New Monoterpene Glycoside Esters and Phenolic Constituents of Paeoniae Radix, and Increase of Water Solubility of Proanthocyanidins in the Presence of Paeoniflorin

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Seven new monoterpene glycoside esters related to paeoniflorin were isolated from Paeoniae Radix, together with polymeric proanthocyanidins, polygalloylglucoses and 48 known compounds (a benzoylsucrose, seven aromatic acids, adenosine, nine monoterpene glycosides, eight flavan-3-ols, a catechin dimer formed by oxidation, seven proanthocyanidins, three galloylsucroses, five galloylglucoses, and six ellagitannins). The structures of the new compounds were determined by spectral investigation including two-dimensional NMR techniques. In addition, increased water solubility of polymeric proanthocyanidin in the presence of paeoniflorin was examined by *n*-octanol–water partition and ^{1}H-NMR spectral experiments.

Key words *Paeonia obovata*; monoterpene glucoside; tannin; Paeoniae Radix; proanthocyanidin; hydrophobic association

Paeoniae Radix (Shaoyao) is an important crude drug in Japanese and Chinese traditional medicine. Although the roots of *Paeoniae lactiflora* PALLAS and related species are usually used after removal of cortex, the root with cortex is also used medicinally for different purposes, for example improvement of blood flow. It was inferred from this that a difference in the constituents of these two crude drugs was responsible for the difference in the medicinal usage. Hence, we compared these two types of Paeoniae Radix by reversed phase HPLC and chemically investigated in detail the constituents of commercial Paeoniae Radix with cortex originating from *Paeoniae obovata* MAXIM. In addition, the change in water solubility of polymeric proanthocyanidin in the presence of paeoniflorin, the major monoterpene glycoside of the crude drug, was also examined.

Results and Discussion

Figure 1 shows reversed phase HPLC chromatograms (Fig. 1) of hot water extracts of two typical commercial Paeoniae Radix samples (A, commercial Paeoniae Radix without the cortex part originating from *P. lactiflora* cultivated in Japan; B, commercial Paeoniae Radix with the cortex part originating from *P. obovata* imported from China). A rise of baseline in chromatogram B (crude drug with cortex) suggested the presence of polymeric proanthocyanidins. This was also supported by thin-layer chromatography showing a reddish-orange coloration at the origin with the *p*-anisaldehyde–H_2SO_4 reagent. In addition, many peaks arising from minor constituents appeared in chromatogram B. It was not clear whether the difference between the two chromatograms arose from the difference in the plant species, or from the presence of the cortex part.

Commercial Paeoniae Radix with cortex (dried root of *P. obovata*) was extracted with a mixture of acetone and water, and the extract was partitioned successively with ethyl ether and ethyl acetate. The ethyl acetate extract and water layer were separately subjected to a combination of column chromatography on Sephadex LH-20, MCI gel CHP20P, Chromatorex ODS, TSK-gel Toyopearl HW40F, and silica gel to yield a total of 55 compounds, together with polygalloylglucoses[1] and polymeric proanthocyanidins. Among these compounds, eight (**1**—**7**) were found to be new compounds and one (**8**) was isolated for the first time from natural source. The remaining compounds were identified as seven aromatic carboxylic acids (gallic acid, benzoic acid, vanillic acid, syringic acid, *p*-hydroxybenzoic acid, 4,5-dihydroxy-3-methoxybenzoic acid, and an equilibrium mixture of *m*- and *p*-digallate[2]), adenosine, nine monoterpene glycosides [paeoniflorin (**9**),[3] oxypaeoniflorin (**10**),[3] benzoylpaeoniflorin (**11**),[3] benzoyloxypaeoniflorin (**12**),[4] galloylpaeoniflorin (**13**),[4] galloyloxypaeoniflorin (**14**),[4] mudanpiosides E (**15**),[5] and F (**17**),[5,6] and desbenzoylpaeoniflorin (**16**)[3]], 6-*O*- (**18**), 1′-*O*- (**19**), and 6′-*O*- (**20**) galloylsucroses,[7] (+)-catechin, catechin 5-*O*-, 7-*O*-, 3′-*O*-, and 4′-*O*-glucosides,[8] catechin 7-*O*-gallate,[9] catechin 3′(4′)-*O*-gallate (an equilibrium mixture),[10] epicatechin 3-*O*-gallate,[11] catechin dimer formed by oxidation (**21**),[12] seven proanthocyanidins [procyanidins B-3 (**22**),[13] B-1 (**23**),[14] B-1 3-*O*-gallate (**24**),[14] B-2 3′-*O*-gallate (**25**),[11] and B-7 (**26**),[15] AC-trimer (**27**),[15] and arecatannin A-1 (**28**)[15]], 1,2,3-tri-,[16] 1,2,6-tri-,[13] 1,2,3,4-tetra,[13] and 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucoses,[2] 2,3,4,6-tetra-*O*-galloyl-D-glucose,[13] and six ellagitannins [2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (**29**),[17] eugeniin (**30**),[18]

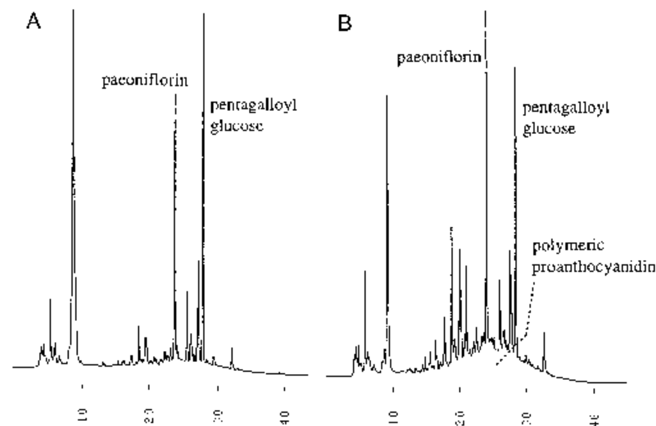

Fig. 1. HPLC Chromatograms of Hot-Water Extract of Paeoniae Radix

A, crude drug from the root of *P. lactiflora* without the cortex part; B, crude drug from the root of *P. obovata* with the cortex part. Conditions: Cosmosil 5C_{18}-AR, 5%→35% (30 min)→75% (20 min) CH_3CN in 50 mM H_3PO_4, 0.8 ml/min, 280 nm.

