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PHENOLIC GLUCOSIDES FROM *PRUNUS GRAYANA*

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Key Word Index - *Prunus grayana*; Rosaceae; phenylpropanoid glucosides; tannin-related compound; caffeic acid esters; 3,4,5-trimethoxybenzoic acid ester.

Abstract—A new bitter phenylpropanoid glucoside, 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside and a new bitter tannin-related compound, 3,4,5-trimethoxybenzoyl-β-D-glucopyranoside, have been isolated together with known compounds, 2-(3,4-dihydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside, 2-(3,4-dihydroxyphenyl)-ethyl-β-D-glucopyranoside and 6-*O*-caffeoyl-D-glucopyranose, from the bark of *Prunus grayana*. The structures of these compounds have been established on the basis of spectroscopic studies and chemical evidence.

INTRODUCTION

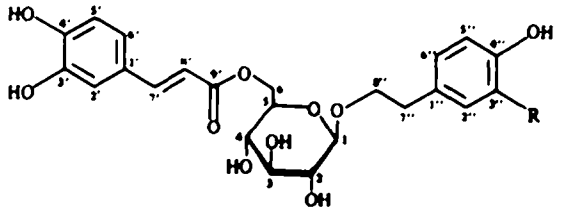
Prunus grayana Maxim. is endemic to Japan and common in the low mountain area. The bark of this plant tastes bitter. In this paper, we describe the isolation and structural elucidation of the bitter substances.

RESULTS AND DISCUSSION

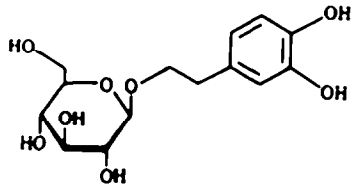
From the methanol extract of the bark of *P. grayana*, five phenolic glucosides were isolated as amorphous powders.

Compound **1** analysed for $C_{23}H_{26}O_{10}$ [secondary ion mass spectrometry (SIMS), *m/z* 463 $[M+H]^+$]. The ^1H NMR spectrum of **1** showed the existence of a *trans*-olefin system, aromatic protons of ABC and AA'BB' systems, sugar protons and the two methylene groups typical of phenethyl alcohol. The electron-impact mass spectrum of **1** showed peaks at *m/z* 163, a characteristic fragment ion peak due to caffeic acid, and at *m/z* 121, due to a *p*-hydroxyphenethyl moiety. Acid hydrolysis of **1** in refluxing aqueous 2 M hydrochloric acid–methanol (1:1) yielded D-glucose and caffeic acid. Alkaline hydrolysis of **1** with sodium methoxide gave methyl caffeate and an amorphous powder which was identical to 2-(4-hydroxyphenyl)-ethyl-β-D-glucopyranoside (**1a**) from ^1H NMR and ^{13}C NMR spectral data (see Table 1 and Experimental). Comparison of the ^{13}C NMR spectrum of **1a** with that of **1** revealed that the signals assignable to C-5 and C-6 of the glucose moiety were shifted by −2.7 and +1.9 ppm by deacylation, respectively. Consequently, the caffeoyl group was located at C-6 in the glucose moiety [1]. Thus, the structure of **1** was established as 2-(4-hydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside.

Compound **2**, $C_{23}H_{26}O_{11}$ (SIMS *m/z* 479 $[M+H]^+$), has spectral data similar to those of **1**. The ^1H NMR spectrum indicated the presence of caffeoyl, phenethyl alcohol and sugar moieties. Acid hydrolysis of **2** yielded D-glucose and caffeic acid. Alkaline methanolysis of **2** with sodium methoxide gave methyl caffeate and 2-(3,4-dihydroxyphenyl)-ethyl-β-D-glucopyranoside (**2a**). The ^{13}C NMR spectrum of **2** showed that the caffeoyl moiety was located at glucose C-6, as in the case of **1**. These data indicate that the structure of **2** is 2-(3,4-dihydroxyphenyl)-ethyl-(6-*O*-caffeoyl)-β-D-glucopyranoside [2].



