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Monoamine Oxidase Inhibitory Flavonoids from the Root Bark of *Cudrania tricuspidata*

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Abstract – Two new benzylated flavonoids, 5,7,4'-trihydroxy-6-*p*-hydroxybenzylflavanone (**1**) and 5,7,4'-trihydroxy-6,8-di-*p*-hydroxybenzylflavanone (**2**) together with six known flavonoids, kaempferol (**3**), artocarpesin (**4**), cycloartocarpesin (**5**), cudraflavone D (**6**), gericudranin E (**7**), and leachianone G (**8**) have been isolated from the root bark of *Cudrania tricuspidata*. The structures of **1** and **2** were characterized based on spectroscopic data including 1D- and 2D-NMR. All the isolates were evaluated for their inhibitory effects of monoamine oxidase (MAO). Among them, kaempferol (**3**), artocarpesin (**4**), and cudraflavone D (**6**) showed moderate inhibitory effects with IC_{50} values of 82.3, 30.8, and 71.8 μM, respectively.
Keywords – *Cudrania tricuspidata*, Moraceae, Benzylated flavonoid, Monoamine oxidase inhibitor

Introduction

Cudrania tricuspidata (Carr.) Bur. (Moraceae) has been traditionally used as a folk medicine to treat jaundice, hepatitis, neuritis, dysmenorrheal, and rheumatism (Jung and Shin, 1990). Previous studies on the stems or root barks of *C. tricuspidata* have revealed various types of isoprenylated xanthones and flavonoids with numerous biological activities such as cytotoxic, hepatoprotective, anti-lipid peroxidative, anti-atherosclerotic, and anti-inflammatory activities (Fujimoto *et al.*, 1984a, 1984b; Hano *et al.*, 1991; Lee *et al.*, 1996, 2005, 2009; Zou *et al.*, 2004, 2005; Tian *et al.*, 2005; Park *et al.*, 2006; Seo *et al.*, 2007).

Monoamine oxidase (MAO, EC 1.4.3.4, amine-oxygen oxidoreductase) is a flavin-containing and membrane-bound enzyme responsible for the oxidative deamination of a number of monoamine neurotransmitters such as norepenephrine, dopamine and 5-hydroxytryptamine in the brain and the peripheral tissues (Shih *et al.*, 1999; Edmondson *et al.*, 2009). MAO inhibitors represent a useful tool for the treatment of several psychiatric and neurological disorders. MAO A inhibitors are used in anxiety and depression, while MAO B inhibitors have been found to be potentially beneficial in the treatment of Parkinson's disease and Alzheimer's disease (Yamada *et al.*, 2004; Youdim *et al.*, 2006). Recently, we have described isolation of prenylated xanthones and isoflavonoids and their MAO inhibitory activities from *C. tricuspidata* (Han *et al.*, 2005; Hwang *et al.*, 2007).

In this study, we identified two new benzylated flavonoids **1** and **2**, and six known flavonoids (**3** - **8**) from the MeOH extract of the root bark of *C. tricuspidata*. All the isolates were examined for their inhibitory effects on the mouse brain MAO.

Materials and Methods

General experimental procedures – UV and IR spectra were obtained on a JASCO UV-550 and JASCO Report-100 spectrometer, respectively. CD spectra were recorded on a JASCO J-715 spectrometer. NMR spectra were acquired with a Bruker AMX 500 instrument at room temperature. ESI-MS and HRFAB-MS were measured on a Finnigan LCQ Fleet and a JEOL JMS-HX/HX110A Tandem Mass spectrometer, respectively. Silica gel (70 - 230 mesh, Merck, Germany), Lichroprep RP-18 (40 - 63 μM, Merck, Germany), and Sephadex LH-20 (25 - 100 μM, Amersham Biosciences, Sweden) were used for open column chromatography. Preparative HPLC was carried out on a Waters system (two 515 pumps and 2996

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photodiode array detector) and a YMC J'sphere ODS-H80 column (4 μm, 150 × 20 mm), using the mixed solvent system of acetonitrile-water at a flow rate of 6.0 mL/min. Thin layer chromatography (TLC) was performed on a pre-coated silica gel 60 F_{254} (0.25 mm, Merck, Germany). All other chemicals and reagents were analytical grade. Kynuramine, 4-hydroxyquinoline, and iproniazid were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Plant material – The root barks of *C. tricuspidata* were collected from the herb garden at Chungbuk National University, Cheongju, Korea, in October 2005 and identified by Emeritus Professor Kyong Soon Lee. A voucher specimen (CBNU 0502) has been deposited at the Herbarium of College of Pharmacy, Chungbuk National University, Korea.

