Natural Product Sciences
7(1) : 5~8 (2001)

Minor Constituents from the Roots of *Sophora flavescens*

Ju Sun Kim, Sang Jun Han, Ji Hye Byun, Yong Nan Xu, Sang Woo Yoo, Sam Sik Kang*, Kun Ho Son[1], Hyeun Wook Chang[2], and Hyun Pyo Kim[3]

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea
[1]Department of Food and Nutrition, Andong National University, Andong 760-749, Korea
[2]College of Pharmacy, Yeungnam University, Kyongsan 712-749, Korea
[3]College of Pharmacy, Kangwon National University, Chuncheon 200-749, Korea

Abstract – Lupenone, hexadecyl ferulate, (–)-sophocarpine and three isoflavonoids such as genistein, 3'-methoxydaidzein and calycosin were isolated from the roots of *Sophora flavescens*.
Key words – *Sophora flavescens*, Leguminosae, triterpene, ferulate, alkaloid, isoflavonoid.

Introduction

The roots of *Sophora flavescens* Ait. (Leguminosae) is a well-known Chinese herbal medicine used as a diuretic and for the treatment of acute dysentery, gastrointestinal hemorrhage, and eczema (Perry and Metzger, 1980; Huang, 1993). It is known to a number of quinolizidine alkaloids and flavonoids as major constituents (Tang and Eisenbrand, 1992; Kang *et al.* 2000). In a previous paper,[1)] we have reported the isolation of seven flavonoids and a coumarin, umbelliferone (Kang *et al.*, 2000). Further study of the root constituents led to the isolation of lupenone, hexadecyl ferulate, (–)-sophocarpine and three isoflavonoids such as genistein, 3'-methoxydaidzein and calycosin from this plant. This paper deals with the isolation and identification of these compounds on the basis of spectroscopic analysis.

Materials and Methods

General experimental procedures – Melting points were uncorrected. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer in KBr method. EI mass spectra were obtained on a Hewlett-Packard 5989B spectrometer. NMR spectra were measured on a Varian 2000 (300 MHz) instrument, and the chemical shifts were referenced to TMS. TLC was performed on silica gel $60F_{254}$ (Merck).

Plant material – The roots of *S. flavescens* Ait. were collected in August 1998, Yangku in Kangwon Province, Korea and authenticated by Dr. T.J. Kim of Korea Research Institute of Bioscience and Biotechnology in Taejon. A voucher specimen (SSK98002) was deposited in the herbarium of the Natural Products Research Institute, Seoul National University.

Extraction and isolation – The dry roots (3.65 kg) of *S. flavescens* were extracted three times with MeOH at room temperature. The MeOH extract was evaporated to dryness, and the dry residue was partitioned in succession between H_2O and CH_2Cl_2, and then EtOAc affording 128.4 g and 25.4 g of the respective extracts. A portion of the CH_2Cl_2 fraction (127 g) was subjected to silica gel column (10×150 cm) chromatography. Elution with CH_2Cl_2 with increasing amounts of MeOH (0.5%, 1-5%, 10%) and then MeOH gave 43 subfractions. The subfractions Nos. 4 and 15 were further purified by recrystallization from MeOH-CH_2Cl_2 to give **1** (16 mg) and sterol mixture, respectively. Subfraction No. 9 (0.1 g) was chromatographed over silica gel using hexane and then hexane-EtOAc (gradient) as eluant to yield **2** (72 mg). Subfraction No. 29 (5.6 g) was chromatographed over silica gel using hexane-EtOAc (gradient) as eluant to yield 22 subfractions (No. 29-1~29-22). Subfractions No. 29-12, 29-15 and 29-18 were recrystallized to give **3** (10 mg), **4** (8 mg) and **5** (12 mg), respectively. Subfractions No. 34 (6.1 g) was further chromatographed over silica gel using benzene-EtOAc (3:2) and then EtOAc to give **6** (15 mg).

*Author for correspondence.
[1)]The description of the isolation of (2*S*)-7,4'-dihydroxy-5-methoxy-8-(γ,γ-dimethylallyl)-flavanone as one of the new compound was erroneous. The compound has already been isolated from *S. angustifolia* (Komatsu *et al.*, 1970b).

