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Inhibition of Monamine Oxidase by a Flavone and Its Glycoside from *Ixeris dentata* Nakai

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Abstract

***Ixeris dentata* Nakai (Compositae) is a perennial herb which has been used as a folk medicine for treating diabetes and gastroenteric troubles in Korea. Active compounds were isolated from the aerial parts of *Ixeris dentata* through the bioassay-guided fractionation and isolation method evaluated for inhibition of monoamine oxidase (MAO) *in vitro*. The compounds were identified as 5,7,3′,4′-tetrahydroxyflavone (1) and 5,7,3′,4′-tetrahydroxyflavone 7-glucoside (2), based on physical and spectroscopic characteristics. Compounds 1 and 2 showed a selective inhibition of type B MAO (MAO-B) activity, with IC_{50} values of 15.3 μM and 36.4 μM, respectively, but did not inhibit type A MAO (MAO-A) activity.**

Key words: monoamine oxidase, *Ixeris dentata* Nakai, flavone, luteolin, cynaroside

INTRODUCTION

Monoamine oxidase (MAO, EC 1.4.3.4), a flavoprotein found exclusively in the mitochondrial outer membrane, plays a central role in the metabolism of amines, such as neurotransmitter monoamines. MAO-A and MAO-B are two isoenzymes involved in the degradation of many biological amines in the nervous system and in peripheral organs. MAO inhibitors have been used in psychiatry for the treatment of depressive disorders and in neurology for the treatment of Parkinson's disease (1). The investigation of bioactive natural products has, in recent years, assumed a greater sense of urgency in response to the expanding human population and its subsequent demands for food, good health, and increasing areas of land on which to live; which have resulted in a decrease in biodiversity and loss of plant species with medicinal potential. The isolation of natural compounds with biological activity that can be administered in reproducible, accurate doses, presents obvious benefits from experimental and therapeutic points of view (2). Flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers as well as tea and wine and are important constituents of the human diet. The average Western diet contains approximately 1.0 g/d of mixed flavonoids; a quantity that may result in pharmacologically significant concentrations in body fluids and tissues. Flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antiviral, anti-proliferative and anticarcinogenic activities as well as to affect some aspects of mammalian metabolism (3). It has been reported that four natural flavonoids of quercitrin, isoquercitrin, rutin, and quercetin exhibit an inhibitory effect on MAO-B, with IC_{50} values of 19.06, 11.64, 3.89, and 10.89 μM, respectively (4). Whole plants of *Ixeris dentata* Nakai, when properly prepared, are edible as a bitter appetizing vegetable. It was previously reported that the plants contain sesquiterpene lactones and its glycosides, and flavone glycoside as bitter and antidiabetic principles (5-7).

As a part of a continuing study on bioactive phytochemicals in plants, two flavones, luteolin (1) and cynaroside (2) with inhibitory activity on MAO-B, were isolated from the ethylacetate (EtOAc) soluble fraction of aerial parts of *Ixeris dentata* Nakai. It has been recently reported that luteolin attenuates asthma symptoms in experimental mice (8). Luteolin has been shown to affect human T24 cell NAT activity and gene expression (NAT1 mRNA), DNA-AF adduct formation (9), and to inhibit proliferation of HepG2 human hepatocellular carcinoma cells (10).

MATERIALS AND METHODS

Experimental section

General experimental procedures: The UV and IR spectra were recorded on a Hitachi 3100 UV-vis and JASCO FT-IR-5300 spectrophotometer, respectively. EI-MS was obtained on a Hewelett Packard Model 5985B GC/MS spectrometer. ^{1}H and ^{13}C NMR spectra were measured, with tetramethylsilane as an internal standard, using a Bruker CXP-500 spectrometer operating at 500 and 125 MHz to perform ^{1}H NMR and ^{13}C NMR experiments,

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respectively.

Plant material: The fresh whole plants of *Ixeris dentata* were collected in Yeuju, Kyungkido and a voucher specimen is deposited in the Kwangnung Arboretum, Chungbu Forestry Experiment Station.

