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Pyranocoumarins from *Seseli gummiferum* subsp. *corymbosum* Growing in Turkey

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Seseli gummiferum Pall. ex Sm. subsp. *corymbosum* (Boiss. & Heldr.) P.H. Davis (Syn: *S. corymbosum* Boiss. & Heldr.) (Umbelliferae) collected in southern Anatolia was investigated for the presence of coumarins. A new angular-type pyranocoumarin, corymbocoumarin (**1**), along with 5 known coumarins (**2-6**) were isolated from the aerial parts of this plant. Corymbocoumarin (**1**) was established to be (−)-(3′*S*,4′*S*)-3′-acetoxy-4′-isovaleryloxy-3′,4′-dihydroseselin (**1**) and coumarins **2-6** were identified as (−)-(3′*S*,4′*S*)-3′-acetoxy-4′-angeloyloxy-3′,4′-dihydroseselin (**2**), (+)-(3′*S*,4′*S*)-3′-hydroxy-4′-angeloyloxy-3′,4′-dihydroseselin (d-laserpitin) (**3**), (−)-(3′*S*,4′*S*)-3′-angeloyloxy-4′-hydroxy-3′,4′-dihydroseselin (**4**), 3′-acetoxy-4′-isobutyloxy-3′,4′-dihydroseselin (**5**) and osthole (**6**), respectively, by spectroscopic methods. The structural elucidation and absolute configurations were determined by chemical correlations with known compounds.

Key Words: Umbelliferae, corymbocoumarin, angular-type pyranocoumarin, *Seseli gummiferum* Pall. ex Sm. subsp. *corymbosum* (Boiss. & Heldr.) P.H. Davis

Introduction

The genus *Seseli* L. is represented by 12 taxa (11 species and 1 subspecies) in the *Flora of Turkey*, of which 4, including the title species, are native to the region. *S. gummiferum* subsp. *corymbosum* is a perennial or monocarpic plant growing in southern Anatolia, Turkey[1−4]. There is no record of the chemical constituents of this plant.

This paper describes the isolation of a new angular-type dihydropyranocoumarin, corymbocoumarin (**1**), along with 4 known angular-type pyranocoumarins (**2-5**) and a simple coumarin (**6**), all isolated from the *n*-hexane extract of the aerial parts of *S. gummiferum* subsp. *corymbosum*.

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Experimental

General experimental procedures

Chromatographic separations were carried out on a silica gel open column (0.063-0.200 mm, 70-230 mesh Merck 1.07734). TLC (Silica gel 60 $F_{254,}$ Merck 1.05554) was observed under UV at 366 nm and 254 nm, and then sprayed with sulfuric acid and 5% KOH solution. Prep. HPLC was performed on a TOSOH-Prep-HPLC equipped with an HPLC packed column of Senshu-Pak silica 4251-N (250 mm x 10 mm) and TOSOH UV-8010, 320 nm. Melting points were measured on a BUCHI SMP-20, electrothermal melting point apparatus and a YANACO micro melting point apparatus. Optical rotations were determined on a JASCO DIP-140 automatic polarimeter at 20 °C. ^{1}H and ^{13}C NMR spectra were measured in $CDCl_3$, on a JEOL JNM-EX 270 FT-NMR spectrometer. Chemical shifts are expressed in δ units relative to TMS (δ = 0) as internal standard. Mass spectra were recorded on a JEOL-JMS DX 302 MS spectrometer operating at 70 eV in electron impact mode.

Plant material

Aerial parts of *Seseli gummiferum* Pall. ex Sm. subsp. *corymbosum* (Boiss. & Heldr.) P.H. Davis (Umbelliferae) were collected from the Village of Pınarbaşı, Kadife Mountains, Akseki-Antalya, at an altitude of 1650-1900 m, during the flowering season of August, 2000. A voucher specimen identified by Prof. Hayri Duman is deposited in the Herbarium of Ankara University's Faculty of Pharmacy (AEF 21701).

Extraction and Isolation

Air-dried aerial parts (1 kg) of *S. gummiferum* subsp. *corymbosum* were successively extracted with *n*−hexane, Et_2O, EtOAc and MeOH in a Soxhlet apparatus over 8 h. The organic solvents were evaporated to dryness in vacuo to yield the corresponding *n*−hexane (85 g), Et_2O (31.66 g), EtOAc (13.14 g) and MeOH extracts (110 g), respectively. The *n*−hexane extract was chromatographed on a silica gel column using an *n*-hexane:EtOAc gradient elution system.

