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Original Article

Isolation and identification of osthol from the fruits and essential oil composition of the leaves of *Prangos asperula* Boiss.

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Abstract

Coumarins are bioactive secondary metabolites and could be found in the fruits of Umbelliferae. *Prangos asperula* Boiss. is an Iranian native plant which is found wild in many regions of Iran. Osthol, a prenylated coumarin, was isolated from the hexane extract of the fruits of *P. asperula* and its structure was elucidated using ^{1}HNMR, ^{13}CNMR, IR and MS spectra. The essential oil of the aerial parts of the plant obtained by hydrodistillation was also investigated by GC-MS. Forty-seven constituents have been identified of which 2,3,6-trimethyl benzaldehyde (18.4%), δ-3-carene (18.0%) and α-pinene (17.4%) were the main constituents of the oil.

Keywords: *Prangos asperula*; Essential oil; Coumarin; Osthol

INTRODUCTION

The genus *Prangos* (Jashir in Persian), which belongs to Umbelliferae family, consists of about 30 species (1). Fifteen species are found wild in many regions of Iran among which five are endemic (2). Fruits of some *Prangos* species have been traditionally used in Iran as emollient, carminative and tonic (3). There are also some reports on antibacterial (4,5) and antioxidant (6,7) activities of different *Prangos* species.

Phytochemical investigations on different species of *Prangos* have led to the isolation of coumarins (8-10) and volatile oils (11-15) from different parts of the plants.

Prangos asperula Boiss. is a native plant growing wild in many parts of Iran (16). Fruits of the plant are used as provender for mutton in Iran (17). Continuing our previous investigation on *Prangos*, (13) here we report essential oil composition of the leaves of *P. asperula* as well as the occurrence of a coumarin, osthol, in the fruits for the first time.

MATERIALS AND METHODS

General procedures for osthol identification

The IR spectrum was recorded on a Rayleigh WQF-510 FTIR instrument. The ^{1}HNMR was recorded on a Brucker (500 MHz) instrument, using CDCl3 as solvent and TMS as internal standard. EI-MS spectrum was recorded on QP-1000EX mass spectrometer. Compounds on the TLC (Silicagel $60GF_{254}$ precoated plates, Merck) were detected at 365 nm by KOH as spraying reagent.

Plant material

Fruits of *P. asperula* were collected from Dena Mountains, west of Iran, in July 2005. The plant identity was confirmed by Botany Department of Yasouj University. Voucher specimens of the plant is deposited at the Herbarium of School of Pharmacy and Pharmaceutical Sciences, Isfahan, Iran (No. 1126).

Coumarin isolation

The air-dried grounded fruits (200 g) of *P. asperula* were extracted with *n*-hexane, using

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a soxhlet apparatus for 4 h. The solvent was evaporated under reduced pressure to 50 ml. The hexane extract was cooled to 4°C in a refrigerator for several days to render a semi pure white to pale yellow mass. For further purification, they were washed with chilled *n*-haxane for several times. Later, the sample was subjected for recrystallization process until resulted pure crystalline.

Essential oil isolation:

Plant material was hydrodistilled in a Clevenger-type apparatus for 3 h according to the method recommended in the British Pharmacopoeia (18). The volatile oil was dried over anhydrous sodium sulfate and stored in sealed vial at 4 °C until analysis. The yield of oil was calculated based on dried weight of plant material.

GC-MS analysis

Gas chromatography combined with mass spectrometry (GC-MS) was used for identification of the components. The analysis was performed on a Hewlett-Packard 5972A mass selective detector coupled with a Hewlett-Packard 6890 GC, equipped with a HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven temperature was programmed from 60-280 °C at 4 °C/min. Helium was used as carrier gas at a flow rate of 2 ml/min. Injector and detector temperatures were 280 °C. The MS operating parameters were: ionization voltage, 70 eV; ion source temperature, 200 °C.

