Constituents of *Geranium thunbergii* Sieb. *et* Zucc. Part 14.[1] Structures of Didehydrogeraniin, Furosinin, and Furosin

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Three novel tannins, dehydrogeraniin, furosinin, and furosin, members of the dehydroellagitannins, were isolated from the aerial part of *Geranium thunbergii* Sieb. *et* Zucc., and their structures were elucidated on the basis of spectral evidence and chemical reactions. Dehydrogeraniin and furosinin were found to form equilibrium mixtures composed of four and eight tautomers, respectively, due to the equilibration of two dehydrohexahydroxydiphenoyl groups and the anomerization of the glucose moiety in the latter compound.

Geraniin (**1**) is a crystalline tannin which forms an equilibrium mixture of six- and five-membered hemiacetal structures of the didehydrohexahydroxydiphenoyl (DHHDP)† group in aqueous solution.[2] It was found to be the main component of *Geranium thunbergii* Sieb. *et* Zucc. (Geraniaceae), which is one of the most commonly used folk medicines and also an official antidiarrhoeal in Japan.[3] Further investigation of tannins from this plant revealed the presence of three dehydroellagitannins[4] in which the DHHDP group shows a remarkable complexity in its equilibrium states. This paper deals with the isolation and structure elucidation,[5] including that of the equilibrium states, of these new tannins, didehydrogeraniin (**2**), furosinin (**12**), and furosin (**13**), whose names are associated with the Japanese name *furoso* for *Geranium* species.

† Throughout, the 'dedihydro' prefix has been used in accordance with IUPAC recommendations for compounds where the 'dehydro' prefix has been formerly employed in the literature.

Results and Discussion

The ethyl acetate-soluble portion from the crude extract of *G. thunbergii*, from which geraniin crystallized out, was fractionated by droplet counter-current chromatography[6] followed by gel-column chromatography to give didehydrogeraniin (**2**), furosinin (**12**), and furosin (**13**). All these tannins were positive to $FeCl_3$ and 2,4-dinitrophenylhydrazine reagents on t.l.c. plates, but they did not give the characteristic colour of ellagitannin with the $NaNO_2$–AcOH reagent.[7]

Didehydrogeraniin (**2**), $C_{41}H_{28}O_{28}\cdot 6H_2O$, $[\alpha]_D$ −137°, was isolated as a yellow amorphous powder. The ^1H n.m.r. spectrum of compound (**2**) exhibited two singlets, at δ 7.24 and 7.23 (2 H in total), which are attributable to a galloyl group, and also signals assignable to two methine protons [δ 4.92 (d, *J* 1.5 Hz, $\frac{2}{5}$ H), 5.33 (s, $\frac{3}{5}$ H); 5.28 (s, $\frac{2}{5}$ H), 5.30 (s, $\frac{3}{5}$ H)], two vinyl protons [δ 6.29 (d, *J* 1.5 Hz, $\frac{2}{5}$ H), 6.60 (s, $\frac{3}{5}$ H); 6.72 (s, $\frac{2}{5}$ H), 6.74 (s, $\frac{3}{5}$ H)], and two aromatic protons [δ 7.24—7.28, 2 H in total)] which are those in the two didehydrohexahydroxydiphenoyl (DHHDP) groups. The presence of a monosaccharide core (δ 6.42, 6.37, and 5.83—4.35) in the molecule was also shown by the ^1H n.m.r.

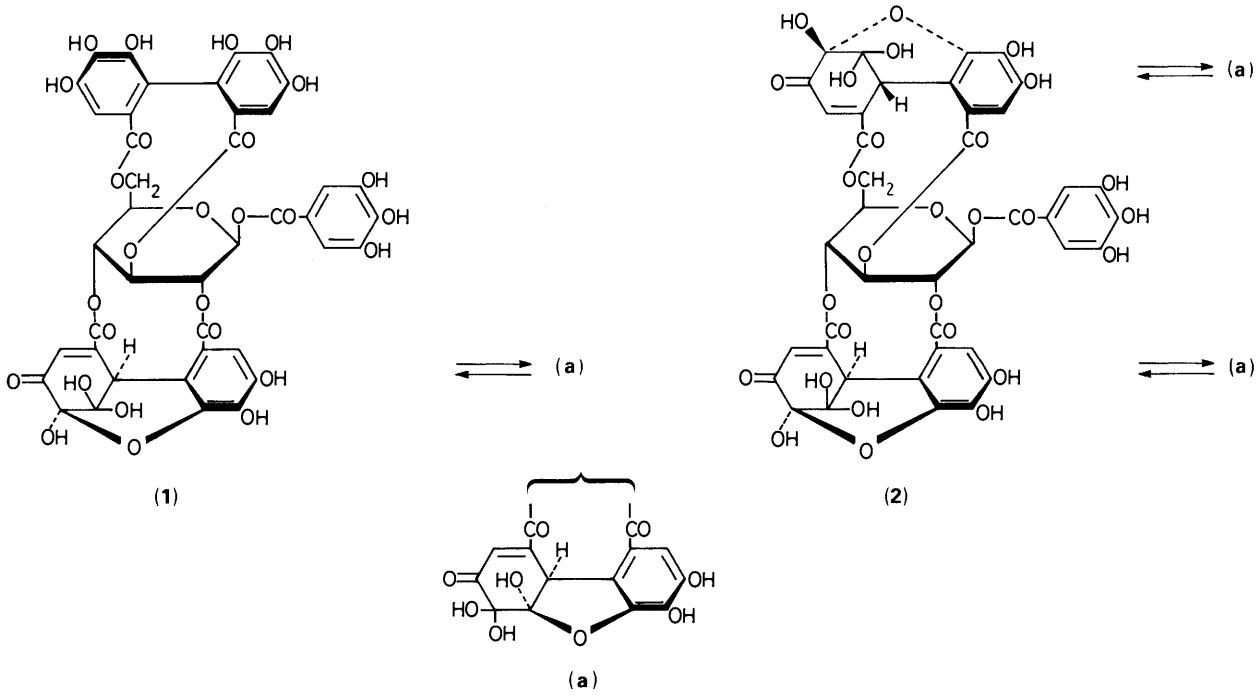
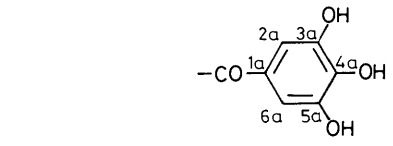
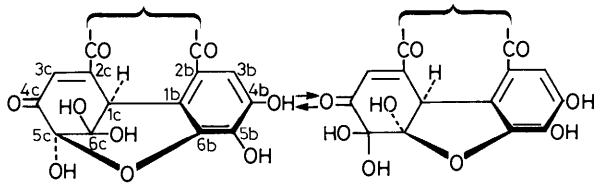


Table. ^{13}C N.m.r. (50 MHz) signals of compounds (**2**) and (**13**) measured in CD_3COCD_3–D_2O[a]

