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Furanocoumarins from *Heracleum rawianum* in Iran

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Abstract

The species *Heracleum rawianum* that belongs to Apiaceae is one of the native species in Iran. In the present work, the extraction of aerial parts of this plant with acetone is described by maceration. Furocoumarins such as angelicin (in remarkable amount), allobergapten and sphondin were isolated and identified by IR, ^{1}H NMR, ^{13}C NMR and DEPT-135 spectroscopic data, MS spectrometery and camparison with the literature. To the best of our knowledge, no phytochemical investigations on this species have been reported.

Keywords: Allobergapten; angelicin; apiaceae; furanocoumarins; *Heracleum rawianum*; sphondin.

Introduction

The genus *Heracleum* with more than 120 species in the world is one of the largest genera of the Apiaceae family (Umbelliferae). It is widely distributed in Asia [1] and is represented by eight species in the flora of Iran. Three of them, *Heracleum rechingeri*, *Heracleum gorganicum* and *Heracleum anisactis* are endemic in Iran [2].

The leaves and fruits of this genus are used as a flavoring agent, antiseptic, carminative, digestive and analgesic in the Iranian folk medicine [3-5]. Essential oils and extracts of different species of *Heracleum* have shown different biological properties such as analgesic and anti-inflammatory effects of essential oil of *H. persicum*, anticonvulsant activity of acetone extract of the seeds of this species [6], cytotoxic activity for *H. sibiricum* [7], and antimicrobial and antioxidant activity for *H. nepalense* [8].

Various secondary metabolites including coumarins, furocoumarins, furocoumarin dimers, coumarin glycosides, anthraquinones, stilbene derivatives, and flavonoids have been isolated and identified from different species of this genus [9-12].

Heracleum rawianum (Figure 1) is a native species growing in the Shabil mountain around Meshkinshahr in northwestern of Iran [2].

The leaves and fruits of *Heracleum rawianum* are used traditionally in Iran as a flavoring agent for food. A literature survey showed that no phytochemical investigation about this plant has been performed. Our phytochemical analysis of the

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chloroform portion of acetone extract of the aerial parts of this plant led to the isolation of three condensed furocoumarins, 1−3 (Figure 2). A remarkable amount of angelicin was isolated from *Heracleum rawianum* in 8% yield.

Figure 1. *H. rawianum*

Experimental

Materials

All reagents were purchased from Merck. Column chromatography was conducted with Silica gel 230-400 mesh, Merck. Silica TLC analysis was performed on Merck F254 silica gel plates (20×20 cm).

Plant Material

The aerial parts of *H. rawianum* were collected (5Kg) from the Shabil mountain around Meshkinshahr in northwestern of Iran at the full flowering stage in July 2012. The plant was identified by plant systematic expert Mr. Shahram Bahadori and a voucher specimen (MPH-2003) has been deposited in the herbarium of Medicinal Plants and Drugs Research Institute (MPH) of Shahid Beheshti University, Tehran, Iran.

Characterization

Melting points were determined on an Electrothermal melting point instrument. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer. NMR spectra were measured on a Bruker DRX-300 spectrometer and were recorded in $CDCl_3$ (300 MHz for ^1H NMR and 75 MHz for ^{13}C NMR). Mass analysis was carried out using a Micromass spectrometer with analysis condition as follows: 3.5kv capilary voltage, 60 v conevoltage, dessolvation temprature 350 °C, 400 l/h dessolvation gas and 300 l/h cone gas.

Isolation and purification

Aerial parts of the plant were air-dried at room temperature in the shade, powdered and extracted with acetone (4 ×15 L) by maceration and the resulting solution was concentrated under reduced pressure to yield dark gummy residue acetone extract (160 g). Then, the acetone crude extract was suspended in water and partitioned with n-hexane (500 mL) and then with chloroform (500 mL) to yield n-hexane soluble extract and chloroform soluble extract respectively. The chloroform