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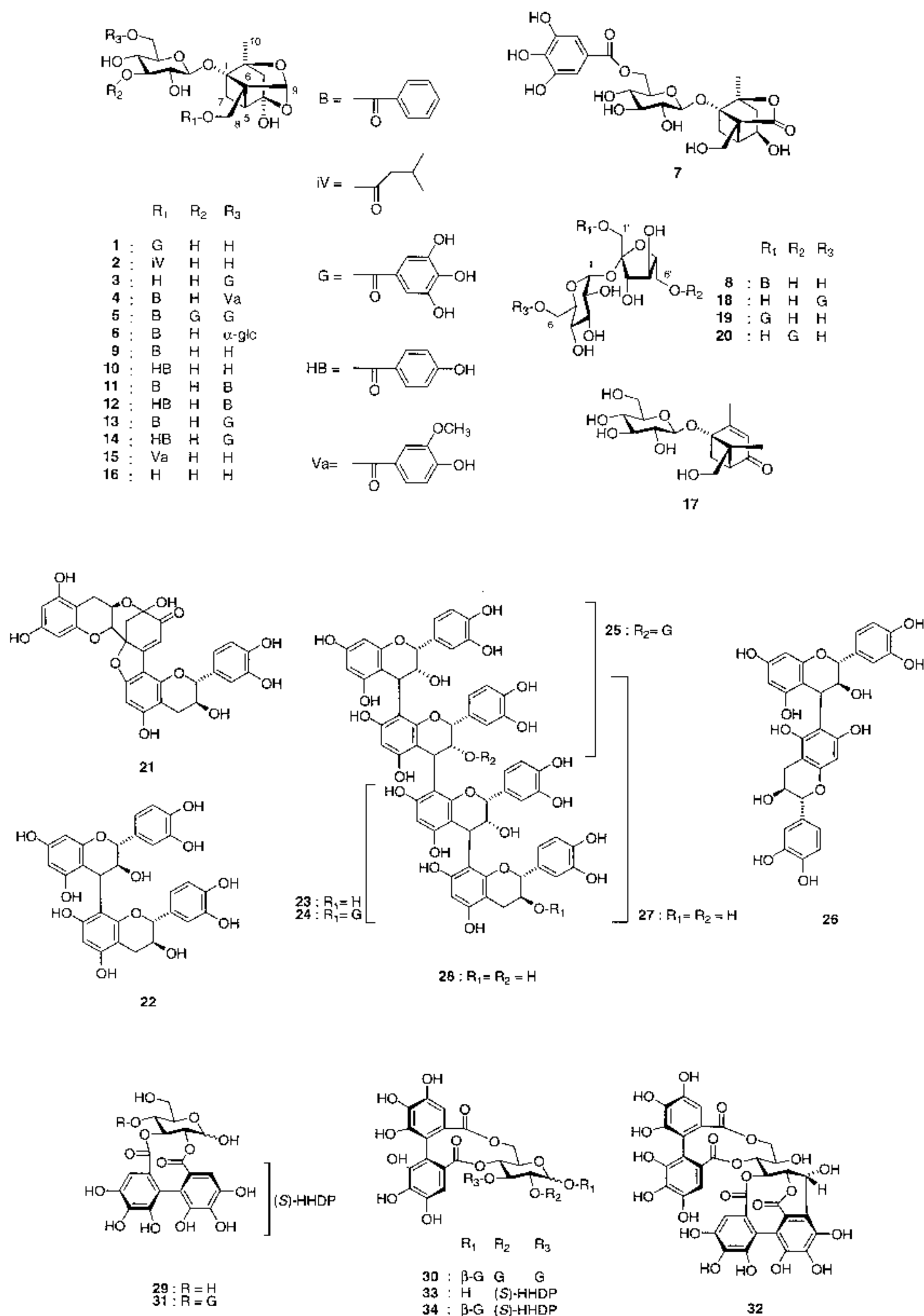

pterocaryanin B (**31**),[19] casuariin (**32**),[20] pedunculagin (**33**),[17] and 1(*β*)-*O*-galloylpedunculagin (**34**)[17)]. These known compounds were identified by comparison of physical and ^{1}H-NMR data with those of authentic samples or described in literature. The mixture of polymeric proanthocyanidins (Fig. 2) was characterized by the thiol degradation method[21] and ^{13}C-NMR spectral analysis (see Experimental). The extension units were suggested to be (−)-epicatechin (65%, estimated by HPLC analysis of thiol degradation products), (±)-catechin (21%), and (−)-epicatechin 3-*O*-gallate (14%), and the terminal units were (+)-catechin (69%) and (−)-epicatechin 3-*O*-gallate (31%). Furthermore, the ratio of extension/terminal units was estimated to be about 7.6. The structural characteristics seemed to be an extension of those of procyanidin dimer–tetramers (**22**—**28**).

Compound **1** was obtained as a tan amorphous powder and

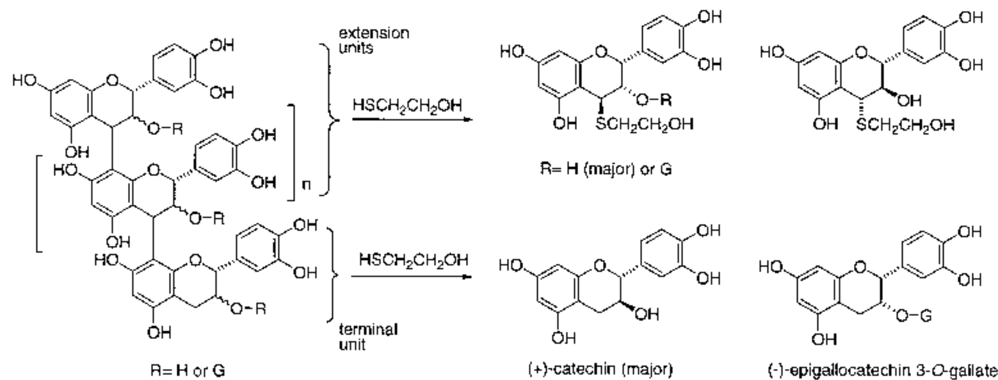

Fig. 2. Thiol Degradation of Polymeric Proanthocyanidin

Table 1. ^{1}H-NMR Spectral Data for Compounds **1**—**7** (in MeOH-d_4)

