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Tannins and Related Compounds. XLVII.[1] Rhubarb. (6). Isolation and Characterization of New *p*-Hydroxyphenylbutanones, Stilbenes and Gallic Acid Glucosides

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Along with the previously reported phenylbutanone glucosides, *i.e.*, lindleyin (**7**), isolindleyin (**8**) and 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-*β*-D-glucopyranoside (**9**), three new related compounds (**1, 2** and **3**) have been isolated from a rhubarb of high quality (commercial name: 長吉黄). The structures of **1**—**3** were established on the basis of chemical and spectroscopic data to be 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-*β*-D-(2′′,6′′-di-*O*-galloyl)-glucopyranoside (**1**), 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-*β*-D-(2′′-*O*-galloyl-6′′-*O*-cinnamoyl)-glucopyranoside (**2**) and 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-*β*-D-(2′′-*O*-galloyl-6′′-*O*-*p*-coumaroyl)-glucopyranoside (**3**). In addition, 3,4′,5-trihydroxystilbene 4′-*O*-*β*-D-(2′′-*O*-galloyl)-glucopyranoside (**4**), gallic acid 3-*O*-*β*-D-glucopyranoside (**5**) and gallic acid 4-*O*-*β*-D-glucopyranoside (**6**) have been newly isolated.

Keywords——rhubarb; *Rheum* sp.; Polygonaceae; phenylbutanone; stilbene; gallic acid glucoside; gallate; cinnamate; *p*-coumaroate

As a result of the chemical examination of Gao (雅黄), which is one of the common rhubarbs in Japan and China, we previously isolated two *p*-hydroxyphenylbutanone glucoside gallates, lindleyin[2] and isolindleyin,[3] which may be responsible for the analgesic and anti-inflammatory action of rhubarb. Further chemical work on various commercial Chinese rhubarbs has now resulted in the isolation, from a rhubarb of high quality (commercial name: 長吉黄), of three additional phenylbutanone glucoside gallates (**1**—**3**), together with a new stilbene glucoside gallate (**4**) and two gallic acid glucosides (**5** and **6**). In this paper, we wish to present details of the isolation and characterization of these compounds.

By a combination of Sephadex LH-20, MCI-gel CHP 20P, Avicel cellulose, Bondapak C_{18}/Porasil B, Fuji-gel ODS G3 and silica gel chromatographies, compounds **1**—**10** were isolated from the 80% aqueous acetone extract. Among these compounds, **7**—**10** were identified as previously reported lindleyin (**7**),[2] isolindleyin (**8**),[3] 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-*β*-D-glucopyranoside (**9**),[2] and 3,4′,5-trihydroxystilbene 4′-*O*-*β*-D-(6′′-*O*-galloyl)-glucopyranoside (**10**)[4] by comparisons of their physical and spectral data with those of authentic samples.

Compound **1**, an off-white amorphous powder, $[\alpha]_D$ $-21.2°$ (acetone), $C_{30}H_{30}O_{15}$ $3/2H_2O$, was strongly positive to the ferric chloride reagent (a dark blue coloration). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** revealed the presence of an acetyl group (δ 2.07, 3H, s), two methylenes (δ 2.72, 4H, m) and an aromatic ring with a *p*-substitution system (δ 6.87 and 7.04, each 2H, d, $J = 8$ Hz); these features correspond well to those of *p*-hydroxyphenyl-2-butanone. The spectrum also showed signals due to two galloyl groups (δ 7.12 and 7.26, each 2H, s) and sugar protons (δ 3.2—5.4). On enzymatic hydrolysis with tannase, **1** yielded gallic acid and the hydrolysate, which was shown to be identical with 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-*β*-D-glucopyranoside (**9**) by comparison of the physical and ^{1}H-NMR data with those of an authentic sample obtained previously.[2]

