Pergamon 0031-9422(95)00625-7 Phytochemistry, Vol. 41, No. 2, pp. 525–530, 1996

0031 9422/96 $15.00 + 0.00

# COUMARINS AND ANTIPLATELET AGGREGATION CONSTITUENTS FROM FORMOSAN *PEUCEDANUM JAPONICUM*

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(*Received in revised form* 12 *June* 1995)

**Key Word Index**—*Peucedanum japonicum*; Umbelliferae; coumarin; khellactone; chromone; antiplatelet aggregation.

**Abstract**—Four new khellactone esters, ( − )-*trans*-3′-acetyl-4′-senecioylkhellactone, ( ± )-*cis*-3′-acetyl-4′-tigloylkhellactone, ( ± )-*cis*-4-tigloylkhellactone, ( + )-*trans*-4′-tigloylkhellactone, together with 14 known coumarins, isoimperatorin, psoralen, bergapten, xanthotoxol, cnidilin, ( − )-selinidin, ( − )-deltoin, ( + )-pteryxin, ( + )-peucedanocoumarin III, xanthotoxin, imperatorin, ( + )-marmesin, ( + )-oxypeucedanin hydrate, ( + )-peucedanol and three chromones, eugenin, ( − )-hamaudol, ( + )-visamminol, have been isolated from the root of Formosan *Peucedanum japonicum*. The structures of the new compounds were elucidated by spectral data. The identities of ( + )-*trans*-3′-tigloyl-4′-acetylkhellactone, formerly reported as a new compound, and ( + )-*cis*-3′-angeloyl-4′-acetylkhellactone, with the known ( + )-peucedanocoumarin III and ( + )-pteryxin, respectively, are discussed. Among the isolates, seven compounds, eugenin, ( − )-selinidin, ( + )-pteryxin, imperatorin, bergapten, cnidilin and ( + )-visamminol, show strong antiplatelet aggregation activity *in vitro*.

## INTRODUCTION

*Peucedanum japonicum* Thunb. is a perennial herb distributed in Japan, Philippines, mainland China and Taiwan. In Taiwan, it is abundant in the coastal area of the northern part and Lanyu Island [1]. The root was used as a folk medicine in the treatment of cold [2]. The chemical constituents of this plant have been studied to some extent [3–12] and the khellactone coumarins were shown to be the characteristic components. Studies searching for antiplatelet aggregation principles from Formosan plants revealed that the methanol extract of the root of this plant, collected in Lanyu Island, exhibited strong inhibitory activity on platelet aggregation *in vitro*. Examination of the chloroform-soluble part led to the isolation of four new khellactones esters (**1–4**) and 17 compounds known as isoimperatorin (**5**) [13], psoralen (**6**) [13], bergapten (**7**) [3], xanthotoxol (**8**) [13], eugenin (**9**) [14], cnidilin (**10**) [15], ( − )-selinidin (**11**) [16], ( − )-deltoin (**12**) [17], ( + )-pteryxin (**13**) [18, 19], ( + )-peucedanocoumarin III (**14**) [18], xanthotoxin (**15**) [13], imperatorin (**16**) [17], ( − )-hamaudol (**17**) [13], ( + )-visamminol (**18**) [13], ( + )-marmesin (**19**) [20], ( + )-oxypeucedanin hydrate (**20**) [13] and ( + )-peucedanol (**21**) [17]. Several compounds among these show antiplatelet aggregation activity. We report herein the structural elucidation of the new compounds and the active principles with antiplatelet aggregation activity.

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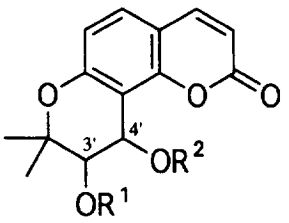
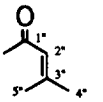
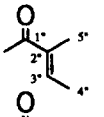
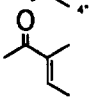
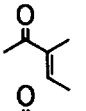
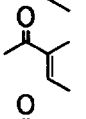
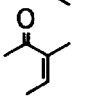
## RESULTS AND DISCUSSION

Compound **1**, $[\alpha]_D^{25}$: − 7.0° ($CHCl_3$, *c* 0.14), was isolated as an oil. The IR spectrum showed a ketone absorption at 1740 $cm^{-1}$. The UV spectrum showed maximal absorptions at 204, 220 *sh*, 255 *sh*, 295 *sh*, 323 nm and indicated the presence of a 7-oxygenated coumarin moiety. In the $^1H$ NMR spectrum, the presence of two pairs of doublets ($\delta$6.23, 7.60 (each 1H, *d*, $J$ = 9.5 Hz, H-3 and H-4), $\delta$7.37, 6.82 (each 1H, *d*, $J$ = 8.6 Hz, H-5 and H-6)), two methyl singlets ($\delta$1.38, 1.46 (each 3H, *s*)), doublets of two vicinal methine protons ($\delta$5.31, 6.20 (each 1H, *d*, $J$ = 3.4 Hz, H-3′ and H-4′)), one acetoxy singlet ($\delta$2.10 (3H, *s*)) and one senecioyloxy group ($\delta$5.62 (1H, *m*, H-2″), $\delta$2.24 (3H, *d*, $J$ = 1.2 Hz, 5″-Me), $\delta$1.89 (3H, *d*, $J$ = 1.1 Hz, 4″-Me)), suggested a khellactone diester, acetylsenecioylkhellactone. The EI mass spectrum showed $[M]^+$ at *m/z* 386, the important ion fragments at *m/z* 326 $[M - 60]^+$, 311 $[M - 75]^+$, 287 $[M - 99]^+$, indicated the location of the acetoxy group at C-3′ and the senecioyloxy group at C-4′ [21]. The relative configuration of this khellactone ester was indicated as *trans*-form owing to the coupling constant $J_{3',4'}$ as 3.4 Hz [22]