	1[a)]	**2**[a)]	**3**[a)]	**4**[b)]	**5**[a)]	**6**[b)]		**7**[b)]
H-3	2.19 (d, 13)	2.16 (d, 12)	1.90 (d, 12)	1.88 (d, 13)	1.94 (d, 13)	2.32 (d, 13)		1.92 (dd, 6, 15)
	1.80 (dd, 1, 13)	1.78 (dd, 1, 12)	1.65—1.69 (m)	1.71 (d, 13)	1.71 (d, 13)	1.78 (dd, 1, 13)		1.80 (d, 15)
H-4								3.97 (dd, 5, 6)
H-5	2.57 (dd, 1, 6)	2.46 (dd, 1, 7)	2.30—2.37 (m)	2.51 (d, 7)	2.55 (d, 6)	2.58 (dd, 1, 7)		2.54 (dd, 5, 8)
H-7	2.50 (dd, 6, 11)	2.41 (dd, 7, 11)	2.30—2.37 (m)	2.47 (dd, 7, 11)	2.48 (dd, 6, 11)	2.52 (dd, 7, 11)		2.64 (dd, 8, 11)
	1.95 (d, 11)	1.90 (d, 11)	1.65—1.69 (m)	1.77 (d, 11)	1.79 (d, 11)	2.02 (d, 11)		1.62 (d, 11)
H-8	4.69 (d, 12)	4.50 (d, 12)	3.95 (d, 12)	4.71 (2H, s)	4.70 (2H, s)	4.77 (d, 12)		3.92 (2H, s)
	4.61 (d, 12)	4.47 (d, 12)	3.87 (d, 12)	—	—	4.73 (d, 12)		—
H-9	5.38 (s)	5.27 (s)	5.23 (s)	5.38 (s)	5.39 (s)	5.41 (s)		
H-10	1.36 (s)	1.33 (s)	1.22 (s)	1.26 (s)	1.28 (s)	1.35 (s)		1.34 (s)
Glc-1	4.53 (d, 7.5)	4.48 (d, 8)	4.55 (d, 8)	4.57 (d, 8)	4.69 (d, 8)	4.56 (d, 8)	4.82 (d, 4)	4.59 (d, 8)
Glc-2	3.17—3.31	3.19 (dd, 8, 9)	3.22—3.32 (m)	3.26 (dd, 8, 9)	3.51 (dd, 8, 9)	3.22 (dd, 8, 9)	3.40 (dd, 4, 9)	3.17—3.33 (m)
Glc-3	3.17—3.31	3.24 (m)	3.22—3.32 (m)	3.38 (t, 9)	5.15 (t, 9)	3.32 (t, 9)	3.67 (t, 9)	3.17—3.33 (m)
Glc-4	3.17—3.31	3.24 (m)	3.22—3.32 (m)	3.32 (t, 9)	3.61 (t, 9)	3.25 (t, 9)	3.34 (t, 9)	3.17—3.33 (m)
Glc-5	3.17—3.31	3.35 (m)	3.22—3.32 (m)	3.58 (m)	3.68 (m)	3.49 (ddd, 2, 7, 9)	3.65 (ddd, 2, 5, 9)	3.17—3.33 (m)
Glc-6	3.86 (dd, 2, 12)	3.85 (dd, 2, 12)	4.50 (dd, 3, 12)	4.59 (dd, 2, 12)	4.48 (dd, 6, 13)	3.82 (dd, 7, 11)	3.78 (dd, 2, 12)	4.52 (dd, 8, 12)
	3.60 (dd, 5, 12)	3.60 (dd, 6, 12)	4.44 (dd, 6, 12)	4.44 (dd, 8, 12)	4.54 (dd, 3, 13)	3.71 (dd, 2, 11)	3.69 (dd, 5, 12)	4.47 (dd, 3, 12)
Benzoyl-2, 6				8.02 (br d, 7)	8.03 (br, d, 8)	8.05 (br d, 8)		
Benzoyl-3, 5				7.47 (br t, 7)	7.48 (br, t, 8)	7.49 (br t, 8)		
Benzoyl-4				7.61 (br t, 7)	7.61 (br t, 8)	7.62 (br t, 8)		
Galloyl	7.08 (s)		7.07 (s)		7.09 (s)			7.08 (s)
				7.13 (s)				
Isovaleryl-2		2.25 (d, 7)						
Isovaleryl-3		2.09 (m)						
Isovaleryl-4, 5		0.97 (d, 7)						
Vanillyl-2				7.55 (d, 2)				
Vanillyl-5				6.84 (d, 8)				
Vanillyl-6				7.58 (dd, 2, 8)				
OMe				3.87 (s)				

δ in ppm from TMS. Coupling constants in Hz are given in parentheses. *a*) 300 MHz. *b*) 500 MHz.

showed a dark blue coloration with the $FeCl_3$ reagent. The ^{1}H-NMR spectrum (Table 1) was closely related to that of **9**, except for the appearance of an aromatic two-proton singlet at δ 7.08 instead of benzoyl signals. This observation indicated the presence of a galloyl group at C-8 of the monoterpene moiety and the ^{13}C-NMR spectrum also confirmed the presence of a galloyl group in place of the benzoyl group of **9**. This was further supported by the $[M+Na]^+$ peak at *m/z* 551 in the FAB-MS. Therefore, the structure of this compound was determined as shown in formula **1**, and named 8-*O*-galloyl desbenzoylpaeoniflorin.

The ^{1}H-NMR spectrum of compound **2** (Table 1) was also related to that of **1** and **9**, showing similar signals due to monoterpene and glucopyranosyl moieties. The distinctive features of the spectrum were the appearance of two methyls (δ 0.97, 6H, d, $J=7$ Hz), methine (δ 2.09, 1H, m), and methylene (δ 2.25, 2H, d, $J=7$ Hz) signals, instead of the aromatic signals of **1** and **9**. These aliphatic signals indicated the presence of an isovaleryl group at the C-8 of monoterpene moiety. Furthermore, the ^{13}C-NMR spectrum [isovaleryl: δ 174.68 (C-1), 44.16 (C-2), 26.84 (C-3), and 22.76 (C-4, 5)] and FAB-MS (*m/z* 483, $[M+Na]^+$) of **2** verified the structure. This compound was named 8-*O*-isovaleryl desbenzoylpaeoniflorin.

Compound **3** showed a dark blue coloration with the $FeCl_3$ reagent, and the negative ion FAB-MS (*m/z* 527, $[M-H]^-$) indicated that the molecular weight of **3** was the same as that of **1**. ^{1}H-NMR spectral comparison of **3** and **1** (Table 1) indicated that these two were positional isomers differing in the location of the galloyl group (**3**: δ 7.07, 2H, s). In the spec-

trum of **3**, the glucose H-6 signals appeared at lower field (δ 4.44, dd, J=6, 12 Hz; 4.50, dd, J=3, 12 Hz) and the H-8 signals of the monoterpene moiety were shifted upfield (δ 3.87 and 3.95, each d, J=12 Hz) compared with those of **1**. From this spectral analysis, compound **3** was determined to be 6′-*O*-galloyl desbenzoylpaeoniflorin (**3**).