Lupenone (**1**): colorless needles, recrystallized from MeOH-CH_2Cl_2. mp 169-170 °C, $[\alpha]_D^{23}$ + 63.5° (c 0.5, $CHCl_3$); IR ν_{max} (KBr): 1707 (CO), 1638 (C=C), 1458 (CH_2), 1381 (CH_3), 882 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.79 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.95 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.07 (6H, s, 2×CH_3), 1.68 (3H, s, CH_3), 4.57 (1H, d, *J* = 1.2 Hz, H-29), 4.69(1H, d, *J* = 1.8 Hz, H-29); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 218.23 (C-3), 150.86 (C-20), 109.37 (C-29), 54.88 (C-5), 49.75 (C-9), 48.20 (C-18), 47.93 (C-19), 47.32 (C-4), 42.97 (C-17), 42.86 (C-14), 40.74 (C-8), 39.95 (C-22), 39.59 (C-1), 38.12 (C-13), 36.85 (C-10), 35.49 (C-16), 34.14 (C-7), 33.53 (C-2), 29.79 (C-21), 27.39 (C-15), 26.62 (C-23), 25.11 (C-12), 21.43 (C-11), 21.02 (C-24), 19.65 (C-6), 19.28 (C-30), 17.99 (C-28), 15.96 (C-25), 15.76 (C-26), 14.45 (C-27); EI-MS *m/z* (rel. int., %): 424 (M^+, 100%), 409 (36), 381 (8), 368 (12), 355 (5), 342 (5), 313 (33), 245 (18), 218 (21), 205 (54), 189 (20), 175 (11).

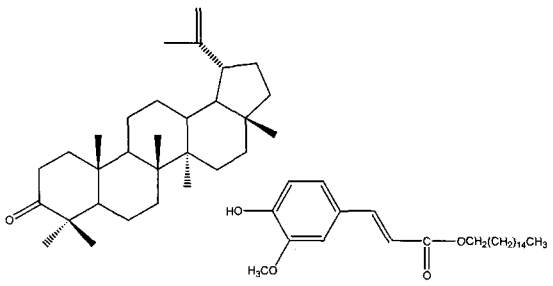
Hexadecyl ferulate (**2**): colorless needles, recrystallized from MeOH-CH_2Cl_2. mp 61-62°C, UV, λ_{max} (MeOH): 236 (log ε 4.23), 297 (sh, 4.32), 326 (4.48); IR, ν_{max} (KBr): 3410 (OH), 1717 (ester), 1636 (α,β-unsaturated C=O), 1607, 1512 (aromatic C=C), 1468, 1431, 1265, 1177, 976 (*trans* C=C), 843, 814, 721$(CH_2)_n$ cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, *J* = 6.3 Hz, CH_3), 1.26 [26H, s, $(CH_2)_{13}$], 1.70 (2H, m, CH_2CH_2O), 3.93 (3H, s, OCH_3), 4.19 (2H, t, *J* = 6.6 Hz, CH_2O), 6.29 (1H, d, *J* = 15.9 Hz, H-8), 6.92 (1H, d, *J* = 8.1 Hz, H-5), 7.03 (1H, d, *J* = 1.8 Hz, H-2), 7.07 (1H, dd, *J* = 1.8, 8.1 Hz, H-6), 7.61 (1H, d, *J* = 15.9 Hz, H-7); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 127.04 (C-1), 109.26 (C-2), 146.73 (C-3), 147.87 (C-4), 115.67 (C-5), 123.02 (C-6), 144.60 (C-7), 114.68 (C-8), 167.37 (C-9), 64.60 (C-1'), 22.67 (C-15'), 25.98 (C-3'), 28.76, 29.29, 29.34, 29.53, 29.58, 29.64, 29.68 (CH_2), 31.91 (C-14'), 14.10 (C-16'), 55.91 (OCH_3); EI-MS *m/z* (rel. int., %): 418 (M^+, 100%), 194 [(M - $C_{16}H_{32}$)$^+$, 75], 177 [(M - $C_{16}H_{32}O$)$^+$, 27], 150 [(M - $C_{17}H_{32}O_2$)$^+$, 19], 137 (24), 117 (8), 89 (9).

