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Chemical Constituents from the Fruits of *Prunus mume*

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Abstract – The chromatographic separation of a methanol extract of *Prunus mume* (Rosaceae) led to the isolation of 5-hydroxymethyl-2-furaldehyde (**1**), 4-*O*-caffeoylquinic acid methyl ester (**2**), prunasin (**3**), 5-*O*-caffeoylquinic acid methyl ester (**4**), benzyl-*O*-β-D-glucopyranoside (**5**), and liquiritigenin-7-*O*-β-D-glucopyranoside (**6**). Their structures were determined by 1D, 2D-NMR and MS data analysis as well as by comparison of their data with the published values.
Keywords – *Prunus mume*, Rosaceae, liquiritigenin-7-*O*-β-D-glucopyranoside

Introduction

Prunus mume Sieb. et Zucc. (Rosaceae) is a deciduous tree which is native to China and other eastern Asian countries and has been widely cultivated and its fruits are consumed as preserved fruits and drink in Korea. The immature fruit has been used in traditional Korean medicinal preparations as a remedy for fever, cough and intestinal disorder (Jung and Shin, 1990). It has recently been reported that *P. mume* has blood fluidity improving activity (Chuda *et al*., 1999), anti-fatigue activity (Kim *et al*., 2008), immune function enhancing activity (Tsuju *et al*., 2011), anti-microbial activity (Xia *et al*., 2011), and cancer cell inhibitory activities (Jeong *et al*., 2006, Tada *et al*., 2012).

Previous phytochemical investigations of *P. mume* have reported to the isolation of citric acid as the major component and other diverse compounds, such as benzyl glucoside, chlorogenic acid derivatives, and 5-hydroxymethyl-2-furaldehyde derivatives (Tang and Eisenbrand, 2011, Choi *et al*., 2007, Ina *et al*., 2004, Chuda *et al*., 1999).

However, limited studies on the polar fractions of this plant have been performed. Therefore, this work deals with the isolation and identification of six known compounds, 5-hydroxymethyl-2-furaldehyde (**1**), 4-*O*-caffeoylquinic acid methyl ester (**2**), prunasin (**3**), 5-*O*-caffeoylquinic acid methyl ester (**4**), benzyl-*O*-β-D-glucopyranoside (**5**), and liquiritigenin-7-*O*-β-D-glucopyranoside (**6**) from the water extract of *P. mume*. Liquiritigenin-7-*O*-β-D-glucopyranoside (**6**) was isolated from this plant for the first time.

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Experimental

General experimental procedures – Optical rotations were determined on JASCO DIP-370 polarimeter at 25 °C. UV spectra were obtained using a JASCO UV-550. NMR spectra were obtained using a Bruker AMX-500 MHz NMR spectrometer. ESI-MS was recorded on Waters Q-TOF micro mass spectrometer. Open column chromatography was performed using a silica gel 60 (Kiesel gel 60, 700 - 230 mesh, Merck), a Sephadex LH-20 (25 - 100 μM, Pharmacia), and MCI gel (particle size 75 - 150 μm, Mitsubishi). HPLC was performed using a Waters system (515 pump and 2996 photodiode array detector) and a YMC J'sphere ODS-H80 preparative column (4 μm, 20 × 250 mm i.d.). Thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 (0.25 mm, Merck). All other chemicals and reagents were analytical grade.

Plant material – The fruit of *P. mume* was purchased from a commercial supplier (KwangMyungDang, Ulsan, Korea) in 2010. It was identified by the Herbal Quality Control Team and deposited at the Herbal Medicine Research Division, Korea Institute of Oriental Medicine (Korea).