The ^{1}H-NMR spectrum of compound **4** (Table 1) was similar to that of **9** and showed signals due to benzoyl, glucosyl and monoterpene moieties. However, the glucose H-6 signals (δ 4.44, dd, J=8, 12 Hz; 4.59, dd, J=2, 12 Hz) were observed at lower field, and additional ABX-type aromatic proton signals (δ 7.55, d, J=2 Hz; 6.84, d, J=8 Hz; 7.58, dd, J=2, 8 Hz), along with a methoxyl signal (δ 3.87, 3H, s) appeared. The ^{13}C-NMR spectrum exhibited two carboxyl carbon signals at δ 168.08 and 167.69 together with two oxygen bearing aromatic carbon signals at δ 148.76 and 152.99, suggesting the presence of a vanillyl group. This was supported by the $[M+Na]^+$ peak at *m/z* 653 in the FAB-MS. The locations of the benzoyl and vanillyl groups were unequivocally confirmed by the heteronuclear multiple bond correlation (HMBC) spectrum, in which both glucose H-6 signals were correlated with the carboxyl signal at δ 167.7, which also correlated with an aromatic doublet at δ 7.55 (d, J=2 Hz), indicating that the vanillyl group was attached to the C-6 position of glucose. Thus, compound **4** was characterized as 6′-*O*-vanillylpaeoniflorin (**4**).

Compound **5** showed a dark blue coloration with the $FeCl_3$ reagent and a $[M-H]^-$ peak in the negative FAB-MS at *m/z* 783, which is 152 mass units larger than galloylpaeoniflorin. This difference coincided with the mass of a galloyl group. The ^{1}H-NMR spectrum (Table 1), which was closely related to that of **9**, indicated the presence of two galloyl groups (δ 7.09 and 7.13) in the molecule of **5**. On hydrolysis with tannase, **5** yielded **9** and gallic acid, confirming that **5** is a digalloyl ester of **9**. The locations of the galloyl groups were determined to be the C-3 and C-6 positions of the glucosyl moiety, since the proton signals at these positions resonated at lower field compared to those for **1** and **2** (Table 1). On the basis of these observations, compound **5** was established as 3′,6′-di-*O*-galloylpaeoniflorin.

The ^{1}H-NMR spectrum of compound **6** (Table 1) also resembled that of paeoniflorin (**9**), except for the appearance of extra sugar proton signals. The $[M+Na]^+$ peak at *m/z* 665 in the FAB-MS revealed that this compound contains an additional hexose in the molecule, and this was also supported by ^{13}C-NMR spectral analysis. The coupling constants for the sugar proton signals (Table 1) suggested the presence of α- and β-glucopyranoses. In addition, a large low field shift for C-6 (δ 68.1) of the β-glucose in the ^{13}C-NMR spectrum indicated glycosidation at this position. The connection of the two sugars and the aglycone was confirmed by the HMBC experiment. The anomeric proton of α-glucose correlated with the C-6 of the β-glucose, and the anomeric proton of the β-glucose was coupled with the C-1 of the monoterpene moiety. Other HMBC correlations were consistent with the structure shown in formula **6**. Thus, this compound was characterized as 6′-*O*-α-glucopyranosylpaeoniflorin, and named isomaltopaeoniflorin (**6**).

Compound **7** showed a $[M-H]^-$ peak at *m/z* 527 in the FAB-MS, and indicated that this compound is an isomer of **1** and **3**. Appearance of a two-proton singlet signal at δ 7.08 in

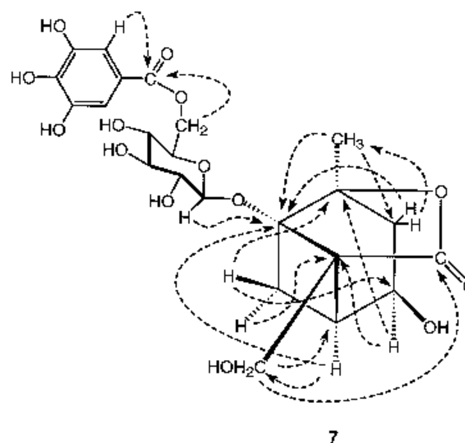

Fig. 3. Selected HMBC Correlations (H→C, ^3J) for Compound **7**

the ^{1}H-NMR spectrum, in addition to a dark blue coloration with the $FeCl_3$ reagent, indicated the presence of a galloyl group in the molecule. In the ^{13}C-NMR spectrum, the chemical shifts of ten carbon signals including one methyl group, three methylenes, two methines, three quaternary carbons, and a single carboxyl carbon, were similar to those of the aglycone part of albiflorin,[22] except for the absence of benzoyl signals. In comparison with the spectrum of **3**, signals due to a carboxyl carbon (δ 178.7, C-9) and a secondary alcohol (δ 68.3, C-4) were observed in place of two acetal carbons (δ 106.34, C-4; 102.09, C-9) for **3**. These spectral observations strongly suggested that **7** is a galloyl ester of desbenzoylalbiflorin. The location of the galloyl group was determined to be at the glucose C-6 position because the glucose H-6 signals (δ 4.52, dd, J=8, 12 Hz; 4.47, dd, J=3, 12 Hz) appeared at lower field compared with those of **1** and **2**. Final confirmation of the structure was achieved by analysis of the HMBC spectrum, as shown in Fig. 3, and hence, this compound was characterized as 6′-*O*-galloyl desbenzoylalbiflorin (**7**). In this investigation of the constituents of Paeoniae Radix, albiflorin was not isolated.

The ^{1}H-NMR spectrum of compound **8** was closely related to that of 1′-*O*-galloylsucrose (**19**),[7] except for the appearance of signals due to a benzoyl group instead of a galloyl group. The sugar carbon signals of **8** in the ^{13}C-NMR were also superimposable to those of **19**. From this spectral data, compound **8** was deduced to be 1′-*O*-benzoylsucrose. Although **8** has been synthesized,[23] this is the first report of isolation from plants.

As shown by HPLC analysis, the presence of polymeric proanthocyanidin is one of the distinctive features of Paeoniae Radix with cortex, compared to that without cortex. In addition, a considerable number of the minor peaks observed in the HPLC chromatogram of the sample with cortex (Fig. 1, B) were probably attributable to proanthocyanidins with lower molecular weight (**19**—**25**), catechin and its derivatives, sucrose esters, galloyl esters of monoterpene glycoside, and ellagitannins. Hence, the difference in medicinal applications may be related to the presence of these phenolic substances. Proanthocyanidins and the related flavan-3-ol are known to be excellent antioxidants and radical scavengers and increase the resistance of blood plasma towards oxidative stress.[24] It was also reported that proanthocyanidins inhibit oxidation of low-density lipoprotein and show antiatheroscle-

Table 2. Solubility of Polymeric Proanthocyanidins in the Presence of Paeoniflorin (**9**), Amygdarin, Apiosyl liquiritin, Glycyrrhizin, and Sucrose (Proanthocyanidin 2 mg in 1 ml of Water, 28 °C)

	Conc. (M)	Solubility (%, relative value[a])
50% MeOH		100
None		75
Paeoniflorin	0.01	81
	0.02	84
Amygdalin	0.01	82
	0.02	87
Apiosyl liquiritin	0.01	87
	0.02	98
Glycyrrhizin	0.01	106
	0.02	104
Sucrose	0.01	79
	0.02	79

a) Relative solubility was estimated by comparison of the HPLC peak area with that of 50% MeOH solution (100%).