1 R = H
2 R = OH



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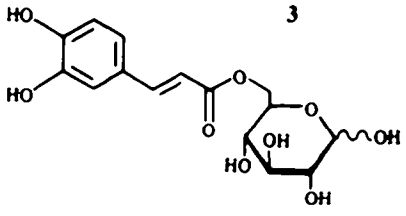

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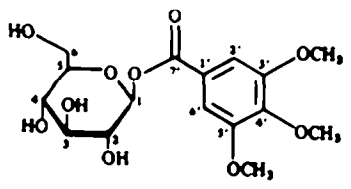

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Table 1. ^{13}C NMR spectral data of compounds 1, 1a, 2 and 2a (3) (CD_3OD, ppm)

	1	1a	2	2a(3)
Glucose moiety 1	104.5	104.3	104.5	104.4
2	75.1	75.1	75.1	75.2
3	77.9	77.9	77.9	78.0
4	71.8	71.6	71.8	71.7
5	75.4	78.1	75.4	78.1
6	64.7	62.8	64.7	62.8
Caffeoyl moiety 1′	127.7		127.7	
2′	114.9		114.9	
3′	149.6		149.6	
4′	146.7		146.7	
5′	116.6		116.6	
6′	123.1		123.2	
7′	147.2		147.3	
8′	115.2		115.2	
9′	169.1		169.2	
Phenethyl alcohol moiety 1″	130.6	130.7	131.5	131.6
2″	130.9	130.9	116.4	116.4
3″	116.2	116.1	146.1	146.1
4″	156.7	156.7	144.6	144.7
5″	116.2	116.1	117.1	117.2
6″	130.9	130.9	121.3	121.3
7″	36.5	36.3	36.7	36.6
8″	72.4	72.1	72.4	72.1

The ^1H NMR and ^{13}C NMR spectra showed compound 3, $C_{14}H_{20}O_8$ (EIMS *m/z* 316 $[M]^+$), to have 3,4-dihydroxyphenethyl alcohol and glucose moieties. The absence of a caffeoyl group in 3 was deduced from ^1H NMR and EIMS. The ^{13}C NMR signal (δ 104.4) of the anomeric carbon of the glucose indicated that the 3,4-dihydroxyphenethyl alcohol moiety was located at C-1 of the glucose [3]. Therefore, 3 is 2-(3,4-dihydroxyphenyl)-ethyl-β-D-glucopyranoside.

The ^{13}C NMR spectrum of compound 4, $C_{15}H_{18}O_9$ (SIMS *m/z* 342 $[M]^+$), exhibited a duplicated signal pattern of sugar carbons. The chemical shifts of the anomeric carbon signals (δ 94.0 and 98.3) were in agreement with those of D-glucose. Alkaline methanolysis of 4 with sodium methoxide afforded methyl caffeate and D-glucose. It was clear from the chemical shift value (δ 65.0, C-6β; 64.8, C-6α) of 4 in the ^{13}C NMR spectrum that the caffeic acid was located at C-6 of glucose. Thus, 4 is 6-*O*-caffeoyl-D-glucopyranose.

The ^1H NMR spectrum of 5, $C_{16}H_{22}O_{10}$ (EIMS *m/z* 374 $[M]^+$), showed the existence of a β-linked sugar and three aromatic methoxyl groups. The ^{13}C NMR spectrum indicated the presence of a β-glucopyranosyl moiety. Alkaline methanolysis of 5 with sodium methoxide yielded methyl 3,4,5-trimethoxybenzoate (5a) and D-glucose. As was evident from the chemical shift value (δ 96.1) of the glucose C-1, the 3,4,5-trimethoxybenzoyl moiety was attached to the glucose C-1. Thus, 5 is 3,4,5-trimethoxybenzoyl-β-D-glucopyranoside.

Among the compounds described above, 1, 2 and 5 have a strong bitter taste. Although there have been many reports on the isolation of phenylpropanoid glycosides from the Labiatae [4, 5], Orobanchaceae [6, 7] and Oleaceae [8, 9], this is the first report of their isolation from the Rosaceae. Flavonoids are widely distributed in the genus *Prunus*, but we could not detect these compounds in *P. grayana*.

EXPERIMENTAL

NMR spectra were measured at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR. Chemical shifts are given on the δ (ppm) scale with TMS as internal standard.

Isolation. The air-dried bark of *Prunus grayana* (1.0 kg), collected in the botanic garden of this college in April 1985, was extracted with hot MeOH under reflux. The MeOH extract was concentrated under reduced pressure and the residue was suspended in H_2O. The suspension was extracted with $CHCl_3$ and then with *n*-BuOH. The *n*-BuOH extract was repeatedly chromatographed on silica gel and Sephadex LH-20 to give compounds 1–5.

