in North Africa. The genus comprises *ca.* 150 species, several of which are aromatic, herbal medicines, insecticides, and dyes. Some 35 species are distributed in Greece, from which 14 are endemic [1–3]. *Anthemis melanolepis* BOISS. (Asteraceae) is a procumbent, subglabrous annual species growing on the Greek islands Rhodes and Crete, as well as in Syria, Lebanon, Palestine, and Cyprus [4]. Characteristic constituents of *Anthemis* species, many of which are used in folk medicine, are elemanolides, eudesmanolides, germacranolides, and guaianolides [2][3].

In continuation of our research on Greek *Anthemis* species [5–7], we herein report the isolation and identification of two new sesquiterpene lactones, melanolepins B (**1**) and C (**2**), together with the known compounds desacetyllaurenobiolide [8], dentatin A [9], (3*S*,5*R*)-loliolide [10], and taraxasterol [11], from the lipophilic extract of the aerial parts of *A. melanolepis*.

Results and Discussion. – Compound **1** showed in its ESI mass spectrum the $[M-H]^-$ peak at *m/z* 281 and the $[M+AcO]^-$ signal at *m/z* 341, in accord with the molecular formula $C_{15}H_{22}O_5$. The IR spectrum showed absorption bands typical of OH (3357 cm^{-1}) and a γ-lactone C=O group (1753 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra (*Table*) of **1** showed signals typical of an eudesmane skeleton [12]. The ^{13}C-NMR spectrum displayed the signals of 15 C-atoms, which were fully assigned by HSQC, HMBC, and DEPT-135° experiments to the resonances of four quaternary C-atoms,



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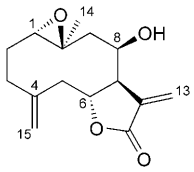

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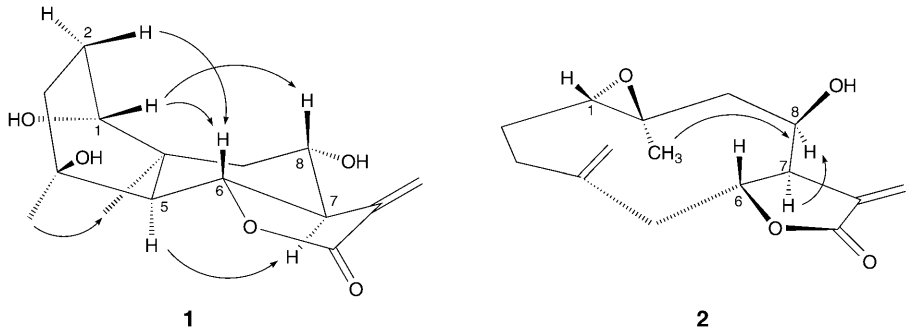
five CH, four CH_2 (one of them olefinic), and two Me groups. The presence of an α-methylidene-γ-lactone moiety was confirmed by the ^{13}C-NMR signals at δ(C) 168.2 (O–C=O), and 140.1 and 129.8 (C=CH_2). COSY Experiments enabled us to assign H–C(7) to the signal at δ(H) 2.31, with allylic couplings to H_a–C(13) [δ(H) 6.32 (*d*, J = 2.2 Hz)] and H_b–C(13) [δ(H) 5.72 (*d*, J = 2.9 Hz)]. Moreover, the coupling constant between H–C(7) (*t*, J = 10.3 Hz) and H–C(6) (*dd*, J = 11.4, 10.3 Hz) indicated a *trans* attachment of the γ-lactone unit to a *cis*-decalin ring system [13].

Table *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**. In CD_3OD at 295 K; δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	3.74 (*dd*, J = 12.4, 5.1)	69.7	2.73 (br. *d*, J = 12.4)	58.2
2	1.93 (*dd*, J = 14.1, 3.3)	29.9	2.20 – 2.15 (*m*)	28.2
	1.70 – 1.66 (*m*)	–	1.73 – 1.70 (*m*)	–
3	1.84 – 1.76 (*m*)	36.9	2.16 – 2.11 (*m*)	36.3
	1.42 (*dd*, J = 12.4, 12.0)	–	1.66 – 1.60 (*m*)	–
4	–	73.8	–	
5	1.51 (*d*, J = 11.5)	59.9	3.06 – 2.99 (*m*)	59.4
	–	–	2.47 (*dd*, J = 13.7, 11.6)	–
6	4.34 (*dd*, J = 11.4, 10.3)	72.2	4.26 (*ddd*, J = 11.5, 10.0, 3.2)	78.8
7	2.31 (*t*, J = 10.3)	60.1	3.03 (*ddd*, J = 10.0, 6.8, 3.3)	57.3
8	4.05 (*dt*, J = 11.3, 4.0)	65.7	4.14 (*dd*, J = 7.0, 3.6)	71.1
9	2.25 (*dd*, J = 12.2, 3.7)	46.2	2.78 (*dd*, J = 9.5, 2.9)	43.8
	1.13 (*t*, J = 12.0)	–	1.63 – 1.57 (*m*)	–
10	–	40.5	–	41.2
11	–	140.1	–	139.2
12	–	168.2	–	170.2
13	6.32 (*d*, J = 2.2)	129.8	6.14 (*d*, J = 3.3)	121.2
	5.72 (*d*, J = 2.9)	–	5.95 (*d*, J = 3.3)	–
14	1.08 (*s*)	24.9	1.48 (*s*)	20.4
15	1.48 (*s*)	32.2	4.98 (br. *s*)	109.2
	–	–	4.91 (br. *s*)	–