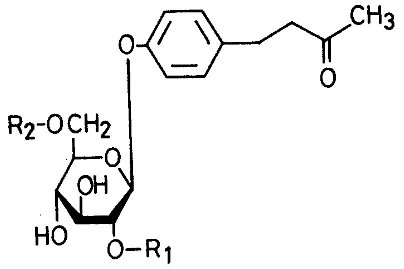
The ^{1}H-NMR spectrum of **1** exhibited deshielded signals [δ 4.40 (1H, dd, $J=6$, 12 Hz) and 4.72 (1H, dd, $J=2$, 12 Hz)] assigned by analogy with those of lindleyin (**7**) to the sugar C-6 methylene protons, implying that one of the two galloyl groups was present at the C-6 position of the glucose moiety. Furthermore, the appearance of two overlapped methine signals at δ 5.12—5.36, the chemical shifts and the multiplicity being similar to those of the C-1 and C-2 proton signals found in isolindleyin (**8**), suggested that another galloyl group was located at the glucose C-2 position. The above observations were supported by the results of carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy, which showed a downfield shift (+2.5 ppm) of the glucose C-6 methylene signal and an upfield shift (−3.0 ppm) of the C-5 methine signal as compared with those of **8**. The positions of the galloyl groups were determined unequivocally to be the C-2 and C-6 positions of the glucose moiety; on ordinary phenol methylation, **1** gave the hexamethylate (**1a**), colorless needles, mp 196—197 °C, $[\alpha]_D$ $-61.5°$ ($CHCl_3$), $C_{36}H_{42}O_{15}$, which was subsequently methylated by the Kuhn method to

TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**—**3**, **2a**, **3a**, **7** and **8**

		1$^{a)}$	**2**$^{a)}$	**2a**$^{a)}$	**3**$^{a)}$	**3a**$^{b)}$	**7**$^{b)}$	**8**$^{a)}$
Aglycone	C-1	29.3	29.3	29.4	29.3	28.1	28.1	29.2
	C-2	210.0	209.4	208.9	209.5	208.2	207.7	209.3
	C-3	29.3	29.3	29.4	29.3	29.5	29.6	29.2
	C-4	45.2	45.2	45.3	45.2	44.1	43.9	45.3
	C-1′	136.2	136.3	135.8	136.2	134.3	134.0	136.3
	C-2′	130.0 (2C)	129.9 (2C)	129.9 (2C)	131.0 (2C)	128.9 (2C)	128.6 (2C)	130.0 (2C)
	C-3′	117.4 (2C)	117.5 (2C)	117.4 (2C)	117.5 (2C)	116.1 (2C)	115.7 (2C)	117.5 (2C)
	C-4′	156.3	156.3	156.8	156.3	155.2	154.9	156.6
Glucose	C-1	100.0	100.0	101.7	100.0	100.2	100.1	100.3
	C-2	75.0	74.9	74.3	74.9	73.0	72.8	74.7
	C-3	75.4	75.4	77.5	75.4	86.1	76.0	75.5
	C-4	71.5	71.5	71.3	71.5	70.0	69.7	71.2
	C-5	74.6	74.5	74.8	74.6	73.6	73.5	77.6
	C-6	64.6	64.4	64.6	64.2	63.3	63.0	62.1
Galloyl	C-1	121.1 (2C)	121.2		121.2		119.1	121.3
	C-2	110.0 (4C)	110.0 (2C)		110.0 (2C)		108.4 (2C)	110.0 (2C)
	C-3	145.9 (2C) 146.0 (2C)	145.9 (2C)		145.9 (2C)		145.0 (2C)	145.9 (2C)
	C-4	139.1 (2C)	139.1		139.1		137.9	138.9
Cinnamoyl	C-1		135.0	135.2				
	C-2		129.0 (2C)	129.1 (2C)				
	C-3		129.9 (2C)	129.9 (2C)				
	C-4		131.4	131.3				
	C-α,β		118.5 145.9	118.8 145.7				
p-Coumaroyl	C-1				126.5	125.0		
	C-2				129.9 (2C)	130.3 (2C)		
	C-3				116.7 (2C)	115.7 (2C)		
	C-4				160.8	159.6		
	C-α,β				114.8 146.1	114.0 144.8		
	–COO–	166.6 167.3	166.5 167.5	167.3	166.5 167.9	166.4	165.3	166.4

a) Measured in acetone-d_6+D_2O. *b)* Measured in DMSO-d_6+D_2O.

yield the permethylate (**1b**), a white amorphous powder, $[\alpha]_D$ $-8.1°$ ($CHCl_3$). Alkaline hydrolysis of **1b**, followed by treatment with methanolic hydrochloric acid, afforded a methyl sugar which was found to be methyl 3,4-di-*O*-methyl glucopyranoside. Accordingly, **1** was characterized as 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-β-D-(2″,6″-di-*O*-galloyl)-glucopyranoside.



