Chemistry of Natural Compounds, Vol. 48, No. 3, July, 2012 [Russian original No. 3, May–June, 2012]

PHENOLIC COMPOUNDS FROM THE LEAVES OF *Psidium guajava* II. QUERCETIN AND ITS GLYCOSIDES

Byoung-Jae Park,[1] Tomohiko Matsuta,[2] Tsutomu Kanazawa,[3] Cheol-Ho Park,[4] Kwang-Jin Chang,[5] and Michio Onjo[1]*

UDC 547.972

Psidium guajava L.(Myrtaceae) has been used widely as food and traditional medicine [1]. The leaves of *P. guajava* have been reported to have some pharmacological activities such as anti-inflammatory, antidiarrhea, antioxidant, antimutagenic, anticough, antimicrobial, antiplaque, antirotavirus activity, etc. [1–5]. In addition, the leaves are used at present as beverage and tea.

In the previous studies, we have isolated the tannin and benzophenone glycosides. To continue, we investigated the composition of the phenolic compound, especially quercetin and its glycosides, from the leaf of *P. guajava*.

The leaves of *P. guajava* were obtained from plants cultivated in the Ibuski Experimental Botanical Garden, Faculty of Agriculture, Kagoshima University, Japan, in May 2008, dried at room temperature, and ground into a fine powder. Eighty grams of powder 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 extract (20.56 g). The crude extract (15 g) was suspended in 500 mL water and then defatted with 500 mL *n*-hexane 3 times to give 0.57 g of the hexane-soluble layer. The hexane-insoluble layer was partitioned by EtOAc, then by *n*-BuOH solution, to give an EtOAc-soluble fraction (3.08 g) and an *n*-BuOH-soluble fraction (4.15g). The remaining water fraction was concentrated to give 7.16 g of a water-soluble fraction.

The EtOAc-soluble fraction (2.5 g) was applied to an MCI GEL CHP-20 (4.0 cm i.d × 18 cm) column using a H_2O–MeOH mixture (2 L each), then collected 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 a Shimadzu (Kyoto, Japan) preparative HPLC system comprising an LC-6A pump, and an SPD-10A detector. The separation column was TSK gel ODS-80TM (21.5 mm i.d. × 30 cm, Tosoh) with solvent H_2O–acetonitorile–formic acid (95:5:1) and/or H_2O–acetonitrile–formic acid (85:15:1).

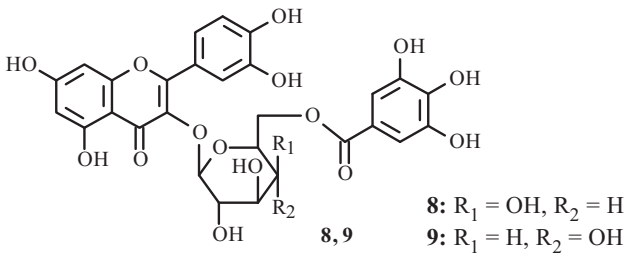
^1H and ^{13}C NMR spectra were measured with a JEOL α-500 spectrometer in methanol-d_4 at 30°C. HR-ESI-TOF-MS spectra were recorded on a JEOL JMS-T100LC spectrometer.

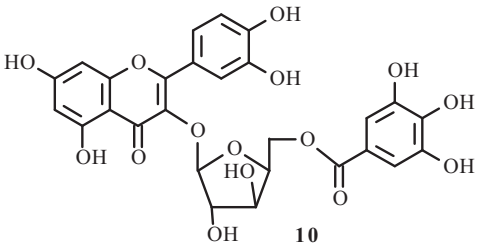
Quercetin (1). Yellow powder, HR-ESI-TOF-MS *m/z* 303.0429 $[M + H]^+$ [6, 7].

