Chemical Evidence for the De-astringency (Insolubilization of Tannins) of Persimmon Fruit

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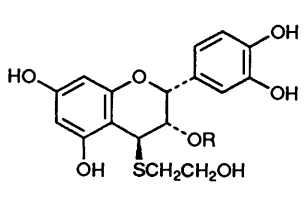
After artificial removal of the astringency from persimmon fruit by treatment with ethanol, thiol-promoted degradation of the insolubilized proanthocyanidin polymers with 2-sulfanylethanol yielded 4β-(2-hydroxyethylsulfanyl)-6- and -8-[1-(2-hydroxyethylsulfanyl)ethyl]-flavan-3-ols **9**–**14**. Furthermore, when deuteriated ethanol was used for de-astringency, the deuterium atoms were incorporated into the C_2 unit attached to the A-ring of these compounds. These findings evidently show that acetaldehyde formed *in situ* from ethanol plays an important role in polymerization (insolubilization) of water-soluble proanthocyanidins, causing the loss of astringency.

Japanese persimmon cultivars (*Diospyros kaki*) are usually classified into two groups, an astringent and a non-astringent type, depending on the astringency at the mature stage.[1] The fruits of astringent cultivars are edible after artificial removal of the astringency by treatment with alcohol vapour,[2] carbon dioxide gas,[3] or warm water. During these anaerobic treatments, acetaldehyde is known to be accumulated in the flesh, and concomitantly the water-soluble tannins which are responsible for the strong astringent taste are gradually changed into insoluble forms to decrease the astringency.[4] Matsuo and Itoo showed that the tannins of persimmon fruit are polymeric proanthocyanidins with a large molecular weight (~ 1.38×10^4 daltons on average),[5] and suggested that the insolubilization of the tannin is caused by condensation with acetaldehyde, based on the finding that the water-soluble proanthocyanidins form a gel on treatment with acetaldehyde *in vitro*, in analogy with the case of phenol–formaldehyde condensation.[6] The chemical evidence for the mechanism of de-astringency *in vivo*, however, still remains obscure. This is probably because the insolubilized tannins cannot be extracted with any organic solvent. In this study, we have extended the thiol-promoted degradation reaction using 2-sulfanylethanol directly to the insoluble tannins remaining in the fleshy debris, and unequivocally showed that the de-astringency (insolubilization) of the persimmon tannin is mainly due to the condensation of proanthocyanidins with endogenous acetaldehyde.

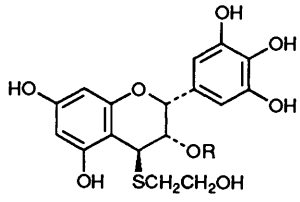
The interflavanoid bonds of proanthocyanidin oligomers have so far been shown to be cleaved by reaction with phenylmethanethiol[7] or phloroglucinol[8] in the presence of acetic acid in refluxing ethanol. However, in this case, taking into account the direct reaction in the homogenized flesh, and analysis of the degradation products by high-performance liquid chromatography (HPLC), it is desirable to use a water-soluble reagent which does not have UV absorption; therefore, 2-sulfanylethanol was selected for the thiol-promoted degradation of insoluble proanthocyanidins.[9] Preliminary degradation of prodelphinidin B-2, a typical proanthocyanidin dimer, with 2-sulfanylethanol in 0.2 mol dm^{-3} hydrochloric acid at 50 °C successfully yielded (−)-epigallocatechin and (−)-4β-(2-hydroxyethylsulfanyl)epigallocatechin **3**, *vide infra*, which were derived from the lower 4-non-substituted and the upper 4-substituted flavan unit, respectively.

Results and Discussion

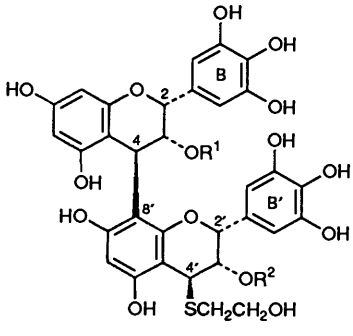
Thiol-promoted Degradation of Soluble Proanthocyanidin Polymer.—First, to characterize the structures of the water-soluble persimmon tannins, we have attempted their isolation. However, several attempts to separate proanthocyanidin polymers even from the aqueous acetone extract of the astringent persimmon fruits by column chromatography were unsuccessful, because a large portion of the soluble tannins was not adsorbed on polystylene gel nor on Sephadex LH-20.[10] This was probably due to the complexation of tannins with other macromolecules such as carboyhydrates and proteins. Hence, the thiol-promoted degradation reactions was directly applied to the aqueous acetone extract. The products were separated by a combination of Sephadex LH-20, high-porosity-polystyrene gel (MCI gel CHP 20P) and reversed-phase silica gel chromatography to give compounds **1**–**8**. The ^1H NMR spectra of compounds **1**–**4** (Table 1) are closely related to that of (−)-4β-benzylsulfanylepicatechin, except for the appearance of signals due to the hydroxyethyl group instead of the benzyl group. The small coupling constants of the heterocyclic proton resonances



1 R = H
2 R = galloyl



3 R = H
4 R = galloyl



5 $R^1 = R^2 = H$
6 R^1 = galloyl, $R^2 = H$
7 $R^1 = H$, R^2 = galloyl
8 $R^1 = R^2$ = galloyl

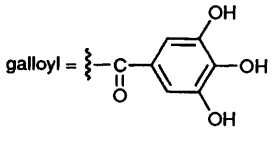


Table 1 ^{1}H NMR data (δ_H) for the sulfides **1**–**4** in $(CD_3)_2CO$ + D_2O at 100 MHz. Splitting patterns and *J* values (Hz) are given in parentheses

	1	**2**	**3**	**4**
2-H	5.23 (s)	5.52 (s)	5.17 (s)	5.42 (s)
3-H	4.09 (br s)	5.30 (d, 2)	4.07 (s)	5.34 (d, 2)
4-H	4.09 (br s)	4.25 (d, 2)	4.07 (s)	4.24 (d, 2)
6-, 8-H	5.92 (d, 2) 6.03 (d, 2)	6.03 (d, 2) 6.07 (d, 2)	5.91 (d, 2) 6.03 (d, 2)	6.05 (2 H, s)
B-ring 2-H	7.10 (d, 2)	7.11 (d, 2)	6.62 (2 H, s)	6.56 (2 H, s)
5-H	6.81 (d, 8)	6.81 (d, 8)		
6-H	6.90 (dd, 8, 2)	6.89 (dd, 8, 2)		
SCH_2	2.63–3.15 (2 H, m)	2.79–3.31 (2 H, m)	2.64–3.31 (2 H, m)	2.68–3.30 (2 H, m)
OCH_2	3.62–3.95 (2 H, m)	3.70–4.10 (2 H, m)	3.66–4.01 (2 H, m)	3.84–4.12 (2 H, m)
galloyl-H		7.00 (2 H, s)		6.99 (2 H, s)

Table 2 ^{1}H NMR data (δ_H) for the sulfides **5**–**8** (at 100 MHz), and compounds **15**, **16a** and **16b** (at 400 MHz) in $(CD_3)_2CO$ + D_2O. Splitting patterns and *J* values (Hz) are given in parentheses

	5	**6**	**7**	**8**	**15**	**16a**	**16b**
2-H	5.00 (s)	5.49 (s)	5.19 (s)	5.58 (s)	5.02 (2 H, s)	4.98 (s)	4.98 (s)
3-H	4.15 (br s)	5.04 (br s)	3.98 (br s)	5.52 (br d, 3)	5.55 (2 H, br s)	5.52 (br s)	5.52 (br s)
4-H	4.71 (s)	4.66 (d, 2)	4.89 (s)	4.83 (d, 3)	2.98 (4 H, m)	2.99 (2 H, m)	3.01 (2 H, m)
6-H	6.02 (2 H, s)	5.93 (2 H, br s)	6.01 (2 H, s)	5.93 (d, 2)			
8-H				5.97 (d, 2)	6.16 (2 H, s)	6.14 (s)	6.17 (s)
B-ring 2-, 6-H	6.48 (2 H, s)	6.52 (2 H, s)	6.51 (2 H, s)	6.50 (2 H, s)	6.58 (4 H, s)	6.59 (2 H, s)	6.59 (2 H, s)
galloyl-H		6.93 (2 H, s)		6.96 (2 H, s)	6.98 (4 H, s)	6.99 (2 H, s)	6.99 (2 H, s)
2′-H	5.28 (s)	5.33 (s)	5.38 (s)	5.26 (br s)		5.23 (s)	5.24 (s)
3′-H	3.97 (br s)	4.05 (br s)	5.56 (br s)	5.36 (br s)		5.57 (br s)	5.57 (br s)
4′-H	4.15 (br s)	4.10 (s)	4.31 (d, 2)	4.27 (d, 2)		2.99 (2 H, m)	3.01 (2 H, m)
6′-H	6.02 (s)	6.13 (s)	6.01 (s)	6.10 (s)		6.10 (s)	6.11 (s)
B′-ring 2-, 6-H	6.71 (2 H, s)	6.52 (2 H, s)	6.76 (2 H, s)	6.59 (2 H, br s)		6.75 (2 H, s)	6.75 (2 H, s)
SCH_2CH_2OH	2.62–3.96 (m)	2.60–3.96 (m)	2.60–3.97 (m)	2.60–4.00 (m)			
galloyl-H			7.07 (2 H, s)	7.06 (2 H, s)		7.09 (2 H, s)	7.10 (2 H, s)
CH					4.88 (q, 7)	5.05 (q, 8)	5.00 (q, 7)
Me					1.79 (d, 7)	1.79 (q, 8)	1.83 (d, 7)

are in accord with those of the 2,3-*cis* and 3,4-*trans* configurations in the C-ring.[11] In the spectra of compounds **1** and **2**, the observation of ABX-type aromatic signals indicated that the B-ring is of catechol type, whereas the appearance of aromatic two-proton singlet signals in the spectra of compounds **3** and **4** implied that the B-ring is of pyrogallol type. The spectra of compounds **2** and **4** exhibited additional two-proton singlets accompanied by large downfield shifts of the flavan 3-H, indicating the presence of a galloyl group on the C-3 hydroxy group. Furthermore, desulfurization[7] of compound **4** with Raney nickel afforded (−)-epigallocatechin 3-*O*-gallate. Based on these findings, compounds **1**–**4** were determined to be 4β-(2-hydroxyethylsulfanyl) derivatives of (−)-epicatechin, (−)-epicatechin 3-*O*-gallate, (−)-epigallocatechin, and (−)-epigallocatechin 3-*O*-gallate, respectively, and, judging from the yields, the proportions of these extension units in the persimmon proanthocyanidins are estimated to be ~4:1:22:6.