soluble extract was concentrated using a rotary evaporator at 40 °C to obtain 70 g of oily substance. The chloroform extract was subjected to column chromatography using Silica gel (230−400 mesh, 600 g) as stationary phase and a gradient of n-hexane:EtOAc (100:0 to 0:100), followed by increasing concentrations of MeOH (up to 10%) in EtOAc as eluent. On the basis of TLC analysis, fractions with similar composition were pooled to yield 23 combined fractions. Fraction 8 eluted with EtOAc:n-hexane (25:75) was further purified by triturated with MeOH:chloroform (1:5) to afford remarkable amount, 5.9g of colorless crystals of 1. Further purification of fraction 11 using EtOAc:*n*-hexane (3:7) as eluent resulted in 28 mg of 2 as a crude white powder. Recrystallization of the crude product from ethanol:water afforded 26 mg pure crystals of 2. Needle crystals of 3 (22 mg) was obtained from the elution of fraction 12 using EtOAc: *n*-hexane (35:65). All compounds showed dark spots at 366 nm under UV light and were not visible to the naked eye.

Results and discussion

Compound **1** was obtained as pure colorless crystals, m.p 136-137 ^{o}C. The IR spectrum revealed the presence of carbonyl group (1740 cm^{-1}) and an aromatic moiety (1617 and 1535 cm^{-1}). The broad band decoupled (BB) and distortionless enhancement by polarization transfer (DEPT) ^{13}C NMR spectra showed 11 signals attributed to six methine and five quaternary carbons. ^{1}H NMR spectrum of **1** showed the presence of three pairs of methines which is expected for unsubstituted furocoumarin. The structure of **1** was identified as angelicin (Figure 2) by comparison of its spectroscopic data and melting point with those reported on literature [13,14].

IR spectrum of 2 indicated the presence of a carbonyl group (1707 cm^{1}). Twelve signals were observed in the ^{13}C NMR spectrum which corresponds to 12 carbon atoms in this compound. The DEPT-135 spectrum shows six resonances for hydrogen substituted carbon atoms, of which five accords to olefinic carbons (CH=) and one belongs to a methyl group (OMe). Signals at δ_H= 6.31, 7.7, 6.7 and 7.66 ppm and their related coupling constants in ^{1}H NMR spectrum showed the presence of two -CH=CH- moiety in the structure of compound **2**. Comparison of spectroscopic data and melting point of this compound with literature data indicated that **2** is allobergapten (Figure 2) [14,15].

The ^{13}C NMR spectrum of **3** was similar to those of compound **2** and 12 signals were observed. ^{13}C NMR and DEPT-135 spectra of this compound showed the presence of six protonated C atoms and six quaternary carbons. The presence of a methyl group (OMe) was confirmed from the signals at δ_C: 56.5 and δ_H: 4.05. The ^{1}H NMR spectrum of **3** revealed the presence of a furocoumarin nucleus, [(δ_H 6.4 and 7.76 (each 1H, *d*, *J*=9.5 Hz, H-4, H-3), 7.13 and 7.7 (each 1H, *d*, *J*=2.10 Hz, H-2′, H-3′), and 6.8 (*s*, 1H, H-5). After the comparison of these results and melting point of compound **3** with reported data, this compound was considered to be sphondin (Figure 2) [15,16].

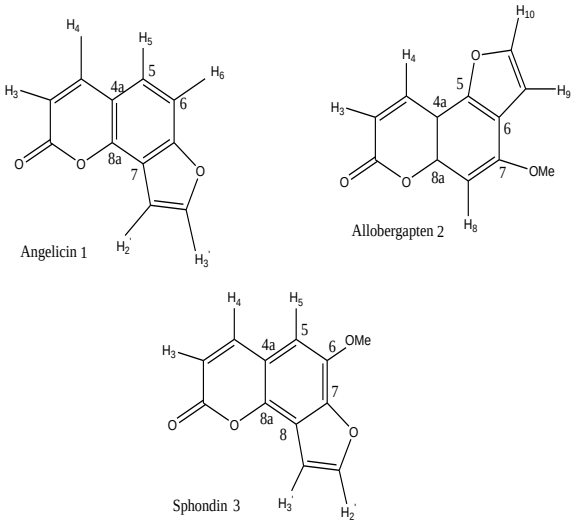

Figure 2. Furocoumarins isolated from *H. rawianum*

Structure elucidation of isolated compounds

Angelicin (1)

