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A New Furfural Diglycoside and Other Carbohydrate Derivatives from Fermented Beverage of *Prunus mume* Fruit

Xi-Tao Yan, Wei Li, Ya-Nan Sun, Seo Young Yang, Gyu Yong Song, and Young Ho Kim*

*College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea. *E-mail: yhk@cnu.ac.kr*
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Prunus mume Sieb. et Zucc. (Rosaceae) is a deciduous fruit tree that is distributed widely in East Asian. Its fruit, commonly known as Maesil in Korea, is a traditional raw ingredient used in herbal medicines and healthy foods in Korea, China, and Japan. In particular, a beverage produced by the fermentation of a mixture of green *P. mume* fruit and sugar is popular in Korea. Recent studies have shown that this fermented beverage, which contains a variety of probiotics, enhances immune activity and suppresses the development of atopic dermatitis-like skin lesions and 7,12-dimethylbenz[α]anthracene (DMBA)- and 12-*O*-tetradecanoyl phorbol-13-acetate (TPA)-induced skin carcinogenesis in mice.[1-3] Despite these findings, the chemical constituents of fermented *P. mume* beverage have yet to be characterized. In the present study, 19 compounds, including a new furfural diglycoside, 5-[β-D-fructopyranosyl-(2→6)-α-D-glucopyranosyloxymethyl]-2-furancarboxaldehyde (**1**), 16 carbohydrate derivatives (**2**-**10** and **12**-**18**), and two additional compounds (**11** and **19**) were isolated from samples of fermented *P. mume* fruit beverage. This is the first report detailing the chemical composition of fermented *P. mume* beverage and provides a useful reference for further biological investigation. This report describes the isolation and structural elucidation of compounds **1**-**19**.

The known compounds that were isolated from the fermented beverage of *P. mume* fruit were identified by comparisons of their physical and spectroscopic data with those reported in the literature. They were identified as 5-hydroxymethyl-2-furfural (**2**),[4] α-methoxy-2,5-furandimethanol (**3**),[5] benzyl-β-D-glucopyranoside (**4**),[6] benzyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (**5**),[7] benzyl-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (**6**),[8] benzyl-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**7**),[7] benzyl-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (**8**),[9] (2*E*)-7-hydroxy-3,7-dimethyl-2-octenyl α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**9**),[10] (2*E*)-7-hydroxy-3,7-dimethyl-2-octenyl α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (**10**),[11] methyl (*E*)-*p*-coumarate (**11**),[12] 3-*O*-*p*-coumaroylsucrose (**12**),[13] 3-*O*-feruloylsucrose (**13**),[14] *n*-butyl-β-D-glucopyranoside (**14**),[15] sucrose (**15**),[16] methyl β-D-fructopyranoside (**16**),[17] D-glucopyranose (an equilibrium mixture of 64% β-D-glucopyranose and 36% α-D-glucopyranose in solution, **17**),[18] D-fructose (an equilibrium mixture of 70% β-D-fructopyranose and 22% β-D-fructofuranose in solution, **18**),[19] and tyrosol (**19**)[20] (Figure 1).

Compound **1** was obtained as a colorless syrup with a positive optical rotation of $[\alpha]_D^{18}$ +47.2 (*c* = 1.0, MeOH). Its molecular formula was determined to be $C_{18}H_{26}O_{13}$ by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) at *m*/*z* 473.1295 $[M+Na]^+$ (calcd for $C_{18}H_{26}O_{13}Na$,