In the ^{1}H NMR spectrum (Table 2), the product **5** showed two-proton singlets attributable to the flavan B-ring, suggesting it to have a prodelphinidin dimer structure, and the aliphatic signals due to a hydroxyethyl group, indicating that this compound is also a 2-hydroxyethyl sulfide. Treatment of compound **5** with Raney nickel afforded prodelphinidin B-2; thus, the product **5** was concluded to be 4β-(2-hydroxyethylsulfanyl)prodelphinidin B-2. The ^{1}H NMR spectra (Table 2) of the products **6**, **7** and **8** are related to that of compound **5**, and showed additional aromatic two-proton singlets due to galloyl groups. Enzymic hydrolyses of compounds **6**, **7** and **8** with tannase gave compound **5**, together with gallic acid. The location of the ester(s) in each compound was confirmed by observation of the downfield shift of 3-H and/or 3′-H of the heterocyclic ring. Accordingly, these compounds were characterized as 4′β-(2-hydroxyethylsulfanyl)prodelphinidin B-2 3-*O*-gallate **6**, 3′-*O*-gallate **7** and 3,3′-di-*O*-gallate **8**. The production of compounds **5**–**8** as major sulfides of dimeric proanthocyanidin suggested that the C-4–C-8′ interflavanoid linkage is predominant in the parent proanthocyanidin polymer. In this experiment, any flavan-3-ol derived from the lower terminal 4-non-substituted flavan unit could not be detected. HPLC analysis of the degradation products (Fig. 1A) also did not show the presence of free flavan-3-ols. These results suggest that the lower terminal unit is different from that of the usual proanthocyanidins, or that the relative molecular mass of persimmon tannin is much too large to allow detection of the lower terminal unit.

Thiol-promoted Degradation of Insoluble Proanthocyanidin.—After complete removal of the astringency in persimmon fruit by treatment with 35% ethanol, the aq. acetone extract no longer yielded any sulfides on thiol-promoted degradation (Fig. 1D), indicating the absence of proanthocyanidins in the extract. On the other hand, direct thiol-promoted degradation of the remaining fleshy debris afforded the sulfides **1**–**8** in reasonable yields which are comparable to those obtained from the extract of the astringent fruit (Fig. 1B). This fact indicated that the proanthocyanidins were insolubilized in the fruit's flesh without appreciable chemical changes. The HPLC profile (Fig. 1B), however, showed some small peaks designated as being due to compounds **9a**, **b**; **10a**, **b**; **11a**, **b**; **12a**, **b**; **13a**, **b**; and **14a**, **b**, which

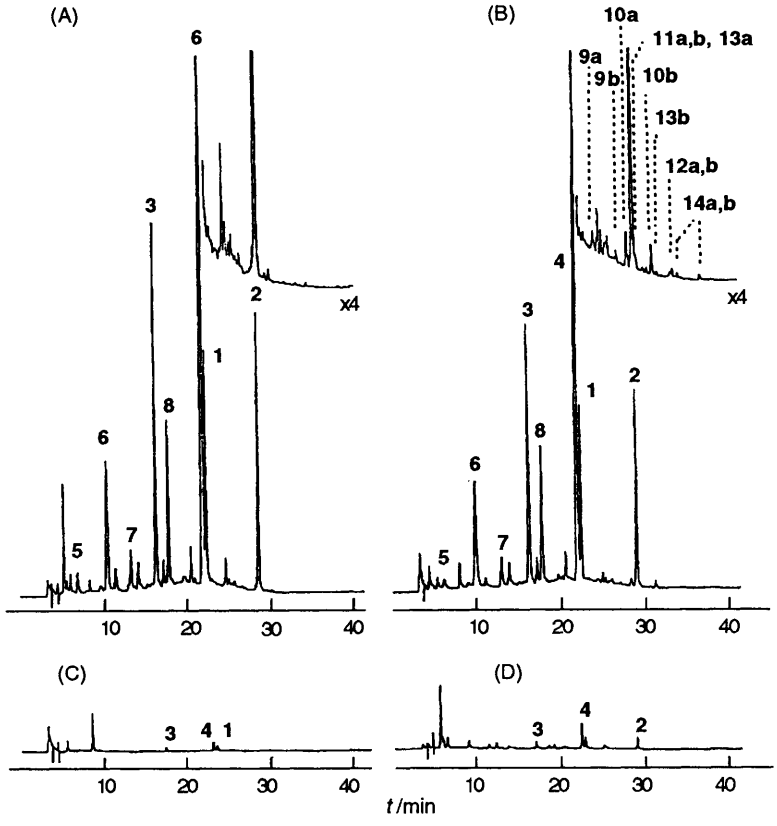
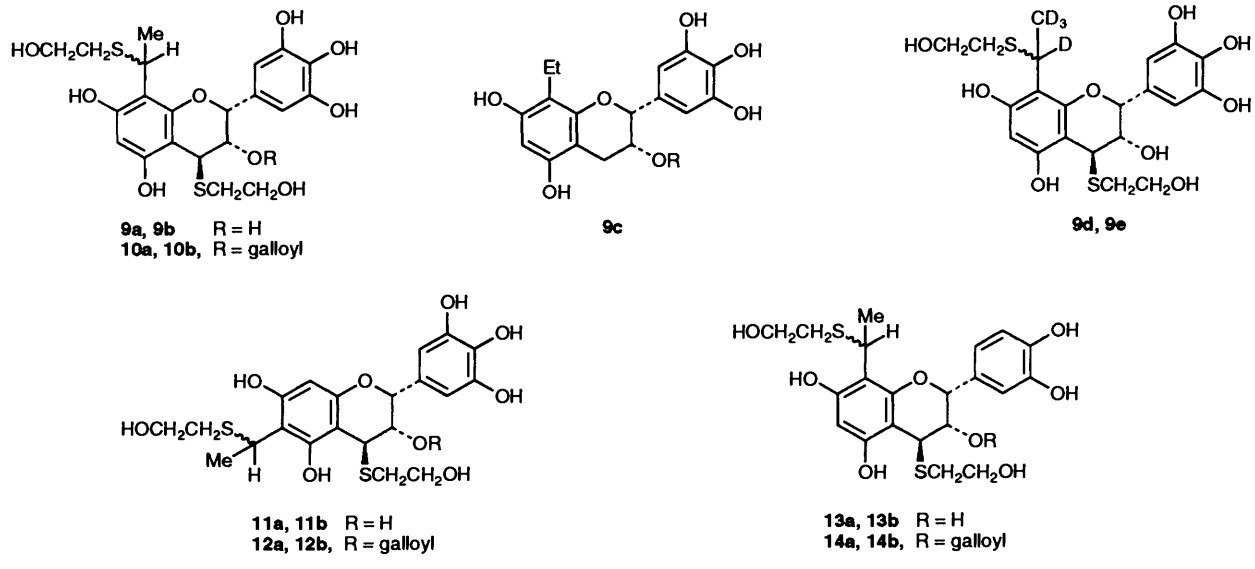


Fig. 1 HPLC chromatograms of the thiol-promoted degradation products. A: aq. acetone extract of astringent fruit, B: debris of the fruit treated with ethanol, C: debris of astringent fruit, D: aq. acetone extract of the fruit treated with ethanol.



were observed only in the chromatogram of the products from the insolubilized tannin. Isolation of these products was achieved by repeated chromotography using Sephadex LH-20 and MCI-gel CHP 20P, and preparative reversed-phase HPLC. On purification by preparative HPLC, despite complete separation of the peaks, isomer **9a** was always contaminated by its diastereoisomer **9b**, the amount of which gradually increased, and *vice versa*. These phenomena suggested the occurrence of isomerization between these compounds. Similar interconversions were also observed between diastereoisomers **10a**, **b**; **11a**, **b**; **12a**, **b**; **13a**, **b**; and **14a**, **b**; hence, these compounds could not be purified completely.

