New benzophenone and quercetin galloyl glycosides from *Psidium guajava* L.

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**Abstract** New benzophenone and flavonol galloyl glycosides were isolated from an 80% MeOH extract of *Psidium guajava* L. (Myrtaceae) together with five known quercetin glycosides. The structures of the novel glycosides were elucidated to be 2,4,6-trihydroxybenzophenone 4-O-(6"0"0-galloyl)-β-D-glucopyranoside (1, guavinoside A), 2,4,6-trihydroxy-3,5-dimethylbenzophenone 4-O-(6"0"0-galloyl)-β-D-glucopyranoside (2, guavinoside B), and quercetin 3-O-(5"0"0-galloyl)-α-L-arabinofuranoside (3, guavinoside C) by NMR, MS, UV, and IR spectroscopies. Isolated phenolic glycosides showed significant inhibitory activities against histamine release from rat peritoneal mast cells, and nitric oxide production from a murine macrophage-like cell line, RAW 264.7.

**Keywords** *Psidium guajava* L. · Benzophenone galloyl glycoside · Flavonol galloyl glycoside

**Introduction**

*Psidium guajava* (Myrtaceae) is a small medicinal tree that is native to South America. All parts of this tree, including fruits, leaves, bark, and roots, have been used for treating stomachache and diarrhea in many countries. Previous studies of this plant have led to the isolation of tannins [1, 2], tannins and other phenolic compounds [3], flavonol glycosides [4, 5], triterpenoids [6], terpenoids [7], and carotenoids [8]. It is well known that an extract of the leaves of *P. guajava* improves symptoms of allergic disease, and we are interested in small molecules from the leaves of *P. guajava*, other than tannins, and their potential anti-inflammatory activities.

In this investigation, two new benzophenone galloyl glycosides, guavinosides A (1) and B (2), and a quercetin galloyl glycoside, guavinoside C (3), were isolated from the leaves of *P. guajava* together with known quercetin glycosides (4–8). These structures of the novel glycosides were established through detailed analysis of their spectroscopic data and chemical evidence, and their inhibitory activities against histamine release from rat mast cells and nitric oxide (NO) from RAW 264.7 were also determined.

**Results and discussion**

Guavinoside A (1) was obtained as a yellow powder. The positive ion mode FAB-MS of 1 showed a quasimolecular ion peak at *m/z* 545 [M + H]+, and the molecular formula was determined to be C26H24O13 based on its high-resolution (HR)-FAB-MS (found 545.8418, calcd. 545.8413 for C26H25O13). In the IR spectrum, the strong absorbance at 1,700 cm−1 indicated the presence of conjugated carbonyl groups in 1. Acid hydrolysis of 1 furnished d-glucose which was identical with HPLC analysis, using an optical rotation (OR) detector. In the 1H-NMR spectrum of 1, two aromatic signals at δH 6.11 (2H, s) and 6.98, (2H, s) indicated the presence of two 3,5,4-tetrasubstituted phenyl groups, and the spin system of δH 7.68 (2H, dd, J = 7.0, 1.0 Hz), 7.46 (2H, t, J = 7.0 Hz), and 7.57 (1H, t,
showed the presence of a phenyl group. Additionally, an anomeric proton signal at $\delta_H$ 4.86 (1H, d, $J = 7.6$ Hz) indicated that the glucose residue was in the $\beta$-form. Correlations in the $^1$H-$^1$H-COSY spectrum were observed from the anomeric proton to $\delta_H$ 3.15 (1H, m), 3.20 (1H, m), 3.50 (1H, m), 3.30 (1H, m), and 4.39 (2H, br s) indicating the presence of $\beta$-glucose. The signal pattern in the aromatic region of the $^{13}$C-NMR spectrum indicated the presence of three aromatic rings. In addition, the $^{13}$C-NMR and DEPT spectra showed an anomeric carbon ($\delta_C$ 100.5), four oxymethines ($\delta_C$ 73.0, 76.1, 68.9, and 73.6), an oxymethylene ($\delta_C$ 62.5), and two carbonyl carbons ($\delta_C$ 165.7 and 195.3). All proton–carbon connectivities assigned by using HMQC experiments are summarized in Table 1. The HMBC correlations from 2$''$, 6$''$-H ($\delta_H$ 6.98, 2H, s) to C-1$''$ ($\delta_C$ 119.6), C-3$''$, 5$''$ ($\delta_C$ 145.3), C-4$''$ ($\delta_C$ 138.3), and a carbonyl carbon ($\delta_C$ 165.7) revealed the presence of a galloyl moiety. A phenyl proton signal at $\delta_H$ 7.68 (2H, dd, $J = 7.0, 1.0$ Hz, 2$'$, 6$'$-H) correlated with a carbonyl carbon signal at $\delta_C$ 195.3, and an aromatic proton at $\delta_H$ 6.11 (2H, s, 3, 5-H) correlated with C-4 ($\delta_C$ 159.8) and the carbonyl carbon by weak $^4$J correlation. Furthermore, the anomeric proton signal correlated with C-4. NOE enhancement was observed between the anomeric proton signal and the signal of 3, 5-H. These data suggested that the aglycone of 1 was 2,4,6-trihydroxybenzophenone, and a sugar was attached at C-4. The signal of 6$''$-H, however, was downfield shifted at $\delta_H$ 4.39 (2H, br s), and correlated with the galloyl carbonyl carbon signal at $\delta_C$ 165.7 in the HMBC experiment. From these data, the structure of 1 was established to be 2,4,6-dihydroxybenzophenone 4-O-($\beta$-O-galloyl)-$\beta$-D-glucopyranoside (Figs. 1, 2).

