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Terpenes from *Inula verbascifolia*

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Abstract

The aerial parts of *Inula verbascifolia* afforded two new xanthanes and a new germacranolide derivative, together with the known compounds inusoniolide, 4-*O*-dihydroinusoniolide and 9β-hydroxyparthenolide. The structures were determined by spectral methods (IR, HRMS,^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC).

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1. Introduction

In continuation of our investigation of the medicinal plants of the family Asteraceae, we investigated the chemical constituents of *Inula verbascifolia* (Willd.) Hausskn. subsp. *methanea* (Hausskn.) Tutin, an endemic species of Central and South Greece. (Ball et al., 1976). The taxonomy of the large genus *Inula*, (family Asteraceae, tribe Inuleae) which comprises ca. 90 species, is problematical and represents a badly delineated complex (Bremer, 1994). Members of this genus are used in herbal medicine and especially elecampane (*I. helenium*) is reported to possess expectorant, antitussive, diaphoretic and bactericidal properties (Newall et al., 1996).

2. Results and discussion

Separation of the MeOH–CH_2Cl_2 extract of the Greek endemic species *I. verbascifolia* subsp. *methanea* on a silica open column yielded two new xanthanes (**1**, **2**) and a new germacranolide derivative (**5**), in addition to the known compounds inusoniolide **3** (Bloszy et al., 1990), 4-*O*-dihydroinusoniolide (**4**) (Marco et al., 1993) and 9β-hydroxyparthenolide (**6**) (Abdel Sattar et al., 1996). The EI mass spectrum of **1** exhibited a $[M-CH_3COOH]^+$ ion at *m*/*z* 248 and exact mass determination of this ion, 248.1390, established the elemental composition as $C_{15}H_{20}O_3$. Compound **1** had a close NMR spectral data to compound **3** (Tables 1 and 2). However, the ^{1}H NMR revealed the presence of two broad singlet signals at δ 5.60 and 6.20. The absence of a correlation between those two signals and H-7, in ^{1}H–^{1}H COSY, suggested that compound **1** had an acid function (C=O, IR: $\nu = 1720$ cm^{-1}). Additionally, the ^{1}H NMR spectrum revealed the presence of an acetyl group at δ 1.90 (C=O, IR: $\nu = 1758$ cm^{-1}), while the two methyls of the sesquiterpene moiety were found at δ 1.03 (d, $J = 7.5$ Hz) and 2.15 (s). The ^{13}C NMR spectrum with the aid of DEPT experiment displayed 17 carbon signals; one oxygen bearing carbon at δ 70.81 (d), three carbonyl carbons at δ 169.60, 214.52, 167.41, four olefinic carbons at δ 146.65 (s), 115.41 (d), 157.021 (s), 124.90 (t), four methylene carbons at δ 29.35, 29.80, 33.60, 37.54 and two methine groups at δ 43.06 and 34.49. All signals were determined by ^{1}H–^{1}H COSY and HMQC. The stereochemistry of **1** was proved by the coupling constants and NOEs experiments. The *trans* orientation of H-6 and H-7 was deduced from the

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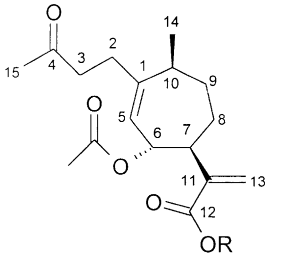
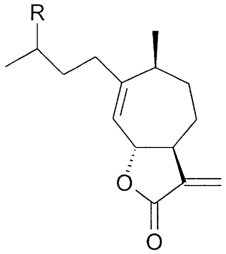
coupling constant (J = 10.2 Hz). Additionally, irradiation of the signal at δ 5.33 (H-6) enhanced the signal at δ 1.03 (H-14). The structure of **1** was establish to be 6-α-acetyl-4-*O*-oxobedfordiaic acid.