	R_1	R_2
1	G	G
2	G	cin
2a	H	cin
3	G	*p*-coum
3a	H	*p*-coum
7	H	G
8	G	H
9	H	H

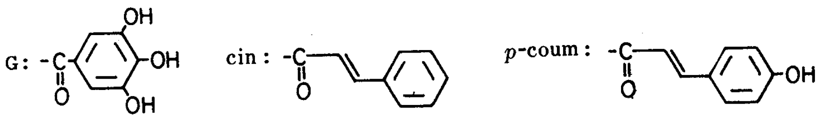


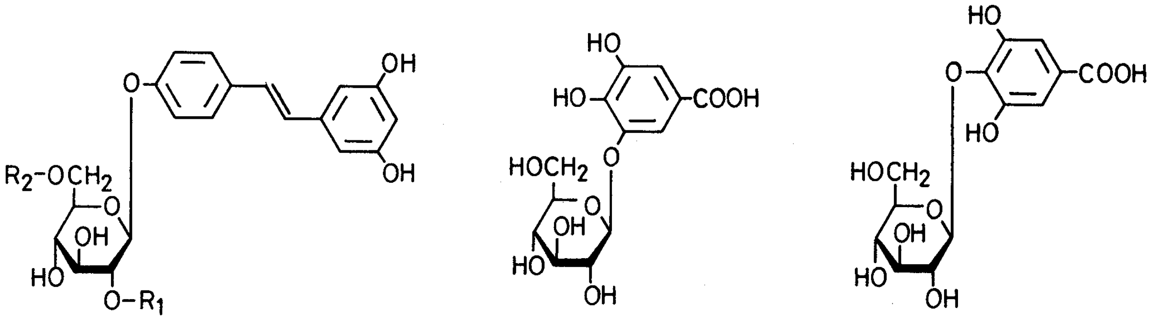
Chart 1

Compound **2**, colorless needles, mp 125—127 °C, $[\alpha]_D$ $-34.0°$ (acetone), $C_{32}H_{32}O_{12}$, and compound **3**, colorless needles, mp 134—136 °C, $[\alpha]_D$ $-38.2°$ (acetone), $C_{32}H_{32}O_{13} \cdot H_2O$, showed blue colorations with the ferric chloride reagent. The ^{1}H-NMR spectra of **2** and **3** were closely correlated with that of **1** in showing signals arising from a *p*-hydroxyphenylbutanone and a sugar moiety. However, in the case of **2**, *trans*-coupled olefinic signals (δ 6.59 and 7.71, each 1H, d, $J=16$ Hz) and aromatic multiplets (δ 7.36—7.78, m) corresponding to five protons were readily discerned, together with one galloyl peak (δ 7.14, 2H, s), while the spectrum of **3** showed the presence of a *p*-substituted aromatic ring (δ 6.90 and 7.56, each 2H, d, $J=8$ Hz), a *trans* olefin (δ 6.40 and 6.64, each 1H, d, $J=16$ Hz) and a galloyl group (δ 7.16, 2H, s). These ^{1}H-NMR observations suggested that one of the two galloyl groups in **1** was replaced by a cinnamoyl group and by a *p*-coumaroyl group in **2** and **3**, respectively. In the ^{13}C-NMR spectra of **2** and **3**, the chemical shifts for the sugar carbons were quite consistent with those observed in **1**, suggesting that the acyl groups were attached to the C-2 and C-6 positions of the glucose moiety.

Hydrolyses of **2** and **3** with tannase furnished gallic acid and partial hydrolysates (**2a**, colorless needles, mp 148—149 °C, $[\alpha]_D$ $-37.7°$ (acetone), $C_{25}H_{28}O_8 \cdot H_2O$, and **3a**, colorless needles, mp 149—150 °C, $[\alpha]_D$ $-14.4°$ (70% aq. acetone), $C_{25}H_{28}O_9 \cdot H_2O$, respectively). Subsequent alkaline methanolyses of **2a** and **3a** with sodium methoxide in methanol yielded methyl cinnamate and methyl *p*-coumaroate, respectively, together with the phenylbutanone glucoside (**9**). The locations of the cinnamoyl and *p*-coumaroyl esters in **2a** and **3a** were determined in both cases to be the C-6 position of the glucose moiety from the downfield shift of the glucose C-6 methylene signals in the ^{1}H- and ^{13}C-NMR spectra: δ 4.41 (1H, dd, $J=6$, 12 Hz), 4.65 (1H, dd, $J=2$, 12 Hz); δ 64.6 in **2a** and δ 4.19 (1H, dd, $J=6$, 12 Hz), 4.42 (1H, dd, $J=2$, 12 Hz); δ 63.3 in **3a**. On the basis of these findings, the structures of **2** and **3** were established as 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-β-D-(2″-*O*-galloyl-6″-*O*-cinnamoyl)-glucopyranoside and 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-β-D-(2″-*O*-galloyl-6″-*O*-*p*-coumaroyl)-glucopyranoside, respectively.