Genistein (**3**): yellowish needles, recrystallized from hexane-EtOAc. mp 297-298°C, IR ν_{max} (KBr): 3414 (OH), 1653 (α,β-unsaturated C=O), 1616, 1570 (aromatic C=C), 1520, 1505, 1312, 1204, 1181, 1044, 841, 791 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 6.21 (1H, d, *J* = 2.1 Hz, H-6), 6.38 (1H, d, *J* = 2.1 Hz, H-8), 6.80 (2H, d, *J* = 8.7 Hz, H-3′, 5′), 7.36 (2H, d, *J* = 8.7 Hz, H-2', 6'), 8.32 (1H, s, H-2), 9.58, 10.87, 12.95 (1H each, br s, OH); EI-MS *m/z* (rel. int., %): 270 (M^+, 100%), 241 (4), 153 [$(A_1+H)^+$, 43], 118 (B_1^+, 20), 84 (46).

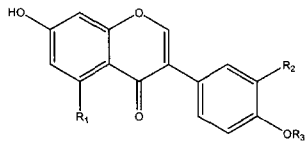
3'-Methoxydaidzein (**4**): yellowish needles, recrystallized from hexane-EtOAc. mp 251-253°C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.78 (3H, s, OCH_3), 6.80 (1H, d, *J* = 8.4 Hz, H-5'), 6.85 (1H, d, *J* = 2.4 Hz, H-8), 6.93 (1H, dd, *J* = 2.4, 8.7 Hz, H-6), 6.98 (1H, dd, *J* = 2.1, 8.4 Hz, H-6'), 7.15 (1H, d, *J* = 2.1Hz, H-2'), 7.96 (1H, d, *J* = 8.7 Hz, H-5), 8.32 (1H, s, H-2), 9.09, 10.78 (1H each, br s, OH); ^{13}C- NMR (75.5 MHz, DMSO-d_6) δ: 55.91 (OCH_3), 102.32 (C-8), 113.49 (C-2'), 115.41 (C-6, 5'), 116.87 (C-10), 121.75 (C-6′), 123.22 (C-1'), 123.74 (C-3), 127.54 (C-5), 146.67 (C-4′), 147.39 (C-3'), 153.29 (C-2), 157.61 (C-9), 162.73 (C-7), 174.93 (C-4); EI-MS *m/z*: 284 (M^+, 100%), 269 (6), 241 (10), 213 (19), 148 (B_1^+, 12), 137 [$(A_1+H)^+$, 27], 105 (20).

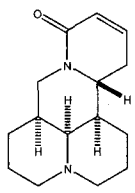
Calycosin (**5**): yellowish needles, recrystallized from hexane-EtOAc. mp 249-250°C, ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.88 (3H, s, OCH_3), 6.90 (1H, d, *J* = 2.1 Hz, H-8), 6.98 (1H, d, *J* = 8.4 Hz, H-5'), 7.00 (1H, dd, *J* = 2.1, 8.7 Hz, H-6), 7.07 (1H, dd, *J* = 2.1, 8.4 Hz, H-6'), 7.17 (1H, d, *J* = 2.1Hz, H-2′), 8.07 (1H, d, *J* = 8.7 Hz, H-5), 8.16 (1H, s, H-2), 7.59, 9.56 (1H each, br s, OH); ^{13}C-NMR (75.5MHz, DMSO-d_6) δ: 56.27 (OCH_3), 103.16 (C-8), 112.12 (C-5'), 115.63 (C-6), 116.89 (C-2'), 118.61 (C-10), 121.08 (C-6′), 125.05 (C-1'), 126.30 (C-3), 128.49 (C-5), 147.05 (C-3'), 148.26 (C-4'), 153.43 (C-2), 158.72 (C-9), 163.14 (C-7), 175.56 (C-4); EI-MS *m/z* (rel. int., %): 284 (M^+, 100%), 269 (16), 241 (21), 213 (34), 148 (B_1^+, 5), 137 [$(A_1+H)^+$, 29], 105 (34).