Further analysis of the ^{1}H- and ^{13}C-NMR spectra of **1**, as well as a NOESY experiment (*Figure*), revealed the presence of an unusual *cis*-eudesmane ring. NOE Signals between H–C(8)/H–C(6) indicated that they had the same relative orientation (β), while NOE interactions between H–C(5)/H–C(7), and H–C(5)/Me(14) indicated them to be orientated on the other face (α) of the molecule. In particular, the presence

of an NOE cross-peak between H–C(5) and Me(14) suggested *cis*-fused rings *A* and *B*, forming the eudesmane skeleton. The NOE cross-peaks between Me(14) and H–C(5), H_a–C(9), and H_b–C(9) indicated an equatorial orientation of Me(14), which, on the other hand, did not interact with H–C(1). Thus, H–C(1) [δ(H) 3.74 (*dd*, J = 5.1, 12.4 Hz)] was placed in axial position at C(10), and, therefore, is spatially close to H–C(6) and H–C(8), in accord with the observed strong NOEs (*Figure*). As a result of the *cis* configuration of the eudesmane nucleus, H_a–C(2) showed a weak interaction with H–C(6). Particularly notable was the low-field shift of Me(14) (δ(C) 24.9) in the ^{13}C-NMR spectrum, which, in *trans*-eudesmanolides [5][14–16], appears at higher field. From these data, the structure of melanolepin B (**1**) was determined as 1α,4β,8α-trihydroxyeudesm-11(13)-en-12,6α-olide.



1 **2**

Figure. *Key NOE correlations of* **1** *and* **2**

Compound **2** showed the $[M-H]^-$ peak at *m/z* 263 in its ESI mass spectrum, compatible with the molecular formula $C_{15}H_{20}O_4$. The IR spectrum showed absorption bands for OH (3398 cm^{-1}) and γ-lactone C=O (1758 cm^{-1}) groups. Detailed analyses of the ^{1}H- and 2D-NMR data of **2** revealed the presence of a germacranolide skeleton, bearing a $\Delta^{4(15)}$ unsaturation and a 1,10-epoxy ring. A COSY experiment was helpful to assign the coupling system of CH_2(13)/H–C(7) typical for sesquiterpene α-methylidene-γ-lactones. The same spectrum exhibited cross-peaks that enabled us to identify the ^{1}H-NMR spin system H–C(7)/H–C(8)/CH_2(9), H–C(7)/H–C(6)/CH_2(5)/Me(15), and H–C(1)/CH_2(2)/CH_2(3). From a HSQC experiment, it was evident that C(1), C(6), and C(8) (δ(C) 58.2, 78.8, and 71.1, resp.) were oxygenated. The strong downfield shift of C(6) indicated that lactonization occurred between C(6) and C(12), and the presence of a Me *singlet* at δ(H) 1.48 suggested a 1,10-epoxide. This was in accord with the ESI-MS data, which indicated six degrees of unsaturation.

The coupling constant between H–C(6)/H–C(7), J(6,7) = 10.0 Hz, helped us to determine the relative *trans* configuration of the lactone ring. NOE Cross-peaks between H–C(7)/H–C(8) indicated a different orientation of the OH group at C(8) (*Fig.*), which was further supported by a constant J value for H–C(8) [δ(H) 4.14 (*dd*, J = 3.6, 7.0 Hz)]. NOE Interactions between Me(14)/H–C(8)/H–C(7) proved them to be on the same side (α), whereas H–C(6) and H–C(1) were placed on the other side (β). From these data, the structure of melanolepin C (**2**) was determined as 1α,10β-epoxy-8β-hydroxygermacra-4(15),11(13)-dien-12,6α-olide.

