ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10): 949-953
http://www.chinaphar.com

In vitro inhibition of rat monoamine oxidase by liquiritigenin and isoliquiritigenin isolated from *Sinofranchetia chinensis*[1]

PAN Xuan[2,3], KONG Ling-Dong[2,4], ZHANG Yong[2], CHENG Christopher HK[4], TAN Ren-Xiang[2,5]
([2]*Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210093, China*; [3]*Department of Pharmacy, Lanzhou Medical College, Lanzhou 730000, China*; [4]*Department of Biochemistry, The Chinese University of Hong Kong, Shatin, N T, Hong Kong, China*)

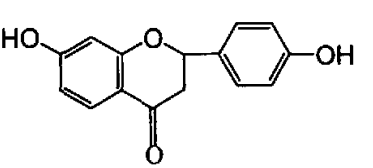
KEY WORDS liquiritigenin; isoliquiritigenin; *Sinofranchetia chinensis*; *Lardizabalaceae*; monoamine oxidase

ABSTRACT

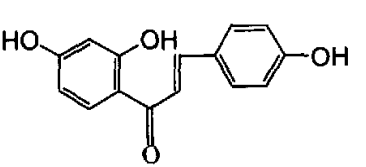
AIM: To study the inhibition of liquiritigenin (1) and isoliquiritigenin (2) isolated from *Sinofranchetia chinensis* on rat monoamine oxidase A and B (MAO A and B). **METHODS**: Rat brain mitochondrial fraction, prepared by differential centrifugation, was utilized as a source of MAO activity. MAO activity was determined radiochemically with [^{14}C]5-hydroxytryptamine (5-HT) and [^{14}C]β-phenylethylamine (β-PEA) used as MAO A or B specific radiolabled substrates, respectively. The K_i and K_I values were obtained from Lineweaver-Burk plot using linear regression analysis. **RESULTS**: Liquiritigenin and isoliquiritigenin were found to be inhibitory against both MAO A and B in a dose-dependent manner. IC_{50} (95 % of confidence limits) of liquiritigenin and isoliquiritigenin were 32 (26 - 36) and 13.9 (12.8 - 15.6) μmol/L for the inhibition of MAO A, and 104.6 (89.0 - 118.9) and 47.2 (39.5 - 54.5) μmol/L for that of MAO B, respectively. Lineweaver-Burk transformation of the MAO A inhibition data indicated that the inhibition was non-competitive for both liquiritigenin and isoliquiritigenin whereas their inhibition of MAO B was of mixed type. Regarding MAO A inhibition, the K_i values of liquiritigenin and isoliquiritigenin were 31.5 μmol/L and 14.3 μmol/L, respectively. As to the inhibition of MAO B, the K_i and K_I data for liquiritigenin were 164.7 and 15.2 μmol/L, and those for isoliquiritigenin were 62.2 and 9.3 μmol/L, respectively. **CONCLUSION**: Liquiritigenin and isoliquiritigen inhibited the activity of MAO A and B in rat brain mitochondria, and the latter was more active than the former.

INTRODUCTION

The monoamine oxidase (MAO, EC.1.4.3.4) A and B catalyze the oxidative deamination of monoamines in the central nervous system and peripheral tissues. The inhibitors of MAO A are expected to be clinically useful to treat anxiety and depression, while the inhibition of MAO B appears to be an effective approach for the prevention and adjunct treatment of Parkinson's disease[1,2]. Prompted by our observation that some traditional Chinese medicines are being used very frequently for the treatment of Parkinson's disease, anxiety, and depression, we therefore aim at characterizing plant



Liquiritigenin (1)



Isoliquiritigenin (2)

[1] Project co-supported by the National Natural Science Foundation of China (No 39725033 and 39900189) and Doctoral Project of High Education (No 1999028419).
[5] Correspondence to Prof TAN Ren-Xiang. Phn 86-25-3593-201. Fax 86-25-3593-201. E-mail rxtan@netra.nju.edu.cn
Received 2000-03-08 Accepted 2000-07-13