Quercetin 3-*O*-β-Glucoside (Isoquercitrin) (2). Yellow powder. HR-ESI-TOF-MS *m/z* 465.1009 $[M + H]^+$ (calcd for $C_{21}H_{21}O_{12}$, 465.1033), 303 $[M + H – 162]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.70 (d, J = 2.0, H-2′), 7.57 (dd, J = 2.0, 8.3, H-6′), 6.86 (d, J = 8.3, H-5′), 6.38 (d, J = 2.0, H-8), 6.19 (d, J = 2.0, H-6), 5.23 (d, J = 7.8, H-1″), 3.70 (dd, J = 2.4, 11.7, H-6′), 3.57 (dd, J = 5.4, 11.7, H-6″), 3.47 (dd, J = 7.8, 8.8, H-2″), 3.42 (t, J = 8.8, H-3″), 3.34 (dd, J = 8.8, 9.3, H-4″), 3.21 (ddd, J = 2.4, 5.4, 9.3, H-5″). ^{13}C NMR (125 MHz, δ, ppm): 179.5 (C-4), 166.1 (C-7), 163.0 (C-5), 159.0 (C-2), 158.4 (C-9), 149.8 (C-4′), 145.9 (C-3′), 135.6 (C-3), 123.2 (C-12), 123.1 (C-6′), 117.6 (C-5′), 116.0 (C-2′), 105.6 (C-10), 104.4 (C-1″), 99.9 (C-6), 94.8 (C-8), 78.3 (C-5″), 78.1 (C-3″), 75.7 (C-2″), 71.2 (C-4″), 62.6 (C-6″) [8, 9].

Quercetin 3-*O*-β-Galactoside (Hyperoside) (3). Yellow powder. HR-ESI-TOF-MS *m/z* 465.1008 $[M + H]^+$ (calcd for $C_{21}H_{21}O_{12}$, 465.1033), 303 $[M + H – 162]^+$. ^1H NMR (500 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.66 (dd, J = 2.0, 8.3, H-6′), 7.52 (d, J = 2.0, H-2′), 6.81 (d, J = 8.3, H-5′), 6.39 (d, J = 1.5, H-8), 6.19 (d, J = 1.5, H-6), 5.36 (d, J = 7.8, H-1″), 3.64 (br.d, J = 3.4, H-4″), 3.56 (dd, J = 7.8, 9.3, H-2″), 3.45 (dd, J = 5.4, 10.2, H-6″), 3.36 (dd, J = 3.4, 9.3, H-3″), 3.31 (m, H-5″), 3.28

1) Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, 890-0065, Kagoshima, Japan, e-mail: parkbj71@mail.goo.ne.jp; 2) Meikai University School of Dentistry, 1-1 Keyakidai, Sakado, 350-0283, Saitama, Japan; 3) Harunire Bio Laboratory Co., Ltd., 603-2 Hakuyo-cho, 090-0013, Kitami, Japan; 4) Dept. of Bio-Health Technology, Kangwon National University, 200-701, Chuncheon, Korea; 5) Korea National University of Agricultural & Fisheries, 445-893, Hwasung, Korea. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 429–431. Original article submitted February 1, 2011.





(m, H-6″). ^{13}C NMR (125 MHz, δ, ppm): 177.4 (C-4), 164.6 (C-7), 161.0 (C-5), 156.4 (C-2), 156.2 (C-9), 148.4 (C-4′), 144.8 (C-3′), 133.6 (C-3), 122.0 (C-1′), 121.2 (C-6′), 116.0 (C-5′), 115.2 (C-2′), 103.8 (C-10), 102.0 (C-1″), 98.8 (C-6), 93.6 (C-8), 75.8 (C-5″), 73.2 (C-3″), 71.2 (C-2″), 67.9 (C-4″), 60.1 (C-6″) [7–9].