Extraction and isolation: The dried and ground aerial parts of *Ixeris dentata* Nakai (1.0 kg) were extracted in hot ethyl alcohol (EtOH) for 3 hours and concentrated under reduced pressure to yield a dried EtOH extract. This extract was partitioned with *n*-hexane, chloroform ($CHCl_3$), EtOAc and *n*-BuOH, successively. The EtOAc layer was concentrated for chromatography on a silica gel column with a $CHCl_3$-MeOH gradient system (98 : 2→90 : 10) to afford seven subfractions. Subfractions 3 and 7 exhibited inhibitory effects on MAO-B, with IC_{50} values of 26.6 and 41.8 μM, respectively. The two fractions were then further chromatographed on Sephadex LH-20 by elution with MeOH in order to purify compounds **1** (57.9 mg) and **2** (34.3 mg), respectively.

5,7,3′,4′-Tetrahydroxyflavone (1): R_f: 0.23 ($CHCl_3$: MeOH =7 : 1); $FeCl_3$, Mg-HCl, Zn-HCl test: positive; m.p. >320°C, pale yellow plates (MeOH); UVmax (MeOH) (log ε): 254 (4.5), 267 (4.4), 297 (sh, 4.3), 344 (4.5); (NaOMe) (log ε): 268 (4.5), 328 (sh, 3.9), 402 (4.5); (NaOAc) (log ε): 270 (4.3), 328 (sh, 4.1), 378 (4.2); (NaOAc + H_3BO_3) (log ε): 260 (4.2), 297 (sh, 3.7), 378 (4.4); ($AlCl_3$) (log ε): 273 (4.4), 300 (sh, 4.3), 331 (3.8), 426 (4.8); ($AlCl_3$ + HCl) (log ε): 262 (4.4), 275 (4.2), 296 (sh, 4.1), 360 (4.5), 385 (4.5) nm; IR (KBr) ν max: 3380 (OH), 1665 (α,β-unsaturated C=O), 1610, 1505, 1460 (aromatic C=C) cm^{-1}; EI-MS (70 eV) *m/z* 286 $[M]^+$ (100.0), 258 $[M - CO]^+$ (24.5), 229 $[M- CO - CHO]^+$ (9.0), 153 $[A_1+H]^+$ (49.3), 152 $[A_1]^+$ (10.1), 137 $[B_2]^+$ (4.9), 134 $[B_1]^+$ (18.5), 124 $[A_1\text{-}CO]^+$ (15.2), 123 $[A_1\text{-}CHO]^+$ (9.0); ^1H NMR and ^{13}C NMR spectral data, see Table 1.

5,7,3′,4′-Tetrahydroxyflavone 7-glucoside (2): R_f: 0.14 ($CHCl_3$: MeOH : H_2O = 25 : 6 : 0.6); $FeCl_3$, Mg-HCl, Zn-HCl Molisch test: positive; m.p. 250 °C, pale yellow plates (MeOH); UVmax (MeOH) (log ε): 256 (4.5), 267 (sh, 4.4), 298 (sh, 4.3), 345 (4.7); (NaOMe) (log ε): 264 (4.5), 402 (4.7); (NaOAc) (log ε): 258 (4.5), 288 (sh, 4.3), 352 (4.4), 401 (4.4); (NaOAc + H_3BO_3) (log ε): 259 (4.5), 295 (sh, 4.2), 370 (4.5); ($AlCl_3$) (log ε): 274 (4.5), 298 (sh, 4.3), 340 (sh, 4.2), 427 (4.6); ($AlCl_3$ + HCl) (log ε): 276 (4.4), 294 (4.3), 348 (4.4), 390 (4.5) nm; IR (KBr) ν max: 3400 (OH), 1665 (α,β-unsaturated C=O), 1610, 1510, 1450 (aromatic C=C), 1130-1030 (glycoside) cm^{-1}; EI-MS (70 eV) *m/z* 286 $[M\text{-}C_6H_{12}O_6]^+$ (100.0), 258 $[M - C_6H_{12}O_6 - CO]^+$ (19.6), 229 $[M\text{-}C_6H_{12}O_6 - CO - CHO]^+$ (8.6), 153 $[A_1\ C_6H_{12}O_6+H]^+$ (39.1), 152 $[A_1 - C_6H_{12}O_6]^+$ (8.2), 134 $[B_1 - C_6H_{12}O_6]^+$ (18.0), 124 $[A_1 - C_6H_{12}O_6 - CO]^+$ (13.8), 123 $[A_1 - C_6H_{12}O_6 - CHO]^+$ (8.1); ^1H NMR and ^{13}C NMR spectral data, see Table 1.

