An Antiplatelet Aggregation Principle and X-Ray Structural Analysis of cis-Khellactone Diester from Peucedanum japonicum

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AN ANTIPLATELET AGGREGATION PRINCIPLE AND X-RAY STRUCTURAL ANALYSIS OF *CIS*-KHELLACTONE DIESTER FROM *PEUCEDANUM JAPONICUM*

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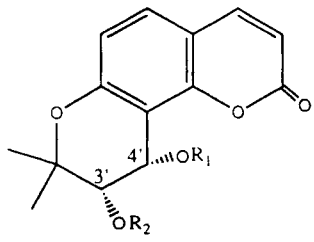
ABSTRACT.—Three structurally related khellactone coumarins, **1–3**, were isolated from the aerial parts of *Peucedanum japonicum* (Umbelliferae). Compound **2** was identified as a new coumarin (*cis*-3′-isovaleryl-4′-senecioylkhellactone) by spectral and chemical analysis. Conformation of the dihydropyrano ring of *cis*-3′,4′-disenecioylkhellactone [**3**] was elucidated by X-ray crystallographic analysis. These three natural khellactone esters were subjected to the antiplatelet aggregation bioassay where *cis*-3′,4′-diisovalerylkhellactone [**1**] showed significant activity (at 50 μg/ml).

Peucedanum japonicum Thunb. (Umbelliferae) is widely distributed in Taiwan, Japan, China, and the Philippines. The root of this plant has been used as a folk medicine in the treatment of colds, gout, and tussis in Taiwan (1). Several coumarins have been isolated from the root of this plant (2–4). In our continuing search for novel bioactive natural products, we report herein the structural elucidation and antiplatelet aggregation activity of three coumarin compounds **1–3** from the aerial parts of this plant.

RESULTS AND DISCUSSION

The *n*-hexane extract of *P. japonium* was repeatedly chromatographed on Si gel columns to afford successively compounds **1**, **2**, and **3**. By comparison of the uv, ir, and nmr spectra of **1–3** (Table 1) with peuformosin [**5**] (2), the structures were suggested as khellactone-type coumarins. Since the values of the vicinal coupling constants (H-3′, H-4′, $J = 5.0$ Hz) in these compounds are about the same as reported for related compounds (5), the cis configurations were also suggested.



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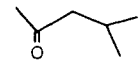

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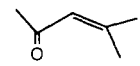
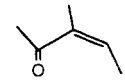


angeloyl=



1 $R_1 = R_2$ = isovaleryl
2 R_1 = senecioyl, R_2 = isovaleryl
3 $R_1 = R_2$ = senecioyl
5 R_1 = senecioyl, R_2 = angeloyl

TABLE 1. ^{1}H-nmr Data for Coumarins **1–4** of *Peucedanum japonicum* (300 MHz, $CDCl_3$, in ppm).

Proton	Compound			
	1	**2**	**3**	**4**
H-3	6.16 (9.5)[a]	6.14 (9.5)	6.21 (9.5)	6.26 (9.5)
H-4	7.59 (9.5)	7.56 (9.5)	7.57 (9.5)	7.67 (9.5)
H-5	7.34 (8.7)	7.33 (8.6)	7.34 (8.6)	7.34 (8.7)
H-6	6.75 (8.7)	6.75 (8.6)	6.79 (8.6)	6.80 (8.7)
H-3′	5.25 (5.0)	5.25 (4.9)	5.36 (4.9)	5.21 (2.2)
H-4′	6.46 (5.0)	6.52 (4.9)	6.62 (4.9)	4.43 (2.2)
H-5′/H-6′	1.36	1.36	1.42	1.43
	1.39	1.40	1.46	1.47
4′-OMe				3.76
Isovaleryl	2.0–2.27 (6H, m)	2.01–2.23 (3H, m)		2.18–2.20 (3H, m)
	0.92–0.95 (12H, m)	0.89–0.93 (6H, m)		0.92 (6H, d, J = 6.9 Hz)
Senecioyl		1.83 (3H, d, J = 0.9 Hz)	1.87 (3H, s)	
			1.88 (3H, s)	
		2.16 (3H, s)	2.15 (3H, d, J = 1.1 Hz)	
		5.58 (1H, br s)	2.19 (3H, d, J = 1.1 Hz)	
			5.62 (1H, br s)	
			5.66 (1H, br s)	

[a]Coupling constants (J in Hz) are given in parentheses.

