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PHENOLIC COMPOUNDS FROM THE LEAVES OF *Psidium guajava*. I. HYDROLYSABLE TANNINS AND BENZOPHENONE GLYCOSIDES

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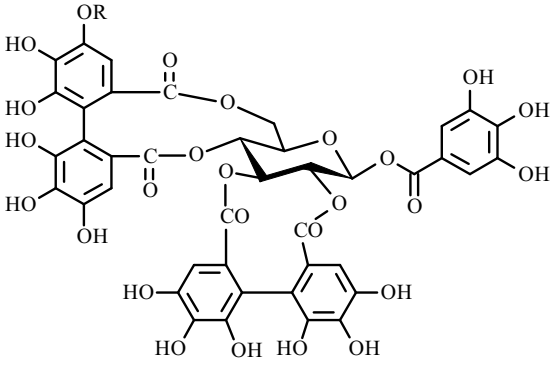
Psidium guajava L. (Myrtaceae) has been used widely as food and traditional medicines [1]. The leaves of *P. guajava* are used in the treatment of diarrhea, gastroenteritis, dysentery, pulmonary diseases, cough, etc. [1–4]. Herein, we investigated the chemical composition of the leaves of *P. guajava*.

The leaves of *P. guajava* were obtained from plants cultivated in the Ibusuki experimental botanical garden, Faculty of Agriculture, Kagoshima University, Japan, in May 2008. The leaves were dried at room temperature and ground into a fine powder. The powder (80 g) was extracted 2 times with 70% MeOH and after that 2 times with 80% acetone. The extract solvent was removed by evaporation under reduced pressure (ca. 40°C) to give the crude extracts (20.56 g). The crude extracts (15 g) were suspended in 500 mL water and then defatted with 500 mL *n*-hexane 3 times to give 0.57 g of hexane-soluble layer. The hexane-insoluble layer was partitioned between EtOAc and *n*-BuOH solution to give an EtOAc-soluble fraction (3.08 g) and an *n*-BuOH-soluble fraction (4.15 g), and the remaining water fraction was concentrated to 7.16 g of a water-soluble fraction.



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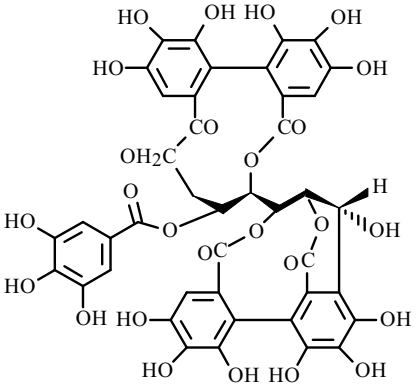

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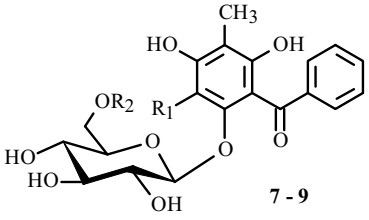

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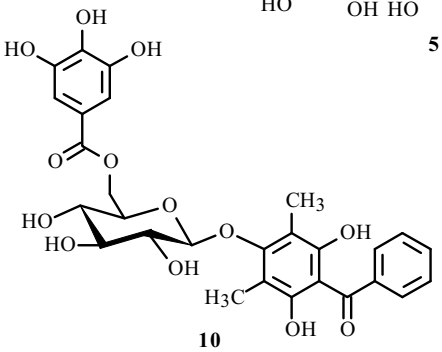

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4: R = H; **6:** R = methylgalloyl; **7:** $R_1 = CH_3$, $R_2 = H$
8: $R_1 = CH_3$, R_2 = galloyl; **9:** $R_1 = H$, R_2 = galloyl

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The EtOAc-soluble fraction (2.5 g) was applied to an MCI GEL CHP-20 (4.0 cm i.d × 18 cm) column using H_2O–MeOH mixture (2 L each) to give fraction I (50% MeOH), fraction II (100% MeOH), and fraction III (100% MeOH). Fraction I (1.48 g) was further separated by preparative HPLC to give compounds **1–10**. HPLC was carried out using the Shimadzu (Kyoto, Japan) preparative HPLC system comprising an LC-6A pump and an SPD-10A detector. The separation column was TSKgel ODS-80TM (21.5 mm i.d. × 30 cm, Tosoh) with solvent H_2O–acetonitrile–formic acid (95:5:1) and/or H_2O–acetonitrile–formic acid (85:15:1).

