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ORIGINAL PAPER

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

Keiichi Matsuzaki · Rie Ishii · Kaori Kobiyama · Susumu Kitanaka

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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″-*O*-galloyl)-*β*-D-glucopyranoside (**1**, guavinoside A), 2,4,6-trihydroxy-3,5-dimethylbenzophenone 4-*O*-(6″-*O*-galloyl)-*β*-D-glucopyranoside (**2**, guavinoside B), and quercetin 3-*O*-(5″-*O*-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 $C_{26}H_{24}O_{13}$ based on its high-resolution (HR)-FAB-MS (found 545.8418, calcd. 545.8413 for $C_{26}H_{25}O_{13}$). 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 ^{1}H-NMR spectrum of **1**, two aromatic signals at δ_H 6.11 (2H, s) and 6.98, (2H, s) indicated the presence of two 1,3,4,5-tetrasubstituted phenyl groups, and the spin system of δ_H 7.68 (2H, dd, J = 7.0, 1.0 Hz), 7.46 (2H, t, J = 7.0 Hz), and 7.57 (1H, t,

K. Matsuzaki · K. Kobiyama · S. Kitanaka (✉)
Research Unit of Pharmacognosy, College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan
e-mail: kitanaka.susumu@nihon-u.ac.jp

R. Ishii
Saitama Institute of Public Health, Kami-Ohkubo, Saitama 338-0824, Japan

$J = 7.0$ Hz) showed the presence of a phenyl group. Additionally, an anomeric proton signal at δ_H 4.86 (1H, d, $J = 7.6$ Hz) indicated that the glucose residue was in the β-form. Correlations in the ^{1}H–^{1}H-COSY spectrum were observed from the anomeric proton to δ_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 β-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 (δ_C 100.5), four oxymethines (δ_C 73.0, 76.1, 68.9, and 73.6), an oxymethylene (δ_C 62.5), and two carbonyl carbons (δ_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 (δ_H 6.98, 2H, s) to C-1‴ (δ_C 119.2), C-3‴, 5‴ (δ_C 145.4), C-4‴ (δ_C 138.4), and a carbonyl carbon (δ_C 165.7) revealed the presence of a galloyl moiety. A phenyl proton signal at δ_H 7.68 (2H, dd, $J = 7.0$, 1.0 Hz, 2′, 6′-H) correlated with a carbonyl carbon signal at δ_C 195.3, and an aromatic proton at δ_H 6.11 (2H, s, 3, 5-H) correlated with C-4 (δ_C 159.8) and the carbonyl carbon by weak ^4J 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 δ_H 4.39 (2H, br s), and correlated with the galloyl carbonyl carbon signal at δ_C 165.7 in the HMBC experiment. From these data, the structure of **1** was established to be 2,4,6-dihydroxybenzophenone 4-*O*-(6″-*O*-galloyl)-β-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 $C_{28}H_{28}O_{13}$ 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_2H_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**)

	1		**2**	
	δ_C	δ_H	δ_C	δ_H
Aglycone				
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				
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)
				4.32 (1H, d, $J = 11.0$ Hz)
Galloyl				
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

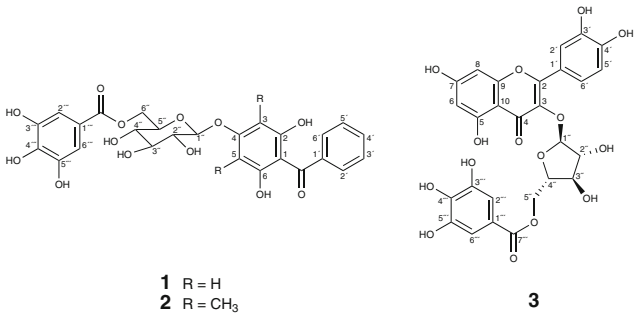

Fig. 1 Structures of guavinosides A (**1**), B (**2**), and C (**3**)

