

## Phytochemical analysis of *Ferulago Bernardii* Tomk & M.Pimen

<sup>1,2</sup>Khalighi-Sigaroodi F., <sup>\*1</sup>Hadjiakhoondi A., <sup>3</sup>Shafiee A., <sup>4</sup>Mozaffarian V.A.,  
<sup>5</sup>Shahverdi A.R., <sup>1</sup>Alavi S.H.R.

<sup>1</sup>Department of Pharmacognosy, Faculty of Pharmacy, and Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, <sup>2</sup>Institute of Medicinal Plants, Iranian Academic Center for Education, Culture and Research (ACECR), Karaj, <sup>3</sup>Department of Chemistry, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, <sup>4</sup>Research Institute of Forests and Rangelands, <sup>5</sup>Department of Pharmaceutical Biotechnology, Faculty of Pharmacy Tehran University of Medical Sciences, Tehran, Iran

Received : 25 Feb. 2006 ; Revised : 1 May 2006 ; Accepted : 12 May 2006

### ABSTRACT

From the hexane extract of the aerial parts of *Ferulago Bernardii* (Apiaceae) four coumarins, namely prantschimgin **1**, oxypeucedanin **2**, psoralen **3** and umbelliferone **4**;  $\beta$ -sitosterol **5**; and nonacosane **6** were isolated by Column Chromatography (CC), Preparative Thin Layer Chromatography (PTLC) and crystallization. The structures were elucidated by melting point, UV, IR, MS, <sup>1</sup>H and <sup>13</sup>C-NMR spectra. The presence of compounds **1**, **2**, **3** and **5** in some others *Ferulago* species could be used as chemotaxonomic marker in genus *Ferulago*. This is the first report on phytochemical analysis of *Ferulago Bernardii* Tomk. & M. Pimen.

**Keywords:** *Ferulago Bernardii*, Apiaceae, Prantschimgin, Oxypeucedanin, Psoralen, Umbelliferone,  $\beta$ -Sitosterol, Nonacosane

### INTRODUCTION

*Ferulago* W. D. Koch, is a perennial genus of the Apiaceae family which is distinguished by the presence of persistent bracts and bracteoles (1). The genus *Ferulago* is represented by 40 species in the world. Eight species exist in Iran of which three are endemic. *Ferulago Bernardii* Tomk. & M. Pimen. is a glabrous plant distributed in southeast Turkey and west of Iran (1,2).

*Ferulago*, *Ferula* and *Prangos* species have been used in folk medicine in different regions of Turkey for their sedative, tonic, digestive, aphrodisiac properties and for treatment of intestinal worms and hemorrhoids (3,4). Moreover the plants of the genus *Ferulago* have been used since antiquity in folk medicine against ulcers, snake bite and for treatment of headache and diseases of the spleen (5).

The chemical compositions of some *Ferulago* species have previously been reported. Coumarins have been found in *F. meoides*, *F. turcomanica*, *F. granatensis*, *F. aucheri*, *F. asparagifolia*, *F. nodosa*, *F. capillaris* and *F. brachyloba* (6-13). Isolation of flavonoids from *F. aucheri* and *F. asparagifolia* (10,11),  $\beta$ -sitosterol from *F. granatensis* (9), aromatic compounds from *F. F. aucheri* (10), monoterpenes from *F. nodosa*