The ^{1}H NMR spectra (Table 3) of diastereoisomers **9a** and **9b** showed the close relationship between compound **3** and these compounds. The appearance of a doublet methyl (δ 1.59, J 7 Hz) and a quartet methine signal (δ 4.72, J 7 Hz), along with a one-proton singlet (δ 6.13) due to the flavan A-ring proton, indicated the presence of a 1-substituted ethyl group at the C-6 or C-8 position of the A-ring. The aliphatic proton signals (SCH_2CH_2OH) appeared at δ 2.5–4.0 corresponding to a total of eight protons, and the observation of the $(M - H)^-$ ion peak at m/z 485 in the negative-ion fast-atom bombardment (FAB) mass spectra indicated that an additional hydroxyethylsulfanyl group is attached to the ethyl group. Furthermore, the

Table 3 ^{1}H NMR data (δ_H) for the sulfides **9a, b–14a, b** in $(CD_3)_2CO + D_2O$ at 100 MHz. Splitting patterns and J values (Hz) are given in parentheses

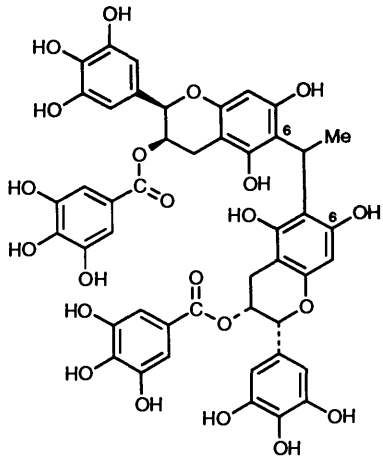
	9a	**9b**	**10a**	**10b**	**11a**	**11b**	**12a**	**12b**	**13a**	**13b**	**14a**	**14b**
2-H	5.24 (s)	5.22 (s)	5.53 (s)	5.48 (s)	5.14 (s)	5.13 (s)	5.40 (s)	5.40 (s)	5.31 (s)	5.28 (s)	5.58 (s)	5.54 (s)
3-H	4.09 (br s)	4.09 (br s)	5.29 (br s)	5.31 (d, 2)	4.07 (d, 2)	4.06 (d, 2)	5.20 (br s)	5.20 (br s)	4.10 (br s)	4.10 (br s)	5.34 (br s)	5.31 (br s)
4-H	4.09 (br s)	4.09 (br s)	4.26 (br s)	4.27 (d, 2)	4.11 (d, 2)	4.11 (d, 2)	4.29 (br s)	4.29 (br s)	4.10 (br s)	4.10 (br s)	4.26 (d, 2)	4.28 (d, 2)
6-H	6.13 (s)	6.13 (s)	6.13 (s)	6.12 (s)					6.14 (s)	6.13 (s)	6.14 (s)	6.14 (s)
8-H					6.04 (s)	6.01 (s)	6.16 (s)	6.16 (s)				
2′-H	6.75 (2 H, s)	6.68 (2 H, s)	6.86 (2 H, s)	6.73 (2 H, s)	6.62 (2 H, s)	6.62 (2 H, s)	6.68 (2 H, s)	6.68 (2 H, s)	7.21 (d, 2)	7.18 (d, 2)	7.29 (d, 2)	7.20 (d, 2)
5′-H									6.86 (d, 8)	6.85 (d, 8)	6.81 (d, 8)	6.84 (d, 8)
6′-H									7.00 (dd, 2, 8)	6.93 (dd, 2, 8)	7.08 (dd, 2, 8)	6.98 (dd, 2, 8)
CH	4.72 (q, 7)	4.71 (q, 7)	4.80 (q, 7)	4.80 (q, 7)	4.75 (q, 7)	4.77 (q, 7)	4.72 (q, 7)	4.73 (q, 7)	4.71 (q, 7)	4.70 (q, 7)	4.80 (q, 7)	4.76 (q, 7)
Me	1.59 (3 H, d, 7)	1.59 (3 H, d, 7)	1.63 (3 H, d, 7)	1.62 (3 H, d, 7)	1.57 (3 H, d, 7)	1.57 (3 H, d, 7)	1.54 (3 H, d, 7)	1.57 (3 H, d, 7)	1.58 (3 H, d, 7)	1.59 (3 H, d, 7)	1.61 (3 H, d, 7)	1.62 (3 H, d, 7)
SCH_2	2.52–3.10 (4 H, m)	2.52–3.10 (4 H, m)	2.62–3.2 (4 H, m)	2.61–3.2 (4 H, m)	2.58 (2 H, t, 7) 2.70–3.11 (2 H, m)	2.56 (2 H, t, 7) 2.69–3.10 (2 H, m)	2.56 (2 H, t, 7) 2.77–3.2 (2 H, m)	2.54 (2 H, t, 7) 2.77–3.2 (2 H, m)	2.51–3.07 (4 H, m)	2.54–3.11 (4 H, m)	2.54–3.2 (4 H, m)	2.60–3.2 (4 H, m)
OCH_2	3.50–4.01 (4 H, m)	3.50–4.01 (4 H, m)	3.4–4.02 (4 H, m)	3.4–4.03 (4 H, m)	3.64 (2 H, t, 7) 3.65–4.03 (2 H, m)	3.64 (2 H, t, 7) 3.65–3.99 (2 H, m)	3.4–4.15 (4 H, m)	3.4–4.15 (4 H, m)	3.40–3.96 (4 H, m)	3.40–4.01 (4 H, m)	3.4–4.04 (4 H, m)	3.4–4.02 (4 H, m)
galloyl			7.03 (2 H, s)	7.03 (2 H, s)			7.02 (2 H, s)	7.00 (2 H, s)			7.02 (2 H, s)	7.03 (2 H, s)

observation of the above mentioned isomerization between compounds **9a** and **9b**, and the difference in the chemical shifts of the 2-H and B-ring proton signals of these compounds suggested that compounds **9a** and **9b** are epimers with respect to the methine carbon attached to C-8 or C-6 of the flavan A-ring. This was confirmed by desulfurization of compounds **9a** and **9b** with Raney nickel to give a single product **9c**, whose ^{1}H NMR spectrum resembled that of epigallocatechin, showing a singlet signal (δ 6.09) due to the A-ring proton, and additional signals due to an ethyl group [δ 1.07 (3 H, t, J 7 Hz) and 2.61 (2 H, q, J 7 Hz)]. The location of the substituent in compounds **9a** and **9b** was considered to be C-8 on the basis of the downfield shift of 2-H ($\Delta\delta$ +0.07 ppm for **9a**; +0.05 ppm for **9b**) and B-ring proton signals ($\Delta\delta$ +0.13 ppm for **9a**; +0.06 ppm for **9b**)[12] compared with that of compound **3**. This was supported by observation of a nuclear Overhauser effect (NOE) between the methine proton of the hydroxyethylsulfanylethyl group and the B-ring proton in the NOESY spectrum indicating that the substituent is situated close to these protons. Furthermore, treatment of compound **3** with acetaldehyde and 2-sulfanylethanol in 2% acetic acid yielded almost equimolar amounts of compounds **9a** and **9b** as major products. Since electrophilic substitution on (−)-epicatechin under aq. acidic conditions is known to occur almost exclusively at the C-8 position,[13] this result strongly supports the C-8 substitution. Taken in conjugation, these findings indicated that compounds **9a** and **9b** are epimers with respect to the methine carbon attached to C-8 of (−)-4β-(2-hydroxyethylsulfanyl)-8-[1-(2-hydroxyethyl(sulfanyl)ethyl]epigallocatechin.

Compounds **11a** and **11b** were shown to have the same constitution as compounds **9a** and **9b** by negative-ion FAB mass spectroscopy [m/z 485 (M − H)$^-$]. The ^{1}H NMR spectra also resembled those of compounds **9a** and **9b**, except for the chemical shifts of the signals due to 2-H and the B-ring proton which were almost identical with those of compound **3**, indicating that the hydroxyethylsulfanylethyl group attached to the A-ring did not influence these protons. From these spectral data, compounds **11a** and **11b** were assigned as epimers of (−)-4β-(2-hydroxyethylsulfanyl)-6-[1-(2-hydroxyethylsulfanyl)ethyl]epigallocatechin, which are positional isomers of compounds **9a** and **9b**.

In the ^{1}H NMR spectra (Table 3), compounds **10a**, **b** and **12a** and **12b** showed two-proton singlets attributable to a galloyl group which were accompanied by large downfield shifts of 3-H. Hydrolysis of esters **10a** and **10b** with tannase yielded compounds **9a** and **9b**, whereas hydrolysis of isomers **12a** and **12b** afforded compounds **11a** and **11b**. These results confirmed that esters **10a**, **b** and **12a**, **b** are 3-*O*-galloyl esters of compounds **9a**, **b** and **11a**, **b**, respectively. The ^{1}H NMR spectra of **13a** and **13b** were similar to those of compounds **9a** and **9b**; however, the appearance of ABX-type signals due to the B-ring protons indicated the presence of a (−)-epicatechin unit. This was supported by the negative-ion FAB mass spectra which showed the same (M − H)$^-$ ion peak at m/z 469, sixteen mass units less than those of compounds **9a** and **19b**. Furthermore, treatment of compound **1** with acetaldehyde and 2-sulfanylethanol afforded a mixture of products **13a** and **13b**. These findings indicated that the hydroxyethylsulfanylethyl group is attached to the C-8 position of the A-ring. Compounds **14a** and **14b** afforded the alcohols **13a** and **13b** on hydrolysis with tannase. Thus, **13a**, **b** and **14a**, **b** were concluded to be the epicatechin analogues of compounds **9a**, **b** and **10a**, **b**, respectively.

As mentioned above, products **9**–**14** were detected only in the thiol-promoted degradation reaction mixture obtained from the fleshy debris of the fruit whose astringency was removed by treatment with ethanol (Fig. 1). This fact suggested that the C_2 unit attached to the flavan A-ring arises from acetaldehyde which was formed by enzymic oxidation of ethanol in the flesh.



