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THE DISTRIBUTION OF GERANIIN AND MALLOTUSINIC ACID IN THE ORDER GERANIALES*

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Key Word Index—Geraniaceae; Euphorbiaceae; geraniin; mallotusinic acid; ellagitannins; hyperin; HPLC; chemosystematics.

Abstract—The distribution of geraniin and mallotusinic acid in Geraniaceae, Euphorbiaceae and other families in the Geraniales were investigated by HPLC. Geraniin composes the main part of the tannin in all of the investigated species of *Geranium*, but not in species of *Pelargonium*. The species of Geraniaceae lack mallotusinic acid. Geraniin was also detected in most of the species in the subfamily Euphorbioideae of Euphorbiaceae, although the amount was generally smaller than in *Geranium*, except for some woody species. Several species of Euphorbioideae contained mallotusinic acid. Characteristic of the subfamily Phyllanthoideae is the absence of mallotusinic acid and poor distribution of geraniin. Geraniin and mallotusinic acid were not detected in most of the species of other families in the Geraniales, except in *Erythroxylum coca*. Hyperin was found to be the main flavonol glycoside in most species of *Geranium*.

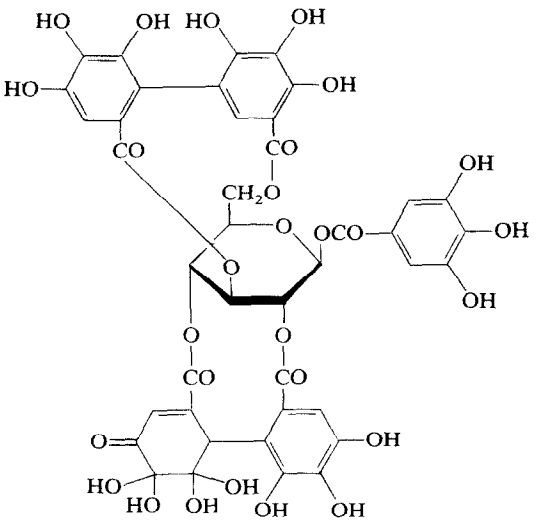
INTRODUCTION

Among the families belonging to the Geraniales, Geraniaceae and Euphorbiaceae are known to be rich in tannins. Although the structures of the tannins in most of these species are unknown, the presence of ellagitannins in numerous species of these families has been based on the chromatographic detection of ellagic acid [1, 2] and on the colorimetry with nitrous acid [3]. Ellagic acid thus detected has been utilized as a chemotaxonomic marker for ordinal phylogeny in dicotyledons [1, 2]. We have recently found by extraction and isolation that geraniin for which we assign structure (**1**) [4] is the main tannin in several species of *Geranium* (Geraniaceae) [5], and *Triadica sebifera* (= *Sapium sebiferum*) (Euphorbiaceae) [5], and that geraniin and mallotusinic acid (**2**) compose the main tannins in *Mallotus japonicus* (Euphorbiaceae) [6]. We have also found that several ellagitannins, viz. geraniin, mallotusinic acid, mallotinic acid, corilagin, chebulinic acid, chebulagic acid, are clearly separated from each other, and from co-existing flavonols and flavonol glycosides by HPLC on a column of hydrocarbon polymer, by reversed-phase development [7]. In this study, we have examined the distribution of geraniin and mallotusinic acid in the order Geraniales by HPLC, since only HPLC permits the detection and quantitative determination of these tannins in complex mixtures. Of these two tannins, the latter may be the more specific marker because of the complexity of its structure. Co-existing flavonol glycosides have also been examined.

Ellagic acid in the plant extracts is regarded mostly as a product of hydrolysis of ellagitannins in the plants. We have found that extracts of *Geranium thunbergii* and several other species show either presence or absence of ellagic acid on PC and HPLC, depending on the method of sample preparation. The sample solution carefully prepared from fresh aerial tissues of *G. thunbergii*, homogenized in a mixture of Me_2CO and H_2O immediately after collection to avoid hydrolysis of geraniin, did not show any detectable ellagic acid. The same sample solution prepared at elevated temperature, or by prolonged treatment, however, showed presence of ellagic acid. We have also observed marked differences in stability among the ellagitannins. For instance, geraniin is quickly hydrolysed during hot water extraction of the plant [8, 9], while corilagin is fairly stable [7]. These observations show that absence of ellagic acid in the extract does not necessarily indicate absence of ellagitannin, and that extracts from each species of plant should be prepared uniformly. The extracts in this study were therefore prepared as follows. The plant materials were dried quickly upon collection, homogenized in a mixture of Me_2CO and H_2O, and then evaporated *in vacuo* below 40°.

