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Relationship between Hydrophobicity and Structure of Hydrolyzable Tannins, and Association of Tannins with Crude Drug Constituents in Aqueous Solution

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The hydrophobicity values of hydrolyzable tannins were evaluated by measuring the distribution of the compounds between *n*-octanol and water. Of 8 gallotannins and 13 ellagitannins examined, pentagalloylglucose (7), the major polyphenol of Paeoniae Radix, showed the largest partition coefficient value. In aqueous solution, pentagalloylglucose associated with various crude drug constituents, such as paeoniflorin, glycyrrhizin potassium salt, aconitine trifluoroacetate, liquiritin apioside and amygdalin. The ^{1}H-NMR spectroscopic examination suggested that the association occurred preferentially at the most hydrophobic sites of the molecules. The association with these compounds inhibited the distribution of pentagalloylglucose into the *n*-octanol phase and adsorption on hide powder. In addition, the water solubility of the biologically active polymeric proanthocyanidins of rhubarb was increased by association with rhein 8-*O*-glucoside potassium salt, the major anthraquinone glycoside of rhubarb.

Key words tannin; hydrophobic association; polyphenol; pentagalloylglucose; proanthocyanidin

The majority of biological activities of tannins are attributable to their abilities to scavenge radicals and to associate with proteins, metals, amines and polysaccharides through hydrophobic, ionic and hydrogen-bonding interaction. Haslam and coworkers have extensively studied the interaction of tannins with caffeine, cyclodextrin and peptides.1) Their results suggested that intrinsic properties of tannins, such as precipitating proteins, are affected by interaction with coexisting compounds, and consequently the apparent behavior of tannin may be different from that of tannin alone. In our preliminary examination, for example, the precipitate formed by hydrophobic and hydrogen-bonding interactions between caffeine and pentagalloylglucose (**7**) in aqueous solution were easily destroyed by addition of paeoniflorin (**23**), which coexists with **7** in the extract of Paeoniae Radix, while addition of sucrose instead of **23** was ineffective.

Recently it was reported that the bioavailability of an active principle of Japanese and Chinese traditional medicine using decoctions of the mixtures of medicinal herbs was significantly higher than that observed when the pure active principle was administered.2) Although the reason for the increase of bioavailability has not been fully clarified, the increase of water solubility of the active principle caused by interaction with coexisting constituents may be an important factor. As for tannins, the polymeric proanthocyanidins of crude drugs, such as rhubarb,3) have been reported to be active principles of some biological and pharmacological activities such as decrease of blood urea nitrogen,4) antihypertensive effect5) and inhibition or activation of various enzymes.6) Although the polymeric proanthocyanidins (PPA) are practically insoluble in water when the samples are purified, the aqueous extracts of crude drugs contain significant amounts of these tannins. This solubilization may also be accounted for by interaction with coexisting substances contained in the crude drug. The purpose of this work was to understand 1) the relationship between water solubility of tannins and their structures, 2) the nature of the interaction between tannins and coexisting substances and 3) the change of properties of tannins as a consequence of the interaction.

Results and Discussion

Relationship between the Water Solubility of Hydrolyzable Tannins and Their Structures In order to clarify the relation between water solubility of tannins and their structures, first of all, the partition coefficients between *n*-octanol and water of 21 hydrolyzable tannins of various structures were compared. The partition coefficients of mono- (**1**), di- (**2**), tri- (**3**), tetra- (**4** and **5**) and pentagalloylglucoses (**7**), a methyl tetragalloyl-β-glucoside (**6**)7) and hamamelitannin (**8**)8) simply depended on the number of galloyl groups and a plot of the logarithm of the partition coefficients *versus* the number of galloyl groups gave a linear correlation (Fig. 1).

On the other hand, ellagitannins showed smaller partition coefficients than that of **7**. For example, the partition coefficient of eugeniin (**9**),9) which has one hexahydroxydiphenoyl (HHDP) group, is almost one-

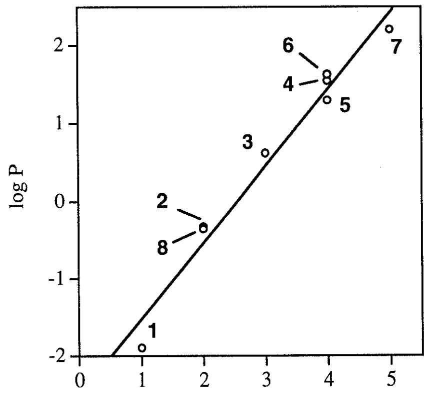

Fig. 1. Relationship between Number of Galloyl Groups of Gallotannins and Partition Coefficient (*P*) between *n*-Octanol and Water at 15 °C

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tenth of that of **7**. Furthermore, ellagitannins with similar molecular weights but two HHDP groups, that is, 1(β)-*O*-galloylpedunculagin (**12**)[10] and casuarinin (**13**),[11,12] showed much smaller partition coefficient values, one-tenth of that of **9**. This result suggested that the water solubility of these hydrolyzable tannins depends on the number of biphenyl bonds in the molecule. A plot of the logarithm of partition coefficient of **7** and thirteen ellagitannins (**9**—**21**) *versus* the number of biphenyl bonds[13] in the molecules is shown in Fig. 2. Although the presence of free alcoholic hydroxyl group(s) [*e.g.* pedunculagin (**11**)[14] and corilagin (**15**)[15]] significantly decreased the partition coefficient, the logarithm of the partition coefficient of these tannins seems to correlate linearly to the number of biphenyl bonds in the molecule. This result suggested that the hydrolyzable tannins with rigid molecular structures showed higher water solubility than tannins with flexible structure such as **7**.