Extraction and isolation – The dried fruit of *P. mume* was pulverized and extracted with distilled water (2 kg/8 L) for 2 hours below 100 °C in an ultra sound assisted extractor (OM30-EP, Sonimedi). All extracts were

concentrated under vacuum using a rotary evaporator after filtration, and were then dried for 48 hours at 40 ºC using an extract vaccum drier (Exdryer, Sonimedi) to yield an extract powder (32.5 g, yield 16.2%). The water extract was successively partitioned between hexane and EtOAc, respectively. The EtOAc-soluble extract of the fruit of *P. mume* (8 g) were chromatographed on Silica gel, eluting with a gradient of *n*-hexane : CH_2Cl_2 (1 : 1 - 0 : 1) and CH_2Cl_2 : MeOH (1 : 1 - 0 : 1), to give 11 fractions. Compound **1** (20 mg) was isolated from Fr.2 by Sephadex LH-20 column chromatography eluted with MeOH. Fr.7 (500 mg) was further separated on Sephadex LH-20 (MeOH) and prep HPLC (20% MeCN) to give compound **2** (5 mg). Fr.8 (450 mg) was further separated on MCI gel (10% MeOH-100% MeOH) and prep HPLC (5% MeCN-15% MeCN) to give compounds **3** (4 mg), **4** (5 mg) and **5** (4 mg). Fr.9 (120 mg) was further separated on MCI gel (10% MeOH-100% MeOH) and prep HPLC (15% MeCN) to give compound **6** (3 mg).

5-Hydroxymethyl-2-furaldehyde (1) – Colorless oil; ESI-MS *m/z* 149 $[M + Na]^+$; UV (MeOH) λ_{max} nm: 280; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 4.60 (2H, s, H-6), 6.56 (1H, d, J = 3.5 Hz, H-4), 7.36 (1H, d, J = 3.5 Hz, H-3), 9.53 (-CHO); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 56.1 (C-6), 109.4 (C-4), 123.3 (C-3), 152.4 (C-2), 161.7 (C-5), 177.9 (C-1).

4-*O*-Caffeoylquinic acid methyl ester (2) – Amorphous powder; $[\alpha]_D^{25}$ –60.5 (c = 0.1, MeOH); ESI-MS *m/z* 367 $[M - H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 2.01 - 2.28 (4H, m, H-2, 6), 3.77 (3H, s, -OCH_3), 4.29 (2H, m, H-3, 5), 4.84 (1H, dd, J = 3.5, 8.5, H-4), 6.38 (1H, d, J = 16.0, H-8'), 6.80 (1H, d, J = 8.0, H-5'), 6.99 (1H, dd, J = 2.0, 8.0, H-6'), 7.08 (1H, br d, J = 2.0, H-2'), 7.66 (1H, d, J = 16.0, H-7'); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 37.0 (C-6), 40.7 (C-2), 51.5 (OMe), 64.3 (C-3), 67.6 (C-5), 75.0 (C-1), 77.1 (C-4), 113.7 (C-2'), 113.9 (C-8'), 115.1 (C-5'), 121.5 (C-6'), 126.5 (C-1'), 145.4 (C-3'), 145.7 (C-7'), 148.2 (C-4'), 167.5 (C-9'), 174.3 (COO).

Prunasin (3) – Amorphous powder; $[\alpha]_D^{25}$ –54.5 (c = 0.1, MeOH); ESI-MS *m/z* 318 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 3.22 - 3.95 (6H, m, H-2', 3', 4', 5', 6'), 4.27 (1H, d, J = 7.5 Hz, H-1'), 5.93 (1H, s, H-2), 7.48 - 7.62 (5H, m, H-4, 5, 6, 7, 8); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 61.4 (C-6'), 67.0 (C-2), 70.1 (C-4'), 73.4 (C-2'), 76.5 (C-3'), 76.9 (C-5'), 100.6 (C-1'), 118.0 (C-1), 127.6 (C-4, 8), 128.7 (C-5, 7), 129.6 (C-6), 133.5 (C-3).

5-*O*-Caffeoylquinic acid methyl ester (4) – Amorphous powder; $[\alpha]_D^{25}$ –27.6 (c = 0.1, MeOH); ESI-MS *m/z* 391 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 2.00 - 2.24 (4H, m, H-2, 6), 3.75 (3H, s, -OCH_3), 3.70 (1H, m, H-4), 4.13 (1H, m, H-3), 5.38 (1H, m, H-5), 6.33 (1H, d, J = 16.0, H-8'), 6.89 (1H, d, J = 8.0, H-5'), 6.97 (1H, dd, J = 2.0, 8.0, H-6'), 7.06 (1H, br d, J = 2.0, H-2'), 7.61 (1H, d, J = 16.0, H-7'); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 34.9 (C-6), 39.4 (C-2), 51.4 (OMe), 67.2 (C-3), 71.2 (C-5), 73.9 (C-1), 72.4 (C-4), 115.0 (C-2'), 113.7 (C-8'), 114.3 (C-5'), 121.5 (C-6'), 126.5 (C-1'), 145.4 (C-3'), 145.4 (C-7'), 148.1 (C-4'), 167.5 (C-9'), 175.0 (COO).