The present study began with the *Geranium* species grown in Japan, some of which are used as *G. thunbergii* in folk medicine. Species of *Pelargonium* was then examined to determine whether the ellagitannin in this species [2] is geraniin. The investigation was then extended to Euphorbiaceae, which is a taxonomically

* Part 10 in the series "Constitution of *Geranium thunbergii* Sieb. et Zucc." For Part 9, see ref. [5].



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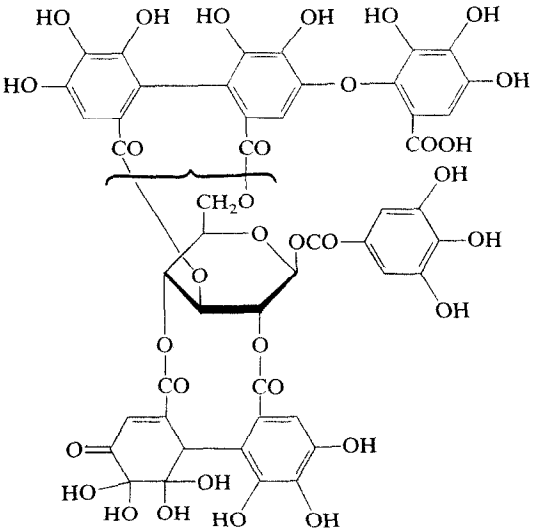

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complex family. Although ellagic acid has been detected by PC in extracts of species of *Acalypha*, *Ricinus* and *Euphorbia* [1], HPLC was erected to reveal ellagitannins in species of other genera of this family, as well as in the species of the three genera described above. Daphniphyllaceae, which had been included in Euphorbiaceae as a subfamily [10], and other families of Geraniales were also examined. As the tannin contents in the species of *Geranium* is high at flowering [11], the plants of this genus were collected at this time. Seasonal variation of geraniin content in *G. thunbergii* was also determined. Our plant taxonomy is based on Engler's system [12] unless otherwise mentioned.

RESULTS AND DISCUSSION

Leaves of all of the investigated species of *Geranium* showed the presence of geraniin, its content being about 10% of the dried leaves on average (Table 1). The highest content of geraniin was in *G. thunbergii*. The gerannin contents in dried stems were 1–2%. Comparisons of these values with those obtained by relative astringency [13], RMB [14] and RE [11] indicate that the main tannin in these species is geraniin. Seasonal variation of geraniin content in *G. thunbergii* determined by HPLC is shown in Table 2. The variation approximately parallels the seasonal variations in tannin content as determined by the hide powder method and relative astringency measurement [11]. Mallotusinic acid was not found in these species.

Among the investigated species of *Pelargonium*, i. e. *P. graveolens* L'Her., *P. odoratissimum* Ait., *P. tomentosum* Jaquin and *P. zonale* Ait., only *P. zonale* showed a peak corresponding to genaniin, but the identification could not be confirmed when spectral measurements were made on it. Other species of this genus did not show this peak. Mallotusinic acid was absent from all of these species.

Table 1. Geraniin and hyperin content of the dry leaves of *Geranium* species

Species	Geraniin (%)	Hyperin (%)	Month of collection
G. eriostemon Fisch. var. *reinii* Maxim.	7.5	0.15	July
G. erianthum DC.	7.6	0.13	August
G. soboliferum Komar.	6.8	0.16	October
G. krameri Franch. et Savat.	6.8	0.19	October
G. yoshinoi Makino	9.8	0.55	September
G. yesoense Franch. et Savat.	12	0.18	August
G. yesoense Franch. et Savat. var. *nipponicum* Nakai	12	0.09	August
G. shikokianum Matsum.	6.0	0.59	October
G. sibiricum L. var. *glabrius* Ohwi	8.1	—	August
G. thunbergii Sieb. et Zucc.	12	—	August
G. wilfordii Maxim.	9.5	0.21	September
G. wilfordii Maxim. var. *hastatum* Hara*	0.50	0.03	September
G. tripartitum R. Knuth	12	1.3	September
G. robertianum L.	9.8	—	September
G. carolinianum L.	11	1.6	May

*Fresh aerial tissue.

Table 2. Seasonal variation of geraniin content in fresh aerial tissue of *Geranium thunbergii*

Month of collection	Geraniin (%)
May	0.6
June	1.1
July	1.4
August	1.8
September	1.6
October	1.2

Corilagin can be produced by partial hydrolysis of geraniin [8, 9, 15], and detection of this tannin in the extract does not necessarily indicate its presence in the plant. However, small amounts of corilagin have been shown to be present in each species of *Geranium*, since the extracts of fresh plants prepared with caution to avoid hydrolysis of geraniin showed the peak of corilagin. Ellagic acid, by contrast, was not detected in these species when the extracts were carefully prepared.