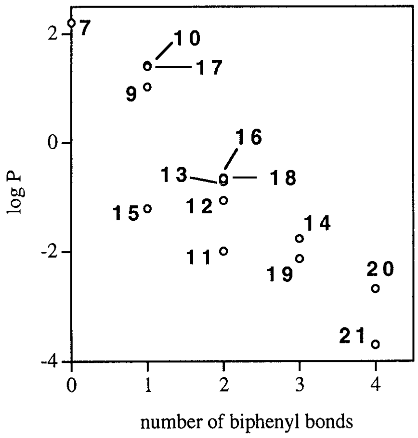
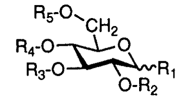


Fig. 2. Relationship between Number of Biphenyl Bonds of Hydrolyzable Tannins and Partition Coefficient (P) between *n*-Octanol and Water at 15 °C

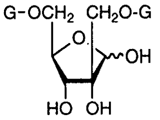
Pentagalloylglucose (**7**), the most hydrophobic tannin used in this experiment, forms a gel in aqueous solution at ambient temperature. This phenomenon has been explained by strong 'self-association' of the molecules caused by hydrophobic stacking of the disk-like molecules and by intermolecular hydrogen-bonding between galloyl groups, accompanied with the localization and exclusion of solvent molecules.[1b] Hence, the preferential distribution of **7** to the *n*-octanol phase is probably associated with an increase of entropy.

Hydrophobic Interaction of Tannins with Coexisting Compounds in Aqueous Solution In Japanese and Chinese traditional medicine, Paeoniae Radix (the root of *Paeonia* spp. containing a mixture of galloylglucoses[16]) and Rhei Rhizoma (rhubarb containing flavan-3-ols and di-polymeric proanthocyanidins[3]) are perhaps the most important tannin-containing crude drugs in terms of the high tannin content and the frequency of use. These crude drugs are usually mixed with other crude drugs such as Glycyrrhizae Radix, Aconiti Tuber and Persicae Semen for medicinal use. In order to study the interaction between tannins and the constituents of these crude drugs, we have used pentagalloylglucose of Paeoniae Radix and dimeric and polymeric proanthocyanidins of Rhei Rhizoma for the following experiments.

Firstly, the change in the ^{1}H-NMR chemical shifts of paeoniflorin (**23**) in deuterium oxide containing 5% of dimethyl sulfoxide (DMSO)-d_6 with variation of the concentration of **7** was examined. As shown in Fig. 3, a large upfield shift of the signals due to the benzoyl group was observed, while the shifts of the signals due to monoterpene and glucose units were smaller in inverse proportion to the distance from the benzoyl group. These upfield shifts were considered to be attributable to the anisotropic effect of the galloyl groups of **7**. This observation indicated that hydrophobic interaction oc-



	R1	R2	R3	R4	R5
1:	β-OG	H	H	H	H
2:	β-OG	H	H	H	G
3:	β-OG	G	H	H	G
4:	β-OG	G	G	H	G
5:	α,β-OH	G	G	G	G
6:	β-OMe	G	G	G	G
7:	β-OG	G	G	G	G



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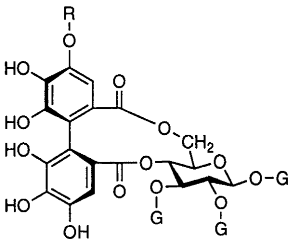
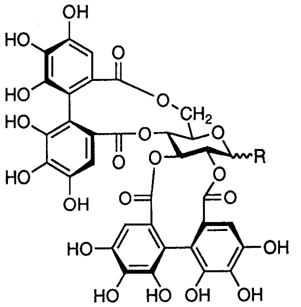
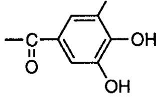


	R
9:	H
10:	PG



	R
11:	α,β-OH
12:	β-OG

G =



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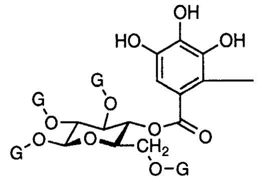

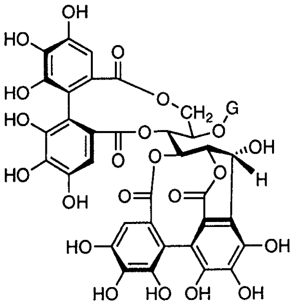

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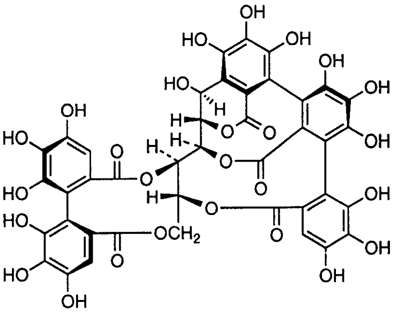

