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NEW FURANOCOUMARINS ISOLATED FROM THE ROOTS OF *FERULAGO ISAURICA* PESMEN GROWING IN TURKEY

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Abstract—New furanocoumarins, 4'-*O*-benzoyl rutaretin (**1**) and 4'-*O*-3-methyl-2-butenoyl rutaretin (**2**) along with five known coumarins, felamedin (**3**), prantschimgin (**4**), bergapten (**5**), xanthotoxin (**6**) and isoimperatorin (**7**), have been isolated from the chloroform extract of the roots of *Ferulago isaurica* Pesmen. These structures have been elucidated on the basis of spectral and physical methods.

Ferulago isaurica (Umbelliferae) is a perennial endemic species, growing only in Alanya, South Anatolia, Turkey.[1] Turkish local names for these plants are Caksir or Cagsir. Species of the genera *Ferula* and *Prangos* are also known by these names and though they are reported to have other usages, are mainly used as aphorodisiac in Turkey.[2] This paper describes the isolation of new furanocoumarins along with known compounds from the aforesaid species.

The roots were grounded and macerated with methanol in a water bath, nor exceeding 60℃. The extract was filtered and concentrated till dryness, then was dispersed in Methanol : Water (1:9) and partitioned with chloroform and ethyl acetate in a separating funnel respectively. Each fraction was then concentrated to dryness. Chloroform fraction was applied to column chromatography with silica gel. Elution started with *n*-hexane, continued with ethyl acetate and finished with methanol and then further purified by

preparative HPLC to yield seven compounds.

Compound 1 (**1**), colorless needles, mp 161-164℃, $[\alpha]_D$ – 112.6° (c= 0.68, $CHCl_3$) , showed a molecular ion peak at *m/z* 366 (18.7 %) in its electron ionization mass spectrum (EI-MS). The molecular formula was determined as $C_{21}H_{18}O_6$ on the basis of high-resolution (HR)-EI-MS (calc. 366.1103; obs. 366.1108, error, +0.5 mmu). The ^{1}H-NMR spectrum in the aromatic proton region of **1** contained a pair of doublet at δ 6.25 (1H, d, *J*= 9.57 Hz) and 7.64 (1H, d, *J*= 9.57 Hz), which are in agreement with the H-3 and H-4 signals at the α-pyrone ring system. One proton-triplet at δ 5.21 and two proton-double doublet at δ 3.39 are attributable to the methin proton and methylen protons on the furan ring, and two three proton signals at δ 1.72 and 1.75 for the *gemi*-dimethyl group were appeared in its spectrum. A proton signal at δ 6.89 (1H, s) bonded with ^{13}C-NMR signal at δ 114.3 was assigned to the H-5 by ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) spectrum, which showed the correlation peak with H-3' (3-bonds), this suggests OH-group is exist in C-8. Three complex signals at δ 7.28~7.36, 7.47~7.57 and 7.72~7.78 were assigned to those of benzoyl group by the comparison with ^{1}H-NMR spectrum of methyl benzoate. The presence of benzoyl group was also supported by the fragment peak at *m/z* 105 (72.8 %) in EI-MS. By the comparison of ^{1}H- and ^{13}C-NMR with compound (**3**) and rutaretin, benzoyl group was attached to C-4' OH group of rutaretin, which was shifted at δ 82.9 (rutaretin ca.70 ppm)[3]

in its ^{13}C-NMR spectrum. All the ^{13}C-NMR signals are reasonably assigned as shown in Table 1. Therefore, **1** was established as 4'-*O*-benzoyl rutaretin.

Compound 2 (**2**), colorless needles, mp 195-197 ℃, showed a molecular ion peak at *m/z* 344 (20.6 %) in its EI-MS. The molecular formula was determined as $C_{19}H_{20}O_6$ on the basis of HR-EI-MS (calc. 344.1260; obs. 344.1261, error, +0.1mmu). ^{1}H- and ^{13}C-NMR spectral data showed a similar spectral data with **1** except for ester group (see Table 1). The presence of an ester group was substantiate by the appearance of signals at δ 1.85 and δ 2.10 (each 3H, d, *J*= 1.22 Hz) and 5.55~5.59 (1H, m, olefinic proton). A proton signals at δ 6.85 (1H, s) bonded with signal at δ 114.3 was assigned to the H-5 by HMBC spectrum, which showed the correlation peak with H-4 and H-3' (3-bonds, respectively), this suggests that OH-group is exist in C-8. The ester group was proved to be 3-methyl-2-butenoyl from ^{1}H- and ^{13}C-NMR spectral data, which was supported by the mass fragment peak *m/z* at 83 (63.6 %) due to butenoyl group. By the comparison of ^{1}H- and ^{13}C-NMR with **4** and rutaretin, ester group was attached to C-4' OH group of rutaretin, which was shifted at δ 81.2 (rutaretin ca. 70 ppm) in its ^{13}C-NMR. On the basis of above data, therefore, **2** was established as 4'-*O*-3-methyl-2-butenoyl rutaretin.

