BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 297-301 (2005)

Studies of Chemical Constituents and Their Antioxidant Activities From *Astragalus mongholicus Bunge*

DE-HONG YU, YONG-MING BAO, CHAO-LIANG WEI, AND LI-JIA AN[1]

Department of Environment and Biology, Dalian University of Technology, Dalian 116024, Liaoning, China

Objective To evaluate the antioxidant activities of different chemical constituents from *Astragalus mongholicus Bunge* and their protection against xanthine (XA)/xanthine oxidase (XO)-induced toxicity in PC12 cells. **Methods** The compounds of *Astragalus mongholicus Bunge* were isolated by chromatography and the structures were elucidated on the basis of spectral data interpretation. Their antioxidant activities were detected by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities in a cell-free system. Meanwhile, the effects against XA/XO-induced toxicity were assessed using MTT assay in PC12 cells. **Results** Ten principal constituents were isolated and identified as formononetin (I), ononin (II), calycosin (III), calycosin-7-O-β-D-glucoside (IV), 9,10-dimethoxypterocarpan-3-O-β-D-glucoside (V), adenosine (VI), pinitol (VII), daucosterol (VIII), β-sitoster (IX) and saccharose (X) from *Astragalus mongholicus Bunge.* The compounds I, III, and IV scavenged DPPH free radicals *in vitro*. Formononetin and calycosin were found to inhibit XA/XO-induced cell injury significantly, with an estimated EC_{50} of 50 ng/mL. **Conclusion** Compound Ⅱ, Ⅵ, and Ⅶ are first reported in this plant. Calycosin exhibits the most potent antioxidant activity both in the cell-free system and in the cell system.

Key words: *Astragalus mongholicus Bunge*; Constituents; Antioxidant; PC12; Toxicity

INTRODUCTION

Astragalus membranaceus Bunge, known as Huangqi in China, is the most important tonic in Traditional Chinese Medicine to reinforce "qi" (vital energy), to strengthen the superficial resistance, and to promote the discharge of pus and the growth of new tissues[1]. It is also an antiperspirant, a diuretic, and is used for treatment of nephritis and diabetes[2-3]. Polysaccharides, saponins, flavonoids, amino acid and choline have been isolated from this plant[4-6]. Recently there are reports on its neuroprotective activities[7-8]. We attempted to evaluate the antioxidant activities of different chemical constituents from *Astragalus mongholicus Bunge* and their protection against xanthine (XA)/xanthine oxidase (XO)-induced toxicity in PC12 cells. Meanwhile the relationship between the structures and the antioxidant activities was analyzed in the paper.

MATERIALS AND METHODS

Materials

The plant materials used in this study were the dried roots of *A. mongholicus* obtained from Dalian Pharmacy (Dalian, China). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylterazolium bromide (MTT) and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were obtained from Gibco BRL. Silica gel GF_{254} for thin layer chromatography and Silica gel for column chromatography were from Qingdao Haiyang Chemical Co. Ltd. Sephadex LH-20 was from Amersham Pharmacia Biotech. All the solvents used in the isolation were of analytical purity.

^{1}H NMR and ^{13}C NMR spectra were recorded using INOVA-400 with TMS as the inner standard. IR analysis was completed by Nicolet 20D×B FT-IR spectrograph with KBr planar. FAB-MS was obtained on a HP1100 MSD spectrometer. Mps were determined by X-4 digital melting apparatus and uncorrected.

Extraction and Isolation[9]

The powdered materials (10 kg) were extracted with 90% ethanol by maceration at room temperature. The alcoholic solution was concentrated at 60℃ under vacuum. The concentrated extract was diluted with water. The water solution was successively

[1]Correspondence should be addressed to Li-Jia AN, E-mail: bioeng@dlut.edu.cn

Biographical note of the first author: De-Hong YU, female, born in 1971, Ph. D. degree, majoring in the fields of phytochemistry and pharmacology.

[1]Correspondence should be addressed to Li-Jia AN, E-mail: bioeng@dlut.edu.cn

Biographical note of the first author: De-Hong YU, female, born in 1971, Ph. D. degree, majoring in the fields of phytochemistry and pharmacology.