Carbon	Partial structure[b]	(2)	(13)	Carbon	Partial structure[b]	(2)	(13)
Ring A 1a		120.1 (1)	120.7 (1)	3c	6	129.4 ($\frac{1}{2}$)	129.1 ($\frac{1}{2}$)
2a, 6a		110.5 (2)	110.7 (2)		6	129.3 ($\frac{1}{2}$)	
3a, 5a		146.2 (2)	146.5 (2)		6	128.8 ($\frac{1}{2}$)	
4a		139.9 (1)	140.7 (1)		5	125.8 ($\frac{1}{2}$)	126.1 ($\frac{1}{2}$)
Ring B 1b	6	117.1 ($\frac{1}{2}$)	117.7 ($\frac{1}{2}$)	4c	6	191.6 ($\frac{1}{2}$)	193.1 ($\frac{1}{2}$)
	6	116.1 ($\frac{1}{2}$)			6	191.2 (1)	
	6	115.03 ($\frac{1}{2}$)			5	194.1 ($\frac{1}{2}$)	195.9 ($\frac{1}{2}$)
	5	114.99 ($\frac{1}{2}$)	116.2 ($\frac{1}{2}$)	5c	6	96.6 (1)	96.2 ($\frac{1}{2}$)
2b	6	120.03 ($\frac{1}{2}$)	120.2 ($\frac{1}{2}$)		6	96.2 ($\frac{1}{2}$)	
	6	119.94 ($\frac{1}{2}$)			5	92.3 ($\frac{1}{2}$)	92.3 ($\frac{1}{2}$)
	6	119.86 ($\frac{1}{2}$)		6c	6	92.0 ($\frac{3}{2}$)	92.9 ($\frac{1}{2}$)
	5	118.8 ($\frac{1}{2}$)	119.2 ($\frac{1}{2}$)		5	109.3 ($\frac{1}{2}$)	109.1 ($\frac{1}{2}$)
3b	6	114.0 (1)	114.3 ($\frac{1}{2}$)	Glucose 1		95.8 ($\frac{1}{2}$)	92.7 ($\frac{1}{2}$)
	6	113.7 ($\frac{1}{2}$)				94.5 ($\frac{1}{2}$)	92.1 ($\frac{1}{2}$)
	5	113.3 ($\frac{1}{2}$)	114.1 ($\frac{1}{2}$)			78.2 ($\frac{1}{2}$)	78.5 ($\frac{1}{2}$)
4b	6	145.8 ($\frac{3}{2}$)	146.1 ($\frac{1}{2}$)			77.8 ($\frac{1}{2}$)	77.8 ($\frac{1}{2}$)
	5	147.7 ($\frac{1}{2}$)	148.0 ($\frac{1}{2}$)			73.7 ($\frac{1}{2}$)	73.6 ($\frac{1}{2}$)
5b	6	139.2 ($\frac{1}{2}$)	139.6 ($\frac{1}{2}$)	2—5		72.8 ($\frac{1}{2}$)	72.3 ($\frac{1}{2}$)
	6	138.52 ($\frac{1}{2}$)				69.8 ($\frac{1}{2}$)	72.2 ($\frac{1}{2}$)
	6	138.46 ($\frac{1}{2}$)				67.7 ($\frac{1}{2}$)	71.1 ($\frac{1}{2}$)
	5	137.6 ($\frac{1}{2}$)	137.7 ($\frac{1}{2}$)			67.39 ($\frac{1}{2}$)	63.0 (1)
6b	6	143.5 ($\frac{1}{2}$)	143.5 ($\frac{1}{2}$)			67.36 ($\frac{1}{2}$)	
	6	143.03 ($\frac{1}{2}$)		6		66.1 (1)	62.6(1)
	6	142.99 ($\frac{1}{2}$)		Ester		168.4 ($\frac{1}{2}$)	167.1 ($\frac{1}{2}$)
	5	147.3 ($\frac{1}{2}$)	147.5 ($\frac{1}{2}$)			168.2 ($\frac{1}{2}$)	166.8 ($\frac{1}{2}$)
Ring C 1c	6	46.5 ($\frac{1}{2}$)	45.5 ($\frac{1}{2}$)			166.3 ($\frac{1}{2}$)	166.6 ($\frac{1}{2}$)
	6	45.0 ($\frac{1}{2}$)				166.2 ($\frac{1}{2}$)	166.4 (1)
	6	44.8 ($\frac{1}{2}$)				165.1 ($\frac{1}{2}$)	166.1 ($\frac{1}{2}$)
	5	52.2 ($\frac{1}{2}$)	51.7 ($\frac{1}{2}$)			165.0 ($\frac{1}{2}$)	
2c	6	153.6 ($\frac{1}{2}$)	155.2 ($\frac{1}{2}$)			164.8 ($\frac{1}{2}$)	
	6	152.5 ($\frac{1}{2}$)				164.7 ($\frac{1}{2}$)	
	6	152.0 ($\frac{1}{2}$)				164.6 ($\frac{1}{2}$)	
	5	148.7 ($\frac{1}{2}$)	149.8 ($\frac{1}{2}$)			164.4 ($\frac{1}{2}$)	

[a] Chemical shifts are given in δ-values with 1,4-dioxane (δ 67.4) as internal standard. Number in parentheses is the number of carbon atoms. [b] 6 Indicates the carbon assignable to DHHDP moiety with six-membered hemiacetal structure, and 5 indicates those with the five-membered hemiacetal structure.

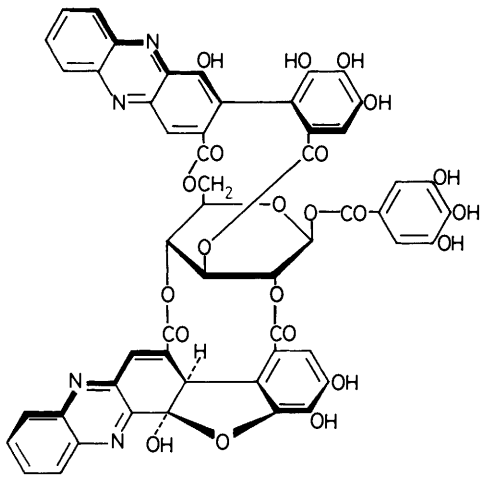




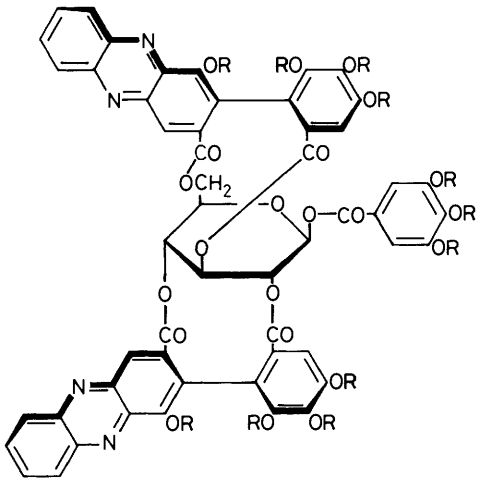
spectrum. These proton signals indicated that the equilibration between the six- and five-membered hemiacetal structures [the latter is illustrated by the partial structure (**a**) besides formulae (**1**) and (**2**)] similar to that in the structure of geraniin (**1**),[2] occurs mainly for one of the two DHHDP groups in the approximate ratio 3:2. Other small peaks observed in the spectrum, however, indicated that the equilibration is also occurring for the other DHHDP group, to a small extent. The ratios of each tautomeric structure in the equilibrium mixture for these two DHHDP groups appear to be easily affected by temperature or the preparation procedure of this compound, as shown in the ^1H n.m.r. spectrum.