Compound 3 (**3**), colorless needles, mp 133-135 ℃, $[\alpha]_D$- 92.3° (c= 2.27, $CHCl_3$), $C_{21}H_{18}O_5$, showed a molecular ion peak at *m/z* 350 (6.1%) in its EI-MS. The ^{1}H-NMR spectrum in the aromatic proton region of **3** contained a pair of doublet at δ 6.34 and 7.73 (each 1H, d, *J*= 9.57 Hz), which are in

agreement with H-3 and H-4, and the singlet signals at δ 6.88 and 7.36 were assigned to H-8 and H-5, respectively. The signal at δ 3.40~3.50 (2H, m) to H-3' and the signal at δ 5.24 (1H, dd) to H-2' proton were also supported to the linear furanocoumarin skeltone by comparison of ^{13}C-NMR spectral data such as marmesinin and nodakenetin. The presence of benzoyl group was also shown from ^{1}H- and^{13}C-NMR and a fragment peak at *m/z* 105 (36.7 %) in its EI-MS spectrum. By the comparison of $[\alpha]_D$, therefore, **3** was identified as 4'-*O*-benzoyl marmesinin, felamedin.[4]

Compound 4 (**4**), colorless needles, mp 136-139 ℃, $C_{19}H_{20}O_5$, $[\alpha]_D$- 45.8° (*c*= 0.85 , $CHCl_3$) , showed a molecular ion peak at *m/z* 328 (5.8 %) in its EI-MS. EI-MS also showed the fragment peak at *m/z* 83 (25.6 %) due to that ester group. The ^{1}H- and ^{13}C-NMR showed a similar spectral data of coumarin skelton with **3** and the presence of 3-methyl-2-butenoyl group as an ester group. By the comparison of $[\alpha]_D$, therefore, **4** was identified to be 4'-*O*-3-methyl-2-butenoyl marmesinin, prantschimgin.[5]

Compound 5 (**5**), colorless needles, mp 185-188 ℃, $C_{12}H_8O_4$, showed a molecular ion peak at *m/z* 216 (100 %) in its EI-MS. ^{1}H-NMR spectrum showed a pair of doublet at δ 6.28 and 8.16 (each 1H, d, *J*= 9.57 Hz), a pair of signals at δ 7.02 (1H, dd, *J*= 0.99 and 2.31 Hz) and 7.59 (1H, d, *J*= 2.64 Hz) due to furan ring and a singlet proton at δ 7.15, and revealed the presence of a methoxyl at δ 4.27. Therefore, the structure of **5** was identified by comparison with MS and ^{1}H-NMR spectral data of the authentic sample of bergapten.[6,7]

Compound 6 (**6**), colorless needles, mp 142-144 ℃, $C_{12}H_8O_4$, were identified by comparison with MS, ^{1}H- and ^{13}C-NMR spectral data of the authentic sample of xanthotoxin.[6]

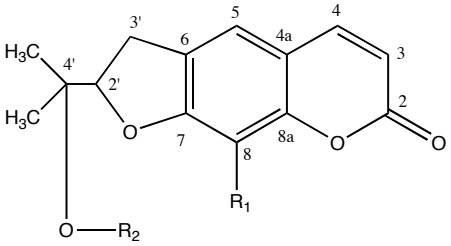
Compound (**7**), colorless needles, mp. 103-107 ℃, $C_{16}H_{14}O_4$, showed a similar ^{1}H-NMR spectral data of coumarin skelton with compound **5 e**xcept for methoxyl group. The presence of a 2-methyl-2-butenyl group was substantiate by the appearance of signals at δ 1.70 and 1.80 (each 3H, s) and 5.54 (1H, m, olefinic proton) and 4.92 (2H, d, *J*=6.93 Hz). The ester group was proved to be 2-methyl-2-butenyl from ^{1}H- and ^{13}C-NMR spectral data, which was supported by a mass fragment peak *m/z* at 69 (30.2 %) due to butenyl group. Therefore, **7** was identified to be isoimperatorin,[6] by comparison with MS, ^{1}H-NMR and ^{13}C-NMR spectral data of the authentic sample.

