Iranian Journal of Pharmaceutical Research (2009), 8 (1): 71-75
Received: November 2007
Accepted: February 2008



*Original Article*

# Phenolic Compounds from *Peucedanum ruthenicum* M. Bieb

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**Abstract**

From methanolic extract of aerial parts of *Peucedanum ruthenicum* M. Bieb (*Apiaceae*) collected from Mazandaran province of Iran, four flavonoids namely Isorhamnetin 3-0-rutinoside 1, rutin 2, quercetin 3, morin 4 and two phenolic acids namely caffeic acid 5 and *p*-coumaric acid 6 have been isolated by Paper Chromatography (PC) and crystallization. Their structures were elucidated by MS, $^{1}H$, $^{13}C$ NMR spectra.

**Keywords:** Aerial parts; *Peucedanum ruthenicum*; *Apiaceae*; Phenolic compound.

## Introduction

There are some 120 species of *Peucedanum* L. (fam. *Apiaceae* subfam. *Apioideae* trib. *Peucedaceae*) widespread in Europe, Mediterranean region and South, Western and central Asia (1). Four Species of *Peucedanum* growing in Iran are: *P. glaucopruinosum* Rech., *P. knappii* Bornm., *P. translucens* KH. Rechinger (2) and *P. ruthenicum*. They are distributed in Iran (3), Europe, Russia and Turkey (4).

*P. ruthenicum* (*Apiaceae*) is a glabrous perennial plant that distributed in the north and central part of Iran (3). Some species of this genus have been used traditionally in treatment of colds (5), coughs due to pathogenic wind-heat, accumulation of phlegm, and heat in the lung (6), anti-tussive, and are used as anti-asthmatic and as a remedy for angina (7). Previous phytochemical studies on this species were indicated the presence of furanocoumarins and their glycoside derivatives, linear-type furanocoumarin glucosides and simple coumarin glucosides (8). From *P. ruthenicum*, a Bulgarian *Umbelliferae*, peucedanin (furanocoumarin) and a coumarin (peuruthenicin) in the roots and rutin (flavonol glycoside) in the flowers (9) have been isolated. Several new coumarins from *P. praeruptorum* Dunn. have been reported (6).

There were some reports related to the chemical analysis of voltile oil of this genus in the literature. The major components of herb and rhizome essential oil of *P. ostruthium* were sabinene (35.2%), 4-terpineol (26.6%), β-caryophyllene (16.1%) and α-humulene (15.8%) (10). The major constituents of *P. verticillare* leaf and branch oil were sabinene and trans-anethole. β-Caryophyllene, α-Phellandrene, cis-β-farnesene and β-bisabolene were components of *P. verticillare* dried fruit oil and sabinene was the constituent of *P. verticillare* fresh fruit oil (11).

In this work the structures of phenolic

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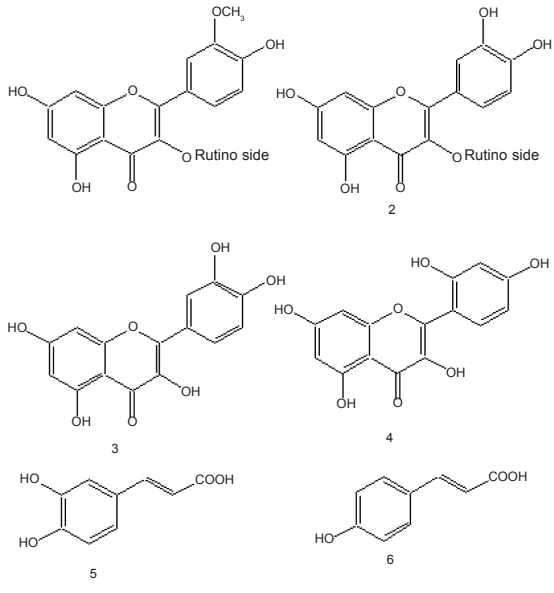


**Figure 1.** The structure of phenolic compounds from *Peucedanum ruthenicum.*

compounds from the aerial part of *P. ruthenicum* M. Bieb is reported.