The ^{13}C n.m.r. spectrum of didehydrogeraniin (**2**) shown in the Table strongly supported the notion that (**2**) possesses a galloyl group and two DHHDP groups on the sugar core, in accord with the observation of its ^1H n.m.r. spectrum, although the equilibrium ratio of the equilibrated DHHDP group in the molecule was almost 1:1 when measured by the ^{13}C n.m.r. spectrum [which was measured with a higher concentration of (**2**) than that for the ^1H n.m.r. spectrum].

In order to eliminate the spectral complexity due to the equilibration of DHHDP groups, didehydrogeraniin (**2**) was condensed with *o*-phenylenediamine (2 mol equiv.) to give a phenazine derivative (**3**), $C_{53}H_{32}N_4O_{22}\cdot 6H_2O$. This compound showed, in its ^1H n.m.r. spectrum, only one methine proton, at δ 5.42 (1 H, d, *J* 1.5 Hz), indicating aromatization of one of the two newly formed tricyclic groups to a phenazine. This compound (**3**) was slowly converted into another phenazine derivative (**4**), $C_{53}H_{32}N_4O_{22}\cdot 6H_2O$, in acidic medium. The ^1H n.m.r. spectrum of the latter showed an absence of the proton signal attributable to a methine and thus indicated that the second phenazine group was aromatized. The upfield shift (~0.4 p.p.m.) of the 1-H signal of the glucose moiety upon the conversion of compound (**3**) into its isomer (**4**) should be due to the anisotropic effect of the phenazine at O-2 ~ O-4 as illustrated by structure (**4a**), in a manner analogous to that observed upon the aromatization of 'phenazine A' (**5**) to 'phenazine B' (**6**), both of which were derived from geraniin (**1**).[8,2] Therefore, based on stereochemical considerations of structure (**4a**), one of the two DHHDP groups in dehydrogeraniin, which aromatized later

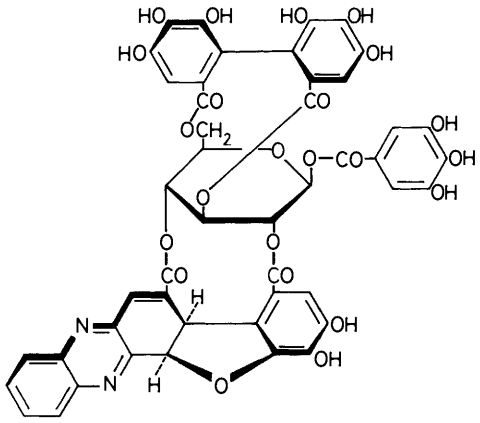


(3)



(4) R = H

(7) R = Me



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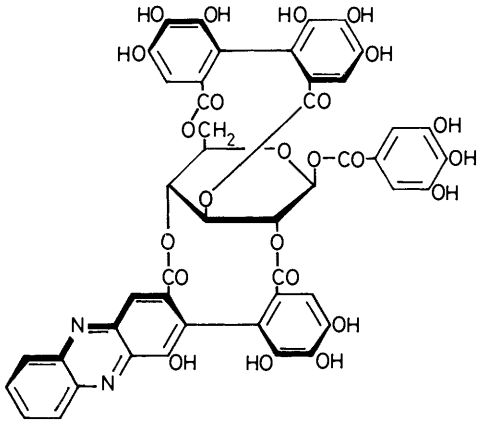

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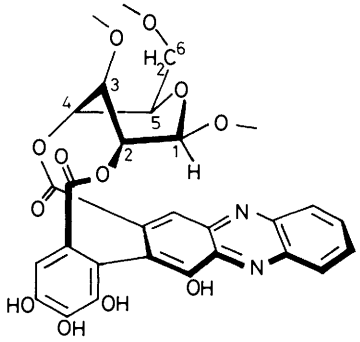
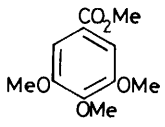


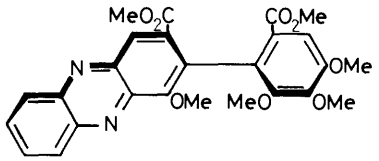
(4a)

upon the conversion of (**3**) into (**4**), was concluded to be located on O-2 ~ O-4 with the same orientation as that of the DHHDP group in geraniin (**1**).

Upon methylation of compound (**4**) with diazomethane, the undeca-*O*-methyl derivative (**7**), $C_{64}H_{54}N_4O_{22}\cdot 4H_2O$, of (**4**) was obtained, and this was methanolysed to give methyl tri-*O*-methylgallate (**8**) and (+)-methyl 4-methoxy-3-(2,3,4-trimethoxy-6-methoxycarbonylphenyl)phenazine-2-carboxylate (**9**),[8] which were identical with authentic samples derived from geraniin (**1**).[2] Therefore, the absolute configuration at C-1c (see Table) of both DHHDP groups was determined to be *R*. Glucose in the mother liquor of methanolysis was identified by gas chromatography of the trimethylsilyl derivative. Hydrolysis of compound (**4**) in boiling water produced 'phenazine C' (**10**)[8] which was formed from one of the two phenylphenazine groups in (**4**), and was identical with that produced from geraniin (**1**). The residual phenazine derivative (**11**), $C_{33}H_{24}N_2O_{16}\cdot 2.5H_2O$, showed a large upfield shift of 2-H (δ 5.88 → 4.30) of the glucose moiety, as well as that of 4-H (δ 5.83 → 4.40) in the ^{1}H n.m.r. spectrum. This result indicated that the location of one of the



(8)



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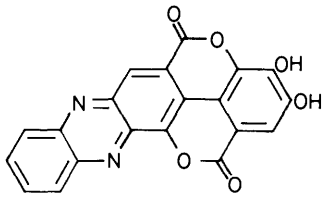

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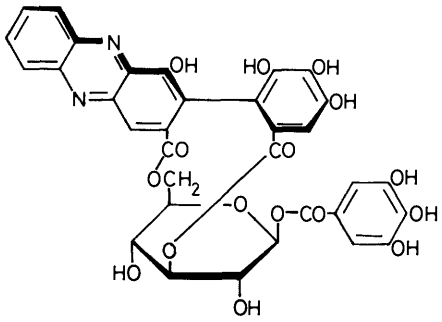

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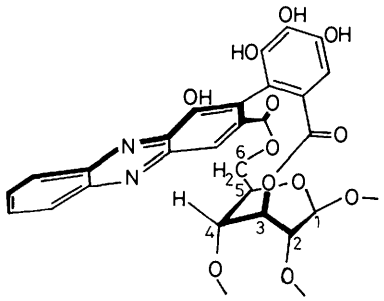
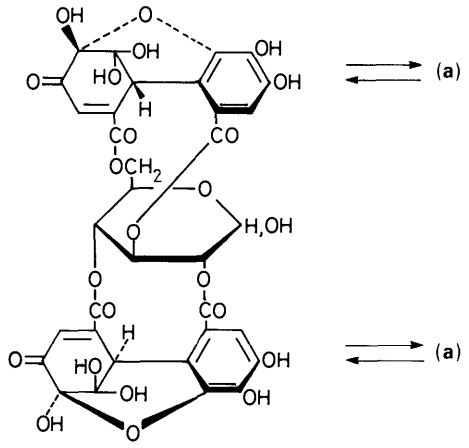