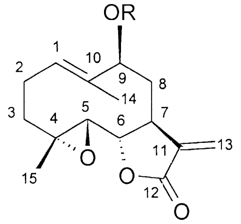
1 R = H

2 R = CH_3



3 R = CO

4 R = α–OH



5 R = $COCH_2\text{-}C(CH_3)\text{=}CH(CH_3)$

6 R = H

The ^{1}H NMR spectral data of compound **2** were identical with those of **1** except the presence of an additional methyl signal at δ 3.70, which showed a correlation with a carbon signal at δ 51.06 in HMQC. The isolation of a large amount of compound **2** (10 mg) allowed us to run the HMBC, which showed important correlations: H-2 and H-15 with C-1, the methoxyl signal at δ 3.70 with C-11 and H-13 with C-11. The other proton and carbon signals were almost identical with those of **1** (Tables 1 and 2). Therefore, compound **2** was the methyl ester of **1**. The EI mass spectrum of **2** was similar to the corresponding spectrum of compound **1**. The $[M]^+$ was below detection limit and fragment ions are all shifted by 14 mu. Exact mass determination of the $[M–CH_3COOH]^+$ ion at *m*/*z* 262.1550 established the molecular formula of **2** as $C_{18}H_{26}O_5$. The structure of **2** was establish to be 6-α-acteyl-4-*O*-oxobedfordiaic methyl ester. The fragmentation pattern of **1** and **2** was given in Fig. 1, and can be explained as following: a fragment at *m*/*z* 237 (15%) is due to the elimination of C_2H_5 from the $[M–CH_2CO]^+$ ion. This ion can be formed by cleavage of the C9–C10 bond, transfer of a hydrogen from C6 to C9 and breaking of the C7–C8 bond. The fragment ion at *m*/*z* 230 (12%) may be produced by elimination of water from the $[M–CH_3CO_2H]^+$ ion. The strong peak at *m*/*z* 194 (71%)

Table 1
^{1}H NMR spectral data of compounds **1, 2** and **5** (400 MHz, $CHCl_3$, δ-values)

	1	**2**	**5**
H-1			5.46 *dd* (J = 12.0, 3.8 Hz)
H-2a[a]	1.69 *m*	1.71	2.25
H-2b	2.30 *m*	2.25	2.45
H-3a[a]	1.69 *m*	1.70	1.25
H-3b	2.31 *m*	2.30	2.15
H-5	5.71 *br s*	5.72	2.69 *d* (J = 8.9 Hz)
H-6	5.33 *dd* (J = 10.2, 1.5 Hz)	5.32	3.84 *d* (J = 11.0 Hz)
H-7	2.50 *ddd* (J = 10.2, 10.2, 3.1 Hz)	2.50	2.91
H-8a[a]	1.55 *m*	1.51	2.00
H-8b	1.86 *m*	1.87	2.15
H-9a[a]	1.70 *m*	1.67	5.20 *dd* (J = 10.7, 2.5 Hz)
H-9b	2.42 *m*	2.45	
H-10	2.02 *m*	2.0	
H-13a	5.60 *br s*	5.50	5.68 *d* (J = 3.0 Hz)
H-13b	6.20 *br s*	6.05	6.36 *d* (J = 2.9 Hz)
H-14	1.03 *d* (J = 7.5 Hz)	1.0	1.72 s
H-15	2.15 *s*	2.14	1.31
OCH_3		3.70 *s*	
AcO	1.90 *s*	1.86	

H-2′, 3.06 (s), H-4′, 4.41 (*dq*, J = 6.4, 1.5 Hz), H-5′, 1.61 (*d*, J = 6.4 Hz), H-6′, 1.76 (s).

[a] Overlapped.