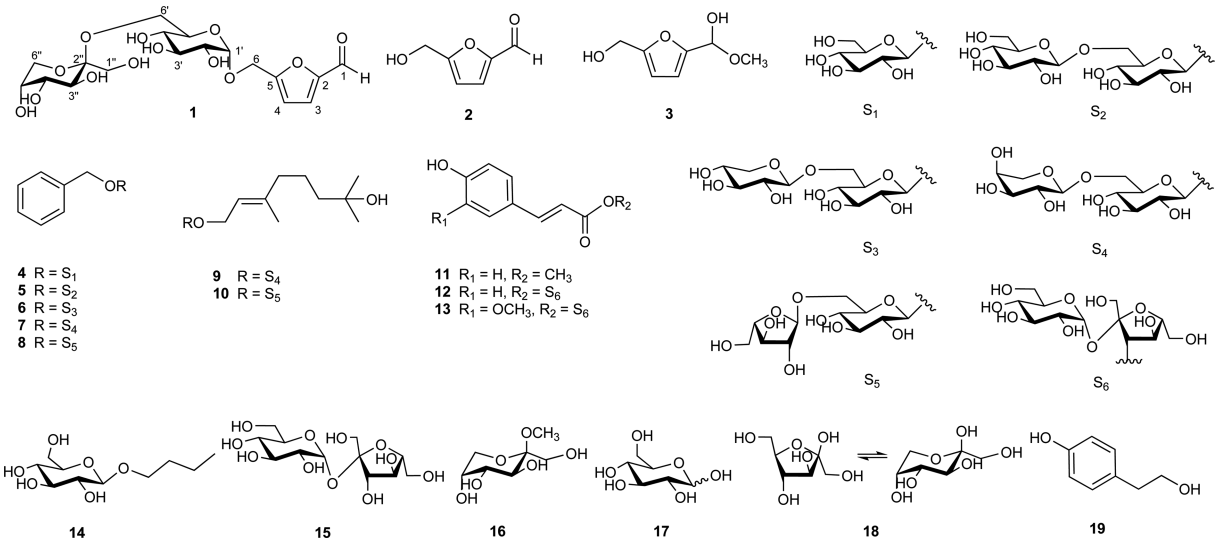

Figure 1. Chemical structures of compounds **1**-**19**.

Table 1. NMR data (600/150 MHz) of compound **1** in CD_3OD

Position	δ_C	δ_H (J in Hz)	HMBC	COSY
1	179.7	9.56, s	C-2	
2	154.4			
3	124.5	7.39, d (3.7)	C-2, 4, 5	H-4
4	113.2	6.72, d (3.7)	C-2, 3, 5	H-3
5	159.7			
6	62.5	4.67, d (13.8)	C-4, 5, 1'	H-6β
		4.76, d (13.8)	C-4, 5, 1'	H-6α
1'	100.2	4.92, d (3.7)	C-6, 2', 3'	H-2'
2'	73.6	3.42, dd (10.1, 3.7)	C-3'	H-1', 3'
3'	75.0	3.64, m	C-2', 4'	H-2', 4'
4'	71.8	3.30, m	C-2', 3', 6'	H-3', 5'
5'	74.3	3.60, m	C-4', 6'	H-4', 6'
6'	62.7	3.66, m	C-4', 5', 2"	H-5', 6'β
		3.78, m	C-4', 5', 2"	H-5', 6'α
1"	63.6	3.69, d (11.0)	C-2", 3"	H-1"β
		3.74, m	C-2", 3"	H-1"α
2"	101.9			
3"	70.6	3.90, d (10.1)	C-4"	H-4"
4"	71.7	3.78, m	C-3", 5", 6"	H-3", H-5"
5"	71.3	3.84, br s	C-3"	H-4", H-6"
6"	65.3	3.66, m	C-2", 4", 5"	H-5", H-6"β
		3.75, m	C-2", 4", 5"	H-5", H-6"α

473.1266). The IR spectrum contained clear absorption bands corresponding to hydroxyl groups (3362 cm^{-1}), a conjugated aldehyde group (2927 cm^{-1}), and conjugated double bonds (1666 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Table 1) contained signals corresponding to an aldehyde proton at δ_H 9.56 (1H, s, H-1), two coupled olefinic protons at δ_H 7.39 (1H, d, J = 3.7 Hz, H-3) and 6.72 (1H, d, J = 3.7 Hz, H-4), and two coupled oxygenated protons at δ_H 4.76 and 4.67 (each 1H, d, J = 13.8 Hz, H-6β and 6α). The ^{13}C-NMR spectrum showed signals corresponding to a carbonyl carbon atom at δ_C 179.7, two quarternary carbon atoms at δ_C 159.7 and 154.4, two methine carbon atoms at δ_C 124.5 and 113.2, and an oxygenated methylene carbon atom at δ_C 62.5. These spectral data implied the presence of a 5-hydroxymethylfurfural moiety in the molecule of **1**.[4] The ^{13}C-NMR and DEPT spectra of **1** also revealed two glycosyl moieties with two anomeric carbon signals at δ_C 101.9 and 100.2 and ten oxygenated carbon signals at δ_C 62.7-75.0 including three methylene carbon atoms and seven methine carbon atoms, although only one anomeric proton signal was evident in the ^{1}H-NMR spectrum at δ_H 4.92 (1H, d, J = 3.7 Hz). Analysis of COSY and HMBC data of the disaccharide moiety (Table 1 and Figure 2) suggested the presence of glucopyranosyl and fructopyranosyl units. This was confirmed by a comparison of the NMR data with previously published data.[21-23] The α-configuration at the anomeric center of the glucopyranosyl unit is supported by a relatively small J value (J = 3.7 Hz) and the β-configuration at the anomeric center of the fructopyranosyl unit is supported by a comparison of its δ_C values with those of α- and β-configured methyl-D-fructopyranoside.[24] Acid hydrolysis of **1** yielded D-glucose and D-fructose, which were identified by GC analysis. The HMBC spectrum showed a correlation between H-6' (δ_H 3.66 and 3.78) and C-2" (δ_C 101.9), indicating a 2"→6' linkage in the disaccharide moiety. The linkage between this disaccharide and the 5-hydroxymethylfurfural moiety was determined by HMBC correlations between H-1' (δ_H 4.92) and C-6 (δ_C 62.5) and between H-6 (δ_H 4.67 and 4.76) and C-1' (δ_C 100.2). Thus, the compound **1** was identified as a new furfural diglycoside, 5-[β-D-fructopyranosyl-(2→6)-α-D-glucopyranosyloxymethyl]-2-furancarboxaldehyde.