(6a)

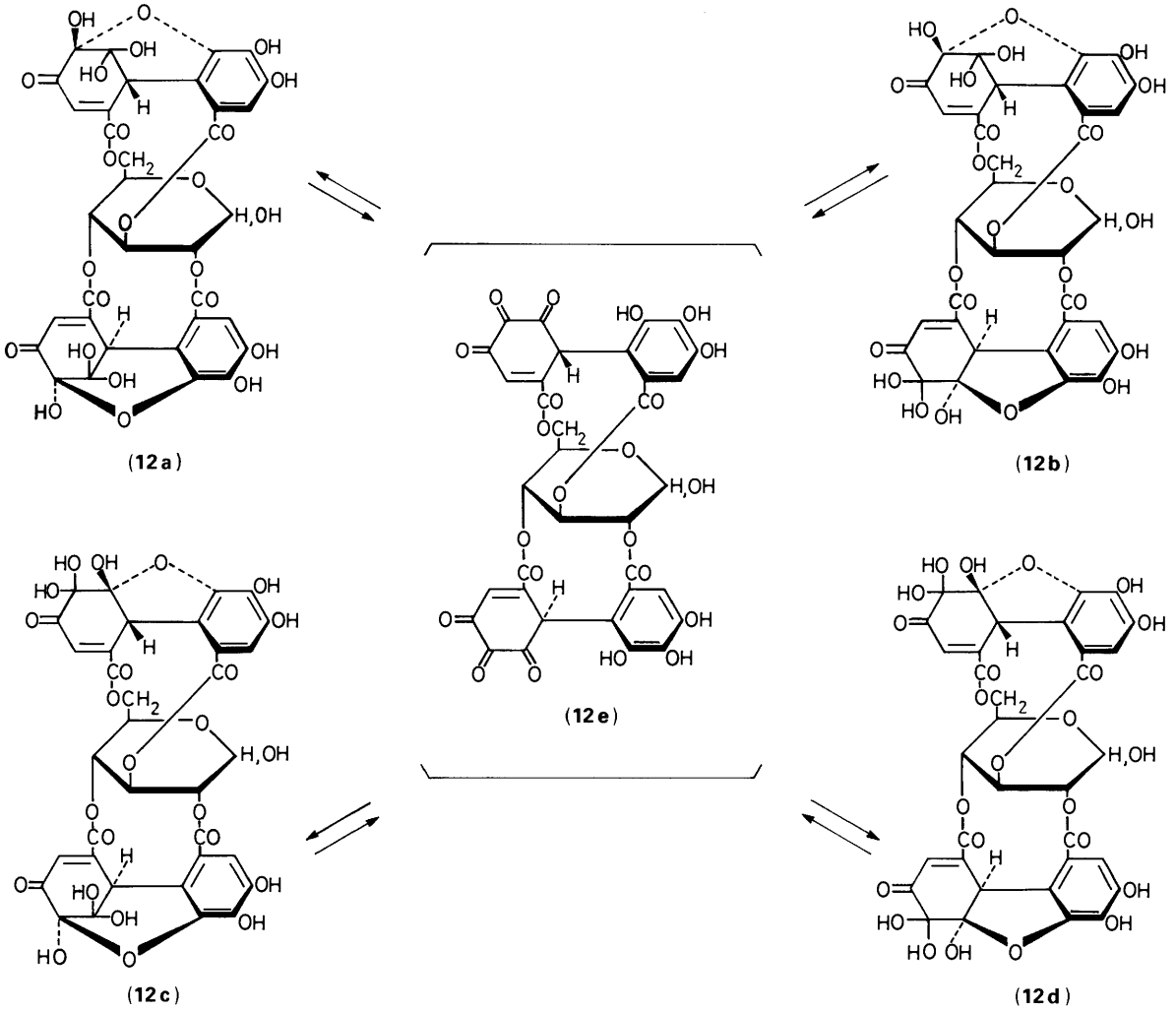


(12)

DHHDP groups, which formed the phenazine group and was then eliminated upon hydrolysis, is at O-2 ~ O-4 of the glucose. The galloyl group in didehydrogeraniin (**2**) was shown to be at O-1 of the glucose by selective degalloylation with tannase, to give the degradation product (**12**) whose ^{1}H n.m.r. spectrum showed a large upfield shift (>0.7 p.p.m.) of the glucose 1-H [δ 6.37 and 6.42 in (**2**)].

Based on the identity of the pyranose-ring conformation for compounds (**4**) and (**6**), which is exhibited by the ^{1}H n.m.r. spectra, the orientation of the DHHDP group at O-3 ~ O-6 of the glucose was assigned as in formula (**2**), since 4-H of (**4**) (δ 5.83) shifts markedly downfield from that of 4-H in (**6**) (δ 5.48),[2,8] while the shifts of 3-H [δ 5.44 in (**4**), 5.48 in (**6**)] and 6-H, -H′ [δ 4.17, 4.96 in (**4**); 4.03, 4.72 in (**6**)] do not differ by so much. This specific downfield shift of 4-H is attributable to the anisotropic effect of the phenazine moiety bound to O-6 of the glucose as shown in structure (**6a**).

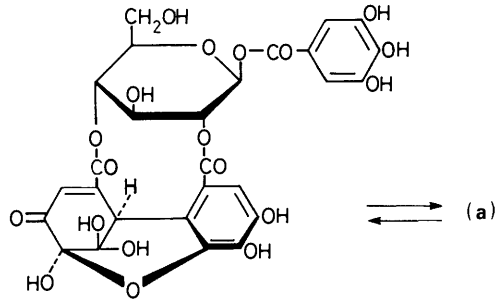
Furosinin (**12**), $C_{34}H_{24}O_{24}\cdot 3H_2O$, $[\alpha]_D$ −58°, forms a yellow amorphous powder. Although its ^{1}H and ^{13}C n.m.r. spectra are extremely complicated, they show, as do those of didehydrogeraniin (**2**), the presence of two DHHDP groups and a sugar core in the molecule of furosinin (**12**). The obvious difference between compounds (**2**) and (**12**) in their n.m.r. spectra is the absence of a galloyl group at O-1 of furosinin (**12**), which should be responsible for the remarkable spectral complication of compound (**12**) due to the anomerization. The presence of α and β anomers of furosinin (**12**) was shown by its ^{13}C n.m.r. signals at δ_C 88.9 (α); δ_C 96.3, 97.0 (β). The structure of compound (**12**) was confirmed by identifying it with the product of degalloylation of compound (**2**), mentioned above. These results established the structure of furosinin as (**12**).



