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SESQUITERPENE LACTONES AND ELEMANE DERIVATIVES FROM *ONOPORDON MYRIACANTHUM*

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Key Word Index—*Onopordon myriacanthum*; Compositae; sesquiterpene lactones; elemanolides; germacranolides; eudesmanolides; elemane derivatives.

Abstract—The aerial parts of *Onopordon myriacanthum* afforded, in addition to several known flavonoids and sesquiterpene lactones a new elemanolide, a new germacranolide, two new eudesmanolides and a new elemane derivative. The structures of the new compounds were elucidated by spectroscopic methods, particularly highfield NMR spectroscopy as 8α-sarracinoyl dehydromelitensin and its methylester derivative, 8α-(4′-hydroxysenecioyl)-salonitenolide and 8α-sarracinoyl sonchucarpolide and its 4-*epi*-derivative.

INTRODUCTION

Phytochemical studies of the genus *Onopordon* (Compositae) have led to the isolation of flavonoids [1–3], sesquiterpene lactones [4, 5] and several unusual hydroxyester sesquiterpene derivatives [6–10]. These latter seem to characterize *Onopordon* and most likely are the biosynthetic precursors of the sesquiterpene lactones [5]. In continuation of our work on the chemical constituents of this genus [2, 3, 9, 10] we have investigated *O. myriacanthum* Boiss [11] a species distributed in the Peloponnese (Greece).

We now report the isolation of several known flavonoids (chrysoeriol, hispidulin, apigenin, eriodictyol, sorbifolin, luteolin, onopordin, eupafolin and vitexin [12, 13]), four elemanolides (**1–4**), five germacranolides (**5–9**), three eudesmanolides (**10–12**) and two hydroxyester elemane derivatives (**13** and **14**). Compounds **2**, **6** and **11–13** are new naturally occurring sesquiterpenes, and their structures were elucidated by extensive highfield NMR studies.

RESULTS AND DISCUSSION

The NMR spectra of **1–4** showed typical lowfield signals that clearly indicated the presence of an elemane framework [9]. The mass spectrum (CI) of **2** showed a peak at *m/z* 363.1797 corresponding to $[M + H]^+$, which agreed with a molecular formula of $C_{20}H_{26}O_6$. In its ^1H NMR spectrum the signals at δ5.76 *dd*, 5.04 *d*, 4.99 *d*, 5.42 *br s* and 4.97 *br s* were assigned to H-1, H-2a, H-2b, H-3a and H-3b, respectively. From a pair of doublets at δ4.09 and 3.99 a hydroxymethyl group as substituent at C-4 was evident. A typical doublet at δ2.56 (J = 11.6 Hz) for H-5 and a typical *dddd* signal at δ2.93 (J = 11.2, 10.8, 3.2 and 2.8 Hz) for H-7 indicated a *trans*-disposition of H-5/H-6, H-6/H-7 and H-7/H-8 and so the oxygenated functions at C-6 and C-8 should be α-oriented. An α-methylene-γ-lactone (1766 cm^{-1} in the IR spectrum and two doublets for H-13 at δ6.14 and 5.58) were also evident. The lowfield double triplet at δ5.29 corresponding to H-8 indicated that an ester of an 8α-hydroxyl derivative was present. The acid part was a 2-hydroxymethyl-2-butenoate (sarracinoate) moiety. This was indicated in the ^1H NMR spectrum by a characteristic quartet at δ7.01 for an olefinic proton and a doublet at δ1.94 for the vinyl methyl group, together with a broad singlet at δ4.38 for $-CH_2OH$ [14]. The presence of the sarracinoyl moiety was also confirmed by the peak in the mass spectrum (CI) at *m/z* 247 (76%) corresponding to the loss of the sarracinoyl side chain from the molecular ion $[M + H - RCO_2H]^+$. Consequently, **2** is the new 8α-(2′-hydroxymethyl-2′-butenoyloxy) derivative of dehydromelitensin.

In a similar way, **3**, **1** and **4** were identified as dehydromelitensin [15, 16], its 8-(4-hydroxymethacrylate) [4, 17] and melitensin [16, 18], respectively.