(−)-Sophocarpine (**6**): colorless needles, recrystallized from hexane. mp 45°C, $[\alpha]_D^{19}$-22.5° (c 0.5, EtOH); IR ν_{max} (KBr): 2945, 2868, 2810 (Bohlman band), 1659 (α,β-unsaturated lactam), 1595 (C=C), 1437, 1096, 824 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.16 (1H, dddd, *J* = 2.4, 3.8, 8.7, 18.6 Hz, H-12β), 2.59 (1H, dddd, *J* = 1.8, 5.1, 6.6, 18.6 Hz, H-12α), 2.98 (1H, dd, *J* = 12.6, 12.6 Hz, H-17β), 3.83 (1H, m, H-11), 3.89 (1H, dd, *J* = 4.7, 12.6 Hz, H-17α), 5.71 (1H, dt, *J* = 1.8, 9.9 Hz, H-14), 6.54 (1H, ddd, *J* = 3.9, 4.8, 9.9 Hz, H-13); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 57.2 (C-2, 10), 20.9 (C-3), 27.6 (C-4), 34.5 (C-5), 63.6 (C-6), 41.4 (C-7), 26.4 (C-8), 20.6 (C-9), 51.4 (C-11), 27.2 (C-12), 137.4 (C-13), 124.5 (C-14), 165.6 (C-15), 41.9 (C-17); EI-MS *m/z* (rel. int., %): 246 (M^+, 81%), 245 (100), 231 (2), 217 (8), 203 (21), 177 (18), 160 (8), 150 (34), 138 (34), 136 (29), 122 (15), 96 (31).



1 2





3 R_1 = OH, R_2 = R_3 = H

4 R_1 = H, R_2 = OCH_3, R_3 = H

5 R_1 = H, R_2 = OH, R_3 = CH_3

6

Results and Discussion

The MeOH extract of the roots of *S. flavescens* was suspended in water and then successively partitioned with CH_2Cl_2 and EtOAc. The CH_2Cl_2 fraction was subjected to silica gel column chromatography to give 43 subfractions. Repeated column chromatography followed by recrystallization of the subfractions gave six minor compounds in addition to the sterol mixture. Three minor flavonoids were identified as genistein (**3**) (Arisawa *et al.*, 1980), 3'-methoxydaidzein (**4**) (Yahara *et al.*, 1989) and calycosin (**5**) (Kobayashi *et al.*, 1985) by spectral data. Genistein (**3**) and calycosin (**5**) were isolated from several *Sophora* species (Min *et al.*, 1999; Tanaka *et al.*, 1998; Shirataki *et al.*, 1997; 1991; 1988; Iinuma *et al.*, 1995b; Komatsu *et al.*, 1970a), but the isolation of 3'-methoxydaidzein (**4**) is the first report from this species. A sterol mixture, mp 149-150 °C, was also identified as a mixture of stigmasterol, β-sitosterol and campesterol by GC/MS. An alkaloid was identified as (−)-sophocarpine (**6**) by means of spectral analysis (Xiao *et al.*, 1999; Kang and Son, 2000a).

Compound **1** was obtained as colorless needles, mp 169-170 °C. The IR spectrum of **1** showed strong absorptions at 1707 (C=O), 1638 (C=C) and 882 cm^{-1} (exo cyclic double bond) which suggested to be a triterpenoid skeleton. The ^1H NMR spectrum revealed the presence of six tertiary methyl groups and an isopropylidene group (δ 1.68, 3H, s; δ 4.57, 1H, d, J = 1.2 Hz and δ 4.69, 1H, d, J = 1.8 Hz). EI-MS gave a fragmentation pattern typical of lupane-type triterpenes at *m/z* 218 and 189 derived from rings D/E and *m/z* 205 from rings A/B (Kang and Son, 2000b). Therefore, **1** was identified as lupenone which was further supported by ^{13}C-NMR data (Ahmad and Atta-ur-Rahman, 1994).