^1H and ^{13}C NMR spectra were measured with a JEOL α-500 spectrometer in methanol-d_4 at 30°C. HR-ESI-TOF-MS spectra were recorded on a JEOL JMS-T100LC spectrometer.

Compounds **1–3** were identified as gallic acid (**1**), methylgallate (**2**), and catechin (**3**) from the NMR spectra data [5, 6].

Casuarictin (4), white powder. HR-ESI-TOF-MS *m/z* 959.0781 $[M + Na]^+$ (calcd for $C_{41}H_{28}O_{26}Na$, 959.0767). ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): galloyl, 7.11 (2H, s, H-2, 6); 2,3-HHDP, 6.43 (1H, s, H-3), 6.39 (1H, s, H-3′); 4,6-HHDP, 6.61 (1H, s, H-3), 6.52 (1H, s, H-3′); Glc, 6.12 (d, J = 8.4, H-1), 5.44 (dd, J = 9.1, 10.3, H-3), 5.35 (dd, J = 6.6, 13.2, H-6), 5.18 (dd, J = 8.6, 9.1, H-2), 5.17 (t, J = 10.1, H-4), 4.36 (ddd, J = 1.3, 6.6, 9.8, H-5), 3.89 (dd, J = 1.3, 13.2, H-6). ^{13}C NMR (125 MHz, δ): galloyl, 166.1 (C-7), 146.7 (C-3, 5), 140.8 (C-4), 119.8 (C-1), 110.5 (C-2, 6); 2,3-HHDP, 170.6 (C-7′), 169.9 (C-7), 146.0 (C-4), 145.9 (C-4′), 145.1 (C-6), 144.9 (C-6′), 137.6 (C-5), 137.3 (C-5′), 126.3 (C-2), 126.2 (C-2′), 116.7 (C-1), 115.6 (C-1′), 107.6 (C-3′), 106.0 (C-3); 4,6-HHDP, 169.5 (C-7′), 169.0 (C-7), 145.9 (C-4′), 145.8 (C-4), 144.9 (C-6′), 144.8 (C-6), 137.5 (C-5′), 137.5 (C-5), 126.0 (C-2), 125.9 (C-2′), 116.6 (C-1′), 115.0 (C-1), 107.7 (C-3′), 107.6 (C-3); Glc, 92.7 (C-2), 77.9 (C-3), 76.2 (C-2), 74.0 (C-5), 71.3 (C-4), 61.4 (C-6) [6, 8, 9].

Casuarinin (5), white powder. HR-ESI-TOF-MS *m/z* 959.0707 $[M + Na]^+$ (calcd for $C_{41}H_{28}O_{26}Na$, 959.0767). ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): galloyl, 7.07 (2H, s, H-2, 6); 2,3-HHDP, 6.37 (1H, s, H-3′); 4,6-HHDP, 6.81 (1H, s, H-3), 6.49 (1H, s, H-3′); Glc, 5.50 (d, J = 4.9, H-1), 5.44 (dd, J = 1.2, 9.8, H-4), 5.36 (m, H-3), 5.30 (dd, J = 2.4, 8.5, H-5), 4.90 (dd, J = 3.7, 13.4, H-6), 4.68 (dd, J = 2.4, 4.9, H-2), 4.04 (d, J = 13.4, H-6). ^{13}C NMR (125 MHz, δ): galloyl, 167.2 (C-7), 146.7 (C-3), 140.3 (C-4), 120.8 (C-1), 110.4 (C-2, 6); 2,3-HHDP, 171.0 (C-7′), 167.0 (C-7), 147.0 (C-4), 146.5 (C-4′), 145.0 (C-6), 144.9 (C-6′), 140.1 (C-5), 135.8 (C-5′), 125.2 (C-2′), 120.2 (C-2), 118.0 (C-3), 117.0 (C-1), 116.7 (C-1′), 105.3 (C-3′); 4,6-HHDP, 170.4 (C-7′), 169.5 (C-7), 146.0 (C-4′), 145.9 (C-4), 144.5 (C-6′), 144.4 (C-6), 137.9 (C-5), 137.1 (C-5′), 127.6 (C-2), 127.1 (C-2′), 116.7 (C-1), 116.0 (C-1′), 109.2 (C-3), 107.7 (C-3′), Glc; 78.0 (C-2), 74.8 (C-4), 71.7 (C-5), 70.7 (C-2), 67.8 (C-1), 65.1 (C-6) [6–8].

