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EUDESMANOLIDES AND OTHER CONSTITUENTS OF *INULA THAPSOIDES**

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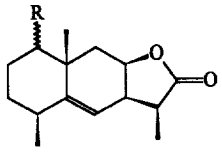
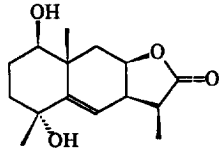
Key Word Index—*Inula thapsoides*; Compositae; sesquiterpene lactones; eudesmanolides; diterpenes; steroids.

Abstract—Three new eudesmanolides, 1β-hydroxy-11α,13-dihydroalantolactone, 1α-hydroxy-11α,13-dihydroalantolactone and 1β,4α-dihydroxy-11α,13-dihydroalantolactone, along with the known compounds sclareol, 7,11,15-trimethyl-3-methylenehexadecan-1,2-diol, β-sitositerol, stigmasterol and stigmasterol 3β-D-glucoside have been isolated from the whole plant extract of *Inula thapsoides*. © 1997 Elsevier Science Ltd

INTRODUCTION

In our studies on Turkish *Inula* species, we have isolated guaianolides and eudesmanolides from *Inula anatolica* [1] and *I. graveolens* [2, 3] as well as some sesquiterpene acids, steroids and flavonoids. The whole plant extract of *Inula thapsoides* (Bieb. ex Willd. Sprengel) subsp. *thapsoides* (Syn: Conyza *thapsoides* Bieb. ex Willd.) gave seven new guaianolides [4]. Further investigation of *I. thapsoides* has led to the isolation of three new eudesmanolides, 1β-hydroxy-11α,13-dihydroalantolactone (**1**), 1α-hydroxy-11α,13-dihydroalantolactone (**2**) and 1β,4α-dihydroxy-11α,13-dihydroalantolactone (**3**), in addition to the known compounds, sclareol [5], 7,11,15-trimethyl-3-methylene-hexadecan-1,2-diol [6], β-sitosterol, stigmasterol and stigmasterol 3β-D-glucoside. We now report on the structure determination of the three new eudesmanolides (**1**–**3**) by spectroscopic methods including X-ray analysis for compound **1**.





1 R=β-OH
2 R=α-OH
1a R=β-OAc
2a R=α-OH

3

RESULTS AND DISCUSSION

Compound **1** was the most abundant eudesmanolide of the plant extract. The IR spectrum contained absorptions bands for a hydroxyl at 3440 cm^{-1}, a lactone at 1780 cm^{-1} and unsaturation at 1675 cm^{-1}. The ^1H NMR spectrum showed a methyl singlet at δ 1.21 and two methyl doublets at δ 1.12 (J = 7.5 Hz) and 1.36 (J = 7.5 Hz). However, the characteristic doublets of exocyclic methylene protons were not observed. The absorption of the lactone carbonyl in the IR spectrum at 1780 cm^{-1} and the signal at δ 179.8 in the ^{13}C NMR spectrum of **1** (Table 1) established the presence of a five membered saturated lactone. In the ^1H NMR spectrum (Table 1), an olefinic proton at δ 5.26 (*d*, J = 3.5 Hz), a lactone proton at δ 4.90 (*ddd*, J = 2, 3.5 and 6.5 Hz) and an oxymethine proton at δ 3.29 (*ddd*, J = 1, 4 and 11.2 Hz, H-1) were observed. Irradiation of H-7 at δ 2.64 led to the sequence of H-6 to H-9 as shown by the collapse of the protons at δ 5.26 (*dd*, H-6), 4.90 (*ddd*, H-8) and 2.49 (*d*, H-9) into a singlet, a doublet of doublet and a doublet, respectively, while on irradiation of H-1 at δ 3.29, the C-2 proton signals were simplified. Compound **1** gave a monoacetyl derivative (**1a**). In the ^1H NMR spectrum of **1a** (see Experimental) the signal at δ 3.29 was shifted to δ 4.53 and an acetyl methyl singlet appeared at δ 2.08 verifying the presence of a secondary hydroxyl group. Its β orientation was deduced from the splitting pattern of H-1 as well as

* Dedicated to Professor Ayhan Ulubelen (University of Istanbul, Turkey) on the occasion of her sixtyfifth Birthday.

