Two New Coumarins from the Chloroform Extract of *Angelica urumiensis* from Iran

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The aerial parts of several *Angelica* species of the Apiaceae as natural medicine, are rich sources of various coumarins with biological, to a lesser extent, toxicological activities. From the chloroform extract of the aerial parts of *Angelica urumiensis* Mozaff. two new coumarins (1, 2), together with six known coumarins and two known flavonoids were isolated. On the basis of comprehensive spectroscopic analyses, including electron ionization-mass spectra (EI-MS), ^{1}H-NMR, ^{13}C-NMR, 1D nuclear Overhauser effect (NOE), distortionless enchancement by polarization transfer (DEPT), H, H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), rotating frame Overhauser enhancement spectroscopy (ROESY) spectra and comparison with spectral data of known compounds, the structure of new compounds were established as pyranocoumarin dimmer (1) and (+)-8,9-dihydro-8-(2-hydroxypropan-2-yl)-2-oxo-2*H*-furo[2,3-*h*]chromen-9-yl-3-methylbut-2-enoate (2). The eight known compounds (3—10) were isosamidin, laserpitin, pteryxin, isolaserpitin, *cis*-khellactone, angelicin, genkwanin and salvigenin, respectively. These known structures are isolated from the aerial parts of *A. urumiensis* for the first time. Antioxidant activities of the two new coumarins were evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and exhibited a moderate antioxidant activity.

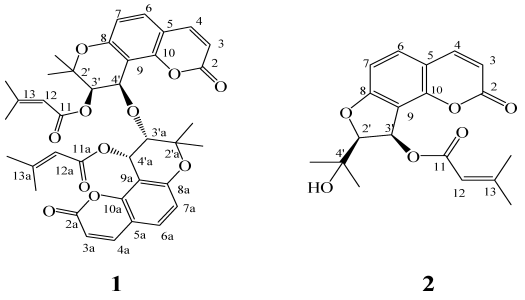
Key words flavonoid; Apiaceae; pyranocoumarin dimmer

Angelica is a genus of about 60 species of tall biennial and perennial herbs in the family Apiaceae, native to temperate and subarctic regions of the Northern Hemisphere. *A. urumiensis* is grown only in a limited region of Uremia Province in Iran (a small region with 1 km^2 area).

As *Angelica* species are one of the most important genera of medicinal plants that are still in use in traditional medicine, numerous scientific studies have been carried out on several species of this genus and also on the commercial preparations of *Angelica*.[1)] Most of these studies have been focused on identification of active components present in these plants and evaluation of their biological properties. From various phytochemical studies, it has now been established that majority of *Angelica* species contain quite high amounts of biologically active coumarins, predominantly simple and furano-coumarins.[1—6)]

Many species of this genus have been used for purifying the blood, anti-inflammatory, diuretic, stimulant, cordial, appetizer, dyspepsia, cardioactive, carminative, diaphoretic and also in stomach troubles, bilious complaints, infantile atrophy, menorrhiza, for treating rinderpest and constipation, expectorant and diaphoretic, and remedy for nervousness, insomnia, intestinal disturbances, cold, influenza, pleurisy, hepatitis, indigestion, typhoid, arthritis, coughs, chronic bronchitis, wind, headaches, fever, colic, travel sickness, rheumatism, toothache, leucorrhoea, boils, abscesses, bacterial and fungal infections and diseases of the urinary organs.[1,7—12)]

There have been no attempts to study the chemical constituents of *A. urumiensis* grown in Iran. Therefore, in continuation of our researches to find new natural products from the Iranian medicinal plants, we report the isolation and structural elucidation of two new coumarins (**1**, **2**) (Fig. 1), together with six known coumarins and two known flavonoids. The natural occurrence of these compounds can be conclusive for the chemotaxonomic characterization of this genus.



1 **2**

Fig. 1. Structures of Compounds **1** and **2**

Experimental

Instrumentation NMR spectra were measured on a Bruker AVANCE 300 (Bruker Biospin, Rheinstetten, Germany). ^{1}H-NMR, ^{13}C-NMR, distortionless enchancement by polarization transfer (DEPT), H, H-correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) were measured using a 5 mm probe. The operating frequencies were 300.13 MHz for acquiring ^{1}H-NMR and 75.46 MHz for ^{13}C-NMR spectra. Samples were measured at 298 K in $CDCl_3$. The optical rotation was measured on a polarimeter using a sodium lamp (589 nm). Direct electron ionization-mass spectrum (EI-MS) was recorded with an Agilent 5973 at 70 eV. Preparative TLC was performed on silica gel 60 mesh GF_{254} plates (20×20 cm) while silica gel (70—230 mesh) was used for column chromatography. Elemental analysis was performed by CHNSO elemental analyzer, Elementar Valio, EL-III. UV–Vis spectra were obtained using a Shimadzu UV-2100 spectrophotometer. IR spectra were recorded on a Shimadzu IR-470 spectrometer.