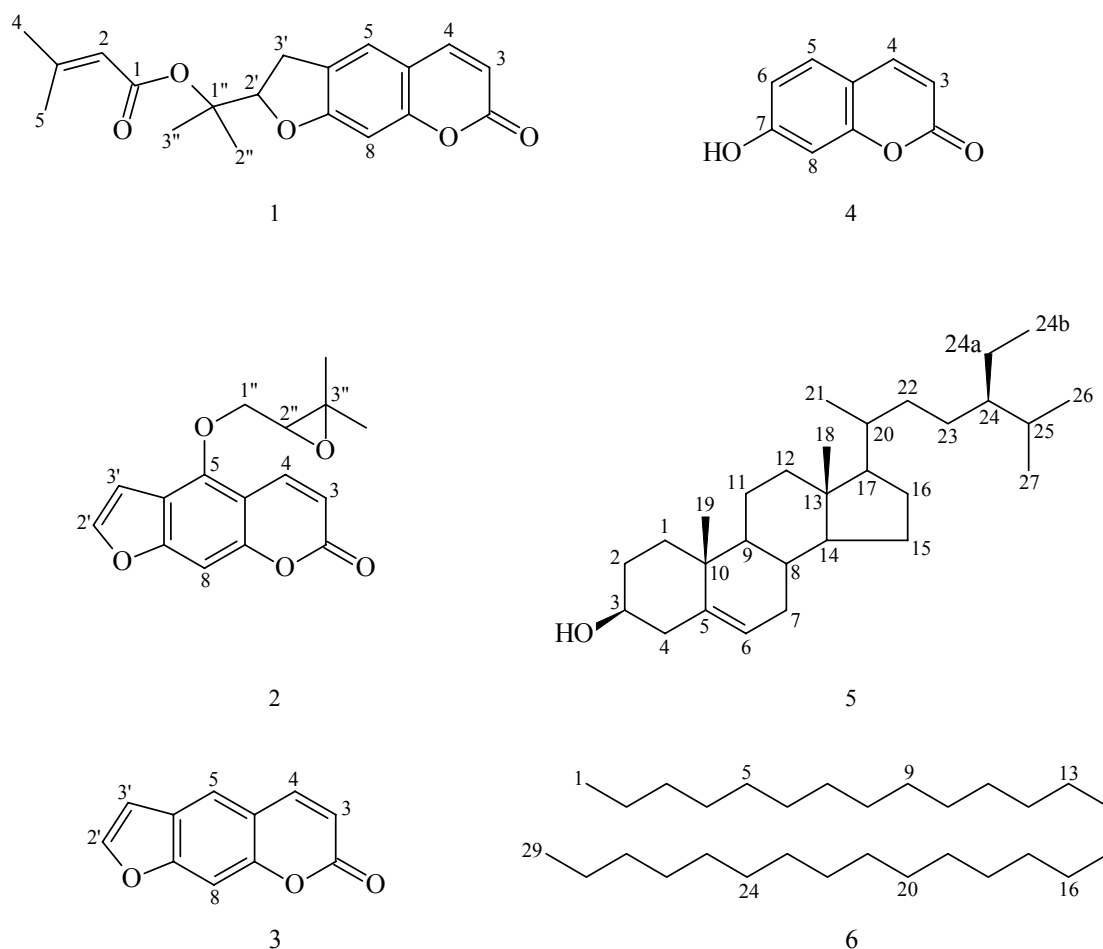
(12), phenylpropanoid and sesquiterpene aryl esters from *F. antiochia* have also been reported (14).

A number of *Ferulago* species have previously been investigated for their essential oil compositions and antimicrobial activities (3,5,15-27). In addition the chemical composition and antimicrobial activity of the essential oil of *Ferulago Bernardii* Tomk. & M. Pimen have been reported (28). In this report the isolation of four coumarins, prantschimgin (1), oxypeucedanin (2), psoralen (3) and umbelliferone (4);  $\beta$ -sitosterol (5); and nonacosane (6) from the aerial parts of *Ferulago Bernardii* of Iran (Figure 1) is described.

### MATERIALS AND METHODS

#### General

Melting points were recorded by a Reichert-jung apparatus and are uncorrected. EIMS spectra were recorded on a Finnigan MAT TSQ-70. <sup>1</sup>H and <sup>13</sup>C-NMR spectra were measured on a Varian FT-400 unity plus spectrometer with tetramethylsilane as an internal standard. Chemical shifts are given in  $\delta$ . The FT-IR spectra were recorded on a Nicolet 550 instrument. The UV spectra were obtained using a Shimadzu 160A



**Figure 1.** Structures of compounds 1-6 isolated from *Ferulago Bernardii* (Apiaceae)

spectrophotometer. Silica gel for column chromatography (Mesh 70 – 230) was purchased from Merck Company. Preparative TLC was conducted on Merck 60F<sub>254</sub> silica gel plates (absorbent thickness: 0.75 mm). Silica gel 60F<sub>254</sub> precoated plates (Merck) were used for TLC. Spots were detected under UV (254 and 365 nm) and by spraying KOH 5-10% in EtOH as reagent.