Guavinoside B (2) was obtained as a brownish solid. The negative ion mode FAB-MS of 2 showed quasimolecular ion peak at $m/z$ 571 [M − H]$^-$, and the molecular formula was determined to be C$_{28}$H$_{27}$O$_{13}$ based on its HR-FAB-MS (found 571.1474, calcd. 571.1451 for C$_{28}$H$_{27}$O$_{13}$). The molecular weight was 28 mass units (C$_2$H$_4$) greater than that of 1. The $^1$H- and $^{13}$C-NMR spectra of 2 were very similar to those of 1 except for

| Table 1 | $^{13}$C- and $^1$H-NMR spectral data of guavinosides A (1) and B (2) |
|---------|-----------------|-----------------|-----------------|-----------------|
| Aglycone | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 108.9 s | 113.0 s |
| 2, 6 | 157.3 s | 151.8 s |
| 3, 5 | 94.9 d | 6.11 (2H, s) | 110.8 s |
| 4 | 159.8 s | 155.6 s |
| 1$'$ | 138.4 s | 138.7 s |
| 2$, 6'$ | 128.7 d | 7.68 (2H, dd, $J = 7.0, 1.0$ Hz) | 128.6 d | 7.65 (2H, dd, $J = 7.0, 1.0$ Hz) |
| 3$, 5'$ | 128.2 d | 7.46 (2H, t, $J = 7.0$ Hz) | 128.1 d | 7.45 (2H, t, $J = 7.0$ Hz) |
| 4$'$ | 132.5 d | 7.57 (1H, t, $J = 7.0$ Hz) | 132.3 d | 7.54 (1H, t, $J = 7.0$ Hz) |
| C=O | 195.3 s | 196.7s |
| 3, 5-CH$_3$ | 9.9 q | 2.00 (6H, s) |