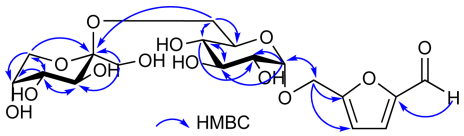

Figure 2. Key HMBC correlations of compound **1**.

Experimental

Plant Materials. Fresh green fruits of *P. mume* (10 kg) were purchased from local markets (Yuseong, Daejeon, Korea) in June 2012 and authenticated by one of the authors, Prof. Young Ho Kim. Thick, fermented *P. mume* beverage was obtained by mixing cleaned fruits of *P. mume* (10 kg) with sugar (7 kg) and storing the mixture in the dark at room temperature for 3 months. During this time, the mixture was stirred weekly. To obtain the final beverage, fermentation residue and seeds were filtered from the fermentation broth.

Extraction and Isolation. Thick fermented beverage of *P. mume* fruit (4.5 L) were firstly diluted with distilled water (10.5 L) to 15 L. The dilution was subjected to adsorptive macroporous resins HP-20 column chromatography (CC) and eluted successively with distilled water, 25% aqueous MeOH, 50% aqueous MeOH, 75% aqueous MeOH, and 100% MeOH to yield 5 fractions, respectively. The 25% MeOH elute (9.4 g) was further subjected to silica gel CC eluted with CH_2Cl_2/MeOH/H_2O (8:1:0.1 – 2:1:0.1) to afford 12 fractions (Frs. W1 – W12). Fr. W1 was separated by repeated RP-C_{18} CC (MeOH-H_2O, 1:10) to yield **2** (11 mg) and **3** (5 mg). Fr. W4 was purified on a RP-C_{18} column (Me_2CO-H_2O, 1:20) to yield **14** (110 mg). Fr. W5 was subjected to RP-C_{18} CC (MeOH/H_2O, 1:30) to yield three subfractions (Frs. W5-1 – W5-3). Compound **1** (6 mg) was obtained by the separation of Fr. W5-2 on a silica gel column (EtOAc/MeOH, 10:1) and **16** (7 mg) was obtained by the separation of Fr. W5-3 on a silica gel column ($CHCl_3$/94% EtOH, 5:2). **18** (426 mg), **17** (266 mg), and **15** (39 mg) were obtained by the separation of Frs. W9, W10, and W12 on a RP-C_{18} column (MeOH/H_2O, 1:25), respectively. The 50% MeOH elute (5.1 g) was subjected to silica gel CC eluted with CH_2Cl_2/MeOH/H_2O in a gradient (8:1:0.1 – 3:1:0.1) to afford 11 fractions (Frs. M1 – M11). **11** (3 mg) and **19** (5 mg) were obtained by the separation of Fr. M1 on a RP-C_{18} column (MeOH/H_2O, 1:15 – 1:5) and a silica gel column ($CHCl_3$/94% EtOH, 30:1 – 10:1). Fr. M4 was subjected to

RP-C_{18} CC (Me_2CO /H_2O, 1:20) to yield **4** (200 mg). Fr. M7 was purified on a silica gel column ($CHCl_3$/MeOH, 5:1) and a RP-C_{18} column (MeOH/H_2O, 1:4) to yield **8** (9 mg). Fr. M8 was subjected to silica gel CC (EtOAc/94% EtOH, 5:1 – 5:2) and RP-C_{18} CC (MeOH/H_2O, 1:12) to yield **12** (4 mg), **6** (105 mg), and **7** (135 mg). Fr. M9 was separated on a RP-C_{18} column (MeOH/H_2O, 1:7 – 1:2) to afford **10** (3 mg) and **9** (5 mg). Fr. M10 was subjected to RP-C_{18} CC (Me_2CO/MeOH/H_2O, 0.2:1:10 – 0:1:3) and silica gel CC ($CHCl_3$/94% EtOH, 4:1 – 3:2) to yield **5** (12 mg) and **13** (2 mg).

5-[β-D-Fructopyranosyl-(2→6)-α-D-glucopyranosyloxymethyl]-2-furancarboxaldehyde (**1**)**:** Colorless syrup; $[\alpha]_D^{18}$ +47.2 (*c* = 1.0, MeOH); UV (MeOH) λ_{max} (logε): 278 nm (3.65); IR (KBr) ν_{max}: 3362, 2927, 1666, 1027 cm^{-1}; HR-ESI-MS (positive mode): *m*/*z* 473.1295 $[M+Na]^+$ (calcd for $C_{18}H_{26}O_{13}Na$, 473.1266); ^1H- and ^{13}C-NMR (600/150 MHz, CD_3OD) data, see Table 1.

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Supporting Information. General experimental procedures, hydrolysis procedure and NMR spectra of compound **1** are available as supporting information.

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