0895-3988/2005
CN 11-2816/Q

extracted with petroleum ether, ethyl acetate, and finally *n*-butanol. The petroleum ether layer (20 g) was subjected to chromatography using a silica gel column eluted with a step wise gradient from petroleum ether–acetone (100:1) to acetone, compounds VII (339.2 mg) and IX were obtained, respectively. The n-butanol extract (25 g), in which sugar was removed by foramen magnum resin D101, was purified by the column chromatography over silica gel and Sephadex LH20 and repeated crystallization, compounds II (22.6 mg), IV (39.4 mg) and VI (28.5 mg) were gained. The ethyl acetate extract (60 g) was repeatedly subjected to silica gel column chromatography and yielded compounds I (243.7 mg), III (324.0 mg), V (76.2 mg), VII (339.5 mg) and VIII. Compound X in water extract came out for long time static putting.

Assay for DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of the isolated isoflavonoids were tested according to the method previously described by Dok-Go *et al.*[10]. In brief, reaction mixtures containing various concentrations of the principal constituents dissolved in DMSO and 50 μmol/L DPPH ethanol solutions in a 96-well microtiter plate were incubated at 37℃ for 30 min and absorbance was measured at 517 nm. Percent scavenging activity was determined in comparison with the vehicle treated control group and calculated according to the equation below.

Inhibition (%) = $(1\text{-}OD_{sample}/OD_{control}) \times 100$

Cell Culture and Viability Assessment[11]

PC12 cells, a rat pheochromocytoma cell line, were cultured in DMEM supplemented with 5% fetal bovine serum, 10% horse serum and 50 μg/mL penicillin/streptomycin in a humidified incubator at 37℃ and 5% CO_2. For measurement of viability and generation of reactive oxygen intermediates, cells were seeded in 96-well plates. Tested principal constituents were dissolved in dimethylsulfoxide (DMSO) (final culture concentration in culture was less than 0.1%). For the cell viability assays, 24 h after seeding, the cultures were pretreated with a test compound 12 h before exposure to XA (100 μmol/L)/XO (25 mU/mL) to induce injuries and then incubated for a further 9 h. The cultures were assessed using the MTT assay, which could reflect the cellular integrity by estimating mitochondrial succinate dehydrogenase function. The absorption values at 570-630 nm were detected using an automatic microtiter plate reader. Cell viability was calculated as 100× (OD of XA/XO and principles-treated cultures−OD of XA/XO-insulted cultures)/ (OD of control cultures−OD of XA/XO-nsulted cultures).

RESULTS

Compound Identification

In the present study, the 10 principal constituents isolated from *Astragalus mongholicus Bunge* were identified. They were formononetin (I), ononin (II), calycosin (III), calycosin-7-O-β-D-glucoside (IV), 9,10-dimethoxypterocarpan-3-O-β-D-glucoside (V), adenosine (VI), pinitol (VII), daucosterol (VIII), β-sitosterol (IX) and saccharose (X), respectively. The compound II, VI and VII were first reported in this plant.

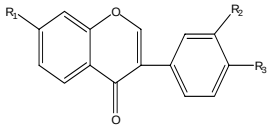
Formononetin: Yellowish needle. mp. 267℃. FAB-MS m/z:269[M+H]. IR$\nu_{max}cm^{-1}$: 3129 (-OH), 1639 (conjugation＞C=O), 1608, 1569, 1513 (-Ar), 2980, 2835, 1453, 1385 (-CH_3). ^{1}H-NMR (DMSO- d_6, δppm): 8.34 (1H, s, H-2,), 7.97 (1H, d, J=8.8Hz, H-5), 7.50 (2H, d, J=8.8Hz, H-2', 6'), 6.98 (2H, d, J=8.8Hz, H-3', 5'), 6.94 (1H, dd, J=8.6Hz, 2.4Hz, H-6), 6.88 (1H, d, J=2.0Hz, H-8), 10.84 (7-OH), 3.79 (3H, s, OCH_3). The data of ^{13}C-NMR are summarized in Table 1. The physical properties and spectral data were the same as reported in literature[12-13].

Ononin: White raphide. mp. 214℃-216℃. FAB-MS m/z: 431 [M+H]. IR$\nu_{max}cm^{-1}$: 3398(-OH), 1638 (conjugation＞C=O), 1608, 1569, 1513(-Ar), 2980, 2835, 1453, 1385(-CH_3). ^{1}H-NMR (DMSO- d_6, δppm): 8.44 (1H, s, H-2), 8.05 (1H, d, J=8.8Hz, H-5), 7.52 (2H, d, J=8.8Hz, H-2', 6'), 7.25 (1H, d, J=0.8, H-8), 7.14 (1H, dd, J=8.8Hz, 2.0Hz, H-6), 6.99 (2H, d, J=8.4Hz, H-3', 5'), 3.79 (3H, s, OCH_3), Glu: 5.11 (1H, d, J=6.8Hz, H-1). The data of ^{13}C-NMR are shown in Table 1. The physical properties and spectral data were almost the same as reported in reference[13].