#### *Plant Material and Isolation Procedure*

The aerial parts of *F. Bernardii* in fruiting stage were collected in September 2001 from west of Iran, the height of Ariz (in the road of Sanandaj to Marivan), with an altitude ca. 2140 m. The plant was identified by the Department of Botany of the Research Institute of Forests and Rangelands (TARI), Tehran. A voucher specimen No. 71608 has been deposited at the Herbarium of TARI. The air-dried and finely grounded aerial parts (500 g) were extracted with n-hexane at room temperature. The extract was concentrated in vacuo (16 g) and a part of it was subjected to

preparative thin layer chromatography (PTLC) using petroleum ether-EtOAc (60:40) as solvent system. Several bands were separated from PTLC under UV light (254 and 365 nm). Bands 3 and 4 contained two main compounds showing a violet fluorescence. These bands were subjected to PTLC for further purification using petroleum ether-EtOAc (70:30) as solvent system. After separation of compounds from silica gel, they were further purified by crystallization from MeOH to yield compound **1** (2 mg) and **3** (1.2 mg). The hexane extract (2.8 g) was chromatographed on a silica gel column (3.5×60 cm) eluted with a petroleum ether-EtOAc-MeOH gradient. The volume of each fraction was 20 ml. Fractions 121-132 (0.8 g) were subjected to second silica gel column (2×30 cm) eluted with a petroleum ether-EtOAc-MeOH gradient. Fractions 10-14 of the second CC were combined and further purified by crystallization from EtOH and water to yield compound **2** (1.8 mg). Fractions 134-150 (0.9 g) of the first CC were subjected to third silica gel

column (2×30 cm) eluted with a petroleum ether-EtOAc-MeOH gradient. Fractions 15-21 of the third CC were combined and further purified by crystallization from EtOH and water to yield compound **4** (2.2 mg). Fractions 79-85 of the first CC were combined and further applied to PTLC over silica gel, using toluene-ether (1:1) saturated with 10% acetic acid as solvent system. Three bands were separated from silica gel. Band **1** was dissolved in EtOAc from which after a few hours compound **5** (2.6 mg) as white needles crystallized. Some of the hexane extract (1 g) was dissolved in CHCl<sub>3</sub> and subjected to PTLC over silica gel, using petroleum ether-EtOAc (50:50) as solvent system. Band **6** (R<sub>f</sub> 0.74) was separated from silica gel, by using EtOAc as solvent. Compound **6** (20 mg) was isolated and further purified by crystallization from CHCl<sub>3</sub>.

Compound **1** (Prantschimgin): White needle crystal (2 mg); Mp 137-139°C (lit. (29) Mp: 138-140°C); R<sub>f</sub> 0.47 toluene-ether (1:1 saturated with 10% acetic acid); UV λ<sub>max</sub> nm 244 and 335 nm; FTIR (CHCl<sub>3</sub>) ν<sub>max</sub> cm<sup>-1</sup>: 2919, 2851, 1727 (lactone C=O), 1627, 1570, 1263, 1229, 1123; EIMS (70 eV) m/z (%): 328 [M]<sup>+</sup> (40), 228 [M-Osen]<sup>+</sup> (87), 213 (100), 187 (32), 159 (5), 131 (15), 83 (85), 55 (50); formula: C<sub>19</sub>H<sub>20</sub>O<sub>5</sub>; <sup>1</sup>H-NMR, see Table 1; <sup>13</sup>C-NMR, see Table 2. The spectral data of the compound **1** were in agreement with the reported data for prantschimgin (13,29,30).

Compound **2** (Oxypeucedanin): Yellow powder (1.8 mg); Mp 138-140°C (lit. (31) Mp: 139-140°C); R<sub>f</sub> 0.43 toluene-ether (1:1 saturated with 10% acetic acid); UV λ<sub>max</sub> nm 251 and 306 nm; FTIR (CHCl<sub>3</sub>) ν<sub>max</sub> cm<sup>-1</sup>: 1726 (lactone C=O), 1603-1451 (aromatic C=C), 1257, 1209, 1082, 821, 750; EIMS (70 eV) m/z (%): 286 (100), 202 (42), 173 (20), 145 (25), 85 (35), 59 (60); formula: C<sub>16</sub>H<sub>14</sub>O<sub>5</sub>; <sup>1</sup>H-NMR, see Table 1; <sup>13</sup>C-NMR, see Table 2. The spectral data of the compound **2** were in agreement with the reported data for oxypeucedanin (13,31,32).