| Glucosyl | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
|---------|-----------------|-----------------|-----------------|-----------------|
| 1$''$ | 100.5 d | 4.86 (1H, d, $J = 7.6$ Hz) | 104.2 d | 4.63 (1H, d, $J = 8.5$ Hz) |
| 2$''$ | 73.0 d | 3.15 (1H, m) | 74.2 d | 3.36 (1H, m) |
| 3$''$ | 76.1 d | 3.20 (1H, m) | 76.1 d | 3.30 (1H, m) |
| 4$''$ | 68.9 d | 3.50 (1H, m) | 69.3 d | 3.44 (1H, m) |
| 5$''$ | 73.6 d | 3.30 (1H, m) | 73.5 d | 3.42 (1H, m) |
| 6$''$ | 62.5 t | 4.39 (2H, br s) | 62.7 t | 4.23 (1H, d, $J = 11.0, 3.0$ Hz) |
| Galloyl | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| $1''''$ | 119.2 s | 119.6 s |
| $2''''$, 6$''''$ | 108.6 d | 6.98 (2H, s) | 108.5 d | 6.96 (2H, s) |
| $3''''$, 5$''''$ | 145.4 s | 145.3 s |
| $4''''$ | 138.4 s | 138.3 s |
| C=O | 165.7 s | 165.6 s |

$^1$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) spectra were measured in DMSO-$d_6$ with TMS as internal standard.
the presence of hydroxyl (3,400 cm\(^{-1}\)) were characteristic of flavonol. The IR spectrum indicated the structure of 3,5-dimethylbenzophenone 4-

Additionally, the signals of \(\delta_h = 6.89, 2\text{H}, s\) correlated with \(\delta_c = 118.9\) (C-1\(^{\prime\prime}\)), \(108.5\) (C-2\(^{\prime\prime}\)), \(6\text{H}, s\), \(145.3\) (C-3\(^{\prime\prime}\)), \(5\text{H}, s\), \(138.4\) (C-4\(^{\prime\prime}\)) and \(165.4\) (carbonyl), indicating the presence of a galloyl moiety the same as 1 and 2. Acid hydrolysis of 3 with 2 M HCl afforded (+)-l-arabinose that was identical by HPLC analysis using an OR detector comparison to an authentic sample of l-arabinose. The small coupling constant of the anomeric proton \((\delta_h = 5.56, d, J = 1.4\text{ Hz})\) indicated the presence of the \(\alpha\)-form of arabinose. Correlations in the \(^1\text{H}-^1\text{H}\) COSY spectrum were observed for a spin system from the anomeric signal to three oxymethine signals at \(\delta_h = 4.18\) (br s, 2\(^{-}\text{H}\)), \(3.81\) (m, 3\(^{-}\text{H}\)), and \(3.74\) (m, 4\(^{-}\text{H}\)), and methylene signals \((\delta_h = 4.11\) and \(4.02\), 5\(^{-}\text{H}\)) was observed. Furthermore, two hydroxy signals at \(\delta_h = 5.72\) (br s) and \(5.48\) (br s) both coupled with 2\(^{-}\text{H}\) and 3\(^{-}\text{H}\), respectively. Additionally, the signals of 5\(^{-}\text{H}\) of 3 were shifted downfield compared with those of 4 \((\delta_h = 3.36\text{ and } 3.32)\), and correlated with a galloyl carbon signal in the HMBC experiment. From these observations, the sugar moiety was determined to be 1\(-\alpha\)-arabinofuranoside. Thus, the structure of 3 was determined to be quercetin 3\(-\text{O}(5\text{-}\\text{O-galloyl})-\alpha\)-l-arabinofuranoside.

The structures of 4–8 were elucidated to be quercetin 3\(-\text{O}-\text{arabinofuranoside} (4), quercetin 3\(-\text{O}-\text{xylopyranoside} (5), quercetin 3\(-\text{O}-\text{beta-d-xylopyranoside} (6), quercetin 3\(-\text{O}-\text{beta-d-galactopyranoside} (7), and quercetin 3\(-\text{O}-\text{beta-d-glucopyranoside} (8) by comparison with spectroscopic data [10, 11] and chemical degradation methods.

Benzophenone glycosides have been isolated from many kind of plants, but this is first reported isolation of a dimethylbenzophenone glycoside from a natural source. The substitution pattern is the well-known A ring of flavonoid, a possible biosynthesis pathway to the aglycone moiety of 2 would be methylation of the benzophenone skeleton.