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Experimental Part

General. Vacuum liquid chromatography (VLC): silica gel *60 H* (*Merck*). Column chromatography (CC): silica gel *60* (*SDS*, 40–63 μm), gradient elution with the solvent mixtures indicated in each case. Thin-layer chromatography (TLC): silica gel *60* F_{254} (*Merck*) or *RP-18* F_{254} (*Merck*), visualization under UV light (254 and 365 nm) and by spraying with anisaldehyde/H_2SO_4 reagent. Reverse-phase (RP)-HPLC: *CE-1100 Liquid Chromatography* pump, *Kromasil* C_{18} (250×10 mm) column, flow rate 2 ml/min. Optical rotations: *Perkin-Elmer 341* polarimeter; in $CDCl_3$ at 20°. IR Spectra: *Perkin-Elmer Paragon 500 FT-IR* spectrophotometer; in cm^{-1}. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker DRX-400* and *AC-200* instruments, at 295 K; δ in ppm rel. to Me_4Si, *J* in Hz. ESI-MS: *TSQ-7000* mass spectrometer, spray voltage 4 kV, heated capillary at 200°. HR-FAB-MS: *JEOL JMS-AX505HA* mass spectrometer; in *m*/*z*; recorded at the Department of Chemistry and Biochemistry, University of Notre Dame, South Bend, Indiana.

Plant Material. The aerial parts of *A. melanolepis* were collected at Rethimnon/Herakleion, island of Crete, in June 2002. The plant was authenticated by Dr. *Z. Kypriotakis*, and a voucher specimen (Kypriotakis 8607) was deposited at the Herbarium of the Technological Education Institute, School of Agricultural Production, Laboratory of Taxonomy and Management of Wild Flora, Crete.

Extraction and Isolation. The fresh aerial parts of *A. melanolepis* (360 g) were finely ground and extracted at r.t. with cyclohexane/Et_2O/MeOH 1:1:1. The extract was washed with brine, the aq. layer was re-extracted with AcOEt, and the org. layer was dried (Na_2SO_4), and concentrated under reduced pressure. The residue (9.9 g) was fractionated by VLC (SiO_2; 10.0×5.0 cm; cyclohexane/AcOEt/Me_2CO gradient of increasing polarity) to afford nine main fractions, *Fr. A–I*, 500 ml each. *Fr. B* (cyclohexane/AcOEt 75:25; 964 mg) was subjected to CC (SiO_2; 3.3×14.5 cm; CH_2Cl_2/AcOEt/MeOH 10:0:0 → 0:0:10), which afforded taraxasterol (53.1 mg). *Fr. C* (cyclohexane/AcOEt 50:50; 649 mg) was subjected to CC (SiO_2; 2.7×16 cm; CH_2Cl_2/AcOEt/MeOH 10:0:0 → 0:0:10) to yield twelve subfractions, *Fr. C1–C12*. Further purification of *Fr. C9* (80 mg) by RP-HPLC (MeOH/H_2O 3:2) gave (3*S*,5*R*)-loliolide (4.5 mg; t_R 13.3 min). *Fr. C10* (53 mg) was subjected to RP-HPLC (MeOH/H_2O 1:1) to afford dentatin A (1.5 mg; t_R 8.4 min). *Fr. D* (cyclohexane/AcOEt 25:75; 861.0 mg) was subjected to CC (SiO_2; 2.7×17 cm; CH_2Cl_2/AcOEt/MeOH 10:0:0 → 0:0:10) and yielded 22 subfractions, *Fr. D1–D22*. *Fr. D4* (56 mg) was subjected to RP-HPLC (MeOH/H_2O 9:11) to yield desacetyllaurenobiolide (1.6 mg; t_R: 9.4 min). *Fr. D18* (61 mg) was subjected to RP-HPLC (MeOH/H_2O 42:58) to provide **2** (2.6 mg; t_R 30.5 min). Further purification of *Fr. D19* (80 mg) RP-HPLC (MeOH/H_2O 1:1) gave **1** (3.7 mg; t_R 14.5 min).

Melanolepin B (=*1α,4β,8α-Trihydroxyeudesm-11(13)-en-12,6α-olide*; **1**). Colorless oil. $[\alpha]_D^{20} = +5.6$ ($c = 0.09$, MeOH). IR (CaF_2): 3357, 2932, 2871, 1753, 1703. ^{1}H- and ^{13}C-NMR: see *Table.* ESI-MS (neg.): 281 (15, $[M-H]^-$), 341 (70, $[M+AcO]^-$).

Melanolepin C (=*1α,10β-Epoxy-8β-hydroxygermacra-4(15),11(13)-dien-12,6α-olide*; **2**). Colorless oil. $[\alpha]_D^{20} = +10.0$ ($c = 0.04$, MeOH). IR (CaF_2): 3417, 2922, 1758. ^{1}H- and ^{13}C-NMR: see *Table.* ESI-MS (neg.): 263 (13, $[M-H]^-$), 323 (67, $[M+AcO]^-$). HR-FAB-MS: 265.1440 ($[M+H]^+$, $C_{15}H_{21}O_4^+$; calc. 265.1440).

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