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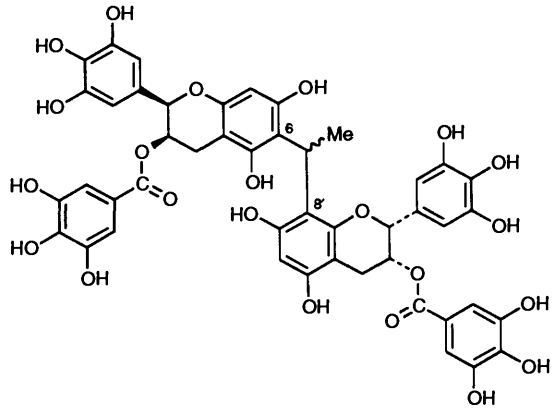
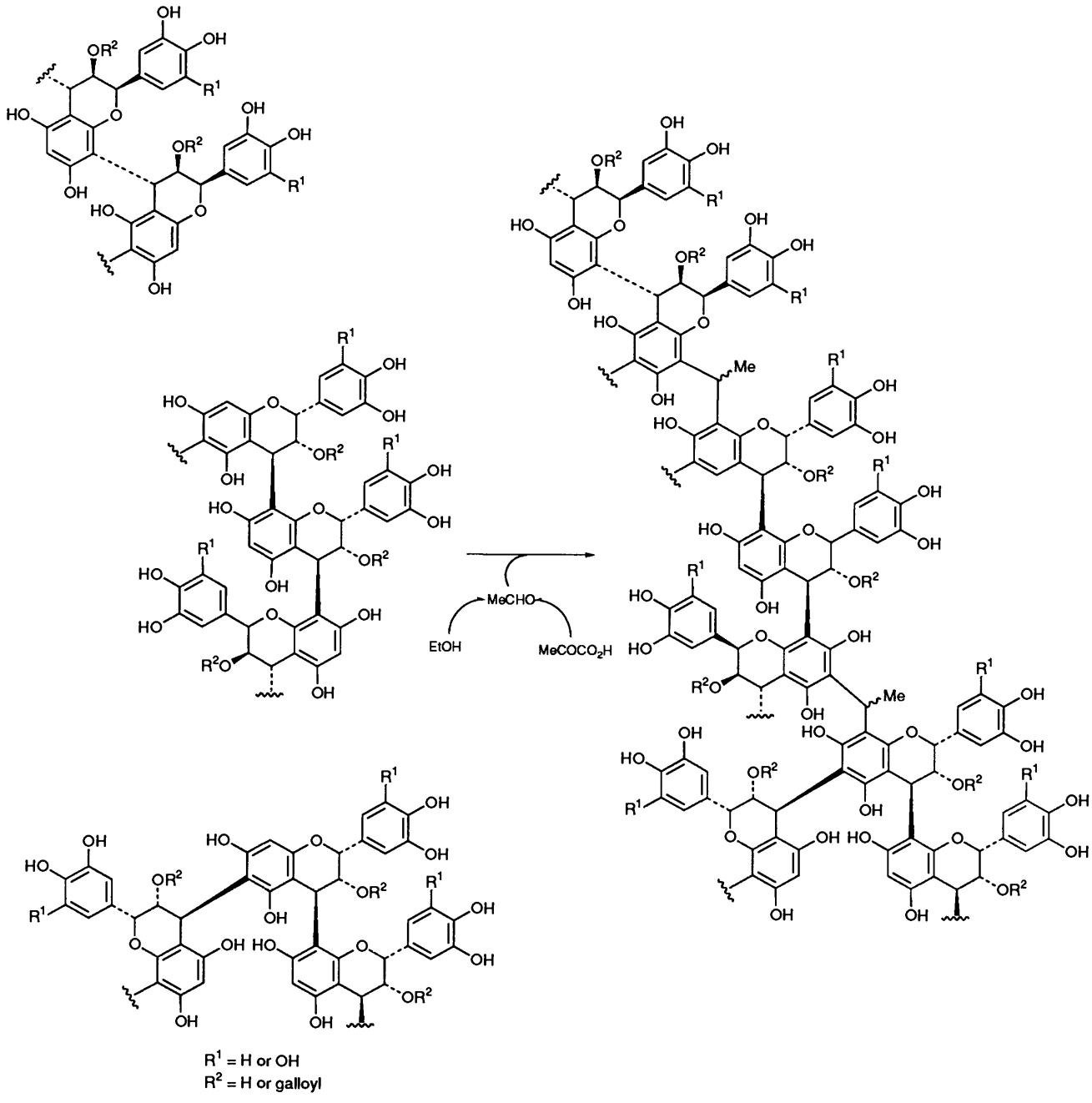


16a, 16b

To confirm this, the following experiments were attempted. First, the extract of the astringent fruit, which did not afford compounds **9a** and **9b** on degradation by thiol, was treated with acetaldehyde to form a gel, subsequent treatment of which with 2-sulfanylethanol in dil. hydrochloric acid yielded products **9a**, **b** and **11a**, **b**. Furthermore, removal of the astringency by treatment with 30% hexadeuterioethanol ([$^2H_6$]ethanol), and subsequent thiol-promoted degradation of the flesh afforded deuterio compounds **9d** and **9e**, which are products corresponding to compounds **9a** and **9b**, respectively. The negative-ion FAB mass spectra and the integration curves in their ^{1}H NMR spectra revealed that ~ 50% of the molecules have a C_2 unit in which hydrogen atoms were replaced by their deuterium analogues. These results evidently show that the C_2 unit is derived from endogenous and exogenous ethanol *via* acetaldehyde.

To confirm more definitively the connection of the proanthocyanidin molecules through the C_2 unit, (−)-epigallocatechin 3-*O*-gallate was treated with acetaldehyde. The resulting complex mixture consisted of somewhat unstable compounds, liberating the starting material; however, three major products, **15**, **16a** and **16b**, could be isolated. The negative-ion FAB mass spectra of these compounds exhibited the same (M − H)$^-$ ion peak at m/z 941, suggesting their dimeric structure. The ^{1}H NMR spectra showed signals due to methyl and methine protons (Table 2) attributable to a C_2 unit derived from acetaldehyde. In the spectrum of compound **15**, the signals arising from the two galloylepigallocatechin moieties completely overlapped, indicating the symmetrical structure of compound **15**. In addition, the chemical shifts of the B-ring protons and 2-H of the C-ring, which were in good agreement



Scheme 1

with those of epicatechin 3-*O*-gallate (δ 6.63 and 5.07, respectively), implied that the methine carbon of the C_2 unit is attached to the C-6 positions of both flavan units.[12] In contrast, two sets of flavan resonances appeared separately in the spectra of compounds **16a** and **16b**, and a pair of the B-ring proton and 2-H proton signals were shifted downfield, and the chemical shifts of the remaining signals of the B-ring proton and 2-H were consistent with those of structure **15**. Thus, the locations of the C-2 unit were concluded to be at the C-6 and C-8′ positions of the flavan A-rings, and these two products were considered to be epimers with respect to the methine carbon of the C_2 unit. The isomer having a C-8 to C-8′ linkage could not be isolated because of its instability, probably caused by steric effect. The products **15**, **16a** and **16b** were also gradually decomposed to give a complex mixture containing epigallocatechin 3-*O*-gallate. White and Foo reported on the analogous cleavage of the linkage between acetaldehyde and phloroglucinol in similar acid conditions.[14] These results suggested that the linkage between the C_2 unit and the proanthocyanidin A-ring is somewhat unstable and easily cleaved.

Conclusions.—The chemical evidence described above showed that, during anaerobic treatment to remove astringency, the originally water-soluble proanthocyanidins become insoluble by condensation with acetaldehyde (Scheme 1). Acetaldehyde is known to be generated *in situ* by oxidation of endogenous and exogenous ethanol and by decarboxylation of pyruvic acid.[15] The HPLC profiles of the thiol-promoted degradation products of dried fruits and of fruits treated with warm water were similar to that shown in Fig. 1B, indicating that the mechanism of the de-astringency is the same as that for the ethanol-treated fruit. Furthermore, in early winter, the astringent fruits naturally lose astringency on softening and finally become sweet. This phenomenon is also considered to be

the result of the condensation of tannins with acetaldehyde, because the peaks due to compounds **10a** and **10b** were detected on HPLC after thiol-promoted degradation of the fruits' flesh.

The fruit of the other type of persimmon, *i.e.* non-astringent type which naturally loses astringency on ripening, also has significant astringency at the immature stage. HPLC analysis of the thiol-promoted degradation products of immature fruit (picked on June 25th) of the non-astringent cultivar showed the presence of soluble tannins (~4%, estimated from the yields of compounds **1**, **2**, **3** and **4**) in amounts comparable to those of the astringent cultivar (7%). The predominance of the epigallocatechin unit was also similar to that of the astringent cultivar (proportions of the yields of **1**, **2**, **3** and **4** ~3:1:10:3). However, on September 7th, the amount of soluble and insoluble tannins in the non-astringent cultivar (0.4 and 0.1%, respectively) is significantly lower than that of the astringent cultivar (1.6%). Taking into account the growth of the fruit of about ten times in weight, this result indicated that the tannin in the fruit of the non-astringent type did not increase during summer. This observation was in accord with the results of the morphological study of tannin cells,[16] which suggested that the major factor of de-astringency is dilution due to fruit enlargement, and the tannin coagulation is a minor factor in the non-astringent cultivar.

