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Artigo

Coumarins from the aerial parts of *Prangos uloptera* (Apiaceae)

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RESUMO: "Cumarinas das partes aéreas de *Prangos uloptera* (Apiaceae)". Os estudos fitoquímicos das partes aéreas de *Prangos uloptera*, uma espécie do gênero *Prangos* endêmica do Irã, forneceram cinco cumarinas, xantotoxina (**1**), prangenina (**2**), escopoletina (**3**), deltoína (**4**) e prangolarina (**5**). As estruturas destas cumarinas foram elucidas através de métodos espectroscópicos e o potencial antioxidante de **1-5** foi avaliado pelo ensaio de DPPH. O significado quimiotaxonômico de **1-5** também é discutido.

Unitermos: *Prangos uloptera*, Apiaceae, Umbelliferae, cumarina, furanocumarina, marmesina, antioxidante, quimiotaxonomia.

ABSTRACT: Phytochemical studies on the aerial parts of *Prangos uloptera*, an endemic Iranian species of the genus *Prangos*, yielded five coumarins, xanthotoxin (**1**), prangenin (**2**), scopoletin (**3**), deltoin (**4**) and prangolarin (**5**). The structures of these coumarins were elucidated by spectroscopic means, and the antioxidant potential of **1-5** was evaluated by the DPPH assay. The chemotaxonomic significance of **1-5** is also discussed.

Keywords: *Prangos uloptera*, Apiaceae, Umbelliferae, coumarin, furanocoumarin, marmesin, antioxidant, chemotaxonomy.

INTRODUCTION

Prangos uloptera DC. (family: Apiaceae *alt.* Umbelliferae) is an endemic Iranian species of the genus *Prangos* that comprises *ca.* 30 species of perennial herbs, distributed in the Mediterranean region, Caucasia, Central Asia, Turkey, Iraq and Iran (Evans, 1989; Mazloomifar et al., 2004; GRIN Databases, 2008). *Prangos* species, commonly known as 'Djashir' in Iran, are widely used in folk medicine as tonic, and for the treatment of flatulence, haemorrhoids, wounds and leukoplakia (Dokonic et al., 2004; Yasuhiro et al., 2001). Previous phytochemical investigations on the fruits and roots of *P. uloptera* revealed the presence of various coumarins, monoterpenes and sesquiterpenes (Abyshev and Denisenko, 1970, 1973; Sefidkon and Navaii, 2001; Mazloomifar et al., 2004). The essential oils obtained from the umbels of this species have recently been shown to contain various terpenoidal compounds (Nazemieyh et al., 2007). As part of our on-going phytochemical and bioactivity studies on Iranian medicinal plants, we now report on the isolation, identification and antioxidant properties of five coumarins from the aerial parts of *P. uloptera*.

MATERIAL AND METHODS

General procedures

UV spectra were obtained in MeOH using a Hewlett-Packard 8453 UV-Vis spectrometer (Agilent, Germany). Optical rotations were measured on a Bellingham Stanley ADP220 polarimeter. CIMS (Chemical Ionisation Mass Spectrometry) analyses were performed in EPSRC Central Mass Spectroscopy Facility in Swansea, UK, on a Micromass Quattro II triple quadrupole instrument (Waters, UK) in chemical desorption mode using ammonia as CI gas. Mass accuracy was within 0.4 Da. CI source temperature was 170 °C and electron energy was 59 eV. NMR spectra were recorded in $CDCl_3$ on a Varian Unity INOVA 400 MHz NMR Spectrometer 400 (400 MHz for ^1H

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and 100 MHz for ^{13}C) using the residual solvent peaks as internal standard. Chemical shifts δ were in ppm. The HMBC (Heteronuclear Multiple Bond Coherence) experiment used J = 9 Hz and had a 55 ms long-range coupling delay. A NOESY experiment was carried out with a mixing time of 0.8 s. Spectra were recorded with a probe temperature of 25 °C. 2,2-Diphenyl-1-picrylhydrazyl (molecular formula $C_{18}H_{12}N_5O_6$, DPPH) and trolox were purchased from Fluka (UK) and were used without further purification. Precoated aluminium sheets silica gel 60 F_{254} (0.25 mm thickness) TLC plates (Merck, Germany) were used. TLC mobile phases were *n*-hexane-ethylacetate (EtOAc) mixtures of various proportions, e.g. 5% EtOAc in *n*-hexane, 10% EtOAc in *n*-hexane, etc. Silica 60G was used for vacuum liquid chromatography (VLC).

Plant material

The aerial parts of *Prangos uloptera* DC., were collected in May 2005, from Mishov-Dagh mountain (altitude 2056 m) in the northwest of Iran. A Voucher specimen (No. 1384-1) was deposited in the herbarium of the Department of Plant Sciences, Faculty of Natural Sciences, Tabriz University, Iran, and also in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

Preparation of plant extracts

The dried and ground aerial parts of *P. uloptera* (270 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane and methanol (0.7 L each). The extracts were concentrated by rotary evaporator at 45 °C.