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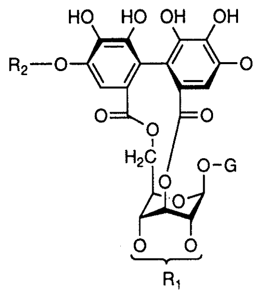
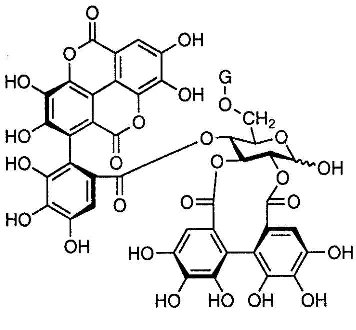


	R_1	R_2
15:	H, H	H
16:	DHHDP	H
17:	H, H	PG
18:	DHHDP	PG



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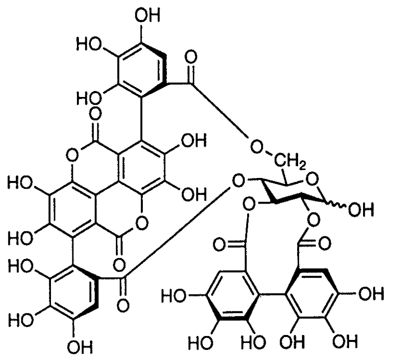

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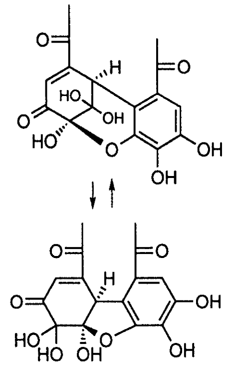
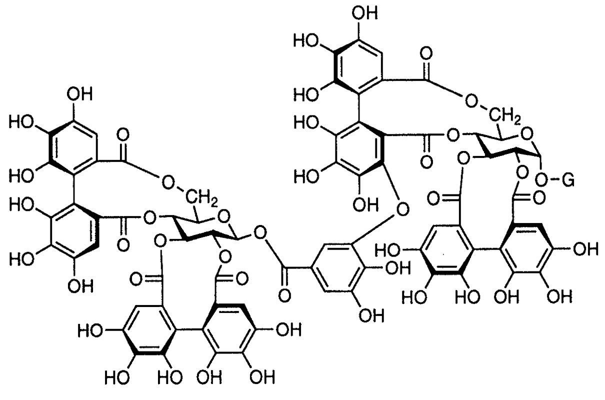
DHHDP =





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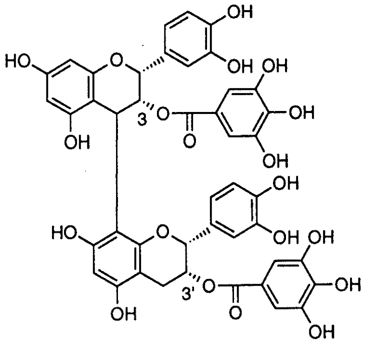

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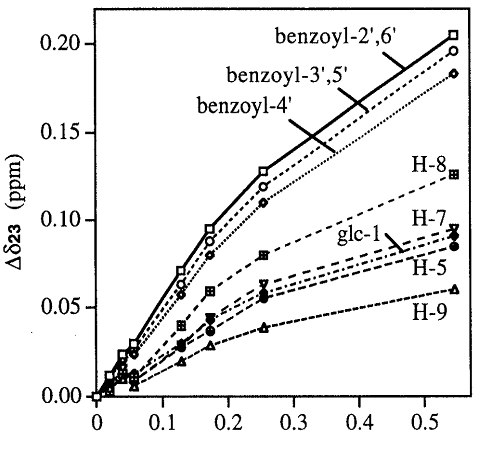


Fig. 3. Chemical Shift Change ($\Delta\ \delta$) of the Proton Signals of **23** in the Presence of **7** (20 °C, in D_2O–DMSO-d_6, 95 : 5, v/v)

Positive $\Delta\ \delta$ value indicates an upfield shift.

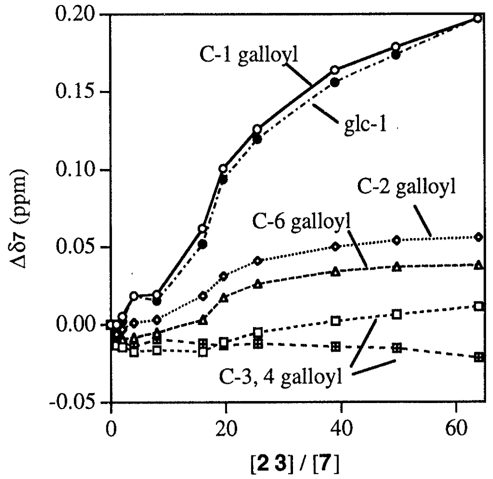


Fig. 4. Chemical Shift Change ($\Delta\ \delta$) of the Signals due to Galloyl Groups of **7** in the Presence of **23** (20 °C, in D_2O–DMSO-d_6, 95 : 5, v/v)

Positive $\Delta\ \delta$ value indicates an upfield shift.

