Journal of Food and Drug Analysis

Volume 5 | Issue 4 Article 14

1997

Tannins and related compounds from Erodium moschatum (L.) L'Her

Journal of Food and Drug Analysis
1997. 5(4) : 347-354

藥物食品分析
第五卷 第四期

Tannins and Related Compounds from *Erodium moschatum* (L.) L'Her

JER-HUEI LIN* AND MEI-FAN LIN

National Laboratories of Foods and Drugs,
Department of Health, Executive Yuan,
161-2, Kuen Yang Street, Nankang, Taipei, Taiwan, R. O. C.

ABSTRACT

***Erodium moschatum* is a newly naturalized plant in Taiwan. From the aqueous acetone extract of the fresh herb, seventeen tannins and related compounds were isolated. They included five phenolcarboxylic acids and ester including: protocatechuic acid (1), gallic acid (2), methyl gallate (3), caffeic acid (4), brevifolincarboxylic acid (5); four gallotannins: 3-*O*-galloylshikimic acid (6), 3,4-di-*O*-galloylshikimic acid (7), 3,5-di-*O*-galloylshikimic acid (8), 1-*O*-galloyl-β-D-glucose (9); six ellagitannins and other related compounds which include corilagin (10), furosin (11), geraniin (12), acetonylgeraniin A (13), methyl gallate 3-*O*-β-D-glucoside (14), gallic acid 3-*O*-β-D-(6'-*O*-galloyl)-glucoside (15) and two flavonoids: kaempferol (16), quercetin (17). These structures were identified on the basis of their physical data and spectroscopic evidence.**

***Key words: Erodium moschatum*, Geraniaceae, hydrolyzable tannin, flavonoid.**

INTRODUCTION

Since ancient times, Geraniaceous plants including genus *Geranium* and *Erodium* were used for the treatment of diarrhea[1,2]. The active principles are tannins and the main component has been shown to be geraniin[3]. In Taiwan, only the genus *Geranium* has been described in the "Flora of Taiwan"[4]. In 1994, Ou and Kao reported *Erodium moschatum* as a newly recorded plant in Taiwan[5]. *E. moschatum* was originally distributed in southern and western Europe, Africa, America, Indonesia and Japan, and naturalized at the Ching-Ch'ing farm, Nantou, Taiwan[5]. From this plant, seventeen compounds were isolated. This paper describes the isolation procedures and structural elucidation of these compounds.