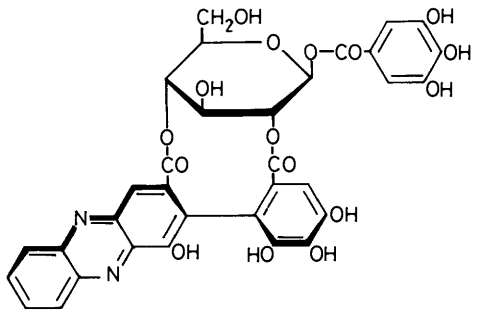
Scheme. Tautomeric forms of furosinin (**12**)

Furosinin (**12**) is present as an equilibrium mixture of many isomers in solution. Four isomers can be formed by the presence of two DHHDP groups which are equilibrated between six- and five-membered ring hemiacetal structures as indicated in the Scheme. The average proportions of these in aqueous acetone solution are 8 (**12a**): 12 (**12b**): 2 (**12c**): 3 (**12d**), although they are often variable. Further complication of the equilibrium state can be induced theoretically by the anomerization of the glucose core. Although the ratio of α form to β form is very small, small peaks in the ^{1}H and ^{13}C n.m.r. spectra of furosinin (**12**) clearly indicated the presence of eight tautomeric forms, which are basically hydrated hemiacetal forms of the potential tricarbonyl structure (**12e**) illustrated in the Scheme. This is a rare example of a natural organic compound forming many isomeric structures in the equilibrium state.

Furosin (**13**), $C_{27}H_{22}O_{19}\cdot 4H_2O$, $[\alpha]_D$ −146°, was obtained as a yellow crystalline powder. This compound showed mutarotation, $[\alpha]_D$ −154° → −146°, in the presence of 10% water in acetone solution, and the value reached a plateau in 6 h after addition of water. This phenomenon is similar to that of geraniin (**1**).[2] The ^{1}H n.m.r. spectrum of furosin (**13**) in [$^{2}H_6$]-acetone showed peaks characteristic of a galloyl group (δ 7.26), a DHHDP group (δ 7.30, 6.55, and 5.37), and a glucose moiety (δ 6.47 and 5.5—3.9). Comparison of the signals of the DHHDP group with those of crystalline geraniin before equilibration[2,8] indicated that this group in unequilibrated furosin forms the



(**13**)



(**14**)

six-membered hemiacetal structure. Occurrence of the five-membered hemiacetal structure (**13a**) upon mutarotation of furosin (**13**) was shown by the peaks at δ 4.98 (d, *J* 1.5 Hz, methine) and δ 6.28 (d, *J* 1.5 Hz, vinyl) in the ^{1}H n.m.r. spectrum measured in [$^2H_6$]acetone containing D_2O, and the ratio of six- and five-membered hemiacetal structures, 6 h after addition of D_2O, was *ca.* 1: 1. When crystallized from the solution of the equilibrium mixture, furosin formed the six-membered hemiacetal structure in a way analogous to geraniin. The ^{13}C n.m.r. spectrum in [$^2H_6$]acetone–D_2O also showed peaks attributable to a galloyl group, a DHHDP group, and a glucose in the equilibrium state, but no signal assignable to the hexahydroxydiphenoyl (HHDP) group.[9]

Condensation of furosin (**13**) with *o*-phenylenediamine gave a phenazine derivative (**14**), $C_{33}H_{24}N_2O_{16}\cdot 3H_2O$. The ^{1}H n.m.r. signals assignable to 3-H and 6-H_2 of the glucose, which are shifted upfield from those of compound (**6**), were observed at δ 4.46 and 3.95, respectively. The upfield shift of the 1-H of glucose to δ 6.15, from that of furosin (**13**) at δ 6.47, is analogous to that of compound (**6**) produced from geraniin, and is attributable to the anisotropic effect of the phenazine moiety bound to O-4 of the glucose.[2] These results indicated that the location and the orientation of the *R*-DHHDP group in furosin (**13**) are the same as in geraniin (**1**). The absolute configuration *R* at C-1c was confirmed by a positive Cotton effect at 202 nm and a negative one at 350 nm in the c.d. spectrum.[4] Therefore, the structure of furosin should be (**13**), which is an analogue of geraniin (**1**) lacking its HHDP group.

Among these three new tannins, furosinin (**12**) is presumed to have been produced upon drying of the plant, as it was isolable only from dried plants. However, all these compounds, which have the DHHDP group and lack the HHDP group, can be classified as didehydroellagitannins. The didehydroellagitannins are remarkable among the various types of tannins, not only because of the complexity of their equilibrium states, and their complex behaviour in high-performance liquid chromatography due to acetal formation in alcoholic solution,[10] but also by their high reactivities with various compounds as found for the condensations with *o*-phenylenediamine and ascorbic acid.[1]

Experimental

M.p.s were measured with a Yanagimoto micro melting point apparatus. Optical rotations were measured on a JASCO DIP-4 Digital Polarimeter. I.r. spectra were recorded on a JASCO A-102 spectrophotometer and u.v. spectra on a Hitachi 200-10 spectrophotometer. If not specified, ^{1}H n.m.r. (90 MHz) and ^{13}C n.m.r. (22.6 MHz) spectra were measured on a Hitachi R-22FTS instrument using $SiMe_4$ as standard for ^{1}H n.m.r. spectra, and $SiMe_4$ or dioxane as standard for ^{13}C n.m.r. 200 MHz ^{1}H n.m.r. and 50 MHz ^{13}C n.m.r. spectra were measured on a JEOL FT-NMR FX-200 spectrometer. G.l.c. was performed on a Hitachi Gas Chromatograph 163 equipped with a glass column (3 mm i.d. × 2 m) packed with 1% OV-1 on Chromosorb W. Paper-partition chromatography (p.p.c.) was done on Toyo No. 50 paper in the solvent system (A) butan-1-ol–acetic acid–water (4:1:5 v/v; upper layer), (B) 7% acetic acid in water (v/v), or (C) butan-1-ol–acetic acid–water (5:2:6 v/v), and spots were visualized by spraying with aqueous iron(III) chloride, with $NaNO_2$–acetic acid reagent,[7] or with 2,4-dinitrophenylhydrazine reagent. T.l.c. was performed on glass plates coated with Avicel SF microcrystalline cellulose (Funakoshi), and solvent systems and detection were the same as those applied to p.p.c. Merck Kieselgel PF_{254} was used for analytical and preparative t.l.c. Normal-phase h.p.l.c. was run on a column of Nomura Develosil 60-5, 4 × 150 mm, with hexane–methanol–tetrahydrofuran(THF)-formic acid (55:33:11:1 v/v) containing oxalic acid (450 mg^{-1} l^{-1}) as eluant. Reversed-phase h.p.l.c. was carried out on a column of Merck LiChrosorb RP-18 (10 μm) 4 × 300 mm, with 0.1 M-H_3PO_4–0.1M-KH_2PO_4–acetonitrile (43:43:14 v/v) as eluant at 40 °C. Detection was effected by u.v. absorption at 280 nm. Avicel microcrystalline cellulose (Funakoshi), and Sephadex LH-20 and G-25 (Pharmacia Fine Chemicals), were used for column chromatography. Extracts were evaporated under reduced pressure below 40 °C. Light petroleum refers to the fraction boiling in the range 85—120 °C.

