Arch Pharm Res Vol 28, No 12, 1324-1327, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Monoamine Oxidase Inhibitory Constituents from the Fruits of *Cudrania tricuspidata*

Xiang Hua Han[1], Seong Su Hong[1], Ji Sang Hwang[1], Seon Hwa Jeong[1], Ji Hye Hwang[1], Min Hee Lee[1], Myung Koo Lee[1,2], Dongho Lee[3], Jai Seup Ro[1], and Bang Yeon Hwang[1]

[1]College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea, [2]Research Center for Bioresource and Health, Chungbuk National University, Cheongju 361-763, Korea, and [3]Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-600, Korea

(Received September 6, 2005)

A methylene chloride soluble fraction of the fruits of *Cudrania tricuspidata* significantly inhibited the mouse brain monoamine oxidase (MAO). Three known prenylated isoflavones were isolated and identified by activity-guided fractionation. Gancaonin A (**1**), 4'-*O*-methylalpinumisoflavone (**2**), and alpinumisoflavone (**3**) inhibited MAO activity in a concentration-dependent manner with IC_{50} values of 19.4, 23.9, and 25.8 μM, respectively. Of these, gancaonin A (**1**) showed a selective and potent inhibitory effect against MAO-B (IC_{50} 0.8 μM) than MAO-A (IC_{50} >800 μM). The kinetic analysis using Lineweaver-Burk plots indicated that gancaonin A (**1**) competitively inhibited MAO-B.

Key words: *Cudrania tricuspidata*, Moraceae, Prenylated isoflavone, Gancaonin A, Monoamine oxidase inhibitor

INTRODUCTION

Cudrania tricuspidata (Carr.) Bur. (Moraceae) is a deciduous and dioecious tree widely distributed in Korea, China, and Japan. The cortex and root bark of this species have been used as a traditional medicine for the treatment of neuritis and inflammation (Jung and Shin, 1989). Previous phytochemical studies on the stems or root barks of *C. tricuspidata* resulted in the isolation of various isoprenylated xanthones and flavonoids, some of which have cytotoxic, hepatoprotective, and anti-lipid peroxidative activities (Fujimoto *et al.*, 1984a, 1984b; Hano *et al.*, 1991, 1990a, 1990b; Lee *et al.*, 1996; Lee *et al.*, 2005; Tian *et al.*, 2005; Zou *et al.*, 2004).

The MAOs are responsible for the degradation of neurotransmitters including, noradrenaline, dopamine, and 5-hydroxytryptamine in the central nervous system and peripheral tissues (Benedetti *et al.*, 1992). Selective and reversible inhibitors of MAO-A and MAO-B, respectively, have been thought to be useful therapeutic approach for the treatment of depressive and anxiety disorders, and Parkinson's disease and Alzheimer's disease (Thomas, 2000; Yamada and Yasuhara, 2004; Youdim and Riederer, 2004).

In the course of our studies searching for monoamine oxidase inhibitors from higher plants, the CH_2Cl_2 soluble extract of the fruits of *C. tricuspidata* was found to show significant inhibitory effect on mouse brain MAO. In this study, we identified MAO inhibitors in *C. tricuspidata* and characterized their inhibitory activities against MAO-A and MAO-B.

MATERIALS AND METHODS

General experimental procedures

Melting points were measured on a Büchi model B-540 without correction. UV and IR spectra were obtained on a JASCO UV-550 and JASCO Report-100 spectrometer, respectively. NMR spectra were taken on a Bruker AMX 500 MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are expressed in δ values. EI-MS was recorded on Hewlett-Packard MS 5988 mass spectrometer. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck), and thin layer chromatography (TLC) using a pre-coated silica gel 60

Correspondence to: Bang Yeon Hwang, College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea
Tel: 82-43-261-2814, Fax: 82-43-268-2732
E-mail: byhwang@chungbuk.ac.kr

F_{254} (0.25 mm, Merck).

Kynuramine, clorgyline, *l*-deprenyl, 4-hydroxyquinoline, and iproniazid were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Plant material

The fruits of *C. tricuspidata* were collected from the herb garden at Chungbuk National University, Cheongju, Korea, in September 2003 and identified by Emeritus Prof. Kyong Soon Lee, a plant taxonomist at Chungbuk National University. A voucher specimen (CBNU 03017) has been deposited at the Herbarium of College of Pharmacy, Chungbuk National University, Korea.