The new coumarin, corymbocoumarin (**1**) (1.5 g), and coumarin **6** (3.2 g) were isolated from the fractions eluted with *n*-hexane:EtOAc (9:1). The fractions obtained by elution with *n*-hexane:EtOAc (8:2) were purified by HPLC eluted with *n*-hexane:EtOAc (3:1), to give coumarins **2** (20.5 mg), **3** (77.8 mg), **4** (48.3 mg) and **5** (10.2 mg).

Coumarin 2 (2): white oil, $[\alpha]_D$ - 36.45° ($CHCl_3$); EIMS: $C_{21}H_{22}O_7$ (M^+ , m/z: 386; 13.07%), 326 (1.80), 311 (5.45), 303 (1.70), 286 (9.25), 244 (40.24), 229 (89.47), 191 (19.72), 83 (base peak, 100), 55 (50.31), 43 (37.11); ^{1}H NMR (270 MHz, in $CDCl_3$, δ ppm; see Table 1); ^{13}C NMR (see Table 2).

Coumarin 3 (3): white oil, $[\alpha]_D$ + 84.61° ($CHCl_3$). EIMS: $C_{19}H_{20}O_6$ (M^+, m/z: 344; 18.99%), 326 (7.64), 311 (20.60), 245 (26.58), 244 (21.16), 229 (35.17), 203 (40.57), 83 (base peak, 100), 55 (39.37); ^{1}H NMR (270 MHz, in $CDCl_3$, δ ppm; see Table 1); ^{13}C NMR (see Table 2).

Coumarin 4 (4): glass - like substance, m.p. 71 °C, $[\alpha]_D$ - 38.84° ($CHCl_3$). EIMS: $C_{19}H_{20}O_6$ (M^+, m/z: 344; 10.91%), 229 (31.22), 83 (base peak, 100), 71 (18.34), 55 (34.62), 43 (27.26); ^{1}H NMR (270 MHz, in $CDCl_3$, δ ppm; see Table 1); ^{13}C NMR (see Table 2).

Coumarin 5 (5): glass - like substance. EIMS: $C_{19}H_{22}O_7$ (M^+, m/z: 374; 12.18%), 314 (8.62), 299 (13.83), 229 (base peak, 100), 71 (16.68), 43 (16.14); ^{1}H NMR (270 MHz, in $CDCl_3$, δ ppm; see Table 1); ^{13}C NMR (see Table 2).

Coumarin 6 (6): colorless needles, m.p. 83 - 84 °C, $C_{15}H_{16}O_3$ (M^+, m/z: 244; 100%), 229 (72.7), 213 (25.94), 201 (32.50), 189 (44.53); ^{1}H NMR (270 MHz, in $CDCl_3$, δ ppm): 6.23 (1H, d, J = 9.6 Hz, H - 3), 7.61 (1H, d, J = 9.6 Hz, H - 4), 7.29 (1H, d, J = 8.6 Hz, H - 5), 6.83 (1H, d, J = 8.6 Hz, H - 6), 3.53 (2H, d, J = 7.3 Hz, H - 1′), 5.22 (1H, t - like, J = 7.3 Hz, H - 2′), 1.66 (3H, s, H - 4′), 1.84 (3H, s, H - 5′), 3.92 (3H, s, - OCH3).

Table 1. ^{1}H NMR spectral data of *Seseli* pyranocoumarins **1-5** (ppm from TMS, in $CDCl_3$).