The mass spectrum of compound **6** showed a molecular peak at *m/z* 362.1718, which agreed with the molecular formula $C_{20}H_{26}O_6$. The features of the ^1H NMR spectrum suggested a germacrane ring with two double bonds at $\Delta^{1(10)}$ and Δ^4. The broadened doublet of doublets at δ4.97 (J = 4.6 and 11.0 Hz) and the broadened doublet at δ4.82 (J = 10.0 Hz) were assigned to H-1 and H-5, respectively. In the ^{13}C NMR spectrum the signals at δ129.3, 143.7, 128.8 and 132.8 were assigned to C-1, C-4, C-5 and C-10, respectively. From a pair of doublets at δ4.31 and 4.08 (J = 14.0 Hz) a hydroxymethyl group (at δ61.4 in the ^{13}C NMR spectrum) as substituent at C-4 was evident. The presence of an

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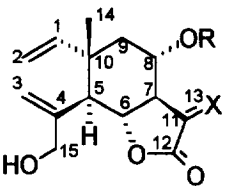
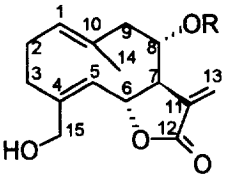
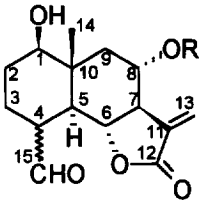


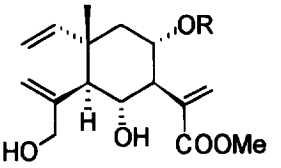
1 X= CH_2 , R= **A**
2 X= CH_2 , R= **B**
3 X= CH_2 , R= H
4 X= αMe,βH , R= H



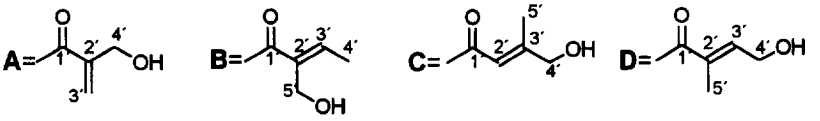
5 R= **B**
6 R= **C**
7 R= **D**
8 R= **A**
9 R= H



10 R= **A**, 4αH
11 R= **B**, 4αH
12 R= **B**, 4βH



13 R= **B**
14 R= **A**



oxygenated group at C-6α (δ76.6) was inferred from the double doublet at δ5.07 (J = 8.4 and 10.0 Hz), which showed coupling with the signal of H-5 (δ4.82) and H-7 (δ3.02). An α-methylene-γ-lactone (δ169.8 in the ^{13}C NMR spectrum, and 1760 cm^{-1} in the IR spectrum) and a methylene at δ 135.3 (C-11) and 125.7 (C-13) and at δ6.30 and 5.85 (H-13) were also evident. An ester moiety was located at C-8α (δ71.6) as was deduced from the position and pattern of the signal at δ5.13 (*br dd*, J = 8.4 and 10.4 Hz). The identity of the ester side chain was inferred from the following signals: a one-proton signal at δ6.03, a two-proton broad singlet at δ4.18 and a three-proton singlet at δ2.09. The chemical shifts of these signals strongly suggested the presence of a 4-hydroxysenecioate moiety. This was confirmed by the presence in the mass spectrum of a base peak at *m/z* 99 [19]. Full analysis of the NMR spectra by decoupling experiments, together with heteronuclear two-dimensional shift correlation ^{1}H–^{13}C (HMQC) led to structure **6**, a new 8α-(4′-hydroxy-3′-methylbutenoyloxy) derivative of salonitenolide (**9**). Compounds **5** [14], **7** [14] and **8** (onopordopicrin) [5,6,20,21] are also esters of salonitenolide (**9**) [7,22].

