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Cyclooxgenase Inhibitory Components from *Portulaca oleracea*

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Abstract – Five triterpenoids, epifriedelanol (**1**), friedelin (**2**), lupeol (**3**), β-sitosterol (**4**), daucosterol (**5**), and one phenyl propanoids ester, *trans*-docosanoyl ferulate (**6**) were isolated from the whole parts of *Portulaca oleracea*. They were determined using a combination of spectroscopic analyses (^{1}H-, ^{13}C-NMR, and MS data) and evaluated for their cyclooxygenase inhibitory activity. Compound **6** exhibited inhibitory effect with IC_{50} values of 40.2 μM and 1.6 mM on COX-1 and COX-2 activities, respectively.
Keywords – *Portulaca oleracea*, cyclooxygenase inhibitory activity, *trans*-docosanoyl ferulate

Introduction

Cyclooxygenase (COX) is an enzyme that is responsible for the formation of important biological mediators, prostanoids (prostaglandins, prostacyclins, and thromboxanes) which are each involved in the inflammatory response (Dannhardt *et al*., 2001). The COX-1, which is constitutively expressed in the most tissues and in blood platelets, is responsible for the physiological production of prostaglandins whereas the expression of COX-2 isoform is induced in response to inflammatory stimuli such as cytokines (Suh *et al*., 2001). The enzyme COX-1 has been comprehensively studied as a mean of plant extracts and plant-derived compounds on the anti-inflammatory effects (Noreen *et al*., 1998). In addition, the discovery of the isoenzyme COX-2 which is strongly associated with prostanoids (Jouzeau *et al*., 1997), demands interest in a comparative study of COX-1 and COX-2, and development of COX inhibitors as a novel anti-inflammatory agent.

Portulaca oleracea L. (Portulacaceae) is a widespread and well-known medicinal herb, which has been used as folk medicine in several countries for a diuretic, antiseptic, antispasmodic, and vermifuge (Liu *et al*., 2011, Xin *et al*., 2008). Recently, extensive investigations have revealed various pharmacological effects such as antibacterial, anti-inflammatory, antioxidant, and wound-healing activities (Elkhayat *et al*., 2008, Yang *et al*., 2009). The plant contains a range of biological constituents, including unsaturated fatty acids, terpenoids, coumarins, flavonoids, and alkaloids (Xiang *et al*., 2005; Xu *et al*., 2006).

Since our aim was to obtain naturally occurring compounds which exhibit potent anti-inflammatory effects, the ethanol extract of *P. oleracea* was isolated by several column chromatographies and the isolates were evaluated using COX-1 assay *in vitro*. Subsequently, activity-guided chromatographic separation resulted in the isolation of five triterpenes (**1 - 5**) and one phenyl propanoid ester (**6**). The COX inhibitory effect of compounds **1 - 6** was studied to result that *trans*-docosanoyl ferulate (**6**) showed potent inhibition on COX-1 and COX-2 activities.

Experimental

General – Melting points were obtained with an Electrothermal 9100 melting point apparatus (Electrothermal Ltd.). NMR spectra were obtained at room temperature on Bruker DRX 400 NMR spectrometers with tetramethylsilane (TMS) as internal standard. Fast atom bombardment mass spectroscopy (FABMS) measurements utilized a JMS-T100TD spectrometer (Tokyo, Japan). ESI-MS data were obtained on an 1100 LC-MSD Trap spectrometer (Agilent). Column chromatography (CC) was performed on silica gel (70 - 230 and 230 - 400 mesh; Merck, Germany) and YMC C-18 resins (30 - 50 μm; Fuji Silysia Chemical Ltd.). Thin layer chromatography analyses were run on Kieselgel 60 F_{254}

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and RP-18 F_{254s} plates; spots were visualized by spraying with 10% aqueous H_2SO_4 followed by heating.

Plant Material – The whole parts of *P. oleracea* were collected in Gongju, Chungnam province, Korea, in October 2000, and were taxonomically identified by one of us (Young Ho Kim). Voucher specimens (CNU00105) have been deposited at the College of Pharmacy, Chungnam National University.