Isolated compounds were evaluated for inhibitory activities against histamine release from rat peripheral mast cells [12] and nitric oxide (NO) production from a murine macrophage-like cell line, RAW264.7 cells [13]. Compounds 3–8 (at 100 \(\mu\text{g/mL}\)) inhibited histamine release from mast cells with inhibition ratios of 94.4, 21.9, 30.5, 23.9, 100, and 93.5\%, respectively. But 1 and 2 did not show inhibitory activity against histamine release at this concentration. Compounds 3–8 (at 100 \(\mu\text{g/mL}\)) inhibited...
NO production by RAW 264.7 cells stimulated with lipopolysaccharide and interferon gamma with inhibition ratios of 50.0, 33.2, 32.4, 65.1, 55.3, and 52.1%, respectively. The isolated compounds therefore inhibited chemical mediators, such as histamine and NO, and increased IL-12 release from RAW 264.7 cells.

In conclusion, phenolic compounds isolated from *P. guajava* might be valuable candidates for treating various inflammatory diseases.

**Experimental**

**General**

The UV spectra were recorded on a Shimadzu model UV-160 spectrophotometer. IR spectra were recorded on a Horiba FT-210 diffraction infrared spectrometer. FAB-MS were obtained with a JEOL model JMS-AX505 HA spectrometer. 1H-NMR (600 MHz) and 13C-NMR (150 MHz) spectra were obtained with a Varian InovaTM 600 spectrometer. 1H- and 13C-NMR data, see Table 1.

**Plant material**

Leaves of *P. guajava* were donated by OS Industrial Co. Ltd. (Tokyo, Japan).

**Extraction and isolation**

The dried leaves of *P. guajava* (5 kg) were extracted with 15 l of 80% MeOH at room temperature for 7 days. The solution was filtered and concentrated under reduced pressure to give a crude extract. The extract was dissolved in water and passed through a Diaion HP-20 column (Mitsubishi Kasei, Tokyo, Japan), and eluted stepwise with 50, 70, and 100% MeOH. The 70% MeOH eluate was dissolved in EtOH and passed through a Sephadex LH-20 column (Pharmacia, Uppsala, Sweden). The effluent was chromatographed on a Diaion CHP-20P column (Mitsubishi Kasei, Tokyo, Japan), and eluted with 50 and 70% MeOH. All fractions were monitored by TLC, and the fractions containing the same compound(s) (as evidenced by TLC) were combined to give four fractions. Fraction 3 (900 mg) was chromatographed on a Sephadex LH 20 column developed with CHCl₃–MeOH (1:1) to give four fractions. Fraction 2 (770 mg) was further applied to a reversed-phase column (SSC ODS, Senshu Scientific Co. Ltd., Tokyo, Japan) eluted stepwise with 0–25% MeOH, and recrystallized from MeOH to give 1 (47 mg). Fraction 4 (124 mg) was purified by medium pressure liquid chromatography (Yamazen Baker-bond ODS Yamazen, Kyoto, Japan) column eluted with MeCN–MeOH–H₂O (5:3:560) to give 2 (44 mg). Fraction 3 (130 mg) was purified by reversed-phase HPLC [column: Shiseido Capcell pak C18 UG120 (10-mm i.d. × 250 mm, Shiseido, Tokyo, Japan); mobile phase: MeCN–MeOH–H₂O (5:30:65); flow rate: 3.0 ml/min.; detection: UV at 254 nm] to give 3 (14 mg). The 100% MeOH eluate of Diaion HP-20 was dissolved in MeOH, and chromatographed on a Sephadex LH-20 column (2.5 × 100 cm) to give ten fractions. Fraction 3 (1.9 g) was chromatographed on a silica gel column developed with CHCl₃–MeOH to give eight fractions. Fraction 6 (515 mg) was purified by reversed-phase HPLC [column: Shiseido Capcell pak C18 UG120 (10-mm i.d. × 250 mm); mobile phase: MeCN–H₂O (18:82); flow rate: 3.0 ml/min.; detection: UV at 250 nm] to give 4 (20 mg), 5 (50 mg), 6 (62 mg), 7 (62 mg), and 8 (30 mg), respectively.