Compounds **10**–**12** had ^{1}H NMR spectra that suggested an eudesmane framework. The spectrum of **11** showed a singlet at δ0.92 for C-14 and signals close to those of a C-6 lactonized eudesmanolide functionalized at C-1 and C-8 (δ3.39, *dd*, H-1; 4.52, *dd*, H-6 and 5.31, *ddd*, H-8) [23]. However, no signals characteristic of aliphatic or olefinic protons at C-15 were observed. The signal of H-15 was a low-field singlet at δ9.94 indicating the presence of an aldehyde group. As in **2**, typical signals of a sarracinoyl moiety indicated the nature of the ester residue at C-8 {which was also confirmed by the peak at *m/z* 263 (100%) corresponding to [M + H − RCOOH]$^+$}. For this compound the coupling patterns and the magnitude of the coupling constants of H-5 to H-8 and H-1 were in full agreement with a *trans*-disposition of H-5/H-6, H-6/H-7 and H-7/H-8 and a H-1α stereochemistry for C-1. The similarity of this ^{1}H NMR spectrum to that of 8α-[4′-hydroxymethacryloyloxy]-4-*epi*-sonchucarpolide (**10**) [6] indicated that **11** was the new 8α-[2′-hydroxymethyl-2-butenoyloxy]-4-*epi*-sonchucarpolide. Compounds **11** and **12** were isomers of molecular formula $C_{20}H_{26}O_7$ (*m/z* 379.1753, corresponding to [M + H]$^+$) and their ^{1}H NMR spectra

Table 1. ^{1}H NMR chemical shifts of compounds **2**, **6** and **11**–**13** (400 MHz, $CDCl_3$, δ values)

H	2	6	11	12*	13
1	5.76 *dd* (10.8, 17.6)	4.97 *br dd* (4.6, 11.0)	3.39 *dd* (4.5, 11.2)	3.44 *dd* (4.2, 10.8)	5.68 *dd* (10.8, 17.6)
2a	5.04 *d* (10.8)	2.30–2.20 *m*	1.80–1.70 *m*	1.70–1.50 *m*	4.95 *d* (10.8)
2b	4.99 *d* (17.6)		1.61 *dddd* (3.6, 11.1, 12.0, 13.2)		4.91 *d* (17.6)
3a	5.42 *br s*	2.58 *br d* (11.2)	2.50–2.40 *m*	1.81–1.66 *m*	5.38 *br s*
3b	4.97 *br s*	1.97 *br dt* (6.4, 11.2)	1.47 *ddt* (3.6, 4.2, 12.0)	1.70–1.50 *m*	5.03 *br s*
4	—	—	2.81 *br dd* (4.2, 5.4)	2.60–2.50 *m*	—
5	2.56 *d* (11.6)	4.82 *br d* (10.0)	2.02 *dd* (5.6, 11.8)	1.89 *t* (11.1)	2.10 *d* (10.8)
6	4.23 *dd* (11.2, 11.6)	5.07 *dd* (8.4, 10.0)	4.52 *dd* (11.4, 11.8)	4.01 *t* (11.1)	4.20 *t* (10.8)
7	2.93 *dddd* (2.8, 3.2, 10.8, 11.2)	3.02 *ddt* (2.8, 3.2, 8.4)	2.87 *ddt* (3.0, 11.4, 11.2)	2.88 *dddd* (2.7, 3.2, 10.8, 11.1)	2.71 *t* (10.8)
8	5.29 *td* (4.0, 10.8)	5.13 *br dd* (8.4, 10.4)	5.31 *ddd* (4.5, 10.5, 11.2)	5.31 *td* (4.2, 10.8)	5.38 *ddd* (4.4, 10.8, 11.2)
9a	1.65 *dd* (10.8,12.8)	2.43 *br dd* (10.4, 12.4)	1.31 *br dd* (11.2, 12.6)	1.32 *dd* (10.8, 12.9)	1.60 *dd* (11.2, 12.8)
9b	2.05 *dd* (4.0, 12.8)	2.59 *br d* (12.4)	2.50 *dd* (4.5, 12.6)	2.58 *dd* (4.2, 12.9)	1.79 *dd* (4.4, 12.8)
13a	6.14 *d* (3.2)	6.30 *d* (3.2)	6.18 *d* (3.0)	6.13 *d* (3.3)	6.29 *s*
13b	5.58 *d* (2.8)	5.85 *d* (2.8)	5.60 *d* (3.0)	5.56 *d* (2.7)	5.74 *s*
14	1.17 *s*	1.50 *br s*	0.92 *s*	1.05 *s*	1.18 *s*
15a	4.09 *br d* (13.2)	4.31 *d* (14.0)	9.94 *s*	9.68 *d* (3.9)	4.06 *d* (13.2)
15b	3.99 *br d* (13.2)	4.08 *d* (14.0)	—	—	3.93 *d* (13.2)
3′	7.01 *q* (7.2)	6.03 *br d* (1.2)	7.00 *q* (7.2)	7.00 *q* (7.2)	6.87 *q* (7.2)
4′	1.94 *d* (7.2)	4.18 *br s*	1.94 *d* (7.2)	1.94 *d* (7.2)	1.86 *d* (7.2)
5′	4.38 *br s*	2.09 *s*	4.38 *br s*	4.39 *br s*	4.27 *br s*
MeO-	—	—	—	—	3.76 *s*