Plant Material The aerial parts of *A. urumiensis* were collected from Soluk, Uremia, Province of West Azerbaijan, Iran in June 2007 during the flowering stage. It was identified by Dr. Mozaffarian[13)] (Research Institute of Forest and Rangelands). Voucher specimens (No. 883525 TARI) have been deposited at the herbarium of Research Institute of Forests and Rangelands.

Extraction and Analysis The air-dried powder of the aerial parts of *A. urumiensis* (250 g) was extracted with chloroform at room temperature. The

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$CHCl_3$ extract was concentrated *in vacuo* to yield a gummy extract. This residue treated with MeOH to remove waxy compounds. The MeOH soluble portion (8.5 g) was subjected to a silica gel column chromatography (70—230 mesh, 240 g) with a gradient of hexane–EtOAc and then MeOH as eluent. Twelve fractions were collected according to TLC analysis.

The fraction of hexane–EtOAc (90 : 10) was subjected to another column chromatography using hexane–EtOAc (90 : 10) as the eluent. After recrystallization from EtOH, 40 mg of compound **9** was obtained. The fraction of hexane–EtOAc (80 : 20) was rechromatographed over silica gel and eluted with hexane–EtOAc (85 : 15) to give 50 mg of compound **10**. The fraction of hexane–EtOAc (75 : 25) was separated on another column chromatography and eluted with hexane–EtOAc (80 : 20) to afford 20 mg of compound **8** and 150 mg of compound **5**. Further purification on fraction of hexane–EtOAc (70 : 30) was carried out by thin layer chromatography with hexane–EtOAc (70 : 30) for several times and yielded 20 mg of compound **4**, 20 mg of compound **6** and 15 mg of compound **3**. The fraction of hexane–EtOAc (65 : 35) was further rechromatographed eluting with hexane–EtOAc (70 : 30) to yield 200 mg of compound **1**. Finally the fraction of hexane–EtOAc (60 : 40) was applied to another column eluting with hexane–EtOAc (65 : 35) to give 10.3 mg of compound **2** and 25 mg of compound **7**.

Pyranocoumarin Dimmer (Compound 1) $C_{38}H_{38}O_{11}$: White oil; $[\alpha]_D^{25}$ 7.4° (c=1, $CHCl_3$); EI-MS: m/z (%): 672 ($M^{+\cdot}$, 5%), 344 (10), 327 (8.3), 287 (82), 229 (100), 83 (83); UV λ_{max} (nm) CH_3Cl: 325, 234; IR (KBr) cm^{-1} 1727, 1605, 1140; ^{1}H-NMR (CD_3Cl, 300 MHz) δ: 7.65 (1H, d, J=9.5 Hz, H-4), 7.6 (1H, d, J=9.5 Hz, H-4a), 7.35 (2H, d, J=8.6 Hz, H-6, H-6a), 6.79 (2H, d, J=8.6 Hz, H-7, H-7a), 6.45 (1H, d, J=4.5 Hz, H-4′a), 6.25 (1H, d, J=9.5 Hz, H-3), 6.22 (1H, d, J=9.5 Hz, H-3a), 5.81, 5.73 (2H, s, H-12, H-12a), 5.44 (1H, d, J=4.6 Hz, H-4′), 5.22 (1H, d, J=4.6 Hz, H-3′), 4.05 (1H, d, J=4.5 Hz, H-3′a), 2.25, 2.21, 1.93 (12H, s, Senecioyloxy group), 1.5, 1.47 (6H, s, 2′a-*gem*-Me), 1.45, 1.42 (6H, s, 2′-*gem*-Me); ^{13}C-NMR (CD_3Cl, 75 MHz) δ: 167.5 (C-11a), 165.6 (C-11), 160.6 (C-2a), 160.2 (C-2), 159.7 (C-13a), 159.2 (C-13), 157 (C-8a), 156.1 (C-10), 154.4 (C-8), 154.2 (C-10a), 144 (C-4), 143.3 (C-4a), 129.2 (C-6a), 128.7 (C-6), 115.1 (C-12a), 114.9 (C-12), 114.6 (C-7), 114.5 (C-7a), 113 (C-3), 112.5 (C-3a), 112.4 (C-9), 112.3 (C-5a), 110.7 (C-5), 107.2 (C-9a), 78.7 (C-2′a), 77.8 (C-4′), 71.6 (C-3′), 71.3 (C-3′a), 62.9 (C-4′a), 60.22 (C-4′), 27.6, 20.59 (13a-*gem*-Me), 27.5, 20.47 (13-*gem*-Me), 22.6, 21.2 (2′-*gem*-Me), 25.5, 25.4 (2′a-*gem*-Me); *Anal.* Calcd for $C_{38}H_{38}O_{11}$: C, 68.05; H, 5.71; O, 26.24. Found: C, 67.90; H, 5.60; O, 26.23.