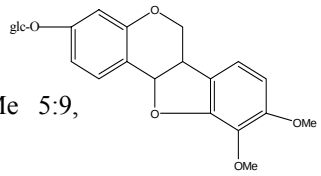
Calycosin: Yellowish needle. mp. 254℃-256℃. FAB-MS m/z: 285[M+H]. IRν_{max}: 3168(-OH), 1624 (conjugation＞C=O), 1572, 1509(-Ar), 2972, 2844, 1445, 1380(-CH_3). ^{1}H-NMR (DMSO-d_6, δppm): 8.29 (1H, s, H-2), 7.97 (1H, d, J=8.8Hz, H-5), 7.06 (1H, s, H-2'), 6.94 (1H, d, J=8.8Hz, H-8), 6.88 (1H, s, H-5', H-6'), 3.80 (3H, s, -OCH_3). The data of ^{13}C-NMR are shown in Table 1. The structure was confirmed in comparison with literature data[13].

Calycosin-7-O-β-D-glucoside: Yellowish crystal. mp. 232℃-235℃. FAB-MS m/z [M+Cl^-]: 481. Molecular formula: $C_{22}H_{22}O_{10}$. ^{1}H-NMR(DMSO- d_6, δppm): 8.40 (1H, s, H-2), 8.06 (1H, dd, J=8.8Hz, H-5), 7.16 (1H, dd, J=8.8 Hz, 2.2 Hz, H-6), 7.24 (1H, d, J=8.8Hz, H-8), 7.07 (1H, s, H-2'), 6.97 (1H, s, H-5',

H-6'), 3.80 (3H, s, OCH_3), Glu: 5.11 (1H, d, J=4.4Hz, H-1"). The data of ^{13} C-NMR are shown in Table 1. The physical properties and spectral data were almost the same as presented in references[13].



1:Formononetin R_1=OH R_2=H R_3=OMe
2:Ononin R_1=Oglc R_2=H R_3=OMe
3:Calycosin R_1=R_2=OH R_3=OMe
4:Calycosin-7-O-glc R_1=Oglc R_2=OH R_3=OMe 5:9, 10-dimethoxypterocarpan-3-O-β-D-glucoside
Structures of constituents 1---5



9, 10-dimethoxypterocarpan-3-O-β-D-glucoside: White amorphous powder, mp. 132-135℃. API-ES m/z [M+H]: 463. IRν_{max}cm^{-1}: 3525, 3413, 1618, 1585, 1497, 1459, 1268, 1081. ^{1}H-NMR (DMSO-d_6, δppm): 7.41 (1H, d, J=8.5Hz, 1-H), 7.00 (1H, d, J=8.0Hz, 7-H), 6.71 (1H, dd, J=8.8 Hz, 2.2Hz, 2-H), 6.55 (1H, d, J=2.2Hz, 4-H), 6.52 (1H, d, J=8.2Hz, 8-H), 5.62 (1-H, d, J=6.4Hz, 11a-H), 4.27 (1H, d, J=6.4Hz, 6-H), 3.72 (3H, s, OMe), 3.70 (3H, s, OMe), 3.65 (1H, m, 6a-H).^{13} C-NMR (DMSO-d_6, δppm): 132.02 (C-1), 110.46 (C-2), 158.52 (C-3), 103.97 (C-4), 156.16 (C-4a), 66.15 (C-6), 39.9 (C-6a), 121.58 (C-6b), 118.78 (C-7), 105.05 (C-8), 152.71 (C-9), 133.32 (C-10), 151.00 (C-11), 78.21 (C-11a), 114.02 (C-11b), 56.06 (OMe), 59.90 (OMe), 100.25 (C-1'), 73.17 (C-2'), 76.51 (C-3'), 69.64 (C-4'), 77.05 (C-5'), 60.63 (C-6'). The physical properties and spectral data were almost the same as published in references 14 and 15.