## Experimental

Melting points were taken on a Reichert-Jung apparatus (Vienna, Austria). Ultraviolet spectra were recorded on a Shimadzu 160A spectrometer (Kyoto, Japan). Electron Ionization Mass Spectra (EIMS) were determined on a Finnigan MAT TSQ 70 (California, USA) at 70eV.$^{1}$H NMR and $^{13}$C NMR spectra were measured in DMSO and $CDCl_3$ with tetramethylsilane (TMS) as an internal standard using a Varian 400 Unity plus spectrometer. FTIR spectra were recorded on a Nicolet 550 spectrometer (Madison, WI, USA). Chromatographic papers were purchased from Whatman Company. All compounds for UV spectral shift reagents, natural product reagent and sugar indicator, solvents, glacial acetic acid, authentic samples of sugars and concentrated $NH_3$ were purchased from Merck Company.

The aerial parts of *P. ruthenicum* were collected in October 2004 from Roodbarak (Mazandaran province) north of Iran and identified by Dr. H. Akhani (Dept. of Plant Biology, Faculty of Science, Tehran University, Tehran, Iran). A voucher specimen is deposited in the private herbarium of Dr. H. Akhani (hb. Akh. Salimian 39).

Dried powdered aerial parts (0.1 kg) of the plant were extracted with pure methanol (2.5 l) by percolation (one week). The solvent was evaporated and dried in vacuum at 45°C to give a gummy residue (13.5 g). This residue was then treated with 10 ml. water and gave a cloudy suspension, which was extracted with chloroform (3×50 ml). The solvent (methanol-water) was evaporated and dried in vacuum at 45°C and the residue (11.2 g) was analyzed on paper chromatography (PC). The chromatograms were developed descendingly in the long direction of Whatman 3 MM chromatographic papers in the chromatocab using BAW (n-butanol: acetic acid: water, 4:1:5) as eluent for 12 h to obtain phenolic compound lines as follow: line1 (17 mg, $R_f$=0.63, purple before and after using $NH_3$ at 366 nm), line 2 (32 mg, $R_f$=0.61, purple color before and after using $NH_3$ at 366 nm), line 3 (27 mg, $R_f$=0.75, purple color before and after using $NH_3$ at 366 nm), line 4 (12 mg, $R_f$=0.88, purple color before and after using $NH_3$ at 366 nm), line 5 (16 mg, $R_f$= 0.53, blue color before and after using $NH_3$ at 366 nm), line 6 (14 mg, $R_f$=0.49, blue before and after using $NH_3$ at 366 nm). Each line extracted with methanol maceration.

For further purification, compounds were subjected to PC with 15% acetic acid as eluent to separate compounds 1 ($R_f$ = 0.81 purple before and after using $NH_3$ at 366 nm), 2 ($R_f$=0.74, purple before and after using $NH_3$ under 366 nm), 3 ($R_f$= 0.32, purple before and after using $NH_3$ under 366 nm), 4 ($R_f$= 0.45, purple before and after using $NH_3$ under 366 nm), 5 ($R_f$= 0.41, blue before and after using $NH_3$ under 366 nm) 2 and 6 ($R_f$= 0.38, blue before and after using $NH_3$ at 366 nm). Detection of flavonoids was carried out under ultraviolet lamp (366 nm).