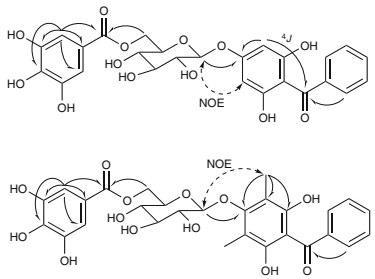

Fig. 2 Key HMBC and NOE correlations of guavinosides A (**1**) and B (**2**)

absence of the aromatic methine signal [δ_H 6.11 (s); δ_C 94.9] in **1**, and a new aryl methyl signal [δ_H 2.00 (6H, s); δ_C 9.9] and a quaternary carbon (δ_C 110.8) were observed. In the ^{13}C-NMR spectrum, the appearance of a high-field region shifted methyl signal suggested that the methyl is linked to a benzene ring in the *ortho*-position and attached via an oxygen atom [9]. HMBC correlations were observed from the methyl proton signal to δ_C 110.8 (C-3, 5), 151.8 (C-2, 6), and 155.6 (C-4). NOE enhancement was also observed between the methyls and an anomeric proton at δ_H 4.63. These data suggested that the aglycone of **2** was 2,4,6-trihydroxy-3,5-dimethylbenzophenone. Absolute configuration of the glucose moiety was determined to be D by using HPLC analysis with an OR detector. From the above data, the structure of **2** was identified to be 2,4,6-trihydroxy-3,5-dimethylbenzophenone 4-*O*-(6″-*O*-galloyl)-*β*-D-glucopyranoside.

Guavinoside C (**3**) was obtained as a yellow powder. Its HR-FAB-MS showed a quasimolecular ion peak at *m/z* 585.0868, corresponding to the molecular formula $C_{27}H_{22}O_{15}$. The UV absorbances at 211, 265, and 355 nm were characteristic of flavonol. The IR spectrum indicated the presence of hydroxyl (3,400 cm^{-1}), ester (1,710 cm^{-1}), and conjugated carbonyl group (1,690 cm^{-1}). In the ^{1}H-NMR spectrum, *meta*-coupled signals at δ_H 6.20 and δ_H 6.41 and a hydrogen-bonded hydroxyl signal at δ_H 12.62 indicated the presence of a 5,7-dihydroxy A ring system in flavonol. A spin system of three aromatic signals at δ_H 7.46 (1H, d, J = 2.2 Hz), δ_H 6.85 (1H, d, J = 8.8 Hz), and δ_H 7.49 (1H, dd, J = 8.8, 2.2 Hz) indicated the presence of a 3′,4′-dihydroxy B ring system in flavonol. In the ^{13}C-NMR spectrum, significant flavonol signals at δ_C 157.3 (C-2), 133.0 (C-3), and 177.4 (C-4) were observed. In the HMBC experiment, the correlations from 2′-H to C-2, δ_C 144.9 (C-3′), 148.3 (C-4′), and 121.3 (C-6′), from 6′-H to C-2, C-4′ and δ_C 115.6 (C-2′), and from 5′-H to C-3′, C-4′, and δ_C 120.8 (C-1′) were observed. From these data, the aglycone of **3** was determined to be quercetin. In addition, an aromatic methine (δ_H 6.89, 2H, s) correlated with δ_C 118.9 (C-1‴), 108.5 (C-2‴, 6‴), 145.3 (C-3‴, 5‴), 138.4 (C-4‴), 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 OR detector comparison to an authentic sample of L-arabinose. The small coupling constant of the anomeric proton (δ_H 5.56, d, J = 1.4 Hz) indicated the presence of the α-form of arabinose. Correlations in the ^{1}H–^{1}H COSY spectrum were observed for a spin system from the anomeric signal to three oxymethine signals at δ_H 4.18 (br s, 2″-H), 3.81 (m, 3″-H), and 3.74 (m, 4″-H), and methylene signals (δ_H 4.11 and 4.02, 5″-H$_2$) was observed. Furthermore, two hydroxy signals at δ_H 5.72 (br s) and 5.48 (br s) both coupled with 2″-H and 3″-H, respectively. Additionally, the signals of 5″-H$_2$ of **3** were shifted downfield compared with those of **4** (δ_H 3.36 and 3.32), and correlated with a galloyl carbonyl carbon signal in the HMBC experiment. From these observations, the sugar moiety was determined to be L-α-arabinofuranose. Thus, the structure of **3** was determined to be quercetin 3-*O*-(5″-*O*-galloyl)-α-L-arabinofuranoside.

