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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*-