Compound **4**, a white amorphous powder, $[\alpha]_D$ $+25.2°$ (acetone), $C_{27}H_{26}O_{12} \cdot H_2O$, was shown to be structurally related to **10** by ^{1}H-NMR spectral comparison, which showed signals ascribable to a galloyl group (δ 7.14, 2H, s), A_2B_2-type aromatic protons (δ 6.94 and 7.42, each

2H, d, $J=8$ Hz), *trans*-coupled olefinic protons (δ 6.86 and 7.02, each 1H, d, $J=16$ Hz) and AB_2-type aromatic protons [δ 6.28 (1H, t, $J=2$ Hz) and 6.54 (2H, d, $J=2$ Hz)], together with sugar signals (δ 3.6—5.4). Enzymatic hydrolysis of **4** with tannase gave gallic acid and 3,4′,5-trihydroxystilbene 4′-*O*-β-D-glucopyranoside.[4] In the ^{1}H- and ^{13}C-NMR spectra of **4**, the signal patterns of the glucose moiety were almost the same as those observed in **8**, indicating the location of the galloyl group to be the C-2 position of the glucose moiety. From these observations, **4** was concluded to be 3,4′,5-trihydroxystilbene 4′-*O*-β-D-(2″-*O*-gallyl)-glucopyranoside.



	R_1	R_2
4	G	H
10	H	G

5

6

Chart 2

Compound **5**, colorless granules, mp 244—245 °C, $[\alpha]_D$ $-73.6°$ (MeOH), $C_{13}H_{16}O_{10}$, and compound **6**, colorless needles, mp 192—194 °C, $[\alpha]_D$ $-13.7°$ (MeOH), $C_{13}H_{16}O_{10} \cdot 1/2H_2O$, exhibited anomeric signals (δ 4.90, d, $J=7$ Hz, in **5** and δ 4.70, d, $J=7$ Hz; δ 107.2 in **6**) in the ^{1}H- and ^{13}C-NMR spectra, the chemical shifts of which were consistent with a glycosidic nature. Acid hydrolyses of **5** and **6** to yield gallic acid and glucose confirmed their constitution. The ^{1}H-NMR spectrum of **5** exhibited two *meta*-coupled aromatic resonances (δ 7.30 and 7.42, each 1H, d, $J=2$ Hz), indicating an unsymmetrical molecule. Thus, the glucosyl residue was assumed to be attached to the C-3 hydroxyl group of gallic acid. On the other hand, a symmetrical signal pattern (δ 7.08, 2H, s) of the gallic acid moiety in the ^{1}H-NMR spectrum of **6** suggested that the glucosyl residue was located at the C-4 hydroxyl group of gallic acid. Confirmation of the structures of **5** and **6** was obtained by comparison of the physical and spectral data with those of authentic samples[2] previously prepared by tannase hydrolysis of the corresponding 6′-*O*-gallates. Consequently, **5** and **6** were assigned as gallic acid 3-*O*-β-D-glucopyranoside and gallic acid 4-*O*-β-D-glucopyranoside, respectively.

In conclusion, the contents of lindleyin and isolindleyin in this rhubarb were found to be the highest among rhubarbs so far examined, indicating that this rhubarb has excellent quality when utilized as an analgesic and anti-inflammatory drug. In addition, since compounds **1**—**3** are structurally related to lindleyin and isolindleyin, similar analgesic and anti-inflammatory activities might be expected.

Experimental

The instruments and chromatographic conditions used throughout this work were the same as described in the preceding paper[1b] except for the following. Avicel micro-crystalline cellulose (Funakoshi) was used for cellulose column chromatography.

Isolation——Details of fractionation of the 80% aqueous acetone extract of commercial rhubarb (長吉黄) (3.2 kg) were described in the previous paper,[1b] and the following fraction numbers correspond to those given in that paper.