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

derived inhibitors of both types of MAO[3]. The medicinal plant *Sinofranchetia chinensis* (Franch) Hemsl (*Lardizabalaceae*) is used as a substitute of "Mu Tong" (the stem of *Akebia trifoliata* (Thunb) Koidz var *australis* (Diels) Rehd, which is applied to treat dysuria and painful joints of the limbs in China[4,5]. Our preliminary bioassay indicated that the methanol extract of the title species was active against the enzyme. We hereby report the characterization of liquiritigenin (5-deoxyflavanone, **1**) and isoliquiritigenin (6′-deoxychalcone, **2**) isolated from *S chinensis* as MAO inhibitors.

MATERIALS AND METHODS

Reagents [^{14}C]5-hydroxytryptamine (5-HT) and [^{14}C] β-phenylethylamine (β-PEA) (DuPont NEN, USA). Dimethyl sulfoxide (Me_2SO, Sigma). All other chemicals used in the study were of analytical grade.

Plant materials The stems of *S chinensis* were collected in August 1995 in Wudu Prefecture, Gansu Province, China. A voucher specimen (WD-957211) was identified by Prof X Pan and deposited in the Herbarium of Lanzhou Medical College, Lanzhou, China.

Fractionation and identification The pulverized plant material (1.3 kg) was extracted twice with MeOH at room temperature. The concentrated extract (42 g) was chromatographed over CC (Si gel, 200 – 300 mesh 700 g), eluted with $CHCl_3$ containing gradually increasing amounts of MeOH. The CC fractions (300 mL each) were combined according to the TLC monitoring into 5 pools (P-1: 2.5 g, P-2: 1.6 g, P-3: 1.0 g, P-4: 0.8 g, P-5: 2.5 g). P-4, shown to be inhibitory to MAO A and B, was further separated successively by CC over Si gel (270 g, using a $CHCl_3$-MeOH gradient) and by gel filtration over Sephadex LH-20 with MeOH to afford pure liquiritigenin (**1**, 128 mg) and isoliquiritigenin (**2**, 147 mg). The structures of **1** and **2** were ascertained by means of spectroscopic methods (IR, UV, MS, ^{1}H and ^{13}C NMR)[6,7].

Enzyme preparation Rat brain mitochondrial fraction was prepared as a source of MAO activity following the procedure detailed earlier[8]. Briefly, the mitochondrial fraction and sodium phosphate buffer (50 mmol/L, pH 7.4) were mixed in a proportion of 1:20 by stirring gently at 4 ℃ for 60 min. The mixture was centrifuged immediately at 16 000 × g at 0 ℃ for 30 min and the pellets were resuspended in the same buffer containing additional sucrose at a concentration of 250 mmol/L.

Assay of MAO activity MAO activity was assayed radiochemically by slightly modifying the method described previously[9,10]. Thus, the assay mixtures contained 50 μmol/L [^{14}C]5-HT or 10 μmol/L [^{14}C]β-PEA as specific substrates for MAO A and B, respectively, 10 μL solution of test samples in Me_2SO at different concentrations, and 100 mmol/L sodium phosphate buffer (pH 7.4) up to a final volume of 200 μL. After 20-min preincubation at 37 ℃, the reaction was started by adding 50 μg of the mitochondrial fraction. The reaction was allowed to proceed at 37 ℃ for 20 min, and terminated by addition of 2 mol/L HCl (1 mL), the radioactive product was extracted with 2 mL of toluene/ethyl acetate (v/v, 1:1). The radioactivity of the organic phase was counted in a liquid scintillation spectrometer. Blank samples were prepared by adding 2 mol/L HCl (1 mL) prior to reaction, and worked up subsequently in the same manner. Enzyme activity was expressed as nmol product formed per mg protein per min[11].