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Table 1. ^1H and ^{13}C NMR data of eudesmanolides **1**–**3** ($CDCl_3$)

	1 (^{13}C)	**1** (^1H)	**2** (^1H)	**3** (^1H)	**3** (^{13}C)
1	80.3	3.29 *dd* (4, 11.2)	3.46 *dd* (5.5, 10)	3.29 *ddd* (1.5, 4.5, 12)	77.9
2	29.9	1.86 *m*, 1.56 *m*	1.95 *dd*, 1.52 *m*	1.88 *m*, 1.53 *m*	30.1
3	39.3	1.61 *m*, 1.72 *m*	1.70 *m*	obscured	41.7
4	37.8	2.45 *m*	2.42 *m*	—	72.3
5	147.7	—	—	—	147.9
6	121.4	5.26 *d* (3.5)	5.32 *d* (3.5)	5.61 *d* (3.5 Hz)	122.1
7	42.3	2.64 *ddd* (1.5, 3.5, 6)	2.67 *ddd* (1.5, 3.5, 6)	2.69 *m*	43.6
8	76.0	4.90 *ddd* (2, 3.5, 6.5)	4.88 *ddd* (2.5, 3.5, 6.5)	4.91 *ddd* (2.5, 3.5, 7.5)	78.1
9	39.3	2.49 *dd* (3.5, 15 Hz) 1.58 *dd* (4, 15 Hz)	2.24 *dd* (2.5, 15.5) 1.60 *dd* (3.5, 15.5)	2.53 *dd* (4.5, 15), 1.63 *m*	40.3
10	38.6	—	—	—	39.7
11	43.5	2.42 *dq* (7, 7.5)	2.46 *dq* (7, 7.5)	2.46 *dq* (7, 7)	43.6
12	179.8	—	—	—	177.3
13	16.1	1.36 *d* (7.5)	1.37 *d* (7.5)	1.39 *d* (7)	14.6
14	22.0	1.21 *s*	1.30 *s*	1.26 *s*	22.4
15	22.4	1.12 *d* (7.5)	1.13 *d* (7.5)	1.37 *s*	16.5

by comparing the ^1H NMR spectral data with those of 1β-hydroxyalantolactone [7] and similar eudesmanolides [8]. The placement of a double bond between C-5 and C-6 was also compatible with the dihydroalantolactone derivatives [9]. The ^{13}C NMR (APT) spectrum displayed 15 carbons (3 methyl, 6 methine, 3 methylene and 3 quaternary). The HREI mass spectrum proved the proposed structure i.e. $[M]^+$ at m/z 250.1585 in agreement with the molecular formula $C_{15}H_{22}O_3$. HETCOR experiments allowed the unambiguous assignment of all proton-bearing carbons. The orientation of the C-11 methyl group was deduced from the J value of H-11 with H-7 and by comparison with 11,13-dihydroeudesmanolides [9, 10]. Since the acetate of **1** (**1a**) gave suitable crystals, an X-ray analysis was performed. Thus, the stereochemistry of all centres was clearly deduced. Based on all the spectral data, the structure of **1** was determined as 1β-hydroxy-11α,13-dihydroalantolactone.

Compound **2** showed ^1H NMR spectral properties very similar to those of **1**. A proton geminal to a secondary hydroxyl group was observed at δ 3.46 with a splitting pattern of $J = 5.5$ and 10.5 Hz and attributed to an α hydroxyl group at C-1. The data given in the literature [11] for 1α-hydroxylated eudesmanolides were in a good agreement with the chemical shift and multiplicity of H-1. The EI mass spectrum gave the same molecular ion peak ($[M]^+ = m/z$ 250) as compound **1**, while the acetate of **2** (**2a**) showed a $[M]^+$ peak at m/z 292 confirming the presence of one secondary hydroxyl group in the molecule. Thus, **2** was identified as 1α-hydroxy-11α,13-dihydroalantolactone.

