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Galloyl, caffeoyl and hexahydroxydiphenoyl esters of dihydrochalcone glucosides from *Balanophora tobiracola*

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Abstract

Seven galloyl, caffeoyl and *(S)*-hexahydroxydiphenoyl (HHDP) esters of dihydrochalcone glucosides were isolated from *Balanophora tobiracola*; based on spectroscopic and chemical evidence, their structures were determined to be 6″-*O*-galloyl-, 3″,4″-di-*O*-galloyl-, 4″,6″-di-*O*-galloyl-, 4″,6″-*O*-(*S*)-HHDP-, 3″-*O*-galloyl-4″,6″-*O*-(*S*)-HHDP-, 3″-*O*-caffeoyl-4″,6″-*O*-(*S*)-HHDP-3-hydroxyphloretin 4′-*O*-β-D-glucosides and 3″-*O*-galloyl-4″,6″-*O*-(*S*)-HHDP-phloretin 4′-*O*-β-D-glucoside, respectively. By contrast, these compounds were not found in the taxonomically related *B. japonica*. The 3″-galloyl-4″,6″-HHDP esters of the dihydrochalcone glucosides showed strong inhibitory activities against α-glucosidase. Four known compounds were also isolated namely, (±)-eriodictyol 7-*O*-β-D-glucoside, 1-*O*-caffeoyl-3-*O*-galloyl-β-D-glucose, phloretin 4′-*O*-β-D-glucoside, and 3-hydroxyphloretin 4′-*O*-β-D-glucoside.

Keywords: *Balanophora tobiracola*; Balanophoraceae; Phloretin; Dihydrochalcone; Tannin

1. Introduction

Previously, we reported 34 caffeoyl, coumaroyl, galloyl, and hexahydroxydiphenoyl (HHDP) glucopyranose esters from *Balanophora japonica*, a parasitic plant growing on the roots of *Symplocos* plants (Jiang et al., 2001). Its major phenolic constituents were 1-*O*-caffeoyl-4, 6-(*S*)-HHDP-β-D-glucopyranose (0.22% from fresh aboveground parts) and the 3-*O*-gallate ester (0.12%), the latter perhaps representing a new class of ellagitannins in terms of possessing a caffeoyl ester moiety. From a chemotaxinomical interest, we next examined constituents of *Balanophora tobiracola* Makino, a parasitic plant growing on *Pittosporum* and *Rhaphiolepis* and distributed in the islands of Kyushu, Okinawa and Taiwan. From the aeriel tissues of this plant, seven new galloyl, caffeoyl and HHDP esters of dihydrochalcone glucosides were the major constituents, more, which were present in *B. japonica*. This paper deals with their isolations at structure determination, as well as the α-glucosidase inhibitory propositions.

2. Results and discussion

Fresh aeriel from of *B. tobiracola* was extracted with MeOH and then 70% aq. acetone, with the resulting extracts combined and partitioned between water and Et_2O. The aqueous and Et_2O layers were separately fractionated by Sephadex LH-20 column chromatography, water fractions positive to $FeCl_3$ reagent subjected to additional MCI-gel CHP20P, Chromatorex ODS, and Sephadex LH-20 column chromatographic steps to give (±)-eriodictyol 7-*O*-β-D-glucoside (Mun'im et al.,

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2003), 1-*O*-caffeoyl-3-*O*-galloyl-β-D-glucose (Jiang et al., 2001), phloretin 4′-*O*-β-D-glucoside (**1**) (Tanaka et al., 1980), 3-hydroxyphloretin 4′-*O*-β-D-glucoside (**2**) (Ito et al., 1980), and seven new compounds **3**–**9**.