The structures of **4**–**8** were elucidated to be quercetin 3-*O*-α-L-arabinofuranoside (**4**), quercetin 3-*O*-α-L-arabinopyranoside (**5**), quercetin 3-*O*-*β*-D-xylopyranoside (**6**), quercetin 3-*O*-*β*-D-galactopyranoside (**7**), and quercetin 3-*O*-*β*-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 dimethylbenzophene 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 μg/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 μg/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. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were obtained on a Varian Inova^{TM} 600 spectrometer and a JEOL Delta 600 spectrometer. NMR spectra were measured in DMSO-d_6 with TMS as internal standard. Optical rotation was measured with a Jasco DIP-370 polarimeter. The inhibitory activities against histamine release from rat mast cells and NO production were carried out as described in the literature method [14].

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 $CHCl_3$–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–H_2O (5:35:60) 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–H_2O (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 $CHCl_3$–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–H_2O (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. $[\alpha]_D^{26}$ -114° ($c = 0.1$, MeOH); FAB-MS *m/z* 545 $(M + H)^+$; HR-FAB-MS (found 545.8418, calcd for $C_{26}H_{25}O_{13}$: 545.8413); UV λ_{max}^{MeOH} nm (ε): 218 (26,800), 288 (16,200); IR υ_{max}^{KBr} cm^{-1}: 3,390 (OH), 1,700 (ester). ^{1}H- and ^{13}C-NMR data, see Table 1.

Guavinoside B (**2**) was obtained as a yellow powder; $[\alpha]_D^{26}$ -83° ($c = 0.5$, MeOH); FAB-MS *m/z* 571 $(M - H)^-$; HR-FAB-MS (found 571.1474, calcd for $C_{28}H_{27}O_{13}$: 571.1451); UV λ_{max}^{MeOH} nm (ε): 218 (26,400), 283 (15,100); IR υ_{max}^{KBr} cm^{-1}: 3,410 (OH), 1,710 (ester), 1,690 (C=O). ^{1}H- and ^{13}C-NMR data, see Table 1.

Guavinoside C (**3**) was obtained as a yellow powder; $[\alpha]_D^{26}$ -73° ($c = 1.0$, MeOH); FAB-MS *m/z* 585 $(M - H)^-$; HR-FAB-MS (found 585.0868, calcd for $C_{27}H_{21}O_{15}$: 585.0880); λ_{max}^{MeOH} nm (ε): 211 (32,000), 265 (16,200), 355 (10,000); IR υ_{max}^{KBr} cm^{-1}: 3,400 (OH), 1,710 (ester), 1,690 (C=O). ^{1}H- and ^{13}C-NMR data, see Table 2.^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) spectra were measured in DMSO-d_6 with TMS as internal standard

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 H_2O, and extracted with an equal volume of EtOAc, and the water layer was evaporated to dryness. The residue was dissolved in H_2O (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–H_2O (75:25); flow rate: 1.0 ml/min.; detection: OR detector (Shodex

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

No.	δ_C	δ_H
2	157.3 s	
3	133.0 s	
4	177.4 s	
5	161.9 s	
6	98.5 d	6.20 (1H, d, J = 1.5 Hz)
7	164.1 s	
8	93.5 d	6.41 (1H, d, J = 1.5 Hz)
9	156.3 s	
10	103.9 s	
1′	120.8 s	
2′	115.6 d	7.46 (1H, d, J = 2.2 Hz)
3′	144.9 s	
4′	148.3 s	
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)
		4.02 (1H, dd, J = 12.0, 5.8 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	
C=O	165.4 s	

OR-2, Showa Denko, Tokyo, Japan) and RI (Shodex RI-72, Showa Denko, Tokyo, Japan), column temperature: 40°C]. Retention time and optical rotation of these samples (**1**, **2**, and **3**) were found to be 8.5 min (positive), 8.5 min (positive), and 6.0 min (positive), respectively. Retention time of standard samples of (+)-D-glucose, (+)-L-arabinose, (−)-D-arabinose, and (+)-D-xylose were found to be 8.5 min (positive), 6.0 min (positive), 6.0 min (negative), and 6.3 min (positive), respectively.

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