Quercetin 3-*O*-α-Rhamnoside (Quercitrin) (4). Yellow powder. HR-ESI-TOF-MS *m/z* 449.1061 $[M + H]^+$ (calcd for $C_{21}H_{21}O_{11}$, 449.1084), 303 $[M + H - 146]^+$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.33 (d, J = 1.5, H-2′), 7.30 (dd, J = 1.5, 8.3, H-6′), 6.90 (d, J = 8.3, H-5′), 6.36 (br.s, H-8), 6.20 (br.s, H-6), 5.35 (br.s, H-1″), 4.21 (br.s, H-2″), 3.74 (dd, J = 2.9, 9.3, H-3″), 3.41 (m, H-5″), 3.30 (m, H-4″), 0.93 (3H, d, J = 5.9, H-6″). ^{13}C NMR (125 MHz, δ, ppm): 179.7 (C-4), 165.8 (C-7), 163.2 (C-5), 159.3 (C-2), 158.5 (C-9), 149.8 (C-4′), 146.4 (C-3′), 136.2 (C-3), 123.0 (C-1′), 122.9 (C-6′), 117.0 (C-2′), 116.4 (C-5′), 105.9 (C-10), 103.6 (C-1″), 99.8 (C-6), 94.7 (C-8), 73.3 (C-4″), 72.1 (C-3″), 72.0 (C-2″), 71.9 (C-5″), 17.6 (C-6″) [9].

Quercetin 3-*O*-α-Arabinopyranoside (Guaijaverin) (5). Yellow powder. HR-ESI-TOF-MS *m/z* 435.0907 $[M + H]^+$ (calcd for $C_{20}H_{19}O_{11}$, 435.0927), 303 $[M + H - 132]^+$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.73 (d, J = 2.0, H-2′), 7.57 (dd, J = 2.0, 8.4, H-6′), 6.86 (d, J = 8.4, H-5′), 6.39 (d, J = 2.0, H-8), 6.20 (d, J = 2.0, H-6), 5.15 (d, J = 6.4, H-1″), 3.89 (dd, J = 6.4, 8.2, H-2″), 3.81 (m, H-4″), 3.81 (m, H-5″), 3.64 (dd, J = 3.1, 8.2, H-3″), 3.43 (dd, J = 3.1, 13.5, H-5″). ^{13}C NMR (125 MHz, δ, ppm): 179.5 (C-4), 166.0 (C-7), 163.1 (C-5), 158.7 (C-2), 158.4 (C-9), 150.0 (C-4′), 146.0 (C-3′), 135.7 (C-3), 123.1 (C-1′), 122.9 (C-6′), 117.5 (C-2′), 116.2 (C-5′), 105.7 (C-10), 104.6 (C-1″), 99.9 (C-6), 94.7 (C-8), 74.1 (C-2″), 72.9 (C-3″), 69.1 (C-4″), 66.9 (C-5″) [6, 9].

Quercetin 3-*O*-α-Arabinofuranoside (Avicularin) (6). Yellow powder. HR-ESI-TOF-MS *m/z* 435.0907 $[M + H]^+$ (calcd for $C_{20}H_{19}O_{11}$, 435.0927), 303 $[M + H - 132]^+$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.52 (d, J = 2.0, H-2′), 7.48 (dd, J = 2.0, 8.3, H-6′), 6.90 (d, J = 8.3, H-5′), 6.39 (d, J = 2.0, H-8), 6.20 (d, J = 2.0, H-6), 5.46 (br.s, H-1″), 4.32 (br.d, J = 2.9, H-2″), 3.90 (dd, J = 2.9, 4.9, H-3″), 3.86 (br.q, J = 4.9, H-4″), 3.51 (dd, J = 3.4, 11.7, H-5″), 3.48 (dd, J = 4.4, 11.7, H-5″). ^{13}C NMR (125 MHz, δ, ppm): 180.0 (C-4), 166.0 (C-7), 163.1 (C-5), 159.4 (C-2), 158.6 (C-9), 149.8 (C-4′), 146.4 (C-3′), 134.9 (C-3), 123.1 (C-1′), 123.0 (C-6′), 116.9 (C-2′), 116.5 (C-5′), 109.6 (C-1″), 105.6 (C-10), 99.9 (C-6), 94.8 (C-8), 88.0 (C-4″), 83.3 (C-2″), 78.7 (C-3″), 62.6 (C-5″) [7, 8].

