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Inhibitory Effects of Bioactive Fractions Containing Protoberberine Alkaloids from the Roots of *Coptis japonica* on Monoamine Oxidase Activity

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Abstract – The effects of bioactive fractions containing protoberberine alkaloids from *Coptis japonica* on monoamine oxidase (MAO) activity were investigated. The MAO was obtained from the mitochondrial fraction of mouse brain. The butanol fraction from *Coptis japonica* was fractionated into separate bioactive fraction (Fr I-IV) by silica gel column chromatography. MAO activity was strongly inhibited by Fr III and IV, which mainly contain protoberberine alkaloids such as berberine, palmatine and coptisine. These results indicated that the protoberberine alkaloids from *Coptis japonica* had an inhibitory effect on MAO activity.

Key words – Inhibition of Monoamine Oxidase, Protoberberine Alkaloids, *Coptis japonica*, Ranunculaceae

Introduction

The root of *Coptis japonica* Makino (Coptidis Rhizoma, Ranunculaceae) has been widely used in traditional medicine as anxiolytic, antibacterial, antihypertensive and CNS depressant activities (Tang and Eisenbrand, 1992).

Monoamine oxidase (EC 1.4.3.4; MAO), present in the outer mitochondrial membrane containing a flavin adenine dinucleotide, plays an important role in the catabolism of catecholamines and serotonin, and participates in regulating their levels in brain. Therefore, MAO plays a protective role by inactivating potentially toxic exogenous amines.

Recently, we have investigated the effects of methanol extracts from 130 kinds of natural products on MAO activity (Lee *et al.*, 1998a; 1998b). Among them, the methanol extract from *Coptis japonica* exhibited a strong inhibition of MAO activity. The main components of *Coptis japonica* are protoberberine isoquinoline alkaloids (Tang and Eisenbrand, 1992).

In this study, we have examined the inhibitory effects of bioactive fractions from *Coptis japonica* on MAO activity and have proved that their bioactive fractions mainly contain the protoberberine alkaloids.

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Experimental

Materials – Kynuramine, 4-hydroxyquinoline, zinc sulfate, berberine chloride, iproniazid and sucrose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade.

Preparation of mouse brain mitochondria – Mouse brain mitochondria was isolated by the method of Thull and Testa (1994) with a slight modification. Mice (ICR, male, 20-25 g) were decapitated, and the brains (8.8 g) were homogenized in an ice-cold 20 ml of 0.25 M sucrose containing 10 mM potassium phosphate buffer (pH 7.4) and centrifuged at 1,200 g for 5 min at 4°C. The supernatant was collected and further centrifuged at 16,000 g for 20 min. The crude mitochondrial pellet was washed using 10 mM sodium phosphate buffer (pH 7.4) and suspended in the same buffer. Protein amounts were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. MAO activity obtained from the final was adjusted to 0.30 ± 10.020 nmol/min/mg protein.

Preparation of active fractions from *Coptis japonica* – The dried roots of *Coptis japonica* (300 g) were identified and extracted using MeOH as

described previously (Lee and Kim, 1996). The MeOH extract was successively extracted with CH_2Cl_2, EtOAc, BuOH and H_2O, and the BuOH extract was fractionated into fractions (Fr) I-IV using silica gel column chromatography (SiO_2, 70-230 mesh, Merck, 4.5×50 cm) as described previously (Lee and Kim, 1996). The alkaloidal spots from each fraction were detected by TLC (Kieselgel 60 F_{254}, Merck) using Dragendorff reagent.

Assay for MAO activity – MAO activity was measured fluorimetrically using kynuramine as a substrate according to the method of Kraml (1965). 5-Hydroxyquinoline level formed by enzyme reaction for 30 min at 37°C was measured using fluorospectrophotometer (λex., 315 nm/λem., 380 nm, Hitachi Model F-3000, Tokyo, Japan)

Results and Discussion

We have previously reported that MeOH extract of *Coptis japonica* shows an inhibitory effect on MAO activity (Lee *et al.*, 1998b). Therefore, the MeOH extract was successively partitioned into CH_2Cl_2, EtOAc, BuOH and H_2O fractions. Among them, the EtOAc and BuOH fractions showed 37.5% and 49.8% inhibition on MAO activity at 100 μg/ml, respectively (Table 1). BuOH fraction provided a greater inhibition than EtOAc fraction.