Fraction I was chromatographed over MCI-gel CHP 20P [solvent: H_2O–MeOH (1:0—2:3)] and then over Sephadex LH-20 (solvent: acetone) to afford two fractions (I-1 and I-2). Fraction I-1 was purified by silica gel chromatography [solvent: AcOEt–Acetone–H_2O (5:2:0.2)] to give the *p*-hydroxyphenylbutanone glucoside (**9**)2) (7.6 g). Chromatographies of fraction II-1 over Sephadex LH-20 [solvent: H_2O–MeOH (1:0—4:1)] and MCI-gel CHP 20P [solvent: H_2O–MeOH (1:0—4:1)] yielded **5** (280 mg) and **6** (460 mg). Fraction IV was subjected to chromatography over MCI-gel CHP 20P [solvent: H_2O–MeOH (4:1—0:1)] to give four further fractions (IV-1—IV-4). Fraction IV-2 was a mixture of lindleyin and isolindleyin, which were separated successfully by chromatography over Avicel cellulose (solvent: 2% AcOH) to give isolindleyin (**8**)3) (5.8 g) and lindleyin (**7**)2) (3.2 g). Fraction IV-3, consisting of a mixture of stilbene gallates, was chromatographed over Sephadex LH-20 (solvent: EtOH) to furnish **4** (110 mg) and 3,4′,5-trihydroxystilbene 4′-*O*-*β*-D-(6″-*O*-galloyl)-glucopyranoside (**10**)4) (6.5 g). Fraction V (160 g) was fractionated by chromatography over Sephadex LH-20 (solvent: EtOH) to afford five fractions (V-1—V-5). Subsequent chromatography of fraction V-1 over MCI-gel CHP 20P [solvent: H_2O–MeOH (3:2—1:4)] gave two fractions, V-1a and V-1b, containing **2** and **3**, respectively, which were purified individually by chromatography over Sephadex LH-20 (solvent: 60% aq. MeOH) (yields; 4.0 and 7.9 g, respectively). Chromatography of fraction V-2 over Sephadex LH-20 (solvent: 80% aq. MeOH) gave **1** (920 mg).

Compound 1——An off-white amorphous powder, $[\alpha]_D^{27}$ −21.2° (c=0.94, acetone). *Anal.* Calcd for $C_{30}H_{30}O_{15}\cdot 3/2H_2O$: C, 55.01; H, 5.06. Found: C, 55.01; H, 5.12. Field desorption mass spectrum (FD-MS) *m/z*: 669 $[M+K]^+$, 653 $[M+Na]^+$, 630 $[M]^+$, 517 $[M+K+H-\text{galloyl}]^+$, 501 $[M+Na+H-\text{galloyl}]^+$, 164. ^{1}H-NMR (acetone-d_6+D_2O): 2.07 (3H, s, $COCH_3$), 2.72 (4H, m, 3, 4-H), 3.5—4.1 (3H, m, sugar-H), 4.40 (1H, dd, J=6, 12 Hz, 6″-H), 4.72 (1H, dd, J=2, 12 Hz, 6″-H), 5.12—5.38 (2H, m, 1″, 2″-H), 6.87, 7.04 (each 2H, d, J=8 Hz, 2′, 3′-H), 7.14, 7.20 (each 2H, s, galloyl-H). ^{13}C-NMR: Table I.

Tannase Hydrolysis of 1——An aqueous solution of **1** (80 mg) was incubated with tannase at room temperature for 2 h. The solvent was evaporated off under reduced pressure, and the residue was treated with EtOH. The EtOH-soluble portion was subjected to chromatography over Sephadex LH-20. Elution with EtOH yielded 4-(4′-hydroxyphenyl)-2-butanone 4′-*O*-*β*-D-glucopyranoside (**9**) (33 mg) as colorless needles (AcOEt), mp 110—112 °C, $[\alpha]_D^{27}$ −50.6° (c=0.5, MeOH). Further elution with EtOH afforded gallic acid (35 mg).

Methylation of 1——A mixture of **1** (100 mg), dimethyl sulfate (0.7 ml) and anhydrous potassium carbonate (1 g) in dry acetone (20 ml) was refluxed for 4 h with stirring. After removal of inorganic salts by filtration, the filtrate was concentrated, and the residue was chromatographed over silica gel. Elution with benzene–acetone (4:1) furnished the hexamethyl ether (**1a**) (75 mg) as colorless needles ($CHCl_3$–MeOH), mp 196—197 °C, $[\alpha]_D^{27}$ −61.5° (c=0.49, $CHCl_3$). *Anal.* Calcd for $C_{36}H_{42}O_{15}$: C, 60.50; H, 5.92. Found: C, 60.51; H, 5.95. ^{1}H-NMR (acetone-d_6+D_2O): 2.10 (3H, s, $COCH_3$), 2.69 (4H, m, 3, 4-H), 3.90, 3.95, 3.80 (18H in total, s, 6×OCH_3), 3.6—4.1 (3H, m, sugar-H), 4.52 (1H, dd, J=6, 12 Hz, 6″-H), 4.85 (1H, dd, J=2, 12 Hz, 6″-H), 6.84, 6.92 (each 2H, d, J=8 Hz, 2′, 3′-H), 7.36 (4H, s, 2×galloyl-H).