Experimental

^1H (100 and 400 MHz) and ^{13}C (25 and 100 MHz) NMR spectra were recorded on a JEOL FX-100 and a JEOL GX-400 instrument, and ^1H–^1H COSY and NOESY spectra were measured with a JEOL GX-270 spectrometer. *J* Values are given in Hz. Optical rotations were measured with a JASCO DIP-4 digital polarimeter and $[\alpha]_D$ units are 10^{-1} deg cm^2 g^{-1}. FAB mass spectra were obtained with a JEOL JMS DX-300 machine with glycerol as a matrix. Column chromatography was carried out with Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP 20P (Mitsubishi Chemical Industries, Ltd.), Cosmosil 75 C_{18}-OPN (Nacalai tesque Inc.), and Bondapak C18/Porasil B (Waters Associates, Inc.). TLC was performed on a precoated Kieselgel 60 F_{254} plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (1:7:1, v/v), and spots were detected by UV illumination and by spraying 2% ethanolic iron(III) chloride or anisaldehyde–sulfuric acid reagents. Analytical HPLC was conducted on a Tosoh apparatus equipped with a CCPM solvent delivery system, a UV-8 model II spectrometer and a Cosmosil 5C_{18}-AR (Nacalai tesque) column (4.6 mm i.d. × 250 mm) [mobile phase, acetonitrile–0.05 mol dm^{-3} H_3PO_4 (gradient elution of 10%–50% acetonitrile for 60 min); flow rate, 0.8 cm^3/min; detection at 280 nm]. Preparative HPLC was performed on a Tosoh apparatus equipped with a TSK-gel ODS-80TM (21.5 mm i.d. × 300 mm) [mobile phase, methanol–water (3:7 or 4:6, v/v)]. The persimmon fruits were kindly supplied by Dr. A. Morita and Mr. S. Hayashida of the Nagasaki Fruit-tree Experiment Station at Ohmura city, Nagasaki.

Thiol-promoted Degradation of Prodelphinidin B-2.—Prodelphinidin B-2 (2 mg) was dissolved in a mixture of 5% 2-sulfanylethanol in 0.2 mol dm^{-3} HCl (1 cm^3) and the mixture was stirred at 50 °C and analysed by HPLC [gradient elution of 10–40% acetonitrile for 45 min]. At 4 h, 70% of the starting material (t_R 5.6 min) was degraded into almost equimolar amounts of epigallocatechin (t_R 10.6 min) and (−)-4β-(2-hydroxyethylsulfanyl)epigallocatechin **3**, *vide infra*, (t_R 16.4 min), on the basis of the peak areas. After 20 h, the starting material had disappeared.

Isolation of Thiol-promoted Degradation Products from the Extract of the Astringent Fruit.—Astringent fruit (100 g) picked on September 7th 1992 was macerated with water (50 cm^3) in a Waring blender and was extracted with acetone–water (4:1; 250 cm^3) at room temperature three times. Concentration of the extract under reduced pressure (~40 °C) gave an aqueous solution (250 cm^3), to which 2-sulfanylethanol (10 cm^3) and conc. HCl (5 cm^3) were added and the mixture was stirred for 48 h at room temperature. After filtration, the mixture was directly applied to a column of MCI-gel CHP 20P (2.7 cm i.d. × 15 cm) and washed with water. The phenolic compounds adsorbed on the gel were eluted with 20–60% methanol to afford a fraction (3.3 g) positive to iron(III) chloride and anisaldehyde–sulfuric acid reagents. Rechromatography of the fraction over Sephadex LH-20 (4.0 cm i.d. × 35 cm) with ethanol gave six fractions; fractions 1–5 were separately purified on a Cosmosil 75 C_{18}-OPN (3.0 cm i.d. × 30 cm) with water containing increasing proportions of methanol to yield compounds **1** (100.1 mg), **3** (637.5 mg), **2** (60.6 mg), **4** (479.8 mg) and **5** (65.2 mg), respectively. Fraction 6 was further separated by MCI-gel CHP 20P (2.5 cm i.d. × 24 cm) chromatography with methanol–water (1:4–1:2) to give three fractions, which were separately purified by Cosmosil 75 C_{18}-OPN chromatography to furnish compounds **6** (106.8 mg), **7** (98.3 mg) and **8** (100.2 mg).

(−)-4β-(2-*Hydroxyethylsulfanyl*)*epicatechin* **1** was obtained as an amorphous powder, $[\alpha]_D^{18}$ −65.6 (*c* 0.7, acetone) (Found: C, 55.7; H, 5.2. $C_{17}H_{18}O_7S$ requires C, 55.7; H, 4.95%); *m/z* 365 [(M − H)$^-$, negative ion FAB]; δ_H (Table 1); δ_C[(CD$_3$)$_2$CO–D$_2$O] 35.1 (CH_2S), 43.7 (C-4), 62.8 (CH_2OH), 72.0 (C-3), 75.1 (C-2), 95.6 and 96.9 (C-6 and -8), 99.9 (C-4a), 115.2 and 115.5 (B-ring C-2 and -5), 119.2 (B-ring C-6), 131.7 (B-ring C-1), 145.3 and 145.4 (B-ring C-3 and -4) and 156.8, 158.5 and 158.8 (C-5, -7 and -8a).

(−)-4β-(2-*Hydroxyethylsulfanyl*)*epicatechin* 3-O-*gallate* **2** was obtained as an amorphous powder, $[\alpha]_D^{18}$ −136.8 (*c* 0.7, acetone) (Found: C, 54.9; H, 4.6. $C_{24}H_{22}O_{11}S$ requires C, 54.65; H, 4.4%); *m/z* 517 [(M − H)$^-$, negative ion FAB]; δ_H (Table 1); δ_C[(CD$_3$)$_2$CO–D$_2$O] 35.2 (CH_2S), 40.8 (C-4), 62.6 (CH_2OH), 73.9 (C-2 and -3), 95.6 and 97.3 (C-6 and -8), 99.0 (C-4a), 110.0 (galloyl C-2 and -6), 114.9 and 115.8 (B-ring C-2 and -5), 119.1 (B-ring C-6), 120.9 (galloyl C-1), 130.5 (B-ring C-1), 139.2 (galloyl C-4), 145.6, 145.8 and 145.9 (B-ring C-3 and -4, galloyl C-3 and -5), 156.6, 158.4 and 159.2 (C-5, -7 and -8a) and 166.5 (CO_2).

4β-(2-*Hydroxyethylsulfanyl*)*epigallocatechin* **3** was obtained as an amorphous powder, $[\alpha]_D^{18}$ −38.9 (*c* 1.0, acetone) (Found: C, 53.6; H, 5.1. $C_{17}H_{18}O_8S$ requires C, 53.4; H, 4.7%); *m/z* 381 [(M − H)$^-$, negative ion FAB]; δ_H (Table 1); δ_C[(CD$_3$)$_2$CO–D$_2$O] 35.1 (CH_2S), 43.6 (C-4), 62.8 (CH_2OH), 72.0 (C-3), 75.0 (C-2), 95.7 and 97.0 (C-6 and -8), 99.9 (C-4a), 106.9 (B-ring C-2 and -6), 131.1 (B-ring C-1), 133.0 (B-ring C-4), 146.1 (B-ring C-3 and -5) and 156.7, 158.5 and 158.8 (C-5, -7 and -8a).

4β-(2-*Hydroxyethylsulfanyl*)*epicatechin* 3-O-*gallate* **4** was obtained as an amorphous powder, $[\alpha]_D^{18}$ −121.3 (*c* 0.7, acetone) (Found: C, 53.6; H, 4.5. $C_{24}H_{22}O_{12}S$ requires C, 53.9; H, 4.2%); *m/z* 533 [(M − H)$^-$, negative ion FAB]; δ_H (Table 1); δ_C[(CD$_3$)$_2$CO–D$_2$O] 35.2 (CH_2S), 40.8 (C-4), 62.5 (CH_2OH), 73.9 (C-2 and -3), 95.6 and 97.2 (C-6 and -8), 99.0 (C-4a), 106.6 (B-ring C-2 and -6), 110.0 (galloyl C-2 and -6), 120.9 (galloyl C-1), 130.0 (B-ring C-1), 133.2 (B-ring C-4), 139.2 (galloyl C-4), 145.9 and 146.3 (B-ring C-3 and -5, galloyl C-3 and -5), 156.5, 158.3 and 159.1 (C-5, -7 and -8a) and 166.6 (CO_2).

This compound (200 mg) was desulfurized with Raney nickel (W-4) in 2% ethanolic acetic acid (2 cm^3) for 2 h at room temperature. After removal of the reagent by filtration, the mixture was concentrated and subjected to MCI-gel CHP 20P column (1.0 cm i.d. × 20 cm) chromatography with methanol–

water (3:7) to yield (−)-epigallocatechin 3-*O*-gallate (34.2 mg) as needles, m.p. 223–224 °C; $[\alpha]_D^{28}$ −175.1 (*c* 0.1 in acetone).

4′β-(2-*Hydroxyethylsulfanyl*)*prodelphinidin B-2* **5** was obtained as an amorphous powder, $[\alpha]_D^{18}$ +36.8 (*c* 1.4, acetone) (Found: C, 56.3; H, 4.1. $C_{32}H_{30}O_{15}S$ requires C, 56.0; H, 4.4%); *m/z* 685 [(M − H)$^-$, negative ion FAB]; δ_H (Table 2); δ_C[$(CD_3)_2CO$–D_2O] 35.2 (CH_2S), 36.7 (C-4), 43.8 (C-4′), 62.6 (CH_2OH), 71.1 (C-3′), 72.8 (C-3), 75.0 (C-2′), 76.6 (C-2), 95.9, 96.4 and 97.8 (C-6, -8 and -6′), 100.4 (C-4a and -4a′), 106.5 and 106.8 (C-8′, B- and B′-ring C-2 and -6), 130.8 and 131.3 (B- and B′-ring C-1), 132.9 (B- and B′-ring C-4), 146.1 (B- and B′-ring C-3 and -5) and 153.9, 156.7, 157.3, 157.4, 157.9 and 158.1 (C-5, -7, -8a, -5′, -7′ and -8a′).