Compound 1. Amorphous pale yellow powder (1.2 g), $[\alpha]_D^{25}$ − 33.9° (MeOH; *c* 1.10); IR ν_{max}^{KBr} cm^{-1}: 3400, 1690, 1630, 1600, 1515; ^1H NMR (CD_3OD): caffeoyl moiety: δ 7.04 (1H, *d*, *J* = 2.1 Hz, H-2′), 6.77 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.89 (1H, *dd*, *J* = 8.2, 2.1 Hz, H-6′), 7.57 (1H, *d*, *J* = 15.9 Hz, H-7′), 6.29 (1H, *d*, *J* = 15.9 Hz, H-8′); glucose moiety: δ 4.33 (1H, *d*, *J* = 7.7 Hz, H-1), 3.35–3.39 (2H, overlapping H-2, H-3), 3.22 (1H, *t*-like, H-4), 3.52 (1H, *m*, H-5), 4.50 (1H, *dd*, *J* = 11.9, 2.2 Hz, H-6a), 4.34 (1H, *dd*, *J* = 11.9, 6.1 Hz, H-6b); phenethyl alcohol moiety: δ 7.03 (2H, *d*, *J* = 8.2 Hz, H-2″, H-6″), 6.65 (2H, *d*, *J* = 8.2 Hz, H-3″, H-5″), 2.83 (2H, *t*-like, H-7″), 3.95 (1H, *m*, H-8″a), 3.72 (1H, *m*, H-8″b).

Alkaline methanolysis of 1 *with* NaOMe. Compound 1 (100 mg) was dissolved in methanolic 3% NaOMe (5 ml) and the soln was allowed to stand 1 hr at room temp. The mixture was passed through an Amberlite IR-120 (H^+) column and the eluate was concentrated under reduced pressure. The residue was purified by Sephadex LH-20 CC to give methyl caffeate and 2-(4-hydroxyphenyl)-ethyl-β-D-glucopyranoside (1a) (61 mg). 1a: Amorphous powder; IR ν_{max}^{KBr} cm^{-1}: 3440, 1615, 1520; ^1H NMR (CD_3OD): glucose moiety: δ 4.29 (1H, *d*, *J* = 7.8 Hz, H-1), 3.29–3.33 (2H, overlapping H-2, H-3), 3.18 (1H, *dd*, *J* = 8.9, 7.9 Hz, H-4), 3.86 (1H, *dd*, *J* = 11.9, 2.0 Hz, H-6a); phenethyl alcohol moiety: δ 7.06 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″), 6.69 (2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 2.83 (2H, *t*-like, H-7″), 4.03 (1H, *m*, H-8″a), 3.64 3.73 (3H, overlapping H-5, H-6b and H-8″b).

Acetylation of 1. Compound 1 (33 mg) was dissolved in pyridine (0.5 ml) and Ac_2O (2.0 ml) and left at room temp. overnight to afford the hexaacetate (1b) (47 mg), amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1755, 1720, 1640, 1510; ^1H NMR ($CDCl_3$): caffeoyl moiety: δ 7.37 (1H, *d*, *J* = 1.9 Hz, H-2′), 7.23 (1H, *d*, *J* = 8.3 Hz, H-5′), 7.40 (1H, *dd*, *J* = 8.3, 1.9 Hz, H-6′), 7.64 (1H, *d*, *J* = 16.0 Hz, H-7′), 6.40 (1H, *d*, *J* = 16.0 Hz, H-8′); glucose moiety: δ 4.50 (1H, *d*, *J* = 7.9 Hz, H-1), 5.01 (1H, *dd*, *J* = 9.4, 7.9 Hz, H-2), 5.20 (1H, *dd*, *J* = 9.4, 9.4 Hz, H-3), 5.11 (1H, *dd*, *J* = 9.4, 9.4 Hz, H-4), 3.76 (1H, *m*, H-5), 4.33 (2H, *m*, H-6); phenethyl alcohol moiety: δ 7.19 (2H, *d*, *J* = 8.5 Hz, H-2″, H-6″), 6.97 (2H, *d*, *J* = 8.5 Hz, H-3″, H-5″), 2.88 (2H, *m*, H-7″), 4.13 (1H, *m*, H-8″a), 3.66 (1H, *m*, H-8″b); acetoxyl groups: δ 2.31, 2.30, 2.27, 2.03, 2.00 and 1.91 (each 3H, *s*, 6 × OAc).