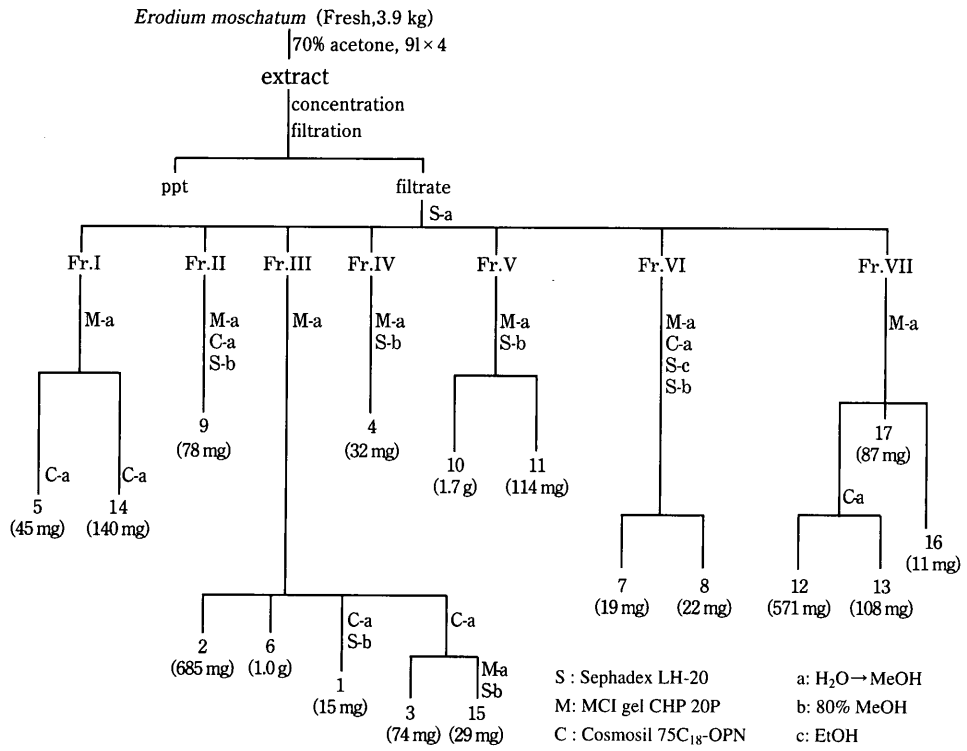
MATERIALS AND METHODS

I. *Instruments and Reagents*

Melting points were determined with a Fisher-Johns melting-point apparatus and were uncorrected. Optical rotation was measured with a Jasco DIP-140 polarimeter. Mass spectra were obtained on a JEOL JMS HX110 spectrometer. ^{1}H(300 MHz) and ^{13}C(75 MHz) NMR spectra

Correspondence to: Jer-Huei Lin

Accepted for Publication: Apr. 8, 1997



Scheme 1. Isolation of tannins and related compounds from *E. moschatum*

were recorded on a Bruker AM-300WB spectrometer, using the solvent peak as reference standard. Column chromatography was carried out with Sephadex LH-20(25-150 μm, Pharmacia Fine Chemical Co. Ltd.), MCI gel CHP 20P (75-150 μm, Mitsubishi Chemical Industries, Ltd.), and Cosmosil 75 C_{18}-OPN columns. Thin-layer chromatography was conducted on precoated Kiesel gel 60 F_{254} plates (0.2 mm, Merck) and Cellulose F plates (0.1 mm, Merck) and spots were detected by UV illumination before spraying with ethanolic ferric chloride.

II. *Plant Material*

The fresh *Erodium moschatum* were collected at the Ching-Ch'ing Farm, Nantou, Taiwan, Republic of China, and verified by Mr. Muh-Tsuen Kao, Department of Botany, National Taiwan University. The voucher specimen is deposited in the National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, Republic of China.

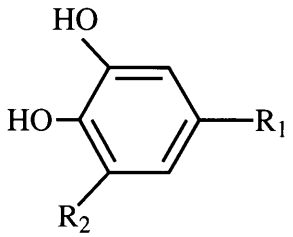
III. *Extraction and Separation*

The fresh *Erodium moschatum* (3.9 kg), as shown in Scheme 1, were extracted four times with 70% acetone (36 L) at room temperature. The acetone was removed by evaporation under reduced pressure (ca 40℃), and the resulting precipitate removed by filtration. The filtrate, after concentration, was subjected to a Sephadex LH-20 column chromatography using H_2O containing increasing amounts of MeOH (0-100%) to yield seven fractions (Fr.I-VII). Repeated column chromatography of each fraction over Sephadex LH-20 (80% MeOH), MCI gel CHP 20P (0-100% MeOH) and Cosmosil 75 C_{18}-OPN (0-100% MeOH) yielded the compounds 1-17.

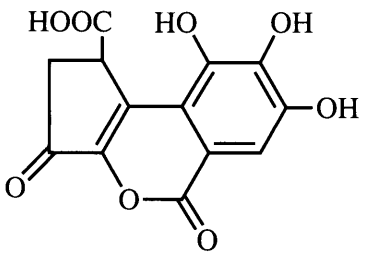
Protocatechuic Acid (1)

White needles (H_2O), mp 197-199℃, $FeCl_3$ reagent: dark green. IR (KBr) υ_{max} cm^{-1}: 2200-3500 (OH), 1650 (C=O). ^{1}H-NMR (acetone-d_6): δ 6.90 (1H, d, J=8.0 Hz, H-5), 7.47 (1H,dd, J=2.0, 8.0 Hz, H-6), 7.53 (1H, d, J=2.0 Hz, H-2).

