Korean J. Medicinal Crop Sci. 13(2) : 75 - 79 (2005)

The Constituents Isolated from *Peucedanum japonicum* Thunb. and their Cyclooxygenase (COX) Inhibitory Activity

MingShan Zheng*, WenYi Jin*, Kun Ho Son, Hyeun Wook Chang***, Hyun Pyo Kim****, KiHwan Bae*†, and Sam Sik Kang*******

**College of Pharmacy, Chungnam Natl. Univ., Daejeon 305-764, Korea.*
***Dept. of Food & Nutrition, Andong Natl. Univ., Andong 760-749, Korea.*
****College of Pharmacy, Yeungnam Univ., Gyongsan 712-749, Korea.*
*****College of Pharmacy, Kangwon Natl. Univ., Chuncheon 200-749, Korea.*
******Natural Products Research Institute & College of Pharmacy, Seoul Natl. Univ., Seoul 110-640, Korea.*

ABSTRACT : Five coumarins, psoralen (1), scopoletin (2), isoimperatorin (4), (+)-marmesin (5) and xanthotoxin (6), three chromones, cimifugin (3), hamaudol (7) and *sec-O*-glucosylhamaudol (10), one sterol, daucosterol (8) and one aliphatic alcohol, galactitol (9) were isolated from the root of *Peucedanum japonicum*. Their chemical structures were identified by the physicochemical and spectroscopic data by comparing literature values. Among them, compounds 9 and 10 were isolated for the first time from this plant. The anti-inflammatory effects of isolated compounds were examined on cyclooxygenase (COX), compounds 1, 2 and 7 showed inhibitory activity on COX-1 with IC_{50} values of 0.88, 0.27 and 0.30 mM, respectively. In the test for COX-2 activity, only compound 7 showed significant inhibitory activity with the IC_{50} value of 0.57 mM. The other compounds exhibited weak inhibitory or no inhibitory activity.

Key words **:** *Peucedanum japonicum*, coumarins, chromones, anti-inflammatory, COX-1, COX-2

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are well-known as COX inhibitors, but have the particular disadvantage of causing gastrointestinal toxicity. The discovery of an inducible COX-2, which is markedly up regulated at the inflammatory sites, has opened the possibility for discovering selective inhibitors with reduced gastrointestinal side effects. In accordance with this respect, development of COX-2 inhibitors or modulators of COX-2 expression may be a significant subject to find new anti-inflammatory agents.

Peucedanum japonicum Thunb. is a perennial herb distributed in Japan, Philippines, China and Korea. The root is used for cough, cold, headache and as an anodyne (Ikeshiro *et al.*, 1992). In previous study, some bioactivities were reported such as antiplatelet aggregation (Chen *et al.*, 1996), inhibitory of nitric oxide (Choi *et al.*, 1999), cytotoxicity (Duh *et al.*, 1992), antioxidant activity (Hisamoto *et al.*, 2003), antiallergic effects (Aida *et al.*, 1998), and so on except the anti-inflammatory activity. During the course of our research for new COX-2 inhibitors from natural sources, we found that $CHCl_3$ fraction of *P. japonicum* showed significant anti-inflammatory activity on COX-1 and COX-2. The BuOH fraction showed inhibitory activity on COX-1, but inactivity on COX-2. In this study, we described the isolation, structure elucidation and inhibitory activity of isolated compounds against COX-1 and COX-2.

MATERIALS AND METHODS

Plant material

Peucedanum japonicum was purchasedat Deajeon, Korea in February 2004. The plant was identified by an author K. Bae. A voucher specimen (CNU 1560) is deposited in the herbarium of the College of Pharmacy, Chungnam National University, Deajeon, Korea.

Instruments and reagents

Melting point were measured by an electrothermal melting point apparatus, UV spectra were recorded on a Milton Roy Spectronic 3000 spectrophotometer and IR spectra were determined on an IR Report-100 spectrophotometer (JASCO). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker NMR DRX 400, with the chemical shift being represented in parts per million (ppm,) with tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out on silica gel Kieselgel 60 (230-400 mesh, ASTM, Merck, U.S.A.)