In the kinetic analyses, the reaction mixture consisting of different concentrations of [^{14}C]5-HT (20 – 200 μmol/L) or [^{14}C]β-PEA (3.3 – 20 μmol/L) were used as MAO A or B substrates, respectively, in the absence and presence of inhibitors.

Estimation of protein Protein concentration was estimated by the Lowry method[12] using bovine serum albumin as the standard.

Data analysis Data were presented as $\bar{x} \pm s$. The IC_{50} value was calculated using computer software 'GraphPad InPlot'. The K_i and K_I values were determined by consulting Lineweaver-Burk's plot using linear regression analysis[13]. Specifically, K_i was calculated from the slope of the inhibition curve by the equation $[slope = \frac{K_m}{V_{max}}(1 + \frac{[I_0]}{K_i})([I_0]$, K_m and V_{max} representing inhibitor's initial concentration, Michaelis constant and maximum initial velocity, respectively), and K_I was calculated from the y-intercept of the inhibition curve using the equation $[\text{y-intercept} = \frac{1}{V_{max}}(1 + \frac{[I_0]}{K_I}]$.

RESULTS

Inhibition of MAO A by liquiritigenin and isoliquiritigenin Liquiritigenin (**1**) and isoliquiritigenin (**2**) inhibited the activity of MAO A in a dose-dependent manner with IC_{50} (95 % of confidence limits) of 32 (26 – 36) and 13.9 (12.8 – 15.6) μmol/L, respectively (Fig 1). In our study, the IC_{50} value of clorgy-

line, an MAO A inhibitor used as a positive control, was 0.198 μmol/L. The inhibition mode of both liquiritigenin and isoliquiritigenin was non-competitive to the substrate 5-HT with the K_i values of 31.5 and 14.3 μmol/L, respectively (Fig 2, 3).

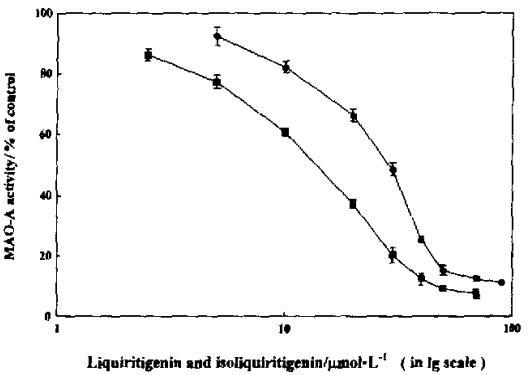


Fig 1. Dose-dependent inhibitory actions of liquiritigenin (●) and isoliquiritigenin (■) on rat brain mitochondrial MAO-A activity. Values are expressed as $\bar{x} \pm s$ of 5 replicates.

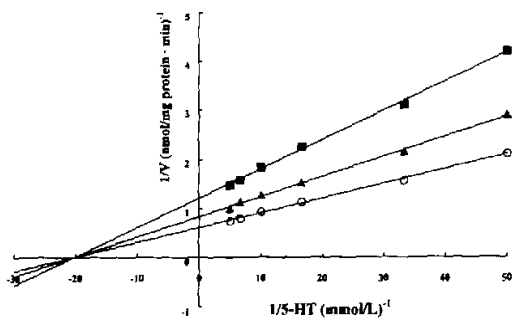


Fig 2. Lineweaver-Burk plot of inhibition of rat brain mitochondrial MAO A by liquiritigenin. MAO assay was performed at different concentrations of the substrate [^{14}C]5-HT. Control without any inhibitor (○), in the presence of 16 (▲) and 32 μmol/L (■) liquiritigenin. The values are expressed as the average of triplicates.