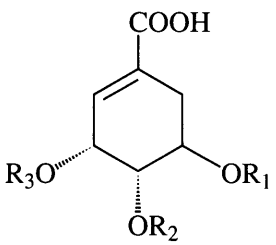
Caffeic Acid (4)



	R_1	R_2
1.	COOH	H
2.	COOH	OH
3.	$COOCH_3$	OH
4.	CH=CH-COOH	H

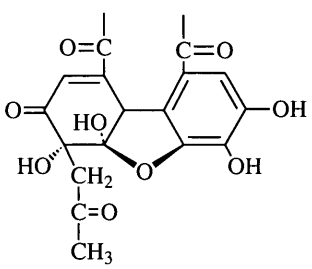


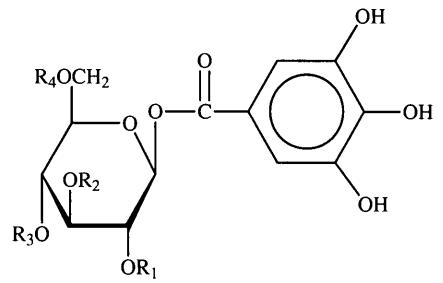
5.



6.	R_1=G	R_2=R_3=H
7.	R_1=R_2=G	R_3=H
8.	R_1=R_2=G	R_2=H

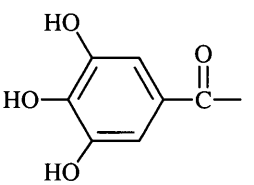
Acetonyl DHHDP



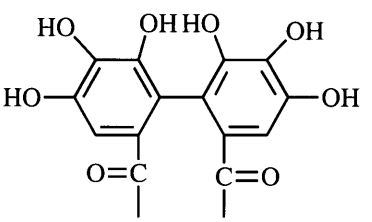


	R_1	R_3	R_2	R_4
9.	H	H	H	H
10.	H	H	(R)HHDP	
11.	DHHDP		H	H
12.	DHHDP		(R)HHDP	
13.	Acetonyl DHHDP		(R)HHDP	

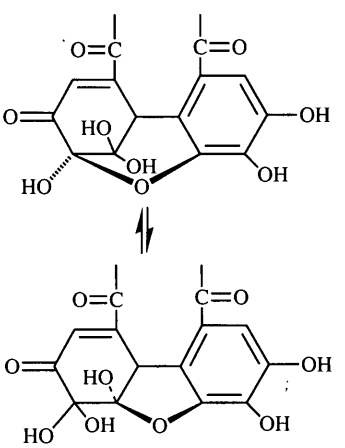
G.