† Corresponding author: (Phone) +82-42-821-5925 (E-mail) baekh@cnu.ac.kr
Received February 22, 2005 / Accepted March 31, 2005

and Sephadex LH-20 (Amersham Biosciences, U.S.A.). TLC was performed on precoated silica gel Kieselgel 60 F254 (Art. 5715, Merck, U.S.A.), RP-18 F254 (Art. 15685, Merck, U.S.A.).

COX-1, COX-2, hematine, *l*-epinephrine, reduced-glutathion and *tris*-buffer were purchased from the Sigma Chemical Co. (U.S.A.), [1-^{14}C] arachidonic acid (NEC-661-2) was provided by BMS (U.S.A.). All other chemicals used were reagent grade.

Extraction and isolation

The dried root of *Peucedanum japonicum* (4.9 kg) was extracted three times with MeOH at room temperature for 3 days. The combined MeOH solution was concentrated under reduced pressure and obtained the MeOH extract (960 g). The extract was suspended in H_2O and then fractionated sequentially with $CHCl_3$ and water-saturated butanol 3 times, respectively. The $CHCl_3$ fraction (242 g), exhibiting inhibitory effect on the COX activation, was chromatographed on a silica gel column eluting with a step gradient of $CHCl_3$-MeOH (100:10 0:1) to give 8 fractions (C.1~C.8). Active C.4 was subjected to a silica gel column chromatography with mixtures of $CHCl_3$-MeOH (50:1 0:1) as eluents in a stepwise gradient to afford compound 1 (20 mg), 2 (25 mg) and 3 (23 mg). C.6 was chromatographed on a silica gel column with gradient of $CHCl_3$-MeOH (25:1 0:1) to give 4 (15 mg), 5 (30 mg) and 6 (15 mg). Compound 7 (10 mg) and 8 (45 mg) were isolated from C.7 by rechromatographyed and recrytallizated in MeOH.

The BuOH fraction (250 g) was chromatographed on silica gel column using $CHCl_3$-MeOH and $CHCl_3$-MeOH-H_2O step gradient system to give 4 fractions (fr. B-1~fr. B-4). Fr. B-2 was repeated column chromatography on silica gel ($CHCl_3$-MeOH-H_2O, 70:30:2) and afforded compound 9 (50 mg). Fr. B-3 was chromatographed on Sephadex LH-20 column eluted with MeOH to give compound 10 (20 mg).

Compound 1 – white crystal, mp 165~167℃. IR $(KBr)_{max}$ cm^{-1}: 1720 (C = O), 1020 (C-O). UV (MeOH) λ_{max}: 209, 247, 350 nm. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.38 (1H, d, *J* = 9.6 Hz, H-3), 7.80 (1H, d, *J* = 9.6 Hz, H-4), 7.47 (1H, s, H-5), 7.68 (1H, s, H-8), 7.69 (1H, d, *J* = 2.0 HzH-2'), 6.38 (1H, d, *J* = 2.2 HzH-3'). ^{13}C-NMR (100 MHz, $CDCl_3$): 161.3 (C-2), 114.6 (C-3), 144.0 (C-4), 106.3 (C-5), 114.6 (C-8), 152.0 (C-8a), 99.8 (C-4a), 124.8 (C-6), 156.3 (C-7), 146.8 (C-2'), 119.8 (C-3').

Compound 2 – white crystal, mp 202~204℃. IR $(KBr)_{max}$ cm^{-1}: 3350 (-OH), 1700 (C=O), 1080 (C-O). UV (MeOH) λ_{max}: 209, 230, 299, 350 nm. ^{1}H-NMR (400 MHz, $CDCl_3$): 6.28 (1H, d, *J* = 9.6 Hz, H-3), 7.60 (1H, d, *J* = 9.6 Hz, H-4), 6.84 (1H, s, H-5), 6.91 (1H, s, H-8), 6.23 (1H, s, 7-OH), 3.96 (3H, s, OCH3). ^{13}C-NMR (100 MHz, $CDCl_3$): 161.3 (C-2), 113.3 (C-3), 143.2 (C-4), 107.5 (C-5), 103.1 (C-8), 150.1 (C-8a), 111.4 (C-4a), 143.9 (C-6), 149.6 (C-7), 56.4 (OCH_3).