curred between the benzoyl group of **23** and the galloyl groups of **7**. Selective hydrolysis of the galloyl groups of **7** by addition of tannase in the NMR tube significantly decreased the upfield shift of the benzoyl proton signals [$\Delta\ \delta_{H\text{-}2,6}=0.13$ ([**7**] : [**23**] = 1 : 4)→$\Delta\ \delta_{H\text{-}2,6}=0.04$ (after addition of tannase)] indicating that the bound form of gallic acid on glucose is essential for the hydrophobic interaction. On the other hand, when the concentration of **23** was varied, the chemical shifts of the anomeric proton and the galloyl group attached to the anomeric position of **7** were markedly shifted upfield (Fig. 4). This observation suggested that contribution of the galloyl group at the anomeric position to the hydrophobic interaction is larger than that of other galloyl groups. This result is consistent with the site of association of **7** with caffeine.[1a)] A differential nuclear Overhauser effect (NOE) experiment was conducted to probe the preferential association between the benzoyl groups of **23** and the C-1 galloyl group of **7** by seeking intermolecular NOE (Fig. 5). Irradiation of the benzoyl H-2 and H-6 showed NOE with the galloyl groups and anomeric proton of **7**. Conversely, irradiation of the glucose anomeric proton of **7** revealed the NOE with aromatic protons of **23**. In this experiment, negative NOEs were observed because the molecular motion of **7** in water was restricted by strong self-association.[17)]

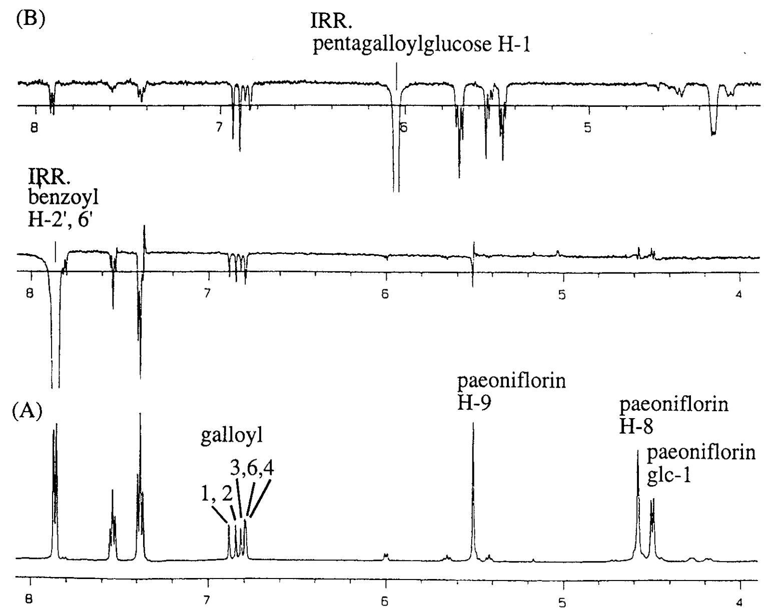
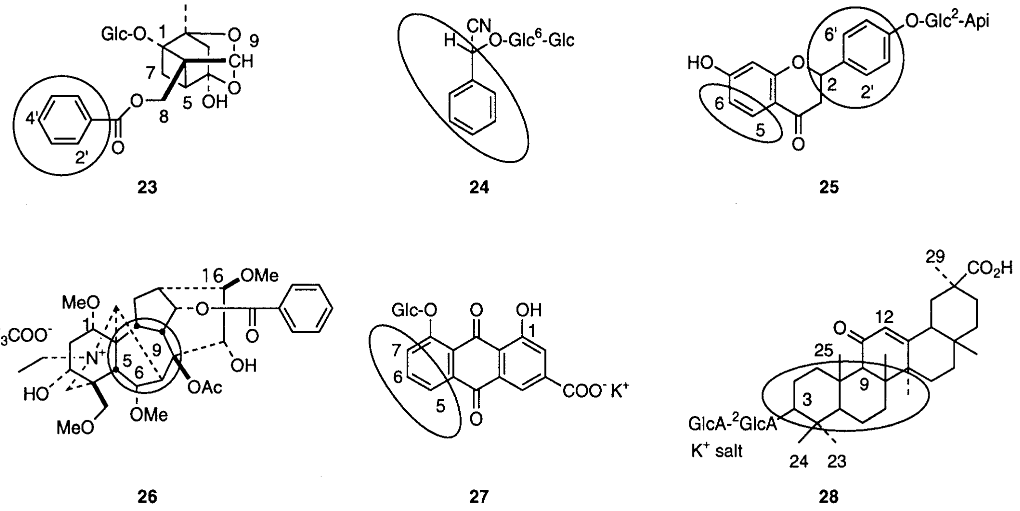


Fig. 5. ^{1}H-NMR Spectrum (A) and NOE Difference Spectra (B) of a Mixture of Paeoniflorin (**23**) and Pentagalloylglucose (**7**) [**23** (0.056 M) and **7** (0.0071 M) in D_2O at 33 °C]



23 **24** **25**

26 **27** **28**

Chart 1. The Preferential Sites (Circle) of Association of **23**—**28** with **7**