Galloyl 2,3-HHDP-(4′-methylgalloyl)-4,6-HHDP-β-glucoside (rugosin C methyl ester) (6), white powder. HR-ESI-TOF-MS *m/z* 1141.1104 $[M + Na]^+$ (calcd for $C_{49}H_{34}O_{31}Na$, 1141.0982). ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): galloyl, 7.10 (2H, s, H-2, 6); methylgalloyl, 7.00 (1H, s, H-6), 3.71 (3H, s, OCH_3); 2,3-HHDP, 6.40 (1H, s, H-3′), 6.19 (1H, s, H-3); 4,6-HHDP, 6.50 (1H, s, H-3′), 6.42 (1H, s, H-3); Glc, 6.10 (d, J = 8.6, H-1), 5.41 (dd, J = 9.1, 10.1, H-3), 5.23 (dd, J = 6.6, 13.4, H-6), 5.17 (dd, J = 8.6, 9.1, H-2), 5.10 (t, J = 10.1, H-4), 4.30 (ddd, J = 1.5, 6.6, 10.1, H-5), 3.81 (dd, J = 1.5, 13.4, H-6). ^{13}C NMR (125 MHz, δ): galloyl, 166.0 (C-7), 146.7 (C-3, 5), 140.8 (C-4), 119.8 (C-1), 110.6 (C-2, 6); methylgalloyl, 167.7 (C-7), 143.8 (C-5), 140.8 (C-3), 140.8 (C-4), 137.7 (C-2), 115.5 (C-1), 52.5 (OCH_3); 2,3-HHDP, 170.6 (C-7′), 169.9 (C-7), 147.7 (C-4), 145.8 (C-4′), 145.5 (C-6), 144.8 (C-6′), 138.2 (C-5), 137.3 (C-5′), 126.3 (C-2), 125.7 (C-2′), 118.5 (C-1), 115.1 (C-1′), 107.6 (C-3′), 106.1 (C-3); 4,6-HHDP, 169.1 (C-7′), 168.9 (C-7), 146.1 (C-4′), 145.9 (C-4), 145.1 (C-6′), 144.9 (C-6), 137.6 (C-5′), 137.5 (C-5), 126.0 (C-2′), 125.9 (C-2), 116.5 (C-1′), 115.0 (C-1), 107.8 (C-3′), 107.7 (C-3); Glc, 92.7 (C-1), 77.8 (C-3), 76.2 (C-2), 74.0 (C-5), 71.3 (C-4), 61.5 (C-6) [9, 10].

2,4-Dihydroxy-3,5-dimethyl-6-*O*-glucosyl-benzophenone (7), yellow powder, HR-ESI-TOF-MS *m/z* 421.1421 $[M + H]^+$ (calcd for $C_{21}H_{25}O_9$, 421.1499). ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.61 (2H, dd, J = 1.3, 8.2, H-2′, 6′), 7.47 (1H, t, J = 7.3, H-4′), 7.36 (2H, t, J = 7.9, H-3′, 5′), 4.20 (1H, d, J = 7.7, H-1″), 3.71 (1H, dd, J = 2.7, 11.9, H-6″), 3.60 (1H, dd, J = 5.7, 11.9, H-6″), 3.18 (1H, dd, J = 8.6, 9.3, H-3″), 3.02 (1H, dd, J = 8.6, 9.7, H-4″), 2.99 (1H, ddd, J = 2.7, 5.9, 9.7, H-5″), 2.50 (1H, dd, J = 7.7, 9.3, H-2″), 2.14 (3H, s, 3-CH_3), 2.11 (3H, s, 5-CH_3). ^{13}C NMR (125 MHz, δ): 202.6 (C-7′), 160.7 (C-4), 157.7 (C-2), 153.1 (C-6), 142.9 (C-1′), 132.5 (C-4′), 130.7 (C-2′, 6′), 128.5 (C-3′, 5′), 113.9 (C-5), 111.4 (C-3), 109.8 (C-1), 105.0 (C-1″), 77.7 (C-3″), 77.6 (C-5″), 75.0 (C-2″), 71.8 (C-4″), 63.3 (C-6″), 9.2 (3-CH_3), 8.5 (5-CH_3).