Table 3. Change of the Chemical Shifts of Paeoniflorin (**9**) in the Presence of Polymeric Proanthocyanidin (**9**, 0.013 M; Proanthocyanidin <7.5 mg/ml in D_2O,[a] at 20 °C)

	Paeoniflorin	+Polymer	+Tannase (30 min)	+Tannase (3 h)
B-2, 6	8.058	8.040	8.043	8.044
B-4	7.699	7.684	7.687	7.688
B-3, 5	7.553	7.532	7.536	7.537
H-9	5.625	5.621	5.620	5.619

a) After dissolution in D_2O by heating, the solution was cooled to 20 °C, and the resulting precipitate was removed by filtration.

rotic activity.[25] These beneficial properties seem to be consistent with those of Paeoniae Radix with cortex, and are different to those from plant without cortex containing lesser amounts of proanthocyanidins, which are mainly used for restoration of blood flow circulation.

In addition to the above structural investigation, we are interested in the water solubility of polymeric proanthocyanidin, which showed poor water solubility in a purified form. Previously, we have demonstrated the increase in water solubility of rhubarb polymeric proanthocyanidins in the presence of an anthraquinone glycoside.[26] Since large amounts of paeoniflorin coexist with proanthocyanidin in the extract of Paeoniae Radix, the change in water solubility of polymeric proanthocyanidin from Paeoniae Radix in the presence of **9** was examined. After dissolution in water at 80 °C, 25% of the polymeric proanthocyanidin precipitated from solution as the temperature was cooled down to 28 °C, and 75% remained in solution. When **9** (0.02 M) was present, the amount of precipitate was decreased and 84% of the proanthocyanidin remained in solution (Table 2). On the other hand, the presence of sucrose did not affect the water solubility. The increase in water solubility was attributable to the hydrophobic association between **9** and polymeric proanthocyanidins. This was deduced from the ^{1}H-NMR chemical shift change of **9** in the presence of polymeric proanthocyanidin (Table 3). When polymeric proanthocyanidin coexists in aqueous solution, the aromatic proton signals shifted upfield. This phenomenon was similar to that observed for association between anthraquinone glycoside and polymeric proanthocyanidin from rhubarb. In addition, treatment with tannase partially restored the chemical shift to lower field. This observation suggested that the presence of galloyl groups in the polymeric proanthocyanidins was important for the association with **9**. Other glycosides contained in major Japanese traditional crude drugs having amphipathic structure also increased the water solubility (Table 2). Water solubility is an important factor for bioavailability and affects the biological activities especially with orally administered drugs. Hence, the increased water solubility of polymeric proanthocyanidins by coexisting compounds may be important in oriental medicine.

Experimental

Column chromatography was performed with Sephadex LH-20 (25—100 mm, Pharmacia Fine Chemical Co. Ltd.), Diaion HP-20 and MCI-gel CHP 20P (75—150 mm, Mitsubishi Chemical Industries, Ltd.), Bondapak ODS (Waters), TSK-gel Toyopearl HW40F (TOSOH Co.), Chromatorex ODS (Fuji Silysia), and Silica gel 60 (Merck). Thin layer chromatography was performed on precoated Silica gel 60 F_{254} plates (0.2 mm thick, Merck) with $CHCl_3$–MeOH–H_2O (70 : 30 : 5, v/v) or benzene–HCO_2Et–HCO_2H (1 : 7 : 1 or 1 : 7 : 2, v/v), and spots were detected by ultraviolet (UV) illumination, by spraying with 5% H_2SO_4 followed by heating, by spraying with 2% ethanolic $FeCl_3$ reagent, and by *p*-anisaldehyde–H_2SO_4 reagent. Analytical HPLC was performed on a Cosmosil 5C_{18}-AR (Nacalai Tesque Inc.) column (4.6 i.d. ×250 mm) (mobile phase, CH_3CN–50 mM H_3PO_4, gradient elution; flow rate, 0.8 ml/min, detection, UV absorption at 280 nm). Negative FAB-MS were recorded on a JEOL JMX DX-303 spectrometer with glycerol as a matrix. ^{1}H- and ^{13}C-NMR spectra were obtained with Varian Unity plus 500 and Varian Gemini 300 spectrometers operating at 500 and 300 MHz for ^{1}H, and 125 and 75 MHz for ^{13}C, respectively; chemical shifts are reported in parts per million on the δ scale with tetramethylsilane (TMS) as the internal standard, and coupling constants are in Hertz.

