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Note

Inhibitory Activity of Monoamine Oxidase by Coumarins from *Peucedanum japonicum*

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Four coumarins were isolated from chloroform extract of the root of *Peucedanum japonicum* and identified as praeruptorin A (**1**), xanthotoxin (**2**), psoralen (**3**) and bergapten (**4**) on the basis of spectroscopic methods. The inhibitory activities of these coumarins on monoamine oxidase prepared by mouse brain were tested. The IC_{50} values of them were shown to be 27.4 μM (**1**), 40.7 μM (**2**), 35.8 μM (**3**), and 13.8 μM (**4**), *in vitro*.

Key words : *Peucedanum japonicum*,Umbelliferae, Coumarins, Monoamine oxidase

INTRODUCTION

Monoamine oxidase (MAO; EC 1.4.3.4) catalyzes oxidation of endogenous neurotransmitter monoamines and various exogenous physiological amines. Great interests in inhibitors of MAO from plant and microbial sources have been made due to their possible use in the treatment of depression (Rocha *et al.*, 1994). A number of MAO inhibitors have been identified, including alkaloids (Rosazza *et al.*, 1992), xanthones (Schaufelberger *et al.*, 1987), azaphilones (Yoshida *et al.*, 1996) and coumarins (Hossain *et al.*, 1996). Several naturally occurring coumarins and synthetic coumarin derivatives have reported to show potent inhibitory activities against MAO with strong selectivities against MAO-A and MAO-B (Rendenbach-Muller *et al.*, 1994). From this standpoint of view, this paper deals with MAO inhibitory effects of coumarins isolated from chloroform extract of the root of *Peucedanum japonicum*.

MATERIALS AND METHODS

Plant material

The root of *P. japonicum* was purchased from Ilshin drug store, Taejon, Korea in 1997. A voucher specimen are deposited at the herbarium of the College of Pharmacy, Chungnam National University.

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Instruments

Thin layer chromatography was carried out on precoated TLC plate silica gel $60F_{254}$ (Merck, Art. 5554) and RP-$18F_{254s}$ (Merck). Kieselgel 60 (70~230 mesh, Merck), YMC-gel (ODS-A, 60-230 mesh), and Sephadex LH-20 (Pharmacia) were used for the stationary phases of column chromatography. ^{1}H- and ^{13}C-NMR were recorded on a Varian Unity 300 or Bruker DRX 300 spectrometers. FAB-MS and EI-MS spectra were taken on a Kratos Concept-1S and Hewlett-Packard MS Engine 5989A mass spectrometer, respectively.

Extraction and isolation

Four coumarins were isolated from the dried roots of *P. japonicum* as reported previously (Choi *et al.*, 1999). Their structures were determined by physicochemical and spectral data and identified as praeruptorin A, xanthotoxin, psoralen, and bergapten.

Compound 1 (praeruptorin A): white crystal, mp 156~158°C, $[\alpha]_D$+3.3°, $C_{21}H_{22}O_7$; FAB-MS, *m/z* 387 [M^++H]; ^{1}H- and ^{13}C-NMR : see reference (Okuyama *et al.*, 1981).

Compound 2 (xanthotoxin): colorless needles, mp 146~148°C, $C_{12}H_8O_4$; EI-MS, m/z 216 [M^+]; ^{1}H-NMR (300 MHz, $CDCl_3$) see reference (Masuda *et al.*, 1998); ^{13}C-NMR (75 MHz, $CDCl_3$) : see reference (Elgamal *et al.*, 1979).

Compound 3 (psoralen): colorless needles, mp 165~166°C, $C_{11}H_6O_3$; ^{1}H-NMR (300 MHz, $CDCl_3$) : see reference (Masuda et al., 1998)

Compound 4 (bergapten): colorless needles, mp 188~190°C, $C_{12}H_8O_4$; ^{1}H-NMR (300 MHz, $CDCl_3$) : (Masuda *et al.*, 1998); ^{13}C-NMR (75MHz, $CDCl_3$) : see reference (Elgamal *et al.*, 1979).

Enzyme preparation

Mice (male, ICR, 25~30 g) were purchased from Samyook Animal Center (Soowon, Korea). The animals were fed with laboratory chow and water *ad libitum* and killed by cervical dislocation. A crude mitochondrial fraction was prepared from mouse brain according to the reported method (Naoi *et al.*, 1989).

Assays

MAO activity was measured fluorometrically using kynuramine as an amine substrate according to the reported method with slight modification (Kraml *et al.*, 1965; Naoi *et al.*, 1989).

RESULTS AND DISCUSSION

The dried roots of *Peucedanum japonicum* (Umbelliferae) have been used for treatment of coughs, colds, headaches and a medicine for treating diseases of the bladder and intestinal diseases. It has been reported for the properties of eliminative, diuretic, bechic, tonic, and nerve sedative actions in Asian area (Perry *et al.*, 1980). The chemical constituents of this plant have been extensively studied. A number of coumarins were isolated from the roots and aerial parts from this plant (Ikeshiro *et al.*, 1994, Duh *et al.*, 1992). Several compounds among these showed antiplatelet aggregation activity (Chen *et al.*, 1996) and significant cytotoxic activity aginst P-388 lymphocytic leukemia system in cell cultures (Duh *et al.*, 1992).

In previous study, we isolated five known coumarins from the root of this plant, which showed inhibitory activities on nitric oxide production (IC_{50} values : 0.3~25.0 μg/ml) by LPS-activated macrophage RAW 264.7 cells (Choi *et al.*, 1999). In general, coumarins have been known to have strong inhibitory activities against MAO. Therefore, we tested inhibitory effects of coumarins from *P. japonicum* on MAO from mouse brain. As shown in Table I, the coumarins exhibited potent MAO inhibitory activities with the IC_{50} values of 13.8~40.7 μM. Bergapten (**4**) in which methoxy group is substituted at C-5 of psoralen (**3**) showed the most potent inhibitory activity (IC_{50} : 13.8 μM) for mouse brain MAO though the activity was less than those of clorgyline and iproniazid, a selective inhibitor of type A and type B MAO, respectively. As far as the nerve sedative actions of *P. japonicum* is concerned, definite conclusion can not be drawn from this result, but these coumarins may have a more important contribution to this activity. Further investigations are required to establish structure-activity relationship and *in vivo* studies of naturally occurring coumarins.

Table I. Inhibitory effects of coumarins from *Peucedanum japonicum* on mouse brain monoamine oxidase activities

Compound	IC_{50} (μM)
Praeruptorin A (**1**)	27.4
Xanthotoxin (**2**)	40.7
Psolaren (**3**)	35.8
Bergapten (**4**)	13.8
Iproniazid	3.2
Clorgyline	3.8

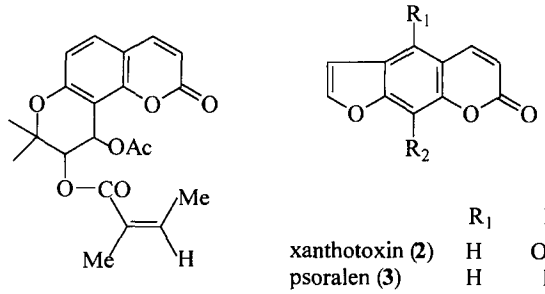

Fig. 1. Structures of compounds isolated from *P. japonicum*.

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