Compound **2** showed the presence of OH (3410 cm^{-1}), ester (1717 cm^{-1}), α,β-unsaturated C=O (1636 cm^{-1}), aromatic C=C (1607 and 1512 cm^{-1}) and $(CH_2)_n$ (721 cm^{-1}) in its IR spectrum which suggested to be an aromatic ester of long-chain alcohol. This is further corroborated by its UV spectrum which was virtually identical to that of eicosanoyl caffeate (Kang and Kim, 1987). The ^1H NMR spectrum showed signals for a terminal methyl (δ 0.88), long-chain methylenes $(CH_2)_n$ (δ 1.26), a methoxyl (δ 3.93), a *trans* double bond [δ 6.29 and 7.61 (1H each, d, J = 15.9 Hz)] and 1,3,4-trisubstituted benzene ring protons [δ 6.92 (1H, d, J = 8.1 Hz), 7.03 (1H, d, J = 1.8 Hz) and 7.07 (1H, dd, J = 1.8, 8.1 Hz)]. EI-MS showed a molecular ion at *m/z* 418 and a typical McLafferty rearrangement peak at *m/z* 194 (ferulic acid). Thus, the structure of **2** was concluded to be hexadecyl ferulate (Bernards and Lewis, 1992). Although betulin from *S. japonica* (Shibata and Nishikawa, 1963) and lupeol from *S. subprostrata* (Shibata and Nishikawa, 1961) as well as caffeic acid ester mixture from several *Sophora* species (Tanaka *et al.*, 1998; Iinuma *et al.*, 1995a; Iinuma *et al.*, 1995c; Komatsu *et al.*, 1978; Komatsu *et al.*, 1970a) have been isolated, this is the first report on the isolation of lupenone as well as ferulic acid ester from this species. Very recently, isolation of sinapic acid hexadecyl ester from *S. flavescens* has also been reported (Zhang *et al.*, 2000).

Acknowledgements

This work was supported by the research fund from KOSEF (Project No. 98-0403-11-01-3). SSK gratefully acknowledges the financial assistance of the Ministry of Education through BK21 project.

References

Ahmad, V.U. and Atta-ur-Rahman, Handbook of natural products data, Vol. **2**, Pentacyclic triterpenoids, Elsevier, London, pp. 1029-1030, 1994.

Arisawa, M., Kyozuka, Y., Hayashi, T., Shimizu, M.,

and Morita, N., Isoflavonoids in the roots of *Thermopsis fabacea* D.C. (Leguminosae). *Chem. Pharm. Bull.* **28**, 3686-3688 (1980).

Bernards, M.A. and Lewis, N.G., Alkyl ferulates in wound healing potato tubers. *Phytochemistry* **31**, 3409-3412 (1992).

Huang, K. C., The Pharmacology of Chinese Herbs, CRC Press, Boca Raton, pp. 63-66, 1993.

Iinuma, M., Ohyama, M., and Tanaka, T., Flavonoids in roots of *Sophora prostrata*. *Phytochemistry* **38**, 539-543 (1995a).

Iinuma, M., Ohyama, M., Tanaka, T., Shirataki, Y., and Burandt, C.L., Isoflavonoids in roots of *Sophora secundiflora*. *Phytochemistry* **39**, 907-910 (1995b).

Iinuma, M., Ohyama, M., Kawasaka, Y., and Tanaka, T., Flavonoid compounds in roots of *Sophora tetraptera*. *Phytochemistry* **39**, 667-672 (1995c).

Kang, S.S. and Kim, C.M., Studies on the Korean indigenous plants. Isolation of eicosanoyl cafferate from *Echinosophora koreensis*. *Arch. Pharm. Res.* **10**, 67-68 (1987).

Kang, S.S., Kim, J.S., Son, K.H., Chang, H.W., and Kim, H.P., A new prenylated flavanone from the roots of *Sophora flavescens*. *Fitoterapia* **71**, 511-515 (2000).

Kang, S.S. and Son, K.H., Structure Elucidation of Natural Products by Spectroscopy, Seoul National University Press, Seoul, pp. 757-759, 2000a.

Kang, S.S. and Son, K.H., Structure Elucidation of Natural Products by Spectroscopy, Seoul National University Press, Seoul, pp. 209-211, 2000b.