| *trans* | $R^1$ | $R^2$ |
|---|---|---|
| **1** |  |  |
| **4** | H |  |
| **14** |  |  |
| **23** |  |  |

| *cis* | $R^1$ | $R^2$ |
|---|---|---|
| **2** |  |  |
| **3** | H |  |
| **13** |  |  |
| **22** |  |  |

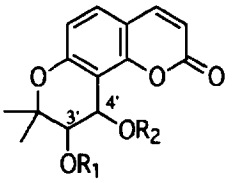
and this was further demonstrated by the chemical shift difference of *gem*-dimethyl signals in the $^{13}C$ NMR spectrum as 0.12 ppm lower than 2 ppm [23]. From above data, the new compound **1** was elucidated as ( − )-*trans*-3′-acetyl-4′-senecioylkhellactone.

Compound **2**, $[\alpha]_D^{25}$: 0° ($CHCl_3$, *c* 0.11), was isolated as prisms. The IR and UV spectra showed close resemblances with those of ( + )-peucedanocoumarin III (**14**). The $^1H$ NMR spectrum was also similar to that of **14** except for a pair of doublets ($\delta$6.62 (1H, *d*, *J* = 4.9 Hz), 5.33 (1H, *d*, *J* = 4.9 Hz)) and the close *gem*-methyl singlets ($\delta$1.43, 1.47) [22]. The EI mass spectrum of **2** showed *m/z* 386 for $[M]^+$ and other diagnostic fragments at *m/z* 326, 311, 287. Thus an acetoxy group and a tigloyloxy group were located at C-3′ and C-4′, respectively, in the pyran ring of dihydroseselin [21]. The structure of **2** was elucidated as ( ± )-*cis*-3′-acetyl-4′-tigloylkhellactone which was further supported by molecular formula $C_{21}H_{22}O_7$, established from the HR mass spectrum, $^{13}C$ NMR, DEPT and HETCOR spectra.

Compound **3**, $[\alpha]_D^{24}$: 0° ($CHCl_3$, *c* 0.11), was obtained as needles and the molecular formula, $C_{19}H_{20}O_6$, determined by EI ($[M]^+$, *m/z* 344) and HR mass spectrometry. The UV absorptions were very similar to those of **1** and a 7-oxygenated coumarin moiety was suggested. The IR spectrum indicated the presence of a ketone group (1720 $cm^{-1}$) and a hydroxyl group (3490 $cm^{-1}$). In the $^1H$ NMR spectrum, the existence of two pairs of doublets [$\delta$6.23, 7.61 (each 1H, *d*, *J* = 9.5 Hz, H-3 and H-4), $\delta$7.37, 6.81 (each 1H, *d*, *J* = 8.6 Hz, H-5 and H-6)), a closer two methyl singlets ($\delta$1.44, 1.48 (each 3H, *s*)), two vicinal methine protons ($\delta$4.07 (1H, *dd*, *J* = 4.7, 2.6 Hz, H-3′), $\delta$6.47 (1H, *d*, *J* = 4.7, H-4′)), one hydroxyl group ($\delta$2.98 (1H, *br d*, *J* = 2.6 Hz, 3′-OH)) and a tigloyloxy group ($\delta$6.90 (1H, *qq*, *J* = 7.1, 1.4 Hz, H-3″), $\delta$1.86 (3H, *m*, 5″-Me), $\delta$1.79 (*dq*, *J* = 7.1, 1.3 Hz, 4″-Me)), a 4′-tigloylkhellactone was suggested. The relative configuration of **3** was determined as the *cis*- form because of the coupling constant $J_{3',4'}$ as 4.7 Hz [21], the *gem*-dimethyl signals appeared as two singlets close together [22, 24] in the $^1H$ NMR spectrum and the chemical shifts of *gem*-dimethyl signals with 4.78 ppm difference in the $^{13}C$ NMR spectrum was larger than 2 ppm in the *trans* form [23]. The structure of new compound **3** thus was elucidated as ( ± )-*cis*-4′-tigloylkhellactone.