Activity-guided isolation

The dried fruits of *C. tricuspidata* (410 g) were extracted three times with MeOH at room temperature. The concentrated methanol extract (30 g) was suspended in water, and then partitioned in turn with hexane, CH_2Cl_2, BuOH, and water. The MAO inhibitory activities for these four extracts were 53.5, 91.5, 62.7, and 38.6% at 200 μg/mL, respectively. The most active CH_2Cl_2 extract (5 g) was chromatographed over silica gel column eluted with a mixture of CH_2Cl_2-MeOH (100:1, 50:1, 20:1, 10:1, 0:1, 1 L each), to afford five combined fractions (Fr.1-Fr.5). Fr.2 fraction (1.0 g, 91.0% MAO inhibition at the concentration of 150 μg/mL) was further purified over silica gel column with hexane-acetone gradient system (50:1, 20:1, 10:1, 2:1, 500 mL each) to afford four subfractions (Fr.21-Fr.24). Gancaonin A (**1**, 100 mg) was obtained as pale yellow needles by recrystallization in hexane-acetone mixture from fraction Fr.23. Repeated silica gel column chromatography of Fr.1 fraction (1.6 g, 85.6% MAO inhibition at the concentration of 150 μg/mL) using hexane-acetone (50:1) as a solvent system gave 4'-*O*-methylalpinumisoflavone (**2**, 27 mg). Fr.5 fraction (1.1 g, 80.5% MAO inhibition at the concentration of 150 μg/mL) was further purified over silica gel column with hexane-acetone (20:1) to afford alpinumisoflavone (**3**, 87 mg).

Gancaonin A (1)

Pale yellow needle crystal; m.p. 215-218°C; UV (MeOH): λ_{max} nm (log ε): 215 (4.5), 265 (4.5), 330 (3.5); EI-MS *m/z* 352 $[M]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ: 13.30 (1H, s, 5-OH), 8.24 (1H, s, H-2), 7.49 (2H, d, *J* = 8.7 Hz, H-2', 6'), 6.98 (2H, d, *J* = 8.7 Hz, H-3', 5'), 6.47 (1H, s, H-8), 5.21 (1H, s, H-2"), 3.80 (3H, s, 4'-OMe), 3.25 (2H, br d, *J* = 7.0 Hz, H-1"), 1.75 (3H, s, 3"-Me), 1.64 (3H, s, 3"-Me); ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 180.1 (C-4), 162.1 (C-7), 159.1 (C-4'), 158.8 (C-5), 155.3 (C-8a), 154.0 (C-2), 130.7 (C-3"), 130.2 (C-2', 6'), 123.1 (C-1'), 122.1 (C-3), 121.8 (C-2"), 113.7 (C-3', 5'), 111.1 (C-6), 104.2 (C-4a), 93.0 (C-8), 55.2 (4'-OMe), 25.2 (C-5"), 21.0 (C-1"), 17.7 (C-4").

4'-*O*-Methylalpinumisoflavone (2)

Pale yellow needle crystal; m.p. 135-136°C; UV (MeOH): λ_{max} nm (log ε): 226 (4.3), 285 (4.8), 325 (3.3); EI-MS *m/z* 350 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 13.15 (1H, s, 5-OH), 7.80 (1H, s, H-2), 7.42 (2H, d, *J* = 8.6 Hz, H-2', 6'), 6.96 (2H, d, *J* = 8.6 Hz, H-3', 5'), 6.70 (1H, d, *J* = 10.0 Hz, H-4"), 6.31 (1H, s, H-8), 5.60 (1H, d, *J* = 10.0 Hz, H-3"), 3.82 (3H, s, 4'-OMe), 1.45 (6H, s, 2"-Me × 2); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 181.6 (C-4), 160.5 (C-7), 160.2 (C-4'), 158.0 (C-5), 157.6 (C-8a), 153.3 (C-2), 130.8 (C-2', 6'), 128.4 (C-3"), 124.2 (C-1'), 123.7 (C-3), 116.2 (C-4"), 114.8 (C-3', 5'), 106.8 (C-4a), 106.3 (C-6), 95.5 (C-8), 78.7 (C-2"), 56.0 (4'-OMe), 29.0 (2"-Me × 2).