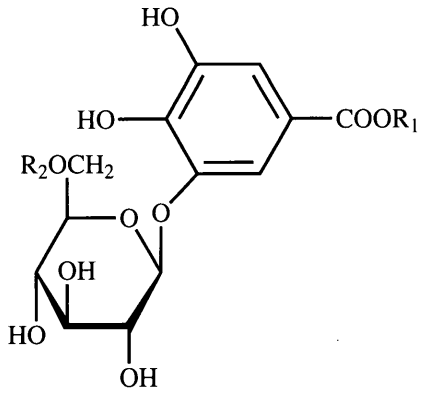
HHDP



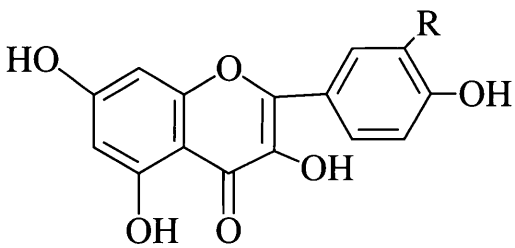
DHHDP



Structures of compounds 1-13



14. R_1=CH_3, R_2=H
15. R_1=H, R_2=G



16. R=H
17. R=OH

Structures of compounds 14-17

Colorless needles (H_2O), mp 214-215℃. ^{1}H-NMR (acetone-d_6 + D_2O): δ 6.22 (1H, d,J = 16.2 Hz, H-α), 6.83 (1H, d,J = 8.4 Hz, H-5), 6.96 (1H,dd,J = 2.1, 8.4 Hz,H-6), 7.13 (1H, d, J = 2.1 Hz, H-2), 7.49 (1H d, J = 16.2 Hz, H-β).

Brevifolincarboxylic Acid (5)

Yellow powder (H_2O-MeOH), mp>300℃, $[\alpha]_D^{25}$-3.0° (c = 0.4, H_2O: acetone = 2:3). ^{1}H-NMR (acetone-d_6 + D_2O): δ 2.58 (1H, dd, J = 18.9, 1.8 Hz, H-5), 3.09 (1H, dd, J = 7.8, 18.9 Hz, H-5), 4.56 (1H, dd, J = 7.8 , 1.8 Hz, H-4), 7.45 (1H, s, H-3').

3-O-Galloylshikimic Acid (6)

Colorless needles (H_2O), mp 255℃ (dec.), $[\alpha]_D^{25}$-110.0° (c = 0.6, acetone). ^{1}H-NMR (acetone-d_6): δ 2.35 (1H, dd, J = 6.0, 18.4 Hz, H-2),2.78(1H, dd, J = 6.0, 18.4 Hz, H-2),3.98 (1H, dd, J = 4.2,7.0 Hz, H-4),4.56(1H, t, J = 4.2 Hz, H-5),5.30(1H,m, H-3),6.82(1H, d, J = 4.2 Hz, H-6),7.12(2H, s, galloyl H).

3,4-Di-O-galloylshikimic Acid (7)

Brown needles (H_2O), mp 268-270℃, $[\alpha]_D^{25}$-168.3° (c = 1.0, acetone). ^{1}H-NMR (acetone-d_6): δ 2.49 (1H, dd, J = 5.4, 18.3 Hz, H-2),2.91(1H, dd, J = 5.9, 18.3 Hz, H-2), 5.34(1H, dd, J = 3.9, 8.0 Hz, H-4),5.56(1H, m, H-3),4.74(1H, t, J = 3.9 Hz, H-5), 6.92(1H, d, J = 3.9 Hz, H-6),7.04,7.06 (each 2H, s, galloyl H).

3,5-Di-O-galloylshikimic Acid (8)

Brown needles (H_2O), mp 237-238℃, $[\alpha]_D^{25}$-168.0°(c = 1.2, MeOH). ^{1}H-NMR (acetone-d_6): δ 2.49 (1H, dd, J = 4.8, 18.6 Hz, H-2),3.04(1H, dd, J = 4.8, 18.6 Hz, H-2), 4.32(1H, dd, J = 4.2, 7.1 Hz, H-4),5.41(1H, m, H-3),5.79(1H, t, J = 4.2 Hz, H-5), 6.85(1H, d, J = 4.2 Hz, H-6),7.11,7.16 (each 2H, s, galloyl H).

Corilagin (10)

White amorphous powder (H_2O), $[\alpha]_D^{25}$-190.2° (c = 0.8, acetone). ^{1}H-NMR (acetone-d_6 + D_2O): δ 4.05(1H, br s, glc H-2), 4.10 (1H, dd, J = 10.7,8.0 Hz, glc H-6), 4.43 (1H, br s, glc H-4), 4.49 (1H, dd, J = 10.7, 8.0 Hz, glc H-5), 4.79 (1H, br s, glc H-3), 4.84 (1H, t, J = 10.7 Hz, glc H-6), 6.38 (1H, br s, glc H-1), 6.67, 6.80 (each 1H, s, HHDP H), 7.08 (2H, s, galloyl H).