Compounds **1** and **3** were identified as *cis*-3′,4′-diisovalerylkhellactone and *cis*-3′,4′-disenecioylkhellactone, respectively, by comparison of their spectral data with those isolated previously from roots of the same plant, and their absolute configurations are known to be 3′*S*,4′*S* based upon the work of Yamada *et al.* (3).

The structure of compound **2** was depicted through its ^{1}H-nmr spectrum. The aromatic portions, at δ 6.14 (1H, d, J = 9.5 Hz), 7.56 (1H, d, J = 9.5 Hz), 7.33 (1H, d, J = 8.6 Hz), and 6.75 (1H, d, J = 8.6 Hz), corresponded to H-3 to H-6, respectively, and signals at δ 5.25 (1H, d, J = 4.9 Hz) and 6.52 (1H, d, J = 4.9 Hz) represent H-3′ and H-4′ on the dihydropyran ring. The presence of a senecioyl group, instead of an angeloyl group due to the higher field chemical shift of the olefinic proton (7), was substantiated by the appearence of signals at δ 1.83 (3H, d, J = 0.9 Hz), 2.16 (3H, s), and 5.58 (1H, m, olenic proton). The peaks at δ 0.89–0.93 (6H, m) and 2.01–2.23 (3H, m) were assigned to an isovaleryl substituent. These two substituents were further confirmed by comparisons of their C-13 nmr spectra (Table 2) with those cited in the literature (6).

As to the position of the two substituent groups, they were mapped as 3′-isovaleryl and 4′-senecioyl based upon the presence of the fragment ion peak at *m/z* 326 $[M - C_5H_{10}O_2]^+$ in the ms, due to the extrusion of isovaleric acid from the C-3′ position (7,8). This was further confirmed by partial solvolysis of **2** with MeOH in the presence of Na_2CO_3 at room temperature to give *trans*-3′-isovaleryl-4′-methoxykhellactone **4** (Scheme 1), since the senecioyl group attached to C-4′ (a benzylic position) can more readily undergo solvolysis. These two ester substituents in **2** were assigned the cis configuration on the basis of the 4.9 Hz coupling constant between H-3′ and H-4′, plus the chemical shift of C-3′ at 70.18 ppm and Δδ (gem Me_2) around 2.9 ppm in the

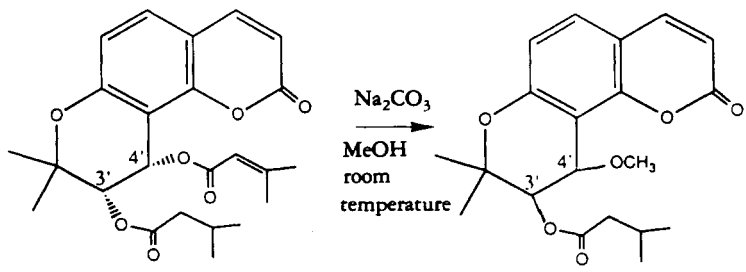
TABLE 2. ^{13}C-nmr Data for Coumarins **1–3** (250 MHz, $CDCl_3$).

Carbon	Compound		
	1	**2**	**3**
C-2 s	160.00	159.90	159.99
C-3 d	113.21	113.20	113.20
C-4 d	143.19	143.18	143.24
C-5 d	129.17	129.06	129.05
C-6 d	114.43	114.35	114.39
C-7[a] s	156.59	156.64	156.82
C-8 s	107.23	107.51	107.62
C-9[a] s	154.00	154.10	154.08
C-10 s	112.46	112.47	112.55
dihydropyran ring			
C-2′ s	77.51	77.58	77.76
C-3′ d	70.14	70.18	69.40
C-4′ d	60.41	59.55	59.83
C-5′/C-6′ (Me)	25.50	25.28	25.09
	22.43	22.40	22.67
Isovaleryl or senecioyl group	171.87 s	171.90 s	165.29 s
	43.20 t	43.04 t	(165.17 s)
	(43.05 t)	25.28 d	115.29 d
	25.35 d	22.40 q	158.31 s
	22.43 q	22.40 q	(157.66 s)
	22.43 q		20.43 q
		165.10 s	(20.30 q)
		115.00 d	27.54 q
		158.27 s	
		20.35 q	
		27.47 q	

[a]May be interchangeable.