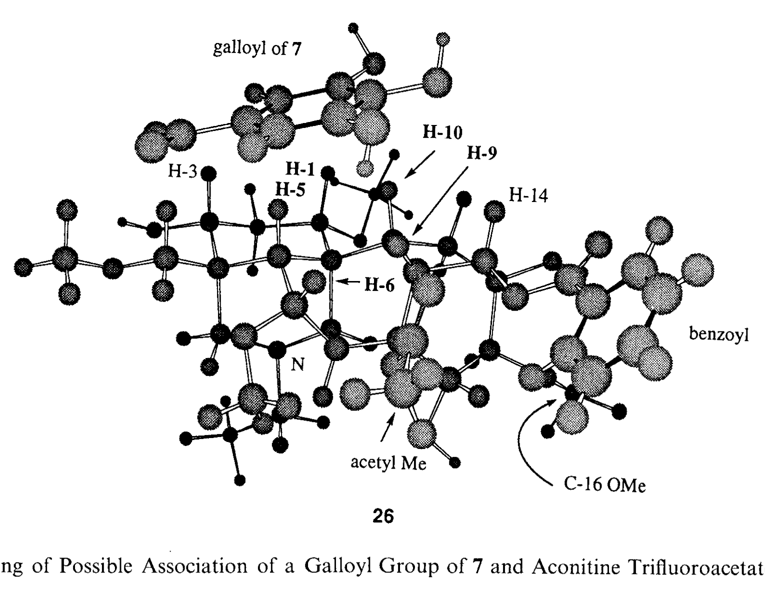


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Chart 2. A Chem 3D Drawing of Possible Association of a Galloyl Group of **7** and Aconitine Trifluoroacetate (**26**)

Similar ^{1}H-NMR experiments with amygdarin (**24**) of Persicae Semen and liquiritin apioside (**25**) of Glycyrrhizae Radix indicated that the hydrophobic association of these compounds with **7** occurred at the aromatic rings, which are the most hydrophobic moieties in these molecules (Chart 1). In addition to hydrophobic interaction, π–π interaction between aromatic rings may also be involved in the association.[18] With aconitine trifluoroacetate (**26**), however, the association seemed to occur preferentially over the β-side of the seven-membered ring (the opposite side to the nitrogen atom) rather than its benzoyl group, because the signals due to H-5, H-10, H-6, H-1 and H-9 of **26** were markedly shifted upfield ($\Delta\ \delta > 0.15$ with 0.0039 M **7**) by addition of **7** (Chart 2). In this association, interaction with the benzoyl group is probably hindered by the steric effect of the neighboring C-8 acetyl and C-16 methoxyl groups. Similar hydrophobic association was also observed between procyanidin B-2 3, 3′-di-*O*-gallate (**22**) and rhein 8-*O*-glucoside potassium salt (**27**), both of which are major constituents of rhubarb.[3] From the $\Delta\delta_H$ value, the interaction was deduced to occur mainly between the 3′-*O*-galloyl group of the **22** and around C-5 and C-6 of the anthraquinone nucleus of **27**. In this experiment, the signals due to H-2 and H-4 of the anthraquinone moiety were shifted to lower field, probably through deshielding by the aromatic rings of **22**. These results suggested that the hydrophobic interaction of tannins and coexisting compounds occurs at the most hydrophobic site of each molecule and the association is modified by steric factors.

In the association of **7** with glycyrrhizin potassium salt (**28**), large $\Delta\ \delta_H$ values of the signals due to H-9 , H-3 and H_3-25 of **28** were observed (Fig. 6), suggesting that the association preferentially occurred at the A and B rings of the aglycone. However, compared to the change of $\Delta\ \delta_H$ of **23** in Fig. 3, the $\Delta\ \delta_H$ of **28** increased almost linearly with the concentration of **7**, suggesting that the mode of association is different from that between **23** and **7**. Since triterpene glucuronides are known to be anionic surfactants,[19] **7** was probably associated with the micelles formed by the aggregation of glycyrrhizin molecules.

Effect of Hydrophobic Association on the Water Solubility of Tannins The distribution of **7** to the *n*-octanol phase from the water phase decreased concentration-dependently in the presence of **23** (Table 1). Similarly, the partition coefficient of **7** was decreased with the presence of **24**, **25**, **26** and **28**. Since sucrose did not affect the partition coefficient of **7**, the decrease of the distribution was presumably accounted for by the association of **7** with coexisting compounds in the aqueous phase. The larger decrease of the partition coefficient in the presence of **28** than those with other compounds is consistent with the difference in the mode of association deduced from the above-mentioned ^{1}H-NMR experiment. The partition coefficient of **7** in the aqueous solution of the extract of Paeoniae Radix was at almost the same level as that observed for **7** in the presence of 25 eq of **23**, which is consistent with the relative concentrations of **23** and **7** in the aqueous extract ([**23**]/[**7**] = *ca.* 40, estimated from their absorbances and peak areas on HPLC analysis).