Values in parentheses are coupling constants in Hz.
*At 300 MHz.

were similar. Spin decoupling indicated that the whole sequence was identical for both compounds as well as for the sarracinoyl ester residue at C-8. However, some significant differences were observed. In addition to differences in the chemical shifts the aldehyde signal was a doublet at δ9.68 (J = 3.9 Hz), the H-4 signal now was a multiplet at δ2.60–2.50 (a *br dd* at δ2.81 in **11**) and the H-5 signal now was a triplet at δ1.89 (J = 11.1 Hz) (a *dd* at δ2.02, J = 5.6 and 11.8 Hz in **11**). These differences in the coupling constants in **12** required an axial proton at C-4 and so the aldehyde group at this carbon should be equatorial [5,6]. Consequently, **12** is the new 8α-[2′-hydroxymethyl-2′-butenoyloxy]-sonchucarpolide, a C-4-epimer of **11**.

The spectra of **13** and **14** were in part close to those of **2** and **1**, respectively. The methoxyl singlets at δ3.76 and 3.75, respectively, as well as slightly broadened singlets for exomethylene protons (H-13), indicated the presence of methyl esters of the hydroxyl acids corresponding to **2** and **1**. Compound **14** was identified as the known elemacarmanin, previously isolated from several *Onopordon* species [6–10], whereas **13** is a new natural product.

The chemistry of *O. myriacanthum* again shows that onopordopicrin, as well as closely related lactones and hydroxyl esters, are characteristic of this genus. However, in contrast to the previously studied *Onopordon* species whose constituents contain mainly a 8-(4′-hydroxymethacrylate) side chain, the chemistry of *O. myriacanthum* is characterized by the occurrence of 8-(2′-hydroxymethyl-2′-butenoyloxy) sesquiterpene lactones.

Table 2. ^{13}C NMR chemical shifts of compounds **6**, **7**, **11** and **13** (75.43 MHz, $CDCl_3$, δ values)

C	6*	7	11	13*
1	129.3	129.6	78.1	146.3
2	26.2	26.3	27.2	111.9
3	34.6	29.7	22.3	114.8
4	143.7	143.7	45.0	?
5	128.8	128.6	48.8	55.2
6	76.6	76.4	76.2	70.9
7	52.8	53.0	53.9	54.4
8	71.6	72.9	69.3	70.7
9	49.0	48.7	44.0	43.5
10	132.8‡	133.2	41.4	40.1
11	135.3‡	135.5‡	136.4	138.0
12	169.8	169.7	169.2	167.2‡
13	125.7	125.2	120.5	128.1
14	16.8	16.7	13.9‡	18.3
15	61.4	61.5	201.7	67.7
1′	165.2	166.1	166.5	166.4‡
2′	112.4	135.0‡	131.6	131.7
3′	159.9	141.3	141.7	140.7
4′	66.8	59.8	14.4‡	14.1
5′	15.7	12.8	56.7	56.7
CH_3O-	—	—	—	51.9

*Assignment by heteronuclear ^{1}H–^{13}C correlation (HMQC).

‡Signals may be interchanged within each column.

EXPERIMENTAL

NMR: 400, 300 MHz (^{1}H) and 100 MHz (^{13}C). Vacuum liquid chromatography (VLC): silica gel (Merck; 43–63 μm); CC: silica gel (SDS; 40–63 μm), gradient elution with the solvent mixts indicated in each case; HPLC: Merck Lichrospher 100RP-18 (250 × 10 mm).

Plant material. Aerial parts of *O. myriacantum* were collected on the Taïgetos mountain, Peloponnese (Greece), in July 1991 and authenticated by Mr Theophanis Constantinidis (Institute of Systematic Botany, University of Patras). A voucher specimen is deposited in the herbarium of the above mentioned institute (no. 4892).