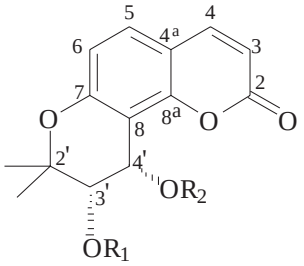
Proton	**1**	**2**	**3**	**4**	**5**
H-3	6.23	6.24	6.20	6.25	6.23
	(d, J = 9.6)	(d, J = 9.6)	(d, J = 9.2)	(d, J = 9.6)	(d, J = 9.6)
H-4	7.59	7.61	7.60	7.65	7.60
	(d, J = 9.6)	(d, J = 9.6)	(d, J = 9.2)	(d, J = 9.6)	(d, J = 9.6)
H-5	7.36	7.36	7.35	7.34	7.36
	(d, J = 8.6)	(d, J = 8.6)	(d, J = 8.6)	(d, J = 8.9)	(d, J = 8.6)
H-6	6.80	6.81	6.80	6.80	6.81
	(d, J = 8.6)	(d, J = 8.6)	(d, J = 8.6)	(d, J = 8.6)	(d, J = 8.6)
H-3′	5.31	5.41	4.10	5.47	5.32
	(d, J = 5.0)	(d, J = 4.6)	(d, J = 3.0)	(br. m)	(d, J = 5.0)
H-4′	6.55	6.60	6.51	5.22	6.53
	(d, J = 4.6)	(d, J = 5.0)	(d, J = 5.0)	(d, J = 5.0)	(d, J = 5.0)
***gem*-Me**	1.45, 1.42	1.48, 1.43	1.50, 1.44	1.51, 1.44	1.45, 1.42
Ester part	2.10	2.11	3.14	3.41	2.09
	(3H s, $OCOCH_3$)	(3H s, $OCOCH_3$)	(1H m, OH)	(1H br.s, OH)	(3H s, $OCOCH_3$)
	2.24-2.30 (2H m)	6.14 (1H m)	6.08 (1H m)	6.15 (1H m)	2.61 (1H m)
	2.15	1.96	1.99	1.99	1.20
	(1H t-like, J = 6.6)	(3H dd, J = 7.3, 1.7)	(3H d, J = 7.3)	(3H d, J = 7.3)	(3H d, J = 6.9)
	1.00, 0.98	1.87	1.89	1.94	1.23
	(each 3H d, J = 6.3)	(3H t-like, J = 1.7)	(3H t-like, J = 1.7)	(3H d-like)	(3H d, J = 6.9)

Table 2. ^{13}C NMR spectral data of *Seseli* pyranocoumarins **1-5** (ppm from TMS, in $CDCl_3$).

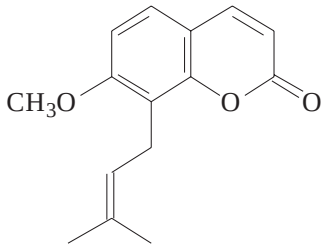
Carbon	**1**	**2**	**3**	**4**	**5**
2	159.71	159.87	159.87	160.61	159.72
3	113.26	113.10	112.85	112.51	113.30
4	143.14	143.27	143.27	143.90	143.20
5	129.24	129.11	129.20	128.68	129.23
6	114.38	114.30	114.47	114.52	114.43
7	156.57	156.69	156.89	155.96	156.64
8	107.19	106.99	107.19	110.82	107.22
4a	112.49	112.49	112.17	112.36	112.54
8a	153.96	153.94	154.12	154.30	154.09
2′	77.84	77.65	78.53	77.58	77.31
3′	70.55	69.74	71.43	72.45	70.55
4′	60.40	60.97	63.20	59.86	60.54
***gem*-Me**	25.34	24.85	25.68	25.70	25.34
	22.14	22.90	20.79	22.52	22.32
Ester part					
CO	169.81	169.76	—	—	169.86
$OCOCH_3$	20.69	20.60	—	—	20.74
others	171.93	166.41	168.09	166.90	175.85
	43.25	139.71	138.72	139.43	34.14
	25.50	126.92	127.37	127.19	18.94
	22.43	20.43	20.34	20.54	18.83
	22.39	15.71	15.65	15.78	—

Results and Discussion

The coumarins **2-6** were identified as (−)-(*3′S,4′S*)-3′-acetoxy-4′-angeloyloxy-3′,4′-dihydroseselin (**2**), (+)-(*3′S,4′S*)-3′-hydroxy-4′-angeloyloxy-3′,4′-dihydroseselin (d-laserpitin) (**3**), (−)-(*3′S,4′S*)-3′-angeloyloxy-4′-hydroxy-3′,4′-dihydroseselin (**4**), 3′-acetoxy-4′-isobutyloxy-3′,4′-dihydroseselin (**5**), and osthole (**6**), by analysis of physical and spectroscopic data and comparison with previously published values[5−11].



	R_1	R_2
1	$COCH_3$	$COCH_2CH(CH_3)_2$
2	$COCH_3$	$COC(CH_3)=CHCH_3$
3	H	$COC(CH_3)=CHCH_3$
4	$COC(CH_3)=CHCH_3$	H
5	$COCH_3$	$COCH(CH_3)_2$



6 Osthole

Structure determination of corymbocoumarin (1)

Corymbocoumarin (**1**), colorless needles; m.p. 105.0-108.0 °C, $[\alpha]_D$−6.502° ($CHCl_3$), showed a molecular ion peak at m/z 388 (EIMS: $C_{21}H_{24}O_7$). The mass spectrum of **1** exhibits a fragmentation pattern almost identical with that of **2**. The ^{1}H NMR spectrum of **1** shows 2 methyl doublets at δ 0.98 and 1.00 (J=6.3), *gem* dimethyl singlets at δ 1.42 and 1.45, and an acetoxy methyl signal at δ 2.10. The complex signals appearing at δ 2.15 (1H, triplet like) and 2.24-2.30 (2H, multiplet) were assigned to the methine and methylene protons.