Quercetin 3-*O*-β-Xylopyranoside (7). Yellow powder. HR-ESI-TOF-MS *m/z* 435.0907 $[M + H]^+$ (calcd for $C_{20}H_{19}O_{11}$, 435.0927), 303 $[M + H - 132]^+$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.60 (d, J = 2.0, H-2′), 7.57 (dd, J = 2.0, 8.3, H-6′), 6.85 (d, J = 8.3, H-5′), 6.37 (d, J = 1.5, H-8), 6.19 (d, J = 1.5, H-6), 5.17 (d, J = 7.4, H-1″), 3.78 (dd, J = 5.3, 11.7, H-5″), 3.51 (t, J = 6.8, H-2″), 3.49 (dd, J = 5.3, 9.3, H-4″), 3.40 (t, J = 8.8, H-3″), 3.10 (dd, J = 9.3, 11.7, H-5″). ^{13}C NMR (125 MHz, δ, ppm): 179.4 (C-4), 166.0 (C-7), 163.0 (C-5), 158.9 (C-2), 158.4 (C-9), 149.7 (C-4′), 146.0 (C-3′), 135.4 (C-3), 123.3 (C-1′), 123.0 (C-6′), 117.2 (C-2′), 116.0 (C-5′), 105.6 (C-10), 104.7 (C-1″), 99.9 (C-6), 94.7 (C-8), 77.5 (C-3″), 75.3 (C-2″), 71.0 (C-4″), 67.2 (C-5″) [10, 11].

Quercetin 3-*O*-(6″-*O*-Galloyl)-β-galactoside (8). Yellow powder. HR-ESI-TOF-MS *m/z* 617.1087 $[M + H]^+$ (calcd for $C_{28}H_{25}O_{16}$, 617.1143), 303 $[M + H - 314]^+$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.64 (dd, J = 2.4, 8.3, H-6′), 7.51 (d, J = 2.4, H-2′), 6.86 (2H, s, H-2‴, 6‴), 6.81 (d, J = 8.3, H-5′), 6.37 (d, J = 2.0, H-8), 6.17 (d, J = 2.0, H-6), 5.34 (d, J = 7.8, H-1″), 4.12 (dd, J = 7.3, 10.7, H-6″), 4.03 (dd, J = 5.9, 10.7, H-6″), 3.72 (m, H-4″), 3.72 (m, H-5″), 3.60 (dd, J = 7.8, 9.3, H-2″), 3.44 (dd, J = 2.9, 9.3, H-3″). ^{13}C NMR (125 MHz, δ, ppm): 177.3 (C-4), 165.4 (C-7‴), 164.4 (C-7), 161.1 (C-5), 156.4 (C-2), 156.3 (C-9), 148.6 (C-4′), 145.5 (C-3‴, 5‴), 144.8 (C-3′), 138.5 (C-4‴), 133.5 (C-3), 121.9 (C-1′), 120.9 (C-6′), 119.0 (C-1‴), 115.9 (C-2′), 115.1 (C-5′), 108.5 (C-2‴, 6‴), 103.7 (C-10), 102.2 (C-1″), 98.7 (C-6), 93.5 (C-8), 72.8 (C-5″), 72.4 (C-3″), 71.0 (C-2″), 67.7 (C-4″), 62.0 (C-6″).

Quercetin 3-*O*-(6″-*O*-Galloyl)-β-glucoside (9). Yellow powder. HR-ESI-TOF-MS *m/z* 617.1147 $[M + H]^+$ (calcd for $C_{28}H_{25}O_{16}$, 617.1143), 303 $[M + H - 314]^+$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.54 (dd, J = 2.4, 8.3, H-6′), 7.52 (d, J = 2.4, H-2′), 6.93 (2H, s, H-2‴, 6‴), 6.72 (d, J = 8.3, H-5′), 6.33 (d, J = 2.0, H-8), 6.17 (d, J = 2.0, H-6), 5.19 (d, J = 7.4, H-1″), 4.33 (dd, J = 5.4, 11.7, H-6″), 4.26 (dd, J = 2.0, 11.7, H-6″), 3.49 (m, H-2″), 3.45 (m, H-3″), 3.38 (m, H-4″), 3.32 (m, H-5″). ^{13}C NMR (125 MHz, δ, ppm): 179.3 (C-4), 165.7 (C-7‴), 166.4 (C-7), 162.0 (C-5), 157.7 (C-2), 157.2 (C-9), 149.0 (C-4′), 145.4