Permethylation of 1a——A mixture of **1a** (60 mg), methyl iodide (3 ml) and freshly prepared silver oxide (0.9 g) in dimethylformamide (3 ml) was stirred for 4.5 h with ice-cooling. The reaction mixture was diluted with $CHCl_3$, and the resulting precipitate was filtered off. The filtrate was evaporated to dryness, and the residue was chromatographed over silica gel using *n*-hexane–AcOEt (3:2) to yield the permethylate (**1b**) (16 mg) as a white amorphous powder, $[\alpha]_D^{27}$ −8.1° (c=0.61, $CHCl_3$). ^{1}H-NMR ($CHCl_3$): 2.12 (3H, s, $COCH_3$), 2.58—2.82 (4H, m, 3, 4-H), 3.55, 3.62, 3.89, 3.91, 3.93 (24H in total, each s, OCH_3), 3.3—3.9 (3H, m, sugar-H), 4.44 (1H, dd, J=6, 12 Hz, 6″-H), 4.72 (1H, dd, J=2, 12 Hz, 6″-H), 5.07 (1H, d, J=8 Hz, anomeric-H), 5.40 (1H, t, J=8 Hz, 2″-H), 6.82, 6.93 (each 2H, d, J=8 Hz, 2′, 3′-H), 7.31, 7.33 (each 2H, s, galloyl-H).

Methanolysis of 1b——**1b** (15 mg) was refluxed in 1% methanolic potassium hydroxide (5 ml) for 1 h. The mixture was neutralized with 1% methanolic hydrochloric acid, and the solution was concentrated to dryness under reduced pressure. The residue was treated with MeOH. The MeOH-soluble portion was passed through a Sephadex LH-20 column using MeOH to give a hydrolysate mixture, which was refluxed in 1 N methanolic hydrochloric acid for 4 h. The reaction mixture was neutralized with 5% methanolic potassium hydroxide, concentrated, and passed through a Sephadex LH-20 column (MeOH). The fraction containing a methyl sugar was directly analyzed by thin layer chromatography (TLC) to detect a spot of *Rf* 0.38 [solvent: benzene–acetone (2:3)] corresponding to methyl 3,4-di-*O*-methyl glucopyranoside (*cf.* methyl 4,6-di-*O*-methyl glucopyranoside, *Rf* 0.34; methyl 3,6-di-*O*-methyl glucopyranoside, *Rf* 0.41; methyl 2,6-di-*O*-methyl glucopyranoside, *Rf* 0.27; methyl 2,4-di-*O*-methyl glucopyranoside, *Rf* 0.34; methyl 2,3-di-*O*-methyl glucopyranoside, *Rf* 0.36).

Compound 2——Colorless needles, mp 125—127 °C, $[\alpha]_D^{26}$ −34.0° (c=0.98, acetone). *Anal.* Calcd for $C_{32}H_{32}O_{12}$: C, 63.15; H, 5.30. Found: C, 62.80; H, 5.15. FD-MS *m/z*: 647 $[M+K]^+$, 631 $[M+Na]^+$, 609 $[M+H]^+$, 445, 164. ^{1}H-NMR (acetone-d_6+D_2O): 2.04 (3H, s, $COCH_3$), 2.64 (4H, m, 3, 4-H), 3.5—4.1 (3H, m, sugar-H), 4.41 (1H, dd, J=6, 12 Hz, 6″-H), 4.65 (1H, dd, J=2, 12 Hz, 6″-H), 5.12—5.36 (2H, m, 1″, 2″-H), 6.59, 7.71 (each 1H, d, J=16 Hz, olefinic-H), 6.86, 7.01 (each 2H, d, J=8 Hz, 2′, 3′-H), 7.14 (2H, s, galloyl-H), 7.36—7.78 (5H, m, aromatic-H). ^{13}C-NMR: Table I.