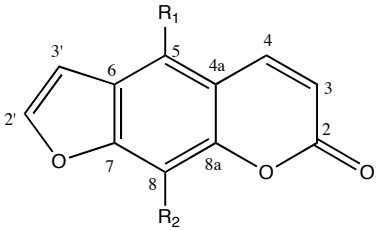
Umbelliferae is a large family containing mainly various coumarins and volatile oils. Species of the genera *Ferulago* are also containing many varieties of coumarin compounds. This plant, *Ferulago isaurica* was the first representitive havining natural benzoylated and 3-methyl-2-butenoylated rutaretin, respectively.

Table 1. ^{1}H and ^{13}C-NMR Spectral data of **1**~ **4**

	1		**2**		**3**		**4**	
C								
2	160.3		160.3		161.3		161.4	
3	112.2	6.25 (1H,d,*J* =9.57)	112.2	6.22 (1H,d,*J* =9.46)	112.3	6.34 (1H,d,*J* =9.57)	112.3	6.21 (1H,d,*J* =9.57)
4	144.2	7.64 (1H,d,*J* =9.57)	144.2	7.61 (1H,d,*J* =9.46)	143.6	7.73 (1H,d,*J* =9.57)	143.6	7.59 (1H,d,*J* =9.57)
4a	113.1		113.1		112.7		112.7	
5	114.3	6.89 (1H,s)	114.3	6.85 (1H,s)	123.2	7.36 (1H,s)	123.2	7.21 (1H,s)
6	125.2		125.2		124.5		124.5	
7	149.9		149.9		163.5		163.4	
8	127.9		127.8		98.0	6.88 (1H,s)	98.0	6.75 (1H,s)
8a	143.0		143.1		155.8		155.8	
2'	89.7	5.21 (1H,t,*J* =8.25;8.58)	90.0	5.19 (1H,t,*J* =8.55)	89.1	5.24 (1H,dd)	88.9	5.14 (1H,dd)
3'	30.4	3.39 (2H,dd,*J* =8.25;0.99)	30.3	3.25~3.28 (2H,m)	29.7	3.40~3.5 0 (2H,m)	29.6	3.14~3.31 (2H,m)
4'	82.9		81.2		82.9		81.3	
CH_3	21.3	1.72 (3H, s)	21.1	1.56 (3H,s)	22.1	1.71 (3H,s)	21.3	1.54 (3H,s)
CH_3	22.4	1.75 (3H,s)	22.5	1.64 (3H,s)	21.4	1.69 (3H,s)	22.3	1.59 (3H,s)
1"	165.4				165.4			
2"	131.0				131.0			
3",7"	128.3	7.72~7.78(2H,m)			128.2	7.80~7.83 (2H,dd)		
4",6"	129.4	7.28~7.36(2H,m)			129.4	7.36~7.43 (2H,m)		
5"	132.8	7.47~7.57(1H,m)			132.8	7.56~7.75(1H,m)		
1"			165.8				165.9	
2"			117.0	5.55~5.59 (1H,m)			116.9	5.54~5.56 (1H,m)
3"			156.4				156.9	
CH_3			20.0	2.10 (d,*J* =1.22)			20.1	2.10 (3H,d,*J* =0.99)
CH_3			27.3	1.85 (d,*J* =1.22)			27.4	1.87 (3H,d,*J* =1.32)

Chemical shifts are in δ-values from TMS and followed by multiplicities and *J* -values (in Hz), R.T. in $CDCl_3$.





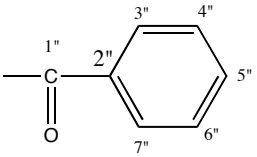
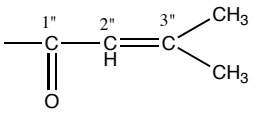
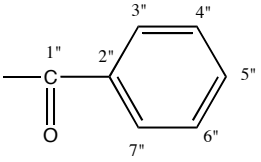
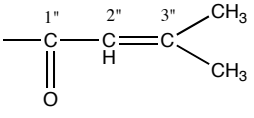
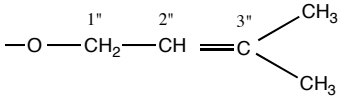
	R_1	R_2
1	—OH	
2	—OH	
3	—H	
4	—H	
5	—OCH_3	—H
6	—H	—OCH_3
7		—H

Chart 1 The Structures of **1**~**7**.