(+)-8,9-Dihydro-8-(2-hydroxypropan-2-yl)-2-oxo-2*H*-furo[2,3-*h*]chromen-9-yl-3-methylbut-2-enoate (Compound 2) $C_{19}H_{20}O_6$: White oil; $[\alpha]_D^{25}$ 5.4° (c=0.6, $CHCl_3$); EI-MS: m/z (%): 344 ($M^{+\cdot}$, 15%), 327 (32), 229 (23), 245 (17), 83 (100), 55 (25); UV λ_{max} (nm) CH_3Cl: 323.5, 246.5; IR (KBr) cm^{-1} 3440, 1720; ^{1}H-NMR (CD_3Cl, 300 MHz) δ: 7.64 (1H, d, J=9.5 Hz, H-4), 7.43 (1H, d, J=8.4 Hz, H-6), 6.97 (1H, d, J=6.5 Hz, H-3′), 6.89 (1H, d, J=8.4 Hz, H-7), 6.25 (1H, d, J=9.5 Hz, H-3), 5.61 (1H, s, H-12), 4.54 (1H, d, J=6.5 Hz, H-2′), 2.26 ,1.91 (6H, s, 13-*gem*-Me), 1.43, 1.41 (6H, s, 4′-*gem*-Me); ^{13}C-NMR (CD_3Cl, 75 MHz) δ: 164.8 (C-2), 161.1 (C-8), 160 (C-10), 143.5 (C-4), 131.4 (C-6), 114.5 (C-5), 113.1 (C-3), 113 (C-9), 107.8 (C-7), 91.7 (C-2′), 71.1 (C-4′), 68.3 (C-3′), 27, 26.1 (4′-*gem*-Me); *Anal.* Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85; O, 27.88. Found: C, 66.10; H, 5.73; O, 27.55.

Free Radical Scavenging Activity Free radical scavenging activity was evaluated by measuring the scavenging activity of the two new compounds on the solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). One milliliter of 500 μM solution of DPPH in methanol was thoroughly mixed with an equal volume of a solution of test compounds at various concentrations and kept in the dark for 30 min. The absorbance of the solutions, including a blank (without sample) and a positive control (BHT, *tert*-butylated hydroxytoluene), was read at 517 nm after 1 h incubation without light at room temperature on a Shimadzu UV-2100 spectrophotometer. Each sample assay was carried out in triplicate and data presented as a mean of the three values. A decrease in absorbance of the DPPH solution indicates increased DPPH radical scavenging activity. The values were calculated as a percentage using the following formula:

$$\%\ \text{DPPH radical scavenging} = [(\text{absorbance of blank} - \text{absorbance of sample})/\text{absorbance of blank}] \times 100$$

Results and Discussion

Herbal remedies used in the traditional folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs. The genus *Angelica* includes a number of popular medicinal herbs that have long been used in traditional medicine systems for the treatment of various illnesses. We have investigated the chemical profile of *A. urumiensis* for the first time.

From the aerial parts of *A. urumiensis* two new coumarins (**1**, **2**), together with six other known coumarins and two known flavonoids were isolated.

Compound **1** had a molecular formula of $C_{38}H_{38}O_{11}$ determined by CHN analyzer, EI-MS (at m/z 672 $[M]^{+\cdot}$) as well as ^{13}C-NMR and DEPT data. The IR spectrum shows absorption band at 1727 assignable to lactones and conjugated ketone functions. The ^{13}C-NMR spectrum displays 38 signals, which are classified into eight methyles, fourteen methines, and sixteen quaternary carbons by DEPT experiments. The ^{1}H-NMR spectrum is a combination of NMR spectrum of two dihydropyranocoumarins linked together *via* an ether bond. This spectrum displays signals at δ 7.65, 7.6 (H-4, H-4a), 7.35 (H-7, H-7a), 6.79 (H-6, H-6a), 6.25, 6.22 ppm (H-3, H-3a) as well as two pairs of doublets: δ 5.44, 5.22 ppm (each 1H, d, J=4.6 Hz) and δ 6.45, 4.05 ppm (each 1H, d, J=4.5 Hz). The former belongs to the H-4′, H-3′ protons which are in *cis* configuration based on their coupling constant ($J_{H3',H4'}$=4.6 Hz)[14—16] and also based on positive signal enhancement in selective 1D NOE experiment. Also, rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum of compound **1** shows correlation between these two protons. The latter associates with two methine protons (H-4′a, H-3′a) which have *cis* configuration with respect to their coupling constant ($J_{H3'a,H4'a}$=4.5 Hz)[14—16] and we can see again a positive signal for one proton by selective irradiation of the other one due to the *cis* configuration of methine protons. On the other hand H-4′ and H-3′a have no correlation with each other based on ROESY and 1D NOE experiment, so they have *trans* configuration. To conclude we can suggest one of the two absolute configurations for compound **1**: 3′*S*, 4′*S*, 3′a*R*, 4′a*R* or 3′*R*, 4′*R*, 3′a*S*, 4′a*S*, that are enantiomers and are not distinguishable based on spectroscopic methods.