Guavinoside A (1) was obtained as a yellow powder. [α]D²⁶ = 114° (c = 0.1, MeOH); FAB-MS *m/z* 545 (M + H)+; HR-FAB-MS (found 545.8418, calcd for C₂₇H₂₁O₁₅: 545.8413); UV *λ*max nm (ε) = 218 (28,600), 288 (16,200), 340 (1,690) (OH), 1,700 (ester). 1H- and 13C-NMR data, see Table 1.

Guavinoside B (2) was obtained as a yellow powder; [α]D²⁶ = 83° (c = 0.5, MeOH); FAB-MS *m/z* 571 (M – H)+; HR-FAB-MS (found 571.1474, calcd for C₂₉H₂₅O₁₃: 571.1451); UV *λ*max nm (ε) = 218 (26,400), 283 (15,100); IR *ν*max cm⁻¹: 3,410 (OH), 1,710 (ester) (C=O). 1H- and 13C-NMR data, see Table 1.

Guavinoside C (3) was obtained as a yellow powder; [α]D²⁶ = 73° (c = 1.0, MeOH); FAB-MS *m/z* 585 (M – H)+; HR-FAB-MS (found 585.0868, calcd for C₃₀H₂₇O₁₃: 585.0880); UV *λ*max nm (ε) = 211 (32,000), 265 (16,200), 355 (10,000); IR *ν*max cm⁻¹: 3,400 (OH), 1,710 (ester), 1,690 (C=O). 1H- and 13C-NMR data, see Table 2.

Acid hydrolysis of 1–3

Compound 1, 2, or 3 (3.0 mg, each) was treated with 0.5 ml of 2 M HCl for 2 h at 110°C in a sealed tube. The reaction mixture was diluted with 1 ml of H₂O, and extracted with an equal volume of EtOAc, and the water layer was evaporated to dryness. The residue was dissolved in H₂O (200 μl) and subjected to HPLC analysis [column: Asahi pak NH2P-50, (4.6-mm i.d. × 250 mm, Showa Denko, Tokyo, Japan); mobile phase: MeCN–H₂O (75:25); flow rate: 1.0 ml/min.; detection: OR detector (Shodex...
Retention time and optical rotation of these samples (1d No. University, and by the Japan–China Medical Association, and Ministry of Education, Science, Sports and Culture of Japan to Nihon of characteristic education and High-Tech Research Centers from the 19980070), by a Special Research Grant-in Aid for the development of Aids from the Ministry of Health and Welfare of Japan (No. 365614057).