TABLE 1

^{13}C-NMR Data for Compound I-IV (solvent: DMSO-d_6)

C	I	II	III	IV
C-2	153.2	153.7	153.1	153.6
C-3	124.2	124.0	123.4	123.6
C-4	174.6	174.7	174.6	174.6
C-5	127.3	127.0	127.3	127.0
C-6	115.2	115.7	115.1	115.6
C-7	162.6	161.5	162.4	161.4
C-8	102.1	103.4	102.1	103.4
C-9	157.4	157.1	157.4	157.0
C-10	116.6	118.5	116.7	118.5
C-1’	123.2	123.4	124.7	124.4
C-2’	130.1	130.1	116.4	116.4
C-3’	113.6	113.7	145.9	146.0
C-4’	159.0	159.0	147.5	147.6
C-5’	113.6	113.7	112.0	112.0
C-6’	130.1	130.1	119.7	119.7
OMe	55.1	55.1	55.7	55.7
glc				
1′′		100.0		100.0
2′′		73.0		73.1
3′′		76.3		76.5
4′′		69.5		69.6
5′′		77.2		77.2
6′′		60.5		60.6

Adenosine: White powder. mp. 232℃-235℃. Molecule formula: $C_{10}H_3N_5O_4$. FAB-MS m/z [M+H]: 268. ^{1}H-NMR (DMSO-d_6, δppm): 8.35 (1H, s, H-2), 8.14 (1H, s, H-2), 7.37 (2H, br.s, NH_2), 5.87 (1H, d, J=6.4Hz, Rib-H-1). ^{13}C-NMR (DMSO-d_6, δppm): 156.16 (C-6), 152.45 (C-2), 149.09 (C-4), 140.01 (C-8), 119.36 (C-5), 87.91 (C-1'), 85.93 (C-4'), 73.46 (C-2'), 70.62 (C-3'), 61.62 (C-5'). The structure was confirmed in comparison with literature data[16].

Pinitol: White powder. mp. 190.5℃. FAB-MS m/z [M+H]: 169. ^{13}C-NMR (DMSO-d_6, δppm): 83.75 (C-3), 72.54 (C-4), 72.36 (C-6), 71.92 (C-1), 70.84 (C-5), 70.02 (C-2), 59.63 (OMe). The spectral data were almost the same as presented in reference[17].

Daucosterol: White powder. mp. 268℃-272℃. API-ES m/z [M+Cl$^-$]: 611. ^{13}C-NMR (DMSO-d_6, δppm): 140.43 (C-10), 121.21 (C-4), 76.90 (C-3), 56.18 (C-17), 55.43 (C-14), 49.60 (C-8), 45.132 (C-24), 41.85 (C-13), 40.2 (C-12), 38.30 (C-5), 36.84 (C-1), 36.21 (C-9), 35.5 (C-20), 33.34 (C-22), 31.40 (C-6, 7), 29.27 (C-16), 28.68 (C-27), 27.81 (C-2), 25.40 (C-23), 23.87 (C-15), 22.60 (C-25), 20.61 (C-11), 19.72 (C-21), 19.10 (C-19), 18.93 (C-28), 18.62 (C-29), 11.79 (C-18), 11.67 (C-26), 100.79 (C-1'), 75.45 (C-2'), 76.75 (C-3'), 70.05 (C-4'), 76.75 (C-5'), 61.06 (C-6'). The physical properties and spectral data were almost the same as published in reference[18].

β-sitosterol: White raphide. mp. 136℃-138℃ which was the same as the R_f of the standard β-sitosterol. The compound had the same R_f as the standard β-sitosterol.

Saccharose: White lamella crystal. mp. 184℃-185℃ which was the same as the R_f of the standard fructose. The compound had the same R_f as the standard fructose.

DPPH Radical Scavenging Activity

The results, shown in Table 2, demonstrated that the five isoflavonoids had their own elimination effects on organic free radicals. Calycosin had the strongest elimination effect with a 44.1% clearance rate of 100 μg/mL. Calycosin-7-O-β-D-glucoside and formononetin were less effective than calycosin, with a 31.9% clearance rate and an 11% clearance rate of

100 μg/mL, respectively. Ononin and 9, 10-dimethoxypterocarpan-3-O-β-D-glucoside did not show any antioxidant activity.

TABLE 2

Elimination Rate of the Principal Constituents at Different Concentrations in DPPH Free Group (% of control, $\bar{x} \pm s$)

	10 μg/mL	20 μg/mL	40 μg/mL	100 μg/mL
I	5.5±0.73	6.8±0.68	10.6±0.17	11±0.64
II	ND	ND	ND	ND
III	13.1±0.86	22.9±0.42	34.7±0.78	44.1±0.47
IV	12.3±0.6	17.7±0.27	23.6±0.13	34.5±0.37
V	ND	ND	ND	ND

Note. ND: not detected. I: formononetin; II: ononine; III: calycosin; IV: calycosin -7-O-glc; V: 9, 10-dimethoxypterocarpan-3-O-β-D-glucoside.