On the basis of ^{1}H-NMR data two senecioyloxy groups were identified, each of them corresponds to a coumarin unit. The connectivity of the carbons in the molecular structure was determined by analysis of the HMQC and HMBC spectra. The latter spectrum shows correlations between H-4′a (6.45) and C-11a (167.5), C-8a (157), C-10a (154.2), C-2′a (78.7), and between H-3′ (5.22) and C-11 (165.6), 2′-*gem*-Me (22.6, 21.2), thereby positioning the two senecioyloxy groups at C-3′ and C-4′a.

The mass spectrum of compound **1** exhibited only weak peak corresponding to $[M]^{+\cdot}$, the main feature being cleavage with hydrogen transfer, to an ion of m/z 344 ($C_{19}H_{20}O_6^+$) which belongs to the pyranocoumarin half and ion of m/z 327 ($C_{19}H_{19}O_5^+$) which corresponds to other half. The remaining important fragments are 287, 229 and 83 in accordance to the literature[16] for dihydropyranocoumarin compounds. The structure of **1** was strongly confirmed by 2D NMR techniques, including H, H COSY, HMQC and HMBC. The arrangement of atom orders was in a good agreement using ^{1}H-NMR, ^{13}C-NMR, COSY and DEPT analysis.

Compound **2** was isolated as an oil. The IR spectrum

Table 1. Antioxidant Activities of the Compounds **1** and **2** against DPPH (IC_{50})

Material	DPPH IC_{50} (μg/ml)
Compound **1**	170
Compound **2**	190
BHT	26

shows a (C=O) bond at 1720 cm^{-1} and (OH) group at 3440. The UV spectrum shows maximal absorptions at 323.5, 246.5 nm. The ^{1}H-NMR spectrum shows three pairs of doublets at δ 7.64, 6.25 (each 1H, d, J=9.5 Hz, H-4, H-3, respectively), 7.43, 6.89 ppm (each 1H, d, J=8.4 Hz, H-6, H-7) and at 6.97, 4.54 ppm (each 1H, d, J=6.5 Hz, H-3′, H-2′, respectively) which are characteristic of the furanocoumarin skeleton. The presence of senecioyloxy group (δ 5.62, (1H, s), 2.26 (3H, s), 1.91 ppm (3H, s)) on C-3′ was identified on the basis of large difference (2.43 ppm) between H-2′ and H-3′ signals. The presence of correlation between H-2′ and H-3′ in the ROESY spectrum, as well as having a large coupling constant $J_{2',3'}$=6.5 Hz[17,18] for these protons confirms the *cis* configuration. The absolute configuration of compound **2** was not determined because of the small amount of the sample. However, after referring to the 2′*S*, 3′*R* configuration of the 8(*S*)-9-isovaleryloxy-8,9-dihydrooroselol,[17] it was reasonable to assign the 2′*S*, 3′*R* configuration regarding to the laevorotatory optical rotation. An extensive study of the NMR data, including HMQC, HMBC and H, H COSY experiments, confirmed unambiguously the structure of suggested coumarin.

On the basis of the close agreement of their physical and spectral data with those already published, compounds **3**, **4**, **5**, **6**, **7**, **8**, **9** and **10** were found to be identical with those of the known compounds, isosamidin,[14] laserpitin,[14] pteryxin,[16] isolaserpitin,[14] *cis*-khellactone,[15] angelicin,[19] genkwanin[20] and salvigenin,[20] respectively.

Antioxidant activities of the compounds **1** and **2** were tested by the DPPH radical scavenging assay. The effect of antioxidant on DPPH radical scavenging was thought to be due to their radical scavenging activity. When a solution of DPPH is mixed with that of a substance, then this gives rise to the reduced form diphenypicrylhydrazine (non radical) with the loss of this violet colour.[21] Free radical scavenging properties of the new compounds are presented in Table 1. Lower IC_{50} value indicates higher antioxidant activity. Compounds **1** and **2** exhibited moderate antioxidant activities. Compound **1** (IC_{50}=170 μg/ml) showed higher scavenging ability on DPPH radicals than the compound **2** (IC_{50}= 190 μlg/ml). Also, DPPH scavenging abilities of them were lower than that of synthetic antioxidant BHT (IC_{50}=26 μg/ml). In this study, DPPH radical scavenging activity of test samples was in the order BHT>compound **1**>compound **2**.

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