Compound **3** was isolated as a white amorphous powder and gave a dark blue coloration with $FeCl_3$ reagent. The ^1H NMR spectrum (Table 1) was closely related to that of compound **2**, suggesting the presence of a phloroglucinol ring [δ 6.09 (2H, s, H-3″and H-5″)], a catechol ring [δ 6.68 (*d*, *J* = 1.9 Hz, H-2), 6.66 (*d*, *J* = 8.2 Hz, H-5), and 6.54 (*dd*, *J* = 1.9, 8.2 Hz, H-6)], and two mutually coupled methylene groups [δ 3.35 (2H, *t*, *J* = 8.2 Hz, H-8) and 2.80 (2H, *t*, *J* = 8.2 Hz, H-7)]. Its ^{13}C NMR spectral comparison with that of **2** supported the presence of a 3-hydroxyphloretin-4′-*O*-β-glucoside moiety, and five additional carbon resources at δ 168.3 (C-7), 146.5 (C-3, 5), 139.9 (C-4), 121.2 (C-1), and 110.1 (C-2, 6) suggested the presence of a galloyl group; this interpretation was further supported by the presence of a two-proton singlet at δ 7.08 in the ^1H NMR spectrum and a dark blue color results with the $FeCl_3$ reagent. The presence of the galloyl group was also confirmed by enzymatic hydrolysis with tannase, which yielded gallic acid and **2**. Location of the galloyl group was determined to be at glucose C-6 on the basis of low field shifts of H-6″[δ 4.55 (*dd*, *J* = 2.4, 12.4 Hz) and 4.46 (*dd*, *J* = 4.7, 12.4 Hz)] compared to those of **2** [δ 3.91 and 3.71]. This was also supported by the resonance of C-6 the glucose at lower field (δ 64.3, Δδ 2.0) compared with that of **2**. Accordingly, compound **3** was 3-hydroxyphloretin 4′-*O*-(6″-*O*-galloyl)-β-D-glucoside.

Compounds **4** and **5** had the same molecular weights by FAB MS, with $(M + H)^+$ ion peaks at *m*/*z* 757. The ^1H NMR spectra of these compounds were similar to those of **2** and **3**, indicating presence of a 3-hydroxyphloretin 4′-*O*-β-glucoside moiety (Table 1). However, two singlet signals attributable to galloyl groups were also observed in each spectrum, and tannase hydrolysis of **4** and **5** yielded gallic acid and 2, confirming that these compounds are galloyl esters of **2**. The location of the galloyl groups were deduced from the chemical shifts of glucose protons: the glucose H-3 and H-4 protons in compound **4** were at δ 5.55 and 5.31, respectively, indicating that the hydroxyl groups at these positions were acylated. As for compound **5**, the glucose H-4 and H-6 were largely shifted to lower field [δ 5.26 (H-4″), 4.53 and 4.19 (H-6″)] compared to those of **2**. Based on these results, **4** and **5** were 3-hydroxyphloretin 4′-*O*-(3″,4″-di-*O*-galloyl)-β-D-glucoside and 3-hydroxyphloretin 4′-*O*-(4″,6″-di-*O*-galloyl)-β-D-glucoside, respectively

Compound **6** had a $(M + H)^+$ ion peak at *m*/*z* 755, two mass units less than either **4** or **5**. The ^1H NMR spectrum indicated that this compound was also an acylated derivative of 3-hydroxyphloretin-4′-*O*-β-glucoside, with resonances arising from glucose and the dihydrochalcone units being similar to those of **5** (Table 1). The acyl group showed two aromatic singlet signals at δ 6.71 and 6.60 in the ^1H NMR spectrum. In addition, chemical shifts of two ester carbonyl and 12 aromatic carbon signals, including six oxygen-bearing ones, in the ^{13}C NMR spectrum coincided with those of the HHDP groups a ellagitannins (Tanaka et al., 2003). This interpretation was supported by partial hydrolysis in hot water yielding **2** and ellagic acid. The location of the HHDP group was determined to be at the glucose C-4 and C-6 hydroxyl groups based on large low field shifts of the protons of these positions [δ 4.90 (H-4″), 5.23 and 3.85 (H-6″)]. One of the H-6 methylene proton signals was at lower field (δ 5.23) compared to those of 4,6-digalloyl derivative **5**, a characteristic feature of ellagitannins having a HHDP groups at glucose 4, and 6-positions (Gupta et al., 1982). Atropisomerism of the HHDP biphenyl bond was concluded to be *S* configuration from analysis, the CD spectrum of **6**, which showed a positive Cotton effect at 238 nm and a negative Cotton effect at 266 nm (Okuda et al., 1982). Therefore, compound **6** was 3-hydroxyphloretin 4′-*O*-[4′,6″-*O*-(S)-HHDP]-β-D-glucoside.