Compound **3** (Psoralen): White powder (1.2 mg); Mp 161-163°C (lit. (33) Mp: 163-164°C); R<sub>f</sub> 0.42 (toluene-ether (1:1, saturated with 10% acetic acid)); UV λ<sub>max</sub> nm 249, 290 and 331 nm; FTIR (CHCl<sub>3</sub>) ν<sub>max</sub> cm<sup>-1</sup>: 1715 (lactone C=O), 1126 (cyclic ether C-O), 1014, 756; EIMS (70 eV) m/z (%): 186 (100), 158 (80), 130 (18), 102 (35), 51 (15); formula: C<sub>11</sub>H<sub>6</sub>O<sub>3</sub>; <sup>1</sup>H-NMR, see Table 1; <sup>13</sup>C-NMR, see Table 2. The spectral data of the compound **3** were in agreement with the reported data for psoralen (13,33-37).

Compound **4** (Umbelliferone): White needle crystals (2.2 mg); Mp 229-231°C (lit. (38) Mp: 230-232°C); R<sub>f</sub> 0.28 toluene-ether (1:1 saturated with 10% acetic acid); UV λ<sub>max</sub> nm 206 and 325 nm; FTIR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 3159, 1709, 1681 (C=O), 1568 (phenolic C-O), 1322 (phenolic C-O), 1235 (C=O), 1134, 835; EIMS (70 eV) m/z (%): 162 (100), 134 (81), 51 (21); formula: C<sub>9</sub>H<sub>6</sub>O<sub>3</sub>; <sup>1</sup>H-NMR, see Table 1; <sup>13</sup>C-NMR, see Table 2. The spectral data of the compound **4** were in agreement with the reported data for umbelliferone (13,38-41).

Compound **5** (β-Sitosterol): White needle crystal (2.6 mg); Mp 135-136°C (lit. (29) Mp: 136-137°C); R<sub>f</sub> 0.25 (EtOH: CHCl<sub>3</sub> (98:2)); UV λ<sub>max</sub> 247 nm; FTIR (CHCl<sub>3</sub>) ν<sub>max</sub> cm<sup>-1</sup>: 3438 (O-H), 2934 (C-H), 2865, 1462, 1047 (C-O), 757; EIMS (70 eV) m/z (%): 414 (75), 396 (20), 381 (10), 329 (10), 255 (7), 213 (35), 199 (10), 69 (21), 57 (45), 43 (100); formula: C<sub>29</sub>H<sub>50</sub>O; <sup>1</sup>H and <sup>13</sup>C-NMR, see Table 3. The spectral data of the compound **5** were in agreement with the reported data for β-sitosterol (29,42-44).

Compound **6** (Nonacosane): White amorphous powder (20 mg); Mp 62-64°C (lit. (29) Mp: 64°C); FTIR (KBr) ν<sub>max</sub> cm<sup>-1</sup>: 2918, 2849, 1467, 1378 (methyl), 724 (long chain); EIMS (70 eV) m/z (%): 408 (5), 351 (3), 295 (5), 239 (5), 211 (7), 141 (15), 113 (20), 99 (30), 85 (70), 57 (100); formula: C<sub>29</sub>H<sub>60</sub>; <sup>1</sup>H-NMR (400 MHz, CDCl<sub>3</sub>) δ ppm: 1.25 (s, methylene groups), 0.88 (t, J=6 Hz, methyl groups). The spectral data of the compound **6** were in agreement with the reported data for nonacosane (29,45).

## RESULTS AND DISCUSSION

From the hexane extract of the aerial parts of *Ferulago Bernardii* (Apiaceae) four coumarins, prantschimgin **1**, oxypeucedanin **2**, psoralen **3** and umbelliferone **4**; one steroid, β-sitosterol **5**; and a normal alkane, nonacosane **6** were isolated by CC, PTLC and crystallization. The structure of these compounds were elucidated by melting point, UV, IR, MS, <sup>1</sup>H and <sup>13</sup>C-NMR spectra and by comparison of their physical data with those reported in literature.