## Results and Discussion

Compound 1 gave the characteristic UV spectrum of isorhamnetin and a free 700 H group (12) and the EIMS spectrum showed a [M-(Glu+Rh)]+ at m/z 316. The 1HNMR confirmed

**Table 1.** $^{1}$H NMR data of flavonol glycosids from *P. ruthenicum.*

| | Compound 1(Isorhamnetin 3-0-rutinoside) | | Compound 2 (Rutin) | |
|---|---|---|---|---|
| | $^{13}$C | $^{1}$H delta, m, *J* (Hz) | $^{13}$C | $^{1}$H delta, m, *J* (Hz) |
| 2 | 156.7 | | 147.1 | |
| 3 | 133.0 | | 133.1 | |
| 4 | 177.2 | | 176.5 | |
| 5 | 161.3 | | 160.1 | |
| 6 | 101.0 | 6.1 (*d*, J=2.4) | 97.7 | 6.2 (*d*, J=2.4) |
| 7 | 165.5 | | 163.0 | |
| 8 | 95.5 | 6.4 (*d*, J=2.4) | 92.5 | 6.4 (*d*, J=2.4) |
| 9 | 156.4 | | 156.1 | |
| 10 | 103.7 | | 102.1 | |
| 1’ | 122.3 | | 120.7 | |
| 2’ | 115.3 | 7.85 (*d*, J=2.4) | 113.3 | 7.6 (*d*, J=2.1) |
| 3’ | 149.6 | | 143.3 | |
| 4’ | 157.1 | | 155.4 | |
| 5’ | 120.1 | 6.92 (*d*, J=8.4) | 115.3 | 6.9 (*d*, J=8.4) |
| 6’ | 122.3 | 7.51 (*dd*, J=8.4, 2,4) | 120.1 | 7.5 (*dd*, J=8.4, 2.1) |
| 3’-OMe | 55.7 | 3.83 (s) | - | - |
| 1” | 104.5 | 5.2 (*d*, J=7.3) | 103.5 | 5.3 (*d*, J=7.3) |
| 2” | 75.9 | 3.2-3.6 (m) | 74.9 | 3.1-3.5 (m) |
| 3” | 77.3 | 3.2-3.6 (m) | 76.3 | 3.1-3.5 (m) |
| 4” | 71.6 | 3.2-3.6 (m) | 70.3 | 3.1-3.5 (m) |
| 5” | 78.2 | 3.2-3.6 (m) | 79.2 | 3.1-3.5 (m) |
| 6” | 65.5 | 3.8 (*d*, J=10) | 65.5 | 3.9 (*d*, J=10) |
| 1”’ | 102.3 | 4.2 (d, J=1.5) | 101.5 | 4.3 (*d*, J=1.5) |
| 2”’ | 71.4 | 3.2-3.6 (m) | 70.6 | 3.1-3.5 (m) |
| 3”’ | 74.2 | 3.2-3.6 (m) | 73.0 | 3.1-3.5 (m) |
| 4”’ | 72.3 | 3.2-3.6 (m) | 71.3 | 3.1-3.5 (m) |
| 5”’ | 69.7 | 3.2-3.6 (m) | 68.7 | 3.1-3.5 (m) |
| 6”’ | 17.8 | 1.09 (d, J=6.2) | 18.8 | 0.9 (d, J=6.2) |

the presence of one isorhamnetin nucleus since methoxy (Delta H=3.83) and two aromatic spin systems, Delta H 7.51 (J, 8.4, 2.4), 6.92 (J, 8.4) and 7.85 (J=2.4 Hz) corresponded to H-6’, H-5’ and H-2’, respectively, and Delta H 6.4 (J= 2.4) and Delta 6.1 (J= 2.4) to H-8 and H-6, respectively (Table 1). The $^{1}$HNMR spectrum of compounds 1 and 2 indicated the presence of one rhamnosyl and one glycosyl moieties with characteristic signals at 4.2 and 4.3 ppm (H-1”’, H-1”’, d, J=1.5 Hz) and 1.09 and 0.9 ppm (H-6”’, H-6”’, d, J= 6.2 Hz) for rhamnose and anomeric proton of β- glucose at 5.2 and 5.3 ppm (H-1”, d, J=7.3 Hz). The rhamonse moiety must be attached to glucosyl, as, the glucose $^{13}$C signals equivalent to those seen in data available from the literature (13, 14). Thus, 1 is isorhamnetin 3-0-rutinoside.