Compound **4**, $[\alpha]_D^{26}$: + 254° ($CHCl_3$, *c* 0.14), was isolated as prisms and the molecular formula was established as $C_{19}H_{20}O_6$ by EI and HR mass spectrometry

Table 1. Comparisons of chemical shifts of H-3′ and H-4′ in some natural *trans*- and *cis*- khellactone and khellactone esters (in $CDCl_3$)




| | *trans* | | | | | | *cis* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | H-3′ | H-4′ | *J*(Hz) | | $R_1$ | $R_2$ | H-3′ | H-4′ | *J*(Hz) |
| | H | H | δ 3.86 | δ 5.11 | 6.6 | | H | H | δ 3.88 | δ 5.22 | 5.0 |
| |  |  | 5.31 | 6.21 | 4.0 | |  |  | 5.34 | 6.55 | 5.0 |
| | — | — | — | — | — | |  |  | 5.49 | 6.69 | 5.0 |
| * |  |  | 5.33 | 6.23 | 3.5 | * |  |  | 5.33 | 6.62 | 4.9 |
| |  |  | 5.28 | 6.18 | 3.5 | |  |  | 5.28 | 6.52 | 4.9 |
| |  |  | 5.33 | 6.28 | 3.5 | * |  |  | 5.35 | 6.64 | 4.9 |
| * |  |  | 5.31 | 6.20 | 3.3 | |  |  | 5.27 | 6.55 | 5.0 |
| * | H |  | 3.94 | 6.10 | 4.8 | * | H |  | 4.07 | 6.47 | 4.7 |
| | — | — | — | — | — | | H |  | 4.02 | 6.40 | 4.9 |
| | — | — | — | — | — | | H |  | 4.06 | 6.46 | 4.7 |
| | — | — | — | — | — | | H |  | 4.02 | 6.42 | 4.7 |

*Isolated in this study.

([M]$^+$ at *m/z* 344). The IR spectrum indicated the presence of a ketone group (1715 $cm^{-1}$) and a hydroxyl group (3425 $cm^{-1}$). Inspection of the $^1H$ NMR spectrum of **3** led to a coumarin of 4′-tigloylkhellactone with the angular dihydropyranocoumarin moiety (δ6.24, 7.62 (each 1H, *d*, *J* = 9.5 Hz, H-3 and H-4), δ7.37, 6.83 (each 1H, *d*, *J* = 8.6 Hz, H-5 and H-6), δ1.40, 1.48 (each 3H, *s*, *gem*-Me), δ3.94 (1H, *dd*, *J* = 4.8, 3.4 Hz, H-3′), δ3.46 (1H, *br d*, *J* = 3.4 Hz, 3′-OH), δ6.10 (1H, *d*, *J* = 4.8 Hz, H-4′)), tigloyloxy group (δ6.87 (1H, *qq*, *J* = 7.1, 1.4 Hz, H-3″), δ1.84 (3H, *m*, 5″-Me), δ1.78 (3H, *dq*, *J* = 7.1, 1.3 Hz, 4″-Me)). The coupling constant $J_{3',4'}$ of **4** is 4.8 Hz and it is difficult to assign the relative configuration of **4** to be the *cis* form again owing to the aformentioned *cis*-khellactone ester (**3**) with $J_{3',4}$ = 4.7 Hz. After comparison of the chemical shifts of natural khellactone esters having an acyl group at C-4′, we found that the chemical shift of H-4′ in the *trans* form is at higher field than that of H-4′ in the *cis* form, and in the case of khellactone esters, the pair of configuration isomers show consistently *ca.* 0.3 ppm difference (Table 1). This observation supported the relative configuration of **4** as the *trans* form with H-4′ (δ6.10) showing 0.37 ppm difference of chemical shift compared with H-4′ (δ6.47) of the *cis* form **3**. The

*gem*-methyl signals in the $^1$H NMR spectrum of **4** showed two separate signals ($\delta$1.40 and $\delta$1.48) like other *trans*-khellactone coumarins. The new khellactone monoester of **3** was elucidated as ( + )-*trans*-4′-tigloylkhellactone.

The *trans*-khellactone 4′-monoester is rare in nature. This study revealed some interesting facts: (1) the indication of *cis* configuration by the $J_{3',4'}$ value (*ca* 5 Hz) is applicable to the khellactone or khellactone esters; (2) the indication of *trans* configuration by the $J_{3',4'}$ value (*ca* 3–4 Hz [22]) is not applicable to *trans*-khellactone 4′-monoester and *trans*-khellactone; (3) the indication of *trans* configuration by greater than 2 ppm difference of chemical shift of the *gem*-methyl signals in the $^{13}$C NMR spectrum [23] is feasible in khellactone diesters but is not applicable to the *trans*-khellactone 4′-monoester.

The absolute configurations of **1** and **4** were not determined because of the small amounts of samples. However, after referring to the 3′*S*,4′*R*-configuration of ( + )-peucedanocoumarin II and ( + )-peucedanocoumarin III (**14**) [18], it was reasonable to assign the 3′*R*, 4′*S*-configuration to compound **1** with its laevorotatory optical activity.