Compound **1**: The mass spectrum of the compound **1** had [M]<sup>+</sup> at m/z 328 suggesting the molecular formula C<sub>19</sub>H<sub>20</sub>O<sub>5</sub>. In the proton nuclear magnetic resonance (<sup>1</sup>H-NMR) spectrum, H-3 and H-4 were observed at δ 6.22 (1H, d, J=9.8 Hz) and 7.61 (1H, d, J=9.8 Hz) as AB-type signals, respectively. The presence of two singlets proton

**Table 1.** <sup>1</sup>H-NMR spectral data for compounds **1**, **2**, **3**<sup>a</sup> and **4**<sup>b</sup>.

H	1 [ <i>mult.</i> , <i>J</i> (Hz)]	2 [ <i>mult.</i> , <i>J</i> (Hz)]	3 [ <i>mult.</i> , <i>J</i> (Hz)]	4 [ <i>mult.</i> , <i>J</i> (Hz)]
3	6.22 <i>d</i> (9.8)	6.32 <i>d</i> (9.6)	6.39 <i>d</i> (9.6)	6.19 <i>d</i> (9.4)
4	7.61 <i>d</i> (9.8)	8.21 <i>d</i> (9.6)	7.81 <i>d</i> (9.6)	7.93 <i>d</i> (9.4)
5	7.22 <i>s</i>	-	7.69 <i>s</i>	7.52 <i>d</i> (8.4)
6	-	-	-	6.78 <i>dd</i> (0.2, 8.4)
8	6.75 <i>s</i>	7.19 <i>s</i>	7.48 <i>m</i>	6.70 <i>d</i> (0.16)
2'	5.14 <i>dd</i> (8.0, 8.8)	7.62 <i>d</i> (2)	7.70 <i>d</i> (2.2)	-
3'	3.23 <i>m</i>	6.96 <i>d</i> (2)	6.84 <i>dd</i> (1.2, 2.2)	-
1''	-	4.62 <i>dd</i> (10.8, 4.2)	-	-
		4.44 <i>dd</i> (10.8, 6.8)		
2''	1.60 <i>s</i>	3.24 <i>dd</i> (4.2, 6.8)	-	-
3''	1.54 <i>s</i>	-	-	-
Me-3''	-	1.42 <i>s</i>	-	-
		1.34 <i>s</i>		
1(OSen)	-	-	-	-
2(OSen)	5.56 <i>s</i>	-	-	-
3(OSen)	-	-	-	-
4(OSen)	2.10 <i>s</i>	-	-	-
5(OSen)	1.86 <i>s</i>	-	-	-

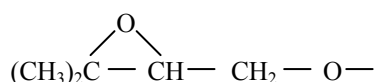
<sup>a</sup> Chemical shifts were determined at 400 (<sup>1</sup>H) MHz in CDCl<sub>3</sub> with TMS as internal standard.<sup>b</sup> Chemical shifts were determined at 400 (<sup>1</sup>H) MHz in DMSO-d<sub>6</sub>.**Table 2.** <sup>13</sup>C-NMR spectral data for compounds **1**, **2**, **3**<sup>a</sup> and **4**<sup>b</sup>.

C	1	2	3	4
2	161.5	161.0	161.0	161.3
3	112.6	113.1	114.6	111.3
4	143.7	139.0	144.1	144.5
4a	112.2	107.3	115.4	111.4
5	123.2	148.3	119.8	129.7
6	124.5	114.1	124.8	113.1
7	163.4	158.0	156.4	160.4
8	97.9	94.8	99.8	102.1
8a	155.7	152.5	152.0	155.5
2'	88.8	145.3	146.9	-
3'	29.6	104.5	106.4	-
1''	81.3	61.1	-	-
2''	22.3	72.2	-	-
3''	21.2	58.3	-	-
Me-3''	-	24.6	-	-
		19.0		
1(OSen)	165.8	-	-	-
2(OSen)	116.9	-	-	-
3(OSen)	156.7	-	-	-
4(OSen)	27.4	-	-	-
5(OSen)	20.1	-	-	-

<sup>a</sup> Chemical shifts were determined at 100 (<sup>13</sup>C) MHz in CDCl<sub>3</sub> with TMS as internal standard.<sup>b</sup> Chemical shifts were determined at 100 (<sup>13</sup>C) MHz in DMSO-d<sub>6</sub>.