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References

1. Okuda T, Yoshida T, Hatano T, Yazaki K, Ashida M (1982) Ellagitannins of the casuarinaceae, stachyurqaceae and myrtaeae. Phytochemistry 21:2871–2874
2. Okuda T, Yoshida T, Hatano T, Yazaki K, Ikegami Y, Shingu T (1987) Guavins A, C and D, complex tannins from Psidium guajava. Chem Pharm Bull 35:443–446
3. Okuda T, Hatano T, Yazaki K (1984) Guavin B, an ellagitannin of novel type. Chem Pharm Bull 32:3787–3788
4. Lozoya X, Meckes M, Abou-zaid M, Tortoriello J, Nozzolillo C, Arnason JT (1994) Quercetin glycosides in Psidium guajava L. leaves and determination of a spasmolytic principle. Arch Med Res 25:17–21
5. Morales MM, Tortoriello J, Meckes M, Paz D, Lozoya X (1994) Calcium-antagonist effect of quercetin and its relation with the spasmolytic propaties of Psidium guajava L. Arch Med Res 25:17–21
6. Osman AM, Younes M, Garby EL, Sheta AE (1974) Chemical examination of local plants. VI. Triterpenoids of the leaves of Psidium guajava. Phytochemistry 14:193–195
7. Smith RM, Siwatibau S (1975) Aequiterpene hydrocarbons of Fijian guavas. Phytochemistry 14:2013–2015
8. Mercadantre AZ, Steck A, Pfander H (1999) Carotenoids from Guava (Psidium guajava L.): isolation and structure elucidation. J Agric Food Chem 47:145–151
9. Matsuzaki K, Tahara H, Inokoshi J, Tanaka H, Masuma R, Omura S (1998) New brominated and halogen-less derivatives and structure-activity relationship of azaphilones inhibiting gp120-CD4 binding. J Antibiot 51:1004–1011
10. Markharm KR, Chari VM (1982) Carbon-13 NMR spectroscopy of flavonoids. In: Harrnorne JB, Mahry TJ (eds) The flavonoids: advances in research. Chapman & Hall, London, pp 19–51
11. Markharm KR, Geiger H (1994) 1H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeutero dimethylsulfoxide. In: Harrnorne JB (ed) The flavonoids, advances in research since 1986. Chapman & Hall, London, pp 441–471
12. Kitanaka S, Nakayama T, Shibano T, Okoshi E, Takido M (1998) Antiallergic agent from natural sources. Structures and inhibitory effect of histamine release of naphthopyrone glycosides from seeds of Cassia obtusifolia L. Chem Pharm Bull 46:1650–1652
13. Wang J, Matsuoka K, Kitanaka S (2007) Antiallergic agents from natural sources. Part I9. Stilbene derivatives from Pholidota chinensis and their antiinflammatory activity. Chem Pharm Bull 54:1216–1218
14. Wang J, Wang N, Yao X, Ishii R, Kitanaka S (2006) Inhibitory activity of Chinese herbal medicines toward histamine release from mast cells and nitric oxide production by macrophage-like cell line, RAW 264.7. J Nat Med 60:73–77

Table 2 13C- and 1H-NMR spectral data of guavinoside C (3)

| No. | δc | δH |
|-----|----|----|
| 2   | 157.3 s | 6.20 (1H, d, J = 1.5 Hz) |
| 3   | 133.0 s | 6.0 min (positive) |
| 4   | 177.4 s | 6.0 min (negative) |
| 5   | 161.9 s | 6.3 min (positive) |
| 6   | 98.5 d  | 8.5 min (positive), 6.0 min (positive), 6.0 min (negative) |
| 7   | 164.1 s | 8.0 min (positive) |
| 8   | 93.5 d  | 8.0 min (positive) |
| 9   | 156.3 s | 6.3 min (positive) |
| 10  | 103.9 s | 8.5 min (positive) |
| 1'  | 120.8 s | 8.5 min (positive) |
| 2'  | 115.6 d | 7.46 (1H, d, J = 2.2 Hz) |
| 3'  | 144.9 s | 8.0 min (positive) |
| 4'  | 148.3 s | 8.0 min (positive) |
| 5'  | 115.4 d | 6.85 (1H, d, J = 8.8 Hz) |
| 6'  | 121.3 d | 7.49 (1H, d, J = 8.8, 2.2 Hz) |
| 5-OH | 12.62 (1H, s) |  |
| Arabinosyl | 1'' | 107.7 d | 5.56 (1H, J = 1.4 Hz) |
| 2'' | 81.7 d  | 4.18 (1H, br s) |
| 3'' | 76.9 d  | 3.81 (1H, m) |
| 4'' | 81.9 d  | 3.74 (1H, m) |
| 5'' | 62.5 t  | 4.11 (1H, dd, J = 12.0, 3.3 Hz) |
| 2''-OH | 5.72 (1H, br s) |  |
| 3''-OH | 5.48 (1H, br s) |  |
| Galloyl | 1''' | 118.9 s |  |
| 2''', 6''' | 108.5 d | 6.89 (2H, s) |
| 3''', 5''' | 145.3 s |  |
| 4''' | 138.4 s |  |
| CeO  | 165.4 s |  |