Table 2
^{13}C NMR spectral data of compounds **1, 2** and **5** (400 MHz, $CHCl_3$, δ-values)

	1[a]	**2**[b]	**5**[c]
C-1	146.65 *s*	146.70	127.60 *d*
C-2	33.60 *t*	33.83	23.90
C-3	37.54 *t*	37.65	36.20
C-4	214.52 *s*	214.50	61.20
C-5	115.41 *d*	115.26	66.10
C-6	70.81 *d*	70.78	81.70
C-7	43.06 *d*	43.05	44.10
C-8	29.80 *t*	29.79	36.00
C-9	29.35 *t*	29.27	80.90 d
C-10	34.49 *d*	34.55	133.10 s
C-11	157.01 *s*	158.20	138.10
C-12	169.60 *s*	165.57	168.60
C-13	124.90 *t*	122.41	121.90 d
C-14	16.70 *q*	16.75	11.70 q
C-15	20.30 *q*	20.29	17.3
OCH_3		51.06 *q*	
AcO	167.41 *s*, 27.29 *q*	167.46, 27.236	

[a] In $CDCl_3$ with drops of CD_3OD.

[b] The assignment was confirmed by HMQC and HMBC.

[c] C-1′, 170.48 (*s*), C-2′, 37.7 (*t*), C-3′, 128.5 (*s*), C-4′,123.4 (*d*), C-5′, 13.0 (*q*), C-6′, 23.8 (*q*).

result from elimination of the side chain at C1 together with a transferred H-atom in the [M–CH_2CO]$^+$ ion.

Compound **5** had a close NMR spectral data to **6.** However, the ^{1}H NMR of **5** showed H-9 more downfield at δ 5.20 (dd, $J = 2.5$, 10.7 Hz), which proved the acylation of H-9 (C=O, IR: $\nu = 1770$ cm^{-1}). This was in accordance with the presence of the following new signals: two olefinic methyl at δ 1.76 (s) and 1.61 (d, $J = 6.5$ Hz), two proton singlet signal at δ 3.06 (*br s*). Additionally, ^{13}C NMR spectrum exhibited six new carbon signals at δ 170.48 (s), 128.50 (s), 123.40 (d), 37.60 (t), 23.80 (q) and 13.00 (q). The acyl moiety could be determined by the aid of ^{1}H–^{1}H COSY, HMQC and HMBC as: $COCH_2C(CH_3){=}CH(CH_3)$. The most important HMBC correlations were observed between H-2′ with C-1′, C-4′ and C-6′, between H-5′ with C-3′. Moreover, all proton and carbon signals of the germacranolide skeleton were determined by ^{1}H–^{1}H COSY, ^{13}C, DEPT, HMQC and HMBC (Tables 1 and 2). Although the coupling constants of **5** were identical with those of the known compound, **6,** the stereochemistry of **5** was supported by NOE experiments. Irradiation of H$_\alpha$-7 (δ 2.91) enhanced H$_\alpha$-9 (δ 5.20). The FAB mass spectrum of **5** exhibited quasi molecular ions [M+Na]$^+$ at *m/z* 383 and [M+H]$^+$ at *m/z* 361, in accordance with the molecular formula $C_{21}H_{28}O_5$. An intense [M+Na]$^+$ ion could also be detected by ESI-ICR mass spectrometry at *m/z* 383, dissolved in methanol–dichloromethane (5:1) containing 1.1% sodium chloride. Exact mass determination, 383.1830, confirmed the elemental composition as $C_{21}H_{28}O_5Na$. Therefore, the structure of **5** was establish to be 9β-(3-methyl-pentoyl-3-ene)-parthenolide.

The structures of known compounds inusoniolide **3** (Bloszyk et al., 1990), 4-*O*-dihydroinusoniolide **4** (Marco et al., 1993) and 9β-hydroxyparthenolide **6** (Abdel Sattar et al., 1996) have been deduced by comparison of their spectral data with those in literature.