2,4-Dihydroxy-3,5-dimethyl-6-*O*-(6″-*O*-galloyl)-glucosyl-benzophenone (8), yellow powder, HR-ESI-TOF-MS *m/z* 573.1579 $[M + H]^+$ (calcd for $C_{28}H_{29}O_{13}$, 573.1608). ^1H NMR (500 MHz, CD_3OD δ, ppm, J/Hz): 7.46 (2H, dd, J = 1.0, 8.3, H-2′, 6′), 7.39 (1H, t, J = 7.3, H-4′), 7.27 (2H, t, J = 7.8, H-3′, 5′), 7.12 (2H, s, H-2‴, H-6‴), 4.32 (1H, dd, J = 4.3, 11.7, H-6″), 4.29 (1H, dd, J = 2.0, 11.7, H-6″), 4.19 (1H, d, J = 7.8, H-1″), 3.25–3.21 (3H, m, H-3″, 4″, 5″), 2.55 (1H, dd, J = 7.8, 9.3, H-2″), 2.14 (3H, s, 3-CH_3), 2.08 (3H, s, 5-CH_3). ^{13}C NMR (125 MHz, δ): 201.6 (C-7′), 168.4 (C-7‴), 160.9 (C-4), 158.6

(C-2), 153.5 (C-6), 146.5 (C-3‴, 5‴), 143.3 (C-1′), 139.9 (C-4‴), 132.0 (C-4′), 130.5 (C-2′, 6′), 128.3 (C-3′, 5′), 121.7 (C-1‴), 113.1 (C-5), 111.2 (C-3), 109.7 (C-1), 105.3 (C-1″), 77.5 (C-3″), 75.3 (C-5″), 74.9 (C-2″), 71.1 (C-4″), 64.5 (C-6″), 9.2 (3-CH_3), 8.4 (5-CH_3).

2,4-Dihydroxy-5-methyl-6-*O*-(6″-*O*-galloyl)-glucosyl-benzophenone (9), yellow powder, HR-ESI-TOF-MS *m/z* 559.1395 $[M + H]^+$ (calcd for $C_{27}H_{27}O_{13}$, 559.1452). ^1H NMR spectrum (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.50 (2H, dd, J = 1.3, 8.2, H-2′, 6′), 7.41 (1H, t, J = 7.5, H-4′), 7.26 (2H, dd, J = 7.5, 8.2, H-3′, 5′), 7.09 (2H, s, H-2‴, 6‴), 6.15 (1H, s, H-3), 4.74 (1H, d, J = 7.7, H-1″), 4.54 (1H, dd, J = 2.0, 11.9, H-6″), 4.36 (1H, dd, J = 4.4, 11.9, H-6″), 3.49 (1H, ddd, J = 2.0, 4.4, 9.1, H-5″), 3.35 (1H, t, J = 9.1, H-4″), 3.30 (1H, m, H-3″), 2.50 (1H, dd, J = 7.7, 9.1, H-2″), 2.01 (3H, s, 5-CH_3). ^{13}C NMR (125 MHz, δ): 201.0 (C-7′), 168.3 (C-7‴), 163.2 (C-4), 162.6 (C-2), 157.8 (C-6), 146.6 (C-3‴, 5‴), 143.1 (C-1′), 140.0 (C-4‴), 132.1 (C-4′), 129.6 (C-2′, 6′), 128.7 (C-3′, 5′), 121.5 (C-1‴), 107.8 (C-1), 107.0 (C-5), 100.9 (C-1″), 95.3 (C-3), 77.7 (C-3″), 75.6 (C-5), 74.2 (C-2″), 70.9 (C-4″), 64.0 (C-6″), 7.6 (5-CH_3).

2,6-Dihydroxy-3,5-dimethyl-4-*O*-(6″-*O*-galloyl)-glucosyl-benzophenone (10), yellow powder, HR-ESI-TOF-MS *m/z* 573.1598 $[M + H]^+$ (calcd for $C_{28}H_{29}O_{13}$, 573.1608). ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.60 (2H, dd, J = 1.3, 8.4, H-2′, 6′), 7.48 (1H, t, J = 7.5, H-4′), 7.36 (2H, dd, J = 7.5, 8.4, H-3′, 5′), 7.04 (2H, s, H-2‴, 6‴), 4.73 (1H, d, J = 7.5, H-1″), 4.45 (1H, dd, J = 2.4, 11.9, H-6″), 4.39 (1H, dd, J = 4.8, 11.9, H-6″), 3.58 (1H, dd, J = 7.7, 9.2, H-2″), 3.55 (1H, br.t, J = 9.2, H-3″), 3.48 (1H, t, J = 9.0, H-4″), 3.46 (1H, ddd, J = 2.4, 4.8, 9.8, H-5″), 2.11 (6H, s, 3-CH_3, 5-CH_3). ^{13}C NMR (125 MHz, δ): 201.5 (C-7′), 168.4 (C-7‴), 160.8 (C-4), 156.3 (C-2, 6), 146.5 (C-3‴, 5‴), 141.8 (C-1′), 139.8 (C-4‴), 132.9 (C-4′), 129.9 (C-2′, 6′), 129.0 (C-3′, 5′), 121.5 (C-1‴), 112.6 (C-3, 5), 111.6 (C-1), 110.2 (C-2″, 6″), 105.5 (C-1″), 77.8 (C-3″), 75.8 (C-5″), 75.5 (C-2″), 71.5 (C-4″), 64.3 (C-6″), 10.0 (3-CH_3, 5-CH_3) [10–12].