Alpinumisoflavone (3)

Pale yellow needle crystal; m.p. 210-213°C; UV (MeOH): λ_{max} nm (log ε): 225 (4.3), 284 (4.7), 326 (3.4); EI-MS *m/z* 336 $[M]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 13.14 (1H, s, 5-OH), 7.79 (1H, s, H-2), 7.32 (2H, d, *J* = 8.6 Hz, H-2', 6'), 6.84 (2H, d, *J* = 8.6 Hz, H-3', 5'), 6.71 (1H, d, *J* = 10.0 Hz, H-4"), 6.31 (1H, s, ,H-8), 5.60 (1H, d, *J* = 10.0 Hz, H-3"), 1.45 (6H, s, 2"-Me × 2); ^{13}C-NMR (125 MHz, $CDCl_3$) d: 181.7 (C-4), 160.3 (C-7), 158.0 (C-5), 157.5 (C-8a), 156.7 (C-4'), 153.3 (C-2), 131.0 (C-2', 6'), 128.9 (C-3"), 124.3 (C-1'), 123.6 (C-3), 116.3 (C-3', 5'), 116.1 (C-4"), 106.8 (C-4a), 106.3 (C-6), 95.6 (C-8), 78.7 (C-2"), 29.0 (2"-Me × 2).

MAO preparation and assay for MAO activity

A crude mitochondrial fraction from mouse brain was isolated by the method of Naoi *et al.* with minor modification (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. The suspension was preincubated with either 1 μM of *l*-deprenyl (type-B inhibitor) or clorgyline (type-A inhibitor) for 15 min to measure MAO-A or MAO-B activity, respectively.

RESULTS AND DISCUSSION

As part of our ongoing search for MAO inhibitors from plants, the methylene chloride-soluble extract of the fruits of *C. tricuspidata* was found to inhibit the mouse brain MAO. Bioactivity-guided fractionation of this extract resulted in the isolation of three known prenylated isoflavones, gancaonin A (**1**), 4'-*O*-methylalpinumisoflavone (**2**) and alpinumisoflavone (**3**) (Fig. 1). The structures of these isolates were identified by physical and spectroscopic

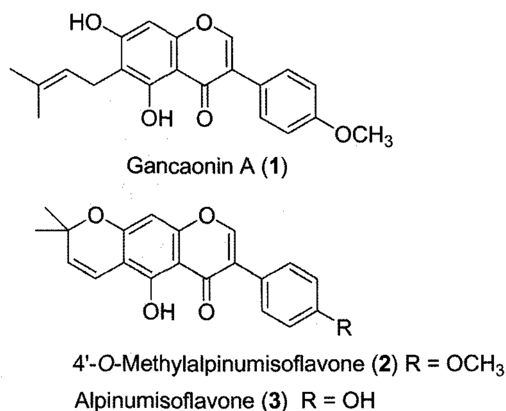

Fig. 1. Structures of isolated compounds from *C. tricuspidata*

data (m.p., UV, MS, ^{1}H−NMR, ^{13}C−NMR, 2D NMR) measurement and by comparison with published values (Fukai *et al.*, 1989; Olivares *et al.*, 1982).

Gancaonin A (**1**), 4'-*O*-methylalpinumisoflavone (**2**), and alpinumisoflavone (**3**) inhibited the total MAO activity of mouse brain homogenates with the IC_{50} values of 19.4, 23.9, and 25.8 μM, respectively. As a positive control, iproniazid also exhibited an IC_{50} value of 19.7 μM.

