Cytotoxic Activity of Diterpenoids Isolated from the Aerial Parts of *Elaeoselinum asclepium* subsp. *meoides*

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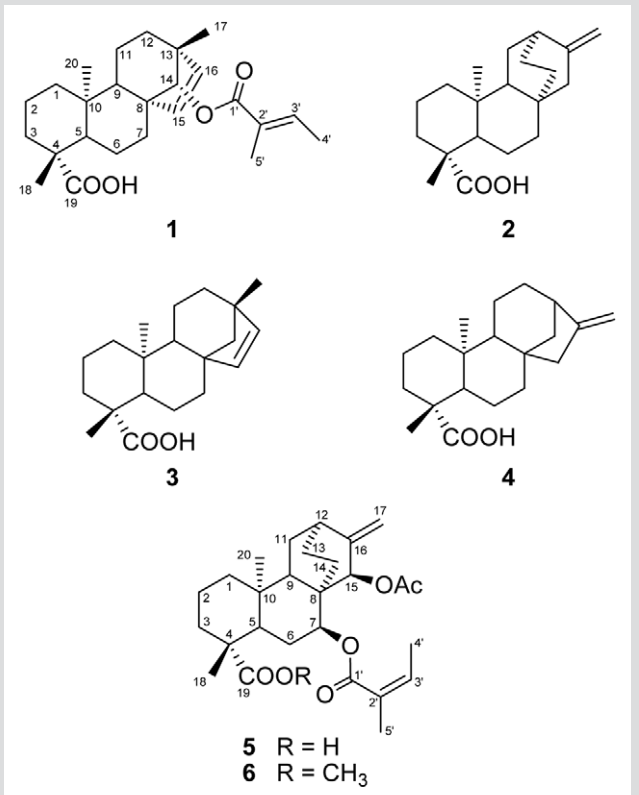
Abstract

The phytochemical investigation of the acetone extract of the aerial parts of *Elaeoselinum asclepium* (L.) Bertol. subsp. *meoides* (Desf.) Fiori afforded several known diterpenoids as well as meoidic acid (**5**), new in the literature. The cytotoxic activities of elasclepic acid (**1**), *ent*-atis-16-en-19-oic acid (**2**), *ent*-beyer-15-en-19-oic acid (**3**), *ent*-kaur-16-en-19-oic acid (**4**) and meoidic acid (**5**) were investigated on rat glioma C6 cells by evaluation of cell growth inhibition.

Key words

Elaeoselinum asclepium subsp. *meoides* · Umbelliferae · diterpenoids · meoidic acid · cytotoxic activity



The former genus *Elaeoselinum* W. D. J. Koch ex DC., (Umbelliferae) *in sensu latu* [1] included two taxa, *Distichoselinum* and *Margotia* [2], [3], that are now segregated as monotypic genera. Only seven species belong to the genus *Elaeoselinum* s. l. [4] and all are confined to the Mediterranean region, mainly in western regions and Morocco.

E. asclepium (L.) Bertol. s. l. is the only taxon present in Italy and no phytochemical investigations have been published on this isotype, in fact, all the species studied so far have a Spanish origin [5].

E. asclepium subsp. *meoides* is a rare species growing in the mountains of Sicily. Investigation of the root of this subspecies, collected in Spain, resulted in the isolation of two *ent*-beyerane diterpenoids: elasclepiol and elasclepic acid (**1**) [6]. In this work, the results of the investigation on the phytochemical composition of the aerial parts, not previously studied, and the cytotoxic activity of some diterpenes (**1 – 5**) are reported.

Examination of the acetone extract of the aerial parts of *Elaeoselinum asclepium* (L.) Bertol. subsp. *meoides* (Desf.) Fiori yielded four known diterpenoids: elasclepic acid (**1**) [6], *ent*-atis-16-en-19-oic acid (**2**) [7], *ent*-beyer-15-en-19-oic acid (**3**) [7] and *ent*-kaur-16-en-19-oic acid (**4**) [8], and the new atisane derivative meoidic acid (**5**).

The structure of compound **5** was determined by spectral methods: positive ESI-MS and homo- and heteronuclear 1 D and 2 D NMR experiments. In fact, its elemental analysis and ESI-MS were in agreement with a formula of $C_{27}H_{38}O_6$. The ^{1}H- and ^{13}C-NMR spectra showed signals for an exocyclic double bond (δ_H = 5.00, 1H, brs, H-17a; δ_H = 4.90, 1H, brs, H-17b; δ_C = 111.5, CH_2, C-17; δ_C = 151.6, C, C-16), a diagnostic quartet of quartets of an angeloxy ester at δ_H = 6.01, 1H, qq, H-3′, whose presence was confirmed by the signals of the two vinylic methyl groups (δ_H = 2.01, 3H, dq, CH_3 – 4'; δ_C = 15.8, CH_3, CH_3 – 4′; δ_H = 1.92, 3H, quint, CH_3 – 5′; δ_C = 21.0 CH_3, CH_3 – 5′) and of the two quaternary carbons at δ_C = 167.0 C, C-1′ and δ_C = 128.8 C, C-2′. Furthermore, there was clear evidence for the presence of two tertiary methyl groups (δ_H = 1.18, 3H, s, Me-18; δ_C = 20.6, CH_3, C-18; δ_H = 0.98, 3H, s, Me-20; δ_C = 12.5 CH_3, C-20), a carboxylic group (δ_C = 182.7, C, C-19), an acetyl and two methines carrying the two esters (δ_H = 5.36, 1H, brs, H-15; δ_C = 75.1, CH, C-15; δ_H = 5.07, 1H, t, H-7; δ_C = 74.8 CH, C-7). Extensive 2 D NMR experiments (^{1}H-^{1}H COSY, HMBC, HSQC) clearly indicated the presence of the atisane skeleton as well as the position of the substituents and allowed to assign all the protons and carbons (**Table 1**).