Furosin (11)

Yellow powder (H_2O), mp 197-198℃ (dec.), $[\alpha]_D^{25}$-142.1° (c=1.0, MeOH). ^{1}H-NMR (acetone-d_6 + D_2O): δ 5.30 (1H, s, DHHDP H-1), 6.41 (1H, d, J=1.6 Hz, glc H-1), 6.48 (1H, s, DHHDP H-3), 7.18 (2H, s, galloyl H), 7.24 (1H, s, DHHDP H-3').

Geraniin (12)

Yellow powder (H_2O), mp 218-221℃ (dec.), $[\alpha]_D^{25}$-147.8° (c=0.9, MeOH). $^1H_{NMR}$ (acetone-d_6 + D_2O): δ 4.28-4.54(1H, m, glc H-6),4.68-4.90 (2H in total , glc H-5,6), 5.15 (1H, s, DHHDP H-1), 5.40-5.60 (3H in total, glc H-2,3,4), 6.48 (1H, s, DHHDP H-3), 6.55 (1H, s, glc H-1), 6.63, 7.06

(each 1H, s, HHDP H), 7.14 (2H, s, galloyl H), 7.18 (1H, s, DHHDP H-3').

Acetonylgeraniin A (13)

White amorphous powder (H_2O), mp 235-238 ℃(dec.), $[\alpha]_D^{25}$ -91.6° (c=1.0, MeOH). Negative FABMS *m/z*: 991 $[M-H]^-$. ^{1}H-NMR (acetone-d_6): δ 2.17 (3H, s, $-CH_3$), 2.97 and 3.46 (each 1H, d, J=15.6 Hz, $-CH_2-$), 4.39 (1H, dd, J=6.0, 12.0 Hz, glc H-6), 4.74-4.86 (2H, m glc H-5, 6), 4.90 [1H, d, J=1.3 Hz, acetonyldehydrohexahydroxydiphenyl (ADHHDP) H-1'], 5.42 (1H, m, glc H-3), 5.50 (1H, m, glc H-4), 5.54 (1H, m, glc H-2), 6.29 (1H, d, J=1.3 Hz, ADHHDP H-3'), 6.55 (1H, br s, glc H-1), 6.65, 7.06 (each 1H, s, HHDP H), 7.15 (2H, s, galloyl H), 7.21 (1H, s, ADHHDP H-3').

Methyl Gallate 3-O-β-D-Glucoside (14)

White amorphous powder, $[\alpha]_D^{25}$ -59.2° (c=0.5, 50% acetone), ^{1}H-NMR (acetone-d_6 + D_2O): δ 3.75 (1H, dd, J=12.5,4.6 Hz, glc H-6), 3.77 (3H, s, $-OCH_3$), 3.84 (1H, dd, J=l 2.5,4.6 Hz, glc H-6), 4.81 (1H, d, J=7.5 Hz, glc H-1), 7.22, 7.37 (each 1H, d, J=1.8 Hz, H-2,6). ^{13}C-NMR (acetone-d_6): δ 52.3 ($-OCH_3$), 61.5 (glc C-6), 70.3 (glc C-4), 73.0 (glc C-2), 76.5 (glc C-3), 77.3 (glc C-5), 103.2 (glc C-1), 111.0,112.5 (galloyl C-2,6), 121.0 (galloyl C-1), 140.9 (galloyl C-4), 146.1, 146.2 (galloyl C-3,5), 167.7 (-COO-).