at  $\delta$  6.75 (1H, *s*, H-8) and 7.22 (1H, *s*, H-5), H-2' at  $\delta$  5.14 (1H, *dd*,  $J=8.0, 8.8$  Hz), H-3' at  $\delta$  3.23 (2H, *m*) and the deshielding of H-4, suggest the structure of a linear dihydrofuranocoumarin (13). A substituent at C-2' was identified by  $^1\text{H-NMR}$  according to the following signals: two singlets at  $\delta$  1.60 (3H, *s*, H-2'') and 1.54 (3H, *s*, H-3'') (methyl groups) and the remaining signals in agreement with the presence of a senecieryl group; three singlets at  $\delta$  5.56 (1H, *s*, H-2), 2.10 (3H, *s*, H-4) and 1.86 (3H, *s*, H-5) (13,30). The  $^{13}\text{C-NMR}$  spectral data (Table 2) showed nineteen signals. The peaks at  $\delta$  165.8 (C-1(OSen)) and 161.5 (C-2) ppm showed a characteristic senecieryl and pyrone carbonyl group, respectively. The infrared spectrum of compound **1** displayed two significant absorptions at 1727 and 1627  $\text{cm}^{-1}$  due to the carbonyl and olefinic functional groups, respectively (30). On the basis of these data and the reported data for prantschimgin (13,29,30), the compound **1** was assigned as prantschimgin.

Compound **2**: The mass spectrum of the compound **2** had  $[\text{M}]^+$  at  $m/z$  286.1 suggesting the molecular formula  $\text{C}_{16}\text{H}_{14}\text{O}_5$  (32). The  $^1\text{H-NMR}$  spectrum shows two AB system at  $\delta$  6.32,  $\delta$  8.21 (1H each, *d*,  $J=9.6$  Hz, H-3, H-4, respectively) and at  $\delta$  6.96,  $\delta$  7.62 (1H each, *d*,  $J=2$  Hz, H-3', H-2', respectively) which are characteristic of the furanocoumarin skeleton. The presence of a singlet proton at  $\delta$  7.19 (1H, *s*, H-8) and the deshielding of H-4, suggest a linear furanocoumarin structure with a substituent at C-5 for compound **2**. This substituent was identified by  $^1\text{H-NMR}$  spectroscopy as



group according to these signals: two singlets at  $\delta$  1.42 and  $\delta$  1.34 (methyl group) and an ABX system with signals at  $\delta$  4.62 (1H, *dd*,  $J=10.8, 4.2$  Hz, H-1''),  $\delta$  4.44 (1H, *dd*,  $J=10.8, 6.8$  Hz, H-1'') and  $\delta$  3.24 (1H, *dd*,  $J=4.2, 6.8$  Hz, H-2'') (13). The  $^{13}\text{C-NMR}$  spectral data (Table 2) showed sixteen signals. The peak at  $\delta$  161.0 (C-2) showed a characteristic pyrone carbonyl group. The signals at  $\delta$  158.0 (C-7), 152.5 (C-8a), 148.3 (C-5) and 145.3 (C-2') were related to  $=\text{C-O}$  whereas the signals at  $\delta$  72.2 (C-2''), 61.1 (C-1'') and 58.3 (C-3'') were related to  $-\text{C-O}$  (13). The infrared spectrum of compound **2** displayed absorption at 1726  $\text{cm}^{-1}$  due to the pyrone carbonyl group. On the basis of these and the reported data for oxypeucedanin (13,31,32), the compound **2** was assigned as oxypeucedanin.

Compound **3**: The mass spectrum of the compound **3** had  $[\text{M}]^+$  at  $m/z$  186 suggesting the molecular formula  $\text{C}_{11}\text{H}_6\text{O}_3$  (34,35). The C-4, C-5

and C-8 protons in coumarin are markedly more deshielded than that on C-3 as a result of the linear fusion of a furan moiety in psoralen (36). The  $^1\text{H-NMR}$  spectrum shows two AB system at  $\delta$  6.39,  $\delta$  7.81 (1H each, *d*,  $J=9.6$  Hz, H-3, H-4, respectively) and at  $\delta$  6.84 (1H, *dd*,  $J=1.2, 2.2$  Hz, H-3'),  $\delta$  7.70 (1H, *d*,  $J=2.2$  Hz, H-2') which are characteristic of the furanocoumarin skeleton. The presence of a multiplet proton at  $\delta$  7.48 (1H, *m*, H-8) and the deshielding of H-4, suggest a linear furanocoumarin structure for compound **3**. H-5 was observed at  $\delta$  7.69 (1H, *s*) (13). The  $^{13}\text{C-NMR}$  spectral data (Table 2) showed eleven signals. The peak at  $\delta$  161.0 ppm shows a characteristic pyrone carbonyl group and  $\delta$  156.4, 152.0 and 146.9 ppm are related to  $=\text{C-O}$  (34). The infrared spectrum of compound **3** displayed absorption at 1715  $\text{cm}^{-1}$  due to the pyrone carbonyl group (37). On the basis of these and the reported data for psoralen (13,33-37), the compound **3** was assigned as psoralen.