^{13}C-nmr spectrum (6). The absolute configuration of 3′*S*,4′*S* was suggested by analogy with the known **1** and **3** from the same plant. Thus, compound **2** was, for the first time, isolated from nature.

The conformation of khellactone derivatives has been described in the literature on the basis of nmr studies by Lemmich *et al.* (5) and also by Yamada *et al.* (3), via the application of the dibenzoate chirality rule. As to the X-ray analysis, only the *trans*-4′-methylkhellactone has been reported (6). The molecular structure of *cis*-3′,4′-disenecioylkhellactone [**3**], as determined by X-ray analysis, is illustrated in Figure 1.



2 **4**

SCHEME 1

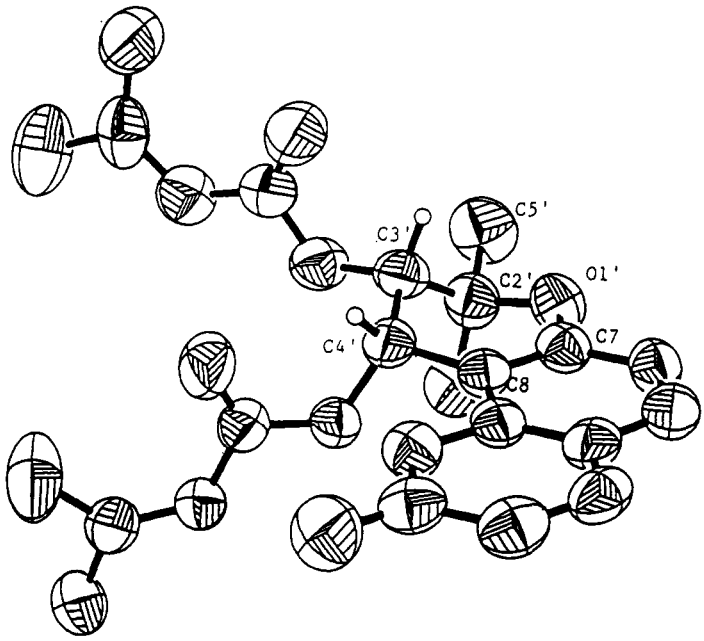
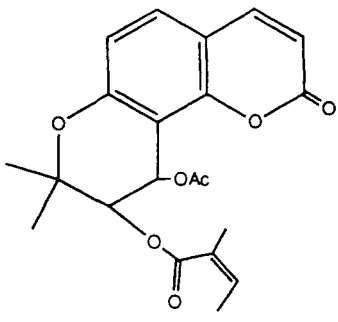


FIGURE 1. Molecular structure of *cis*-3′,4′-disenecioylkhellactone [**3**].

No absolute configuration is implied, as this was not directly determined in the X-ray experiment; however, the relative configuration of the two chiral centers, C-3′ and C-4′, has been established as cis. The ten atoms of the aromatic and lactone rings lie within 0.013 Å of a common plane, while the dihydropyran ring is in the C-3′ half-chair conformation; the torsion angle about C-7 and C-8 is near zero [−5.6°], and C-2′ and C-3′ are alternatively below and above the best plane of the four other atoms [O-1′, C-2′, C-3′, C-4′ torsion angle = −60.8°]. The dihedral angle between C-3′ and C-4′ is 47.5°.

Khellactone coumarins have been reported as possessing various biological activities (9,10). In 1988, Takeuchi *et al.* (9) reported an investigation of 25 natural and synthetic khellactone coumarins for antiplatelet aggregation activity and found that a mixture of compounds **6** (*cis*-3′-angeloyl-4′-acetylkhellactone) and **7** (*cis*-3′,4′-di-angeloylkhellactone) is effective.