Benzyl-*O*-β-D-glucopyranoside (5) – Amorphous powder; ESI-MS *m/z* 293 $[M + Na]^+$; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 3.23 - 3.93 (6H, m, H-2', 3', 4', 5', 6'), 4.37 (1H, d, J = 7.5 Hz, H-1'), 4.68 (1H, d, J = 12.0, H-2b), 4.95 (1H, d, J = 12.0, H-2a), 7.27 - 7.45 (5H, m, H-4, 5, 6, 7, 8); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 61.4 (C-6'), 70.3 (C-2'), 73.6 (C-4'), 76.6 (C-5'), 76.7 (C-3'), 101.9 (C-1'), 127.3 (C-6), 127.8 (C-4, 8), 127.8 (C-5, 6), 137.7 (C-3).

Liquiritigenin-7-*O*-β-D-glucopyranoside (6) – Amorphous powder; ESI-MS *m/z* 417 $[M - H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz) δ_H: 2.75 (1H, dt, J = 17.0, 3.0, H-3b), 3.07 (1H, dd, J = 17.0, 13.0, H-3a), 3.2-3.5 (4H, m, H-2", 3", 4", 5"), 3.72 (1H, dd, J = 12.0, 5.5, H-6"b), 3.92 (1H, dd, J = 12.0, 2.0, H-6"a), 5.48 (1H, dd, J = 13.0, 3.0, H-2), 6.39 (1H, br d, J = 2.0, H-8), 6.53 (1H, dd, J = 8.5, 2.0, H-6), 7.46 (2H, d, J = 8.5, H-2', 6'), 7.17 (2H, d, J = 8.5, H-3', 5'), 7.75 (1H, d, J = 8.5, H-5). ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: 100.8 (C-1"), 73.5 (C-2"), 76.8 (C-3"), 70.0 (C-4"), 76.6 (C-5"), 61.1(C-6"), 79.3 (C-2), 43.6 (C-3), 191.7 (C-4), 133.0 (C-5), 110.5 (C-6), 165.5 (C-7), 113.6 (C-8), 164.0 (C-9), 102.5 (C-10), 128.5 (C-1'), 127.4 (C-2', 6'), 116.4 (C-3', 5'), 157.8 (C-4').

Results and Discussion

Repeated column chromatographic separation of the EtOAc-soluble extract of the fruit of *P. mume* resulted in the isolation of six known compounds, 5-hydroxymethyl-2-furaldehyde (**1**), 4-*O*-caffeoylquinic acid methyl ester (**2**), prunasin (**3**), 5-*O*-caffeoylquinic acid methyl ester (**4**), benzyl-*O*-β-D-glucopyranoside (**5**), and liquiritigenin-7-*O*-β-D-glucopyranoside (**6**).

Compound **1**, which was obtained as a colorless oil, exhibited a mass peak at *m/z* 149 $[M + Na]^+$ corresponding to the molecular formula $C_8H_6O_3$. The ^{1}H- and ^{13}C-NMR spectroscopic data suggested the structure of compound **1** to be a two-substituted furan derivative [δ_H 6.56 (H-4) and 7.36 (H-3), δ_C 109.4 (C-4), 123.3 (C-3), 152.4 (C-2), and 161.7 (C-5)]. The remaining NMR data revealed a methylene at δ_H 4.60 (H-6) and δ_C 56.1 (C-6) and an aldehyde group at δ_H 9.53 (CHO) and δ_C 177.9 (C-1). Therefore, compound **1** was identified as 5-hydroxymethyl-