Compound 3 – white crystal, mp 112~113℃. IR $(KBr)_{max}$ cm^{-1}: 3350 (-OH), 1650 (C = O), 1430 (C = C), 1175 (C-O), 845 (trisubstituted aromatic ring). UV (MeOH) λ_{max}: 217, 246, 300 nm. ^{1}H-NMR (400 MHz, CD_3OD): 1.24 (3H, s, H-4'), 1.28 (3H, s, H-4'), 3.27 (1H, dd, *J* = 16.1, 9.3 Hz, H-3a'), 3.32 (1H, dd, *J* = 16.1, 7.8 Hz, H-3b), 3.77 (3H, s, OCH3-5), 4.45 (2H, br d, *J* = 4.8 Hz, H-11), 6.16 (1H, s, H-3), 6.50 (H, s, H-8). ^{13}C-NMR (100 MHz, CD_3OD): 164.4 (C-2), 108.9 (C-3), 177.6 (C-4), 165.8 (C-5), 93.6 (C-8), 155.5 (C-8a), 111.7 (C-4a), 116.9 (C-6), 159.2 (C-7), 53.4 (OCH_3), 60.8 (2-CH_2OH), 91.3 (C-2'), 27.8 (C-3'), 71.4 (C-4'), 24.5 (CH_3), 26.0 (CH_3).

Compound 4 – white crystal, mp 106~107℃. IR $(KBr)_{max}$ cm^{-1}: 1730 (C = O), 1626 (C = C), 1075 (C-O), 825 (trisubstituted aromatic ring). UV (MeOH) λ_{max}: 210, 255, 345 nm. ^{1}H-NMR (400 MHz, CD_3OD): 8.13 (1H, d, *J* = 9.9 Hz, H-4), 7.58 (1H, d, *J* = 1.5 Hz, H-10), 7.13 (1H, s, H-8), 6.95 (1H, m, H-9), 6.26 (1H, d, *J* = 9.9 Hz, H-3), 5.53 (1H, m, 2'-H), 4.95 (2H, d, *J* = 6.9 Hz, H-1'), 1.79 (3H, s, H-5'), 1.68 (3H, s, H-4'). ^{13}C-NMR (100 MHz, CD_3OD): 18.2 (C-5'), 25.8 (C-4'), 69.4 (C-1'), 111.1 (C-8), 105.5 (C-9), 107.4 (C-4a), 112.4 (C-3), 114.0 (C-6), 119.0 (C-2'), 139.6 (C-4), 139.8 (C-3'), 144.8 (C-10), 148.9 (C-8a), 152.6 (C-5), 158.0 (C-7), 161.3 (C-2).

Compound 5 – white crystal, mp 18s9~191℃. IR $(KBr)_{max}$ cm^{-1}: 3445 (-OH), 1700 (C = O), 1270 (C-H), 1120 (C-O). UV (CHCl3) λ_{max}: 337, 260, 224 nm. ^{1}H-NMR (400 MHz, CD_3OD) : 6.19 (1H, d, *J* = 9.2 Hz, H-3), 7.58 (1H, d, *J* = 9.2 Hz, H-4), 7.21 (1H, s, H-5), 7.72 (1H, s, H-8), 4.74 (1H, dd, *J* = 9.3, 8.4 HzH-2'), 3.19 (1H, dd, *J* = 15.9, 9.3 HzH-3'a), 3.27 (1H, dd, *J* = 15.9, 8.4 HzH-3'b), 1.24 (3H, s, CH_3), 1.37 (3H, s, CH_3). ^{13}C-NMR (100 MHz, CD_3OD): 163.0 (C-2), 112.1 (C-3), 143.6 (C-4), 122.9 (C-5), 97.8 (C-8), 161.3 (C-8a), 112.6 (C-4a), 125.3 (C-6), 155.5 (C-7), 74.8 (C-4'), 91.1 (C-2'), 29.5 (C-3'), 24.3 (C-5'a), 26.0 (C-5'b).