In the aromatic proton region, 2 pairs of doublets at δ 6.23 and 7.59 (J=9.6 Hz) and at δ 6.80 and 7.36 (J=8.6 Hz) were attributed to the protons at C_3, C_4, C_6 and C_5, respectively. The doublets at δ 5.31 (1H, J=5.0 Hz) and 6.55 (1H, J=4.6 Hz) were assigned to the methine protons at $C_{3'}$ and $C_{4'}$ of the *cis*-khellactone diesters, which showed a characteristic splitting pattern. This spectrum is almost identical with that of **2**.

Assigning the ester group positions at $C_{3'}$ and $C_{4'}$ was achieved with mass spectrometry. Based on mass fragmentograms on seselin-type coumarins proposed by Bohlmann et al.[12], Takata et al.[13] were able to analyze the fragmentation patterns of peucedanocoumarins I and II isolated from the crude Chinese drug, "Baihua Qianhu". Similarly, the mass spectral fragmentation pattern of **1** was seen at m/z 328 (7.5%) and 313 (4.3%) for the loss of an acetoxyl group, and m/z 287 (8.3%) for loss of an isovaleryloxyl group (Figure 1). Hence, we conclude that **1** is a khellactone diester with an acetoxy and an isovaleryloxyl group.

As previously described[14], **1** was subjected to alkaline hydrolysis with 1N KOH to yield (-) *cis* and (+) *trans*-khellactones. Alkaline hydrolysis of **1**, with 0.5 N KOH at reflux for 5 min, yielded *cis*-3′-acetoxy-4′-hydroxy-3′,4′-dihydroseselin. This product is also obtained from **2** under the same conditions (Figure 2).

The configuration at $C_{3'}$ and $C_{4'}$ of **1** was determined by the difference in the separation pattern of the 2 geminal methyl carbon signals in the ^{13}C NMR spectrum. Gonzalez et al. determined that the 2′-*gem*-dimethyl groups of dihydropyran give a broad singlet in 3′,4′-*trans* compounds and 2 close singlets in 3′,4′-*cis* compounds[5]. Moreover, previously, the ^{1}H NMR signals appearing at δ 1.42 and 1.45 (Δ 0.03) in **1** and at δ 1.43 and 1.48 (Δ 0.05) in **2** indicated *cis* configurations at $C_{3'}$ and $C_{4'}$, respectively[15].