Isolation and identification of compounds

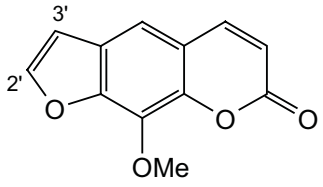
The *n*-hexane extract (2 g) was subjected to vacuum liquid chromatography (VLC) on silica gel, eluting with solvent mixtures of increasing polarity: 100% *n*-hexane, *n*-hexane-EtOAc, 100% EtOAc and 100% MeOH, to yield a number of fractions which upon initial thin layer chromatographic (TLC) analyses were grouped into 16 main fractions. Fractions 14-16 (80-100% EtOAc in *n*-hexane and 100% MeOH) were further analysed by preparative TLC (mobile phase acetone:$CHCl_3$ = 5:95) to afford five coumarins, xanthotoxin (**1**, 10.1 mg, R_f = 0.5), prangenin (heraclenin, **2**, 9.3 mg, R_f = 0.55), 6-methoxy-7-hydroxycoumarin (scopoletin, **3**, 13.0 mg, R_f = 0.43), marmesin angelate (deltoin, **4**, R_f = 0.64, 10.2 mg) and marmesin isovalerate (oxypeucedanin or prangolarin, **5,** 9.2 mg, R_f = 0.60). The structures of the isolated compounds **1**-**5** were elucidated by direct comparison of their UV, specific rotation (where applicable), ESIMS, ^1H and ^{13}C NMR data with published data, and by comprehensive 1D and 2D NMR analyses.

Antioxidant assay

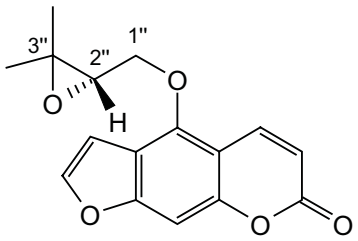
2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemie AG, Bucks. Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Sigma-Aldrich, UK (http://www.sigmaaldrich.com/Area_of_Interest/Europe_Home/UK.html). The method used by Takao et al. (1994) was adopted with suitable modifications (Kumarasamy et al., 2007). DPPH (8 mg) was dissolved in MeOH (100 mL) to obtain a concentration of 80 µg/mL.

Qualitative Analysis: Test samples were applied on a Silica gel TLC plate and sprayed with DPPH solution using an atomiser. It was allowed to develop for 30 min. The colour changes (purple on white) were noted.

Quantitative Analysis: Serial dilutions were carried out with the stock solutions (1 mg/mL) of the compounds (**1**-**5**) to obtain concentrations of 5 x 10^{-1}, 5 x 10^{-2}, 5 x 10^{-3}, 5 x 10^{-4}, 5 x 10^{-5}, 5 x 10^{-6} mg/mL. Diluted solutions (2 mL each) were mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur. The UV absorbance was recorded at 517 nm. The



1



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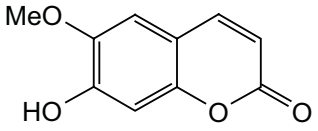
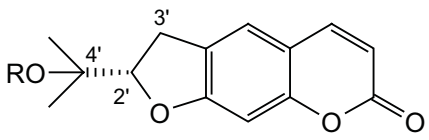


3



4 R = -$COC(CH_3)$=$CHCH_3$

5 R = -$COCH_2CH(CH_3)_2$

Table 1. ^{1}H (coupling constant *J* in Hz in parentheses) coumarins 1-5.

Position	Chemical shift (δ) in ppm				
	1	**2**	**3**	**4**	**5**
3	6.40 d (9.5)	6.32 d (9.8)	6.25 d (9.5)	6.24 d (9.5)	6.24 d (9.5)
4	7.78 d (9.5)	8.24 d (9.8)	7.65 d (9.5)	7.60 d (9.5)	7.60 d (9.5)
5	7.42 s	-	7.44 s	7.24 s	7.24 s
6-OMe	-	-	3.94 s	-	-
8	-	7.19 d (0.5)	6.84 s	6.67 s	6.67 s
8-OMe	4.30 s	-	-	-	-
2’	7.68 d (2.2)	7.63 d (2.3)	-	5.00 t (8.6)	4.98 t (8.6)
3’	6.82 d (2.2)	6.98 d (2.3)	-	3.18 dd (15.8, 8.6)	3.14 dd (15.8, 8.6)
				3.28 dd (15.8, 8.6)	3.20 dd (15.8, 8.6)
4’- 2 x Me	-	-	-	1.50 s	1.49 s
				1.60 s	1.59 s
1’’	-	4.66 dd (6.4, 4.4)	-	-	-
		4.46 dd (6.4, 4.4)			
2’’	-	3.25 d (4.4)	-	-	2.05 d (7.6)
2’’-Me	-	-	-	1.60 s	-
3’’	-	-	-	6.46 q (6.4, 13.2)	1.90 m
3’’-Me	-	-	-	1.56 d (6.4)	-
3’’-2 x Me	-	1.36 s and 1.43 s	-	-	0.80 d (6.6) and 0.80 d (6.6)

Table 2. ^{13}C NMR data of coumarins 1-5.