The ^1H and ^{13}C NMR spectra of compounds **7** and **8** were related to **6**, indicating the presence of a 3-hydroxyphloretin-4′-*O*-β-glucoside and a HHDP ester moiety in each molecule. However, the glucose H-3 signals of these compounds appeared at lower field (δ 5.48 for **7**, δ 5.38 for **8**) compared to that of **6** (δ 3.80), suggesting the presence of additional acyl groups in **7** and **8**. From analysis of the ^1H and ^{13}C NMR signals, the additional acyl group of **7** was deduced to be a galloyl group, this being supported by selective hydrolysis of the galloyl group by treatment with tannase yielding **6** and gallic acid. Accordingly, compound **7** was determined to be 3-hydroxyphloretin 4′-*O*-[3″-*O*-galloyl-4″,6″-*O*-(S)-HHDP]-β-D-glucoside. As for compound **8**, the acyl group at glucose C-3 was concluded to be a caffeoyl group on the basis of observation of the signals due to a conjugated *trans*-double bond [δ 7.53 (H-7) and 6.18 (H-8)] and a trisubstituted benzene ring [δ 7.10 (d, *J* = 1.9 Hz, H-2), 6.98 (dd, *J* = 1.9, 8.2 Hz, H-6), and 6.82 (d, *J* = 8.2 Hz, H-5)]. The presence of a caffeoyl group was also supported by the ^{13}C NMR spectroscopic comparison with those of 1-*O*-caffeoyl-3-*O*-galloyl-β-D-glucose. Therefore, compound **8** was 3-hydroxyphloretin 4′-*O*-[3″-*O*-caffeoyl-4″,6″-*O*-(*S*)-HHDP]-β-D-glucoside.

The ^1H NMR spectrum of compound **9** was closely related to that of **7**, were signals arising from a 3-galloyl-4, 6-HHDP-β-glucoside moiety. However, the molecular mass was 16 mass units less than that of **7** by FAB-MS [*m*/*z* 891 $(M + H)^+$], and had A_2B_2-type aromatic proton signals, instead of the catechol ring proton signals of **7**. This indicated the presence of a *p*-

Table 1
^{1}H NMR spectroscopic data for compounds **3**–**9**

	3[a]	**4**[a]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**9**[b]
2	6.68 (*d*, 1.9)	6.68 (*d*, 1.9)	6.76 (*d*, 1.6)	6.76 (*d*, 1.6)	6.76 (*d*, 1.9)	6.77 (*d*, 1.9)	7.10 (*br d*, 8.5)
3							6.75 (*br d*, 8.5)
5	6.66 (*d*, 8.2)	6.67 (*d*, 7.8)	6.72 (*d*, 8.2)	6.72 (*d*, 8.2)	6.73 (*d*, 8.0)	6.73 (*d*, 8.2)	6.75 (*br d*, 8.5)
6	6.54 (*dd*, 1.9, 8.2)	6.55 (*dd*, 1.9, 7.8)	6.59 (*dd*, 1.6, 8.2)	6.59 (*dd*, 1.6, 8.2)	6.59 (*dd*, 1.9, 8.0)	6.60 (*dd*, 1.9, 8.2)	7.10 (*br d*, 8.5)
7	2.80 (2H, *t*, 8.2)	2.82 (2H, *t*, 8.2)	2.83 (2H, *t*, 8.2)	2.83 (2H, *t*, 8.2)	2.84 (2H, *t*, 8.2)	2.85 (2H, *t*, 8.2)	2.90 (2H, *t*, 8.2)
8	3.32 (2H, *t*, 8.2)	3.31[c]	3.36 (2H, *t*, 8.2)	3.35 (2H, *t*, 8.2)	3.37 (2H, *t*, 8.2)	3.37 (2H, *t*, 8.2)	3.38 (2H, *t*, 8.2)
3′, 5′	6.09 (2H, *s*)	6.15 (2H, *s*)	6.19 (2H, *s*)	6.15 (2H, *s*)	6.19 (2H, *s*)	6.19 (2H, *s*)	6.19 (2H, *s*)
1″	4.98 (*d*, 7.4)	5.22 (*d*, 7.9)	5.24 (*d*, 8.2)	5.14 (*d*, 7.7)	5.34 (*d*, 7.7)	5.31 (*d*, 7.7)	5.34 (*d*, 7.9)
2″	3.45–3.55 (*m*)	3.85 (*dd*, 7.9, 9.6)	3.68 (*br t*, 8.2)	3.65 (*dd*, 7.7, 9.6)	3.93 (*br dd*, 7.7, 9.6)	3.92 (*dd*, 7.7, 9.6)	3.92 (*br t*, 8.0)
3″	3.45–3.55 (*m*)	5.55 (*t*, 9.6)	3.98 (*br t*, 9.9)	3.80 (*t*, 9.6)	5.48 (*t*, 9.8)	5.38 (*t*, 9.6)	5.48 (*t*, 9.6)
4″	3.45–3.55 (*m*)	5.31 (*t*, 9.6)	5.26 (*t*, 10.4)	4.90 (*t*, 9.4)	5.06 (*t*, 9.8)	5.02 (*t*, 9.6)	5.06 (*t*, 9.6)
5″	3.74 (*m*)	3.95 (*m*)	4.24 (*br dd*, 5.2, 10.4)	4.22 (*dd*, 6.3, 9.6)	4.46 (*dd*, 6.0, 9.8)	4.44 (*dd*, 6.0, 9.6)	4.46 (*dd*, 6.0, 9.6)
6″	4.55 (*dd*, 2.4, 12.4)	3.76 (*dd*, 1.6, 12.5)	4.53 (*br dd*, 5.2, 10.4)	5.23 (*dd*, 6.3, 12.9)	5.34 (*dd*, 6.0, 13.5)	5.31 (*dd*, 6.0, 13.0)	5.32 (*dd*, 6.0, 13.7)
	4.46 (*dd*, 4.7, 12.4)	3.63 (*dd*, 5.1, 12.5)	4.19 (*dd*, 5.2, 10.7)	3.85 (*d*, 12.9)	3.85 (*d*, 13.5)	3.88 (*d*, 13.5)	3.89 (*d*, 13.7)
Galloyl H-2, 6	7.08 (2H, *s*)	7.02 (2H, *s*)	7.15 (4H, *s*)		7.02(2H, *s*)		7.04 (2H, *s*)
		6.97 (2H, *s*)					
HHDP H-3, 3′				6.71 (*s*)	6.64 (*s*)	6.62 (*s*)	6.63 (*s*)
				6.60 (*s*)	6.44 (*s*)	6.53 (*s*)	6.43 (*s*)
Caffeoyl H-2						7.10 (*d*, 1.9)	
H-5						6.82 (*d*, 8.2)	
H-6						6.98 (*dd*, 1.9, 8.2)	
H-7						7.53 (*d*, 15.9)	
H-8						6.18 (*d*, 15.9)	