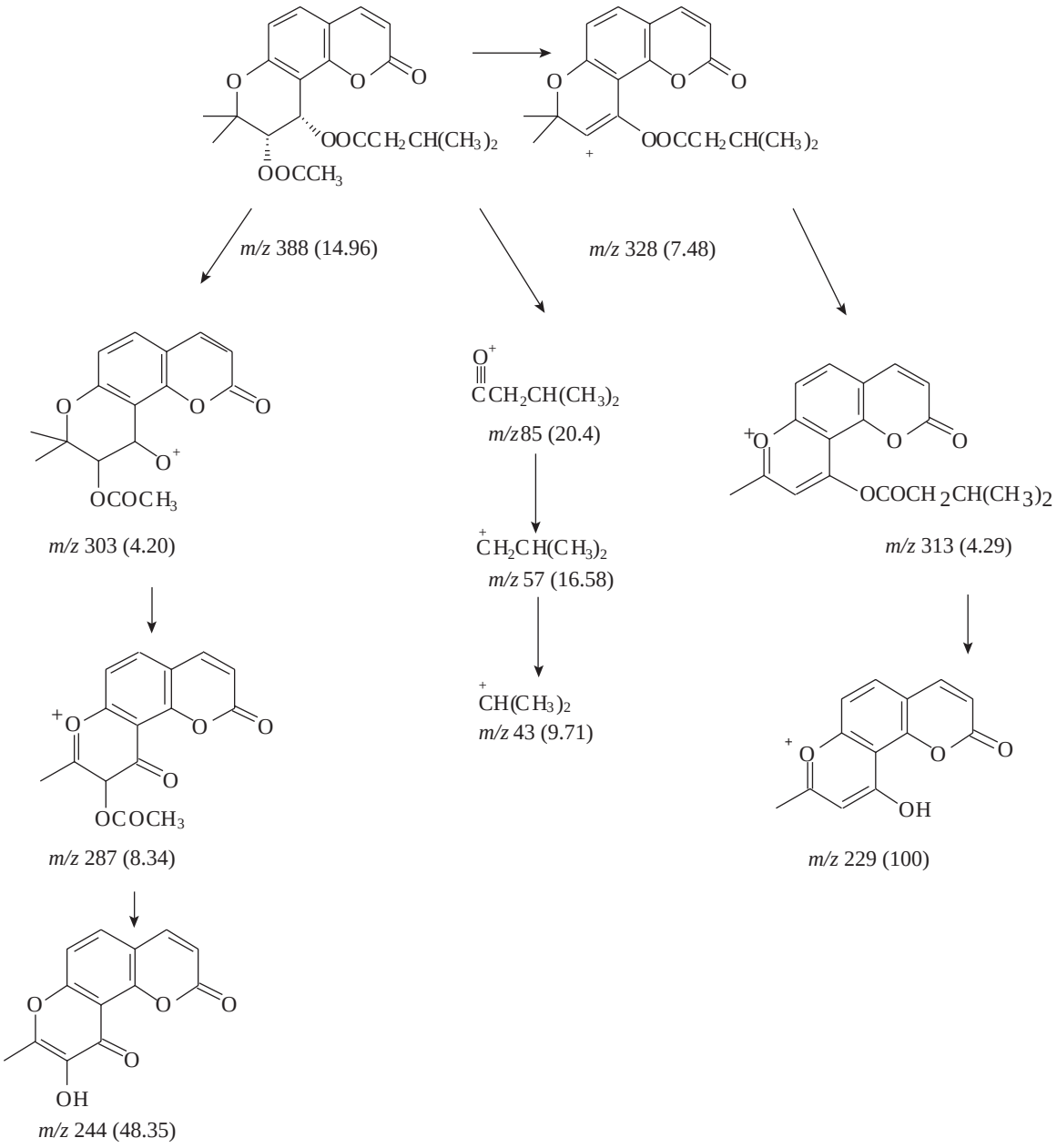


Figure 1. The MS fragmentation pattern of **1.**

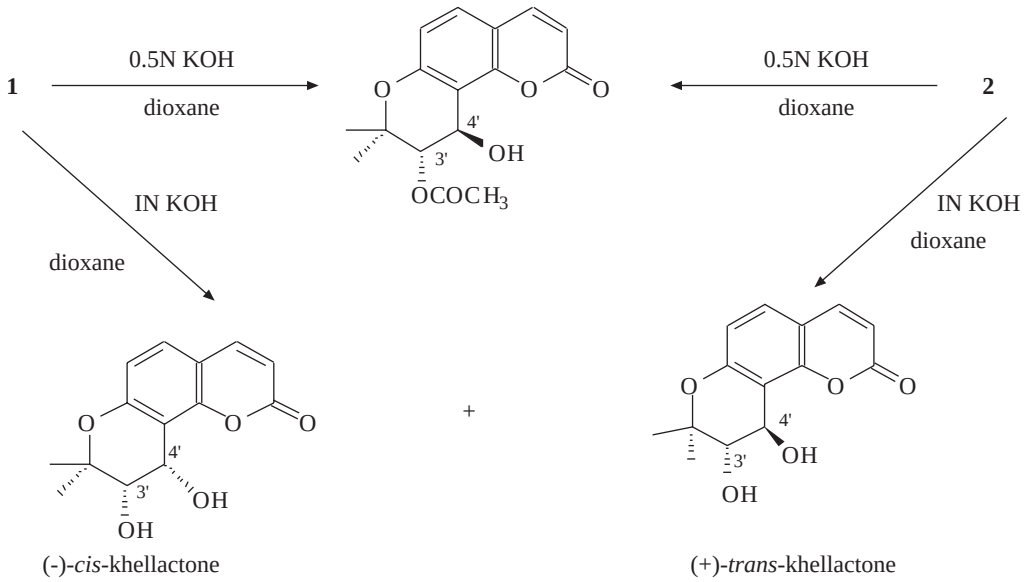


Figure 2. Alkaline hydrolysis of **1** and **2.**

Thus, a new angular-type dihydropyranocoumarin, corymbocoumarin, was determined as (−)-(3′*S*,4′*S*)-3′-acetoxy-4′-isovaleryloxy-3′, 4′-dihydroseselin (**1**).

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