Inhibition of MAO B by liquiritigenin and isoliquiritigenin The inhibitory activity of liquiritigenin and isoliquiritigenin on MAO B activity was also dose-dependent with IC_{50} values of 104.6 (89.0 – 1118.9) and 47.2 (54.5 – 39.5) μmol/L, respectively (Fig 4). In our study, the IC_{50} value of deprenyl, a MAO B inhibitor used as a positive control, was 0.251 μmol/L. However, the mode of inhibition of the

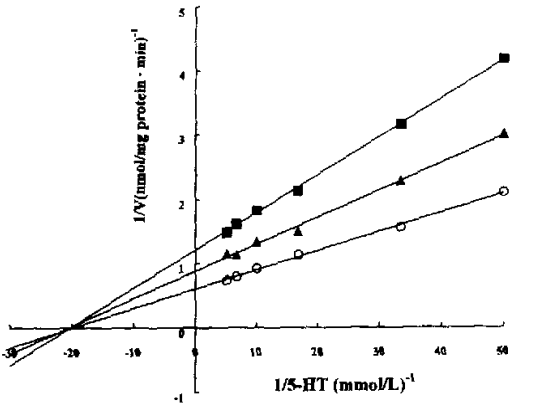


Fig 3. Lineweaver-Burk plot of inhibition of rat brain mitochondrial MAO A by isoliquiritigenin. MAO assay was performed at different concentrations of the substrate [^{14}C]5-HT. Control without any inhibitor (○), in the presence of 7 (▲) and 14 μmol/L (■) isoliquiritigenin. The values are expressed as the average of triplicates.

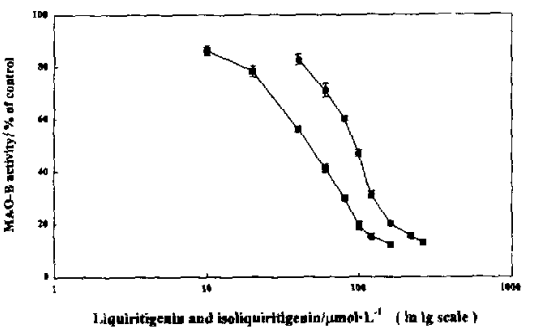


Fig 4. Dose-dependent inhibitory actions of liquiritigenin (●) and isoliquiritigenin (■) in rat brain mitochondrial MAO B activity. Values are expressed as $\bar{x} \pm s$ of 5 replicates.

substrate β-PEA was of a mixed type with K_i values of 164.7 and 62.2 μmol/L, and K_I values of 15.1 and 9.3 μmol/L, respectively (Fig 5, 6).

DISCUSSION

Growing attention has been directed to MAO inhibitors as the regulation of the enzyme is closely associated with incidence and therapy of the disorders in the central nervous system[1,2]. Present investigation disclosed that liquiritigenin (1) and isoliquiritigenin (2) are the two main MAO inhibitors present in the stem of *S chinensis*. To understand the mode of MAO inhibition,

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2000 Oct; 21 (10)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

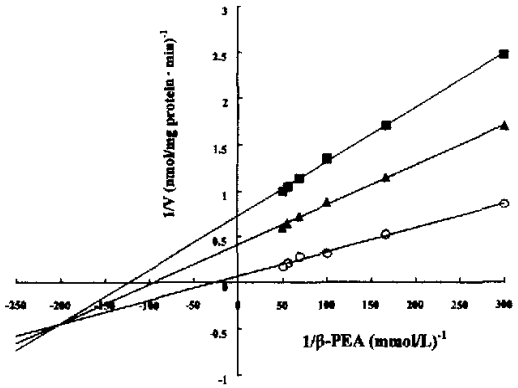


Fig 5. Lineweaver-Burk plot of inhibition of rat brain mitochondrial MAO B by liquiritigenin. MAO assay was performed at different concentrations of the substrate [^{14}C]β-PEA. Control without any inhibitor (○), in the presence of 100 (▲) and 120 μmol/L (■) liquiritigenin. The values are expressed as the average of triplicates.