[a] Measured at 300 MHz in CD_3OD.
[b] Measured in 300 MHz in acetone-d_6.
[c] Overlapped with the solvent signal.

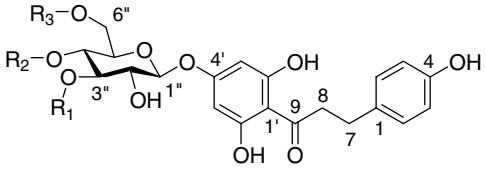
substituted benzene ring in **9**. Comparison of the ^{13}C NMR chemicals shifts with those of **1** also supported that **9** is an ester of phloretin 4′-*O*-glucoside. From these spectral comparison, compound **9** was deduced to be phloretin 4′-*O*-[3′-*O*-galloyl-4′, 6′-*O*-(S)- HHDP]-β-D-glucoside.

Although the ester derivatives of dihydrochalcone glycosides were isolated as major constituents of *B. tobiracola* (total isolation yield of compounds **3**–**9** was 1.4%) from fresh aeriel tissues, neither dihydrochalcone glycosides nor their ester derivatives were isolated from the taxonomically related *B. japonica*. On the other hand, 1-*O*-caffeoyl-3-*O*-galloyl-β-D-glucose, which is a major compound of *B. tobiracola* (1.4%), was also present in *Balanophora iceponica*. This is interesting from a chemotaxonomical viewpoint. In addition, it is known that polyphenolic compounds have inhibitory properties to some enzymes, such as glucosidase (Honda and Hara, 1993). In this work, we found that compounds **5**–**9** (IC_{50} 1.8, 1.6, 0.4, 1.1, and 0.8 μg/mL, respectively) inhibited α-glucoside at a lower concentration than epigallocatechin-3-*O*-gallate (3.1 μg/mL), which is a green tea polyphenol known to have moderate inhibition activity (Honda and Hara, 1993). Compounds **7** and **9**, which have the 3-*O*-galloyl-4,6-*O*-HHDP-glucose structure, were the most potent inhibitors.