This compound (45 mg) was desulfurized with Raney nickel (W-4) as described above. The products were chromatographed on a Sephadex LH-20 column [methanol–water (3:2)], and purified by preparative HPLC [methanol–water (1:4)] to give prodelphinidin B-2 (6.6 mg) as an amorphous powder, $[\alpha]_D^{26}$ +35.3 (*c* 0.5, acetone), δ_H[$(CD_3)_2CO$] 2.85 (2 H, m, 4′-H_2), 3.96 (1 H, br s, 3-H), 4.27 (1 H, br s, 3′-H), 4.71 (1 H, d, *J* 3, 4-H), 4.90 (1 H, s, 2-H), 5.01 (1 H, s, 2′-H), 5.94 (1 H, s, 6′-H), 6.02 (2 H, s, 6- and 8-H), 6.46 (2 H, s, B-ring 2- and 6-H) and 6.63 (2 H, br s, B′-ring 2- and 6-H). The ^{1}H NMR data and $[\alpha]_D$-value were identical with those described in the literature.[17]

4′β-(2-*Hydroxyethylsulfanyl*)*prodelphinidin B-2* 3-O-*gallate* **6** was obtained as an amorphous powder, $[\alpha]_D^{18}$ +11.4 (*c* 1.1, acetone) (Found: C, 54.4; H, 4.7. $C_{39}H_{34}O_{19}S{\cdot}3/2H_2O$ requires C, 54.1; H, 4.3%); *m/z* 837 [(M − H)$^-$, negative ion FAB]; δ_H (Table 2); δ_C[$(CD_3)_2CO$–D_2O] 34.2 (C-4), 35.0 (CH_2S), 42.2 (C-4′), 62.8 (CH_2OH), 71.7 (C-3′), 74.5 (C-3), 75.5 (C-2 and -2′), 95.9, 96.2 and 97.9 (C-6, -8 and -6′), 100.5 and 101.8 (C-4a and -4a′), 106.8 and 107.2 (C-8′, B- and B′-ring C-2 and -6), 110.1 (galloyl C-2 and -6), 121.6 (galloyl C-1), 130.8 and 130.9 (B- and B′-ring C-1), 132.7 and 132.8 (B- and B′-ring C-4), 138.8 (galloyl C-4), 145.7 and 146.0 (B- and B′-ring C-3 and -5, galloyl C-3 and -5), 154.7, 156.8, 157.2 and 157.4 (C-5, -7, -8a, -5′, -7′ and -8a′) and 166.7 (CO_2).

A solution of compound **6** (3 mg) in water (1 cm^3) was incubated with tannase (~ 1 mg) for 3 h at room temperature, and was analysed by TLC and HPLC; the R_f 0.78 and t_R 4.98 min, and the R_f 0.22 and t_R 6.99 min data coincided with those of gallic acid and compound **5**, respectively.

4′β-(2-*Hydroxyethylsulfanyl*)*prodelphinidin B-2* 3′-O-*gallate* **7** was obtained as an amorphous powder, $[\alpha]_D^{18}$ +15.4 (*c* 1.1, acetone) (Found: C, 55.8; H, 4.5. $C_{39}H_{34}O_{19}S$ requires C, 55.85; H, 4.1%); *m/z* 837 [(M − H)$^-$, negative ion FAB]; δ_H (Table 2); δ_C[$(CD_3)_2CO$–D_2O] 35.3 (CH_2S), 36.3 (C-4), 41.0 (C-4′), 62.4 (CH_2OH), 73.0 (C-3 and -3′), 73.9 (C-2′), 76.9 (C-2), 95.7, 96.3 and 97.8 (C-6, -8 and -6′), 99.3 and 101.5 (C-4a and -4a′), 106.4 and 106.7 (B- and B′-ring C-2 and -6), 107.8 (C-8′), 110.4 (galloyl C-2 and -6), 121.0 (galloyl C-1), 130.0 and 131.5 (B- and B′-ring C-1), 132.8 and 133.0 (B- and B′-ring C-4), 139.2 (galloyl C-4), 145.7 and 146.2 (B- and B′-ring C-3 and -5, galloyl C-3 and -5), 154.8, 156.3, 157.1, 157.4, 157.7 and 157.9 (C-5, -7, -8a, -5′, -7′ and -8a′) and 166.7 (CO_2).

Hydrolysis of this compound as described for compound **6** yielded gallic acid and compound **5**.

4′β-(2-*Hydroxyethylsulfanyl*)*prodelphinidin B-2* 3,3′-*di*-O-*gallate* **8** was obtained as an amorphous powder, $[\alpha]_D^{18}$ +8.2 (*c* 1.5, acetone) (Found: C, 54.7; H, 4.4. $C_{46}H_{38}O_{23}S{\cdot}H_2O$ requires C, 54.8; H, 4.0%); *m/z* 989 [(M − H)$^-$, negative ion FAB]; δ_H (Table 2); δ_C[$(CD_3)_2CO$–D_2O] 33.5 (C-4), 35.2 (CH_2S), 41.3 (C-4′), 62.5 (CH_2OH), 73.7 (C-3′), 74.2 (C-3 and -2′), 75.9 (C-2), 95.8, 96.4 and 97.8 (C-6, -8 and -6′), 99.5 and 102.4 (C-4a and -4a′), 106.8 (B- and B′-ring C-2 and -6), 107.2 (C-8′), 110.2 and 110.3 (galloyl C-2 and -6), 121.1 and 121.5 (galloyl C-1), 129.9 and 130.8 (B- and B′-ring C-1), 132.9 (B- and B′-ring C-4), 145.7, 146.0 and 146.1 (B- and B′-ring C-3 and -5, galloyl C-3 and -5), 154.5, 156.7, 157.3 and 157.4 (C-5, -7, -8a, -5′, -7′ and -8a′) and 166.5 (CO_2).

Hydrolysis of this compound as described for compound **6** afforded gallic acid and compound **5**.

HPLC Analysis of Thiol-promoted Degradation Products.—Persimmon fruits (55–65 g) were macerated with water (50 cm^3) in a Waring blender, and extracted over a period of 24 h with acetone–water (4:1; 250 cm^3) (three times). After filtration, the combined filtrate was concentrated to 200 cm^3; aliquots (2 cm^3) of the solution were treated with 20% 2-sulfanylethanol in 0.4 mol dm^{-3} HCl (2 cm^3) for 8 h at 50 °C, and then at room temperature for 15 h. The flesh debris remaining on the filter paper after filtration was washed with acetone and air-dried (~ 10 g). A portion (exactly 1%) of the debris was similarly treated with 10% 2-sulfanylethanol in 0.2 mol dm^{-3} HCl (4 cm^3) for 8 h at 50 °C and then at room temperature for 15 h. Aliquots (1 cm^3) of these reaction mixtures were separately passed through a Sep-pak ODS cartridge (Waters Associates, Inc.) with methanol–water (6:4) and adjusted to 5 cm^3; aliquots (10 mm^3) were analysed by HPLC. Removal of astringency by treatment with warm water was achieved at 40 °C for 30 h. For quantitative analysis, compounds **1** (12.9 mg), **2** (11.2 mg), **3** (14.1 mg) and **4** (9.4 mg) were dissolved in water and diluted stepwise; 10 mm^3 was used for calibration. The calibration curve was made from individual peak areas calculated by a Chromatopac C-R5A (Shimadzu) integrator. The analysis was repeated three or four times and the average value was used for quantitative analysis.

Isolation of the Thiol-promoted Degradation Products of Fleshy Debris of De-astringent Fruits.—Astringent fruits (530 g) picked on September 7th were packed into a polyethylene bag with ethanol–water (3:7; 5 cm^3), and kept for 13 days at room temperature. The fruits, after removal of seeds, were macerated with water in a Waring blender, and extracted with acetone–water (7:3; 1.5 dm^3) three times. The flesh debris was collected by filtration, washed with acetone, and dried *in vacuo*, and the brown powder (90 g) was suspended in 5% 2-sulfanylethanol in 0.2 mol dm^{-3} HCl (500 cm^3) and left for 6 days. After filtration, the filtrate, without concentration, was subjected to MCI-gel CHP 20P (7.5 cm i.d. × 35 cm) chromatography, and the column was washed with water (~ 1 dm^3). The sulfides **1** and **3**–**8** were eluted out with methanol–water (1:9–3:7) (fraction 1, 14 g). Elution with methanol–water (2:3) afforded fraction 2 (0.8 g) containing compounds **2**, **9a** and **9b**. Further elution of the column with methanol–water (1:1–7:3) gave fraction 3 (1.5 g). Fraction 2 was chromatographed over Sephadex LH-20 (2 cm i.d. × 25 cm) with ethanol to afford compound **2** (0.3 g) and a mixture of stereoisomers **9a** and **9b**, which were separated by chromatography on Bondapak C_{18}/Porasil B [methanol–water (1:3)] and preparative HPLC to give compounds **9a** (33.8 mg) and **9b** (30.7 mg). Fraction 3 was applied to a column of Sephadex LH-20 (2.0 cm i.d. × 25 cm) with ethanol to give three fractions; 3-1, 3-2 and 3-3. Each fraction was further separated by Bondapak C_{18}/Porasil B chromatography and preparative HPLC to give stereoisomers **13a** (5.3 mg) and **13b** (4.1 mg) (from fraction 3-1), stereoisomers **11a** (7.8 mg) and **11b** (8.8 mg) (from fraction 3-2), and compounds **10a** (25.6 mg), **10b** (28.4 mg), **14a** (0.9 mg) and **14b** (2.6 mg) and a mixture of stereoisomers **12a** and **12b** (13.4 mg) (from fraction 3-3).