EXPERIMANTAL

General Procedures Melting points were determined on a micro melting point apparatus (Yanako) without correction. Optical rotations were recorded on a JASCO DIP-140 digital polarimeter. NMR spectra were recorded on a JEOL EX-270 (^{1}H: 270 MHz, ^{13}C: 67.5 MHz or LA-500 ^{1}H: 500MHz, ^{13}C: 125 MHz); δ in ppm relative to TMS (internal standard), *J* in Hz. EI- and FAB-MS spectra were measured with a JEOL DX-302 mass spectrometer.

Plant Material Plants materials were collected from Alanya, Antalya, 5.9-6 km far from Derince Turn, rocky slopes facing north, 990-1040 m, 16/7/2000, and identified by Professor M. Koyncu, Ankara University. A voucher specimen (AEF 22956) was deposited to the herbarium of the faculty of pharmacy, Ankara University.

Extraction and Isolation The roots (500 g) were grounded and macerated with 8 hours/3 days with methanol in a water bath, nor exceeding 60℃, using a Heidolph mechanical mixer (300 rpm). The extracts, filtered and concentrated till dryness were dispersed in MeOH : Water (1:9) and fractionated with 400 mL of chloroform, AcOEt and in a separating funnel respectively. Each fraction was then concentrated to dryness. After TLC analysis, $CHCl_3$ fraction was selected for isolation. $CHCl_3$ fraction of 26 g was applied to column chromatography with silica gel 30-70 mesh, Merck 7733. Elution started with *n*-hexane, continued with AcOEt and finished with MeOH and yielded 8 compounds, further purified by preparative HPLC (detector TOSOH UV-8011 (320 nm), pump TOSOH CCPS, recorder Sekonic SS-100F, flow rate 3 mL/min., on Cosmosil packed Column, Silica 10 x 250 mm) and their structures have been identified by means of NMR and MS spectra.

Compound 1 (**1**): colorless needles, mp 161-164 ℃. $[\alpha]_D$ -112.6° (c= 0.68, $CHCl_3$). EI-MS (*m/z*) : 366 (M^+, 18.7), 244 (54.2), 229 (100), 105 (72.8), 77 (18.2). HR-EI-MS: $C_{21}H_{18}O_6$ (calc. 366.1103; obs. 366.1108, error, +0.5 mmu). ^{1}H-NMR and ^{13}C-NMR: See Table 1.

Compound 2 (**2**): colorless needles, mp 195-197 ℃. EI-MS (*m/z*): 344 (M^+, 20.6), 244 (49.7), 229 (100), 83 (63.6). HR-EI-MS: $C_{19}H_{20}O_6$ (calc. 344.1260; cal. 344.1261, error, +0.1 mmu). ^{1}H-NMR and ^{13}C-NMR: See Table 1.

Compound 3 (**3**): colorless needles, mp 133-135 ℃. $[\alpha]_D$ –92.3° (*c*= 2.27 , $CHCl_3$). EI-MS (*m/z*): 350 (M^+, 6.1), 228 (56.9), 213 (100), 105 (36.7), 77 (14.5). $C_{21}H_{18}O_5$, ^{1}H-NMR and ^{13}C-NMR: See Table 1.

Compound 4 (**4**): colorless needles, mp 136-139 ℃. $[\alpha]_D$ – 45.8 ° (c= 0.85 , $CHCl_3$) . EI-MS (*m/z*):

328 (M^+, 5.8), 228 (53.5), 213 (100), 83 (25.6). $C_{19}H_{20}O_5$. ^{1}H-NMR and ^{13}C-NMR: See Table 1.

Compound 5 (**5**): colorless needles, mp 185-188 ℃. EI-MS (*m/z*): 216 (M^+, 100), 201 (26.7), 173 (41.6), 145 (16.0), $C_{12}H_8O_4$.

Compound 6 (**6**): colorless needles, mp 140-142 ℃, EI-MS (*m/z*:): 216(M^+,100), 201 (23.3), 173 (36.0), 145 (11.7) , $C_{12}H_8O_4$.

Compound 7 (**7**): colorless needles, mp 103-107 ℃. EI-MS (*m/z*): 270 (M^+, 3.0), 202 (100), 174 (15.7), 69 (30.2). $C_{16}H_{14}O_4$.

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