(C-3‴, 5‴), 145.3 (C-3′), 138.8 (C-4‴), 135.3 (C-3), 123.0 (C-1′), 122.1 (C-6′), 120.2 (C-1‴), 116.8 (C-2′), 115.4 (C-5′), 108.9 (C-2‴, 6‴), 104.6 (C-10), 103.5 (C-1″), 98.9 (C-6), 94.5 (C-8), 73.8 (C-5″), 73.6 (C-3″), 71.9 (C-2″), 69.6 (C-4″), 62.5 (C-6″).

Quercetin 3-*O*-(5″-*O*-Galloyl)-α-arabinofuranoside (10). Yellow powder. HR-ESI-TOF-MS *m/z* 587.1004 $[M + H]^+$ (calcd for $C_{27}H_{22}O_{15}$, 587.1037), 303 $[M + H - 284]^+$. ^1H NMR (500 MHz, CD_3OD, δ, ppm, J/Hz): 7.50 (d, J = 2.2, H-2′), 7.46 (dd, J = 2.2, 8.4, H-6′), 6.99 (2H, s, H-2‴, 6‴), 6.87 (d, J = 8.4, H-5′), 6.38 (d, J = 2.0, H-8), 6.20 (d, J = 2.0, H-6), 5.49 (d, J = 1.1, H-1″), 4.38 (dd, J = 1.1, 3.1, H-2″), 4.20 (dd, J = 3.3, 11.9, H-5″), 4.12 (dd, J = 5.3, 11.9, H-5″), 3.95 (m, H-3″), 3.95 (m, H-4″). ^{13}C NMR (125 MHz, δ, ppm): 179.8 (C-4), 168.0 (C-7‴), 166.0 (C-7), 163.0 (C-5), 159.8 (C-9), 158.6 (C-2), 149.7 (C-4′), 146.4 (C-3‴, 5‴), 146.2 (C-3′), 139.9 (C-4‴), 134.9 (C-3), 123.1 (C-6′), 123.0 (C-1‴), 121.2 (C-1′), 117.0 (C-2′), 116.5 (C-5′), 110.3 (C-2‴, 6‴), 109.6 (C-1″), 105.7 (C-10), 99.9 (C-6), 94.8 (C-8), 84.5 (C-4″), 83.6 (C-2″), 79.2 (C-3″), 64.6 (C-5″) [11].

In the ^1H NMR spectrum of compound **1**, *meta*-coupled signals at δ 6.37 (d, J = 2.0 Hz) and δ 6.17 (d, J = 2.0 Hz) of the 5,7-dihydroxyl A ring system and three aromatic signals at δ 7.72 (d, J = 2.2 Hz), δ 7.62 (dd, J = 2.2, 8.4 Hz), and δ 6.88 (d, J = 8.4 Hz) of the 3,4-dihydroxy B ring system in flavonol were attributed to quercetin [6, 7]. In addition, the stucture of compounds **2–7** exhibited the aglycone and single sugar moiety characteristic of quercetin. Base on the NMR, MS spectra data, and comparison with literature data, the sugar moieties of compounds **2–7** were assigned to β-glucose, β-galactose, α-rhamnose, α-arabinopyranose, α-arabinofuranose, and β-xylopyranose [6–11]. The COSY, HSQC, and HMBC spectra allowed assignment of all ^1H and ^{13}C NMR signals. In the HMBC spectrum of compounds **2–7**, the H-1″ proton of the sugar moieties showed a correlation with C-3 of quercetin. Therefore, compounds **2–7** were identified as quercetin 3-*O*-β-glucoside (**2**), quercetin 3-*O*-β-galactoside (**3**), quercetin 3-*O*-α-rhamnoside (**4**), quercetin 3-*O*-α-arabinopyranoside (**5**), quercetin 3-*O*-α-arabinofuranoside (**6**), and quercetin 3-*O*-β-xylopyranoside (**7**).

