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This paper is dedicated to Professor Toru Okuyama on his retirement.

Linear-type furocoumarins, 5-[(2''E,6''R)-6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy]psoralen and 5-[(2''E,6''S)-6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy]psoralen (**1**) were first isolated from whole plants of *Seseli hartvigii* together with one new natural product 6-(3'-methyl-2'-oxo-3'-butenyl)-7-methoxycoumarin (**2**), and four known compounds (tamarin, bergaptol, notoptol, and a mixture of β -sitosterol and stigmaterol). The structure of **1** was elucidated by extensive spectroscopic analysis and chemical conversion. The modified Mosher's method and HPLC were applied to determine its stereochemistry. Both R- and S-configurations exist in **1**; after modification by Mosher's reagent, they were effectively separated, and their ratio was deduced to be 59% and 41%, respectively.

Keywords: *Seseli hartvigii*, coumarin, stereochemistry, modified Mosher's method.

Seseli hartvigii belongs to the Umbelliferae family and has previously only been found in southern Turkey [1]. *Seseli* species are very rich sources of coumarins [2], although there has been no report about constituents of this species except from our study team. We are most interested in some of its special constituents. Our previous investigation on the chemical constituents of *Seseli hartvigii* resulted in the isolation of three angular-type pyranocoumarins: (-)-anomalin, (+)-(3'R,4'R)-3',4'-diseneciolyoxy-3',4'-dihydroseselin and (-)-(3'R,4'R)-3'-angeloyloxy-4'-acetoxo-3',4'-dihydroseselin; and two linear-type furocoumarins: bergamottin and isoimperatorin; together with two simple coumarins: suberosin and mexotcin [2,3]. In a continuing search for new compounds in whole parts of this plant, we obtained a linear-type furocoumarin, 5-(6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy)psoralen, together with one new natural product 6-(3'-methyl-2'-oxo-3'-butenyl)-7-methoxycoumarin and four known compounds (tamarin, bergaptol, notoptol and the mixture of β -sitosterol and stigmaterol). The planar structure of 5-(6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy)psoralen was published in 2003 [4], but its

stereochemistry was not determined. We describe herein the structural elucidation and stereochemistry determination of compound **1**. To the best of our knowledge, this is the first report of HPLC application to separate isomers after modification by Mosher's reagent and the determination of the ratio of R and S isomers.

Compound **1** was obtained as a white crystal (mp 100-101°C with positive optical rotation ($[\alpha]_D^{22}$: +16.4°). The molecular ion peak was at m/z 354 (EI-MS: C₂₁H₂₂O₅). The IR spectrum of **1** showed the presence of an ester function (1724.05 cm⁻¹), which also corresponded to δ 161.00 in the carbon spectrum. The tentative structure was presumed by comparing NMR data with reference [4], as listed in Table 1.

As shown in Figure 1, two-dimensional NMR data support the structure. However, from ¹³C-¹H COSY, δ 7.60 was assigned to δ 144.56 and δ 7.60 was assigned to δ 104.74, contrary to the order previously reported [4]. The position of the hydroxyl moiety is determined at C-7'' by acetylated experiment. The double bond at

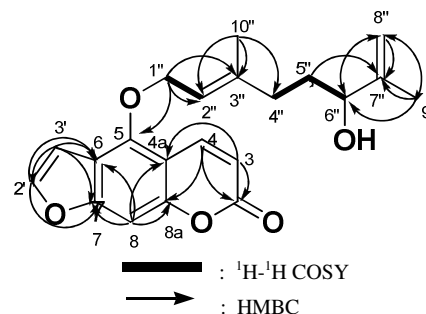
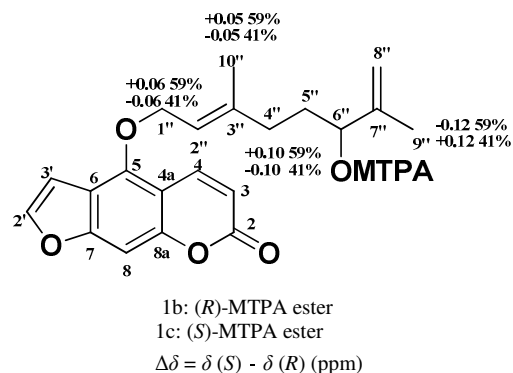
Table 1: NMR data for compound **1**^{b)}, **1b** and **1c** in CDCl₃^{a)}, and reference in CD₃OD^{a)}.

