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Anti-Inflammatory Activity of Compounds from the Whole Plant of *Patrinia saniculaefolia*†

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Abstract – An *in vitro* bioassay-guide revealed that the methanol (MeOH) extract of the whole plant of *Patrinia saniculaefolia* (Valerianaceae) showed cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) dual inhibitory activity by assessing their effects on the production of prostaglandin D_2 (PGD_2) and leukotriene C_4 (LTC_4) in mouse bone marrow-derived mast cells (BMMCs). Phytochemical study of the MeOH extract of this plant led to the isolation of twelve compounds; β-farnesene (**1**), squalene (**2**), nardostachin (**3**), patridoid I (**4**), patridoid II (**5**), patridoid II-A (**6**), oleanolic acid (**7**), oleanonic acid (**8**), 23-hydroxyursolic acid (**9**), oleanolic acid 3-*O*-α-L-arabinopyranoside (**10**), oleanolic acid 3-*O*-β-D-glucopyranoside (**11**), oleanolic acid 3-*O*-[β-D-xylopyranosyl-(1 → 3)-β-D-(6-*O*-butyl)glucuronopyranoside] (**12**). Among the compounds, **4** and **5** strongly inhibited both the COX-2-dependent PGD_2 generation with IC_{50} values of 8.7 and 13.6 μM, respectively, and the generation of LTC_4 in the 5-LOX dependent phase with IC_{50} values of 41.7 and 46.9 μM, respectively, which suggest that the anti-inflammatory activity of *P. saniculaefolia* might occur in part *via* the inhibition of both PGD_2 and LTC_4 generation by **4** and **5**.
Keywords – *Patrinia saniculaefolia*, Valerianaceae, Cyclooxygenase-2, 5-Lipoxygenase, Anti-inflammatory activity

Introduction

Patrinia saniculaefolia Hemsley (Valerianaceae) is a perennial herb and an endemic species in Korea. The genus *Patrinia* is taxonomically classified into four species, *P. saniculaefolia*, *P. scabiosaefolia*, *P. villosa*, and *P. rupestris* (Lee, 1980; Lee, 1996). This genus is one of the valuable crude drugs which have been used in Korea and China as a traditional folk medicine for the treatment of initial stages of edema, appendicitis, endometriosis and inflammation. Saponins, coumarins, iridoids, and flavonoids isolated from this genus have demonstrated sedative, antibacterial, and cytotoxic effects (Bae, 2000; Li *et al*., 2001). We have reported the isolation of iridoids, ursane- and oleanane-type triterpenes, and oleanane-type triterpene saponins from the *P. saniculaefolia*. (An *et al*., 2003, 2008). During the course of our studies on the bioactive constituents derived from the endemic species in Korea (Jung *et al*., 2002; Park *et al*., 2002; Thuong *et al*., 2006), we found that the n-hexane fraction of the MeOH extract from *P. saniculaefolia* showed potent inhibitory effects in the COX-2 and 5-LOX assays. However, the bioactive constituents of *P. saniculaefolia* have not yet been characterized. This paper describes the biological activity of the compounds from the bioactive fractions, along with the inhibitory effects of the isolates on COX-2 and 5-LOX. Therefore, the development of dual inhibitors, which can simultaneously inhibit COX-2/5-LOX, might enhance their individual anti-inflammatory effects and reduce the undesirable side effects associated with nonsteroidal anti-inflammatory drugs (NSAIDs).

Experimental

General experimental procedures – Melting points were measured by an electrothermal melting apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 Digital polarimeter. UV spectra were recorded on a Milton Roy Spectronic 3000 spectro-

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photometer. IR spectra were determined on an IR Report-100 spectrophotometer (JASCO). FAB-MS spectra were measured on an Autospec Mass spectrometer (Micromass). NMR spectra were recorded on a Bruker NMR DRX300, 600 spectrometer, with the chemical shift being represented in parts per million (ppm, δ) with tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out on silica-gel 70 - 230 and 230 - 400 mesh (Merck). Thin layer chromatography (TLC) was performed on precoated silica gel 60 GF_{254} (Merck) and RP-$18F_{254S}$ (Merck) plates, and spot were detected by spraying with 10% H_2SO_4.