Isolation of Tannins from Geranium thunbergii.—(i) Fresh leaves (260 g) of *Geranium thunbergii* were homogenized in acetone–water (7:3 v/v)(1.9 l), and the extract was filtered. The filtrate was concentrated to 190 ml and then washed with diethyl ether (190 ml × 5). The aqueous layer was subsequently extracted with ethyl acetate (100 ml × 10), and the ethyl acetate layer was evaporated to dryness to give a yellowish residue (7.7 g). To this residue (7 g) was added methanol, and insoluble substance was removed by filtration. The same volume of water was added to the filtrate and the mixture was concentrated under reduced pressure. Geraniin (**1**) crystallized from the concentrated solution on seeding, and was obtained as a yellow crystalline powder (3.34 g). Evaporation of the mother liquor afforded a yellowish residue (3.43 g). A portion (1.5 g) of this residue was subjected to droplet counter-current chromatography (d.c.c.c.) of descending mode (65 glass tubes; 2.8 mm i.d. × 60 cm) using chloroform–methanol–propan-1-ol–water (9:12:2:8 v/v), and collecting 15 g portions. Every third fraction was monitored by u.v. absorption at 280 nm, and by t.l.c. (cellulose; solvent B). Fractions 34—62 were combined, and the solvent was evaporated off to give a yellow residue (539 mg). The corresponding fractions, obtained by d.c.c.c. repeated with the rest of the residue (1.9 g) from the mother liquor of the ethyl acetate extract, were combined with the above yellowish residue. The combined fractions (1.2 g in total) were further chromatographed by d.c.c.c. (100 glass tubes; 3.2 mm i.d. × 120 cm) using butan-1-ol–acetic acid–water (4:1:5 v/v) as eluant with ascending development, collecting every 12 g-portion of eluate. Combined fractions 20—41 from this ascending d.c.c.c. were further purified on a short column of Sephadex LH-20 (1.1 cm i.d. × 15 cm) with ethanol as eluant to yield dehydrogeraniin (**2**) (778 mg). Fractions 53—93 were treated in the same way to give furosinin (**12**) (440 mg).

Total yield of each tannin from fresh leaves: geraniin (**1**) 1.28%; dehydrogeraniin (**2**) 0.3%; furosinin (**12**) 0.17%.

(ii) Dried leaves (500 g) were homogenized in acetone-water (1:1; 1.5 l) and treated in a similar way to that in (i). The remaining aqueous solution (500 ml), after extraction with diethyl ether (500 ml × 7), was subsequently extracted with ethyl acetate (250 ml × 35) to yield an ethyl acetate extract (23.4 g) from which geraniin (**1**) (0.8 g) crystallized out as in (i), and the mother liquor was evaporated to dryness. A portion (2 g) of the residue was subjected to d.c.c.c. (100 glass tubes; 3.2 mm i.d. × 120 cm) with butan-1-ol–acetic acid–water (4:1:5 v/v) by ascending development, and every 10 g of eluate was collected. Fractions 36—50 were combined and the solvent was evaporated off. The yellow residue (23 mg) was dissolved in methanol, the same volume of water was added, and the mixture was concentrated under reduced pressure. Furosin (**13**) crystallized from the aqueous solution as a yellow crystalline powder (75 mg). Didehydrogeraniin (**2**) was detected by t.l.c. in fractions 1—35 accompanied by flavonoids and other phenolics. Yield of furosin (**13**) from dried leaves was 0.015%.

Didehydrogeraniin (**2**).—A yellow amorphous powder, p.p.c. R_F (A) 0.36, R_F (B) 0.18; $[\alpha]_D$ −137° (*c* 0.5 in MeOH) (Found: C, 45.7; H, 3.4. $C_{41}H_{28}O_{28}\cdot 6H_2O$ requires C, 45.73; H, 3.74%);

$\nu_{max.}$(KBr) 3 400, 1 700, 1 610, 1 510, 1 440, 1 330, 1 210, 1 080, 1 030, and 755 cm^{-1}; $\lambda_{max.}$(MeOH) 224 (log ε 4.97) and 283 nm (4.56); c.d. (MeOH) $[\theta]_{202}$ $+10.1 \times 10^4$, $[\theta]_{233}$ -0.5×10^4, $[\theta]_{250}$ $+3.4 \times 10^4$, and $[\theta]_{358}$ -1.7×10^4; δ_H (200 MHz; CD_3COCD_3) 7.28—7.24 (2 H in total, ArH), 7.24 and 7.23 (2 H in total, galloyl), 6.74 ($\frac{3}{5}$ H, s) and 6.72 ($\frac{2}{5}$ H, s) (1 H in total, vinyl), 6.60 ($\frac{3}{5}$ H, s) and 6.29 ($\frac{2}{5}$ H, d, *J* 1.5 Hz) (1 H in total, vinyl), 6.42 ($\frac{2}{5}$ H, d, *J* 3.5 Hz) and 6.37 ($\frac{3}{5}$ H, d, *J* 3.5 Hz) (1 H in total, glucose 1-H), 5.83—4.35 (5 H in total, glucose 2—6-H), 5.33 ($\frac{3}{5}$ H, s) and 4.92 ($\frac{2}{5}$ H, d, *J* 1.5 Hz) (1 H in total, methine), and 5.30 ($\frac{3}{5}$ H, s) and 5.28 ($\frac{2}{5}$ H, s) (1 H in total, methine). δ_C Values are shown in the Table.

Condensation of Didehydrogeraniin (**2**) *with o-Phenylenediamine to Produce Compound* (**3**).—To a solution of *o*-phenylenediamine (38 mg) in a mixture of methanol–water–acetic acid (1:1:1 v/v; 7 ml) was added compound (**2**) (80 mg). After the mixture had been stirred for 7 h at room temperature, the methanol was evaporated off under reduced pressure, and the dark yellow precipitate was collected by suction, washed with water, and purified by reprecipitation from acetone–light petroleum to yield the *phenazine derivative* (**3**) as a reddish yellow amorphous powder, t.l.c. R_F (C) 0.85; $[\alpha]_D$ −210° (*c* 0.5 in MeOH) (Found: C, 53.8; H, 3.4; N, 4.4. $C_{53}H_{32}N_4O_{22}\cdot 6H_2O$ requires C, 53.72; H, 3.72; N, 4.73%); $\nu_{max.}$(KBr) 3 400, 1 720, 1 610, 1 505, 1 440, 1 355, 1 220, 1 190, 1 135, and 755 cm^{-1}; c.d. (MeOH) $[\theta]_{248}$ $+11.4 \times 10^4$, $[\theta]_{280}$ -16.9×10^4, and $[\theta]_{360}$ -0.9×10^4; δ_H (CD_3COCD_3) 8.45—7.90 (8 H, m, phenazine ring), 8.31 (1 H, s, ArH), 7.41 (1 H, s, ArH), 7.21 (2 H, s, galloyl), 7.16 (1 H, d, *J* 1.5 Hz, vinyl), 7.02 (1 H, s, ArH), 6.70 (1 H, br s, glucose 1-H), 6.09 (1 H, d, *J* 4 Hz, glucose 4-H), 5.85 (1 H, br s, glucose 2-H), 5.42 (1 H, d, *J* 1.5 Hz, methine), 5.42 (1 H, glucose 3-H), 4.94—4.55 (3 H, m, glucose 5-H and 6-H_2).