Compound 1			Reference ^[4]		1b	1c
2		160.84 s		163.3		
3	6.27 d (1H, d, <i>J</i> = 9.6 Hz)	112.33 d	6.28 d (1H, d, <i>J</i> = 10.0 Hz)	112.8	6.27 (1H, d, <i>J</i> = 9.8 Hz)	6.27 (1H, d, <i>J</i> = 9.8 Hz)
4	8.15 d (1H, d, <i>J</i> = 9.6 Hz)	139.17 d	8.27 d (1H, d, <i>J</i> = 10.0 Hz)	141.2	8.15 (1H, d, <i>J</i> = 9.8 Hz)	8.15 (1H, d, <i>J</i> = 9.8 Hz)
4a		107.27 s		109.2		
5		148.52 s		150.7		
6		113.93 s		116.0		
7		157.72 s		159.8		
8	7.15 (1H, s)	94.04 d	7.21 (1H, brs)	94.6	7.17(1H, s)	7.16(1H, s)
8a		152.27 s		153.9		
2'	7.60 (1H, d, <i>J</i> = 2.4 Hz)	144.56 d	7.79 (1H, d, <i>J</i> = 2.4 Hz)	105.9	7.60 (1H, d, <i>J</i> = 2.4 Hz)	7.59 (1H, d, <i>J</i> = 2.4 Hz)
3'	6.95 (1H, dd, <i>J</i> = 2.4, 1.0 Hz)	104.74 d	7.16 (1H, dd, <i>J</i> = 2.4, 0.9 Hz)	147.0	6.94 (1H, d, <i>J</i> = 1.5 Hz)	6.94 (1H, d, <i>J</i> = 1.5 Hz)
1''	4.95 (2H, d, <i>J</i> = 7.3 Hz)	69.52 t	5.04 (2H, d, <i>J</i> = 7.1 Hz)	70.6	5.0-4.9 (2H, m)	5.0-4.9 (2H, m)
2''	5.57 (1H, dd, <i>J</i> = 5.6, 7.2 Hz)	118.81 d	5.58 (1H, tq, <i>J</i> = 7.1, 1.3 Hz)	120.7	5.48 (0.59H, m)	5.54 (0.59H, m)
					5.54 (0.41H, m)	5.48 (0.41H, m)
3''		142.47 s		143.9		
4''	2.13 (2H, m)	35.37 t	2.07 (2H, m)	36.4	1.97 (0.59H, m)	2.07 (0.59H, m)
					2.07 (0.41H, m)	1.97 (0.41H, m)
5''	1.67 (2H, m)	32.70 t	1.64 (2H, m)	29.9		
6''	4.04 (1H, t, <i>J</i> = 6.4 Hz)	75.20 d	4.15 (1H, t, <i>J</i> = 6.8 Hz)	89.3	5.38 (1H, m)	5.38 (1H, m)
7''		146.88 s		145.7		
8''	4.94 (1H, d, <i>J</i> = 7.2 Hz)	111.01 t	4.92 m	114.2	5.0-4.9 (2H, m)a	5.0-4.9 (2H, m)
	4.86 (1H, d, <i>J</i> = 1.6 Hz)		4.87 m			
9''	1.73 (3H, s)	17.54 q	1.66 brs	16.4	1.75 (1.77H, s)	1.63 (1.77H, s)
					1.63 (1.23H, s)	1.75 (1.23H, s)
10''	1.71 (3H, s)	16.71 q	1.69 brs	17.0	1.65 (1.77H, s)	1.70 (1.77H, s)
					1.70 (1.23H, s)	1.65 (1.23H, s)
Phenyl					7.40-7.37 (5H, m)	7.40-7.37 (5H, m)
OCH ₃					3.54 (3H, s)	3.55 (3H, s)

a) The chemical shifts (δ ppm) were relative to TMS (400 MHz for ¹H NMR and 100 MHz for ¹³C NMR).b) Chemical shifts based on ¹H-¹H COSY, ¹³C-¹H COSY and HMBC data.

the position of C-2'' and C-3'' was determined to be *E* configuration by comparing the chemical shift of H-2'' with a similar type of compound, 5-[(2''*E*,5''*Z*)-7''-hydroxy-3'',7''-dimethyl-2'',5''-octadienoxy]psoralen [6].