Because the three coumarins **1–3** isolated from *P. japonicum* were not included in their study, we examined their anti-platelet aggregation activity (11). All three coumarins showed inhibitory activity (Table 3). Compound **1** showed significant antagonistic



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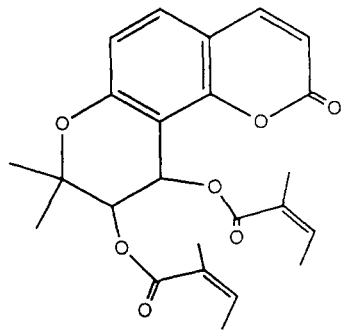

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TABLE 3. Effect of Compounds **1–3** on the Aggregation of Washed Rabbit Platelets.[a]

Compound	% Aggregation			
	ADP	Arachidonic acid	Collagen	PAF
Control	70.9 + 2.3	87.2 + 1.1	89.0 + 0.6	89.3 + 0.7
1	8.8 + 5.6[d]	49.8 + 13.9[b]	39.3 + 12.4[c]	0.0 + 0.0[d]
2	64.0 + 6.7	76.3 + 6.0[b]	75.7 + 3.2[d]	57.4 + 7.9[d]
3	61.7 + 8.8	43.6 + 16.1[b]	72.3 + 3.4[d]	73.2 + 3.9[d]

[a]Platelets were preincubated with each compound (100 μg/ml) or 0.5% DMSO (control) at 37° for 3 min, then the inducer ADP (20 μM), arachidonic acid (100 μM), collagen (10 μg/ml), or PAF (2 ng/ml) was added. Values are presented as means ± S.E.M. (n = 3–6).

[b]$p<0.05$.

[c]$p<0.01$.

[d]$p<0.001$ as compared with the respective control.

effects of the platelet activating factor (PAF) in platelet aggregation induced by several aggregating agents. The concentration-dependent study for the inhibition of percent aggregation by **1** is shown in Figure 2. The results indicate the necessity of a cis-disubstituted dihydropyrano ring and, more importantly, a saturated isovaleryl group at C-3′ or C-4′ or both, for the enhanced PAF-antagonistic activity among these khellactone coumarins.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were determined in MeOH, and ir spectra were recorded as $CHCl_3$ films. ^{1}H-nmr spectra were obtained with a Varian VXR-300 MHz nmr spectrometer in $CDCl_3$, with TMS as an internal standard. Eims were obtained from a 70 eV direct inlet system on a Jeol DX-300 spectrometer. Mp's are uncorrected.

PLANT MATERIAL.—*P. japonicum* was collected in Taipei Hsien, Taiwan, in 1989. The plant was identified by Prof. C.S. Kuoh of the National Cheng-Kung University, Taiwan. A voucher specimen is deposited in the Herbarum of Chia-Nan Junior College of Pharmacy, Tainam, Taiwan.

EXTRACTION AND SEPARATION.—The air-dried and powdered aerial parts (2.9 kg) were extracted exhaustively with hexane. The extract was filtered and concentrated under reduced pressure to furnish a

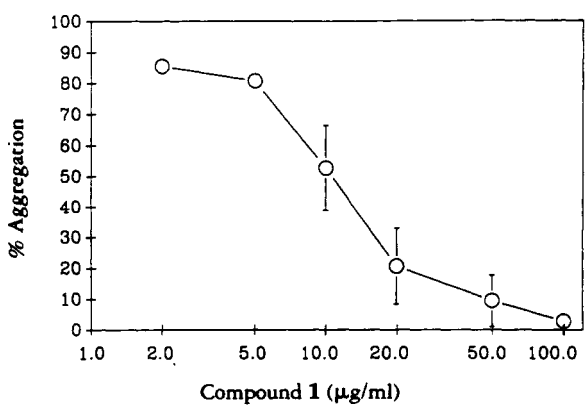

FIGURE 2. Effect of **1** on the platelet aggregation induced by platelet-activating factor.[a]

[a]Washed rabbit platelets were incubated with various concentrations of **1** for 3 min. Then platelet-activating factor (2 ng/ml) was added to trigger the aggregation. Percent aggregation is presented as mean ± SEM ($n = 5$).

brown syrup (60 g), which was then partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated and directly chromatographed on a Si gel column, using *n*-hexane–EtOAc (9:1) as eluent, to afford 15 fractions. Compounds **1–3** were isolated [R_f 0.28, 0.23, 0.18 on Si gel, *n*-hexane–EtOAc (3:1)] from the tenth fraction.