A survey of the literature showed that this compound was the de-methyl derivative of margotianin (**6**), an atisane diterpenoid from *Elaeoselinum gummiferum* [9], isolated several years ago.

This information allowed the structure assignment as depicted in formula **5** and the trivial name of meoidic acid was given to it. Furthermore, treatment of **5** with diazomethane afforded margotianin (**6**) [9] confirming the structure and the absolute configuration of meoidic acid.

In **Table 1**, the full NMR signal assignments of compound **1** are also reported. These data were not reported previously.

The biological activity of the isolated compounds was investigated on the rat glioma cell line (C6 line) by evaluation of cell growth and viability [10]. Since gliomas show a poor response to cytotoxic drugs, they are very resistant to most chemotherapeutic agents. Although many advances in antineoplastic thera-

Table 1 Spectroscopic data of compounds **1** and **5** in $CDCl_3$

	1		5		
	H (J Hz)	C	H (J Hz)	C	HMBC
1	1.72, 0.96	40.0 t	1.72, 1.05	39.9 t	H-3b, Me-20
2	1.46, 1.26	19.1 t	1.87, 1.49	18.7 t	
3	2.18, 1.06	37.7 t	2.24, 1.09	38.0 t	Me-18
4		43.6 s		43.2 s	H-5, H-3b, Me-18
5	1.13	56.2 d	1.67	49.2 d	Me-18, Me-20
6	1.85, 0.94	20.6 t	2.28, 1.92	26.3 t	H-7, H-5
7	1.82, 1.19	32.1 t	5.07 t (3.0)	74.8 d	H-15
8		53.2 s		38.9 s	H-5
9	1.20	52.0 d	2.22	39.9 d	H-15, Me-20
10		37.9 s		37.9 s	
11	1.45, 1.16	19.8 t	1.72, 1.49	26.0 t	
12	1.40 (2 H)	32.4 t	2.36	35.4 d	H-15, H-17a, H-17b
13		48.2 s	1.57, 1.24	26.4 t	
14	4.54 brs	94.3 d	1.19 (2 H)	26.6 t	
15	5.71 d (5.7)	132.1 d	5.36 brs	75.1 d	H-17a, H-17b
16	5.44 d (5.7)	133.5 d		151.6 s	H-15, H-17a, H-17b
17			5.00 brs, 4.90 brs	111.5 t	H-15
Me-17	0.95 s	19.2 q			
Me-18	1.24 s	29.0 q	1.18 s	28.6 q	H-3b
19		183.1 s		182.7 s	H-5
Me-20	0.70 s	14.1 q	0.98 s	12.5 q	H-5, Me-18, H-3b, H-9, H-1a
1′		168.4 s		167.0 s	H-7, Me-4′, Me-5′
2′		128.8 s		128.8 s	Me-4′, Me-5′
3′	6.83 qq (7.0, 1.0)	136.7 d	6.01 qq (7.0, 1.0)	136.5 d	Me-4′, Me-5′
Me-4′	1.78 brd (7.0)	14.3 q	2.01 dq (7.0, 1.0)	15.8 q	
Me-5′	1.81 brd (1.0)	12.1 q	1.92 quint (1.0)	21.0 q	H-3′
Ac				170.4 s	H-15
Me-Ac			1.95 s	21.7 q	

py have taken place, the rat C6 glioma cell line, like most malignant gliomas encountered clinically, is resistant to many pharmacological treatments [11]. Compared to primary neural cells, the C6 cell line offers some major advantages like easy accessibility and culture as well as a good availability of high cell numbers. Thus, the rat C6 glioma-derived cell line is widely used in biological research.

For bioactivity experiments, the C6 cells were continuously treated for 48 h with various concentrations of each compound and, after cell counting, the MTT assay was performed [12]. No cell growth inhibitory activity was observed for compounds **1**, **2**, **4** and **5** at a concentration below 150 μM, whereas compound **3** exhibited an IC_{50} value of 77 ± 16 (results are expressed as mean ± S.D., n = 24). The positive control for cytotoxicity was performed using digitonin (Calbiochem), a non-ionic lytic detergent, which showed an IC_{50} value lower than 1 μM on C6 glioma cells.