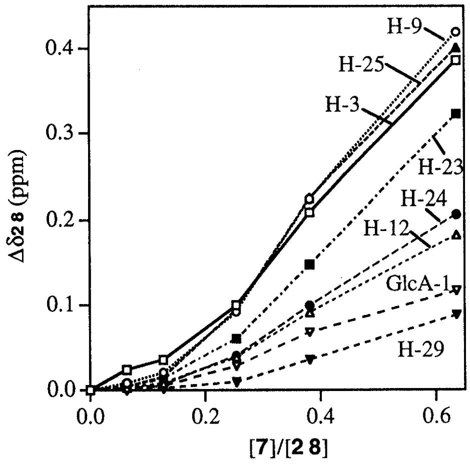

Fig. 6. Chemical Shift Change ($\Delta\ \delta$) of the Proton Signals of **28** in the Presence of **7** (20 °C, in D_2O–DMSO-d_6, 95 : 5, v/v)

In addition, the presence of water-soluble glycosides increased the water solubility of polymeric proanthocyanidins: for example, rhubarb polymeric proanthocyanidins[3] which are substantially insoluble in water, could be dissolved by addition of **27** under neutral conditions (Table 2). Because the addition of sucrose did not change the solubility, it can be considered that the increase of solubility was caused by hydrophobic, hydrogen-bonding or ionic interactions between polymeric proanthocyanidins and the anthraquinone glycoside. When the ^{1}H-NMR spectrum of **27** was measured in the presence of the polymeric proanthocyanidins (**27** 2 mg, polymer 2 mg in

Table 1. Partition Coefficient of **7** between *n*-Octanol and Water in the Presence of **23**, **24**, **25**, **26**, **28** and Sucrose

	[**7**] (M)	Conc. (M)	Partition coefficient (relative value)
Control[a]		—	100
23	0.001[b]	0.005	41 ± 3
	0.002[c]	0.01	41 ± 3
	0.002[c]	0.02	14 ± 1
	0.002[c]	0.05	4 ± 0.3
	0.002[c]	0.1	1 ± 0.02
24[b]	0.001	0.005	77 ± 1
25[b]	0.001	0.005	43 ± 11
26[b]	0.001	0.005	63 ± 3
28[b,d]	0.001	0.005	0.6 ± 0.02
Sucrose[b]	0.001	0.005	103 ± 9

a) Partition coefficient of **7** was 86 ± 5 at 27 °C (0.001 M) and 160 ± 30 at 15 °C (0.002 M). *b*) Measured at 27 °C. *c*) Measured at 15 °C. *d*) pH 6.0. Conc. = concentration.

Table 2. Solubility of Polymeric Proanthocyanidins (PPA) of Rhubarb in the Presence of Rhein 8-*O*-Glucoside Potassium Salt (**27**) (in 1 ml of Water, pH 6, 25 °C)

	Solubility (%, relative value[a])
PPA (2 mg) in MeOH	100
PPA (2 mg)	7
PPA (2 mg) + sucrose (13 mg)	11
PPA (2 mg) + **27** (1 mg)	17
PPA (2 mg) + **27** (4 mg)	68
PPA (2 mg) + **27** (8 mg)	89

a) Relative solubility was estimated by comparison of the HPLC peak area with that of MeOH solution (100%).

Table 3. Inhibition of Adsorption of **7** on Hide Powder in Water by Addition of **23** ([**7**] = 0.002 M, Hide Powder (3 mg/ml), 50 °C, 15 min)

	[**23**] (M)	Adsorption (%[a])
Control	—	48
+**23**	0.02	35
+**23**	0.05	28
+**23**	0.1	17

a) Adsorption (%) was estimated from the concentration of 7 in the supernatant.

0.7 ml of D_2O), H-5 showed the largest upfield shift ($\Delta\ \delta_H$ 0.13). Since C-5 is the position at which the association with **22** occurs preferentially, this result suggests that hydrophobic interaction is the major factor causing the increase of the solubility.

Moreover, the presence of **23** reduced the adsorption of **7** on hide powder, which is used for quantitative determination of tannins (Table 3). Under conditions where 48% of **7** was adsorbed on hide powder, the presence of 25 eq of paeoniflorin reduced the adsorption to 28% . This result could be accounted for by competitive association of **7** with **23** and hide powder.

Conclusion

Our results indicated that the water solubility of tannins is increased by hydrophobic association with water-soluble compounds such as **23**—**28** contained in the crude drugs used in Japanese and Chinese traditional medicine. Since it has been reported that the hydrophobic interaction is most important for association between tannins and proteins,[1d,e] the association of tannins with coexisting compounds may affect competitively their ability to inhibit or activate enzymes.[6]

Although galloylglucoses are well known as constituents of galls formed on leaves of *Rhus japonica*, *Quercus lusitianica* and *Distylium racemosum* by parasitism of insects specific to each plant, there are not many higher plants which accumulate galloylglucoses as major constituents in normal tissue.[20] *Paeonia* spp. and *Arctostaphylos uva-ursi* contains significant amounts of galloylglucoses in their normal tissue together with large amounts of water-soluble glycosides having a hydrophobic aromatic group, that is, **23** and arbutin, respectively. In these plants, the water solubility of the galloylglucoses is probably increased by the association with these glycosides. Most other higher plants accumulating hydrolyzable tannins seem to metabolize galloylglucoses into more water-soluble ellagitannins by formation of biphenyl bonds *via* oxidative coupling between galloyl groups.[21]

Experimental

General Analytical HPLC was performed on a Tosoh apparatus equipped with a CCPM solvent delivery system, a UV-8000 spectrometer (280 nm) and a Cosmosil $5C_{18}$-AR (Nacalai Tesque Inc.) column (4.6 i.d. × 250 mm) (mobile phase, 5—30% acetonitrile in 50 mM phosphoric acid; flow rate, 0.8 ml/min). ^{1}H-NMR spectra were obtained with Varian Unity Plus 500, and Varian Gemini 300 spectrometers operating at 500 and 300 MHz; chemical shifts are reported in ppm on the δ scale from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate. The residual dimethyl sulfoxide signal was used as a reference in the experiments without an internal standard. Hide powder (slightly chromated) was purchased from Merck.