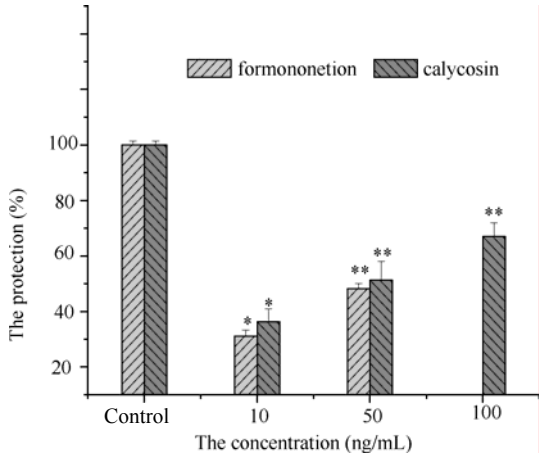


FIG. 1. Effects of formononetin and calycosin on XA/XO-induced toxicity in PC12 cells. Cells were seeded in 96 well plates. After pretreatment for 12 h at different concentrations of formononetin and calycosin, cultures were exposed to XA (100 μmol/L)/XO (25 mU/mL) for 9 h. At the end of each treatment cultures were stained with MTT as described. Data were presented as $\bar{x} \pm s$ (n=3). * P<0.05, ** P<0.01 vs. XA/XO treatment.

PC12 cells were employed to quantify the neuroprotective activity of the isolated compounds (Ⅰ-Ⅴ) using MTT assay (Fig. 1). Formononetin and calycosin could inhibit XA/XO-induced oxidant cell injury, with an estimated EC_{50} of 50 ng/mL. However, the other three isoflavonoids 9, 10-dimethoxypterocarpan-3-O-β-D-glucoside, ononin, calycosin-7-O-glc had no significant protective activity. Furthermore calycosine increased the cellular viability from the concentration 10 ng/mL to 100 ng/mL in a dose-dependent manner. Formononetin demonstrated the highest protective effect at the concentration 50 ng/mL. It deteriorated the cell damage instead of its protection at 100 ng/mL. The results illustrated that calycosin was the most potent neuronprotective compound in XA/XO-injured cells among the isolated principal constituents.

DISCUSSION

Traditionally, astragalus (*Astragalus mongholicus Bunge*) is used as one of the primary tonics in Chinese herbal medicine. In modern Chinese medicine it is widely used as an immune modulator, especially to support the immunity against various chronic degenerative diseases, and it is commonly used as an adjunctive therapy of chemo- and radiation therapy for cancer[1]. In our studies, compound II, VI, and VII were first found in this plant. Meanwhile, our research indicated that the isoflavonoids from *Astragalus mongholicus Bunge* possessed minor antioxidant activity in the cell-free system to DPPH free radicals due to their IC_{50} values more than 100 μg/mL. Calycosin exhibited the most potent scavenging activity with a 44.1% elimination rate at the concentration of 100 μg/mL. Formononetin and calycosin-7-O-β-D-glucoside had 11% and 34.5% activity respectively. However ononin and 9, 10-dimethoxypterocarpan-3-O-β-D-glucoside showed no effect on organic free radical scavenging. Therefore, we can possibly draw a conclusion that the 3'-hydroxy group in the B ring and 7-hydroxy group in the A ring are the crucial moiety to free radical scavenging and the former is more important than the latter[19]. Calycosin possesses the two hydroxy groups and shows the most potent activity. The inhibition of calycosin and formononetin on the XA/XO-induced toxicity illustrates that the antioxidant activity is contributed to their protection only in part because the effective concentrations in the cell system are far less than in the cell-free system. The other mechanisms must be existed and need to be further studied.

ACKNOWLEDGEMENT

We thank the Fine Chemical Laboratory of Dalian University of Technology for its IR, MS, and NMR assay. We are grateful for the great help of associate professor Lan XIANG in Pharmacology College, Shandong University.

REFERENCES

1. Roy, U. and Cathiroe P. (1999). Astragalus Roots. *American Herbal Pharmacopoeia and Therapeutic Conpendium*, August, 1-25.