Acid hydrolysis of compound 2: Compound **2** (20 mg) was dissolved in 6% HCl/MeOH (20 mL) and reacted at 80°C for 1 hr. After dilution with water, the reaction mixture was extracted with EtOAc. The EtOAc layer was washed with water, dried with Na_2SO_4, evaporated and purified by recrystallization with MeOH to yield compound **1**: m.p. >320°C; EI-MS (70 eV) *m/z* 286 $[M]^+$ (45.8); UV, IR and ^1H NMR spectral data were identical with compound **1**. The aqueous layer was neutralized with Ag_2CO_3, filtered, extracted, and identified by co-TLC with aniline hydrogen phthalate reagent to give *D*-glucose: R_f: 0.19 (pyridine : EtOAc : HOAc : H_2O = 36 : 36 : 7 : 21).

Biological activities

Animals: Experiments were performed on adult Sprague-Dawley male rats. Animals were purchased from Samtako Co., Ohsan, Kyungkido, and housed in an air-conditioned animal room at 23°C.

Preparation of enzyme sources: The male rats were anaesthetized with diethylether and blood was collected by

Table 1. ^1H NMR and ^{13}C NMR spectral data of compounds **1** and **2**[1)]

Position	1		2	
	δ H	δ C	δ H	δ C
2		164.2		164.4
3	6.63 (s)[2)]	103.1	6.44 (s)	103.1
4		181.8		181.8
5		161.4		162.9
6	6.17 (d, 1.3)	99.0	6.77 (d, 1.8)	99.5
7		164.3		162.9
8	6.42 (d, 1.3)	94.1	6.88 (d, 1.8)	94.7
9		157.5		157.5
10		113.9		103.1
1′		119.2		119.1
2′	7.38 (d, 1.4)	113.4	7.42 (d, 2.3)	113.5
3′		145.8		145.3
4′		149.7		148.2
5′	6.85 (d, 8.8)	116.2	6.91 (d, 8.2)	116.0
6′	7.43 (dd, 8.8, 1.4)	121.8	7.45 (dd, 8.2, 2.3)	119.2
OH	10.42 (br s)		12.03 (br s)	
	11.56 (br s)		13.02 (br s)	
	12.03 (br s)			
1″			5.08 (d, 6.4)	99.8
2″				73.1
3″				77.1
4″				69.5
5″				76.3
6″				60.6

[1)] ^1H NMR and ^{13}C NMR spectra were run in DMSO-d_6 at 500 and 125 MHz.
Chemical shifts are shown in the scale with *J* values in parentheses.
[2)] Multiplicity; *s* = singlet, *br s* = broad singlet, *d* = doublet, *dd* = double doublet.

cardiocentesis into sodium citrated syringes. The brain and liver tissues were surgically removed for the MAO-A and MAO-B enzyme sources, respectively. The organ tissues were washed with 0.01 M phosphate buffered saline (PBS, pH 7.0), and homogenized at 4°C, 15 min, followed addition of 9 parts by weight cold 0.25 M sucrose. Homogenates were then centrifuged at 700 g at 4°C for 20 min, the supernatant recentrifuged at 18,000 g for 20 min, and the pellet was suspended in 5 parts of PBS for the enzyme analysis.

Monoamine oxidase inhibition assay: Enzyme activity was determined according to Chung et al. (11), improved McEwen method (12), using serotonin and benzylamine hydrochloride as substrates against MAO-A and MAO-B activities, respectively.

Monoamine oxidase-A inhibition assay: A reaction mixture containing 0.5 mL of enzyme solution in 0.01 M phosphate buffered saline (pH 7.0) and 1.0 mL of test solution was preincubated at 37°C for 15 min, after which 0.5 mL of 0.01 M serotonin creatine sulfate in a buffer was added. Following incubation, the enzyme reaction was terminated by heating in a 95°C water bath for 3 min. After centrifugation, 1.6 mL of supernatant was loaded to an Amberlite CG50 (H^+ form) column, and the column was washed with over 40 mL of water. The unreacted substrate was eluted with 3.0 mL of 4.0 N acetic acid solution and subjected to spectrophotometric measurement at 277 nm. MAO-A activity was calculated as follows:

$$\text{Inhibition } \% = \frac{(A_{Sample} - A_{Compensate} - A_{Control})}{(A_{Blank} - A_{Control})} \times 100$$