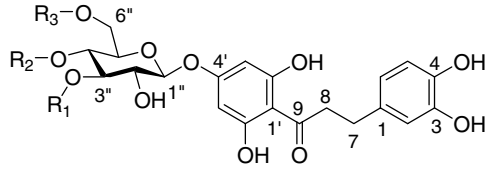
3. Experimental

3.1. General

Optical rotations were measured with a JASCO DIP-370 digital polarimeter, where CD spectra were obtained using a JASCO J-720w apparatus. ^1H and ^{13}C NMR, ^1H–^1H COSY, NOESY, HSQC, and HMBC spectra were recorded with a Unity *plus* 500 spectrometer (Varian Inc, USA) operating at 500 MHz for ^1H, and 125 MHz for ^{13}C, respectively. ^1H and ^{13}C NMR spectra were also measured with a JEOL JMN-AL400 (JEOL Ltd., Japan) operating at 400 MHz for ^1H, and 100 MHz for ^{13}C, respectively. FAB and EIMS were recorded on a JMS DX-303 spectrometer (JEOL Ltd., Japan), with *m*-nitrobenzyl alcohol or glycerol used as a matrix for FABMS. Elemental analysis was obtained with a Perkin–Elmer 2400 II analyzer (PerkinElmer, Inc.). CC was carried out with MCI-gel CHP 20P (Mitsubishi Chemical Co.), Chromatorex ODS (Fuji Silysia Chemical Ltd., Japan), and Sephadex LH-20 (Pharmacia Fine Chemical Co.), respectively. TLC was performed on precoated Kieselgel 60 F_{254} plates, 0.2 mm thick (Merck) with benzene–ethyl formate–formic acid (1:7:1, v/v) or $CHCl_3$–MeOH-H_2O (14:6:1, v/v); Compounds were detected by UV illumination, sprayed with 2% ethanolic $FeCl_3$ or 10% sulfuric acid reagent, and followed by heating.

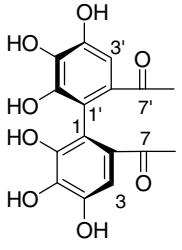


	R_1	R_2	R_3
1 :	H	H	H
9 :	G	(*S*)-HHDP	

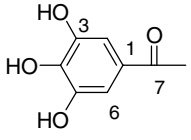


	R_1	R_2	R_3
2 :	H	H	H
3 :	H	H	G
4 :	G	G	H
5 :	H	G	G
6 :	H	(*S*)-HHDP	
7 :	G	(*S*)-HHDP	
8 :	Cf	(*S*)-HHDP	

(*S*)-HHDP =



G =



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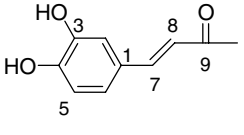

3.2. Plant material

Whole plants of *B. tobiracola* Makino were collected in Nagasaki, Nagasaki Prefecture in November 2002. A voucher specimen (NAP1129-02/11) was deposited at the Medicinal Plants Garden of Nagasaki University.

3.3. Extraction and isolation

Fresh aerial tissues (270 g) of *B. tobiracola* was cut into small pieces and extracted with MeOH (500 mL, 3 times) and then $(CH_3)_2CO$–H_2O (7:3, 500 mL, 2 times). The extracts were combined, concentrated, and partitioned between H_2O and Et_2O. The aqueous layer (40.1 g) was separated by Sephadex LH-20 CC with H_2O containing increasing proportions of MeOH to give 11 fractions. Fr. 5 (0.5 g) was applied to a MCI-gel CHP20P column (H_2O-MeOH) to yield (±)-eriodictyol 7-*O*-β-D-glucoside (142 mg), as was Fr. 6 (7.7 g) to give 1-*O*-caffeoyl-3-*O*-galloyl-β-D-glucose (3.76 g), **1** (92.1 mg) and **2** (559 mg), respectively. Fr. 8 (2.2 g) was separated by MCI-gel CHP20P (H_2O–MeOH) and then by Chromatorex ODS chromotography (H_2O–MeOH) to give compound **3** (50.1 mg). Fr. 9 (3.2 g) contained compound **6** as a major constituent, then being purified by crystallization from water (2.34 g). Fr. 10 (0.9 g) was subjected to MCI-gel CHP20P chromatography (H_2O–MeOH) to yield compounds **5** (113.3 mg) and **6** (57.7 mg). Fr. 11 (2.1 g) was applied to a column of MCI-gel CHP20P to give compound **7** (939 mg) and a mixture of compounds **8** and **9**, which were separated by Chromatorex ODS and further purified by Sephadex LH-20 (80% MeOH) to afford **8** (40.3 mg) and **9** (41.6 mg). The Et_2O layer (2.95 g) was fractionated into 6 fractions by Sephadex LH-20 CC ($CHCl_3$–MeOH, 2:1 – 1:1 – 1:2 – 0:1). Fr. 5 (0.3 g) was separated by MCI-gel CHP20P (H_2O–MeOH), Sephadex LH-20 (60% MeOH), and Chromatorex ODS (H_2O–MeOH) CC to give compound **4** (16.0 mg). Fr. 6 (0.17 g) was identified as compound **7**.