Synthesis of Phenazine Derivative (**4**).—To a solution of *o*-phenylenediamine (48 mg) in acetone–water–acetic acid (1:1:2 v/v; 8 ml) was added compound (**2**) (100 mg). After being stirred for 7 h at room temperature, the mixture was evaporated, and the residue was suspended in water, filtered off, and washed with water. Purification by reprecipitation from acetone–light petroleum afforded the *phenazine derivative* (**4**) (91 mg) as a reddish brown amorphous powder, t.l.c. R_F (C) 0.85 (Found: C, 54.1; H, 3.5; N, 4.3. $C_{53}H_{32}N_4O_{22}\cdot 6H_2O$ requires C, 53.72; H, 3.72; N; 4.73%); $\nu_{max.}$(KBr) 3 400, 1 720, 1 610, 1 505, 1 440, 1 355, 1 220, 1 170, 1 135, and 755 cm^{-1}; c.d. (MeOH) $[\theta]_{246}$ + 24.7×10^4, $[\theta]_{283}$ -22.2×10^4, and $[\theta]_{373}$ -0.5×10^4; δ_H (CD_3COCD_3) 8.40—7.86 (8 H, m, phenazine ring), 8.37 (1 H, s, ArH), 8.29 (1 H, s, ArH), 7.57 (1 H, s, ArH), 7.05 (1 H, s, ArH), 7.02 (2 H, s, galloyl), 6.29 (1 H, d, *J* 5 Hz, glucose 1-H), 5.90 (1 H, d, *J* 5 Hz, glucose 2-H), 5.83 (1 H, d, *J* 4 Hz, glucose 4-H), 4.46 (1 H, d, *J* 4 Hz, glucose 3-H), 5.16—4.80 (2 H, m, glucose 5- and 6-H), and 4.17 (1 H, d, *J* 9 Hz, glucose 6-H′).

Methylation of Phenazine Derivative (**4**).—To a solution of compound (**4**) (50 ml) in anhydrous EtOH (7 ml) was added an excess of ethereal diazomethane. After 10 min, the solvent was evaporated off to give a yellow residue, which was purified by preparative t.l.c. (p.l.c.) with light petroleum–chloroform–acetone (4:6:3 v/v). *Undeca-O-methyl derivative* (**7**) (32 mg) was isolated, from a band at R_F 0.44, as a yellow amorphous powder, $[\alpha]_D$ +49° (*c* 0.5 in dioxane) (Found: C, 58.7; H, 4.4; N, 3.7. $C_{64}H_{54}N_4O_{22}\cdot 4H_2O$ requires C, 58.94; H, 4.78, N, 4.30%); $\nu_{max.}$(KBr) 2 950, 2 840, 1 745, 1 590, 1 500, 1 460, 1 400, 1 335, 1 210, 1 160, 1 120, 1 100, 1 055, and 1 010 cm^{-1}; δ_H ($CDCl_3$) 8.51, 8.47, 7.68, and 7.06 (1 H each, s, ArH), 8.47—7.85 (8 H, m, phenazine ring), 7.16 (2 H, s, galloyl), 6.20 (1 H, d, *J* 5 Hz, glucose 1-H), 5.85 (1 H, d, *J* 5 Hz, glucose 2-H), 5.72 (1 H, d, *J* 4 Hz, glucose 4-H), 5.39 (1 H, d, *J* 4 Hz, glucose 3-H), 5.10—4.40 (3 H, m, glucose 5-H and 6-H_2), and 4.30—3.60 (33 H, 11 × OMe).

Methanolysis of Undeca-O-methyl Derivative (**7**).—A solution of compound (**7**) (22 mg) in 1% sodium methoxide in absolute methanol (5 mg) was left overnight at room temperature. After neutralization with acetic acid, the solvent was evaporated off in a stream of nitrogen at room temperature. The residue was partitioned between chloroform and water. The chloroform layer was evaporated and purified by p.l.c. [light petroleum–dichloromethane–acetone (6:3:1 v/v)] to afford (+)-methyl 4-methoxy-3-(2,3,4-trimethoxy-6-methoxycarbonylphenyl) phenazine-2-carboxylate (**9**), from a band of R_F 0.25, as yellow needles (from aqueous methanol), m.p. 130—132 °C; $[\alpha]_D$ +39° (*c* 1.0 in EtOH); $\nu_{max.}$(KBr) 2 950, 1 725, 1 595, 1 500, 1 460, 1 395, 1 335, 1 240, 1 120, 1 095, and 1 055 cm^{-1}. This was identified by direct comparison with an authentic sample derived from geraniin (**1**). Methyl tri-*O*-methylgallate (**8**) was isolated from a band of R_F 0.50 as needles, and was identical with an authentic specimen by direct comparison.

Glucose in the aqueous phase was identified by g.l.c. of its trimethylsilyl derivative.

Partial Hydrolysis of Compound (**4**).—A suspension of compound (**4**) (60 mg) in water (60 ml) was refluxed for 2.5 h, and the mixture was evaporated to dryness. Methanol was added to the residue and the insoluble residue was collected by filtration, and was crystallized from THF to yield dark red needles (**10**) (8 mg) which were identical with an authentic sample prepared from geraniin.[8] The filtrate was evaporated to afford a reddish brown residue, which was purified by reprecipitation from acetone–chloroform to give the *phenazine derivative* (**10**) as a reddish brown amorphous powder, t.l.c. R_F (C) 0.72 (Found: C, 53.15; H, 3.75; N, 3.3. Calc. for $C_{33}H_{24}N_2O_{16}\cdot 2.5H_2O$: requires C, 52.87; H, 3.90; N, 3.74%), δ_H (CD_3COCD_3) 8.40—7.90 (4 H, m, phenazine ring), 8.05 (1 H, s, ArH), 7.09 (2 H, s, galloyl), 7.00 (1 H, s, ArH), 6.43 (1 H, br s, glucose 1-H), 5.10 (1 H, d, *J* 4 Hz, glucose 3-H), 5.08 (1 H, t, *J* 10 Hz, glucose 6-H), 4.55 (1 H, dd, *J* 8, 10 Hz, glucose 5-H), 4.40 (1 H, d, *J* 4 Hz, glucose 4-H), 4.30 (1 H, br s, glucose 2-H), and 4.28 (1 H, dd, *J* 8, 10 Hz, glucose 6-H′).

Hydrolysis of Didehydrogeraniin (**2**) *with Tannase*.—A solution of compound (**2**) (102 mg) in water (10 ml) was incubated with tannase[11] at 37 °C for 24 h. The reaction mixture was concentrated, and chromatographed on a Sephadex G-25 column (1.1 cm i.d. × 30 cm), eluted with water, and 30 ml portions of eluant were collected. Fractions 7—14 were combined and evaporated to dryness to yield a yellow amorphous powder (42 mg), which was directly identified with furosinin isolated from *G. thunbergii*. Gallic acid was isolated from fraction 2.