Materials 1,2,3,4,6-Penta-*O*-galloyl-β-D-glucopyranose (**7**) was prepared from tannic acid by heating (80 °C for 24 h) in acetate buffer (pH 4.5)–MeOH (1 : 2, v/v) followed by purification by Sephadex LH-20 column chromatography (EtOH–H_2O).[22] 1-*O*-Galloyl-β-D-glucopyranose (**1**), 1,6-di-*O*-galloyl-β-D-glucopyranose (**2**), 1,2,6-tri-*O*-galloyl-β-D-glucopyranose (**3**), 1,2,3,6-tetra-*O*-galloyl-β-D-glucopyranose (**4**), 2,3,4,6-tetra-*O*-galloyl-β-D-glucopyranose (**5**), methyl 2,3,4,6-tetra-*O*-galloyl-β-D-glucopyranoside (**6**), eugeniin (**9**), pedunculagin (**11**), 1(β)-*O*-galloylpedunculagin (**12**) and sanguiin H-6 (**21**) were isolated from Sanguisorba officinalis.[23] Terflavin A (**19**) and punicalagin (**20**) were isolated from *Terminalia cattapa*.[24] Phillyraeoidin A (**10**) and casuarinin (**13**) were isolated from Quercus phillyraeoides.[25] Corilagin (**15**), geraniin (**16**) and bischofianin (**18**) were isolated from *Bischofia javanica*.[26] Dimeric ellagitannin **17** was derived from bischofianin by partial hydrolysis. Hamamelitannin (**8**) and vescalagin (**14**) were isolated from *Castanea crenata*.[8,27] Procyanidin B-2 3,3′-di-*O*-gallate (**22**)[3] (from the AcOEt layer), rhein 8-*O*-glucoside potassium salt (**27**) and polymeric proanthocyanidins[3] (from the final H_2O layer) were isolated from the aqueous acetone extract of commercial rhubarb. The presence of potassium ion in **27** was determined by the negative ion FAB-MS of **27**, showing $[M-H]^-$ at *m/z* 435. Polymeric proanthocyanidin was purified by Sephadex LH-20 column chromatography (H_2O—acetone) followed by precipitation from water. Paeoniflorin (**23**) was isolated from the aqueous acetone extract of commercial Paeoniae Radix by chromatographies over Sephadex LH-20, MCI gel CHP 20P and silica gel. Liquiritin apioside (**25**) and glycyrrhizin were isolated from the MeOH extract of commercial Glycyrrhizae Radix by similar chromatographies. Glycyrrhizin potassium salt (**28**) was prepared by addition of potassium hydroxide, and the pH of the aqueous solution was 6—6.5. Amygdalin (**24**) and aconitine (**26**) were purchased from Nacalai Tesque.

Partition Coefficient (General Procedure) Tannin (2 mg) was dissolved in water (1 ml) and partitioned with *n*-octanol (1 ml) at 25 °C. The upper and lower phases were analyzed by HPLC. The partition coefficient was calculated from peak areas (average of 3—4 injections) obtained by using an integrator connected to a UV detector. Partition coefficient of tannins at 15 °C: **1**, 0.013; **2**, 0.47; **3**, 4.1; **4**, 36; **5**, 20; **6**, 43; **7**, 160; **8**, 0.45; **9**, 10; **10**, 26; **11**, 0.008; **12**, 0.086; **13**, 0.19; **14**, 0.017; **15**, 0.064; **16**, 0.23; **17**, 24.5; **18**, 0.22; **19**, 0.007; **20**, 0.002; **21**, 0.0002; **22**, 27.

Partition Coefficient of 7 in Aqueous Extract of Paeoniae Radix Paeoniae Radix (5 g) was extracted with water (100 ml) at 100 °C for 30 min, filtered, and lyophilized (1.8 g). The extract (10 mg) was dissolved in water (1 ml) and partitioned with *n*-octanol (1 ml) at 15 °C. The upper and lower phases were analyzed by HPLC with gradient elution with 5→35% (30 min) acetonitrile in 50 mM H_3PO_4. The partition coefficient value calculated for **7** was 8.6.