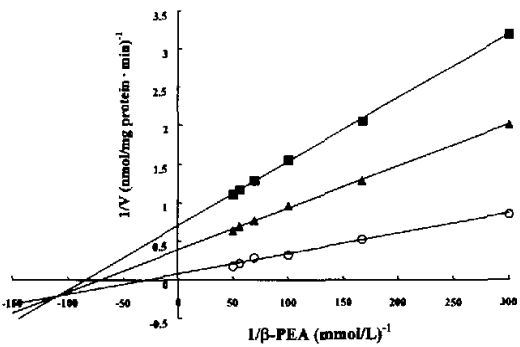


Fig 6. Lineweaver-Burk plot of inhibition of rat brain mitochondrial MAO B by isoliquiritigenin. MAO assay was performed at different concentrations of the substrate [^{14}C]β-PEA. Control without any inhibitor (○), in the presence of 60 (▲) and 80 μmol/L (■) isoliquiritigenin. The values are expressed as the average of triplicates.

the kinetic study was performed with the two phenolic compounds at different concentrations. The observation that both liquiritigenin and isoliquiritigenin were non-competitive inhibitors of MAO A implies that they might be combining to different sites of the enzyme to produce a 'head-end' complex, and are thus independent of the pre-binding of 5-HT.

Biologically, liquiritigenin (**1**) and isoliquiritigen (**2**) have been reported to possess pharmacological properties such as antioxidant action, superoxide scavenging[14], and anti-hyperlipidemic[15] property and inhibitiory action on the enzymes phosphodiesterase, aldose reductase, and hyaluronidase[6,16,17]. Phytochemically, compounds **1** and **2** have been demonstrated to be two of the main constituents of Chinese licorice (*Radix glycyrrhizae*) which is utilized for antiaging and sedative purposes in the traditional medical practice in China and Japan[18]. Interestingly, licorice was also found to be inhibitory against MAO without characterization of the corresponding active constituent[19]. Our findings indicate that both liquiritigenin and isoliquiritigenin could be the main MAO inhibitory principles in licorice, too.

REFERENCES

1 Silverman RB, Ding CZ, Gates KS. Design and mechanism of monoamine oxidase inactivators from an organic chemical perspective. In: Testa B, Kyburz E, Fuhrer W, Giger R, editors. Perspectives in medicinal chemistry. Basel: Verlag Helvetica Chomica Acta; 1993. p 73-86.

2 Kanazawa I. Short review on monoamine oxidase and its inhibitors. Eur Neurol 1994; 34 Suppl 3: 36-9.

3 Kong LD, Cheng CHK, Tan RX. Monoamine oxidase inhibitiors from Rhizoma of *Coptis chinensis*. Planta Med 2000; 66: in press.

4 Jiangsu College of New Medicine. The dictionary of the traditional Chinese medicine. Shanghai: Shanghai Scientific & Technical Publishers; 1977. p 357-9.

5 Wu ZY, Zhou TY, Xiao PG. Xinhua Bencao Gangyao (Book I). Shanghai: Shanghai Scientific & Technical Publishers; 1988. p 163.

6 Aida K, Tawata M, Shindo H, Onaya T, Sasaki H, Yamaguchi T, *et al*. Isoliquiritigenin: a new aldose reductase inhibitor from *Glycyrrhizae radix*. Planta Med 1990; 56: 254-8.

7 Yahara S, Ogata T, Saijo R, Konishi R, Yamahara J, Miyahara K, *et al*. Isoflavan and related compounds from Dalbergia odorifera. Chem Pharm Bull 1989; 37: 979-87.

8 Schurr A, Livne A. Different inhibition of mitochondrial monoamine oxidase from brain by hashish components. Biochem Pharmacol 1976; 25: 1201-3.

9 Fowler CJ, Ekdstedt B, Egashira T, Kinemuchi H, Oreland L. The interaction between human platelet monoamine oxidase its monoamine substrates and oxygen. Biochem Pharmacol 1979; 28: 3063-8.

10 Pizzinat N, Chan SLF, Remaury A, Morgan NG, Parini A. Characterization of monoamine oxidase isoforms in human islets of langerhans. Life Sci 1999; 65: 441-8.