Materials and Methods

General: Optical rotations were determined on a JASCO P-1010 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Bruker AVANCE 300 MHz NMR spectrometer, using the residual solvent signal (δ = 7.27 in ^{1}H and δ = 77.00 in ^{13}C) as reference. ^{1}H-NMR and ^{13}C-NMR assignments were determined by DEPT, ^{1}H-^{1}H COSY, HMBC, HSQC spectra. ESI-MS was obtained with an Applied Biosystem API-2000 mass spectrometer (Applied Biosystem). Elemental analysis was carried out with a Perkin-Elmer 240 apparatus.

Plant material: The aerial parts of *Elaeoselinum asclepium* (L.) Bertol. subsp. *meoides* (Desf.) Fiori were collected in June 2006 near Dingoli, Piana degli Albanesi (Palermo) in Sicily, Italy and voucher specimens (PAL 06 – 597, authenticated by Prof. F. Raimondo) were deposited in the Herbarium of the Botanical Garden of Palermo, Italy.

Extraction and isolation: Dried and finally powdered aerial parts (671 g) of *E. asclepium* subsp. *meoides* were extracted with Me_2CO (5 L) at room temperature for a week. After filtration, the solvent was evaporated to give a gum (22 g) which was chromatographed on silica gel (Merck No. 7734, deactivated with 15 % H_2O, 400 g) column (5 × 20 cm) eluted with petroleum ether, petroleum ether-EtOAc mixtures, EtOAc and EtOAc-MeOH mixtures. 500 mL fractions were collected as follows: 1 – 12, petroleum ether; 13 – 18, petroleum ether-EtOAc (9 : 1); 19 – 22, petroleum ether-EtOAc (4 : 1); 23 – 26, petroleum ether-EtOAc (7 : 3); 27 – 30, petroleum ether-EtOAc (3 : 2); 31 – 34, petroleum ether-EtOAc (1 : 1); 35 – 38, petroleum ether-EtOAc (2 : 3); 39 – 42, petroleum ether-EtOAc (3 : 7); 43 – 46, petroleum ether-EtOAc (1 : 4); 47 – 50, petroleum ether-EtOAc (1 : 9); 51 – 54, EtOAc; 55 – 58, EtOAc-MeOH (9 : 1); 59 – 62, EtOAc-MeOH (4 : 1). Fractions 23 – 26 were re-chromatographed on a silica gel (150 g) column (2 × 20 cm). Elution with *n*-hexane-EtOAc (4 : 1, 2 L), 100 mL fractions being collected, gave a mixture of three compounds (400 mg). Fractions 27 – 34 were re-chromatographed on silica gel (150 g) column (2 × 20 cm). Elution with *n*-hexane-EtOAc (7 : 3, 2 L), 100 mL fractions being collected, gave a mixture of three compounds (1 g) and pure elasclepic acid (**1**).

Radial chromatography of 100 mg of the mixture, using silica gel (45 g, Merck 1.07749.1000) impregnated with $AgNO_3$ (5 % w/w), eluted with *n*-hexane-EtOAc (4 : 1, 800 mL), 20 mL fractions being collected, gave in order of elution *ent*-atis-16-en-19-oic acid (**2**), *ent*-beyer-15-en-19-oic acid (**3**) and *ent*-kaur-16-ene-19-oic acid (**4**).

Fractions 35 – 38 were re-chromatographed on a silica gel column. Elution with *n*-hexane-EtOAc (1 : 1, 1 L), 50 mL fractions being collected, gave meoidic acid (**5**, 100 mg). The physical and spectroscopical data of compounds **1 – 4** and **6** were in complete agreement with those reported in the literature.

Elasclepic acid (**1**): 300 mg; $[\alpha]_D^{25}$: –85.2 (*c* 0.28, EtOH) [6].

ent-Atis-16-en-19-oic acid (**2**): 13 mg; $[\alpha]_D^{25}$: –68.8 (*c* 0.41, $CHCl_3$) [13].

ent-Beyer-15-en-19-oic acid (**3**): 30 mg; $[\alpha]_D^{25}$: + 7.3 (*c* 0.60, $CHCl_3$) [13].

ent-Kaur-16-en-19-oic acid (**4**): 20 mg; $[\alpha]_D^{25}$: –111.4 (*c* 0.19, $CHCl_3$) [14].

Meoidic acid (**5**): 100 mg; oil; $[\alpha]_D^{22}$: –47.5: (*c* 0.6, $CHCl_3$); ESI-MS (positive mode): *m/z* = 497 [M + K] (6), 481 [M + Na] (56), 459 [M + H] (100); anal. calcd. for $C_{27}H_{38}O_6$ (458.59): C 70.71, H 8.35; found: C 70.68, H 8.37; ^{1}H-NMR and ^{13}C-NMR: see **Table 1**.

Margotianin (**6**): 20 mg; obtained after treatment of compound **5** (20 mg) with CH_2N_2; $[\alpha]_D^{25}$: –80.2 (*c* 0.17, $CHCl_3$) [11].

Cell culture and cytotoxic assay **:** The cytotoxic assays were performed according to literature procedures [12].

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