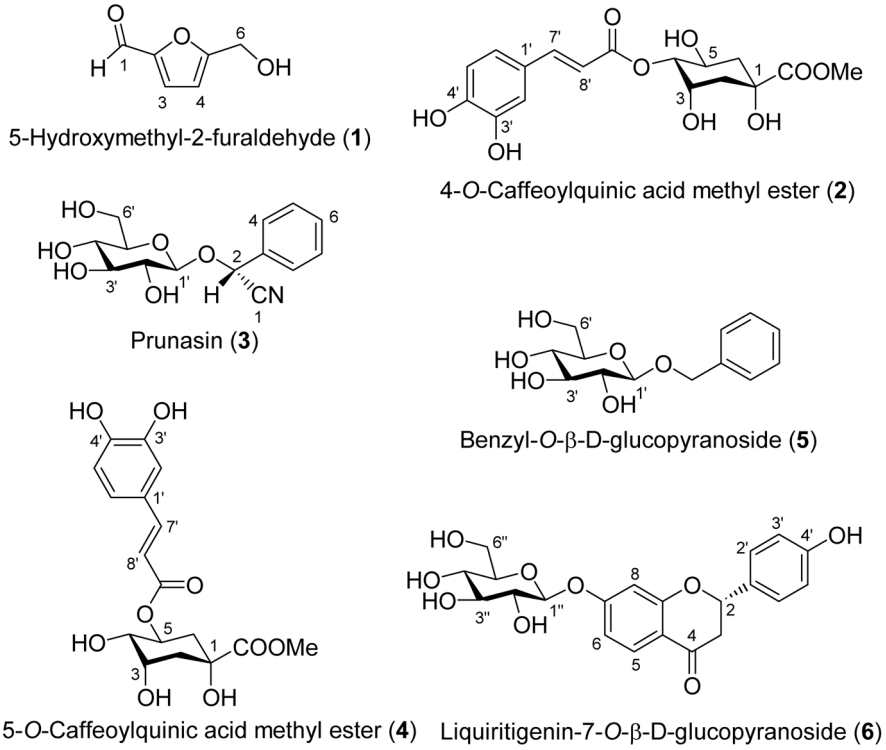

Fig. 1. Structures of compounds **1** - **6**.

2-furaldehyde from the above results and published spectroscopic data (Kang *et al*., 2004).

Compound **2**, an amorphous powder, showed a base peak at *m/z* 367 $[M-H]^-$ in its ESI-MS. The ^{1}H-NMR spectrum exhibited signals belonging to a caffeic acid group at δ_H 7.66 (1H, d, J = 16.0, H-7), 7.08 (1H, br d, J = 2.0, H-2), 6.99 (1H, dd, J = 8.0, 2.0, H-6), 6.80 (1H, d, J = 8.0, H-5), and 6.38 (1H, d, J = 16.0, H-8), a quinic acid group at δ_H 4.84 (1H, dd, J = 8.5, 3.5, H-4), 4.29 (2H, m, H-3, 5), and 2.01-2.28 (4H, m, H-2, 6), and a methoxyl group at δ_H 3.77 (3H, s). The ^{13}C-NMR spectrum of compound **2** showed signals at suggesting the presence of a caffeic acid group at δ_C 126.5 (C-1'), 113.7 (C-2'), 145.4 (C-3'), 148.2 (C-4'), 115.1 (C-5'), 121.5 (C-6'), 145.7 (C-7'), 113.9 (C-8'), and 167.5 (C-9'), a quinic acid group at δ_C 75.0 (C-1), 40.7 (C-2), 64.3 (C-3), 77.1 (C-4), 67.6 (C-5), 37.0 (C-6), 174.3 (COO), and 51.5 (OMe). In the HMBC spectrum, long-range correlations between H-4 (δ_H 4.84) and C-9' (δ_C 167.5) indicated that the caffeic acid group was located at C-4 position of quinic acid. Therefore, compound **2** was identified as 4-*O*-caffeoylquinic acid methyl ester (Nakatani *et al*., 2000, Simoes-Pires *et al*., 2005).