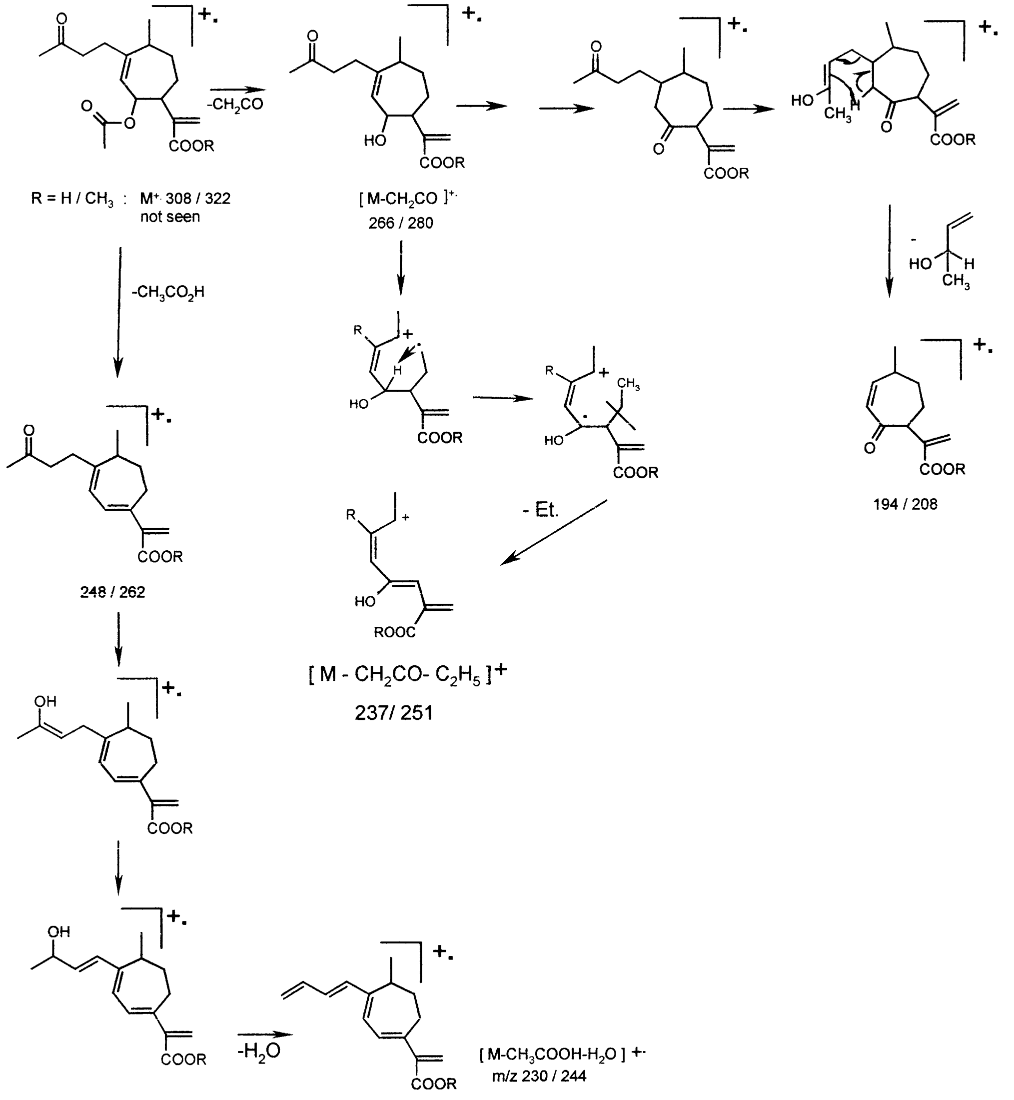


Fig. 1. The proposed fragmentation pattern of compounds **1** and **2**.

In conclusion, the chemistry of *I. verbascifolia* was in agreement with other species of the genus *Inula*, *I. salsoloides* afforded germacranolides (Jeske et al., 1996; Zhou et al., 1994), while *I. aschersoniana* gave xanthanolides (Bloszyk et al., 1990).