Compound **4**: The mass spectrum of the compound **4** had  $[\text{M}]^+$  at  $m/z$  162 suggesting the molecular formula  $\text{C}_9\text{H}_6\text{O}_3$ . In the proton nuclear magnetic resonance ( $^1\text{H-NMR}$ ) spectrum, H-3 and H-4 were observed at  $\delta$  6.19 (*d*,  $J=9.4$  Hz, 1H) and 7.93 (*d*,  $J=9.4$  Hz, 1H) as AB-type signals, respectively. The  $^1\text{H-NMR}$  spectrum showed an ABX system with signals at  $\delta$  7.52 (1H, *d*,  $J=8.4$  Hz, H-5),  $\delta$  6.78 (1H, *dd*,  $J=0.2, 8.4$  Hz, H-6) and  $\delta$  6.70 (1H, *d*,  $J=0.16$  Hz, H-8) (13). The  $^{13}\text{C-NMR}$  spectral data (Table 2) showed eleven signals. The peak at  $\delta$  161.3 ppm shows a characteristic pyrone carbonyl group and  $\delta$  160.4 (C-7) and 155.5 (C-8a) are related to  $=\text{C-O}$  (39,40). The infrared spectrum of compound **4** exhibits two strong bands at 1681 and 1235  $\text{cm}^{-1}$ , which may be assigned to  $\nu_{\text{as}}$  (C=O) and  $\nu_{\text{s}}$  (C=O), respectively. The low carbonyl frequency (1681  $\text{cm}^{-1}$ ) is presumably due to intermolecular hydrogen bonding of the 7-hydroxyl hydrogen either with O(1) or with O(2). The  $\nu_{\text{as}}$  (C=O) (phenolic) and  $\nu_{\text{s}}$  (C=O) (phenolic) have been assigned at 1568 and 1322  $\text{cm}^{-1}$  as very strong bands in umbelliferone, respectively (39). On the basis of these and the reported data for umbelliferone (13,38-41), the compound **4** was assigned as umbelliferone.

Compound **5**: The mass spectrum of the compound **5** had  $[\text{M}]^+$  at  $m/z$  414.3 suggesting the molecular formula  $\text{C}_{29}\text{H}_{50}\text{O}$ . The  $^1\text{H-NMR}$  spectrum of the compound **5** displayed a multiplet at  $\delta$  3.53 for the H-3. This is a deshielded proton as a result of OH group. The  $^1\text{H-NMR}$  spectrum displayed olefinic proton (H-6) at  $\delta$  5.36 as a distorted doublet. The same spectrum showed signals for Me-18 at  $\delta$  0.68 (*s*), Me-19 at  $\delta$  1.01 (*s*), Me-21 at  $\delta$  0.92 (*d*,  $J=6$  Hz), Me-26 and 27 at

**Table 3.** <sup>1</sup>H-NMR & <sup>13</sup>C-NMR spectral data for compound 5<sup>a</sup>.

C/H atoms	$\delta$ C	$\delta$ H [mult., J (Hz)]	C/H atoms	$\delta$ C	$\delta$ H [mult., J (Hz)]
1	37.2	-	16	28.2	-
2	31.6	-	17	56.0	-
3	71.8	3.53 (m, 1H)	18	11.8	0.68 (s, 3H)
4	42.3	-	19	19.8	1.01 (s, 3H)
5	140.7	-	20	36.1	-
6	121.7	5.36 (d, 1H)	21	18.8	0.92 (d, J=6 Hz, 3H)
7	31.9	-	22	33.9	-
8	31.9	-	23	26.0	-
9	50.1	-	24	45.8	-
10	36.5	-	24a	23.0	-
11	21.1	-	24b	12.0	0.84 (brs, 3H)
12	39.7	-	25	29.1	-
13	42.3	-	26	19.0	0.82 (d, J=6.8 Hz, 3H)
14	56.7	-	27	19.4	0.82 (d, J=6.8 Hz, 3H)
15	24.3	-			

<sup>a</sup>Chemical shifts were determined at 400 (<sup>1</sup>H) and 100 (<sup>13</sup>C) MHz in CDCl<sub>3</sub> with TMS as internal standard.