The third eudesmane (**3**) also gave a ^1H NMR spectrum similar to that of compound **1**. However, there was a significant difference in the chemical shift of the C-15 methyl (δ 1.37, *s*) compared with those of the other methyls [δ 1.39 ($J = 7$ Hz, H_3-13) and 1.26 (*s*, H_3-14)]. A secondary hydroxyl group at δ 3.29 with the same J values (*ddd*, $J = 1$, 4, 11.5 Hz) and an olefinic proton at δ 5.61 were observed. The location of the double bond was deduced to be between C-5 and C-6 as in the first two compounds. Its downfield chemical shift compared with that in compound **1** could be explained by the presence of a hydroxyl group at C-4. The quaternary oxygenated carbon at δ 72.3 in the ^{13}C NMR (APT) spectrum confirmed the presence of a tertiary hydroxyl group at C-4. The HREI mass spectrum showed a molecular ion peak at m/z 266.1529 corresponding to molecular formula $C_{15}H_{22}O_4$, and the most prominent fragment ion was observed at m/z 248 $[M\text{-}H_2O]^+$. Compound **3** was deduced to be 1β,4α-dihydroxy-11α,13-dihydroalantolactone.

EXPERIMENTAL

General. IR: $CHCl_3$; ^1H and ^{13}C NMR: 200 and 50.32 MHz; MS: VG ZabSpec GC-MS instruments.

Plant material. Inula thapsoides subsp. *thapsoides* were collected in July 1992 from central Turkey (Yıldızeli, Sivas) and identified by Prof. Semra Kurucu. A specimen is deposited in the Herbarium of Faculty of Pharmacy, University of Ankara (AEF: 17512).

Extraction and isolation. Whole plant (1.7 kg) of *I. thapsoides* was extracted with petrol–Et_2O–EtOH (1:1:1) at room temp. and the solvent evapd. *in vacuo* to give 32 g extract. The extract was chromatographed on silica gel (350 g, 100–200 mesh) eluting with petrol, and a gradient of petrol–EtOAc up to 100% EtOAc, followed by MeOH. From the first frs collected with petrol–EtOAc (9:1), compounds **1** (500 mg), **2** (11 mg) and **3** (18 mg) were isolated and purified by repeated prep. TLC.

1β-Hydroxy-11α,13-dihydroalantolactone (**1**). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3010, 2930, 1780, 1675, 1470, 1390, 1360, 1340, 1280, 1240, 1225, 1200, 1080, 1050, 1030, 990, 940, 920, 900, 880, 855 and 760; HREIMS *m/z*: 250.1585 (calc. 250.1568 for $C_{15}H_{22}O_3$); CIMS *m/z* (rel. int.): 251 $[M+1]^+$ (82), 233 $[M+1\text{-}H_2O]^+$ (58), 205 (19), 187 (12), 159 (23), 57 (100); ^1H and ^{13}C NMR: Table 1.

Acetyl derivative of **1** (**1a**). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 1775, 1730, 1635, 1475, 1380, 1345, 1260, 1240, 1080, 1050, 995, 950, 890 and 760; ^1H NMR ($CDCl_3$) δ: 1.35 (3H, *d*, *J* = 7.5 Hz, H-13), 1.28 (3H, *s*, H-14), 1.13 (3H, *d*, *J* = 7.5 Hz, Me-15), 2.08 (3H, *s*, OAc), 2.43 (1H, *dq*, *J* = 7 and 7 Hz, H-11), 2.23 (1H, *dd*, *J* = 4.5 and 15 Hz, H-9), 1.53 (1H, *dd*, *J* = 3 and 15 Hz, H-9′), 2.63 (1H, *ddd*, *J* = 2 and 3.5 and 6 Hz, H-7), 4.53 (1H, *dd*, *J* = 3.8 and 11.5 Hz, H-1a), 4.87 (1H, *ddd*, *J* = 3, 3.5 and 6 Hz, H-8), 5.30 (1H, *d*, *J* = 3.5 Hz, H-6).

1α-Hydroxy-11α,13-dihydroalantolactone (**2**). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3440, 3020, 2935, 1780, 1670, 1480, 1385, 1360, 1340, 1280, 1245, 1230, 1200, 1080, 1030, 990, 945, 900, 880, 855 and 760; ^1H NMR: Table 1; EIMS (70 eV) *m/z* (rel. int.): 250 $[M]^+$ (3), 232 $[M\text{-}H_2O]^-$ (55), 207 (22), 186 (24), 172 (11), 159 (29), 57 (100).