Gallic Acid 3-O-β-D-(6'-O-galloyl)-Glucoside (15)

White amorphous powder (H_2O), mp 175-178 ℃, $[\alpha]_D^{25}$ -21.0° (c=1.0, acetone). Negative FABMS *m/z*: 483 $[M-H]^-$. ^{1}H-NMR (acetone-d_6 + D_2O): δ 3.4-3.6 (3H, m, glc H-2,3,4) ,3.9 (1H, m, glc H-5), 4.18 (1H, dd, J=7.2, 12.3 Hz, glc H-6), 4.68 (1H, dd, J=1.51,12.3 Hz, glc H-6), 4.96 (1H, d,J=7.4 Hz, glc H-1), 7.20 (2H, s, galloyl H), 7.26,7.45 (each 1H, d, J=1.8 Hz, galloyl H-2', 6'). ^{13}C-NMR (acetone -d_6 + D_2O): δ 64.9 (glc C-6), 70.7 (glc C-4), 73.9 (glc C-2), 75.2 (glc C-5), 76.3 (glc C-3), 103.0 (glc C-1), 109.8 (2C, galloyl C-2,6), 110.4, 113.0 (galloyl C-2',6'), 120.7 (galloyl C-1), 121.9 (galloyl C-1'), 138.9(galloyl C-4), 140.5 (galloyl C-4'), 145.7(2C, galloyl C-3,5), 146.0, 146.4 (galloyl C-3',5'), 167.3 (-COO-), 169.8 (-COOH).

RESULTS AND DISCUSSION

The aqueous acetone extract of fresh *E. moschatum* was subjected to a combination of Sephadex LH-20, MCI gel CHP 20P and Cosmosil 75C_{18}-OPN chromatographies with various solvent systems as shown in Scheme 1 to yield compounds 1-17. Compounds 1-5 were phenolcarboxylic acids or ester and were identified as protocatechuic acid (1)[6], gallic acid (2), methly gallate (3), caffeic acid (4)[7], brevifolincarboxylic acid (5)[8] by direct comparison of their physical and spectral profiles with literature values. Compounds 6, 7 and 8 gave a dark blue coloration with ferric chloride which is a characteristic of gallotannins. ^{1}H-NMR spectra of 6,7 and 8 showed signals in the aromatic region [6:δ 7.12 (2H, s, galloyl H); 7: δ 7.04,7.06 (each 2H, s, golloyl H); 8: δ 7.11, 7.16 (each 2H, s, galloyl H)] indicated the presence of galloyl groups. These three compounds showed two double doublet signals near δ 2.5 and 3.0 (each 1H, J ca. 6, 18 Hz) indicating two H-2 of shikimic acid. Signals in the aliphatic region [6: δ 3.98 (1H, dd, J=4.2, 7.0 Hz, H-4), 4.56 (1H, t, J=4.2 Hz, H-5), 5.30 (1H, m, H-3), 6.82 (1H, d, J=4.2 Hz, H-6); 7: δ 4.74 (1H, t, J=3.9 Hz, H-5), 5.34 (1H, dd, J=3.9, 8.0 Hz, H-4) , 5.56 (1H, m, H-3), 6.92 (1H, d, J=3.9 Hz, H-6); 8: δ 4.32 (1H, dd, J=4.2,7.1 Hz, H-4), 5.41 (1H, m, H-3), 5.79 (1H, t, J=4.2 Hz, H-5), 6.85 (1H, d, J=4.2 Hz, H-6)] showed downfield shifts of the H-3; H-3,4 and H-3,5 of 6,7 and 8 from shikimic acids, respectively, indicating that hydroxyl groups on the corresponding positions were esterified. Therefore, they were determined as 3-*O*-galloylshikimic acid (6), 3,4-di-*O*-galloylshikimic acid (7) and 3,5-di-*O*-galloylshikimic acid (8)[9], respectively.

Compounds 9-12 and 15-17 were identical with 1-*O*-galloyl-β-D-glucose (9), corilagin (10), furosin (11), geraniin (12), gallic acid 3-*O*-β-D-(6'-*O*-galloyl)-glucoside (15), kaempferol (16) and quercetin (17) by direct comparison with authen-

tic samples that were isolated from *Rosa taiwanensis*[10], *Euphoria longana*[8], *Macaranga sinensis*[11] or *M. tanarius*[12, 13] in our laboratory.