Compound 6 – yellow needle, mp 146~147℃. IR $(KBr)_{max}$ cm^{-1}: 1715 (C = O), 1626, 1578, 1456 (aromatic C = C), 1150 (C-O), 820 (trisubstituted aromatic ring). UV (MeOH) λ_{max}: 219, 250, 350 nm. ^{1}H-NMR (400 MHz, CD_3OD): 6.39 (1H, d, *J* = 9.6 Hz, H-3), 7.78 (1H, d, *J* = 9.6 Hz, H-4), 7.36 (1H, s, H-5), 6.83 (1H, d, *J* = 2.4 HzH-3'), 7.70 (1H, d, *J* = 2.4 Hz, H-2'), 4.31 (3H, s, OCH3). ^{13}C-NMR (100 MHz, CD_3OD): 160.8

(C-2), 115.2 (C-3), 144.7 (C-4), 113.3 (C-5), 126.5 (C-6), 148.1 (C-7), 133.2 (C-8), 143.4 (C-8a), 116.9 (C-4a), 147.0 (C-2'), 107.1 (C-3'), 61.7 (OCH_3).

Compound 7 – white amorphous powder, mp 202~203 ℃. IR $(KBr)_{max}$ cm^{-1}: 3450 (-OH), 1720 (C = O), 1650 (C = C), 1130 (C-O), 855 (trisubstituted aromatic ring). UV (MeOH) λ_{max}: 299, 259, 231 nm. ^{1}H-NMR (400 MHz, CD_3OD): 6.34 (1H, s, H-8), 6.01 (1H, s, H-3), 2.90 (1H, dd, J = 18.0, 5.1 Hz, H-4'a), 2.75 (1H, dd, J = 17.1, 5.4 Hz, H-4'b), 3.89 (1H, t, J = 5.4, 5.1 Hz, H-3'), 1.38 (3H, s, 2-CH3), 1.37, 1.41 (6H, s, *gem*-dimethyl). ^{13}C-NMR (100 MHz, CD_3OD): 167.2 (C-2), 108.7 (C-3), 182.9 (C-4), 159.3 (C-5), 103.3 (C-6), 160.1 (C-7), 95.2 (C-8), 156.6 (C-8a), 104.8 (C-5a), 78.8 (C-2'), 69.2 (C-3'), 25.8 (C-4'), 22.4, 25.2 (2'-$2CH_3$), 20.9 (2-CH_3).

Compound 8 – white powder, mp 288~290 ℃. IR $(KBr)_{max}$ cm^{-1}: 3410 (-OH), 2950 (CH2), 1740 (C = C), 1460 (CH3), 1065(C-O), 801 (trisubstituted double bond). ^{1}H-NMR (400 MHz, CD_3OD): 0.67 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 5.06 (1H, d, J = 7.6 Hz, H-1'), 5.36 (1H, br d, J = 4.5 Hz, H-6). ^{13}C-NMR (100 MHz, CD_3OD): 12.2 (C-18), 12.2 (C-29), 19.2 (C-21), 19.4 (C-19), 19.6 (C-26), 20.0 (C-27), 21.3 (C-11), 23.4 (C-28), 24.5 (C-15), 26.4 (C-23), 28.5 (C-16), 29.5 (C-25), 30.3 (C-2), 32.1 (C-8), 32.2 (C-7), 34.2 (C-22), 36.4 (C-20), 37.5 (C-10), 37.5 (C-1), 39.3 (C-4), 39.9 (C-12), 40.8 (C-13), 46.0 (C-24), 50.3 (C-9), 56.2 (C-17), 62.8 (C-6'), 71.7 (C-4'), 75.3 (C-2'), 78.0 (C-3), 78.4 (C-3'), 78.5 (C-5'), 102.5 (C-1'), 121.8 (C-6), 140.3 (C-5).

Compound 9 – white powder, mp 168~169 ℃. IR $(KBr)_{max}$ cm^{-1}: 3350 (-OH), 2950 (C-H), 1065 (C-O). ^{1}H-NMR (400 MHz, CD_3OD): 3.52 (2H, d, CH_2-1 or 6), 4.28 (1H, qui, CH-2 or 5), 4.45 (1H, td, CH-3 or 4), 4.76 (1H, OH). ^{13}C-NMR (100 MHz, CD_3OD): 65.6 (C-1), 72.3 (C-2), 73.4 (C-3), 73.4 (C-4), 72.3 (C-5), 65.6 (C-6).