Colorless crystals, 5.9 g; mp: 136-137 °C (reported: 138-139.5 °C); Rf: 0.6 (Acetone: CH_2Cl_2, 2:98); IR (KBr): 1740, 1402-1535, 1617, 1200-1271, 1057- 1251 cm^{-1}; MS: m/z = 187.2 [M]+; ^1H NMR (300 MHz,$CDCl_3$): 6.38 (1H, d, J= 9.50 Hz, H-3), 7.12 (1H, d, J= 2.10 Hz, H-3′), 7.37 (1H, d, J= 8.50 Hz, H-6), 7.43 (1H, d, J= 8.50 Hz, H-5), 7.69 (1H, d, J= 2.10 Hz, H-2′), 7.8 (1H, d, J=9.50, H-4); ^{13}C NMR (75MHz, $CDCl_3$): 160.8 (C=O), 114.1 (C-3), 144.5 (C-4), 123.8 (C-5), 108.8 (C-6), 157.3 (C-7), 116.9 (C-8), 113.5 (4a), 148.4 (8a), 145.9 (C-2′), 104.1 (C-3′).

Allobergapten (2)

White crystals, 26 mg; mp: 205-207 °C (reported: 207 °C); Rf: 0.7 (Acetone:CH2Cl2, 2:98); IR (KBr): 1707, 1433-1584, 1618, 1215-1297, 1025-1125 cm^{-1}; MS: m/z = 217.4 [M]+; ^1H NMR (300 MHz, $CDCl_3$): 6.31 (1H, d, J=9.5 Hz, H-3), 7.7 (1H, d, J=9.5 Hz, H-4), 6.7 (1H, d, J=2.1 Hz, H-2′), 7.66 (1H, d, J=2.1 Hz, H-3′), 7.31 (1H, s, H-8), 4.24 (3H, s, OMe); ^{13}C NMR (75MHz, CDCl3): 160.5 (C=O), 114.6 (C-3), 144.4 (C-4), 112.9 (C-5), 132.64 (C-6), 142.64 (C-7), 126.13 (C-8), 146.6 (C-10), 104.1(C-9), 116.4 (4a), 147.5 (8a), 61.2 (OMe).

Sphondin (3)

Colorless needle crystals, 22 mg; mp: 190-191°C (reported: 190-192 °C); Rf: 0.7 (Acetone:CH2Cl2, 2:98); IR (KBr): 1735, 1421-1582, 1618, 1025 -1297, 1041-1192 cm^{-1}; MS: m/z = 217.4 [M]+; ^1H NMR (300 MHz, $CDCl_3$) : 4.05 (3H, s, OMe), 6.4 (1H, d, J=9.5 Hz, H-3), 7.13 (1H, d, J=2.1 Hz, H-3′), 6.8 (1H, s, H-5), 7.7 (1H, d, J=2.1 Hz, H-2′), 7.76 (1H, d, J=9.5 Hz, H-4); ^{13}C NMR (75 MHz, $CDCl_3$), δ: 161.1 (C=O), 114.5 (C-3), 144.4 (C-4), 104.6 (C-3′), 146.0 (C-2′), 106.74 (C-4a), 142.85 (C-6), 143.12 (C-8a), 103.64 (C-5), 113.6 (C-8), 143.06 (C-7), 56.5 (OMe).

We demonstrated that *H. rawianum* is rich in furanocoumarin. Furanocoumarins are a group of natural and synthetic compounds used for the photochemotherapeutic treatment of some skin diseases, some lymphomas and autoimmune disorders [17]. Sphondin (**1**) had been previously

isolated from some *Heracleum* species such as *H. pastinacifolium* C. Koch [16], *H. persicum* [18], *H. laciniatum* [19], *H. crenatifolium* [20]. In this work, we report the isolation and structure elucidation of the known three furanocoumarins angelicin (**1**), allobergapten (**2**) and sphondin (**3**). Their structures were established using spectroscopy methods such as IR, ^{1}H NMR, ^{13}C NMR, DEPT-135 and MS spectrometry. This is the first report on phytochemical investigation of *Heracleum rawianum*.

Conclusion

In this study, the aerial parts of the plant were extracted with acetone, by maceration. The chloroform portion of acetone extract of the aerial parts of *H. rawianium* yielded three known furanocoumarins (1-3) after chromatography and purification. A remarkable amount of angelicin was isolated from *Heracleum rawianum* in 8% yield.

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