Extraction and isolation – The air-dried root bark of *Cudrania tricuspidata* (1.5 kg) was extracted three times with MeOH (10 L × 3) at room temperature. The combined extracts were concentrated in vacuo to yield a dried MeOH extract (150 g). This extract was suspended in 90% MeOH and then partitioned with CH_2Cl_2 (2 L × 3) and EtOAc (2L × 3). The CH_2Cl_2 extract (11 g), with 50% inhibitory activity at a concentration of 10 μg/mL in the MAO inhibition assay, was chromatographed on a silica gel column, eluting with a CH_2Cl_2-MeOH gradient system (100 : 0 to 1 : 1, v/v) to give four fractions (CT-A – CT-D). Fraction CT-D (4.1 g) was further chromatographed over silica gel column, eluting with a *n*-hexane-acetone gradient system (5 : 1 to 1 : 1, v/v) to afford four combined fractions (CT-D1 – CT-D4). Fraction CT-D4 was further chromatogrphed over a Sephadex LH-20, eluting with MeOH-H_2O (60 : 40 to 100 : 0, v/v) to yield six sub-fractions (CT-D41 – CT-D46). Fraction CT-D44 was subjected to semi-preparative HPLC (Waters system, YMC J'sphere ODS-H80 column, 4 μm, 150 × 20 mm, i.d., MeCN : H_2O = 45 : 55, flow rate 6.5 mL/min) to yield compounds **1** (3.1 mg), **2** (6.6 mg), **3** (4.1 mg), and **4** (3.8 mg). Fraction CT-D43 was further purified by semi-preparative HPLC using MeCN : H_2O (40 : 60) at a

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of compounds **1** and **2** (Acetone-d_6)

position	**1**		**2**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
2	79.8	5.43 dd (3.0, 13.0)	79.5	5.46 dd (3.0, 12.8)
3	43.5	3.17 dd (13.0, 17.1) 2.72 dd (3.0, 17.1)	43.3	3.16 dd (12.8, 17.1) 2.80 dd (3.0, 17.1)
4	197.3	–	197.8	–
4a	103.0	–	103.1	–
5	162.1	12.53 s	160.5	12.56 s
6	109.5	–	108.8	–
7	164.7	–	162.3	–
8	95.2	6.05 s	108.1	–
8a	162.3	–	159.3	–
1'	128.9	–	128.5	–
2', 6'	130.8	7.38 d (8.5)	130.5	7.35 d (8.5)
3', 5'	116.0	6.89 d (8.5)	115.9	6.88 d (8.5)
4'	158.6	–	158.3	–
1"	27.1	3.79 s	27.1	3.90 s
2"	132.9	–	132.0	–
3", 7"	130.2	7.14 d (8.5)	129.8	7.08 d (8.4)
4", 6"	115.4	6.68 d (8.5)	115.4	6.68 d (8.4)
5"	156.0	–	156.0	–
1"'	–	–	27.7	3.86 d (14.4) 3.89 d (14.4)
2"'	–	–	132.1	–
3"', 7"'	–	–	130.7	7.02 d (8.5)
4"', 6"'	–	–	115.4	6.66 d (8.5)
5"'	–	–	156.0	–

flow rate of 6.5 mL/min to yield compounds **7** (2.6 mg) and **8** (3.2 mg). Fraction CT-D3 was further chromatographed over a RP-18 column, eluting with MeCN : H_2O (50 : 50 to 100 : 0) to yield compounds **5** (63.4 mg) and **6** (6.1 mg).

5,7,4'-Trihydroxy-6-*p*-hydroxybenzylflavanone (1) – Yellow amorphous powder; UV (MeOH): λ_{max} nm (log ε): 295 (4.21), 347 (3.89); IR (KBr) ν_{max} 3439, 1635 cm^{-1}; CD (MeOH) λ_{max} nm (Δε) 289 (–14.1), 333 (+3.3); ESI-MS *m/z*: 378 $[M]^+$; HRFAB-MS: *m/z* 379.1176 $[M + H]^+$, calcd for $C_{22}H_{19}O_6$, 379.1182; ^1H NMR (Acetone-d_6, 500 MHz) and ^{13}C NMR (Acetone-d_6, 125 MHz), see Table 1.

5,7,4'-Trihydroxy-6,8-di-*p*-hydroxybenzylflavanone (2) – Yellow amorphous powder; UV (MeOH): λ_{max} nm (log ε): 296 (4.21), 356 (3.89); IR (KBr) ν_{max} 3502, 1655 cm^{-1}; CD (MeOH) λ_{max} nm (Δε) 291 (–3.7), 353 (+ 0.2); HRFAB-MS: *m/z* 507.1422 $[M + Na]^+$, calcd for $C_{29}H_{24}O_7Na$, 507.1420; ^1H NMR (Acetone-d_6, 500 MHz) and ^{13}C NMR (Acetone-d_6, 125 MHz), see Table 1.