Compound 10 – yellow needle, mp 237~238 ℃. IR $(KBr)_{max}$ cm^{-1}: 3400 (-OH), 1665 (C = O), 1090 (C-O). UV (MeOH) λ_{max}: 300, 260, 230 nm. ^{1}H-NMR (400 MHz, CD_3OD): 1.31 (3H, s, CH_3-2'), 1.36 (3H, s, CH_3-2'), 2.31 (3H, s, CH_3-2), 2.70 (1H, dd, J = 17.4, 6.8 Hz, H-4'a), 2.87 (1H, dd, J = 17.4, 6.8 Hz, H-4'b), 3.12 (1H, ddd, J = 9.2, 7.6, 5.2 Hz, H-2"), 3.19 (1H, td, J = 9.2, 5.2 Hz, H-4"), 3.22 (1H, ddd, J = 9.3, 6.1, 1.9 Hz, H-5"), 3.32 (1H, td, J = 8.8, 4.9 Hz, H-3"), 3.61 (1H, ddd, J = 11.7, 5.9, 5.6 Hz, H-6"a), 3.83 (1H, ddd, J = 11.7, 6.3, 1.9 Hz, H-6"b), 4.06 (1H, dd, J = 17.4, 6.8 Hz, H-3'), 4.32 (1H, d, J = 7.5 Hz, H-1"), 4.37 (1H, dd, J = 6.3, 5.6 Hz, OH-6"), 4.84 (1H, d, J = 5.3 Hz, OH-2"), 4.86 (1H, d, J = 5.1 Hz, OH-4"),5.44 (1H, d, J = 4.9 Hz, OH-3"), 6.01 (1H, s, H-3), 6.27 (1H, s, H-8). ^{13}C-NMR (100 MHz, CD_3OD): 20.4 (C-11), 22.5 (C-4'), 22.2 (2'-CH_3), 26.0 (2'-CH_3)62.9 (C-6"), 71.8 (C-4"), 74.6 (C-3'), 74.9 (C-2"), 78.0 (C-3" or C-5"), 78.1 (C-3" or C-5"), 95.7 (C-8), 101.6 (C-1"), 104.8 (C-6 or C-10), 104.9 (C-10 or C-6), 108.6 (C-3), 157.2 (C-9), 159.5 (C-5 or C-7), 160.1 (C-7 or C-5), 168.0 (C-2), 184.9 (C-4).

Cyclooxygenase (COX) inhibition assay

The experiments were performed according to a slight modification of the method reported by Bohlin (Ylva *et al.*, 1998). Briefly, 10 ㎕ of the enzyme [COX-1 (3.0 units, 0.43 ㎍ protein, Cat # 60100), COX-2 (3.0 units, 0.39 ㎍ protein, Cat # 60120)] was activated with 170 ㎕ of a cofactor solution, which consisted of 1 ㎛ hematin, 1.95 mM *l*-ephedrine and 0.49 mM reduced glutathione in *tris*-HCl buffer (pH 8.0) on ice for 4 min. analiquot (10 ㎕) of the test solution (compound dissolved in DMSO) or the vehicle (DMSO) was added to the reaction tube and preincubated on ice for 10 min. after starting the reaction by adding 10 ㎕ (0.02 μCi) of [1-14C] arachidonic acid (50 μCi, NE-661, NEN), the mixture was incubated for 20 min. at 37 ℃. The reaction was quenched by adding 10 ㎕ of 2 M HCl. The produed prostaglandins and unmetabolized arachidonic acid were extracted with ethylether and separated by TLC (developing system, $CHCl_3$-MeOH-Acetic acid, 18: 1:1). The authentic gross count of the ^{14}C-labelled PGE_2 was measured using electronic autoradiography. The inhibitory effects of the test samples were indicated by the amount of ^{14}C-labelled PGE_2 produced compared with that of the DMSO control (% control). The IC_{50} values were obtained by linear regression analysis of the results from three different concentrations of the test samples.

RESULTS AND DISCUSSION

In the course of isolation, each fraction obtained from various steps was assayed for its inhibitory activity against COX-1 and COX-2. As shown in Table 1, the $CHCl_3$ fraction showed significant inhibitory activity with values of 80.5% and 71.5%

Table 1. COX-1 and COX-2 inhibitory activities of fractions from *Peucedanum japonicum*

Extract / Fractions	Inhibition (%)	
	COX-1*	COX-2*
MeOH extract	50.2	45.3
$CHCl_3$ fraction	80.5	71.5
BuOH fraction	55.6	30.8

*All samples were tested at 200 ㎍/㎖.