Furosinin (**12**). A *yellow amorphous powder*, p.p.c. R_F (B) 0.33; t.l.c. R_F (C) 0.69; $[\alpha]_D$ −58.0° [*c* 0.5 in acetone–water (9:1)] (Found: C, 46.6; H, 3.6. $C_{34}H_{24}O_{24}\cdot 3H_2O$ requires C, 46.90; H, 3.47%); $\nu_{max.}$(KBr) 3 400, 1 705, 1 620, 1 515, 1 440, 1 320, 1 220, 1 140, 1 080, 1 040, 1 000, and 760 cm^{-1}; $\lambda_{max.}$(MeOH) 224 (log ε 4.76) and 277 nm (4.25); c.d. (MeOH) $[\theta]_{210}$ $+10.6 \times 10^4$, $[\theta]_{232}$ -2.7×10^4, $[\theta]_{250}$ $+1.9 \times 10^4$, and $[\theta]_{356}$ -1.9×10^4; δ_H (200 MHz; CD_3COCD_3) 7.33 (s), 7.32 (s), 7.27 (s), 7.25 (s), 7.229 (s), and 7.226 (s) (2 H in total, ArH), 6.70 (s), and 6.52 (s) ($\frac{8}{30}$ H each, vinyl), 6.65 (s) and 6.23 (d, *J* 1.5 Hz) ($\frac{12}{30}$ H each, vinyl), 6.52 (s) and 6.34 (d, *J* 1.5 Hz) ($\frac{2}{30}$ H each, vinyl), 6.33 (d, *J* 1.5 Hz) and 6.25 (d, *J* 1.5 Hz) ($\frac{3}{30}$ H each vinyl), 5.69—4.37 (7 H in total, glucose), 5.42 (s) and 5.38 (s) ($\frac{8}{30}$ H each, methine), and 5.36 (s) and 4.90 (d, *J* 1.5 Hz) ($\frac{12}{30}$ H each, methine); δ_C (50 MHz; CD_3COCD_3–D_2O) 194.0, 193.9, 191.8, 191.5, and 191.0 (C-4c in the partial structure in the Table), 168.3, 168.1, 166.7, 166.0,

164.7, 164.6, 164.5, and 164.4 (ester), 153.9, 152.7, 152.0, 148.8, 147.6, 147.1, 145.7, 143.2, 142.9, 142.8, 139.1, 138.3, 137.3, and 136.5 (C-4b, -5b, -6b, and -2c), 129.0, 128.5, 128.3, 125.3, 124.7, 120.2, 120.0, 119.9, 117.3, 116.1, 114.9, 113.9, 113.0, 109.6, and 109.5 (C-1b, -2b, -3b, and -3c), 97.0, 96.3, 96.0, 92.2, 92.1, 91.9, and 88.9 (C-5c, -6c, and glucose C-1), 77.2, 76.9, 76.3, 75.5, 70.5, 69.8, 68.0, 66.6, 66.4, and 66.2 (glucose C-2—6), and 55.2, 52.0, 51.0, 46.1, 44.8, and 44.5 (C-1c).

Furosin (**13**). A *yellow crystalline powder*, p.p.c. R_F (A) 0.50, R_F (B) 0.32; t.l.c. R_F (C) 0.73; $[\alpha]_D$ −146° (*c* 0.5 in acetone); mutarotation $[\alpha]_D$ −154° → −146° [*c* 0.5 in acetone–water (9:1)] (Found: C, 44.7; H, 4.0. $C_{27}H_{22}O_{19}\cdot 4H_2O$ requires C, 44.88; H, 4.19%); $\nu_{max.}$(KBr) 3 400, 1 705, 1 620, 1 515, 1 440, 1 340, 1 205, 1 085, 1 035, 970, and 760 cm^{-1}; $\lambda_{max.}$(MeOH) 223 (log ε 4.80) and 283 nm (4.43); c.d. (MeOH) $[\theta]_{202}$ +5.1 × 10^4, $[\theta]_{231}$ −2.0 × 10^4, $[\theta]_{250}$ +1.7 × 10^4, $[\theta]_{289}$ −2.9 × 10^4, and $[\theta]_{350}$ −1.1 × 10^4; δ_H (CD_3COCD_3) 7.30 (1 H, s, ArH), 7.26 (2 H, s, galloyl), 6.55 (1 H, s, vinyl), 6.47 (1 H, br s, glucose 1-H), 5.37 (2 H, s, glucose 2-H and methine), 5.22 (1 H, d, *J* 3 Hz, glucose 4-H), 4.50—3.90 (4 H, m, glucose 3-, 5-H and 6-H_2); signals at δ 6.28 (d, *J* 1.5 Hz) and 4.98 (d, *J* 1.5 Hz) assignable to a vinyl proton (½ H) and a methine proton (½ H), respectively, were observed after addition of D_2O which caused equilibration. ^{13}C N.m.r. data are in the Table.

Phenazine Derivative (**14**) *of Furosin*.—To a solution of furosin (14 mg) in a small amount of methanol was added a solution of *o*-phenylenediamine (5 mg) in 50% acetic acid (0.8 ml). After being stirred for 5 h at room temperature, the mixture was evaporated. The residue was suspended in water, collected by suction, washed with water, and reprecipitated from methanol–chloroform to afford the *phenazine derivative* (**14**) (13 mg) as a reddish brown amorphous powder, t.l.c. R_F (C) 0.85; $[\alpha]_D$ −274° (*c* 0.5 in MeOH) (Found: C, 52.6; H, 3.8; N, 3.6. $C_{33}H_{24}N_2O_{16}\cdot 3H_2O$ requires C, 52.25; H, 3.99; N, 3.69%); $\nu_{max.}$(KBr) 3 400, 1 720, 1 610, 1 505, 1 445, 1 355, 1 220sh, 1 190, 1 040, and 750 cm^{-1}; c.d. (MeOH) $[\theta]_{221}$ −2.2 × 10^4, $[\theta]_{247}$ + 7.5 × 10^4, $[\theta]_{279}$ −14.0 × 10^4, and $[\theta]_{324}$ + 0.4 × 10^4; δ_H(CD_3COCD_3) 8.40—7.90 (4 H, m, phenazine ring), 8.22 (1 H, s, ArH), 7.48 (1 H, s, ArH), 7.00 (2 H, s, galloyl), 6.15 (1 H, d, *J* 6 Hz, glucose 1-H), 5.39 (1 H, d, *J* 6 Hz, glucose 2-H), 5.16 (1 H, d, *J* 4 Hz, glucose 4-H), 4.50 (1 H, t, *J* 5 Hz, glucose 5-H), 4.46 (1 H, d, *J* 4 Hz, glucose 3-H), and 3.95 (2 H, d, *J* 5 Hz, glucose 6-H_2).

Detection of compound (**13**) *in Crude Extracts of* G. thunbergii.—Fresh leaves of *G. thunbergii* (240 g) were divided into three portions (80 g fresh wt, each). One of them was homogenized in 50% aqueous acetone immediately after collection, the second portion was dried at 50 °C for 5 h to reduce its weight to 22 g, and the third portion was air-dried to give 25 g of dried leaves. They were extracted with 50% aqueous acetone, and each extract was treated in the same way as described before to give the mother liquors. These were subjected to h.p.l.c. to test for the presence of furosin (**13**).

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