Tannase Hydrolysis of 2——An aqueous solution of **2** (120 mg) was treated with tannase at room temperature for 1 h. The solvent was evaporated off, and the residue was treated with H_2O. The H_2O-soluble portion was chromatographed over Sephadex LH-20 (solvent: EtOH) to give gallic acid. The H_2O-insoluble portion was purified by silica gel chromatography using AcOEt–acetone–H_2O (10 : 1 : 0.1) to furnish the hydrolysate (**2a**) (76 mg) as colorless needles (dil. MeOH), mp 148—149 °C, $[\alpha]_D^{26}$ $-37.7°$ ($c=0.88$, acetone). *Anal.* Calcd for $C_{25}H_{28}O_8 \cdot H_2O$: C, 63.14; H, 6.57. Found: C, 62.99; H, 6.29. ^{1}H-NMR (acetone-d_6 + D_2O): 2.05 (3H, s, $COCH_3$), 2.65 (4H, m, 3, 4-H), 3.4—4.0 (4H, m, sugar-H), 4.37 (1H, dd, $J=6$, 12 Hz, 6''-H), 4.61 (1H, dd, $J=2$, 12 Hz, 6''-H), 4.96 (1H, d, $J=8$ Hz, anomeric-H), 6.58, 7.72 (each 1H, d, $J=16$ Hz, olefinic-H), 6.96, 7.08 (each 2H, d, $J=8$ Hz, 2', 3'-H), 7.20—7.76 (5H, m, aromatic-H). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of 2a——A solution of **2a** (40 mg) in 2% methanolic sodium methoxide (10 ml) was left standing at room temperature for 3 h. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form), and the products were separated by chromatography over MCI-gel CHP 20P [solvent: H_2O–MeOH (2 : 3)] to afford the hydrolysate (20 mg), which was identified as *p*-hydroxyphenylbutanone glucoside (**9**) by comparison of the physical and spectral data with those of an authentic sample.[2] Subsequent elution with H_2O–MeOH (1 : 4) furnished methyl cinnamate (7 mg) as colorless needles (*n*-hexane–benzene), mp 32—33 °C, *Rf* 0.38 (benzene).

Compound 3——Colorless needles, mp 134—136 °C, $[\alpha]_D^{27}$ $-38.2°$ ($c=0.73$, acetone). *Anal.* Calcd for $C_{32}H_{32}O_{13} \cdot 1/2H_2O$: C, 60.60; H, 5.25. Found: C, 60.31; H, 5.41. FD-MS *m/z*: 624 $[M]^+$, 472 $[M+H-\text{galloyl}]^+$, 460, 314, 164. ^{1}H-NMR (acetone-d_6): 2.04 (3H, s, $COCH_3$), 2.64 (4H, m, 3, 4-H), 3.5—4.2 (3H, m, sugar-H), 4.37 (1H, dd, $J=6$, 12 Hz, 6''-H), 4.64 (1H, dd, $J=2$, 12 Hz, 6''-H), 5.10—5.38 (2H, m, 1'', 2''-H), 6.40, 7.64 (each 1H, d, $J=16$ Hz, olefinic-H), 6.86, 7.02 (each 2H, d, $J=8$ Hz, 2', 3'-H), 6.90, 7.56 (each 2H, d, $J=8$ Hz, aromatic-H), 7.16 (2H, s, galloyl-H). ^{13}C-NMR: Table I.

Tannase Hydrolysis of 3——An aqueous solution of **3** (100 mg) was shaken with tannase at room temperature for 2 h. Work-up as described for **1** yielded gallic acid and the hydrolysate (**3a**) (59 mg) as colorless needles (dil. MeOH), mp 149—150 °C, $[\alpha]_D^{27}$ $-14.9°$ ($c=0.6$, 70% aq. acetone). *Anal.* Calcd for $C_{25}H_{28}O_9 \cdot H_2O$: C, 61.21; H, 6.17. Found: C, 61.09; H, 6.26. ^{1}H-NMR (DMSO-d_6): 2.03 (3H, s, $COCH_3$), 2.60 (4H, m, 3, 4-H), 3.1—3.8 (4H, m, sugar-H), 4.19 (1H, dd, $J=7$, 12 Hz, 6''-H), 4.42 (1H, dd, $J=2$, 12 Hz, 6''-H), 4.38 (1H, d, $J=7$ Hz, anomeric-H), 6.38, 7.54 (each 1H, d, $J=16$ Hz, olefinic-H), 6.80, 7.56 (each 2H, d, $J=8$ Hz, aromatic-H), 6.88, 7.02 (each 2H, d, $J=8$ Hz, 2', 3'-H). ^{13}C-NMR: Table I.