The chemical shifts of compound 2 are shown in Table 1. The aromatic carbon shifts of the flovonoid glycoside rutin 2 can be assigned by the use of quercetin 3 as model and the data of sugar were explained before compound 3 was assigned by use of the HNMR spectrum. It showed characteristic signals of a flavonol (3-Hydroxy flavone) with a spin-spin

**Table 2.** $^{1}$H NMR data of flavonol aglycons from *P. ruthenicum*.

| | Compound 3 (Quercetin) | | Compound 4 (Morin) | |
|---|---|---|---|---|
| | $^{13}$C | $^{1}$H delta, m, *J* (Hz) | $^{13}$C | $^{1}$H delta, m, *J* (Hz) |
| 2 | 141.05 | | 147.1 | |
| 3 | 137.1 | | 133.7 | |
| 4 | 177.3 | | 174.09 | |
| 5 | 162.4 | | 160.1 | |
| 6 | 99.2 | 6.1 (*d*, J=2) | 97.2 | 6.2 (*d*, J=2.4) |
| 7 | 165.5 | | 162.7 | |
| 8 | 94.4 | 6.4 (*d*, J=2) | 92.5 | 6.3 (*d*, J=2.4) |
| 9 | 158.2 | | 156.0 | |
| 10 | 104.5 | | 102.94 | |
| 1’ | 124 | | 139.6 | |
| 2’ | 116.05 | 7.8 (*d*, J=2) | 155.1 | |
| 3’ | 146.2 | | 107.2 | 6.3 (*d*, J=2.4) |
| 4’ | 148.7 | | 159.9 | |
| 5’ | 116.2 | 6.88 (*d*, J=8.4) | 108.9 | 6.5 (*dd*=7.4, J=2.4) |
| 6’ | 121.7 | 7.59 (*dd*, J=8.4, 2) | 133 | 7.4 (*d*, J=8.4) |

coupling pattern resulting from the presence of oxygen atoms at C4, C5 and C4 and C7. Thus, an AB pattern was observed for H6 and H8, with J=2 Hz and a pattern typical of a 1, 2, 4- trisubstituted hydrorylated benzene with Delta 7.8 (d, J=2Hz, H-2’), 7.59 (dd, J=8.4 Hz, J=2, H-6’) and 6.88 (d, J=8.4 Hz, H-5’), corresponding to quercetin.

The flavonol morin 4 was assigned by comparison with quercetin 3, a substance possessing the same substitution pattern of rings A and B and a pattern of a 1, 3, 4 trisubstituted hydroxylated benzene was observed for C ring with Delta 7.4 (d, J=8.4 Hz, H-6’), 6.5 (dd, J=7.4, J=2.4 Hz, H-5’) and 6.3 (d, J=2.4, H-3’). The common pathway of fragmentation of flavonoids is a retro-Dicls Alder reaction that, in the case of morin, gives the m/z 152 and 150 ions (15).

Compound 5 and 6 were identified as caffeic acid and p-coumaric acid, by comparing their EI-MS, $^{1}$HNMR and spectra with published data $^{13}$CNMR (16, 17). The chemical shifts of the compounds 5 and 6 are shown in Table 3.

**Table 3.** $^{1}$H NMR data of phenolyc acids from *P. ruthenicum*.

| | Compound 5 (Caffeic acid) | | Compound 6 (*p*-Coumaric acid) | |
|---|---|---|---|---|
| | $^{13}$C | $^{1}$H delta, m, *J* (Hz) | $^{13}$C | $^{1}$H delta, m, *J* (Hz) |
| | 167.9 | | 167.7 | |
| 1 | 144.4 | 7.5 (*d*, J=16) | 143.4 | 7.54 (*d*, J=16) |
| 2 | 120.3 | 6.2 (*d*, J=16) | 114.0 | 6.211 (*d*, J=16) |
| 3 | 125.2 | | 124.4 | |
| 4 | 114.1 | 7.0 (*d*, J=1.6) | 114.8 | 6.82 (*d*, J=8) |
| 5 | 114.7 | | 128.6 | 6.38 (*d*, J=8) |
| 6 | 146.9 | | 158.6 | |
| 7 | 143.9 | 6.8 (*d*, J=8.4) | 128.6 | 7.38 (*d*, J=8) |
| 8 | 113.3 | 6.92 (*dd*, J=8.4, 1.6) | 114.8 | 6.82 (*d*, J=8) |
| 9 | 124 | | 139.6 | |

## Acknowledgments

This research was partially supported by a grant from Iran Chapter of TWAS.