To examine the selectivity of the MAO activity, *l*-deprenyl-pretreated MAO preparation was used for the measurement of MAO-A activity, and a clorgyline-pretreated one was for MAO-B. Gancaonin A (**1**) selectively inhibited MAO-B activity than MAO-A in a concentration-dependent manner with the IC_{50} values of 0.8 and >800 μM, respectively. However, 4'-*O*-methylalpinumisoflavone (**2**) and alpinumisoflavone (**3**) showed a slightly potent inhibitory effect against MAO-B than MAO-A (Table I). These results indicate that gancaonin A (**1**) is a potent inhibitor for MAO-B activity. The only difference is that the 3,3-dimethylallyl side chain at C-6 in gancaonin A (**1**) was cyclized with the hydroxyl group at C-7 to produce a fused dimethylchromene ring in 4-*O*-methylalpinumisoflavone (**2**) and alpinumisoflavone (**3**). Therefore, ring A in gancaonin A (**1**)

Table I. Inhibitory effects of compounds **1-3** on MAO in mouse brain

Compound	IC_{50} (μM)[a]		
	Mixed type MAO	MAO-A	MAO-B
Gancaonin A (**1**)	19.4	>800	0.8
4'-O-methylalpinumisoflavone (**2**)	23.9	37.6	18.7
Alpinumisoflavone (**3**)	25.8	52.6	16.8
Amitriptyline[b]	79.3	316.8	27.6

[a] Inhibitory activity was expressed as the mean of 50% inhibitory concentration (IC_{50}) of triplicate determinations, obtained by interpolation of concentration-inhibition curves. The activities of MAO-A and MAO-B were measured in the presence of 1 μM *l*-deprenyl or clorgyline, respectively.
[b] Positive control

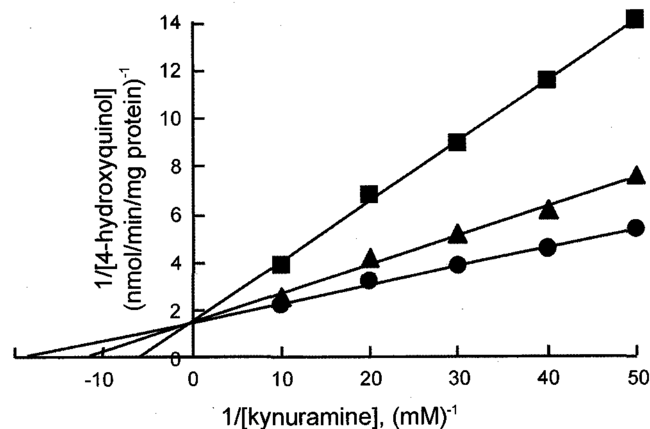

Fig. 2. Inhibition of MAO-B by gancaonin A (**1**) added in the enzyme reaction mixture. The reciprocal of MAO-B activities was plotted against the reciprocal of substrate concentrations (n=5). Concentration of gancaonin A (**1**): ● 0 μM; ▲ 0.5 μM; ■ 1 μM.

appeared to contribute to the selective inhibition of MAO-B.

To further investigate the mode of inhibition of MAO-B enzyme by gancaonin A (**1**), Lineweaver-Burk plot analysis was carried out with different concentrations of kynuramine as a substrate. When the concentration of the substrate was changed, the curves, obtained with the uninhibited enzyme and with the addition of gancaonin A (**1**) crossed at the ordinate. This result indicated that inhibition of mouse brain MAO-B by gancaonin A (**1**) with respect to the substrate kynuramine was competitive (Fig. 2), and Ki value was calculated to be 0.66 μM.

Recently, interest in inhibitors of MAO-B has grown, due to their therapeutic potential in aging-related neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. The selective MAO-B inhibitors, such as selegiline, rasagiline, and lazabemide, have been shown to protective effects on neuronal tissue (Riederer *et al.*, 2004).

As a result, we suggest that the fruit of *C. tricuspidata*, containing potent MAO-B inhibitory prenylated flavones, could be a possible therapeutic candidate for the Parkinson's and Alzheimer's disease. However, further pharmacological investigations and *in vivo* physiological functions remain to be elucidated.

ACKNOWLEDGEMENTS

This work was supported by a Chungbuk National University Grant in 2004. We are grateful to Korea Basic Science Institute for ^{1}H- and ^{13}C-NMR spectral measurements.

REFERENCES

Benedetti, M. S. and Dostert, P., Monoamine oxidase: from physiology and pathophysiology to the design and clinical

application of reversible inhibitors. In Testa, B. (Eds.) Advances in Drug Research, vol. 23. Adademic Press, New York, pp.65-125 (1992).