The structure of compound **8** was assigned to quercetin based on the aglycone moiety and one galactose moiety. In addition, the ^{13}C NMR spectrum exhibited typical signals for a galloyl moiety at δ 165.4 (C-7‴), δ 145.5 (C-3‴, 5‴), δ 138.5 (C-4‴), δ 119.0 (C-1‴), and δ 108.5 (C-2‴, 6‴). In the HMBC spectrum of compound **8**, C-7‴ (δ 165.4) of the galloyl moiety showed long-range correlations with the H-6″ (δ 4.12, 4.03) and H-2‴ (δ 6.86), H-6‴ (δ 6.86). The H-1″ proton of β-galactose (δ 5.34) showed a correlation with C-3 (δ 133.5). On the basis of the NMR and MS spectral data, compound **8** was identified as quercetin 3-*O*-(6″-*O*-galloyl)-β-galactoside.

In the HMBC spectrum of compound **9**, C-7‴ (δ 165.7) of the galloyl moiety showed long-range correlations with the H-6″ (δ 4.33, 4.26), H-2‴ (δ 6.93), and H-6‴ (δ 6.93). The H-1″ proton of β-glucose (δ 5.19) showed a correlation with C-3 (δ 135.3). On the basis of the NMR and MS spectral data, compound **9** was identified as quercetin 3-*O*-(6″-*O*-galloyl)-β-glucoside.

The structure of compound **10** was characterized as quercetin 3-*O*-(5″-*O*-galloyl)-α-arabinofuranoside by comparison with literature data [11].

Although compounds **1–7** and **10** in *P. guajava* [6, 8–11] have already been reported, compounds **8** and **9** were isolated from *P. guajava* for the first time in this investigation.

REFERENCES

1. R. M. P. Guierrez, S. Mitchell, and R. V. Solis, *J. Ethnoparmarcol.,* **117**, 1 (2008).
2. P. Jaiarj, P. Khoohaswan, Y. Wongkrajang, P. Peungvicha, P. Suriyawong, M. L. S. Saraya, and O. Ruangsomboon, *J. Ethnoparmarcol.,* **67**, 203 (1999).
3. O. A. Olajide, S. O. Awe, and J. M. Makinde, *Fitoterapia*, **70**, 25 (1999).
4. X. Lozoya, H. Reyes-Morales, M. A Chavez-Soto, M. C. Martinez-Garcнa, Y. Soto-Gonzalez, and S. V. Doubova, *J. Ethnopharmacol.,* **83**, 19 (2002).
5. J. L. Goncalves, R. C. Lopes, D. B. Oliveira, S. S. Costa, M. M. Miranda, M. T. Romano, N. S. Santos, and M. D. Wigg, *J. Ethnoparmarcol.,* **403**, 3 (2005).
6. H. Arima and G. Danno, *Biosci. Biotechnol. Biochem*., **66**, 1727 (2002).
7. Z. P. Xiao, H. K. Wu, T. Wu, H. Shi, B. Bang, and H. A. Aisa, *Chem. Nat. Comp*., **42**, 736 (2006).
8. Q. Liang, H. Qian, and W. Yao, *Eur. J. Mass Spectrom.*, **11**, 93 (2005).
9. X. Lozoya, M. Meckes, M. Abou-Zaid, J. Tortoriello, C. Nozzolillo, and J. T. Arnason, *Arch. Med. Res.,* **25**, 11 (1994).
10. M. Olszewska and M. Wolbis, *Acta Pol. Pharm.-Drug Res.*, **59**, 133 (2002).
11. K. Matsuzaki, R. Ishii, K. Kobiyama, and S. Kitanaka, *J. Nat. Med*., **64**, 252 (2010).