Extraction and chromatography. The air-dried plant material (1 kg) was finely ground and extracted at room temp. with hexane–Et_2O–MeOH (1:1:1). The extract was washed with brine, the aq. layer re-extracted with EtOAc, and the organic layer dried with Na_2SO_4 and concd under red. press. The residue (41 g) was dissolved in MeOH and cooled at −20°, and the soluble compounds were sepd by VLC [24] on silica gel, using hexane–EtOAc–Me_2CO mixts of increasing polarity as eluents to give several frs. Repeated CC of the fr. eluted with hexane–EtOAc (1:1), over silica gel using $CHCl_3$–MeOH and hexane–Et_2O, followed by further purification on HPLC (MeOH–H_2O, 4:3, 4:3.5 or 1:1) yielded chrysoeriol (12 mg), hispidulin (10 mg), apigenin (5 mg), eriodictyol (8 mg), sorbifolin (2 mg), luteolin (8 mg), onopordin (2 mg), eupafolin (5 mg) and vitexin (2 mg) and **1** (1 mg), **2** (2 mg), **5** (30 mg), **11** (10 mg), **8** (9 mg), **6** (5 mg), **4** (2 mg), **3** (2 mg), **9** (1 mg), **7** (7 mg), **10** (2 mg), **12** (2 mg), **14** (4 mg) and **13** (12 mg).

Compound **2**. Oil; $[\alpha]_D^{24}$ + 51° ($CHCl_3$, *c* 0.10); IR ν_{max}^{NaCl} cm^{-1}: 3600–3300, 1766, 1707, 1660; CIMS *m/z* (rel. int.): 363.1797 $[M + H]^+$(31), $C_{20}H_{27}O_6$ requires 363.1807, 247 $[M + H - RCO_2H]^+$(76), 231 (46), 230 (24), 229 $[M + H - RCO_2H - H_2O]$ (100). ^{1}H NMR spectral data: see Table 1.

Compound **6**. Oil; $[\alpha]_D^{23}$ + 133.5° ($CHCl_3$, *c* 0.39); IR ν_{max}^{NaCl} cm^{-1}: 3600–3300, 1760, 1710, 1650; MS *m/z* (rel. int.): 362.1718 $[M]^+$ (3), $C_{20}H_{26}O_6$ requires 362.1722, 246 $[M - RCO_2H]^+$(10), 228 (12), 99 $[RCO]^+$(100). ^{1}H and ^{13}C NMR spectral data: see Tables 1 and 2, respectively.

Compound **11**. Oil; $[\alpha]_D^{23}$ + 74.8° ($CHCl_3$, *c* 0.18); IR ν_{max}^{NaCl} cm^{-1}: 3550–3300, 1765, 1710, 1660; CIMS *m/z* (rel. int.): 379.1751 $[M + H]^+$(14), $C_{20}H_{27}O_7$ requires 379.1757, 293 (16), 281 (42), 263 $[M + H - RCO_2H]^+$ (100). ^{1}H and ^{13}C NMR spectral data: see Tables 1 and 2, respectively.

Compound **12**. Oil; $[\alpha]_D^{24}$ + 65.7° ($CHCl_3$, *c* 0.175); IR ν_{max}^{NaCl} cm^{-1}: 3600–3300, 1767, 1712, 1660; CIMS *m/z* (rel. int.): 379.1753 $[M + H]^+$(19), $C_{20}H_{27}O_7$ requires 379.1757, 293 (20), 281 (48), 263 $[M + H - RCO_2H]^+$ (100). ^{1}H NMR spectral data: see Table 1.

Compound **13**. Oil; $[\alpha]_D^{24}$ + 21.2° ($CHCl_3$, *c* 0.6); IR ν_{max}^{NaCl} cm^{-1}: 3500–3300, 1700, 1640; CIMS *m/z* (rel. int.): 395.2061 $[M + H]^+$(8) $C_{21}H_{31}O_7$ requires

395.2069, 280 (17), 279 $[M + H - RCO_2H]^+$ (100), 261 $[M + H - RCO_2H - H_2O]^+$(62). ^1H and ^{13}C NMR spectral data: see Tables 1 and 2, respectively.

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