MAO preparation and assay for MAO inhibitory activity – A crude mitochondrial fraction from mouse brain was prepared as a source of MAO activity following the procedure described previously (Naoi and Nagatsu, 1987; Ro *et al.*, 2001). MAO activity was measured fluorometrically using kynuramine as a substrate according to the method of Kraml with a slight modification (Kraml, 1965; Ro *et al.*, 2001). The fluorescence intensity of 4-hydroxyquinoline, which was formed from kynuramine by MAO, was measured at an emission wavelength of 380 nm and an excitation wavelength of 315 nm using a Perkin Elmer LS 50B fluorescence spectrometer.

Results and Discussion

A methanolic extract of the roots of *C. tricuspidata* was partitioned with CH_2Cl_2, EtOAc, and water, successively. Repeated column chromatographic separation of the CH_2Cl_2 extract on a combination of silica gel, RP-18, Sephadex LH-20, and semi-preparative HPLC resulted in the isolation of two new benzylated flavonoids (**1 - 2**), together with six known flavonoids (**3 - 8**), which were identified as kaempferol (**3**) (Kim *et al.*, 2002), artocarpesin (**4**) (Young *et al.*, 1989), cycloartocarpesin (**5**) (Fujimoto and Nomura, 1985), cudraflavone D (**6**) (Hano *et al.*, 1990), gericudranin E (**7**) (Lee *et al.*, 1995), and leachianone G (**8**) (Iinuma *et al.*, 1993).

Compound **1** was obtained as a yellow amorphous powder, and the molecular formula was determined as $C_{22}H_{18}O_6$ by ESI-MS at *m/z* 378 $[M]^+$ and HRFAB-MS at *m/z* 379.1176 $[M + H]^+$ (calcd *m/z* 379.1182). The IR

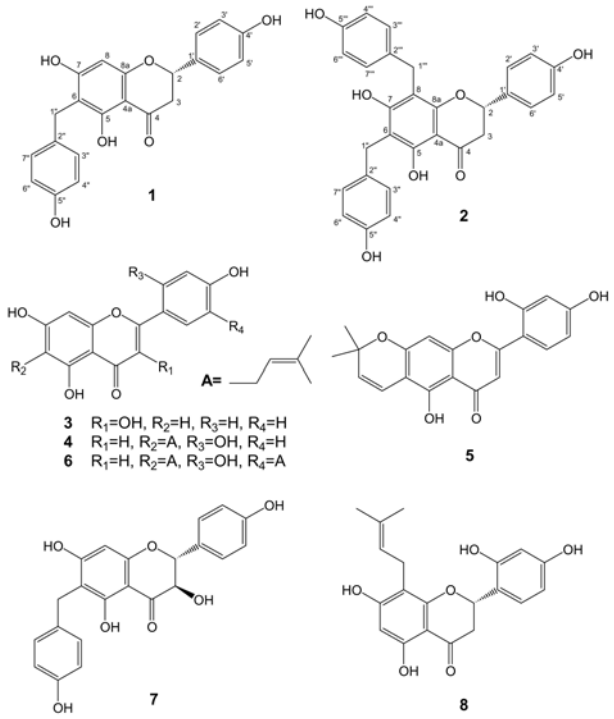

Fig. 1. Structures of compounds **1 - 8**.

absorption bands at 3439 and 1635 cm^{-1} suggested the presence of free hydroxyl and conjugated carbonyl functionalities. The UV spectrum of **1** resembled the spectra of flavanone derivatives (Fujimoto and Nomura, 1985). The ^1H NMR spectrum of **1** showed resonances for an ABX system at δ_H 2.72 (1H, dd, J = 3.0 and 17.1 Hz, H-3b), 3.17 (1H, dd, J = 13.0 and 17.1 Hz, H-3a), and 5.43 (1H, dd, J = 3.0 and 13.0 Hz, H-2), which is diagnostic for H-2 and H-3 of a flavanone skeleton. It also exhibited signals for two sets of *ortho* coupled protons at δ 7.38 (2H, d, J = 8.5 Hz) and δ 6.89 (2H, d, J = 8.5 Hz) characteristic of a 1,4-substituted benzene ring. A sharp singlet at δ_H 6.05 indicated that either C-6 or C-8 ring A was substituted. The remaining proton signals at δ 7.14 (2H, d, J = 8.5 Hz), δ 6.68 (2H, d, J = 8.5 Hz), and δ 3.79 (2H, s) were characteristic of the *p*-substituted benzyl group (Lee *et al.*, 1995, 1996). The skeleton was also supported by the ^{13}C NMR, DEPT, and HMQC spectrum. The ^{13}C NMR spectrum of **1** revealed the presence of 22 carbons including characteristic signals of the naringenin moiety and signals at δ 27.1, 115.4, 130.2, 132.9 and 156.0, assignable to the *p*-hydroxybenzyl group. In the HMBC spectrum, the benzylic methylene proton at δ 3.79 (2H, s) showed long-range correlations with C-5 (161.2), C-6 (109.5), and C-7 (δ 164.7). In addition, the HMBC correlations between H-8 (δ 6.05) and C-7 (δ 164.7), C-6