Kobayashi, M., Noguchi, H. and Sankawa, U., Formations of chalcones and isoflavones by callus culture of *Glycyrrhiza uralensis* with different production patterns. *Chem. Pharm. Bull.* **33**, 3811-3816 (1985).

Komatsu, M., Tomimori, T., Hatayama, K., and Makiguchi, Y., Studies on the constituents of *Sophora* species. III. Constituents of the root of *Sophora subprostrata* Chun et T. Chen. (3). *Yakugaku Zasshi* **90**, 459-462 (1970a).

Komatsu, M., Tomimori, T., Hatayama, K., and Mikuriya, N., Studies on the constituents of *Sophora* species. IV. Constituents of the root of *Sophora angustifolia* Sieb. et Zucc. (1). *Yakugaku Zasshi* **90**, 463-468 (1970b).

Komatsu, M., Yokoe, I. and Shirataki, Y., Studies on the constituents of *Sophora* species. XIII. Constituents of the aerial parts of *Sophora tomentosa* L. (2). *Chem. Pharm. Bull.* **26**, 3863-3870 (1978).

Min, B.K., Oh, S.R., Lee, H.-K., Takatsu, K., Chang, I.-M., Min, K.R., and Kim, Y.S., Sophoricoside analogs as the IL-5 inhibitors from *Sophora japonica*. *Planta Med.* **65**, 408-412 (1999).

Perry, L. M. and Metzger, J., Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses, The MIT Press, Cambridge, p. 226, 1980.

Shibata, S. and Nishikawa, Y., Studies on the constituents of Japanese and Chinese Crude drugs. VII., On the constituents of the roots of *Sophora subprostrata* Chun et T. Chen. (2) and *Sophora japonica* L. (1). *Chem. Pharm. Bull.* **11**, 167-177 (1963).

Shibata, S. and Nishikawa, Y., Studies on the constituents of Japanese and Chinese Crude drugs. V., On the constituents of the root of *Sophora subprostrata*. *Yakugaku Zasshi* **81**, 1635-1639 (1961).

Shirataki, Y., Yokoe, I., Noguchi, M., Tomimori, T., and Komatsu, M., Studies on the Constituents of *Sophora* species. XXII. Constituents of the root of *Sophora moorcroftiana* Benth. *ex* Baker. (1). *Chem. Pharm. Bull.* **36**, 2220-2225 (1988).

Shirataki, Y., Noguchi, M., Yokoe, I., Tomimori, T., and Komatsu, M., Sophora-flavanones H, I and J, flavonostilbenes from *Sophora moorcroftiana*. *Chem. Pharm. Bull.* **39**, 1568-1572 (1991).

Shirataki, Y., Yoshida, S., Sugita, Y., Yokoe, I., Komatsu, M., Ohyama, M., Tanaka, T., and Iinuma, M., Isoflavanones in the roots of *Sophora secundiflora*. *Phytochemistry* **44**, 715-718 (1997).

Tanaka, T., Ohyama, M., Iinuma, M., Shirataki, Y., Komatsu, M., and Burandt, C.L., Isoflavonoids from *Sophora secundiflora*, *S. arizonica* and *S. gypsophila*. *Phytochemistry* **48**, 1187-1193 (1998).

Tang, W. and Eisenbrand, G., Chinese Drugs of Plant Origin, Springer-Verlag, Berlin, pp. 931-943, 1992.

Xiao, P., Kubo, H., Komiya, H., Higashiyama, K., Yan, Y.-N., Li, J.-S., and Ohmiya, S., Lupin alkaloids from seeds of *Sophora viciifolia*. *Phytochemistry* **50**, 189-193 (1999).

Yahara, S., Ogata, T., Saijo, R., Konishi, R., Yamahara, J., Miyahara, K., and Nohara, T., Isoflavan and related compounds from *Dalbergia odorifera*. I. *Chem. Pharm. Bull.* **37**, 979-987 (1989).

Zhang, J.-H., Zhao, Y.-Y., Liu, Q.-X. and Ye, X.-J., Studies on the chemical constituents from *Sophora flavescens* Ait. *China J. Chin. Mat. Med.* **25**, 37-39 (2000).

(Accepted January 23, 2001)