Plant Material – The whole plant of *P. saniculaefolia* was collected during August 1999 at Mt. Chiri, Jeonnam Province, Korea, and identified by Professor KiHwan Bae of the College of Pharmacy, Chungnam National University, Korea. A voucher specimen (CNU 2017) has been deposited in the herbarium of the College of Pharmacy, Chungnam National University, Korea.

Extraction and isolation – The dried whole plant (1.5 kg) of *P. saniculaefolia* was extracted three times with MeOH (2 L) at room temperature for 3 days. The combined MeOH extracts were concentrated under reduced pressure and obtained the MeOH extract (150.0 g). The extract was suspended in H_2O (1.5 L) and then fractionated sequentially with n-hexane, methylene chloride (CH_2Cl_2) and water-saturated n-butanol, 3 times with each solvent. Removal of the solvents afforded 19.0 g, 16.0 g and 35.0 g of the n-hexane, CH_2Cl_2 and n-butanol (BuOH) fractions, respectively. The n-hexane-soluble fraction (19.0 g) was subjected to column chromatography over silica gel eluted with a gradient of n-hexane and acetone to afford three subfractions (Fr. 1–3). Subfraction 1 was rechromatographed on a silica gel column and eluted with n-hexane–EtOAc (40 : 1) to give compounds **1** (96.6 mg) and **2** (235.7 mg). Compounds **3**–**12** see the published data (An *et al*., 2003; An *et al*., 2008).

***β*-Farnesene (1) –** Colourless oil; $[\alpha]_D^{23}$: +64.6 (*c* 0.27, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 220 (2.83) nm; EIMS *m/z*: 204 $[M]^+$; IR ($CHCl_3$) ν_{max} cm^{-1}: 2925 (C-H), 1597 (C = C), 1450 (CH_2), 890 (C =CH_2); ^{1}H-and ^{13}C-NMR data were consistent with the literature values (Andersen *et al*., 1970; Burger *et al*., 1978).

Squalene (2) – Colourless oil; FABMS *m/z*: 411.3 $[M + H]^+$; IR ($CHCl_3$) ν_{max} cm^{-1}: 2960, 2910, 2850, 1450, 1380; ^{1}H-and ^{13}C-NMR data were consistent with the literature values (Burger *et al*., 1978).

Compounds 3 –12 – see the published data (An *et al*., 2003; An *et al*., 2008).

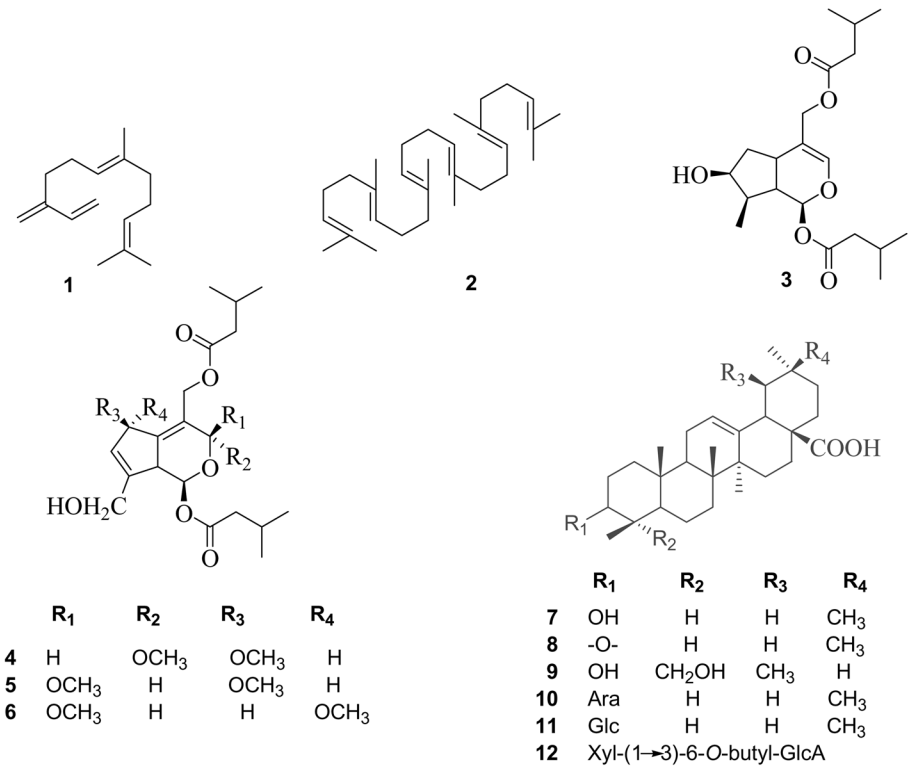
Preparation and activation of bone marrow-derived mast cells (BMMCs) – Bone marrow cells from male Balb/cJ mice were cultured for up to 10 weeks in 50% enriched medium (RPMI 1640 containing 2 mM L-glutamine, 0.1 mM nonessential amino acids, antibiotics and 10% fetal calf serum) and 50% WEHI-3 cell conditioned medium as a source of IL-3. After 3 weeks more than 98% of the cells were found to be BMMCs when checked by the previously described procedure (Muarakami *et al*., 1994).