The BuOH fraction was fractionated into Fr I-IV using silica gel column chromatography as described previously (Lee and Kim, 1996). Fr II-IV showed considerable inhibitory effects on MAO activity. The order of MAO inhibition was Fr IV > Fr III > Fr II (Table 1). By TLC analysis (Rf: berberine, 0.39; palmatine, 0.31; coptisine, 0.26, developing solvent, isopropanol:formic acid : H_2O = 80 : 1 : 20), the main components of Fr II-III were identified as protoberberine alkaloids containing mainly berberine and palmatine, and those of Fr IV were identified as coptisine and other minor alkaloids. Berberine inhibited MAO activity (39.2% inhibition at 50 μM) but was less potent than iproniazid, a selective MAO inhibitor (Table 1). These results indicated that the bioactive fractions containing protoberberine alkaloids from the roots of *Coptis japonica* exhibited inhibitory effects on MAO activity.

Isoquinoline and tetrahydroisoquinoline compounds were reported to inhibit MAO (Bembenck *et al,* 1990). Coumarin, flavone, dibenzofuran, authene, thioxanthone and acridine, which are oxygen containing compounds, were also reported to inhibit MAO activity (Thull and Testa, 1994).

Previously, we have reported that MeOH extract of *Coptis japonica* and its main components such as berberine and palmatine decrease dopamine content in PC12 cells (Lee and Kim, 1996). Berberine and palmatine also inhibit the bovine adrenal tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis (Lee and Zhang, 1996; Lee *at al.*, 1996).

The roots of *Coptis japonica* prove to have anxiolytic, antihypertensive and CNS depressant effects (Tang and Eisenbrand, 1992). Some of these effects, anxiolytic and antihypertensive effects are similar to those of adrenoreceptor agonists. Berberine also has an adrenoreceptor antagonistic action in binding assay

Table 1. Effects of various fractions from the roots of *Coptis japonica* on monoamine oxidase (MAO) activity

Fractions	MAO activity (nmol/min/mg protein) (% of control)	IC_{50} values (μM)
Control	0.301 ± 0.020 (100)	
MeOH extract (100 μg/ml)	0.190 ± 0.008 (63.1)*	
Dichloromethane (100 μg/ml)	0.261 ± 0.009 (86.7)	
Ethyl acetate (100 μg/ml)	0.188 ± 0.004 (62.5)*	
Butanol (100 μg/ml)	0.151 ± 0.011 (50.2)*	
Aqueous layer (100 μg/ml)	0.194 ± 0.005 (64.5)*	
Fr. I (100 μg/ml)	0.196 ± 0.002 (65.1)*	
Fr. II (100 μg/ml)	0.163 ± 0.001 (54.2)*	
Fr. III (100 μg/ml)	0.129 ± 0.003 (42.9)*	
Fr. IV (100 μg/ml)	0.073 ± 0.001 (24.3)**	
Berberine (50 μM)	0.183 ± 0.015 (60.8)*	97.6
Iproniazid (10 μM)	0.153 ± 0.021 (50.8)*	12.6

Control of MAO activity was taken as 0.301 nmol/min/mg protein. Iproniazid was used for the positive control. Results represent the means ± SEM of 4 dishes. Significantly different from the control value: *$p<0.01$; **$p<0.001$ (Student's t test).

(Ölmerz and Ilhan, 1992). These results suggest that *Coptis japonica* has a dual action according to the biologically active sites.

In this study, the roots of *Coptis japonica* have proved to inhibit MAO activity and, therefore, to possess the regulatory activity of catecholamine content. The separation of the main components from *Coptis japonica* and their enzymatic mechanisms need to be studied further and the results will be discussed elsewhere.

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