Acetyl derivative of **2** (**2a**). ^1H NMR ($CDCl_3$) δ 1.15 (3H, *d*, *J* = 7 Hz, H-15), 1.35 (3H, *s*, Me-14) and 1.39 (3H, *d*, *J* = 7 Hz, H-13), 2.06 (3H, *s*, OAc), 4.88 (1H, *dd*, *J* = 5 and 10 Hz, H-1), 4.87 (*m*, H-8), 5.33 (1H, *d*, *J* = 3 Hz, H-6); EIMS (70 eV) *m/z* (rel. int.): 292 $[M]^+$ (1), 291 $[M\text{-}1]^+$ (6), 290 $[M\text{-}2]^+$ (30), 256 (21), 251 $[M\text{-}OAc]^+$ (20), 248 $[M\text{-}OAc\text{-}1]^+$ (100), 230 (37), 215 (18), 185 (17), 159 (47), 131 (22), 119 (23), 97 (26), 83 (31), 57 (44).

1β,4α-Dihydroxy-11α,13-dihydroalantolactone (**3**). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3430, 3010, 2890, 1775, 1660, 1470, 1380, 1350, 1265, 1230, 11020, 1080, 1030, 990, 885, 765; ^1H and ^{13}C NMR: Table 1; HREIMS *m/z*: 266.1529 (calc. 266.1518 for $C_{15}H_{22}O_4$), 248 $[M\text{-}H_2O]^+$ (100), 233 (51), 215 $(233\text{-}H_2O)^+$ (25), 187 (18), 160 (45), 59 (80), 57 (75); EIMS (70 eV) *m/z* (rel. int.): 266 $[M]^+$ (10), 248 (70), 233 (18), 230 (29), 207 (23), 205 (28), 187 (21), 175 (71), 157 (100), 147 (87), 131 (86), 121 (73), 105 (75), 91 (87), 79 (53), 47 (73).

X-ray analysis. All data were collected on a Rigaku AFC6S diffractometer with graphite monochromated Cu-Kα radiation and a constant speed ω-2θ scan technique with weak reflections rescanned a maximum of 4 times. Crystal and refinement data were as follows: formula: $C_{17}H_{24}O_4$; F.W.: 292.37; crystal colour, habit: colourless, prismatic; crystal system: monoclinic; space group: $P2_1$ (#4); crystal dimensions: 0.20 × 0.15 × 0.30 mm; lattice: primitive; lattice parameters: (a) 6.219 (3) Å, (b) 9.112 (1) Å, (c) 14.261 (2) Å; b(°): 96.63 (2); *Z* value: *Z*; V_1: 802.7(4) $Å^3$; D(calc): 1.210 g cm^{-3}; radiation: (1 = 1.54178 Å; abs.coefm: 6.89 cm^{-1}; F(000): 316.00; temp: 23.0°; diffractometer: Rigaku AFC6S; scan mode: w-2θ; 2*q* range (max): 157.4°; total data: 3527; unique data: 1783 (*R* = 0.035); observed data used: 177 8[1 > 0.006 int (I)]; no. of parameters refined: 287; max. shift/error in final cycle: 0.11; max. resid. density: 0.20 e-/$Å^3$; *R Rw*: 0.051; 0.062; Goodness of Fit Indicator: 1.35. Unit cell parameters were obtained from a least-squares refinement of 1 carefully centred reflections in the range 53.13 < 2*q* < 53.13° corresponded to a primitive monoclinic cell. Lorentz-polarization, a *Y*-scan empirical absorption correction and anisotropic extinction corrections were applied. The structure was solved by direct methods [12] and refined and analysed by teXsan [13] and PLATON [14]. The coordinates, distances and angles have been deposited with the Cambridge Structural Data Base.

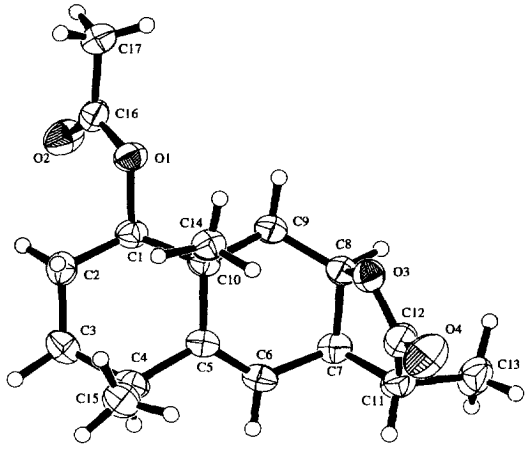

Fig. 1. Molecular structure of **1a**

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