Extraction and Isolation From H_2O Layer: Commercial Paeoniae Radix with the cortex part (2.0 kg, imported from China) originating from the root of *P. obovata* was extracted with a mixture of acetone–water (7 : 3, v/v) three times and then with MeOH two times. The extracts were combined and defatted by partition between water and Et_2O (Et_2O extract, 24.6 g). The aqueous layer was extracted with AcOEt five times (AcOEt extract, 47.5 g), and the water layer was subjected to Diaion HP-20 column chromatography with water containing increasing amounts of MeOH to give four fractions: frs. 1—4. The first fraction was fractionated once again by Diaion HP-20 chromatography to five fractions. Fraction 1-1 was mainly comprised of sugars. Fraction 1-5 contained paeoniflorin as the major compound and was combined together with fr. 3. Fractions 1-2—1-4 were separately subjected to a combination of column chromatography over Sephadex LH-20, MCI-gel CHP 20P, Chromatorex ODS, Toyopearl HW-40 and Bondapak ODS with water–methanol to yield adenosine (47.2 mg), mudanpiosides E (**15**, 6.7 mg) and F (**17**, 81.1 mg), desbenzoylpaeoniflorin (**16**, 26.0 mg), oxypaeoniflorin (**10**, 1.28 g), paeoniflorin (**9**, 3.66 g), 6-*O*-galloylsucrose (**18**, 22.1 mg), 1′-*O*-galloylsucrose (**19**, 29.4 mg), 6′-*O*-galloylsucrose (**20**, 92.1 mg), catechin 7-*O*-glucoside (47.3 mg), catechin 5-*O*-glucoside (37.8 mg), catechin 3′-*O*-glucoside (34.6 mg), catechin 4′-*O*-glucoside (32.7 mg), 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose (**29**, 24.5 mg), pterocaryanin B (**31**, 36.3 mg), casuariin (**32**, 59.7 mg), pedunculagin (**33**, 346 mg), procyanidin B-3 (**22**, 265 mg), 8-*O*-galloyl desbenzoylpaeoniflorin (**1**, 11.9 mg), 8-*O*-isovaleryl desbenzoylpaeoniflorin (**2**, 25.3 mg), 6′-*O*-galloyl desbenzoylpaeoniflorin (**3**, 129.9 mg), and 6′-*O*-galloyl desbenzoylalbiflorin (**7**, 35.7 mg). Fraction 2 was fractionated by Diaion HP-20 (H_2O–MeOH) to give two fractions: frs. 2-1 (19.9 g) and 2-2 (39.9 g). The latter fraction contained mainly **9** and was combined with fr. 3. Fraction 2-1 was separated by Sephadex LH-20, MCI-gel CHP 20P, and Chromatorex ODS to give AC-trimer A (**27**, 454 mg) and arecatannin A-1 (**28**, 76.5 mg), and polymeric proanthocyanidin (2.3 g). Fraction 3 (81.6 g in total) was applied to a column of Sephadex LH-20 with water containing increasing amounts of MeOH and then water–acetone (1 : 1, v/v) to give two fractions: frs. 3-1 and 3-2. Fraction 3-1 was subjected to a combination of chromatography over MCI-gel CHP 20P, Sephadex LH-20, Chromatorex ODS and silica gel 60 to give **10** (0.5 g) and **9** (*ca.* 40 g), isomaltopaeoniflorin (**6**, 30.8 mg), and 1′-*O*-benzoylsucrose (**8**, 51.0 mg). Fraction 3-2 was mainly composed of proanthocyanidins and gallotannins. Fraction 4 (17.5 g) was

separated by Sephadex LH-20 chromatography into two fractions: frs. 4-1 and 4-2. Fraction 4-1 was chromatographed over MCI-gel CHP 20P followed by Chromatorex ODS to yield galloylpaeoniflorin (**13**, 1.05 g), the structure of which was confirmed by spectral comparison and tannase hydrolysis affording **9** and gallic acid. Fraction 4-2, mainly comprised of proanthocyanidins, was combined with fr. 3-2 and applied to a column of Sephadex LH-20 with water–acetone (1 : 1, v/v). The fraction positive to *p*-anisaldehyde–H_2SO_4 reagent was concentrated, and the residue was precipitated from H_2O–MeOH to give a mixture of polymeric proanthocyanidins (10.1 g).

Isolation from AcOEt Layer: The AcOEt layer (47.5 g) was subjected to Sephadex LH-20 column chromatography with water containing increasing amounts of MeOH and then water–acetone (1 : 1, v/v) to give seven fractions: frs. 1′ (5.8 g), 2′ (5.1 g), 3′ (4.7 g), 4′ (1.5 g), 5′ (4.8 g), 6′ (7.4 g), and 7′ (6.23 g). The first fraction contained sugars and **9** as the major components and was not examined further. Fractions 2′—6′ were separately subjected to a combination of column chromatography similar to those described for the aqueous layer to yield gallic acid (759 mg), benzoic acid (78.6 mg), *p*-hydroxybenzoic acid (16.9 mg), 4,5-dihydroxy-3-methoxybenzoic acid (13.6 mg), vanillic acid (29.3 mg), syringic acid (19.0 mg), galloylpaeoniflorin (**13**, 950 mg), galloyloxypaeoniflorin (**14**, 65.6 mg), benzoylpaeoniflorin (**11**, 693 mg), benzoyloxypaeoniflorin (**12**, 141 mg), and 6′-*O*-vanillylpaeoniflorin (**4**, 58.6 mg) from fr. 2′; (+)-catechin (531 mg), procyanidins B-1 (**23**, 63.5 mg) and B-3 (**22**, 34.9 mg) from fr. 3′; a mixture of *m*- and *p*-digallate (8.8 mg), procyanidin B-7 (**26**, 59.4 mg), AC-trimer (**27**, 81.7 mg), 1,2,3-tri-*O*-galloyl-β-glucose (33.8 mg), and pedunculagin (**33**, 55.0 mg) from fr. 4′; catechin 3-*O*-gallate (76.2 mg), 7-*O*-gallate (37.8 mg), and 3′(4′)-*O*-gallate (equilibrium mixture, 14.3 mg), epicatechin 3-*O*-gallate (76.2 mg), catechin oxidation product (**21**, 18.5 mg), procyanidins B-3 (**22**, 14.6 mg), B-1 3-*O*-gallate (**24**, 47.5 mg), B-7 (**26**, 95.8 mg), B-2 3′-*O*-gallate (**25**, 19.7 mg), 1,2,3,4-tetra-*O*-galloyl-β-D-glucose (50.1 mg), and 3′,6′-di-*O*-galloylpaeoniflorin (**5**, 10.9 mg) from fr. 5; 1(β)-*O*-galloylpedunculagin (**34**, 111 mg), eugeniin (327 mg), 2,3,4 6-tetra-*O*-galloylglucose (315 mg), and 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose (2.33 g) from fr. 6. The structure of **14** was confirmed by tannase hydrolysis yielding **10**. The last fraction (fr. 7′) was characterized as a mixture of polygalloylglucoses by ^{1}H-NMR spectral analysis which showed complex aromatic multiplet signals around 6.7—7.5 ppm along with signals due to an acylated sugar core related to that of pentagalloyl-β-D-glucose.

8-*O*-Galloyl Desbenzoylpaeoniflorin (1) A tan amorphous powder, $[\alpha]_D$ −4.9° (c=0.35, MeOH). *Anal.* Calcd for $C_{23}H_{28}O_{14}\cdot 2H_2O$: C, 48.92; H, 5.72. Found: C, 48.60; H, 5.43. FAB-MS *m/z*: 551 [M+Na]$^+$. ^{1}H-NMR (MeOH-d_4, 300 MHz): Table 1. ^{13}C-NMR (MeOH-d_4, 75.5 MHz) δ: monoterpene moiety: 89.28 (C-1), 87.32 (C-2), 44.58 (C-3), 106.40 (C-4), 44.09 (C-5), 72.31 (C-6), 23.72 (C-7), 61.26 (C-8), 102.33 (C-9), 19.56 (C-10); glucose moiety: 100.17 (C-1′), 75.04 (C-2′), 78.01, 77.90 (C-3′, 5′), 71.78 (C-4′), 62.90 (C-6′); galloyl group: 121.22 (C-1″), 110.20 (C-2″, 6″), 147.55 (C-3″, 5″), 139.98 (C-4″), 168.25 (C-7″).