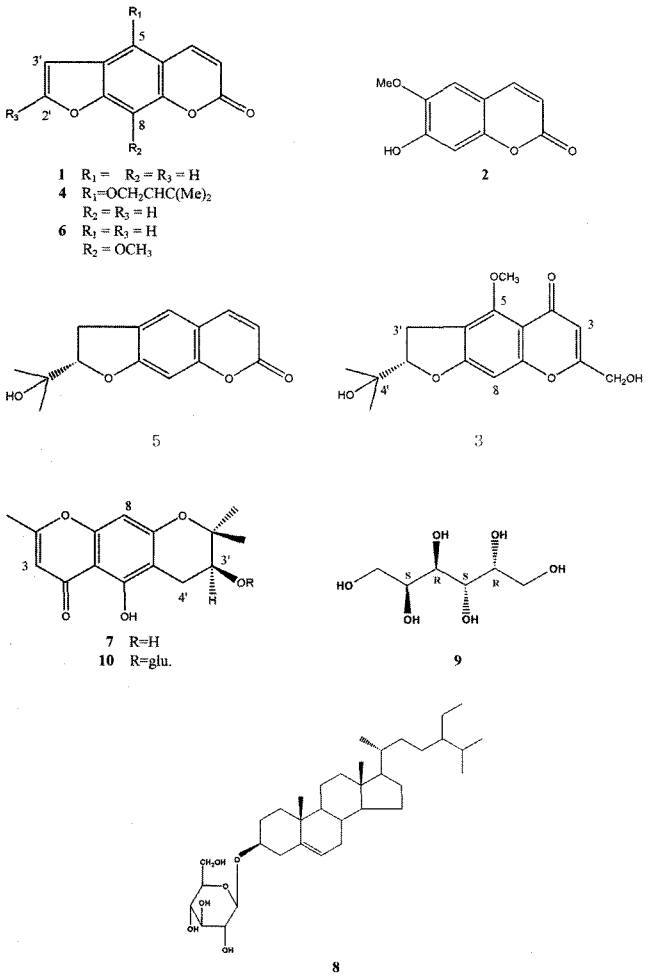

Fig. 1. Structures of compounds 1-10.

against COX-1 and COX-2 at 200 $\mu g/m\ell$, respectively. Therefore, this fraction was further subjected by the silica gel column chromatography to isolate active compounds. Further systematic fractionation of the $CHCl_3$ fraction led to the isolation of eight compounds (Fig. 1). Compounds of 1-8 were identified as five coumarins, psoralen(1) (Choi *et al.*, 1999), scopoletin (2) (Vasconcelos *et al.*, 1998), isoimperatorin (4) (Yang *et al.*, 2000), (+)-marmesin (5) (Trumble & Millar, 1996; Quader. *et al.*, 1992) and xanthotoxin (6) (Takahiro *et al.*, 1998; Xiao *et al.*, 1997), two chromones, cimifugin (3) (Okuyama *et al.*, 2001), hamaudol (7) (Okuyama *et al.*, 2001; Lemmich, 1995) and one sterol, daucosterol (8) (Sang *et al.*, 2002; Yoo *et al.*, 2002). Their chemical structures were determined by comparing the physicochemical and spectroscopic data with the literature values. Bioassay-guided isolates were tested for COX-1 and COX-2 inhibitory activity, the results are summarized in Table 2. Compound 1, 2 and 7 showed COX-1inhibitory activity with IC_{50} values of 0.88, 0.27 and 0.30 mM, respectively. Their inhibitory activities were stronger than positive control, aspirin (IC_{50}: 1.80 mM). In the test of inhibitory activity against COX-2, only compound 7 showed significant activity with IC_{50} value of 0.57 mM, but the inhibitory effect was lower than indomethacin (IC_{50}:0.08 mM), which used as a positive control. The other compounds exhibited no inhibitory activity or inhibitory tendency, but the IC_{50} values are higher than 1 mM.