Among the flavonol glycosides known in *Geranium* species [5], hyperin (quercetin 3-galactoside) has been found to be the major compound in most species. *G. carolinianum* and *G. tripartitum* showed higher hyperin content than the other species. Although hyperin was not detected in *G. thunbergii* and *G. sibiricum* var. *glabrius*, the aglycone quercetin was found in these species. As both the glycosides and the aglycones can be determined by HPLC with high sensitivity, HPLC may be a good tool for correlating 'flavonoid score' [16] with geographical distribution in these species.

Most of the species of Euphorbioideae (Euphorbiaceae) examined (Table 3) showed the presence of geraniin. The geraniin content however, is generally lower than that in *Geranium* species, except for some woody taxa, i. e. *Triadica sebifera*, *Shirakia japonica*, *Mallotus japonicus*, *Alchornea trewioides* and *Dalechampia roezliana*. Also, some herbaceous species grown in the greenhouse lack geraniin. Mallotusinic acid may be a characteristic component of this subfamily, although the amounts in each species are generally low, and it is absent from several. Mallotusinic acid was absent from all species examined in the five genera in the subfamily Phyllanthoideae, for which an independent family 'Phyllanthaceae' has been proposed [17]. However, more than half the species showed the presence of geraniin, although the amounts are small. These results show that this subfamily and the Euphorbioideae are similar in tannin constituents.

The results with the species of other families in the Geraniales and in the 'Euphorbiales' [18] are summarized in Table 4. The absence of both geraniin and mallotusinic acid from the species of *Daphniphyllum*, which has been included in Euphorbiaceae, is in agreement with the contemporary separation of the Daphniphyllaceae from Euphorbiaceae, and also the proposal to establish the separate order Daphniphyllales [19]. The species of Oxalidaceae, Zygophyllaceae and Tropaeolaceae examined in this study did not show presence of either geraniin or mallotusinic acid.

Erythroxylum coca showed the presence of geraniin in the leaf. The identity upon HPLC was confirmed by the UV measurements. The leaf of *Buxus microphylla* var. *suffruticosa* was examined since Buxaceae is included in 'Euphorbiales'. The species showed a peak of identical retention time as that of geraniin, but the identity was not confirmed because of some differences in the UV spectra.

Several species belonging to other orders known to be rich in tannin, were also examined by HPLC. These species are: *Punica granatum* L. (fruit peel), *Coriaria japonica* A. Gray (leaf), *Terminalia chebula* Retz. (dry fruit, Myrobalans), *Alnus firma* Sieb. et Zucc. (unripe fruit), *Rhus javanica* L. (leaf and gall), *Caesalpinia sepiaria* Roxb. var. *japonica* Makino (leaf and seed), *Diospyros kaki* Thunb. (leaf) and *Uncaria gambir* (aqueous extract of leaf and twig, gambir). The initial four species among them are rich in ellagitannins. HPLC showed that geraniin and mallotusinic acid are absent from all these species. This result supports the view that geraniin and mallotusinic acid may be characteristic of certain species in the Geraniales.

EXPERIMENTAL

Preparation of sample solns. Plants were identified by K. Enomoto, Hiroshima Botanical Garden and by G. Murata, Botany Dept., Kyoto University and voucher specimens have been deposited in the herbarium of Okayama University. The plants were air-dried upon collection, homogenized in Me_2CO–H_2O (1:1) ×3, and centrifuged. For the determination of seasonal variation of geraniin content in the species of *Geranium*, fresh aerial tissue was treated in the same way. The combined supernatants were evapd *in vacuo* at room temp. The dried extracts were dissolved in 50% MeOH in the concn of 5 mg/ml. Vols. of 1–6 μl of the solns were injected on to the column of HPLC.

Detection of components by HPLC. HPLC was run on a Shimadzu-Du Pont Model LC-1 equipped with 254 nm UV detector or with Shimadzu spectrometric detector, Model SPD-1. A 100 cm×7.9 mm I. D. stainless steel column packed with Zipax HCP (hydrocarbon polymer, Du Pont, Wilmington, Del., U.S.A.), or a 100 cm×2.1mm I.D. column of the same stationary phase was used at 30–100 kg/cm^2, and at 35°. The former column gave better resolution, and was used to separate mallotusinic acid from corilagin. A mixture of 0.5 M KH_2PO_4 (1 l), EtOH (10 ml) and EtOAc (1 ml) was employed as the mobile phase. Separation of mallotusinic acid from corilagin was also effected by developing with a mixture of 0.5 M KH_2PO_4 (1 l) and EtOAc (2 ml) on a 100 cm×2.1 mm column. The UV spectra of the component tannins were determined by scanning wavelength using SPD-1, in those cases when the species was the only one of its genus or family showing a peak with a retention time corresponding to geraniin.