To determine the absolute configuration of 6''-C, compound **1** was treated with *R*- and *S*- α -methoxy- α -(trifluoromethyl)phenylacetic acids (MTPA), *N,N'*-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) in dry CH₂Cl₂ at room temperature to yield 6''-*O*-(*R*)-MTPA ester **1b** (53.64%) and 6''-*O*-(*S*)-MTPA ester **1c** (63.10%), respectively. Essentially, if compound **1** is in the *R*- or *S*-configuration, the proton signal of the methyl at the 9'' position in **1b** and **1c** should be a single peak with relative integration value about 3H, but actually, in the **1b** and **1c** proton spectra, two single peaks were observed with a relative integration value of about 1.8H and 1.2H (3:2). The shift in the proton relative integration values also occurred on both H-2'' and H-4''. As shown in Figure 2, of the signals due to protons attached to the 2'' and 4'' positions in **1c**, 59% were observed at a lower field ($\Delta\delta > 0$) and 41% were observed at a higher field ($\Delta\delta < 0$) than in **1b**; and at the 9'' position in **1c**, 59% were observed at a higher field ($\Delta\delta < 0$) and 41% were observed at a lower field ($\Delta\delta > 0$) than in **1b**. Consequently, the absolute configuration at the 6'' position was elucidated to be *R* (59%) and *S* (41%). To confirm our conclusion, HPLC on a silica column was performed, and two compounds were obtained from either **1b** or **1c**, with an amount ratio of

**Figure 1:** Key ¹H-¹H COSY and HMBC correlations of compound **1**.**Figure 2:** ¹H NMR chemical shift differences [δ (*S*)-MTPA ester - δ (*R*)-MTPA ester].

about 59:41. On the basis of this evidence, the structures were determined to be 5-[(2''*E*,6''*R*)-6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy]psoralen (59%) and 5-[(2''*E*,6''*S*)-6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy]psoralen (41%).

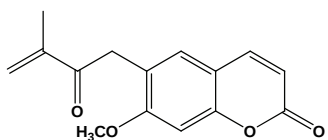


Figure 3: Structure of Compound 2.

Compound **2** was obtained as a white amorphous solid. The ^{13}C NMR and DEPT spectra displayed 15 carbon signals, including one methyl, two methylenes, four methines, and seven quaternary carbons. In the proton spectrum, δ 6.25 (1H, d, $J = 9.2$ Hz) and 7.61 (1H, d, $J = 9.2$ Hz), typical signals of H-3 and H-4 of coumarin, respectively, were observed; and δ 3.86 (3H, s) was the signal belonging to 7-methoxy-coumarin. The chemical shift at δ 7.21 (1H, s) and 6.81 (1H, s) which were assigned to H-5 and H-8 indicated a side chain at the C-6 position. Based on these data, a parent structure of 6-substitute-7-methoxy coumarin was concluded. The carbonyl position in the side chain was determined to be C-2" by comparing proton shifts of two compounds, benzyl isopropenyl ketone and 3-methyl-2-butenophenone, in the references [7,8]. Thus, the structure was determined to be 6-(3'-methyl-2'-oxo-3'-butenyl)-7-methoxycoumarin in Figure 3, previously reported as a synthetic product [7], although this is the first isolation as a natural product.

In addition, other four known compounds were identified by comparison of their spectroscopic data (UV, NMR, MS) with reported values. These compounds were tamarin [9], bergaptol [10], notoptol [11], and a mixture of β -sitosterol and stigmasterol [12], respectively.