Determination of prostaglandin D2 (PGD2) – In order to measure the inhibitory activity on COX-2 by samples, the cells were suspended in enriched medium at a cell density of 5×10^5 cells/ml and preincubated with aspirin (10 μg/ml) for 2 h to irreversibly inactivate any preexisting COX-1. After washing, the BMMC were activated with c-kit ligand (KL, 100 ng/ml), IL-10 (100 U/ml) and LPS (100 ng/ml) at 37 °C for 8 h in the presence or absence of the samples previously dissolved in dimethylsulfoxide (DMSO). All reactions were quenched by centrifugation at 120 g at 4 °C for 5 min. The supernatant and cell pellets were frozen immediately in liquid N_2 and stored at −80 °C until needed for further analysis. Concentrations of PGD_2 in the supernatant were measured using PGD_2 assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to manufacturer's instruction. Under these conditions, the COX-2-dependent phases of PGD_2 generation reached 1.6 ng/10^6 cells. The data is reported as the arithmetic mean of triplicate determinations.

Determination of leukotriene C_4 (LTC_4) – The BMMCs suspended in enriched medium at a density of 1×10^6 cells/ml were pretreated with the samples for 15 min at 37 °C and stimulated with KL (KL; 100 ng/ml). After 20 min stimulation, the supernatants were isolated and analyzed by EIA. The LTC_4 level was determined using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacture's instructions. Under these conditions, the LTC_4 reached up to 500 pg/10^6 cells. The data is reported as the arithmetic mean of triplicate determinations.

Results and Discussion

During our search for biologically active compounds derived from endemic species in Korea, the n-hexane fractions of the MeOH extract from the whole plant of *P. saniculaefolia* were shown to possess COX-2 and 5-LOX dual inhibitory activities by assessing their effects on the production of the PGD_2 and LTC_4 in mouse BMMCs (Table 1). Repeated normal-phase silica gel and ODS



	R_1	R_2	R_3	R_4
4	H	OCH_3	OCH_3	H
5	OCH_3	H	OCH_3	H
6	OCH_3	H	H	OCH_3

	R_1	R_2	R_3	R_4
7	OH	H	H	CH_3
8	-O-	H	H	CH_3
9	OH	CH_2OH	CH_3	H
10	Ara	H	H	CH_3
11	Glc	H	H	CH_3
12	Xyl-(1→3)-6-*O*-butyl-GlcA			

Fig. 1. Chemical structures of compounds **1 - 12**.

Table 1. Inhibitory effect of MeOH extract and solvent fractions against COX-2 and 5-LOX

Extract and fractions	COX-2 inhibitions (%) (12.5 μg/ml)	5-LOX inhibitions (%) (25.0 μg/ml)
MeOH extract	17.8	8.7
n-hexane fraction	70.1	99.4
CH_2Cl_2 fraction	34.4	54.0
n-BuOH fraction	24.5	n. a.[a]

[a]n.a. = not active.

column chromatography lead to the isolation of three triterpenes (**2**, **7** and **8**) and five iridoids (**1**, **3** - **6**) from the n-hexane fraction; one ursane-type triterpene (**9**) and one oleanane-type triterpene saponin (**10**) from the CH_2Cl_2 fraction; two oleanane-type triterpene saponins (**11**, **12**) from the n-BuOH fraction (Fig. 1).