Compounds **4** and **5** showed NMR spectra charateristic of ellagitannins, which have the 2H-singlet of a galloyl and the 1H-singlet of two hexahydroxydiphenoyl (HHDP) moieties [6–9]. In the HMBC spectrum, two HHDP moieties were correlated with H-4, 6 and H-2, 3 of the glucose moiety. A gallyol moiety of compound **4** was correlated with δ 6.12 (H-1) of a glucose moiety. Compound **5** showed ^1H and ^{13}C NMR spectra characteristic of the open-chain form of the glucose moiety, and the formation of the C-glucosidic group with the HHDP moiety [6–8]. Based on NMR, MS, and literature data [6–8], compounds **4** and **5** were identified as casuarictin (**4**) and casuarinin (**5**).

The NMR spectrum of compound **6** showed that this tannin consists of a galloyl, an HHDP, a valoneoyl, and *β*-glucose moiety such as rugosin C [9, 10]. However, the valoneoyl moiety exhibited 4,6-HHDP (δ 6.50, 6.42, 1H each s) and methylgalloyl (δ 7.00, 1H, s; δ 3.71, 3H, s) moieties, and compound **6** was identified as methylated rugosin C (**6**).

In the ^1H NMR spectrum, compound **7** exhibited five proton signals of a phenyl group at δ 7.61 (2H), 7.47 (1H), 7.36 (2H), seven proton signals of the *β*-glucose moiety, and two methyl groups (δ 2.14, 2.11). In the HMBC spectrum, the ketonic carbon of δ 202.6 (C-7′) showed cross-peaks with δ 7.61 (H-2′, 6′). The methyl proton of δ 2.14 correlated with δ 114.4 (C-3), 157.7 (C-2), and 160.7 (C-4), and δ 2.11 correlated with δ 113.9 (C-5), 153.1 (C-6), and 160.7 (C-4). The anomeric proton of δ 4.20 (H-1″) correlated with δ 153.1 (C-6). Based on the NMR and MS spectra data, compound **7** was identified as 2,4-dihydroxy-3,5-dimethyl-6-*O*-glucosyl-benzophenone (**7**).

The ^1H and ^{13}C NMR spectra of compound **8** were similar to those of compound **7**. Compound **8** was showed δ 4.32 and 4.29 (H-6″) of glucose protons correlated with δ 168.4 (C-7‴) of the galloyl moiety in the HMBC spectrum. Compound **9** was similar to the NMR spectrum of compound **8**. However, δ 6.15 (H-3) of the aromatic proton was linked to δ 95.3 (C-3) in the HSQC spectrum. From the above data, compounds **8** and **9** were identified as 2,4-dihydroxy-3,5-dimethyl-6-*O*-(6″-*O*-galloyl)-glucosyl-benzophenone (**8**) and 2,4-dihydroxy-5-methyl-6-*O*-(6″-*O*-galloyl)-glucosyl-benzophenone (**9**).

The ^1H and ^{13}C NMR spectra of compound **10** were similar to those of compound **7**. However, δ 4.73 (H-1″) of the anomeric proton correlated with δ 160.8 (C-4), and δ 4.45, 4.39 (H-6″) of the glucose protons correlated with δ 168.4 (C-7‴) of the galloyl moiety in the HMBC spectrum. From the above data, compound **10** was identified as 2,6-dihydroxy-3,5-dimethyl-4-*O*-(6″-*O*-galloyl)-glucosyl-benzophenone. Although compounds **1–5** and **10** were already known from *P. guajava* [1, 5, 6, 11–13], compounds **6–9** were isolated from *P. guajava* for the first time in this investigation.

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