8-*O*-Isovaleryl Desbenzoylpaeoniflorin (2) A tan amorphous powder, $[\alpha]_D$ −19.6° (c=0.33, MeOH). *Anal.* Calcd for $C_{21}H_{32}O_{11}\cdot 3/4H_2O$: C, 53.20; H, 7.12. Found: C, 53.17; H, 6.88. FAB-MS *m/z*: 483 [M+Na]$^+$. ^{1}H-NMR (MeOH-d_4, 300 MHz): Table 1. ^{13}C-NMR (MeOH-d_4, 75.5 MHz) δ: monoterpene moiety: 89.19 (C-1), 87.21 (C-2), 44.55 (C-3), 106.31 (C-4), 43.95 (C-5), 72.02 (C-6), 23.30 (C-7), 60.93 (C-8), 102.20 (C-9), 19.57 (C-10); glucose moiety: 100.14 (C-1′), 74.98 (C-2′), 78.06, 77.92 (C-3′ and 5′), 72.02 (C-4′), 62.88 (C-6′); isovaleryl group: 174.68 (C-1″), 44.16 (C-2″), 26.84 (C-3″), 22.76 (C-4″, 5″).

6′-*O*-Galloyl Desbenzoylpaeoniflorin (3) A tan amorphous powder, $[\alpha]_D$ −12.8° (c=0.46, MeOH). *Anal.* Calcd for $C_{23}H_{28}O_{14}\cdot 3/2H_2O$: C, 49.92; H, 5.23. Found: C, 49.91; H, 5.49. Negative ion FAB-MS *m/z*: 527 [M−H]$^-$. ^{1}H-NMR (MeOH-d_4, 300 MHz): Table 1. ^{13}C-NMR (MeOH-d_4, 75.5 MHz) δ: monoterpene moiety: 89.34 (C-1), 87.22 (C-2), 44.54 (C-3), 106.34 (C-4), 43.46 (C-5), 73.44 (C-6), 22.95 (C-7), 59.00 (C-8), 102.09 (C-9), 19.53 (C-10); glucose moiety: 99.76 (C-1′), 74.90 (C-2′), 77.91 (C-3′), 72.04 (C-4′), 75.19 (C-5′), 64.69 (C-6′); galloyl group: 121.38 (C-1″), 110.16 (C-2″, 6″), 146.53 (C-3″, 5″), 139.89 (C-4″), 168.08 (C-7″).

6′-*O*-Vanillylpaeoniflorin (4) A white amorphous powder, $[\alpha]_D$ −12.6° (c=0.53, MeOH). *Anal.* Calcd for $C_{31}H_{34}O_{14}\cdot 3/2H_2O$: C, 56.62; H, 5.67. Found: C, 56.77; H, 5.57. FAB-MS *m/z*: 653 [M+Na]$^+$. ^{1}H-NMR (MeOH-d_4, 300 MHz): Table 1. ^{13}C-NMR (MeOH-d_4, 125 MHz) δ: monoterpene moiety: 89.15 (C-1), 87.08 (C-2), 44.41 (C-3), 106.20 (C-4), 43.78 (C-5), 71.93 (C-6), 23.10 (C-7), 61.57 (C-8), 102.17 (C-9), 19.56 (C-10); glucose moiety: 99.95 (C-1′), 74.90 (C-2′), 77.78 (C-3′), 72.06 (C-4′), 75.26 (C-5′), 64.97 (C-6′); benzoyl group: 131.10 (C-1″), 130.62 (C-2″, 6″), 129.60 (C-3″, 5″), 134.39 (C-4″), 167.69 (C-7″); vanillyl group: 122.36 (C-1‴), 113.49 (C-2‴), 148.76 (C-3‴), 152.99 (C-4‴), 116.00 (C-5‴), 125.14 (C-6‴), 168.08 (C-7‴).

3′,6′-Di-*O*-galloylpaeoniflorin (5) A brown amorphous powder, $[\alpha]_D$ −5.7° (c=0.77, MeOH). *Anal.* Calcd for $C_{37}H_{36}O_{19}\cdot 2H_2O$: C, 54.15; H, 4.42. Found: C, 54.13; H, 4.70. Negative ion FAB-MS *m/z*: 783 [M−H]$^-$. ^{1}H-NMR (MeOH-d_4, 300 MHz): Table 1. ^{13}C-NMR (MeOH-d_4, 75.5 MHz) δ: monoterpene moiety: 90.05 (C-1), 87.61 (C-2), 44.93 (C-3), 106.76 (C-4), 44.26 (C-5), 72.48 (C-6), 23.40 (C-7), 62.04 (C-8), 102.68 (C-9), 20.12 (C-10); glucose moiety: 100.57 (C-1′), 73.98 (C-2′), 79.44 (C-3′), 71.05 (C-4′), 75.71 (C-5′), 64.96 (C-6′); benzoyl group: 131.66 (C-1″), 131.07 (C-2″, 6″), 130.11 (C-3″, 5″), 134.89 (C-4″), 168.53 (C-7″); galloyl group: 121.89, 122.22 (C-1‴, 1⁗), 110.72, 110.‴ (C-2‴, 6‴, 2⁗, 6⁗), 146.89, 147.08 (C-3‴, 5‴, 3⁗, 5⁗), 140.23, 140.47 (C-4‴, 4⁗), 168.71, 168.08 (C-7‴, 7⁗). Tannase hydrolysis of **5** (1 mg) yielded gallic acid and **9**, which were identified by TLC and HPLC comparison.

Isomaltopaeoniflorin (6) A white amorphous powder, $[\alpha]_D$ 30.9° (c=0.39, MeOH). *Anal.* Calcd for $C_{29}H_{38}O_{16}\cdot 5/2H_2O$: C, 50.65; H, 6.30. Found: C, 50.92; H, 5.98. FAB-MS *m/z*: 665 [M+Na]$^+$. ^{1}H-NMR (MeOH-d_4, 500 MHz): Table 1. ^{13}C-NMR (MeOH-d_4, 125 MHz) δ: monoterpene moiety: 89.38 (C-1), 87.32 (C-2), 44.57 (C-3), 106.47 (C-4), 44.05 (C-5), 72.32 (C-6), 23.60 (C-7), 61.72 (C-8), 102.34 (C-9), 19.80 (C-10); β-glucose moiety: 100.07 (C-1′), 74.95 (C-2′), 78.00 (C-3′), 71.96 (C-4′), 76.07 (C-5′), 68.09 (C-6′); α-glucose moiety: 99.86 (C-1‴), 73.48 (C-2‴), 71.24 (C-3‴), 71.62 (C-4‴), 73.38 (C-5‴), 62.58 (C-6‴); benzoyl group: 131.20 (C-1″), 130.66 (C-2″, 6″), 129.64 (C-3″, 5″), 134.41 (C-4″), 167.98 (C-7″).