The use of BMMCs as a model appears to be suitable for screening of selective COX-1/COX-2 or 5-LOX and COX-2/5-LOX dual inhibitors from various sources (Lee *et al*., 2004; Moon *et al*., 1999; Murakami *et al*., 1994). The generation of PGD_2 in the COX-2-dependent phase and the generation of LTC_4 in the 5-LOX dependent phase were tested after the BMMCs activated with a combination of KL, IL-10 and LPS in the presence or absence of each compound. As the results, **4** and **5** strongly inhibited both the COX-2-dependent PGD_2 generation with IC_{50} values of 8.7 and 13.6 μM, respectively and the generation of LTC4 in the 5-LOX dependent phase with IC_{50} values of 41.7 and 46.9 μM, respectively (Table 2). Compound **2** showed no inhibition of COX-2-dependent PGD2 generation, but inhibited 5-LOX with an IC_{50} value of 36.3 μM. Compound **3** showed no inhibiton of 5-LOX-dependent LTC_4 generation, but inhibited COX-2 with an IC_{50} value of 3.3 μM. In this experiments, NS398 (COX-2 selective inhibitor) and AA861 (5-LOX inhibitor) were used as positive control (Makino *et al*., 1986; Ouellet *et al*., 1995). Under the same conditions, NS398 and AA861 strongly inhibited PGD_2 and LTC_4 generation of mouse BMMCs in a concentration-dependent manner with an IC_{50} of 1.67×10^4 μM and 3.0×10^2 μM, respectively. Previously anti-inflammatory activity of *P. saiculaefolia* revealed that patridoid II (**5**) and nordostachin (**3**) inhibited nitric oxide (NO) and tumor necrosis factor-α (TNF-α) production in the lipopolysaccharide (LPS)-stimulated Raw 264.7 cells (Ju *et al*., 2003a; Ju *et al*., 2003b). In addition, nordostachin also reduced the iNOS and COX-2 protein levels in LPS-stimulated macrophages (Ju *et al*., 2003a). In present paper, compounds **1** - **12** were examined for their inhibitory

Table 2. Inhibitory effect of isolated compounds against COX-2 and 5-LOX

Compounds	COX-2 inhibition (IC_{50}, μM)	5-LOX inhibition (IC_{50}, μM)
β-farnesene (**1**)	>100	>100
squalene (**2**)	>100	36.3
nardostachin (**3**)	3.3	>100
patridoid I (**4**)	8.7	41.7
patridoid II (**5**)	13.6	46.9
patridoid II-A (**6**)	>100	>100
oleanolic acid (**7**)	>100	>100
oleanonic acid (**8**)	>100	>100
23-hydroxyursolic acid (**9**)	>100	>100
3-*O*-α-L-arabinopyranosyl-oleanolic acid (**10**)	>100	>100
3-*O*-β-D-glucopyranosyl-oleanolic acid (**11**)	>100	>100
oleanolic acid 3-*O*-[β-D-xylopyranosyl-(1 → 3)-β-D-glucuronopyranoside-6-*O*-butyl-ester] (**12**)	>100	>100
NS398[a]	1.67×10^{-4}	–
AA861[a]	–	3.0×10^{-2}

[a]These compounds were used as positive controls.

activity of PGD2 and LTC4 generations in mouse BMMCs. Although the structure-activity relationships of these compounds were not conclusively identified, patridoid I (**4**) showed significant inhibitory effect on two enzymes in mouse BMMCs.

In conclusion, compounds **4** and **5** among those isolated from the roots of *P. saniculaefolia* are principal compounds that inhibit COX-2-dependent PGD2 generation and 5-LOX-dependent LTC4 generation in BMMCs. These results suggest that the anti-in- flammatory activity of *P. saniculaefolia* might occur *via* the inhibited generation of eicosanoids. Further studies are needed to investigate the mechanisms of action of the isolated compounds.

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