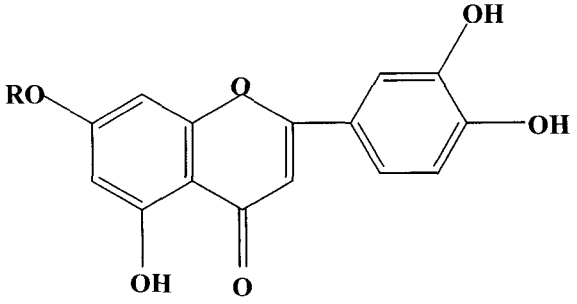
Monoamine oxidase-B inhibition assay: The reaction mixture containing 0.5 mL of enzyme solution in the buffer and 1.0 mL of test solution was preincubated at 37°C for 15 min, after which 0.5 mL of 0.04 M benzylamine hydrochloride was added. Following incubation at 37°C for 90 min, the enzyme reaction was terminated by adding 0.2 mL of 60% perchloric acid. The reaction product, benzaldehyde, was extracted with 4.0 mL of *n*-hexane and subjected to spectrophotometric measurement at 242 nm. MAO-B activity was calculated as follows:

$$\text{Inhibition } \% = \frac{(A_{Control} - A_{Sample} + A_{Compensate})}{(A_{Control} - A_{Blank})} \times 100$$

RESULTS AND DISCUSSION

The dried aerial parts of *Ixeris dentata* were extracted with EtOH and partitioned with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH. The dried EtOAc extract exhibited inhibitory effects on MAO-A and MAO-B, with IC_{50} values of 81.6 M and 27.9 M, respectively, and were then subjected to series of activity-guided chromatographic fractionation steps to afford compounds **1** and **2**. The pure isolates gave characteristic flavonoid color reactions (purplish brown with $FeCl_3$, yellowish orange with Mg-HCl, pale pink with Zn-HCl (13). Compound **1** was isolated as pale yellow plates, mp>320, assigned a molecular formula of $C_{15}H_{10}O_6$ from its EI-MS spectrum, which was consistent with a flavone containing four hydroxyl groups. Compound **1** had UV absorption bands at 254, 267, 297 (sh) and 344 nm; and, α,β-unsaturated carbonyl absorption at 1665, and aromatic absorption at 1610, 1505 and 1460 cm^{-1} in its IR spectra suggesting the flavone skeleton (14). The ^1H NMR spectrum contained a singlet of one proton at δ 6.63 for H-3, and four doublets at δ 6.17, 6.42, 6.85 and 7.38 for H-6, H-8, H-5′ and H-2′, respectively, and *ortho* and a *meta*-coupled double doublet at δ 7.43 for H-6′. Compound **2** was isolated as pale yellow plates, mp 250°C; UV absorption bands at 256, 267 (sh), 297 (sh), 298 and 345 nm, and glycosidic absorptions at 1130~1030 cm^{-1} in its IR spectra. The ^1H NMR spectrum contained a singlet of one proton at δ 6.44 for H-3, and four doublets at δ 6.77, 6.88, 6.91 and 7.42 for H-6, H-8, H-5′ and H-2′, respectively, and *ortho* and a *meta*-coupled double doublet at δ 7.45 for H-6′. On the basis of the spectroscopic data and comparison of its UV-shift pattern, mass spectral retro-Diels-Alder fragmentation and ^{13}C NMR data with literature data (14-16), isolated compounds were characterized to be 5,7,3′,4′-tetrahydroxyflavone, luteolin (**1**) and 5,7,3′,4′-tetrahydroxyflavone 7-glucoside, cynaroside (**2**) (Fig. 1) (Table 1).

The *in vitro* MAO inhibitory activities of the two isolated flavones were evaluated by published methods (11). Compounds **1** and **2** each showed selective moderate inhibition of liver MAO-B activity with IC_{50} values of 15.3 μM and 36.4 μM, respectively, but did not inhibit the brain MAO-A activity (Table 2). Results of this investigation indicate that compounds **1** and **2** can inhibit the deamination of monoamines by MAO-B. It is concluded that *Ixeris dentata* might be useful in the prevention/treatment



1: R = H
2: R = Glucose

Fig. 1. Structure of compounds **1** and **2**.

Table 2. Monoamine oxidase inhibitory activities of EtOAc extract and compounds **1** and **2**[1)]

Samples	IC_{50} (μM)[2)]	
	MAO-A	MAO-B
EtOAc extract	81.6	27.9
1	-	15.3
2	-	36.4

[1)]The activity was determined according to Chung et al. (11).
[2)]Data from 3 experiments of each duplicate.

of neurodegenerative diseases due to its *in vitro* MAO inhibitory activities.

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