Compound 2. Amorphous pale yellow powder (2.4 g), $[\alpha]_D^{25}$ − 32.1° (MeOH; *c* 0.83); IR ν_{max}^{KBr} cm^{-1}: 3420, 1685, 1630, 1605, 1525; ^1H NMR (CD_3OD): caffeoyl moiety: δ 7.03 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.76 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.88 (1H, *dd*, *J* = 8.2, 2.0 Hz, H-6′), 7.56 (1H, *d*, *J* = 15.9 Hz, H-7′), 6.28 (1H, *d*, *J* = 15.9 Hz, H-8′); glucose moiety: δ 4.32 (1H, *d*, *J* = 7.9 Hz, H-1), 3.33–3.38 (2H, overlapping H-2, H-3), 3.21 (1H, *t*-like, H-4), 3.52 (1H, *m*, H-5), 4.49 (1H, *dd*, *J* = 11.9, 2.1 Hz, H-6a), 4.33 (1H,

dd, J = 11.9, 6.0 Hz, H-6b); phenethyl alcohol moiety: δ6.67 (1H, *d*, J = 2.0 Hz, H-2″), 6.63 (1H, *d*, J = 8.0 Hz, H-5″), 6.53 (1H, *dd*, J = 8.0, 2.0 Hz, H-6″), 2.78 (2H, *t*-like, H-7″), 3.96 (1H, *m*, H-8″a), 3.71 (1H, *m*, H-8″b).

Alkaline methanolysis of **2** *with* NaOMe. Compound **2** (114 mg) was treated in the same manner as **1** to give methyl caffeate and 2-(3,4-dihydroxyphenyl)-ethyl-β-D-glucopyranoside (**2a**) (23 mg). **2a**: Amorphous powder; IR ν_{max}^{KBr} cm^{-1}: 3400, 1610, 1530; ^{1}H NMR (CD_3OD): glucose moiety: δ4.28 (1H, *d*, J = 7.8 Hz, H-1), 3.29–3.32 (2H, overlapping H-2, H-3), 3.18 (1H, *dd*, J = 8.9, 7.9 Hz, H-4), 3.86 (1H, *dd*, J = 11.9, 2.0 Hz, H-6a); phenethyl alcohol moiety: δ6.69 (1H, *d*, J = 2.0 Hz, H-2″), 6.67 (1H, *d*, J = 8.0 Hz, H-5″), 6.55 (1H, *dd*, J = 8.0, 2.0 Hz, H-6″), 2.78 (2H, *t*-like, H-7″), 4.02 (1H, *m*, H-8″a), 3.65 3.72 (3H, overlapping H-5, H-6b and H-8″b).

Acetylation of **2**. Compound **2** (18 mg) was acetylated in the same manner as **1** to give the heptaacetate (**2b**) (27 mg), amorphous powder; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1715, 1640, 1510; ^{1}H NMR ($CDCl_3$): caffeoyl moiety: δ7.38 (1H, *d*, J = 2.0 Hz, H-2′), 7.23 (1H, *d*, J = 8.3 Hz, H-5′), 7.41 (1H, *dd*, J = 8.3, 2.0 Hz, H-6′), 7.64 (1H, *d*, J = 16.0 Hz, H-7′), 6.41 (1H, *d*, J = 16.0 Hz, H-8′); glucose moiety: δ4.50 (1H, *d*, J = 8.0 Hz, H-1), 5.01 (1H, *dd*, J = 9.5, 8.0 Hz, H-2), 5.20 (1H, *dd*, J = 9.5, 9.5 Hz, H-3), 5.11 (1H, *dd*, J = 9.5, 9.5 Hz, H-4), 3.76 (1H, *m*, H-5), 4.33 (2H, *m*, H-6); phenethyl alcohol moiety: δ7.06 (2H, *br s*, H-5″, H-6″), 7.03 (1H, *br s*, H-2″), 2.88 (1H, *m*, H-7″), 4.13 (1H, *m*, H-8″a), 3.68 (1H, *m*, H-8″b); acetoxyl groups: δ2.31, 2.30, 2.26, 2.26, 2.03, 2.00 and 1.93 (each 3H, *s*, 7 × OAc).

Acid hydrolysis of **1** *and* **2**. Compounds **1** (20 mg) and **2** (21 mg) were each refluxed in aq. 2 M HCl–MeOH (1:1; 2 ml) at 90° for 2 hr to afford caffeic acid and D-glucose. Both compounds were identified by TLC comparison with authentic samples. Caffeic acid: R_f 0.84, upper layer of MeCOEt–EtOAc–HCO_2H–H_2O–C_6H_6 (4:3:1:1:2). D-Glucose: R_f 0.29, *n*-BuOH–HOAc–H_2O (4:1:2).

Compound **3**. Amorphous pale yellow powder (670 mg). $[\alpha]_D^{15}$ −23.8° (MeOH; *c* 1.00). All spectral data were identical with those of **2a**.