Identification of the components of the essential oil

Identification of components of the oil was based on GC retention indices relative to *n*-alkanes and computer matching with the Wiley275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (19, 20).

RESULTS

The structure of light yellow crystals isolated with melting point of 83 °C (Fig. 1) was elucidated using, ^{1}HNMR, ^{13}CNMR, MS and IR spectra as well as comparison of the data with those reported in the literature (21). The analytical data are as below:

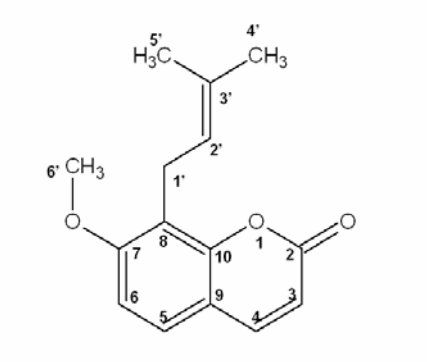

Fig. 1. Structure of osthol

^{1}HNMR (500 MHz, CDCl3, J in Hz) δ: 1.69 (3H, *s*, H-5'), 1.86 (3H, *s*, H-4'), 3.55 (2H, *d*, J=7.3 Hz, H-1'), 3.94 (3H, *s*, H-6'), 5.25 (1H, *t*, J=7.3 Hz, H-2'), 6.2 (1H, *d*, J=9.5 Hz, H-3), 6.8 (1H, *d*, J=8 Hz, H-6), 7.3 (1H, *d*, J=8 Hz, H-5), 7.6 (1H, *d*, J=9.5 Hz, H-4); ^{13}CNMR (125 MHz, CDCl3) δ: 18.34 (C-5'), 22.34 (C-1'), 26.20 (C-4'), 56.46 (C-6'), 107.78 (C-6), 113.34 (C-3), 113.39 (C-9), 118.32 (C-8), 121.57 (C-2'), 126.66 (C-5), 133 (C-3'), 144.19 (C-4), 153.22 (C-10), 160.64 (C-7), 161.78 (C-2); EI-MS *m/z* (rel. int.): 244 $[M]^{+}$ (100), 229 (78), 213 (44), 201 (68), 189 (60), 131 (32), 77 (22); FT-IR (KBr): ν_{max} = 1717, 1604, 1500, 1160, 830 cm^{-1}.

The EI-MS showed a molecular ion peak at m/z 244 (M+, base peak) in agreement with the proposed structure of the known prenylated coumarin, osthol, with $C_{15}H_{16}O_3$ molecular formula. Ion peak at m/z 213 is due to the cleavage of a methoxy moiety from the molecule. The IR spectrum with the peaks at 1717 cm^{-1} (coumarinic carbonyl), 1160 cm^{-1} (C-O streaching) and 1500; 1604 cm^{-1} (aromatic C=C) confirms the skeleton of osthol.

The ^{1}HNMR spectrum displayed characteristic signal for a methoxy group at δ 3.9 (s). The ^{1}HNMR spectrum of the compound showed two proton doublets at δ 6.2 (*J*=9.5 Hz) and 6.8 (*J*=8 Hz) characteristic for the H-3 and H-6 of the isolated compound. The presence of further two proton doublets at δ 7.3 (*J*= 8 Hz) and 7.6 (*J*= 9.5 Hz) indicated the presence of H-5 and H-4 in the furan ring of coumarin, respectively.