Experimental

General: Melting points were determined using a Yanako micro melting point apparatus. UV spectra were obtained on a Shimadzu UV 1600 spectrophotometer, and optical rotation data were obtained using a Horiba SEPA-300 high sensitivity polarimeter. EI-MS and FAB-MS data were obtained from a JEOL JMS-700 mass spectrometer. 1D-NMR and 2D-NMR were measured by a JEOL JNM-AL 400 spectrometer with chloroform- d as the solvent and tetramethylsilane as an internal standard. TLC was performed using silica gel 60 F_{254} TLC plates (Merck) and silica gel 60N (100~200 μm ; Kanto Chemical) was used for open column chromatography. HPLC purification was carried out on a packed column of Cosmosil 5SL (Nacalai, 10 $\times$ 250 mm) with a LC-6AD pump (Shimadzu), monitored by an SPD-20A detector (Shimadzu) at 254 nm.

Plant materials: *Seseli hartvigii* whole plants were collected in August 2002 from Antalya-Saklikent in the Bakirlar Mountains of Turkey. A voucher specimen has

been deposited at the Herbarium of the Faculty of Pharmacy of Ankara University (AEF 21915). The species was identified by Prof. H. Duman.

Extraction and isolation: Air-dried and powdered whole parts of *S. hartvigii* (1.0 kg) were successively extracted with hexane, EtOAc, and MeOH for 8 h under reflux to give a hexane soluble fraction (25 g), an EtOAc soluble fraction (7.5 g), and a MeOH soluble fraction (42 g). Compounds **1** (23.4 mg), **4** (3.4 mg), **5** (15.8 mg) and **6** (40.5 mg) were isolated from the hexane soluble fraction using silica gel column chromatography and silica HPLC. Compounds **2** (1.1 mg) and **3** (38.3 mg) were obtained from the EtOAc soluble fraction by silica gel column chromatography, silica and ODS HPLC.

Acetylation of 1: A solution of **1** (3.00 mg, 8.47 μmol) in dry pyridine (0.1 mL) was cooled with ice water, and acetic anhydride (0.05 mL) was added dropwise. The reaction mixture was stirred for 3.5 h under Ar atmosphere. After dilution with water (10 mL), the mixture was extracted with chloroform (3 $\times$ 10 mL) and chloroform/ n -BuOH (2:1, 3 $\times$ 10 mL). The combined extracts were washed with 5% NaHCO_3 (10 mL), dried with Na_2SO_4 and concentrated *in vacuo* to yield a residue. Chromatography of the residue on a silica gel column (40 mL) with hexane-EtOAc as the eluent yielded **1a** (3.06 mg, 91.34%).

(R)- and (S)-MTPA esters of 1: An (R)-MTPA working solution ((R)-MTPA 7.76 mg and DCC 7.75 mg in 0.5 mL of dry CH_2Cl_2) was prepared. Compound **1** (2.0 mg, 5.6 μmol) and DMAP (1.72 mg) were dissolved in dry CH_2Cl_2 (0.5 mL) and stirred at room temperature for 30 min. Then, (R)-MTPA working solution was added, and the mixture was stirred at room temperature for 1 h. After the addition of 5 mL each of water and CH_2Cl_2 , the solution was washed successively with 5% HCl (5 mL), saturated NaHCO_3 (5 mL) and brine (5 mL). The organic soluble portion was dried with Na_2SO_4 and concentrated *in vacuo* to yield a residue, which was chromatographed on a silica gel column (10 mL) using EtOAc as the eluent and then purified by HPLC on silica gel (hexane: EtOAc 2:1) to obtain **1b** (1.7 mg, 53.64%). Using a similar procedure, **1c** (2.0 mg, 63.10%) was prepared from **1** (2.0 mg, 5.6 μmol) with (S)-MTPA (7.76 mg), DCC (7.75 mg) and DMAP (1.72 mg). **1b** was further purified by HPLC on silica gel (hexane: EtOAc 10:1) whereby two different esters were separated, and the ratio was 59:41. The ^1H NMR data are listed in Table 1 and selected $\Delta\delta$ values are shown in Figure 2.

5-(6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy) psoralen (1)

White crystal.

MP: 100-101°C.