The types of khellactones isolated from Formosan *P. japonicum* were quite different between northern species and Lanyu Island species [5, 8–10]. Two khellactone diesters, ( + )-*trans*-4′-acetyl-3′-tigloylkhellactone (**22**), a new compound, and ( + )-*cis*-4′-acetyl-3′-angeloylkhellactone (**23**), a known compound, were reported from Lanyu Island species in Taiwan [8, 9]. In our study of the same plant collected in Lanyu Island, ( + )-*trans*-3′-acetyl-4′-tigloylkhellactone [( + )-peucedanocoumarin III] (**14**) and ( + )-*cis*-3′-acetyl-4′-angeloylkhellactone [( + )-pteryxin] (**13**) were obtained but without **22** and **23**. Further, re-examination of the spectral data of so-called **22** [8] and **23** [8, 9], did not permit exact identification with respective reference data [25, 26]. In addition, the *m/z* 386 $[M]^+$, 326 $[M - 60]^+$, 311 $[M - 75]^+$ and 287 $[M - 99]^+$ were also observed [21] in the EI mass spectra of **22** and **23**. Therefore the so-called **22** and **23** were reasonably identified and revised as the known compound **14** and **13**, respectively.

The chloroform-soluble fraction of the root of this plant showed strong antiplatelet activity *in vitro* using a turbidimetry method [27]. Bioassay-guided fractionation led to the isolation of seven compounds showing strong inhibition of platelet aggregation. They were psoralen (**6**), eugenin (**9**), ( − )-selinidin (**11**), ( + )-pteryxin (**13**), ( + )-peucedanocoumarin (**14**), xanthotoxin (**15**) and imperatorin (**16**) (Table 2). In addition, bergapten (**7**), cnidilin (**10**) and ( + )-visamminol (**18**) also exhibit significant antiplatelet aggregation activity. The diacyl groups in khellactone are necessary and responsible for the antiplatelet activity, as was shown in this study.

## EXPERIMENTAL

Mps: uncorr. $^1$H NMR (200 MHz) and $^{13}$C NMR (50 MHz): $CDCl_3$ with TMS as int. standard; MS, 70 eV; CC silica gel 60 (70–230 mesh and 230–400 mesh) (Merck); TLC and prep. TLC: silica gel 60 GF 254 (Merck).

*Plant material. Peucedanum japonicum* was collected at Lanyu Island, Taitung Hsien, Taiwan, in July 1991. The

Table 2. Inhibitory effect of **2–4, 6, 9–11, 13–16** and **18** on the aggregation of washed rabbit platelets[a]

| | Percentage | | Aggregation | |
|---|---|---|---|---|
| Treatment | Thrombin | AA | Collagen | PAF |
| Control | 89.2 ± 0.9 | 87.5 ± 1.5 | 86.3 ± 0.7 | 92.4 ± 1.4 |
| **2** | 83.9 ± 1.9** | 68.7 ± 5.8** | 0.0 ± 0.0*** | 24.7 ± 11.7*** |
| **3** | 90.2 ± 0.7 | 73.8 ± 7.1 | 79.8 ± 2.4** | 68.7 ± 9.1* |
| **4** | 86.1 ± 0.7** | 82.8 ± 0.8* | 46.9 ± 15.0* | 74.5 ± 4.9** |
| **6** | 85.3 ± 1.6** | 0.0 ± 0.0*** | 8.9 ± 4.9*** | 73.8 ± 5.1** |
| **9** | 76.4 ± 5.5** | 0.0 ± 0.0*** | 18.2 ± 11.9*** | 65.4 ± 10.2* |
| **10** | 84.3 ± 1.2 | 52.9 ± 4.8*** | 41.2 ± 12.1*** | 64.0 ± 4.1*** |
| **11** | 84.7 ± 2.5 | 0.0 ± 0.0*** | 0.0 ± 0.0*** | 12.6 ± 10.3*** |
| **13** | 85.5 ± 2.7 | 41.7 ± 6.6*** | 0.0 ± 0.0*** | 6.2 ± 5.0*** |
| **14** | 81.7 ± 1.7*** | 63.8 ± 6.4** | 0.0 ± 0.0*** | 20.7 ± 10.7*** |
| **15** | 86.0 ± 0.7** | 0.0 ± 0.0*** | 34.7 ± 12.3** | 72.6 ± 4.7*** |
| **16** | 76.9 ± 4.0*** | 0.0 ± 0.0*** | 8.5 ± 7.3*** | 24.8 ± 11.7*** |
| **18** | 87.7 ± 0.5 | 37.6 ± 18.9* | 78.8 ± 2.0*** | 87.7 ± 0.6 |

[a]Platelets were preincubated with each compound (100 $\mu$g ml$^{-1}$) or 0.5% DMSO (control) at 37° for 3 min, then the inducer thrombin (0.1 U ml$^{-1}$), arachidonic acid (AA, 100 $\mu$M), collagen (10 $\mu$g ml$^{-1}$) or PAF (2 ng ml$^{-1}$) was added to trigger the aggregation. Percentages of aggregation are presented as means ± SEM. ($n$ = 3–5).
*$P$ < 0.05; **$P$ < 0.01; ***$P$ < 0.001 as compared with the respective control.

voucher specimen is deposited in the Herbarium of School of Pharmacy, Kaohsiung Medical College, Taiwan, Republic of China.