2. Wang, Y. P., Li, X.Y., Song, C. Q., and Hu, Z. B. (2002). Effect of Astragaloside Ⅳ on T, B Lymphocyte Proliferation and Peritoneal Macrophage Function in Mice. *Acta phamacol. Sin*. **23**(3), 263-266.
3. Lei, H., Wang, B., Li, W. P., Yang, Y., Zhou, A. W., and Chen, M. Z. (2003). Anti-aging Effect of Astragalosides and its Mechanism of Action. Acta pharmacol Sin. **24**(3), 230-234.
4. Tian, H.Y. (1996). A Review on the Studies of Chemical Constituents of Astragalus. Journal of Yuannan Institute of the Nationalities (Natural Sciences Edition). **5**(1), 75-79.
5. Qi, Z. S. (1987). A Review on Chemical Constituents from Astragalus. Chinese Traditional and Herbal Drugs. **18**(5), 41-43, 23.
6. Chen, C.Y., Lu Y., and Chen, Z. N. (2001). Survey of Studies on Astragalus membranaceus var. mongolicus. Chinese Traditional and Herbal Drugs. **32**(6), 567-569.
7. He, X. H., Cheng, Y. L., and Shao Z. Y. (1998). Selection of Concentration of Radix Astragai against Anoxic Damages of Neurons in Culture. Stroke and Nervous Disease **5**, 1-3.
8. Chen, L. Y., Pe Z. H., Chao Y. T., Yi, Z. W., Yan M. T., and Zhao, J. F. (2002). The Protective Effect of Astragale on the Culture Neuron Cells. Clinical M*edical Journal of China* **9**, 164-165.
9. Long, Z. L., Xian, G. H., Michael, L.G. N., Jie, Y., Michael, C., Sheng, X. Q., and Geoffrey, A. C. (2000). Liquid Chromatography-electrospray Ionization Mass Spectrometry Study of the Flavonoids of the Roots of *Astralus mongholicus* and *A.membrabaceus. Journal of Chromatography A.* **876**, 87-95.
10. Dok-Go, H., Lee, K. H., Kim, H. J., Lee, E. H., Lee, J., Song, Y. S., Lee, Y. H., Jin, C., Lee, Y. S., and Cho, J. (2003). Neuroprotective Effects of Antioxidative Flavonoids, Quercetin, (+)-dihydroquercetin and Qquercetin 3-methyl ether, Isolated from *Opuntia ficus-indica* var. *saboten. Brain Reseach*. **965**, 130-136.
11. Li, J. Z. and Xing, Z. Z. (2000). Reactive Oxygen Species-Induced Apoptosis in PC12 Cells and Protective Effect of Biobalide. *The Journal of Pharmacology and Therapeutics*. **293**(3), 982-988.
12. Wang, Z. X., Ma, Q. F., and He Q. (1983). The Studies of Astragalus Chemical Constituents. *Chinese Traditional and Herbal Drugs*. **14**(3), 1-3
13. Dou, H., Fu T. J., and Zhang, F. (2002). Chemical Constituents of Huangqi Injection. *Natural Product Research and Development* **14**(6),14-17.
14. Lv, G. B., Lv, S. H., and Zhang, G. Q. (1984). Isolation and Identification of Flavone Analog from Astragalus. Chinese *Traditional and Herbal Drugs.* **15**(10), 20-22.
15. Song, C. Q., Zheng, Z. R., and Liu, D. (1997). Pterocarpans and Isoflavans from *Astragalus Membranaceus (Fisch.). Bunge*. *Acta Botanica Sinica.* **39**(12), 1169-1171.
16. Meng, X. Z., Ni, P. F., and Suo, H. (1981). Studies on the KUDIEZI Treatment to the Coronary Heart Disease. *Journal of Shenyang Pharmaceutical University* **14**(2), 32-35.
17. Yu, D. Q. and Yang, J. S. (1999). Analytical Chemistry Handbook: NMR Spectrum analysis. *Chemical Industry Press*, 1999. the 2nd Edition, 909.
18. Gu, X. Y., Zhang, H. Q., and Wang, N. H. (1999). The Studies on the Chemical Constituents from *Chinese Angelica* roots. *Journal of Plant Resources and Environment*. **8**(1), 1-5.
19. Kim, S. R., Park, M. J., Lee, M. K., Sung, S. H., Park, E. J., Kim, J., Kim, S. Y., Oh, T. H., Markelonis, G. J., and Kim, Y. C. (2002). Flavonoids of *Inula Britannica* Protect Culture Cells from Necrotic Cell Death Induced by Glutamate. *Free Radical Biology &Medicine* **32**(7), 506-604.

(*Received November* 20, 2004 *Accepted April* 4, 2005)