[α]_D²²: +16.4° (c 0.20, CHCl₃).IR (in CHCl₃): 1724.05, 1626.66, 1579.41, 1456.96, 1130.08, 1074.16 cm⁻¹.UV $\lambda_{\max}$ (MeOH) (log ϵ): 310 nm (0.90).¹H NMR: Table 1.¹³C NMR: Table 1.MS (EI, 70 eV): C₂₁H₂₂O₅ 354, *m/z* (%) 354 [M]⁺ (0.43), 336 [M-H₂O]⁺ (0.15), 202 (100.00), 174 (15.17), 145 (9.75), 135 (6.27), 107 (11.67), 93 (17.93), 81 (8.55), 55 (7.22).MS (FAB): C₂₁H₂₂O₅ 354, *m/z* (pos.) 355 [M+H]⁺, 377 [M+Na]⁺, 337 [M-H₂O]⁺.**5-(6''-hydroxy-3'',7''-dimethylocta-2'',7''-dienyloxy) psoralen acetate (1a)**

Pale yellow oil.

¹H NMR (CDCl₃, 400 MHz): δ : 6.28 (1H, d, *J* = 10.0 Hz, H-3), 8.16 (1H, d, *J* = 9.6 Hz, H-4), 7.16 (1H, brs, H-8), 7.60 (1H, d, *J* = 2.4 Hz, H-2'), 6.95 (1H, dd, *J* = 2.4, 1.2 Hz, H-3'), 5.15 (1H, d, *J* = 6.4 Hz, H-1''), 4.94 (1H, m, H-1''), 5.55 (1H, dd, *J* = 5.6, 6.8 Hz, H-2''), 2.0 (2H, m, H-4''), 1.76 (2H, m, H-5''), 4.94 (1H, m, H-6''), 4.92 (2H, m, H-8''), 2.07 (3H, s, 6''-OAc), 1.71 (3H, s, H-9''), 1.73 (3H, s, H-10'').¹³C NMR (CDCl₃, 100 MHz): δ : 160.82 (C-2), 112.38 (C-3), 139.17 (C-4), 107.22 (C-4a), 148.51 (C-5),113.84 (C-6), 157.74 (C-7), 94.05 (C-8), 152.31 (C-8a), 144.57 (C-2'), 104.74 (C-3'), 69.43 (C-1''), 119.17 (C-2''), 142.38 (C-3''), 35.07 (C-4''), 30.39 (C-5''), 76.50 (C-6''), 141.57 (C-7''), 112.88 (C-8''), 169.80 (6''-OCOCH₃), 21.14 (6''-OCOCH₃), 18.00 (C-9''), 16.64 (C-10'').MS (EI, 70 eV): C₂₃H₂₄O₆ 396, *m/z* (%) 396 [M]⁺ (1.05), 336 [M-CH₃CO]⁺ (2.18), 202 (100.00), 174 (17.59), 145 (12.01), 135 (47.86), 119 (5.74), 107 (37.34), 93 (55.62), 81 (9.42), 55 (9.52), 43 (15.86).**6-(3'-methyl-2'-oxo-3'-butenyl)-7-methoxycoumarin (2)**

White amorphous solid

¹H NMR (CDCl₃, 400 MHz): δ : 6.25 (1H, d, *J* = 9.2 Hz, H-3), 7.61 (1H, d, *J* = 9.2 Hz, H-4), 7.21 (1H, s, H-5), 6.81 (1H, s, H-8), 4.03 (2H, s, H-1'), 5.85 (1H, m, H-4'), 6.12 (1H, m, H-4'), 1.91 (3H, s, 3'-CH₃), 3.86 (3H, s, OCH₃).¹³C NMR (CDCl₃, 100 MHz): δ : 160.99 (C-2), 113.11 (C-3), 143.17 (C-4), 112.03 (C-4a), 129.46 (C-5), 121.32 (C-6), 160.19 (C-7), 98.94 (C-8), 144.08 (C-8a), 38.26 (C-1'), 198.35 (C-2'), 142.05 (C-3') 125.03 (C-4'), 17.89 (3'-CH₃), 56.08 (OCH₃).**Acknowledgments** - We would like to thank Meiji Pharmaceutical University Onda Scholarship for support of an international exchange program in pharmaceutical science.**References**

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