Fujimoto, T., Hano, Y., and Nomura, T., Components of root bark of *Cudrania tricuspidata* 1. Structures of four new isoprenylated xanthones, Cudraxanthones A, B, C, and D. *Planta Med.,* 50, 218-221 (1984a).

Fujimoto, T., Hano, Y., Nomura, T., and Uzawa., Components of root bark of *Cudrania tricuspidata* 2. Structures of two new isoprenylated flavones, Cudraflavones A and B. *Planta Med.,* 50, 161-163 (1984b).

Fukai, T., Wang, Q. H., and Nomura, T., Four new prenylated flavonoids from aerial parts of *Glycyrrhiza uralensis*. *Heterocycles*, 29, 1369-1378 (1989).

Hano, Y., Matsumoto, Y., Shinohara, K., Sun, J. Y., and Nomura, T., Structures of four new isoprenylated xanthones, Cudraxanthones L, M, N, and O from *Cudrania tricuspidata*. *Planta Med.,* 57, 172-175 (1991).

Hano, Y., Matsumoto, Y., Sun, J. Y., and Nomura, T., Structures of four new isoprenylated xanthones, Cudraxanthones H, I, J, and K. *Planta Med.,* 56, 478-481 (1990a).

Hano, Y., Matsumoto, Y., Sun, J. Y., and Nomura, T., Structures of three new isoprenylated xanthones, Cudraxanthones E, F, and G. *Planta Med.,* 56, 399-402 (1990b).

Jung, B. S. and Shin, M. K., Encyclopedia of illustrated Korean natural drugs. Young Lim Sa, Seoul, pp 544-545 (1989).

Kraml, M., A rapid microfluorimetric determination of monoamine oxidase. *Biochem. Pharmacol.*, 14, 1684-1686 (1965).

Lee, B. W., Gal, S. W., Park, K. M., and Park, K. H., Cytotoxic xanthones from *Cudrania tricuspidata*. *J. Nat. Prod.,* 68, 456-458 (2005).

Lee, I. K., Kim, C. J., Song, K. S., Kim, H. M., Koshino, H., Uramoto, M. and Yoo, I. D., Cytotoxic benzyl dihydroflavonols from *Cudrania tricuspidata*. *Phytochemistry,* 41, 213-216 (1996).

Naoi, M. and Nagatsu, T., Quinoline and quininaldines as naturally occurring inhibitors specific for type A monoamine oxidase. *Life Sci.*, 40, 1075-1082 (1987).

Olivares, E. M., Lwande, W., Monache, F. D., and Bettolo, G. B., A pyrano-isoflavone from seeds of *Milletia thonningii*. *Phytochemistry,* 21, 1763-1765 (1982).

Riederer, P., Danielczyk, W., and Grunblatt, E., Monoamine oxidase-B inhibition in Alzheimer's disease. *NeuroToxicology,* 25, 271-277 (2004).

Ro, J. S., Lee, S. S., Lee, K. S., and Lee, M. K., Inhibition of type A monoamine oxidase by coptisine in mouse brain. *Life Sci.*, 70, 639-645 (2001).

Thomas, T., Monoamine oxidase-B inhibitors in the treatment of Alzheimer's disease. *Neurobiol. Aging*, 21, 343-348 (2000).

Tian, Y. H., Kim, H. C., Cui, J. M., and Kim, Y. C., Hepatoprotective constituents of *Cudrania tricuspidata*. *Arch. Pharm. Res.,* 28, 44-48 (2005).

Yamada, M. and Yasuhara, H., Clinical pharmacology of MAO inhibitors: Safety and future. *Nerurotoxicology,* 25, 215-221 (2004).

Youdim, M. B. and Riederer, P. F., A review of the mechanisms and role of monoamine oxidase inhibitors in Parkinson's disease. *Neurology,* 63, S32-35 (2004).

Zou, Y. S., Hou, A. J., Zhu, G. F., Chen, Y. F., Sun, H. D., and Zhao, Q. S., Cytotoxic isoprenylated xanthones from *Cudrania tricuspidata*. *Bioorg. Med. Chem.,* 12, 1947-1953 (2004).