Position	Chemical shift (δ) in ppm				
	1	**2**	**3**	**4**	**5**
2	161.0	162.4	160.9	161.4	161.3
3	114.7	113.6	112.5	112.7	112.6
4	144.4	144.9	144.3	143.6	143.6
5	113.0	149.0	110.5	123.2	123.2
6	126.3	114.6	146.5	124.6	124.6
6-OMe	-	-	58.7	-	-
7	147.9	158.5	148.4	163.5	163.2
8	132.7	95.3	102.3	97.9	97.9
9	143.8	152.9	148.6	155.8	155.8
10	116.5	107.9	110.8	112.7	112.7
8-OMe	61.0	-	-		
2’	146.7	139.4	-	88.9	88.7
3’	107.2	104.9	-	29.6	29.7
4’	-	-	-	81.9	8.9
4’- 2 x Me	-	-	-	22.1	21.9
				21.3	21.1
1’’	-	-	60.0	167.3	172.3
2’’	-	-	61.5	129.4	44.5
2’’-Me	-	-	-	11.8	25.8
3’’	-		72.8	137.1	-
3’’-Me	-	-	-	14.3	-
3’’- 2 x Me			25.0	-	22.3
			19.4		

experiment was performed in duplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control Trolox®. The RC_{50} value, which is the concentration of the test material that reduces 50% of the free radical concentration, was calculated as mg/mL.

RESULTS AND DISCUSSION

Chromatographic analyses of the *n*-hexane extract of the aerial parts of *P. uloptera* afforded two furanocoumarins, xanthotoxin (**1**, Masuda et al., 1998) and prangenin (**2**, Miftakhova et al., 2001), two dihydrofuranocoumarins, deltoin (**4**, Baba et al., 1989) and prangolarin (**5**, Kapoor et al., 1972; Wei and Ito, 2006), and a simple coumarin, scopoletin (**3**, SDBS Database, 2008). The structures of the isolated coumarins (**1**-**5**) were elucidated by direct comparison of

Table 3. Antioxidant activity of the coumarins (1-5) isolated from *P. uloptera*.

Compounds/Extracts	The DPPH assay (RC_{50} in mg/mL)
1	>1.00
2	>1.00
3	2.43×10^{-2}
4	3.98×10^{-1}
5	3.87×10^{-1}
Trolox	2.60×10^{-3}

their respective UV, specific rotation (where applicable), CIMS, ^{1}H and ^{13}C NMR data with published data, and by comprehensive 1D and 2D NMR analyses. The unambiguous assignment of all ^{1}H and ^{13}C NMR signals for these compounds has been presented in Tables 1 and 2.

Although coumarins have previously been reported from the roots and fruits of *P. uloptera*, to our knowledge, this is the first report on the occurrence of coumarins in the aerial parts of this plant. Also, except xanthotoxin (**1**) and prangenin (**2**), none of these coumarins (**3-5**) have ever been reported from this plant. Xanthotoxin (**1**) and scopoletin (**3**) occur in many genera of the family Apiaceae as well as other families, e.g. Rutaceae. The genus *Prangos* is well-known for producing simple and furanocoumarins with various degrees of oxygenation and prenylations (ISI Database, 2008). Within the genus *Prangos*, prangenin (**2**) occurs in a number of species, including *P. equisetoides* (Kuznetsova et al., 1979), *P. fedtschenkoii*, *P. ferulaceae*, *P. hissarica* (Combined Chemical Dictionary, 2008; ISI Database, 2008), *P. lamellate* (Donchul et al., 1979), *P. pabularia* (Sood et al., 1978; Tada et al., 2002), *P. sarawschanica* (Combined Chemical Dictionary, 2008) and *P. uloptera* (Abyshev and Denisenko, 1973). However, apart from the genus *Prangos*, prangenin (**2**) has also been reported from a few other genera, e.g. *Atalantia*, *Chlamydomonas, Citrus, Eriostemon, Halocnemum, Heracleum*, *Phebalium* and *Stauranthus,* (ISI Database, 2008; Combined Chemical Dictionary, 2008). The occurrence of deltoin (**4**) within the genus *Prangos* is rather restricted mainly to *P. uloptera* and *P. fedtschenkoi,* but it has been isolated from a few other genera, e.g. *Peucedanum, Saposhnikovia, Seseli* and *Zosima.* Marmesin isovalerate (prangolarin or oxypeucedanin, **5**) occurs widely in the family Apiaceae, and also in the genera *Atalantia, Citrus* and *Poncirus* of the family Rutaceae (Combined Chemical Dictionary, 2008).

The antioxidant potential of **1**-**5** was evaluated by the DPPH assay (Kumarasamy et al., 2007). In the qualitative DPPH assay, except **3**, all compounds showed extremely low levels of antioxidant activity by displaying faint white spots against a purple background on the TLC plates. In the quantitative DPPH assay, scopoletin (**3**) was the most active among all compounds, and displayed a significant antioxidant activity with a RC_{50} value of 2.43×10^{-2} (Table 3).

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