SOLVOLYSIS OF COMPOUND **2**.—Compound **2** (10 mg) was dissolved in MeOH (1 ml). Na_2CO_3 (10 mg) was added, and the mixture was stirred for 24 h at room temperature. The Na_2CO_3 was filtered away, and the MeOH was removed by reduced pressure. H_2O and EtOAc were added, and the EtOAc layer was washed with dilute acid and H_2O and dried over Na_2SO_4. The solvent was removed to give a crude product containing three spots on the tlc plate. After purification by preparative tlc, the least polar compound, **4**, was obtained, R_f 0.37 [*n*-hexane–EtOAc (3:1)] (1.5 mg) and was identified as *trans*-3′-isovaleryl-4′-methoxylkhellactone [**4**]: uv λ max nm 326, 293; ir ν max cm^{-1} 1720 (br), 1600; eims *m/z* $[M-C_5H_8O]^+$ 276 (45%), 205 (base), 204 (90%).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF COMPOUND **3**[1].—Crystal data: crystals belong to the orthorhombic system with unit cell parameters (at 25°) $a = 15.525$ (3), $b = 6.780$ (1), $c = 22.001$ (5) Å, and $V = 2315.9$ (8) $Å^3$. The space group is $P2_12_12_1$, with $Z = 4$ formula units/unit cell, and D (calcd) = 1.223 Mg/m^3, F(000) = 904. Intensity data were collected on the Siemens R3m/V diffractometer using monochromatized MoKα radiation (λ = 0.71073 Å) via the θ–2θ scan technique. Those 1837 data points with $I > 3.0\ \sigma I$ were considered observed. The structure was solved by direct methods and refined by full-matrix least-square methods. All nonhydrogen atoms were refined with anisotropic thermal parameters. All hydrogen atoms were added at idealized positions and included in the structure factor calculation. At convergence, $R_F = 4.98\%$, $R_{WF} = 5.84\%$, and GOF = 1.39 for 288 variables. A final difference Fourier map yielded ρ (max) = 0.28 $e^-/Å^3$. Atomic coordinates, thermal parameters, and structure factor lists can be obtained from the authors.

cis-*3′,4′-Diisovalerylkhellactone* [**1**].—Colorless crystal (MeOH): mp 85–86°; $C_{24}H_{30}O_7$; eims *m/z* 430, 328, 313, 229; $[\alpha]^{23}D$ −41.2° (*c* = 0.026, $CHCl_3$); uv λ max nm 247.7, 259.7, 300 (sh), 323.7; ir ν max cm^{-1} 1730, 1603, 1490, 1460, 1370.

cis-*3′-Isovaleryl-4′-senecioylkhellactone* [**2**].—Colorless granules ($CHCl_3$/MeOH): mp 103–104°; $[\alpha]^{23}D$ −13.5° (*c* = 0.20, $CHCl_3$); hreims found 428.1827 (calcd for $C_{24}H_{28}O_7$, 428.1835); *m/z* 428, 326, 311, 229; uv λ max nm 248, 258, 300 (sh), 325; ir cm^{-1} 1730, 1640, 1605, 1488, 1370.

cis-*3′,4′-Disenecioylkhellactone* [**3**].—Colorless needles ($CHCl_3$/*n*-hexane): mp 113–114°; $C_{24}H_{26}O_7$; eims *m/z* 426, 326, 311, 229; $[\alpha]^{23}D$ −21.25° (*c* = 0.069, $CHCl_3$); uv λ max nm 248, 257.9, 300 (sh), 325.4; ir cm^{-1} 1720, 1640, 1605, 1490, 1370.

ACKNOWLEDGMENTS

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[1]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, 12 Union Road, Cambridge CB2 1EZ, UK.