11 Xu SY, Bian RL, Chen X. Methodology of pharmacological experiment. 2nd ed. Beijing: People's Medical Publishing House; 1994. p 510-1.

12 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193: 265-75.

13 Trevor Palmer. Understanding enzyme. 4th ed. Prentice Hall/Ellis Horwood; 1995. p 128-42.
14 Haraguchi H, Ishikawa H, Mizutani K, Tamura Y, Kinoshita T. Antioxidative and superoxide scavenging activities of retrochalcones in *Glycyrrhiza inflata*. Bioorg Med Chem 1998; 6: 339-47.
15 Jahromi MA, Ray AB. Antihyperlipidemic effect of flavonoids from *Pterocarpus marsupium*. J Nat Prod 1993; 56: 989-94.
16 Wegener JW, Nawrath H. Differential effects of isoliquiritigenin and YC-1 in rat aortic smooth muscle. Eur J Pharmacol 1997; 323: 89-91.
17 Kakegawa H, Matsumoto H, Satoh T. Inhibitory effects of some natural products on the activation of hyaluronidase and their anti-allergic actions. Chem Pharma Bull 1992; 40: 1439-42.
18 Nomura T, Fukai T. Phenolic constituents of licorice (*Glycyrrhiza* species). In: Herz W, Kirby, GW, Moore RE, Steglich W, Tamm Ch, editors. Progress in the chemistry of organic natural products; v 73. Wien: Springer-Verlag; 1998. p 1-140.
19 Hatano T, Fukuda T, Liu YZ, Noro T, Okuda T. Phenolic constituents of licorice. IV. Correlation of phenolic constituents and licorice specimens from various sources, and inhibitory effects of licorice extracts on xanthine oxidase and monoamine oxidase. Yakugaku Zasshi 1991; 111: 311-31.

串果藤中甘草素和异甘草素对大鼠单胺氧化酶的体外抑制作用[1]

潘 宣[2,3], 孔令东[2,4], 张 勇[2], 郑汉其[4], 谭仁祥[2,5] ([2]南京大学功能生物分子研究所 国家医药生物技术重点实验室, 南京 210093, 中国; [3]兰州医学院药学系, 兰州 730000, 中国; [4]香港中文大学生物化学系, 中国)

关键词 甘草素; 异甘草素; 串果藤; 木通科; 单胺氧化酶

目的: 研究串果藤中甘草素和异甘草素体外对单胺氧化酶A型和B型的抑制作用. **方法**: 根据不同的离心速度制备大鼠全脑粗线粒体作为单胺氧化酶的酶源; 分别以5-羟基[侧链-2-^{14}C]色胺肌酸硫酸盐([^{14}C]5-HT)和2-苯基[1-^{14}C]乙基胺盐酸盐([^{14}C]β-PEA)为单胺氧化酶A型和B型放射性底物, 用液体闪烁技术, 研究甘草素和异甘草素酶抑制作用和抑制类型. **结果**: 甘草素和异甘草素对单胺氧化酶A型和B型均具有抑制作用, 呈良好的量效关系, 对单胺氧化酶A型的IC_{50}(95 %的可信限)分别为32 (26-36) μmol/L和13.9 (12.8-15.6) μmol/L, 对单胺氧化酶B型的IC_{50}值分别为104.6 (89.0-118.9) μmol/L和47.2 (39.5-54.5) μmol/L. 酶抑制特征曲线显示甘草素和异甘草素对单胺氧化酶A型呈非竞争性抑制, K_i值分别为31.5 μmol/L和14.3 μmol/L, 而对单胺氧化酶B型呈混合竞争性抑制, K_i值分别为164.7 μmol/L和62.2 μmol/L, K_I值分别为15.2 μmol/L和9.3 μmol/L. **结论**: 甘草素和异甘草素体外对单胺氧化酶A型呈非竞争性抑制作用, 对单胺氧化酶B型呈混合竞争性抑制作用.

(责任编辑 吕 静)