3.3.1. 3-Hydroxyphloretin 4′-O-(6″-O-galloyl)-β-D-glucoside (3)

White amorphous powder; $[\alpha]_D$ – 137.4° (*c* 0.1, MeOH); For ^1H NMR (300 MHz, CD_3OD) spectra, see Table 1; ^{13}C NMR (125 MHz, CD_3OD) δ 207.1 (C-9), 168.3 (galloyl C-7), 165.4 (C-2′, 6′), 164.9 (C-4′), 146.5 (galloyl C-3, 5), 146.1 (C-3), 143.9 (C-4), 139.9 (gallyl C-4), 134.7 (C-1), 121.2 (galloyl C-1), 120.7 (C-6), 116.6, 116.3 (C-2, 5), 110.1 (galloyl C-2, 6), 107.0 (C-1′), 101.1 (C-1″), 96.4 (C-3′, 5′), 77.7 (C-3″), 76.0 (C-5″), 74.6 (C-2″), 71.1 (C-4″), 64.3 (C-6″), 47.5 (C-8), 31.3 (C-7); FAB-MS *m/z* 605 $(M + H)^+$. Elemental analysis: Found: C, 50.86; H, 4.91. $C_{28}H_{28}O_{15}3H_2O$ requires: C, 51.07; H, 5.20.

Treatment of **3** (10 mg) with tannase (3 mg) in H_2O (1 mL) at room temperature for 14 h, and subsequent separation of the products by MCI-gel CHP20P CC (1.0 cm i.d. × 15 cm; H_2O–MeOH, gradient elution) gave **2** (5 mg) and gallic acid (1.5 mg). Compound **2** was identified by comparison of the ^1H and ^{13}C NMR spectroscopic data and the $[\alpha]_D$ value [−63.5° (*c* 0.1, EtOH)] with those described in the literature (Ito et al., 1980).

3.3.2. 3-Hydroxyphloretin 4′-O-(3″,4′-di-O-galloyl)-β-D-glucoside (4)

White amorphous powder; $[\alpha]_D$ – 43.2° (*c* 0.1, MeOH); For ^1H NMR (300 MHz, CD_3OD) spectra, see Table 1; ^{13}C NMR (125 MHz, CD_3OD) δ 207.1 (C-9), 167.8, 167.2 (galloyl C-7), 165.4 (C-2′, 6′), 164.8 (C-4′), 146.4, 146.3 (galloyl C-3, 5), 146.1 (C-3), 144.3 (C-4), 140.2, 139.9 (gallyl C-4), 134.6 (C-1), 121.2, 120.5 (galloyl C-1), 120.6 (C-6), 116.6, 116.3 (C-2, 5), 110.3 (galloyl C-2, 6), 107.0 (C-1′), 101.0 (C-1″), 96.4 (C-3′, 5′), 76.24, 76.18 (C-3″, 5″), 73.1 (C-2″), 70.1 (C-4″), 61.7 (C-6″), 47.5 (C-8), 31.3 (C-7); FAB-MS *m/z* 757 $(M + H)^+$. Elemental analysis: Found: C, 51.51; H, 4.60. $C_{35}H_{32}O_{19}$ $3H_2O$ requires: C, 51.86; H, 4.73.

Compound **4** (1 mg) was treated with tannase (1 mg) in H_2O (0.5 mL) at room temperature for 3 h; TLC analysis of the reaction mixture showed presence of **2** and gallic acid.

3.3.3. 3-Hydroxyphloretin 4′-O-(4′,6″-di-O-galloyl)-β-D-glucoside (5)

Tan amorphous powder; $[\alpha]_D$ – 17.2° (*c* 0.1, MeOH); For ^1H NMR (300 MHz, acetone-d_6) spectra, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) δ 206.4 (C-9), 166.4, 165.9 (galloyl C-7), 164.9 (C-2′, 6′), 164.3 (C-4′), 146.0, 145.9 (galloyl C-3, 5), 145.7 (C-3), 143.9 (C-4), 139.0, 138.8 (gallyl C-4), 134.3 (C-1), 121.5, 121.4 (galloyl C-1), 120.4 (C-6), 116.3, 115.9 (C-2, 5), 110.2, 110.0 (galloyl C-2, 6), 106.5 (C-1′), 100.6 (C-1″), 96.3 (C-3′, 5′), 75.3, 74.6 (C-3″, 5″), 73.2 (C-2″), 71.5 (C-4″), 63.2 (C-6″), 46.9 (C-8), 30.6 (C-7); FAB-MS *m/z* 757 $(M + H)^+$. Elemental analysis: Found: C, 53.48; H, 4.71. $C_{35}H_{32}O_{19}$ $3/2H_2O$ requires: C, 53.65; H, 4.50.