Additional, two compounds (9-10) were isolated from BuOH fraction which showed active against COX-1 but inactive on COX-2 in the screening test (Table 1). Compound 9 was identified as galactitol by the physicochemical and spectroscopic

Table 2. Inhibitory activities of isolated compounds from *P. japonicum* on COX assay

	Compounds	Inhibition (%)[a]		IC_{50} (mM)[b]	
		COX-1	COX-2	COX-1	COX-2
1	Psoralen	79.1 ± 8.2	56.0 ± 4.2	0.88 ± 0.23	> 1.0
2	Scopoletin	86.0 ± 6.2	43.3 ± 6.9	0.27 ± 0.10	> 1.0
3	Cimifugin	17.2 ± 5.0	n.a.[c]	> 1.0	> 1.0
4	Isoimperatorin	29.8 ± 3.5	20.3 ± 9.4	> 1.0	> 1.0
5	(+)-Marmesin	36.1 ± 5.2	9.0 ± 3.2	> 1.0	> 1.0
6	Xanthotoxin	40.6 ± 3.6	35.9 ± 8.1	> 1.0	> 1.0
7	Hamaudol	66.8 ± 20.4	58.1 ± 7.6	0.30 ± 0.09	0.57 ± 0.13
8	Daucosterol	25.0 ± 4.5	21.7 ± 3.5	> 1.0	> 1.0
9	Galactitol	n.a.	n.a.	> 1.0	> 1.0
10	Sec-O-glucosylhamaudol	28.6 ± 7.6	15.6 ± 1.3	>1.0	> 1.0
	Aspirin[d]			1.80 ± 0.23	
	Indomethacin[d]				0.08 ± 0.01

[a]Inhibition (%) values were tested at 1 mM and expressed as the mean ± S.D. (n =3).
[b]IC_{50} values are the mean ± S.D. (n =3). It means the 50% inhibition concentration and calculated from regression lines using four different concentrations (1.0, 0.2, 0.04. 0.008 mM).

data with comparing the literatures values (Yamada *et al.*, 1974). It has been isolated from this plant for the first time.

Compound 10, which was obtained as yellow needles. The IR spectrum indicatedthe presence of a hydroxyl group (3400 cm^{-1}), γ-pyrone (1665 cm^{-1}) group and C-O (1090 cm^{-1}). In the ^{1}H-NMR spectrum, the existence of gem-methyl groups at 1.31 (3H, s) and 1.36 (3H, s), ABX pattern signals assignable to adjacent methylene and methane protons at 2.70 (1H, dd, J = 20.0, 6.8 Hz), 2.87 (1H, dd, J= 17.4, 6.8 Hz) and 4.06 (1H, dd, J= 20.0, 17.4 Hz), two aromatic singlets at 6.01 (1H,s) and 6.27 (1H, s) were observed. A set of a sugar moiety signals at 3.12 (1H, ddd, J=9.2, 7.6, 5.2 Hz, H-2"), 3.19 (1H, td, J=9.2, 5.2 Hz, H-4"), 3.22 (1H, ddd, J=9.3, 6.1, 1.9 Hz, H-5"), 3.32 (1H, td, J=8.8, 4.9 Hz, H-3"), 3.61 (1H, ddd, J=11.7, 5.9, 5.6 Hz, H-6"a), 3.83 (1H, ddd, J=11.7, 6.3, 1.9 Hz, H-6"b), 4.06 (1H, dd, J=6.6, 5.4Hz, H-3') and 4.32 (1H, d, J=7.5 Hz, H-1") indicated the presence of glucose. Analysis of the NMR data revealed similarity to compound **7** (hamaudol). The signals at 74.6 (C-3') in the ^{13}C-NMR indicated that the glucose was connected at C-3'. Conclusively, the structure of compound 10 was elucidated as *sec-O*-glucosylhamaudol by comparison of the previously reported in the literature (Okuyama *et al.*, 2001). It also has been isolated from this plant for the first time.

Compounds 9-10, as the main compounds of BuOH fraction were examined inhibitory activity against COX-1 and COX-2, the results shown in Table 2. Compound 10 exhibited weak inhibitory activity against COX-1 and COX-2, but the IC_{50} values was higher than 1 mM. Compound 9 did not exhibit activity against COX-1 and COX-2.

These results suggest that compound **7** was considered to be main inhibitory compound against COX-2 and compound 7 might be a lead compound for development of COX-2 inhibitor.