Quantitation on HPLC. Geraniin was used as the internal standard [7]. Geraniin is the hexahydrate of **1** and mallotusinic acid is the nonahydrate of **2**. The correction factor [7] by which the peak areas of polyphenols in the samples were multiplied are as follows: geraniin, 1.00; mallotusinic acid, 0.84; and hyperin, 2.1.

Preparations of sample solns from Geranium thunbergii. Fresh leaves of *G. thunbergii* was homogenized in Me_2CO–H_2O (1:1) immediately after collection, centrifuged, and the supernatant liquor was injected on to the column of HPLC. The chromatogram obtained showed absence of ellagic acid [7], and presence of geraniin as the main peak. A small peak of corilagin was shown. The plant material frozen

Table 3. Distribution of geraniin and mallotusinic acid in Euphorbiaceae

Tribe, genus and species		Geraniin (%)*	Mallotusinic acid (%)*	
	Phyllanthoideae			
Phyllantheae	*Breynia nivosa* Small. var. *roseo-picta* Hort.	0.01†	—	GH
	B. rhamnoides Muell. Arg.	—	—	GH
	Securinega suffruticosa Rehd. var. *japonica* Hurusawa	—	—	
	Phyllanthus urinaria L.	1.1	—	
	P. embrica L.	0.37	—	GH
	Sauropus androgynus Merr.	—	—	GH
	Glochidion obovatum Sieb. et Zucc.	0.02	—	GH
	Euphorbioideae			
Crotoneae	*Croton tiglium* L.	0.02	—	GH
Chrozophoreae	*Aleurites cordata* R. Br.	0.94	—	
	A. fordii Hemsl.	3.5	1.6	
	A. moluccana Willd.	trace	—	GH
Joannesieae	*Hevea brasiliensis* Muell.	—	—	GH
Acalypheae	*Mallotus japonicus* Muell. Arg.	8.4	7.1	
	M. philippinensis Muell. Arg.	0.28	trace	GH
	Alchornea trewioides Muell.	7.6	2.8	
	Mercurialis leiocarpa Sieb. et Zucc.	—	—	
	Acalypha australis L.	0.71	—	
	Ricinus communis L.	0.02	trace	
Cluytieae	*Jatropha podagrica* Hook.	—	—	GH
	J. hastata Griseb.	—	—	GH
	Codiaeum variegatum Bl. var. *pictum* Muell. Arg.	—	—	GH
Manihoteae	*Manihot esculenta* Crantz var. *variegata* Hort.	—	—	GH
Hippomaneae	*Excoecaria cochinchinensis* Lour.	4.4	—	GH
	Shirakia japonica Hurusawa	10	—	
	Triadica sebifera Small.	12	trace	
	Hura crepitans L.	—	—	GH
Dalechampieae	*Dalechampia roezliana* Muell. Arg.	8.8	—	GH
Euphorbieae	*Euphorbia tirucalli* L.	0.06	—	GH
	E. millii Des Moulin var.	0.09†	tarce	GH
	E. neriifolia L.	0.06		GH
	E. enopla Boiss.	—	—	GH
	E. fulgens Karw. ex Klotzsch	0.03	—	GH
	E. lathyris L.	—	—	
	E. helioscopia L.	0.16	trace	
	E. jolkinii Boiss.	1.0†	0.27†	
	E. pekinensis Rupr.	1.5	trace	
	E. maculata L.	2.3	trace	
	E. supina Rafin.	4.5	trace	
	E. pseudochamaesyce Fisch., Mey., et Lallem.	2.1	trace	
	E. pulcherrima Willd. (red leaf) (green leaf)	0.20 0.04	—	GH

*In the dried leaves or equivalent tissues
†In the fresh leaves.
GH: From the greenhouse.

Table 4. Distribution of geraniin in the order Geraniales and related families

Order	Family	Genus and species	Geraniin
Geraniales	Oxalidaceae	*Oxalis corniculata* Maxim.	—
		Averrhoa carambola L.	—
	Tropaeolaceae	*Tropaeolum majus* L.	—
	Zygophyllaceae	*Tribulus terrestris* L.	—
	Erythroxylaceae	*Erythroxylum coca* Lam.	0.31%
	Daphniphyllaceae	*Daphniphyllum macropodum* Miq.	—
Celastrales	Buxaceae*	*Buxus microphylla* Sieb. et Zucc. var. *japonica* Rehd. et Wils.	+?
		Pachysandra terminalis Sieb. et Zucc.	—
Sapindales	Balsaminaceae†	*Impatiens textori* Miq.	—

*Included in Euphorbiales according to ref. [18].
†Included in Geraniales according to ref. [18].

immediately after collection showed the same result. When the supernatant liquor of the homogenate was concd *in vacuo* at 40°, and the residue was dissolved in MeOH, the soln showed a small peak of ellagic acid on HPLC. Upon concn of the supernatant liquor at higher temp. or for a prolonged time, a larger peak of ellagic acid was observed.

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