*Extraction and separation.* Fresh root (15.5 kg) was chopped, extracted with MeOH and concd under red. pres. The MeOH extract was partitioned with $H_2O$:$CHCl_3$ (1:1) and yielded the $CHCl_3$-soluble fr. (fr. A, 406 g). Fr. A (80 g) was chromatographed over silica gel eluting with $CHCl_3$ and gradually increasing the polarity with MeOH and 19 frs (Fr. A1–A19) were collected. Fr. A5–A9, Fr. A12 and Fr. A13 showed strong antiplatelet aggregation activity *in vitro* and were sepd in advance. Fr. A5 (5.29 g) was washed with MeOH to yield crude crystals (1.63 g). The crude crystals (0.10 g) were purified by prep. TLC (*n*-hexane:EtOAc, 3:1) and recrystallization to give **5** (1.2 mg), **6** (1.4 mg) and **7** (8.1 mg). The above MeOH washing (3.05 g) was sepd by silica gel CC and eluted with *n*-hexane:EtOAc (97:3). The eluate (*n*-hexane:EtOAc, 1:1) (8.2 mg) was repeatedly recrystallized by $CH_2Cl_2$ to yield **8** (1.4 mg). Fr. A6 (0.5254 g) was chromatographed on silica gel using *n*-hexane–EtOAc (97:3) as eluent to yield **6** (34.2 mg) and **7** (52.1 mg) again. Fr. A7 (2.29 g) was washed with MeOH to obtain crude crystals (0.3319 g) and further sepd by silica gel CC using *n*-hexane:EtOAc (99:1) as eluent to give **9** (32 mg). Fr. A8 (15.4 g) was rechromatographed on silica gel CC using *n*-hexane:EtOAc (95:5) as eluting solvent, and gradually increasing the polarity with EtOAc to afford 7 frs (Fr. A8-1–A8-7). Fr. A8-2 (1.701 g) was further sepd by silica gel CC eluting with *n*-hexane:$Me_2CO$ (95:5) to obtain 11 frs (Fr. A8-2-1–Fr. A8-2-11). Fr. A8-2-4 (0.442 g) was purified over silica gel CC eluting with *n*-hexane:$CH_2Cl_2$ (7:3) to yield **10** (12.1 mg) and **11** (82.1 mg). Fr. A8-2-8 (0.161 g) was purified in the same way to give **12** (10.2 mg). Fr. A9 (35.7 g) was washed with MeOH to yield crude crystals (2.27 g) from which 0.5 g was sepd by silica gel CC and the eluate (*n*-hexane:EtOAc, 9:1) was further purified by prep. TLC (*n*-hexane:EtOAc, 2:1) to yield **13** (28.1 mg), **1** (7.2 mg), **14** (32.4 mg) and **4** (7.7 mg). The MeOH washing (32.5 g) from Fr. A9 was sepd with silica gel CC using $CH_2Cl_2$ as the eluting solvent and gradually increasing the polarity with MeOH to obtain 12 frs (fr. A9-1–fr. A9-12). Fr. A9-1 (2.11 g) was rechromatographed over silica gel and eluted with *n*-hexane:$Me_2CO$ (99:1) to give **15** (32.8 mg) and **16** (14.3 mg). Fr. A9-2 (7.30 g) was further sepd with silica gel CC eluting with *n*-hexane:$Me_2CO$ (99:1) to obtain 10 frs (fr. A9-2-1–fr. A9-2-10). Fr. 9-2-9 (0.300 g) was purified by prep. TLC (*n*-hexane–EtOAc, 2:1) and recrystallized with ether to give **2** (27 mg). Fr. A9-5 (1.06 g, $CH_2Cl_2$:MeOH, 99:1) was repeatedly rechromatographed to give **17** (9.2 mg), **3** (23.8 mg), **18** (9.1 mg). Fr. A9-6 (4.551 g) was washed with ether, and the crude crystals (56.9 mg) further recrystallized from MeOH to yield **19** (39 mg). Fr. A12 (1.36 g, $CH_2Cl_2$:MeOH, 9:1) was purified by recrystallization with ether to produce **20** (10 mg), and the fr. A13 (0.563 g, $CH_2Cl_2$:MeOH, 9:1) was washed with ether to yield crude material that which was recrystallized from $Me_2CO$ to give **21** (12.5 mg).