## References

(1) Willis J. *A Dictionary of the Flowering Plants and Ferns*. Cambr. Univ. Press. Cambridge (1973) 1-1245
(2) Pimenov MG. Peucedanum. In: Rechinger KH. (ed.) *Flora Iranica* No. 162, Umbeliferae. Akademische Druck-u. Verlagsanstalt, Graz (1987) 442-444
(3) Salimian M. *Taxonomic Revision of Peucedanum Complex in Iran* [dissertation]. Tehran, Tehran University (2003) 97-99
(4) Frey R. Taxonomische Revision der Gattung *Peucedanum*: section *Peucedanum* und sektion *Palimbioidea* (Umbelliferae). *Candollea* (1989) 44: 257-327
(5) Gan WS. *Manual of Medicinal Plants in Taiwan*. Notional Research Institute of Chinese Medicine, Taichung (1965) 675
(6) Kong LY, Li Y, Min ZD, Li X and Zhu TR. Qianhu coumarin I from *Peucedanum praeruptorum*. *Phytochem.* 42 (1996) 1689 -1691
(7) Tang W and Einsehbrand C. *Chinese Drugs of Plant Origin*. Springer Verlag, Berlin (1992) 753-757
(8) Lu M, Nicoletti M, Battinelli L and Mazzanti G. Isolation of Praeruptorins A and B from *peucedanum praeruptorum* Dunn. and their general pharmacological evaluation in comparison with extracts of the drug. *IL Farmaco.* (2001) 56: 417-420
(9) Soine TO, Zheleva A, Mahandru MM, Erhardt P and Bubeva-Ivanova L. Natural coumarins VII: Isolation and structure of a new coumarin, Peuruthenicin, from *Peucedanum ruthenicum* M.B. *J. Pharm. Sci.* (1973) 62: 1879-1880
(10) Cisowskia DW, Sawickaa U, Mardarowiczb M, Asztemborskac M and Luczkiewizd M. Essential oil from herb and rhizome of *Peucedanum ostruthium* (L. Koch). ex DC. *Z. Naturforsch.* (2001) 56c: 930-2
(11) Danielea F, Lauraa G, Donataa R and Antonioa M. Composition of the essential oil of *Peucedanum verticillare*. *Biochem.* (1978) 28: 143-7
(12) Markham KR. *Techniques of Flavonoid Identification.* Academic Press. London (1982) 36
(13) Tulyaganov TS, Nazarov OM, Makhmudov, OE, Vdorin AD and Abdullaev ND. N- allylisonitrarine and narcissin from plants of the Nitraria genus. Chem. Of Nat. Com. (2001) 37: 470-473
(14) Wenkert E and Gottlieb HE. Carbon-13 nuclear magnetic Resonance spectroscopy of flavonoid and isoflavonoid compounds. *Phytochem.* (1977) 16: 1811-1816
(15) Morita N and Arisawa M. Flavonoids: Chemistry and Biochemistry. Heterocycles. (1976) 4: 373-391
(16) Xu H, Kadota S and Wang H. A new Hydrolysable tannin from *Geum Japanicum* and its antiviral activity. *Hetrocycles* (1994) 38: 167-175
(17) Bergman M, Varshavsky L, Gottlieb HE and Grossman S. The antioxidant activity of aqueous spinach extract: chemical identification of active fractions. *Phytochem.* (2001) 58: 143-52

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