Measurement of ^{1}H-NMR Chemical Shift Change (General Procedure) The ^{1}H-NMR spectra (300 MHz) of **23** (8 mg), **24** (10 mg), **25** (10 mg), **26** (aconitine 2 mg and equimolar trifluoroacetic acid) or **28** (15 mg) in the presence of various amounts of **7** (0.25—10 mg) and those of **7** (1 mg) in the presence of various amounts of **23** (0—32 mg) were measured in D_2O–DMSO-d_6 (95 : 5, v/v, 0.75 ml) at 20 °C, and the $\Delta\ \delta$ value (δ_0—δ_{mix}) was calculated. Assignments of ^{1}H-NMR spectra of **26**, **28** and **7** were achieved by heteronuclear multiple bond correlation (HMBC) spectroscopy. **26**: δ: 8.04 (2H, d, $J=8$ Hz, benzoyl H-2, 6), 7.72 (1H, t, $J=8$ Hz, benzoyl H-4), 7.56 (2H, t, $J=8$ Hz, benzoyl H-3, 5), 5.03 (1H, d, $J=5$ Hz, H-14), 4.51 (1H, d, $J=6$ Hz, H-15), 4.30 (1H, d, $J=6$ Hz, H-6), 4.23 (1H, br s, H-3), 3.68 (3H, s, 16-OMe), 3.62 (1H, br s, H-1), 3.36 (3H, s, 1-OMe), 3.32 (3H, s, 18-OMe), 3.24 (3H, s, 6-OMe), 3.12 (1H, br s, H-7), 3.04 (1H, br t, $J=6$ Hz, H-9), 2.60 (1H, m, H-11), 2.52 (1H, d, $J=6$ Hz, H-5), 2.36 (1H, t, $J=13$ Hz, H-12), 2.27 (1H, d, $J=16$ Hz, H-2), 1.91 (1H, dd, $J=5$, 15 Hz, H-12), 1.64 (1H, br d, $J=16$ Hz, H-2), 1.45 (3H, s, OAc), 1.37 (3H, t, $J=7$ Hz, $CH_2C\underline{H}_3$). **28**: δ: 5.67 (1H, s, H-12), 4.60 (1H, d, $J=7$ Hz, Glc-A H-1), 3.32 (1H, m, H-3), 2.54 (1H, br s, H-9), 1.38 (3H, s, H-27), 1.10 (3H, s, H-26), 1.09 (3H, s, H-25), 1.03 (3H, s, H-29), 1.01 (3H, s, H-23), 0.80 (3H, s, H-24), 0.78 (3H, s, H-28). **7**: δ: 7.09 (2H, s, C-1 galloyl-H), 7.01 (2H, s, C-2 galloyl-H), 6.92 (2H, s, C-6 galloyl-H), 6.91 (2H, s, C-3 galloyl-H), 6.89 (2H, s, C-4 galloyl-H), 6.24 (1H, d, $J=8$ Hz, glc-1).

$\Delta\ \delta$ Values: $\Delta\ \delta$ ($\delta_{23}-\delta_{23+7}$): Fig. 3. $\Delta\ \delta$ ($\delta_7-\delta_{7+23}$): Fig. 4. $\Delta\ \delta$ ($\delta_{24}-\delta_{24+7}$) (**24**, 0.026 M; **7**, 0.014 M): H-3, 4, 5, 0.14; CH, 0.14; H-2,6, 0.13; glc-1, 0.05; glc-6a, 0.04; glc-1′, 0.03; glc-6b, 0.02; glc-6a′, 0.007; glc-6b′, 0.004. $\Delta\ \delta$ ($\delta_{25}-\delta_{25+7}$) (**25**, 0.024 M; **7**, 0.014 M): H-5, 0.17; H-2′, 6′, 0.24; H-3′, 5′, 0.19; H-6, 0.15; H-8, 0.09; api-1, 0.01; H-2, 0.18; glc-1, 0.08. $\Delta\ \delta$ ($\delta_{26}-\delta_{26+7}$) (**26**, 0.0038 M; **7**, 0.0039 M): H-5, 0.26; H-10, 0.22; H-6, 0.22; H-1, 0.18; H-9, 0.17; 6-OMe, 0.14; H-2, 0.13; H-7, 0.11; H-12, 0.10; H-12, 0.09, H-2, 0.09; H-3, 0.09; benzoyl H-3, 5, 0.09; benzoyl H-2, 6, 0.09; benzoyl H-4, 0.08; 1-OMe, 0.07; H-15, 0.07; OAc, 0.07, 18-OMe, 0.06; H-14, 0.05; $CH_2C\underline{H}_3$, 0.04; 16-OMe, 0.03. $\Delta\delta$ ($\delta_{27}-\delta_{27+22}$) (**27**, 0.016 M; **22**, 0.032 M): H-6, 0.41; H-5, 0.37; H-7, 0.25; glc-1, 0.24; glc-6a, 0.1; glc-6b, 0.1; H-4, -0.1; H-2, -0.155. $\Delta\delta$ ($\delta_{22}-\delta_{22+27}$) (**22**, 0.008 M; **27**, 0.16 M): 3′-*O*-galloyl, 0.79; 3-*O*-galloyl, 0.25. $\Delta\delta$ ($\delta_{28}-\delta_{28+7}$): Fig. 6.

Water Solubility of Polymeric Proanthocyanidins Polymeric proanthocyanidins (2 mg) and various amounts of **27** (1—8 mg) or sucrose (13 mg) were suspended in 1 ml of water and homogenized by sonication for 5 min (pH 6). The mixture was centrifuged (3000 rpm) and the supernatant was analyzed by HPLC. The area of the broad peak arising from polymeric proanthocyanidins was compared with that of a methanol solution (Table 2).

Adsorption of 7 on Hide Powder **7** (2 mg), various amounts of **23** (0—50 mg) and hide powder (3 mg) were mixed with water (1 ml) and heated at 50 °C for 15 min. After centrifugation (3000 rpm), the supernatant was analyzed by HPLC (Table 3).

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References and Notes

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