ACKNOWLEDGEMENT

This work was supported by the grant (PF0320302-00) from the Plant Diversity Research Center of the 21st century Frontier Program funded by the Ministry of Science and Technology of Korea.

LITERATURE CITED

Aida Y, Kasama T, Takeuchi N, Chiba M and Tobinaga S (1998) Pharmacological activities of khellactones, compounds isolated from *Peucedanum japonicum* Thunb. and *Peucedanum praeruptorium* Dunn.Methods and Findings in Experimental and Clinical Pharmacology 20(4):343-351.

Chen IS, Chang CT, Sheen WS, Teng CM, Tsai IL, Duh CY, Ko FN (1996) Coumarins and antiplatelet aggregation constituents from Formosan *Peucedanum japonicum*. Phytochemistry 41(2):525-30.

Choi HC, Rho TC, Kim BY, Ko HR, Oh WK, Seong CK, Mheen TI, Ahn JS, Lee HS (1999) Inhibition of nitric oxide production by coumarins from *Peucedanum japonicum* in LPS-activated RAW 264.7 cells. Saengyak Hakhoechi 30(2):99-104.

Duh CY, Wang SK, Wu YC (1992) Cytotoxic pyranocoumarins from roots of *Peucedanum japonicum*. Phytochemistry 31 (5):1829-1830.

Hisamoto M, Kikuzaki H, Ohigashi H, Nakatani N (2003) Antioxidant compounds from the leaves of *Peucedanum japonicum* Thunb. Journal of Agricultural and Food Chemistr 51 (18):5255-5261.

Lemmich J (1995) Monoterpene, chromone and coumarin glucosides of *Diplolophium buchananii*. Phytochemistry 38(2): 427-432.

Trumble JT, Millar JG (1996) Biological activity of marmesin and demethylsuberosin against a Generalist Herbivore, *Spodoptera exigua* (Lepidoptera: Noctuidae). J Agric. Food Chem. 44:2859-2864.

Ikeshiro Y, Mase I, Tomita Y (1992) Dihydropyranocoumarins from roots of *Peucedanum japonicum*. Phytochemistry 31 (12):4303-4306.

Quader MA, Jamila AET, James AA, Alexander IG Peter GW (1992) Coumarins and their taxonomic value in the Genus *Phebalium*. Phytochemistry 31(9):3083-3089.

Okuyama E, Hasegawa T, Matsushita T, Fujimoto H, Ishibashi M, Yamazaki M (2001) Analgesic componments of Saposhnikovia Root (*Saposhnikovia divaricata*). Chem. Pharm. Bull. 49(2):154-160.

Sang SM, Hiroe K, Karen L, Robert T, Rosen NN, Ho CT (2002) Sphingolipid and other constituents from Almond Nuts (*Prunus amygdalus* Batsch). J. Agric. Food Chem. 50:4709-4712.

Takahiro M, Mitsuo T, Masaki A (1998) Psoralen and other linear furanocoumarins as phytoalexins in *Glehnia littoralis*. Phytochemistry 47(1):13-16.

Xiao YQ, Liu XH, Masahiko T, Kimiye B (1997) Bicoumarins from *Pleurospermum rivulorum* Phytochemistry 45(6):1275-1277.

Yamada Y, Hsu CS, Iguchi K, Suzuki M, Hsu HY, Chen YP (1974) Two new khellactone esters from *Peucedanum japonicum*. Tetrahedron Letters 29:2513-16.

Yang FQ, Zhang TY, Liu QH, Xu GQ, Zhang YB, Zhang S, Yoichiro I (2000) Preparative isolation and purification of notopterol and isoimperatorin from *Notopterygium forbessi* boiss (Chinese traditional medicinal herb) by high-speed counter-current chromatography. Journal of Chromatography 67-73.

Ylva N, Therese R, Premila P, Helena D, Lars B (1998) Development of a radiochemical cyclooxygenase-1 and -2 in vitro assay for identification of natural products as inhibitors of prostaglandin biosynthesis. J. Nat. Prod. 61:2-7.

Vasconcelos MJ, Artur MSS, Jose ASC (1998) Chromones and flavanones from *Artemisia Campestris* Subsp. *Maritima*. Phytochemistry 49(5):1421-1424.