(−)-4β-(2-*Hydroxyethylsulfanyl*)-8-[1-(2-*hydroxyethylsulfanyl*)*ethyl*]*epigallocatechin* **9a** was obtained as an amorphous powder, $[\alpha]_D^{20}$ −48.0 (*c* 1.2, acetone) (Found: C, 51.7; H, 5.4. $C_{21}H_{26}O_9S_2$ requires C, 51.8; H, 5.4%); *m/z* 485 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3). The isomer **9b** was also obtained as an amorphous powder, $[\alpha]_D^{20}$ −36.2 (*c* 0.5, MeOH) (Found: C, 51.8; H, 5.5%); *m/z* 485 [(M − H)$^-$,

negative ion FAB]; δ_H (Table 3). Owing to interconversion between these isomers, the ^{13}C NMR data were obtained from a mixture of compounds **9a** and **9b**: δ_C[$(CD_3)_2CO$–D_2O] 20.7 and 21.0 (Me), 34.8, 35.1, 35.3 and 35.5 (CH_2S and CHS), 43.7 (C-4), 61.8, 62.1 and 62.5 (CH_2OH), 71.3 and 71.5 (C-3), 75.1 and 75.3 (C-2), 97.1 and 97.4 (C-6), 100.0 and 100.3 (C-4a), 106.8 (B-ring C-2 and -6), 108.9 (C-8), 131.0 (B-ring C-1), 132.9 (B-ring C-4), 146.2 (B-ring C-3 and -5) and 153.8, 154.2, 156.3, 156.7 and 156.8 (C-5, -7 and -8a).

A mixture of compounds **9a** and **9b** (20 mg) was desulfurized by treatment with Raney nickel (W-4) in 2% ethanolic acetic acid (2 cm^3) for 3 h at room temperature. After removal of reagent by filtration, the mixture was separated by Cosmosil 75 C_{18}-OPN (1.5 cm i.d. × 10 cm) with methanol–water (3:7), and was then further purified by preparative HPLC [mobile phase, methanol–water (1:4)] to yield (−)-8-ethylepigallocatechin **9c** (1.3 mg) as an amorphous powder, $[\alpha]_D^{26}$ −55.7 (*c* 0.1, acetone); *m/z* 333 [(M − H)$^-$, negative ion FAB]; δ_H[$(CD_3)_2CO$–D_2O] 1.07 (3 H, t, *J* 7, Me), 2.61 (2 H, q, *J* 7, CH_2), 2.80 (2 H, m, 4-H), 4.23 (1 H, m, 3-H), 4.81 (1 H, s, 2-H), 6.09 (1 H, s, 6-H) and 6.62 (2 H, s, B-ring 2- and 6-H).

Derivation of isomers **9a** and **9b** from substrate **3** was achieved in the following manner; compound **3** (500 mg) was treated with acetaldehyde (28 mg) and 2-sulfanylethanol (30 mg) in 2% acetic acid (25 cm^3) at room temperature for 10 h. The mixture was chromatographed over MCI-gel CHP 20P with water containing increasing proportions of methanol to give a mixture of compounds **9a** and **9b** (157 mg), which were identified by HPLC and ^1H NMR spectroscopic comparisons.

(−)-4β-(2-*Hydroxyethylsulfanyl*)-8-[1-(2-*hydroxyethylsulfanyl*)*ethyl*]*epigallocatechin* 3-O-*gallate* **10a** was obtained as an amorphous powder, $[\alpha]_D^{20}$ −158.1 (*c* 1.0, MeOH) (Found: C, 51.8; H, 4.8. $C_{28}H_{30}O_{13}S_2 \cdot 1/2H_2O$ requires C, 51.9; H, 4.8%); *m/z* 637 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3); δ_C[$(CD_3)_2CO$–D_2O] 20.6 (Me), 34.6 and 35.3 (CH_2S and CHS), 41.0 (C-4), 62.4 (CH_2OH), 73.5 and 74.0 (C-2 and -3), 97.1 (C-6), 99.4 (C-4a), 106.5 (B-ring C-2 and -6), 108.8 (C-8), 110.0 (galloyl C-2 and -6), 120.7 (galloyl C-1), 130.2 (B-ring C-1), 133.2 (B-ring C-4), 139.2 (galloyl C-4), 145.8 and 146.3 (B-ring C-3 and -5, galloyl C-3 and -5), 154.2, 156.6 and 157.5 (C-5, -7 and -8a) and 166.5 (CO_2).

The isomer **10b** was also obtained as an amorphous powder, $[\alpha]_D^{20}$ −177.0 (*c* 0.9, MeOH) (Found: C, 52.1; H, 4.8%); *m/z* 637 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3); δ_C[$(CD_3)_2CO$–D_2O] 20.9 (Me), 34.6 and 35.3 (CH_2S and CHS), 41.0 (C-4), 62.4 (CH_2OH), 73.5 and 74.3 (C-2 and -3), 98.0 (C-6), 99.0 (C-4a), 106.5 (B-ring C-2 and -6), 108.8 (C-8), 110.0 (galloyl C-2 and -6), 120.7 (galloyl C-1), 129.9 (B-ring C-1), 133.2 (B-ring C-4), 139.2 (galloyl C-4), 145.8 and 146.3 (B-ring C-3 and -5, galloyl C-3 and -5), 153.2, 156.7 and 157.5 (C-5, -7 and -8a) and 166.5 (CO_2). Treatment of a mixture of these compounds (~ 1 mg) with tannase in water afforded the alcohols **9a** and **9b** together with gallic acid, which were identified by HPLC.

(+)-4β-(2-*Hydroxyethylsulfanyl*)-6-[1-(2-*hydroxyethylsulfanyl*)*ethyl*]*epigallocatechin* **11a** was obtained as an amorphous powder, $[\alpha]_D^{16}$ +22.8 (*c* 0.8, MeOH) (Found: C, 51.8; H, 5.5. $C_{21}H_{26}O_9S_2$ requires C, 51.8; H, 5.4%); *m/z* 485 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3); δ_C[$(CD_3)_2CO$–D_2O] 20.6 (Me), 34.9 and 35.2 (CH_2S and CHS), 44.1 (C-4), 61.9 and 62.9 (CH_2OH), 71.9 (C-3), 75.1 (C-2), 96.4 (C-8), 100.5 (C-4a), 106.9 (B-ring C-2 and -6), 109.3 (C-6), 131.0 (B-ring C-1), 133.0 (B-ring C-4), 146.2 (B-ring C-3 and -5) and 155.2, 156.7 and 156.9 (C-5, -7 and -8a).

The isomer **11b** was also obtained as an amorphous powder, $[\alpha]_D^{16}$ −122.7 (*c* 0.9, MeOH) (Found: C, 51.5; H, 5.5%); *m/z* 485 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3); δ_C[$(CD_3)_2CO$–D_2O] 20.3 (Me), 34.8 and 35.3 (CH_2S and CHS), 43.9 (C-4), 61.7 and 62.7 (CH_2OH), 71.8 (C-3), 74.9 (C-2), 96.1 (C-8), 100.6 (C-4a), 106.9 (B-ring C-2 and -6), 109.1 (C-6), 130.9 (B-ring C-1), 133.1 (B-ring C-4), 146.1 (B-ring C-3 and -5) and 155.1, 156.7 and 156.8 (C-5, -7 and -8a).

The isomers of (−)-4β-(2-*hydroxyethylsulfanyl*)-6-[1-(2-*hydroxyethylsulfanyl*)*ethyl*]*epigallocatechin* 3-O-*gallate* **12a** and **12b** could not be separated, and hence the following physical and spectral data were of the mixture which was obtained as an amorphous powder, $[\alpha]_D^{20}$ −77.7 (*c* 0.5, MeOH) (Found: C, 52.5; H, 4.8. $C_{28}H_{30}O_{13}S_2$ requires C, 52.7; H, 4.7%); *m/z* 637 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3); δ_C[$(CD_3)_2CO$–D_2O] 20.3 and 20.5 (Me), 34.9, 35.5 and 35.6 (CH_2S and CHS), 41.3 (C-4), 61.8 and 62.7 (CH_2OH), 73.9 (C-2 and -3), 96.3 (C-8), 99.5 (C-4a), 106.6 (B-ring C-2 and -6), 110.0 (C-6, galloyl C-2 and -6), 120.9 (galloyl C-1), 129.8 (B-ring C-1), 133.2 (B-ring C-4), 139.3 (galloyl C-4), 146.0 and 146.4 (B-ring C-3 and -5, galloyl C-3 and -5), 154.9 and 157.1 (C-5, -7 and -8a) and 166.8 (CO_2).

Hydrolysis of compounds **12a** and **12b** (~ 1 mg) with tannase gave a mixture of compounds **11a** and **11b**, and gallic acid which were identified by HPLC.

(−)-4β-(2-*Hydroxyethylsulfanyl*)-8-[1-(2-*hydroxyethylsulfanyl*)*ethyl*]*epicatechin* **13a** was obtained as an amorphous powder, $[\alpha]_D^{20}$ −29.5 (*c* 0.3, MeOH) (Found: C, 49.1; H, 5.5. $C_{21}H_{26}O_8S_2$ requires C, 49.1; H, 5.4%); *m/z* 469 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3).