Extraction and Isolation – The whole parts of *P. oleracea* (2.0 kg) were extracted in EtOH twice (5.0 L × 2, 50 ºC) and the combined extracts were concentrated *in vacuo* to dryness. The EtOH residue (149.0 g) was subjected to a silica gel column eluted with a gradient of MeOH in CH_2Cl_2 (1 : 100 → 5 : 1, v/v) to give thirteen fractions (Fr. 1 ~ Fr. 13). Fr. 4 (760.0 mg) was purified by recrystallization from CH_2Cl_2 to afford **1** [136.0 mg, 0.091% (w/w) of EtOH extract], while the filtrate was subjected to silica gel column chromatography, eluting with solvent of EtOAc in hexane (1 : 7, v/v), to yield six fractions (Fr. 41 ~ Fr. 46). Fr. 44 (140.0 mg) was purified by a silica gel column with CH_2Cl_2:MeOH (50 : 1, v/v) to obtain compound **6** [36.0 mg, 0.024% (w/w)]. Fr. 3 (1.3 g) was subjected to a silica gel column eluted with solvents of EtOAc in n-hexane (1 : 10 and 1 : 5, v/v) to give eight fractions (Fr. 31 ~ Fr. 38). Fr. 34 (180.0 mg) was purified by recrystallization from CH_2Cl_2 to afford **2** [19.0 mg, 0.012% (w/w]. Fr. 5 (1.4 g) was subjected to a silica gel column eluted with solvents of EtOAc in n-hexane (1 : 5 and 1 : 3, v/v) to give six fractions (Fr. 51 ~ Fr. 56). Fr. 53 (760.0 mg) was purified by a silica gel column with CH_2Cl_2 : MeOH (50 : 1, v/v) to obtain compound **3** [50.0 mg, 0.033% (w/w)]. Fr. 6 (10.8 g) was purified by recrystallization from hexane to afford **4** [37.0 mg, 0.024% (w/w)]. Fr. 12 (7.2 g) was subjected to a silica gel column eluted with solvents of MeOH in $CHCl_3$ (1 : 7, 1 : 5, 1 : 3, and 1 : 1, v/v) to give nine fractions (Fr. 121 ~ Fr. 129). Fr. 123 (500.0 mg) was purified by recrystallization from $CHCl_3$ to afford **5** [50.0 mg, 0.033% (w/w)].

Epifriedelanol (1): White amorphous powder; EI-MS: *m/z* 429 $[M + H]^+$; This compound exhibited comparable spectroscopic data (^{1}H- and ^{13}C-NMR) to published values (Kundu *et al*., 2000).

Friedelin (2): White amorphous powder; EI-MS: *m/z* 427 $[M + H]^+$; This compound exhibited comparable spectroscopic data (^{1}H- and ^{13}C-NMR) to published values (Ali *et al*., 1999; Chang *et al*., 1999).

Lupeol (3): White amorphous powder; mp 199 - 210 ºC; ESI-MS: *m/z* 427 $[M + H]^+$; This compound exhibited comparable spectroscopic data (^{1}H- and ^{13}C-NMR) to published values (Tung *et al*., 2010).

β-Sitosterol (4): White needles; mp 139 - 140 ºC; ESI-MS: *m/z* 415 $[M + H]^+$; This compound exhibited comparable spectroscopic data (^{1}H- and ^{13}C-NMR) to published values (Shen *et al*., 2008; Tung *et al*., 2010).

Daucosterol (5): White needles; mp 284-286 ºC; ESI-MS: 577 $[M + H]^+$; This compound exhibited comparable spectroscopic data (^{1}H- and ^{13}C-NMR) to published values (Shen *et al*., 2008).

***trans*-Docosanoyl ferulate (6):** White amorphous powder; FAB-MS *m/z* (rel. int.): 502.3 $[M]^+$ (20), 194.1 $[M\text{-}C_{22}H_{44}]^+$ (40), 177.1 (100); This compound exhibited comparable spectroscopic data (^{1}H- and ^{13}C-NMR) to published values (Ulubelen *et al*., 1994).

COX inhibitory activity assay – The COX-1 inhibitory activity was measured according to a published protocol (Sankawa *et al*., 1982) with modification. The rabbit renal microsomes were used as the enzyme source. To verify active compound against COX-1, the purified COX-2 enzyme assay was performed according to the protocol reported previously (Noreen *et al*., 1998). The purified COX-2 (prostaglandin endoperoxide H synthetase-2) from sheep placental cotyledons was used as described by the manufacturer (Cayman Chemical Co., Ann Arbor, MI, USA).