Alkaline Hydrolysis of 3a——A solution of **3a** (40 mg) in 2% methanolic sodium methoxide (10 ml) was left standing at room temperature for 2 h. The reaction mixture was treated in the same way as described for **2a** to furnish *p*-hydroxyphenylbutanone glucoside (**9**) and methyl *p*-coumaroate (6 mg) as colorless needles, mp 142—143 °C.

Compound 4——A white amorphous powder, $[\alpha]_D^{27}$ $+25.2°$ ($c=0.69$, acetone). *Anal.* Calcd for $C_{27}H_{26}O_{12} \cdot H_2O$: C, 57.85; H, 5.04. Found: C, 57.45; H, 5.30. FD-MS *m/z*: 542 $[M]^+$, 390 $[M+H-\text{galloyl}]^+$, 314, 228, 170, 153. ^{1}H-NMR (acetone-d_6): 3.6—4.1 (5H, m, sugar-H), 5.12—5.36 (2H, m, 1'', 2''-H), 6.28 (1H, t, $J=2$ Hz, 4-H), 6.54 (2H, d, $J=2$ Hz, 2, 6-H), 6.86, 7.02 (each 1H, d, $J=16$ Hz, olefinic-H), 6.94, 7.42 (each 2H, d, $J=8$ Hz, 2', 3'-H), 7.14 (2H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O): 62.0 (glc. C-6), 71.0 (glc. C-4), 74.6 (glc. C-3), 75.3 (glc. C-2), 77.6 (glc. C-5), 100.0 (C-4), 102.9 (glc. C-1), 110.0 (2C) (galloyl C-2), 117.8 (2C) (C-3'), 121.2 (galloyl C-1), 128.4 (4C) (C-2', C-α and β), 132.8 (C-1'), 139.1 (galloyl C-4), 140.2 (C-1), 145.9 (2C) (galloyl C-3), 157.7 (C-4'), 159.2 (2C) (C-3), 166.6 (–COO–).

Tannase Hydrolysis of 4——An aqueous solution of **4** (25 mg) was treated with tannase at room temperature for 2 h. The solution was concentrated under reduced pressure. The residue was treated with MeOH, and the MeOH-soluble portion was chromatographed over MCI-gel CHP 20P. Elution with H_2O yielded gallic acid. Further elution with H_2O–MeOH (1 : 1) afforded the hydrolysate (15 mg), which was shown to be identical with 3,4',5-trihydroxystilbene 4'-*O*-β-D-glucopyranoside by direct comparison with an authentic sample.[4]

Compound 5——Colorless granules, mp 244—245 °C, $[\alpha]_D^{24}$ $-73.6°$ ($c=0.5$, MeOH). *Anal.* Calcd for $C_{13}H_{16}O_{10}$: C, 46.99; H, 4.85. Found: C, 46.66; H, 5.01. ^{1}H-NMR (acetone-d_6 + D_2O): 3.4—4.2 (6H, m, sugar-H), 4.90 (1H, d, $J=7$ Hz, anomeric-H), 7.30, 7.42 (each 1H, d, $J=2$ Hz, 2, 6-H).

Compound 6——Colorless needles, mp 192—194 °C, $[\alpha]_D^{24}$ $-13.7°$ ($c=1.31$, MeOH). *Anal.* Calcd for $C_{13}H_{16}O_{10} \cdot 1/2H_2O$: C, 45.75; H, 5.02. Found: C, 45.90; H, 5.13. ^{1}H-NMR (acetone-d_6 + D_2O): 3.4—4.2 (6H, m, sugar-H), 4.70 (1H, d, $J=7$ Hz, anomeric-H), 7.08 (2H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O): 61.5 (glc. C-6), 70.1 (glc. C-4), 74.9 (glc. C-2), 76.9 (glc. C-5), 78.2 (glc. C-3), 107.2 (glc. C-1), 109.9 (2C) (C-2), 128.9 (C-1), 137.8 (C-4), 151.0 (2C) (C-3), 168.0 (–COO–).

Acid Hydrolyses of 5 and 6——**5** (10 mg) and **6** (10 mg) were separately hydrolyzed with 5% aqueous sulfuric acid for 4 h under reflux. Each solution was neutralized with barium carbonate, filtered and concentrated under reduced pressure. The residue was directly analyzed by TLC to detect glucose [*Rf* 0.35, *n*-BuOH–pyridine–H_2O (6 : 4 : 3)] and gallic acid [*Rf* 0.72, benzene–ethyl formate–formic acid (2 : 7 : 1)].

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References and Notes

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