Compound **3** obtained as an amorphous powder with $[M+Na]^+$ peak at *m/z* 318 in the ESI-MS spectrum, which was consistent with the molecular formula of $C_{14}H_{17}NO_6$. The ^{1}H- and ^{13}C-NMR spectra exhibited signals belonging to a benzene ring [δ_H 7.48 (H-5, 6, 7) and 7.61 (H-4, 8), δ_C 127.6 (C-4, 8), 128.7 (C-5, 7) 129.6 (C-6), 133.5 (C-3)], a glucose [δ_H 4.27 (H-1'), 3.93 (H-6'a), 3.72 (H-6'b), 3.22-3.31 (H-2', 3', 4', 5'), δ_C 61.4 (C-6'), 70.1 (C-4'), 73.4 (C-2'), 76.5 (C-3'), 76.9 (C-5'), 100.6 (C-1')], and a CH-CN group [δ_H 5.93 (H-2), δ_C 118.0 (C-1) and 67.0 (C-2)]. Therefore, compound **3** was identified as prunasin by comparing the spectroscopic data with those reported in the literature (Fukuda *et al*., 2003, Song *et al*., 2008).

Compound **4**, an amorphous powder, showed a base peak at *m/z* 391 $[M+Na]^+$ in its ESI-MS. Comparison of the ^{1}H- and ^{13}C-NMR spectra of compound **4** with those of compound **2**, showed the only difference to be the position of caffeic acid group. Chemical shifts at δ_H 5.38 (H-5) and HMBC correlations between H-5 (δ_H 5.38) and C-9' (δ_C 167.5) indicated that the caffeic acid group was located at C-5 position of quinic acid. Therefore, compound **4** was identified as 5-*O*-caffeoylquinic acid methyl ester. (Simoes-Pires *et al*., 2005, Kim *et al*., 2010).

Compound **5** was obtained as an amorphous powder with $[M+Na]^+$ peak at *m/z* 293 in the ESI-MS spectrum, which was consistent with the molecular formula of $C_{13}H_{18}O_6$. The ^{1}H-NMR spectrum exhibited a benzylic

methylene at δ_H 4.68 (1H, d, J = 12.0, H-2b) and 4.95 (1H, d, J = 12.0, H-2a), an anomeric proton at δ_H 4.37 (1H, d, J = 7.5 Hz, H-1'), and multiplet signals of an aromatic protons at δ_H 7.27 - 7.45 (5H). 13C-NMR spectrum revealed the signals due to a benzyl group and a glucose moiety. Therefore, compound **5** was identified as benzyl-*O*-β-D-glucopyranoside (Miyase *et al*., 1987, Fukuda *et al*., 2003).

Compound **6**, an amorphous powder, showed a base peak at *m/z* 417 $[M - H]^-$ in its ESI-MS. The ^{1}H- and ^{13}C-NMR spectra showed compound **6** was a flavanone: ring A [δ_H 7.75 (1H, d, J = 8.5, H-5), 6.53 (1H, dd, J = 8.5, 2.0, H-6) and 6.39 (1H, d, J = 8.5, H-8)], ring B [δ_H 7.46 (2H, d, J = 8.5, H-2', 6') and 7.17 (2H, d, J = 8.5, H-3', 5'), δ_C 116.5 (C-3', 6'), 127.4 (C-2', C-6')], ring C [δ_H 5.48 (1H, dd, J = 13.0, 3.0, H-2), 3.07 (1H, dd, J = 17.0, 13.0, H-3a), 2.75 (1H, dt, J = 17.0, 3.0, H-3b), δ_C 79.3 (C-2), 43.6 (C-3), 191.7 (C-4)] and a glucose [δ_H 4.96 (1H, d, J = 7.2, H-1"), 3.92 (1H, dd, J = 12.0, 2.0, H-6"a), 3.72 (1H, dd, J = 12.0, 5.5, H-6"b), δ_C 100.8 (C-1"), 73.5 (C-2"), 76.8 (C-3"), 70.0 (C-4"), 76.6 (C-5"), 61.1(C-6")]. Therefore, compound **6** was identified as liquiritigenin-7-*O*-β-D-glucopyranoside by comparing the spectroscopic data with those reported in the literature (Li *et al*., 1992). Liquiritigenin-7-*O*-β-D-glucopyranoside was isolated from this plant for the first time.

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