Results and Discussion

In the course of studies to find COX-1 inhibitors from the natural plants, the ethanol extract of *P. oleracea* showed potent inhibitory activity in our screening assay system. Subsequently, five triterpenoids (**1** - **5**) and one phenyl propanoid (**6**) were isolated from the ethanol extract of the whole parts of *P. oleracea* using a combination of various column chromatographies. Based on the ^{1}H-, ^{13}C-NMR, and MS data, together with their physical constants, the isolates were characterized as epifriedelanol (**1**), friedelin (**2**), lupeol (**3**), β-sitosterol (**4**), daucosterol (**5**), and *trans*-docosanoyl ferulate (**6**). Two alkaloids (uracil and adenosine), two long chain alcohols (octadecanol and phytol), and two fatty acids (oleic acid and eicosanoic acid) were also isolated from this plant. Among the tested compounds (**1** - **6**), compound **6** showed potent inhibitory effect in the COX-1 activity assay, while the others lacked COX-1 inhibitory activities or did not any activities at the concentration of 250 μg/ml (Table 1). IC_{50} values for compound **6** were investigated using COX-1 and COX-2 inhibitory activity assay *in vitro*. Compound **6** was a dose-dependent inhibitor for both enzymes in our assay conditions, exhibiting IC_{50} values of 40.2 μM and 1.6 mM, respectively. Our

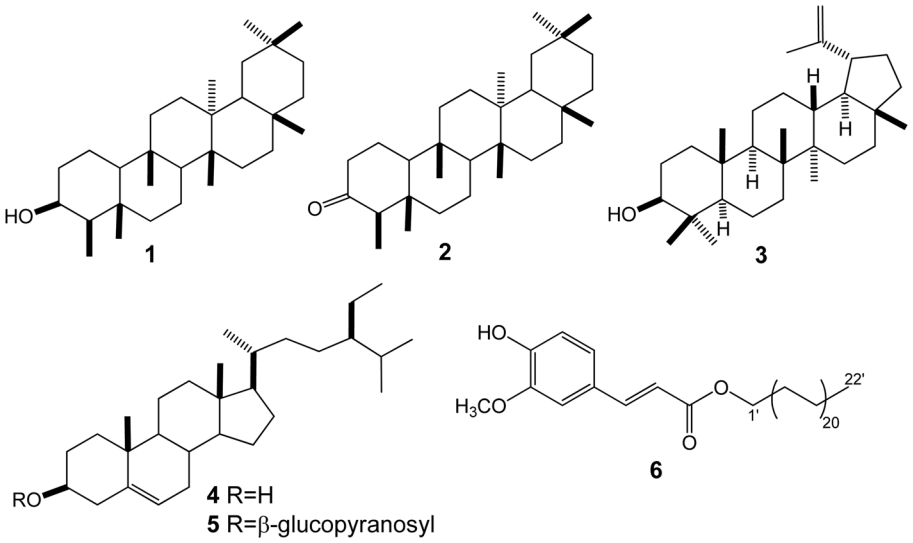

Fig. 1. The structures of components (**1** - **6**) isolated from the whole parts of *P. oleracea*.

Table 1. *In vitro* COX-1 inhibitory activities of compounds (**1** - **6**) from the whole parts of *P. oleracea*

No.	Name	COX-1 inhibition (%) at the concentration of 250 μg/ml
1	epifriedelanol	43.1
2	friedelin	NI[a]
3	lupeol	80.1
4	β-sitosterol	22.3
5	daucosterol	51.1
6	*trans*-docosanoyl ferulate	94.5

[a]NI refers to no inhibition against COX-1 at the concentration of 250 μg/ml.

observations are in agreement with previous studies that ferulic acid and its esters show anti-inflammatory action in carrageenan induced rat paw edema model (Chawla *et al*., 1987) and significantly reduced lipopolysaccharide-induced NO production, mRNA expression of inducible NO synthase, and cyclooxygenese-2 (Nagasaka *et al*., 2007). Although further study about the structure–activity relationship on derivatives of ferulates, such as steryl regions is needed, the results of the present study suggest that *P. oleracea* can be regarded as a source of active metabolites with anti-inflammatory activity and potent inhibitory activity of COX-1 and COX-2.

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