The isomer **13b** was also obtained as an amorphous powder, $[\alpha]_D^{20}$ −63.8 (*c* 0.6, MeOH); *m/z* 469 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3). These compounds were derived from compound **1**: treatment of compound **1** (10 mg) with 0.1% acetaldehyde (1 cm^3) and 1% 2-sulfanylethanol (1 cm^3) gave a mixture of compounds **13a** and **13b** which were identified by HPLC.

(−)-4β-(2-*Hydroxyethylsulfanyl*)-8-[1-(2-*hydroxyethylsulfanyl*)*ethyl*]*epicatechin* 3-O-*gallate* **14a** was obtained as an amorphous powder, δ_H (Table 3). Owing to the small crop, other data for this compound could not be measured.

The *isomer* **14b** was also obtained as an amorphous powder, $[\alpha]_D^{20}$ −98.4 (*c* 0.4, MeOH) (Found: C, 53.9; H, 5.2. $C_{28}H_{30}O_{12}S_2$ requires C, 54.0; H, 4.9%); *m/z* 621 [(M − H)$^-$, negative ion FAB]; δ_H (Table 3).

Hydrolysis of compound **14a** with tannase yielded gallic acid and a mixture of alcohols **13a** and **13b** which were identified by HPLC.

Thiol-promoted Degradation of the Gel Formed by Treatment of Extract with Acetaldehyde.—Astringent fruit (480 g) was macerated in a mechanical mixer and extracted with acetone–water (7:3; 1.5 dm^3) three times. The extract was concentrated to give an aqueous solution (650 cm^3), a portion (80 cm^3) of which was mixed with 5% acetaldehyde (20 cm^3) and kept for 10 h at room temperature. The gel thus formed was stirred with 10% 2-sulfanylethanol in 0.5 mol dm^{-3} HCl (100 cm^3) for 2 days at room temperature. After filtration, the filtrate was applied to a column of MCI-gel CHP 20P (3 cm i.d. × 25 cm) with water containing increasing proportions of methanol. Elution of 10–30% methanol afforded fractions containing sulfides **1** and **3**–**8**. Fractions eluted with 40–60% methanol were collected and further separated by Sephadex LH-20 (2.0 cm i.d. × 25 cm) chromatography with ethanol to give compound **2** and mixtures of stereoisomers **9a** and **9b** (8 mg) and **11a** and **11b** (15 mg), which were identified by HPLC and ^1H NMR spectroscopic comparison.

Removal of Astringency by Treatment with Deuteriated Ethanol.—Astringent fruit (500 g) was packed into a polyethylene bag with 30% aq. [$^2H_6$]ethanol (5 cm^3) and the mixture was left for 10 days at room temperature. The fruit was macerated and extracted as described above, and the flesh

debris was collected by filtration. The debris was treated with 5% 2-sulfanylethanol in 0.2 mol dm^{-3} (1 dm^3) for 3 days at room temperature, and the filtrate, after removal of the debris, was subjected to chromatography on MCI-gel CHP 20P (7.5 cm i.d. × 35 cm) with water–methanol (1:0–1:4). The fractions eluted with 40–60% methanol were collected and rechromatographed over Sephadex LH-20 (2.5 cm i.d. × 25 cm) with ethanol, and purification on preparative HPLC afforded compounds **9d** (1.3 mg) and **9e** (4.3 mg). (−)-4β-(2-hydroxyethylsulfanyl)-8-[1,2,2,2-tetradeuterio-1-(2-hydroxyethylsulfanyl)ethyl]epigallocatechin **9d** was obtained as an amorphous powder, $[\alpha]_D^{19}$ −6.9 (*c* 0.1, MeOH); *m/z* 489 [$(M - H)^-$, 0.26%, negative ion FAB] and 485 (0.29). The ^1H NMR spectrum [$(CD_3)_2CO$–D_2O] was essentially identical with that of unlabelled compound **9a**, except for the integration of the peaks at δ_H 1.59 (3/2H, d, *J* 7, Me) and 4.72 (1/2H, q, *J* 7, CH). The isomer **9e** was also obtained as an amorphous powder, $[\alpha]_D^{20}$ −85.7 (*c* 0.4, MeOH); *m/z* 489 [$(M - H)^-$, 0.43%, negative ion FAB] and 485 (0.42); δ_H[$(CD_3)_2CO$–D_2O] 1.59 (3/2H, d, *J* 7, Me) and 4.71 (1/2H, q, *J* 7, CH). The remaining part of the spectrum was identical with that of unlabelled analogue **9b**.

Treatment of (−)-*Epigallocatechin* 3-O-*Gallate with Acetaldehyde*.—A solution of epigallocatechin 3-*O*-gallate (2.0 g, 4.3 mmol) and acetaldehyde (320 mg, 7.2 mmol) in 2% acetic acid (50 cm^3) was stirred for 40 h at room temperature. The complex mixture formed was directly applied to a column of Bondapak C_{18}/Porasil B (3 cm i.d. × 35 cm) with water containing increasing proportions of methanol to give two fractions, 1 (506 mg) and 2 (347 mg), containing major products, together with the recovery of starting material (320 mg). A portion (50–80 mg) of each fraction was subjected to preparative HPLC to give (−)-6,6′-(*ethane*-1,1-*diyl*)*diepigallocatechin* 3,3′-*di*-O-*gallate* **15** (30 mg) (from fraction 2) and isomers of (−)-6,8′-(*ethane*-1,1-*diyl*)*diepigallocatechin* 3,3′-*di*-O-*gallate* **16a** (5.6 mg) and **16b** (8.9 mg) (from fraction 1). *Compound* **15** was obtained as an amorphous powder, $[\alpha]_D^{26}$ −123.4 (*c* 1.6, acetone) (Found: C, 56.0; H, 4.2. $C_{46}H_{38}O_{22}$·5/2H_2O requires C, 55.9; H, 4.4%); *m/z* 941 [$(M - H)^-$, negative ion FAB]; δ_H (Table 2); δ_C[$(CD_3)_2CO$–D_2O] 18.0 (Me), 26.4 (CH), 27.3 (C-4 and -4′), 69.2 (C-3 and -3′), 78.1 (C-2 and -2′), 96.4 (C-8 and -8′), 101.6 (C-4a and -4a′), 106.8 (B- and B′-ring C-2 and -6), 110.0 (galloyl C-2 and -6), 111.9 (C-6 and -6′), 121.8 (galloyl C-1), 130.6 (B- and B′-ring C-1), 133.2 (B- and B′-ring C-4), 138.9 (galloyl C-4), 145.9 and 146.3 (B- and B′-ring C-3 and -5, galloyl C-3 and -5), 153.5, 155.1 and 155.3 (C-5, -7, -8a, -5′, -7′ and -8a′) and 166.0 (CO_2).

Compound **16a** was obtained as an amorphous powder, $[\alpha]_D^{26}$ −137.5 (*c* 0.7, acetone) (Found: C, 56.1; H, 4.4%); *m/z* 941 [$(M - H)^-$, negative ion FAB]; δ_H (Table 2); δ_C[$(CD_3)_2CO$–D_2O] 19.0 (Me), 26.0 (CH), 26.9 and 27.2 (C-4 and -4′), 69.0 and 69.5 (C-3 and -3′), 78.0 and 79.4 (C-2 and -2′), 97.0 and 97.9 (C-8 and -6′), 100.1 and 100.9 (C-4a and -4a′), 106.8 and 107.0 (B- and B′-ring C-2 and -6), 110.0 and 110.2 (galloyl C-2 and -6), 110.4 (C-8′), 111.7 (C-6′), 121.9 (galloyl C-1), 129.8 and 130.7 (B- and B′-ring C-1), 133.1 and 133.6 (B- and B′-ring C-4), 138.9 (galloyl C-4), 145.8, 145.9, 146.2 and 146.4 (B-and B′-ring C-3 and -5, galloyl C-3 and -5), 153.0, 154.8, 154.9, 155.1 and 155.5 (C-5, -7, -8a, -5′, -7′ and -8a′) and 166.1 (CO_2). *Compound* **16b** was obtained as an amorphous powder, $[\alpha]_D^{26}$ −156.9 (*c* 0.9, acetone) (Found: C, 57.1; H, 4.4. $C_{46}H_{38}O_{22}$·3/2H_2O requires C, 57.0; H, 4.3%); *m/z* 941 [$(M - H)^-$, negative ion FAB]; δ_H (Table 2); δ_C[$(CD_3)_2CO$–D_2O] 19.3 (Me), 26.0 (CH), 27.1 and 27.2 (C-4 and -4′), 68.9 and 69.5 (C-3 and -3′), 78.0 and 79.9 (C-2 and -2′), 96.9 and 97.8 (C-8 and -6′), 100.4 and 101.0 (C-4a and -4a′), 106.8 and 107.3 (B- and B′-ring C-2 and -6), 110.0 and 110.2 (galloyl C-2 and -6), 110.4 (C-8′), 111.7 (C-6′), 121.8 and 121.9 (galloyl C-1), 129.6 and 130.7 (B- and B′-ring C-1), 133.2 and 133.7 (B- and B′-ring C-4), 138.8 and 138.9 (galloyl C-4), 145.8, 145.9, 146.3 and 146.5 (B- and B′-ring C-3 and -5, galloyl C-3 and -5), 153.7, 154.6, 154.7, 154.9, 155.2 and 155.5 (C-5, -7, -8a, -5′, -7′ and -8a′) and 166.0 and 166.2 (CO_2).

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