Compound 13 was obtained as a white amorphous powder, $[\alpha]_D$-91.6° .^{1}H-NMR spectrum showed signals consistent with a combination of an acetonyl group and a geraniin (12) possessing a five member ring DHHDP group. FABMS [m/z 991 $(M-H)^-$] and physical data were agreeable with acetonylgeraniin A (13)[14], and therefore the structure was identified.

The ^{1}H-NMR spectrum pattern of 14 [δ 3.77 (3H, s, $-OCH_3$), 4.81 (1H, d, J = 7.5 Hz, glc H-1), 7.22, 7.37 (each 1H, d, J = 1.8 Hz, galloyl H-2, 6)] was similar to that of 15 except that 14 showed a galloyl group less, but a methoxyl signal more than 15. The signal of H-6 of the glucose moiety appeared at δ 3.75 (1H, dd, J=12.5, 4.6 Hz) and 3.84 (1H, dd, J=12.5, 4.6 Hz) indicated the C-6-OH was not esterified. Furth-ermore, the ^{13}C-NMR spectrum of 14 showed the downfield shift of the carbonyl carbon signal (δ 167.7) analogous to that (δ 169.8) observed in 15. This phenomenon indicated that the methyl group was conjugated to the carboxylic acid of gallic acid. Compound 14 was thus established as methyl gallate 3-*O*-β-D-glucoside. Gallic acid 3-*O*-β-D-glucoside has been reported by Kash-iwada et al in 1986[15]. This methylate may be an artifact.

E. moschatum contained galloylshikimic acid, 3-*O*-,3,4-di-*O*-and 3,5-di-*O*-galloylshikimic acid that differed from *G. thunbergii*[16], which contained many kinds of galloylquinic acid, such as 3-*O*-,4-*O*-,5-*O*-and 3,4-di-*O*-galloylquinic acid. On the other hand, similar to *G. thunbergii*[17], *E. moschatum* possessed many tannins including the active principle, geraniin.

ACKNOWLEDGEMENTS

We thank Mr. M. T. Kao (National Taiwan University) for identification of the plant material. Thanks are also due to Mr. C. Z. Chou, research fellow of National Research Institute of Chinese Medicine and Miss S. L. Ng (Depar-tment of Chemistry, National Taiwan University) for the NMR measurements. This study was supported by a grant from the National Science Council of the Republic of China (NSC83-0412B-043A-003).

REFERENCES

1. Hsu, H. Y. 1986. Oriental Materia Medica, a Concise Guide. pp. 828-829. Oriental Healing Arts Institute. Taipei.
2. Kan, W. S. 1979. Pharmaceutical Botany. pp. 340-342. National Research Institute of Chinese Medicine. Taipei.
3. Namba, I. 1980. Coloured Illustrations of wakan-yaku. Vol. II. pp. 24-26. Hoikusha Publishing CO., Ltd. Japan.
4. Huang, T. C. 1993. "Geraniaceae. In "Flora of Taiwan" . Vol. III. 2nd ed. pp. 403-409. Editorial Committee of the Flora of Taiwan. Taipei.
5. Ou, J. C. and Kao, M. T. 1994. *Erodium moschatum* (L.) L'Her (Geraniaceae)-A newly naturalized plant for Taiwan. Taiwania. 38: 19-21.
6. Pouchert, C. J. 1984. The aldrich library of NMR spectra. Vol. 1. 2nd ed. p. 212. Aldrich Chemical Company. Milwauke, U. S. A.
7. Xu, Y.M., Sakai, T., Nonaka, G. and Nishioka, I. 1991. Tannins and related co-mpounds. CVI. Preparation of amino-alditol derivatives of hydrolyzable tannins having α-and β-glucopyranose cores, and its application to the structure elucidation of new tannins, reginins A and B and flosin A, isolation from *Lagerstroemia flos-reginae* Retz. Chem. Pharm. Bull. 39: 639-646.
8. Lin, J. H. and Tsai, C. C. 1995. Phenolic constituents from the flowers of *Euphoria longana* Lam. Chin. Pharm. J. 47: 113-121.
9. Nonaka, G., Ageta, M. and Nishioka, I. 1985. Tannins and related compounds. XXV. A new class of galloyltannins possesing a(-)-shikimic acid core from *Castanopsis cuspidata* var *sieboldii* Nakai. (1). Chem. Pharm. Bull. 33: 96-101.
10. Lin, J. H. and Huang, Y. F. 1996. Phenolic constituents from the roots of *Rosa taiwanensis*