Table1. Percentage composition of the oil of *Prangos asperula* leaf

No	Compound	Percentage	RI
1	α-thujene	0.1	927
2	α-pinene	17.4	937
3	camphene	1.8	950
4	sabinene	2.7	974
5	β-pinene	0.5	977
6	myrcene	1.3	990
7	mesitylene	0.3	993
8	δ-3-carene	18.0	1010
9	p-cymene	3.2	1025
10	β-phellandrene	3.4	1030
11	*cis*-β-ocimene	t	1037
12	*trans*-β-ocimene	t	1047
13	γ-terpinene	0.1	1059
14	artemisia alcohol	0.1	1083
15	terpinolene	0.5	1086
16	linalool	0.1	1097
17	*cis*-p-menth-2-en-1-ol	t	1121
18	α-campholenal	0.1	1125
19	*cis*-limonene oxide	0.1	1134
20	*trans*-verbenol	0.9	1146
21	menthofuran	0.3	1165
22	terpinene-4-ol	0.5	1176
23	p-cymen-8-ol	0.6	1184
24	pulegone	t	1237
25	verbenone	0.1	1206
26	*trans*-carveol	t	1218
27	cuminal	0.1	1237
28	*cis*-chrysanthenyl acetate	4.9	1262
29	bornyl acetate	8.5	1285
30	*trans*-sabinyl acetate	0.3	1292
31	2,3,6-trimethyl benzaldehyde	18.4	1355
32	β-bourbonene	0.1	1380
33	*trans*-caryophyllene	t	1415
34	*trans*-β-ionone	0.1	1483
35	α-selinene	0.2	1493
36	*cis*- α-bisabolene	0.1	1502
37	δ-cadinene	1.1	1521
38	spathulenol	0.6	1574
39	caryophyllene oxide	0.3	1578
40	T-cadinol	0.7	1637
41	α-cadinol	1.2	1651
42	oplopanone	0.2	1734
43	neophytadiene	0.3	1833
44	osthol	9.5	2140
45	hexadecanoic acid	t	1969
46	n-tricosane	t	2295
47	n-pentacosane	0.1	2526

RI = retention indices on HP-5MS capillary column.

t = trace (< 0.05%).

Aerial parts of *P. asperula* yielded 0.2% v/w of a clear yellow-green volatile oil. The identified components and their percentages are given in Table 1, where the components are listed in order of their elution on the HP-5MS column. Forty-seven components, representing 98.8% of the total oil composition, were identified. 2,3,6-trimethyl benzaldehyde (18.4%), δ-3-carene (18.0%) and α-pinene (17.4%) were the main constituents of the oil. The oil of aerial parts of *P. asperula* consisted of thirty monoterpene

(84.0%), eleven sesquiterpene (4.6%) and six non-terpenic compounds (10.2%). As it could be concluded, the oil is characterized by a high content of monoterpenes and low amount of sesquiterpenes.

DISCUSSION

There are many studies referring to the composition of the essential oil of the fruits or aerial parts in the genus *Prangos* (11-13). For example, β-caryophyllene (18.2%), germacrene D (17.2%) and limonene (8.7%) are reported as the main constituents of the volatile oil of aerial parts of *P. uloptera* (14) and α-pinene (25.1%), limonene (16.1%), myrcene (9.5%) are characterized as the major component of the aerial parts oil of *P. latiloba* (15).

According to our previous work on essential oil of the fruits of *P. asperula*, the oil consist of fifty-two constituents, among them, δ-3-carene (16.1%), β-phellandrene (14.7%), α-pinene (10.5%), α-humulene (7.8%), germacrene D (5.4%) and δ-cadinene (4.2%) are found to be the major components (13). However, regarding to current study, 2,3,6-trimethyl benzaldehyde (18.4%), δ-3-carene (18.0%), α-pinene (17.4%), bornyl acetate (8.5%), osthol (9.5%) and *cis*-chrysanthenyl acetate (4.9%) were detected as the main constituents of the aerial parts volatile oil.

Osthol which had been previously isolated from some Umbelliferae family is a prenylated coumarin compound. Prevention of atherosclerosis, suppression of hepatic lipids (22), antitumor (23) and anti-inflammatory activities (24) are the most important biological activities of osthol. Although osthol has been isolated from some *Prangos* species (10), literature survey indicated that this is the first time it has been reported from *P. asperula*.

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