6′-*O*-Galloyl Desbenzoylalbiflorin (7) A tan amorphous powder, $[\alpha]_D$ −4.9° (c=0.41, MeOH). *Anal.* Calcd for $C_{23}H_{28}O_{14}\cdot 3/2H_2O$: C, 49.92; H, 5.23. Found: C, 49.67; H, 5.41. Negative ion FAB-MS *m/z*: 527 [M−H]$^-$. ^{1}H-NMR (MeOH-d_4, 300 MHz): Table 1. ^{13}C-NMR (MeOH-d_4, 125 MHz) δ: monoterpene moiety: 86.96 (C-1), 93.06 (C-2), 41.43 (C-3), 68.25 (C-4), 40.83 (C-5), 57.84 (C-6), 27.08 (C-7), 59.95 (C-8), 178.74 (C-9), 20.33 (C-10); glucose moiety: 99.27 (C-1′), 74.55 (C-2′), 77.99 (C-3′), 72.27 (C-4′), 75.19 (C-5′), 64.67 (C-6′); galloyl group: 121.37 (C-1″), 110.17 (C-2″, 6″), 146.58 (C-3″, 5″), 139.91 (C-4″), 167.90 (C-7″).

1′-*O*-Benzoylsucrose (8) A tan amorphous powder, $[\alpha]_D$ 43.2° (c=0.48, MeOH). *Anal.* Calcd for $C_{19}H_{26}O_{12}\cdot 5/4H_2O$: C, 48.67; H, 6.13. Found: C, 48.71; H, 5.76. FAB-MS *m/z*: 469 [M+H]$^+$. ^{1}H-NMR (MeOH-d_4, 300 MHz): δ: 3.30—4.05 (m), 4.22 (1H, dd, J=2, 9 Hz, H-3), 4.59, 4.39 (each 1H, d, J=12 Hz, H-1), 5.48 (1H, d, J=4 Hz, H-1′), 7.49 (2H, br t, J=8 Hz, H-3″, 5″), 7.63 (1H, br t, J=8 Hz, H-4″), 8.06 (2H, br t, J=8 Hz, H-2″, 6″). ^{13}C-NMR (MeOH-d_4, 125 MHz) δ: 64.42 (C-1), 104.13 (C-2), 78.91 (C-3), 74.98 (C-4), 83.83 (C-5), 63.25 (C-6), 94.18 (C-1′), 73.03 (C-2′), 74.43, 74.60 (C-3″, 5″), 71.38 (C-4″), 62.21 (C-6″); benzoyl group: 131.08 (C-1″), 130.66 (C-2″, 6″), 129.64 (C-3″, 5″), 134.43 (C-4″), 167.33 (C-7″).

Polymeric Proanthocyanidin A brown powder from H_2O. ^{13}C-NMR (acetone-d_6, 75.5 MHz) δ: 28.4 (C-4 of terminal unit), 36.7 (C-4 of extension unit), 67.5 (C-3 of terminal catechin unit), 71.9 (C-3 of extension units), 76.8 (C-2 of extension units), 81.9 (C-2 of terminal catechin unit), 96.7 (A-ring C-6 and/or 8), 101.2 (A-ring C-4a), 107.8 (A-ring C-8 or 6), 109.8 (galloyl C-2, 6), 114.7, 115.6, 118.9 (B-ring C-2′, 5′, 6′), 120.5 (galloyl C-1), 129.2, 129.8, 131.8 (B-ring C-1′), 139.4 (galloyl C-4), 144.9, 145.7 (B-ring C-3′, 4′; galloyl C-3, 5), 154.1, 155.9, 157.2 (A-ring C-5, 7, 8a), 166—168 (galloyl C-7).

Thiol Degradation of Polymeric Proanthocyanidin Isolation of the Products: Polymeric proanthocyanidin (500 mg) was treated with a mixture of mercaptoethanol (4 ml), 0.5 M HCl (16 ml) and MeOH (10 ml) at 50 °C for 7 h. After concentration to about half volume, the solution was applied to a column of MCI-gel CHP 20P with water containing increasing amounts of MeOH to give (+)-catechin (12.3 mg), $[\alpha]_D$ +4.1° (c=0.8, acetone) and epicatechin 3-*O*-gallate (4 mg), $[\alpha]_D$ −50.0° (c=0.3, acetone) from terminal units; (±)-catechin 4-(2-hydroxyethyl)thio ether (12.8 mg), $[\alpha]_D$ 0° (c=0.2, acetone), epicatechin 4-(2-hydroxyethyl)thio ether (101.0 mg), $[\alpha]_D$ −53.5° (c=0.3, acetone), and epicatechin 3-*O*-gallate 4-(2-hydroxyethyl)thio ether (55.6 mg), $[\alpha]_D$ −91.0° (c=0.4, acetone) from extension units.[21]

HPLC Analysis of Thiol Degradation Products: Polymeric proanthocyanidin (10 mg) was treated with a mixture of mercaptoethanol (0.3 ml), acetic acid (0.3 ml) and ethanol (2.4 m) in N_2 gas at 50 °C for two days. The mixture was diluted with water, passed through Sepak ODS (60% MeOH), and analyzed by reversed phase HPLC [10%→30% (40 min) CH_3CN in 50 mM H_3PO_4]. Retention time (min): (+)-catechin (12.63), epicatechin 3-*O*-gallate (26.36), (±)-catechin 4-(2-hydroxyethyl)thio ether (18.16), (−)-epicatechin 4-(2-hydroxyethyl)thio ether (22.76), (−)-epicatechin 3-*O*-gallate 4-(2-hydroxyethyl)thio ether (30.62). The molar ratio of the products was estimated

by comparison of peak area with those of standard solutions: (−)-epicatechin (65%), (±)-catechin (21%), and (−)-epicatechin 3-*O*-gallate (14%) for extension units, (+)-catechin (69%) and (−)-epicatechin 3-*O*-gallate (31%) for terminal units (average value obtained from four samples with four injections for each sample).

Water Solubility of Polymeric Proanthocyanidin Polymeric proanthocyanidin (2 mg) was dissolved in water (1 ml) containing 0, 0.01 or 0.02 M of test compounds at 80 °C. The solution was cooled to 28 °C and left to stand for 15 h. The resulting precipitates were removed by centrifugation (3000 rpm, 20 min), and the supernatant was analyzed by reversed-phase HPLC (Cosmosil $5C_{18}$-AR, 15—45% (20 min) CH_3CN–50 mM H_3PO_4, 0.8 ml/min).

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