Nakai (1). Chin. Pharm. J. 48: 231-244.

11. Lin, J. H. 1994. Studies on tannins from the bark of *Macaranga sinensis* (Baill.) Muell.-Arg. J. of Food and Drugs Analysis 2: 201-209.
12. Lin, J. H., Nonaka, G. and Nishioka, I. 1990. Tannins and related compounds. XCIV. Isolation and characterization of seven new hydrolyzable tannins from the leaves of *Macaranga tanarius* (L.) Muell.-Arg. CHem. Pharm. Bull. 38: 1218-1223.
13. Lin, J. H. 1993. Studies on tannins of the bark of *Macaranga tanarius* (L.) Muell.-Arg. J. of foods and drugs analysis. 1: 273-280.
14. Hsu, F. L. and Chyn, L. 1991. Studies on the tannins from *Euphoria longana* Lam. Proceedings of the National Science Council, Part A. 15: 541-546.
15. Kashiwada, Y., Nonaka, G. and Nishioka, I. 1986. Tannins and related compounds. XL VII. Rhubarb (6). Isolation and characterization of seven new p-hydroxyphenylbutanones, stilbenes and gallic acid glucosides. Chem. Pharm. Bull. 34:3237-3243.
16. Nonaka, G., Morimoto, S. and Nishioka, I. 1986. Elaeocapsin, a proto-type of geraniin from *Geranium thunbergii*. Chem. Pharm. Bull. 34: 941-943.
17. Okuda, T., Yoshida, T. and Mori, K. 1975. Constituents of *Geranium thunbergii* Sieb. et Zucc. II. Ellagitannins. (1). Yakugaku Zashi. 95:1462-1466.

Journal of Food and Drug Analysis. 1997. 5(4)

麝香牻牛兒苗之單寧及相關化合物之研究

林哲輝* 林美汎

行政院衛生署藥物食品檢驗局
台北市南港區昆陽街161-2號

摘 要

牻牛兒苗科植物*Geranium*屬及*Erodium*屬之地上部做爲止瀉整腸劑，有效成分爲單寧類之Geraniin。麝香牻牛兒苗（*E. moschatum*)爲在台灣新發現之馴化種。

麝香牻牛兒苗之含水丙酮萃取物，利用逆相層析膠質，分離到十七種單寧及相關化合物，包括五種phenolcarboxylic acid及酯類：protocatechuic acid (1), gallic acid (2), methyl gallate (3), caffeic acid(4), brevifolincarboxylic acid (5); 四種 gallotannins:3-*O*-galloylshikimic acid (6), 3,4-di-*O*-galloylshikimic acid (7), 3,5-di-*O*-galloylshikimic acid (8), 1-*O*-galloyl-*b*-D-glucose (9);六種ellagitannins及相關化合物:corilagin (10), furosin (11), geraniin (12), acetonylgeraniin A(13), methyl gallate 3-*O*-*b*-D-glucoside (14), gallic acid 3-*O*-*b*-D-(6'-*O*-galloyl)-glucoside (15)及二種flavonoids: kaempferol (16), quercetin (17)。化合物結構係依其物理性質及核磁共振光譜而決定，並與文獻或標準品比對確認。

關鍵詞：麝香牻牛兒苗，牻牛兒苗科，加水分解型單寧，類黃酮素。