*(−)-trans-3′-Acetyl-4′-senecioylkhellactone* (**1**). Oil, IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 1740 (C=O). UV $\lambda_{max}^{EtOH}$ nm (log ε): 204 (4.67), 220 sh (4.43), 255 sh (3.67), 295 sh (3.87), 323 (4.11). $^1H$ NMR: δ1.38 (3H, *s*, Me), 1.46 (3H, *s*, Me), 1.89 (3H, *d*, *J* = 1.1 Hz, 4″-Me), 2.10 (3H, *s*, OCOMe), 2.24 (3H, *d*, *J* = 1.2 Hz, 5″-Me), 5.31 (1H, *d*, *J* = 3.4 Hz, H-3′), 5.62 (1H, *m*, H-2″), 6.20 (1H, *d*, *J* = 3.4 Hz, H-4′), 6.23 (1H, *J* = 9.5 Hz, H-3), 6.82 (1H, *d*, *J* = 8.6 Hz, H-6), 7.37 (1H, *d*, *J* = 8.6 Hz, H-5), 7.60 (1H, *d*, *J* = 9.5 Hz, H-4), $^{13}C$ NMR (50 MHz): δ20.5 (C-4″), 20.7 (OCOMe), 23.7, 23.8 (*gem*-Me), 27.5 (C-5″), 62.4 (C-4′), 71.4 (C-3′), 77.2 (C-2′), 106.7 (C-8), 112.3 (C-4a), 113.2 (C-3), 114.4 (C-6), 114.9 (C-2″), 129.0 (C-5), 143.2 (C-4), 154.2 (C-8a), 156.6 (C-7), 158.9 (C-3″), 159.9 (C-2), 164.7 (C-1″), 169.4 (OCOMe). EIMS *m/z* (rel. int.): 386 $[M]^+$ (1.3), 326 (3), 310 (10), 287 (4), 245 (10.5), 229 (35). HRMS: $C_{21}H_{22}O_7$, found: 386.1352, calcd: 386.1366. $[\alpha]_D^{25}$: −7.0° ($CHCl_3$, *c* 0.14).

*(±)-cis-3′-Acetyl-4′-tigloylkhellactone* (**2**). Prisms, mp 124–126° ($Et_2O$). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$. 1730 (C=O). UV $\lambda_{max}^{EtOH}$ nm (log ε): 205 (4.79), 221 sh (4.50), 247 sh (3.85), 258 sh (3.73), 296 sh (4.03), 324 (4.25). $^1H$ NMR: δ1.43 (3H, *s*, Me), 1.47 (3H, *s*, Me), 1.78 (3H, *dd*, *J* = 7.1, 1.2 Hz, 4″-Me), 1.85 (3H, *m*, 5″-Me), 2.07 (3H, *s*, OCOMe), 5.33 (1H, *d*, *J* = 4.9 Hz, H-3′), 6.22 (1H, *d*, *J* = 9.5 Hz, H-3), 6.62 (1H, *d*, *J* = 4.9 Hz, H-4′), 6.81 (1H, *d*, *J* = 8.7 Hz, H-6), 6.82 (1H, *qq*, *J* = 7.1, 1.4 Hz, H-3″), 7.36 (1H, *d*, *J* = 8.7 Hz, H-5), 7.60 (1H, *d*, *J* = 9.5 Hz, H-4). $^{13}C$ NMR: δ12.0 (5″-Me), 14.2 (4″-Me), 20.5 (OCOMe), 22.1, 25.1 (*gem*-Me), 60.5 (C-4′), 70.5 (C-3′), 77.6 (C-2′), 107.4 (C-8), 112.6 (C-4a), 113.4 (C-3), 114.5 (C-6), 128.3 (C-2″), 129.3 (C-5), 137.8 (C-3″), 143.3 (C-4), 154.3 (C-8a), 156.8 (C-7), 160.0 (C-2), 167.2 (C-1″), 170.1 (OCOMe). EIMS *m/z* (rel. int.): 386 $[M]^+$ (0.5), 326 (6), 311 (18), 287 (2), 261 (5), 245 (9), 229 (42). HRMS: $C_{21}H_{22}O_7$, found: 386.1339, calcd: 386.1366. $[\alpha]_D^{25}$: ±0.0° ($CHCl_3$, *c* 0.11).

*(±)-cis-4′-Tigloylkhellactone* (**3**). Needles, mp 188–192° (EtOAc). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1720 (C=O), 3490 (OH). UV $\lambda_{max}^{EtOH}$ nm (log ε): 203 (4.58), 220 sh (4.24), 260 sh (3.46), 326 (4.01). $^1H$ NMR: δ1.44 (3H, *s*, Me), 1.48 (3H, *s*, Me), 1.79 (3H, *dq*, *J* = 7.1, 1.3 Hz, 4″-Me), 1.86 (3H, *m*, 5″-Me), 2.98 (1H, *br d*, *J* = 2.6 Hz, OH, disappeared after addition of $D_2O$), 4.07 (1H, *dd*, *J* = 4.7, 2.6 Hz, H-3′), 6.23 (1H, *d*, *J* = 9.5 Hz, H-3), 6.47 (1H, *d*, *J* = 4.7 Hz, H-4′), 6.81 (1H, *d*, *J* = 8.6 Hz, H-6), 6.90 (1H, *qq*, *J* = 7.1, 1.4 Hz, H-3″), 7.37 (1H, *d*, *J* = 8.6 Hz, H-5), 7.61 (1H, *d*, *J* = 9.5 Hz, H-4). $^{13}C$ NMR: δ12.1 (5″-Me), 14.5 (4″-Me), 20.9, 25.7 (*gem*-Me), 63.9 (C-4′), 71.7 (C-3′), 78.6 (C-2′), 107.2 (C-8), 112.2 (C-4a), 113.0 (C-3), 114.5 (C-6), 127.9 (C-2″), 129.3 (C-5), 139.0 (C-3″), 143.3 (C-4), 154.2 (C-8a), 156.9 (C-7), 160.0 (C-2), 169.2 (C-1″). EIMS *m/z* (rel. int.): 344 $[M]^+$ (5.0), 326 (5),. 311 (26), 261 (12), 299 (50). HRMS: $C_{19}H_{20}O_6$, found: 344.1254, calcd: 344.1260. $[\alpha]_D^{25}$: ±0° ($CHCl_3$, 0.11).