Tannase hydrolysis of **5** (10 mg) in a manner similar to that described for **3** yielded **2** (3 mg) and gallic acid (3 mg).

3.3.4. 3-Hydroxyphloretin 4′-O-[4″,6″-O-(S)-HHDP]-β-D-glucoside (6)

Colorless needles (H_2O); mp 214–216 °C (decomp.); $[\alpha]_D$ – 94.9° (*c* 0.2, MeOH); UV (MeOH): λ_{max} (log ε) 278 (4.53), 226 (4.68); CD (EtOH, 1.7×10^{-5} M) Δε (nm): 41.7 (238), −13.9 (266); For ^1H NMR (300 MHz, acetone-d_6) spectra, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) δ 206.2 (C-9), 168.4, 168.1 (HHDP C-7, 7′), 165.0 (C-2′, 6′), 164.4 (C-4′), 145.8, 145.2(2C), 144.5, 144.4, 144.0 (C-3, C-4, HHDP-4, 4′,

6, 6′), 136.5, 136.2 (HHDP C-5, 5′), 134.2 (C-1), 126.8, 126.4 (HHDP C-2, 2′), 120.3 (C-6), 116.3, 115.9 (C-2, 5), 116.1, 115.7 (HHDP C-1, 1′), 108.2, 107.8 (HHDP C-3, 3′), 106.5 (C-1′), 101.1 (C-1″), 96.2 (C-3′, 5′), 75.5, 75.2, 72.6(2C) (C-2″, 3″, 4″, 5″), 63.7 (C-6″), 47.0 (C-8), 30.6 (C-7); FAB-MS *m/z* 755 $(M + H)^+$. Elemental analysis: Found: C, 51.29; H, 4.60. $C_{35}H_{30}O_{19}$ 7/2H_2O requires: C, 51.41; H, 4.56.

An aqueous solution (10 mL) of **6** (80 mg) was heated at 80–90 °C for 4 h. With the resulting pale yellow precipitate collected by filtration and identified as ellagic acid (by IR and TLC comparison). The filtrate was separated by MCI-gel CHP20P column chromatography (1.0 cm i.d. × 15 cm; H_2O–MeOH, gradient elution) to give **2** (15 mg).

3.3.5. 3-Hydroxyphloretin 4′-O-[3″-O-galloyl-4′,6″-O-(S)-HHDP]-β-D-glucoside (7)

Tan amorphous powder; $[\alpha]_D$ – 33.3° (*c* 0.3, MeOH); For ^{1}H NMR (300 MHz, acetone-d_6) spectra, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) δ 206.3 (C-9), 168.1, 167.6, 166.5 (galloyl C-7, HHDP C-7, 7′), 165.0 (C-2′, 6′), 164.2 (C-4′), 145.8 (galloyl C-3, 5), 145.7, 145.2, 145.1, 144.5, 144.4, 144.0 (C-3, C-4, HHDP-4, 4′, 6, 6′), 138.8 (galloyl C-4), 136.5, 136.4 (HHDP C-5, 5′), 134.2 (C-1), 126.5, 126.1 (HHDP C-2, 2′), 121.3 (galloyl C-1), 120.4 (C-6), 116.3, 116.0 (C-2, 5), 115.7, 115.4 (HHDP C-1, 1′), 110.2 (galloyl C-2, 6), 108.1, 107.7 (HHDP C-3, 3′), 106.6 (C-1′), 101.0 (C-1″), 96.3 (C-3′, 5′), 75.4, 73.0, 72.3, 70.6 (C-2″, 3″, 4′, 5″), 63.3 (C-6″), 47.0 (C-8), 30.6 (C-7); FAB-MS *m/z* 907 $(M + H)^+$. Elemental analysis: Found: C, 52.91; H, 4.13. $C_{42}H_{34}O_{23}$ 5/2H_2O requires: C, 53.00; H, 4.13.