**Table 4.** Reports in the literature about compounds in the *Ferulago* species

Compound	Plant	Occurrence
Oxypeucedanin	<i>F. Bernardii</i>	Aerial parts
	<i>F. brachyloba</i> (13)	Roots
	<i>F. capillaris</i> (13)	Aerial parts
	<i>F. capillaris</i> (13)	Roots
	<i>F. granatensis</i> (9)	Umbels
	<i>F. meoides</i> (6)	Roots
	<i>F. turcomanica</i> (8)	Bark
	<i>F. turcomanica</i> (7)	Roots
Prantschimgin	<i>F. asparagifolia</i> (11)	Aerial parts
	<i>F. aucheri</i> (10)	Aerial parts
	<i>F. Bernardii</i>	Aerial parts
	<i>F. brachyloba</i> (13)	Roots
	<i>F. capillaris</i> (13)	Aerial parts
	<i>F. capillaris</i> (13)	Roots
	<i>F. granatensis</i> (9)	Umbels
	<i>F. meoides</i> (6)	Roots
Psoralen	<i>F. Bernardii</i>	Aerial parts
	<i>F. turcomanica</i> (8)	Bark
$\beta$ -Sitosterol	<i>F. Bernardii</i>	Aerial parts
	<i>F. granatensis</i> (9)	Umbels

$\delta$  0.82 (*d*,  $J=6.8$  Hz) and Me-24b at  $\delta$  0.84 (*brs*) (42). The  $^{13}\text{C}$ -NMR spectral data (Table 2) showed twenty-nine signals. The peaks at  $\delta$  140.7 (C-5) and 121.7 (C-6) are related to olefinic carbons. The signal at  $\delta$  71.8 (C-3) ppm may be related to C-O (43,44). The infrared spectrum of compound **5** displayed an absorption at  $3438\text{ cm}^{-1}$  due to the hydroxyl function (42). On the basis of these and the reported data for  $\beta$ -sitosterol (29,42-44), the compound **5** was assigned as  $\beta$ -sitosterol. Compound **6**: The mass spectrum of the compound **6** had  $[\text{M}]^+$  at  $m/z$  408 suggesting the molecular formula  $\text{C}_{29}\text{H}_{60}$ . The absence of an ion at  $m/z$  393  $[\text{M-Me}]^+$  indicated the compound **6** not to be branched having a methyl group as substituent (45). The  $^1\text{H}$ -NMR spectrum of the compound **6** displayed a triplet at  $\delta$  0.88 ( $J=6$  Hz) for the two terminal methyl groups and a singlet at  $\delta$  1.25 for the methylene groups (45). The infrared spectrum of compound **6** displayed two absorptions at  $1378$  (methyl) and  $724\text{ cm}^{-1}$  (long chain). On the basis of these and the reported data for nonacosane (29,45), the compound **6** was assigned as nonacosane.

## CONCLUSION

This is the first report on the phytochemical analysis of *Ferulago Bernardii* Tomk. & M. Pimen. From the hexane extract of the aerial parts of *Ferulago Bernardii* four coumarins, prantschimgin, oxypeucedanin, psoralen and umbelliferone; one steroid,  $\beta$ -sitosterol; and a normal alkane, nonacosane were isolated by CC, PTLC and crystallization were characterized by melting point, and spectral data.

Four of these compounds have been found in some others *Ferulago* species (see Table 4). The presence of these compounds (oxypeucedanin, prantschimgin, psoralen and  $\beta$ -sitosterol) could be used as chemotaxonomic marker in genus *Ferulago*.

## ACKNOWLEDGMENTS

This work was supported by grants from the Research Council of Tehran University of Medical Sciences and by Iran Chapter of TWAS. The authors wish to thank Dr. Amini, Dr. Soori, Miss Javidnia, Mr. Abdi and Mr Dalvandi (Department of Chemistry, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran) for their technical assistance.

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