*(+)-trans-4′-Tigloylkhellactone* (**4**). Prisms, mp 125–127° (EtOAc). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1715 (C=O), 3425 (OH). UV $\lambda_{max}^{EtOH}$ nm (log ε): 206 (4.51), 231 sh (4.01), 245 sh (3.72), 257 sh (3.60), 296 sh (3.86), 322 (4.08). $^1H$ NMR: δ1.40 (3H, *s*, Me), 1.48 (3H, *s*, Me), 1.78 (3H, *dq*, *J* = 7.1,

1.3 Hz, 4″-Me), 1.84 (3H, *m*, 5″-Me), 3.46 (1H, *br d*, $J = 3.4$ Hz, OH, disappeared after addition of $D_2O$), 3.94 (1H, *dd*, $J = 4.8$, 3.4 Hz, H-3′), 6.10 (1H, *d*, $J = 4.8$ Hz, H-4′), 6.24 (1H, *d*, $J = 9.5$, H-3), 6.83 (1H, *d*, $J = 8.6$ Hz, H-6), 6.87 (1H, *qq*, $J = 7.1$, 1.4 Hz, H-3″), 7.37 (1H, *d*, $J = 8.6$ Hz, H-5), 7.62 (1H, *d*, $J = 9.5$ Hz, H-4). $^{13}$C NMR: $\delta$12.1 (5″-Me), 14.5 (4″-Me), 21.4, 24.8 (*gem*-Me), 68.6 (C-4′), 73.2 (C-3′), 79.2 (C-2′), 107.4 (C-8), 112.6 (C-4a), 113.0 (C-3), 114.6 (C-6), 128.0 (C-2″), 129.2 (C-5), 139.0 (C-3″), 143.4 (C-4), 154.3 (C-8a), 157.0 (C-7), 160.1 (C-2), 168.9 (C-1″). EIMS *m/z* (rel. int.): 344 $[M]^+$ (3), 326 (4), 311 (19), 261 (3), 229 (39), HRMS: $C_{19}H_{20}O_6$, found: 344.1269, calcd: 344.1260. $[\alpha]_D^{25}$: + 254.1° ($CHCl_3$, *c* 0.14).

(+)-*Pteryxin* (**13**). Oil. IR $\nu_{max}^{CHCl_3}$ cm$^{-1}$: 1735 (C=O). UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 203 (4.89), 220 sh (4.40), 250 sh (3.68), 323 (4.11), $^1$H NMR: $\delta$1.45 (3H, *s*, Me), 1.46 (3H, *s*, Me), 1.85 (3H, *m*, 5″-Me), 2.00 (3H, *dq*, $J = 7.2$, 1.5 Hz, 4″-Me), 2.10 (3H, *s*, OCOMe), 5.35 (1H, *d*, $J = 4.9$ Hz, H-3′), 6.04 (1H, *qq*, $J = 7.2$, 1.5 Hz, H-3″), 6.23 (1H, *d*, $J = 9.5$ Hz, H-3), 6.64 (1H, *d*, $J = 4.9$ Hz, H-4′), 6.81 (1H, *d*, $J = 8.6$ Hz, H-6), 7.36 (1H, *d*, $J = 8.6$ Hz, H-5), 7.60 (1H, *d*, $J = 9.5$ Hz, H-4). EIMS *m/z* (rel. int.): 386 $[M]^+$ (0.9), 326 (7), 311 (14), 287 (22), 245 (29), 229 (33). $[\alpha]_D^{25}$: + 5.5° (EtOH, *c* 0.13).

(+)-*Peucedanocoumarin III* (**14**). Needles, mp 136–138°. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1735 (C=O). UV $\lambda_{max}^{EtOH}$ nm (log $\varepsilon$): 204 (4.45), 220 sh (4.25), 255 sh (3.45), 324 (4.09). $^1$H NMR: $\delta$1.38 (3H, *s*, Me), 1.46 (3H, *s*, Me), 1.76 (3H, *dq*, $J = 7.0$, 1.5 Hz, 4″-Me), 1.84 (3H, *m*, 5″-Me), 2.09 (3H, *s*, OCOMe), 5.33 (1H, *d*, $J = 3.3$ Hz, H-3′), 6.23 (1H, *d*, $J = 3.3$ Hz, H-4′), 6.23 (1H, *d*, $J = 9.5$ Hz, H-3), 6.81 (1H, *qq*, $J = 7.0$, 1.5 Hz, H-3″), 6.83 (1H, *d*, $J = 8.6$ Hz, H-6), 7.38 (1H, *d*, $J = 8.6$ Hz, H-5), 7.61 (1H, *d*, $J = 9.5$ Hz, H-4). EIMS *m/z* (rel. int.): 386 $[M]^+$ (0.8), 326 (2.5), 311 (9), 287 (2.5), 245 (7), 229 (26). $[\alpha]_D^{25}$: + 23.5° ($CHCl_3$, *c* 0.20).