Compound **7** (5 mg) was treated with tannase (1 mg) in H_2O (1 mL) at room temperature for 8 h, with the reaction mixture directly applied to a Sephadex LH-20 column (eluted with H_2O containing increasing proportions of MeOH) to give gallic acid (0.5 mg) and **6** (2.5 mg).

3.3.6. 3-Hydroxyphloretin 4′-O-[3″-O-caffeoyl-4′,6″-O-(S)-HHDP]-β-D-glucoside (8)

Yellow amorphous powder; $[\alpha]_D$ – 18.4° (*c* 0.3, MeOH); For ^{1}H NMR (300 MHz, acetone-d_6) spectra, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) δ 206.0 (C-9), 168.1, 167.6, 167.1 (caffeoyl C-9, HHDP C-7, 7′), 165.0 (C-2′, 6′), 164.2 (C-4′), 148.8 (caffeoyl C-4), 146.3, 146.2 (caffeoyl C-3, 7), 145.7, 145.3, 145.2, 144.4, 144.3, 144.0 (C-3, C-4, HHDP-4, 4′, 6, 6′), 136.5, 136.4 (HHDP C-5, 5′), 134.2 (C-1), 127.4 (caffeoyl C-1), 126.4, 126.3 (HHDP C-2, 2′), 122.6 (caffeoyl C-6), 120.4 (C-6), 116.3(2C), 116.0(2C) (C-2, C-5, caffeoyl C-2, 5), 115.7, 115.4 (HHDP C-1, 1′), 115.1 (caffeoyl C-8), 108.2, 107.8 (HHDP C-3, 3′), 106.6 (C-1′), 101.0 (C-1″), 96.3 (C-3′, 5′), 75.3, 72.8, 72.2, 70.9 (C-2″, 3″, 4″, 5″), 63.3 (C-6″), 47.0 (C-8), 30.6 (C-7); FAB-MS *m/z* 917 $(M + H)^+$, 939 $(M + Na)^+$; Elemental analysis: Found: C, 54.08; H, 4.51. $C_{44}H_{36}O_{22}$ 7/2H_2O requires: C, 53.94; H, 4.42.

3.3.7. Phloretin 4′-O-[3″-O-galloyl-4′,6″-O-(S)-HHDP]-β-D-glucoside (9)

Tan amorphous powder; $[\alpha]_D$ – 30.1° (*c* 0.2, MeOH); For ^{1}H NMR (300 MHz, acetone-d_6) spectra, see Table 1; ^{13}C NMR (125 MHz, acetone-d_6) δ 206.0 (C-9), 168.0, 167.6, 166.5 (galloyl C-7, HHDP C-7, 7′), 165.0 (C-2′, 6′), 164.2 (C-4′), 145.8 (galloyl C-3, 5), 156.3 (C-4), 145.5, 145.2, 144.5, 144.4 (HHDP-4, 4′, 6, 6′), 138.8 (galloyl C-4), 136.5, 136.4 (HHDP C-5, 5′), 133.2 (C-1), 130.2 (C-2, 6), 126.5, 126.1 (HHDP C-2, 2′), 121.3 (galloyl C-1), 115.9 (C-3, 5), 116.3, 115.7 (HHDP C-1, 1′), 110.2 (galloyl C-2, 6), 108.1, 107.7 (HHDP C-3, 3′), 106.6 (C-1′), 101.0 (C-1″), 96.3 (C-3′, 5′), 75.4, 73.0, 72.3, 70.6 (C-2″, 3″, 4″, 5″), 63.3 (C-6″), 47.0 (C-8), 30.4 (C-7); FAB-MS *m/z* 891 $(M + H)^+$. Elemental analysis: Found: C, 53.37; H, 4.46. $C_{44}H_{34}O_{22}$ 3H_2O requires: C, 53.40; H, 4.27.

3.4. α-Glucosidase inhibition

The activities of α-glucosidase (EC 3.2.1.20, from *Saccharomyces* sp.) were determined in a 96 well plate using maltose as substrate. To the well with a 125 μL of a sample dissolved in 20 mM phosphate buffer (pH 6.8), was added 5 μL of enzyme solution (0.1 mg/mL). Following incubation at 37 °C for 10 min, 25 μL of a 6.25 mM maltose solution was added with incubation at 37 °C for another 10 min. Activity was determined by measuring the liberated glucose from maltose using the Glucose CII-Test Wako (Wako Pure Chemical Institute, Co., Osaka, Japan). The phenolic compounds used in this experiment did not affect coloration generated from liberated glucose. The concentration of the sample required to inhibit 50% of the activity under the assayed condition was defined as the IC_{50} values.

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