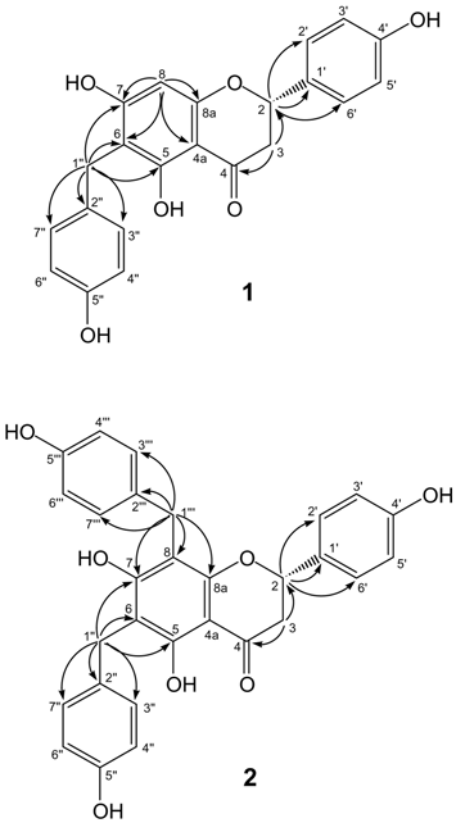

Fig. 2. Selected HMBC correlations of compounds **1** and **2**.

(δ 109.5), C-8a (δ 162.3), and C-4a (δ 103.0) clearly indicated that the attachment of *p*-hydroxybenzyl group was the C-6 position of naringenin. The absolute configuration at C-2 was determined by a positive Cotton effect at 333 nm and a negative Cotton effect at 289 nm in the measurement of the CD spectrum, which is characteristic for the 2*S* configuration of flavanones (Takashima and Ohsaki, 2002). Thus, compound **1** was elucidated as 5,7,4'-trihydroxy-6-*p*-hydroxybenzylflavanone.

Compound **2** was obtained as a yellow amorphous powder, and the molecular formula was determined as $C_{29}H_{24}O_7$ by HRFAB-MS at *m/z* 507.1422 $[M+Na]^+$ (calcd *m/z* 507.1420). The ^{1}H NMR spectrum of **2** were similar to those of **1**, except for the presence of two *p*-substituted benzyl groups in **2**, [δ_H 3.90 (2H, s, H-1"), 7.08 (2H, d, *J* = 8.4 Hz, H-3", 7"), 6.68 (2H, d, *J* = 8.4 Hz, H-4", 6"), 3.86 (1H, d, *J* = 14.4 Hz, H-1'"), 3.89 (1H, d, *J* = 14.4 Hz, H-1'"), 7.02 (2H, d, *J* = 8.5 Hz, H-3'", 7'"), and 6.66 (2H, d, *J* = 8.5 Hz, H-4'", 6'")], instead of one *p*-substituted benzyl group in **1**. The ^{13}C NMR spectrum of **2** showed the presence of 29 carbons including characteristic signals of the naringenin moiety and two *p*-hydroxybenzyl groups in the molecule. The position of additional *p*-hydroxybenzyl group was assigned at C-8 on the basis of the HMBC correlations between the benzylic methylene protons at δ 3.86 (1H, d, *J* = 14.4 Hz) and 3.89 (1H, d, *J* = 14.4 Hz) and carbon signals at C-7 (δ 162.3), C-8 (δ 108.1), and C-8a (δ 159.3). The absolute configuration at C-2 was deduced to be *S* from the measurement of the CD spectrum (Takashima and Ohsaki, 2002). Thus, compound **2** was elucidated as 5,7,4'-trihydroxy-6,8-di-*p*-hydroxybenzylflavanone.

All of the isolates (**1** - **8**) were evaluated for their potential to inhibit mouse brain MAO activity. The MAO activity in the mouse brain mitochondria was measured using the non-selective substrate, kynuramine. The results demonstrated that kaempferol (**3**), artocarpesin (**4**), and cudraflavone D (**6**) showed moderate inhibitory effects with IC_{50} values of 82.3, 30.8, and 71.8 μM, respectively. Iproniazid was used as a positive control (IC_{50} value: 19.2 μM). However, further studies for the selectivity and kinetic analysis for the inhibition of MAO are required to characterize the effects of the compounds in the present study.

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