Compound **4**. Amorphous pale orange powder (1.3 g). $[\alpha]_D^{25}$ +26.8° (MeOH; *c* 0.74); IR ν_{max}^{KBr} cm^{-1}: 3420, 1690, 1630, 1605, 1525; ^{1}H NMR (CD_3OD): caffeoyl moiety: δ7.04 (*d*, J = 1.9 Hz, H-2′), 6.63 (*d*, J = 8.2 Hz, H-5′), 6.94 (*dd*, J = 8.2, 1.9 Hz, H-6′), 7.56 (*d*, J = 15.9 Hz, H-7′), 6.27(*d*, J = 15.9 Hz, H-8′); glucose moiety: δ5.13 (*d*, J = 3.7 Hz, H-1α), 4.53 (*d*, J = 7.8 Hz, H-1β), 3.18 4.50 (sugar protons); ^{13}C NMR (CD_3OD): caffeoyl moiety: δ127.8 (C-1′), 115.0 and 115.1 (C-2′), 149.7 (C-3), 146.9 (C-4′), 116.6 (C-5′), 123.0 (C-6′), 147.2 and 147.1 (C-7′), 115.2 (C-8′), 169.3 and 169.2 (C-9′); glucose moiety: δ94.0 (C-1α), 73.9 (C-2α), 74.9 (C-3α), 71.9 (C-4α), 70.3(C-5α), 64.8 (C-6α), 98.3 (C-1β), 76.4 (C-2β), 77.9 (C-3β), 71.6 (C-4β), 74.9 (C-5β), 65.0 (C-6β).

Alkaline methanolysis of **4** *with* NaOMe. Compound **4** (50 mg) was treated in the same manner as **1** to give D-glucose and methyl caffeate. The sugar was detected by TLC (*n*-BuOH–HOAc–H_2O, 4:1:2).

Compound **5**. Amorphous pale yellow powder (390 mg). $[\alpha]_D^{25}$ −13.1° (MeOH; *c* 1.03); IR ν_{max}^{KBr} cm^{-1}: 3420, 1730, 1590, 1510; ^{1}H NMR (Me_2CO-d_6): δ7.38 (2H, *s*, H-2′, H-6′), 5.72 (1H, *d*, J = 7.8 Hz, H-1), 3.47 3.75 (6H, sugar protons), 3.90 (6H, *s*, OMe-3′, and -5′), 3.81 (3H, *s*, OMe-4′); ^{13}C NMR (Me_2CO-d_6): δ96.1 (C-1), 73.8 (C-2), 78.5 (C-3), 71.2 (C-4), 77.9 (C-5), 62.5 (C-6), 125.5 (C-1′), 108.3 (C-2′, C-6′), 154.2 (C-3′, C-5′), 144.0 (C-4′), 165.2 (C-7′), 56.7 (2 × OCH_3, $O\underline{C}H_3$-3′, -5′), 60.7 (OCH_3-4′).

Alkaline methanolysis of **5** *with* NaOMe. Compound **5** (99 mg) was treated in the same manner as **1** to give methyl 3,4,5-trimethoxybenzoate (**5a**) (41 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1595, 1510; ^{1}H NMR ($CDCl_3$): δ7.30 (2H, *s*, H-2′, H-6′), 3.91 (12H, *s*, 4 × OMe).

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REFERENCES

1. Yoshimoto, K., Itatani, Y. and Tsuda, Y. (1980) *Chem. Pharm. Bull.* **28**, 2065.
2. Matsumoto, M., Koga, S., Shoyama, Y. and Nishioka, I. (1986) *Abstract Papers of the 106th Annual Meeting of the Pharmaceutical Society of Japan*, p. 238.
3. Usui, T., Yamaoka, N., Matsuda, K., Tsujimura, K., Sugiyama, H. and Seto, S. (1973) *J. Chem. Soc. Perkin Trans.* 1, 2425.
4. Miyase, T., Koizumi, A., Ueno, A., Noro, T., Kuroyanagi, M., Fukushima, S., Akiyama, Y. and Takemoto, T. (1982) *Chem. Pharm. Bull.* **30**, 2732.
5. Imakura, Y., Kobayashi, S. and Mima, A. (1985) *Phytochemistry* **24**, 139.
6. Kobayashi, H., Karasawa, H., Miyase, T. and Fukushima, S. (1984) *Chem. Pharm. Bull.* **32**, 3009.
7. Kobayashi, H., Karasawa, H., Miyase, T. and Fukushima, S. (1984) *Chem. Pharm. Bull.* **32**, 3880.
8. Endo, K., Takahashi, K., Abe, T. and Hikino, H. (1981) *Heterocycles* **16**, 1311.
9. Endo, K. and Hikino, H. (1982) *Heterocycles* **19**, 2033.