3. Experimental

3.1. General

^1H, ^{13}C NMR and 2D spectra measured with a Bruker AMX-400 spectrometer, with TMS as an internal standard. EI mass spectra were recorded on a TSQ-70-Triple Stage Quadrupole mass spectrometer (70 eV). ESI-mass spectra were acquired by means of APEX 11-FTICR-MS (4.7 T, Bruker-Daltonik, Bremen, Germany). The IR spectra (oily film, $CHCl_3$) were taken on Perkin Elmer FT-IR- spectrometer. Optical rotations were deduced with a JASCO-20C automatic recording spectropolarimeter.

3.2. Plant material

The aerial parts of *I. verbascifolia* subsp. *methanea* were collected from Mt. Parnitha (Attiki), Greece, in July 1998. The plant material was identified by Dr. Th. Constantinidis, Institute of Systematic Botany, Department of Biotechnology, Agricultural University of Athens. A voucher specimen of the collection (No. I-1) has been deposited in the Herbarium of the University of Patras (UPA).

3.3. Extraction and isolation

The MeOH–CH_2Cl_2 (1:1) extract (10 g) of the aerial parts (600 g) of *I. verbascifolia* was fractionated by flash column chromatography (5×55 cm) over silica gel (1 kg) eluting with *n*-hexane with an increasing amount of CH_2Cl_2. The fraction (100%, *n*-hexane, 1 l) contained hydrocarbons and waxes. The second fraction (*n*-hexane–methylene choride 3:1, 2 l) gave a crude material which was purified by a Sephadex LH-20 (3×35 cm, *n*-hexane-methylene-methanol 7:4:0.5, 300 ml) to give 4-*O*-dihydroinusoniolide **4** (9 mg), **3** (25 mg) and **5** (12 mg). The third fraction (methylene chloride, 100%) was further purified by a Sephadex LH-20 (3×35 cm, *n*-hexane-methylene-methanol, 7:4:1, 500 ml) to afford the compounds **6** (12 mg) and **1** (3 mg).

6-α-acetyl-4-*O*-oxobedfordiaic acid (**1**): obtained as gummy material; $[\alpha]_D^{25}$: −15.5° (MeOH, $c=0.77$); IR (KBr film) cm^{-1}: 3439, 1758, 1720, 1648, 1627, 1262, 1020, 803; EIMS *m/z* (rel. int): 266 $[M–CH_2CO]^+$(7), 248 $[M–CH_3COOH]^+$ (30), 237 $[M–CH_2CO–C_2H_5]^+$ (15), 230 $[M–CH_3COOH–H_2O]^+$ (12), 194 $[M–CH_2CO–C_4H_8O]^+$ (71); HREIMS $[M–CH_3COOH]^+$ *m/z* 248.1390 (calc. for $C_{15}H_{20}O_3$, 248.14120).

6-α-acetyl-4-O-oxobedfordiaic methyl ester (**2**): obtained as oil; $[\alpha]_D^{25}$ −19.53° (MeOH, $c=0.78$); IR (KBr film) cm^{-1}: 753, 1716, 1651, 1626, 1264, 85; EIMS *m/z* (rel. int): 280 $[M–CH_2CO]^+$(5), 262 $[M–CH_3COOH]^+$ (35), 251 $[M–CH_2CO–C_2H_5]^+$ (11), 244 $[M–CH_3COOH–H_2O]^+$ (8), 208 $[M–CH_2CO–C_4H_8O]^+$ (65); HREIMS $[M–CH_3COOH]^+$ *m/z* 262.1550 (calc. for $C_{18}H_{26}O_5$, 262.15690).

9β-(3-methyl-pentoyl-3-ene)-parthenolide (**5**): obtained as white powder; $[\alpha]_D^{25}$: −32.0° (MeOH, $c=0.96$); IR (KBr film) cm^{-1}: 1770, 1722, 1665, 1650, 1140, 996; FABMS $[M+Na]^+$ *m/z* 383, $[M+H]^+$ *m/z* 361; ESI-ICR MS: *m/z* 383.1830 (calc. for $C_{21}H_{28}O_5+Na$, 383.1829).

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