*Acknowledgements*—We are very grateful to Prof. K. Baba (Osaka University of Pharmaceutical Sciences, Japan) for authentic samples of isoimperatorin, psoralen, xanthotoxol, deltoin, xanthotoxin, imperatorin, hamaudol, visamminol, oxypeucedanin hydrate, peucedanol; to Prof. H. Ishii (Chiba University, Japan) for the authentic sample of marmesin; to Prof. H. D. Sun (Kunming Institute of Botany, China) for the authentic sample of pteryxin; to Prof. K. Y. Yen (Taipei Medical College, R.O.C.) for the authentic sample of bargapten and to Dr H. Sasaki (Tsummura Laboratory, Japan) for IR spectrum of cnidilin; to Prof. C. M. Chen (National Tsing Hua University, R.O.C.) for measurement of HR mass spectra. This work was kindly supported by a grant (NSC 81-0420-B-037-16) of the National Science Council of R.O.C.

## REFERENCES

1. Kao, M. T. (1993) *Umbelliferae in Flora of Taiwan*, 2nd edn., Vol. III, p. 1010. Editorial Committe of the Flora of Taiwan, Taipei.
2. Gan, W. S. (1965) *Manual of Medicinal Plants in Taiwan*, Vol. 3, p. 675. National Research Institute of Chinese Medicine, Taiwan.
3. Yen, K. Y., Lai, H. M. and Chen, W. S. (1966) *J. Taiwan Pharm. Assoc.* **18**, 75.
4. Hata, K., Kozawa, M., Ikeshiro, Y. and Yen, K. Y. (1968) *Yakugaku Zasshi* **88**, 513.
5. Yamada, Y., Hsu, C. S., Iguchi, K., Suzuki, M., Hsu, H. Y. and Chen, Y. P. (1974) *Tetrahedron Letters* **29**, 2513.
6. Shigematsu, N., Kouno, I. and Kawano, N. (1982) *Yakugaku Zasshi* **102**, 392.
7. Cisowski, W. (1983) *Pol. J. Chem.* **57**, 1283 [C. A. (1985) **102**, 218357t.]
8. Duh, C. Y., Wang, S. K. and Wu, Y. C. (1991) *Phytochemistry* **30**, 2812.
9. Duh, C. Y., Wang, S. K. and Wu, Y. C. (1992) *Phytochemistry* **31**, 1829.
10. Jong, T. T., Hwang, H. C., Jean, M. Y., Wu, T. S. and Teng, C. M. (1992) *J. Nat. Prod.* **55**, 1396.
11. Ikeshiro, Y., Mase, I. and Tomita, Y. (1992) *Phytochemistry* **31**, 4303.
12. Ikeshiro, Y., Mase, I. and Tomita, Y. (1993) *Phytochemistry* **33**, 1543.
13. Baba, K., Hata, K., Kimura, Y., Matsuyama, Y. and Kozawa, M. (1981) *Chem. Pharm. Bull.* **29**, 2565.
14. Ali, A. A., Makboul, M. A., Attia, A. A. and Ali, D. T. (1990) *Phytochemistry* **29**, 625.
15. Sasaki, H., Taguchi, H., Endo, T. and Yosioka, I. (1980) *Chem. Pharm. Bull.* **28**, 1847.
16. Swager, T. M. and Cardellina II, J. H. (1985) *Phytochemistry* **24**, 805.
17. Baba, K., Yoneda, Y., Kozawa, M., Fujita, E., Wang, N. H. and Yuan, C. Q. (1989) *Shoyakugaku Zasshi* **43**, 216.
18. Takata, M., Shibata, S. and Okuyama, T. (1990) *Planta Med.* **56**, 307.
19. Rao, G. X., Niu, F. D. and Sun, H. D. (1990) *Acta Botanica Yunnanica*, **12**, 434.
20. Ishii, H., Hosoya, K., Ishikawa, T. and Haginiwa, T. (1974) *Yakugaku Zasshi* **94**, 309.
21. Shaath, N. A., Soine, T. O. and Shipchandler, M. T. (1976) *J. Pharm. Sci.* **65**, 1028.
22. Lemmich, J., Lemmich, E. and Nielsen, B. E. (1966) *Acta Chem. Scand.* **20**, 2497.
23. Macias, F. A., Massanet, G. M., Rodriquez-Luis, F., Salvá, J. and Fronczek, F. R. (1989) *Magn. Reson. Chem.* **27**, 653.
24. Bellino, A., Venturella, P., Marino, M. L., Servettaz, O. and Venturella, G. (1986) *Phytochemistry* **25**, 1195.
25. Du, S. J., Gariboldi, P. and Jommi, G. (1986) *Planta Med.* **52**, 317.
26. Takeuchi, N., Kasama, T., Mayuzumi, K., Tamaru, K., Fukaya, H., Ohno, T. and Tobinaga, S. (1988) *Chem. Pharm. Bull.* **36**, 4221.
27. O'Brien, J. R. (1962) *T. Clin. Path.* **15**, 452.