Phytochemistry letters

Two new phenolic glucosides and an ellagitannin from the leaves of *Castanopsis sclerophylla*

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In the course of a chemotaxonomical study of *Castanopsis* species (Fagaceae), detailed investigation of the leaves of *C. sclerophylla* led to isolation of three new phenolic compounds together with 62 known compounds. The structures of the new compounds were elucidated as 2-*O*-galloyl-*O*-4,6-(*S*)-valoneoyl-D-glucose (**1**), 6-*O*-galloyl-1-*O*-vanilloyl-β-D-glucose (**2**), and 4''-*O*-galloylchestanin (**3**) by means of spectroscopic analyses and enzymatic hydrolysis with tannase. Comparison with other *Castanopsis* species indicated that *C. sclerophylla* characteristically accumulates chlorogenic acid and a dimeric ellagitannin, rugosin E. Triterpene hexahydroxydiphenoyl esters, which are major constituents of *C. cuspidata* var. *sieboldii*, *C. hystrix*, and *C. fissa* were not detected.

Key words *Castanopsis sclerophylla*; Fagaceae; phenolic glucoside**;** ellagitannin

1. Introduction

Castanopsis species (Fagaceae) are important as ornamental trees and for use as construction materials in East Asia. The leaves contain a large amount of polyphenols and are promising source of functional natural products. Despite the large number of species (about 120), the phenolic constituents of only few species have been studied (Nonaka et al., 1985; Ageta et al., 1988a; Ageta et al., 1988b; Chen et al., 1993a; Chen et al., 1993b; Huang et al., 2011b). Previously, characteristic triterpene hexahydroxydiphenoyl (HHDP) esters were isolated from the leaves of *C. cuspidata* var. *sieboldii*, *C. hystrix*, and *C. fissa*. More recently, characteristic phenylpropanoid-substituted flavan-3-ols were isolated from the leaves of *C. sclerophylla* (Huang et al, 2011a). The esters have been only found in these plants and seem to have chemotaxonomical significance. Thus, we are continuing phytochemical and chemotaxonomical investigation of the *Castanopsis* species. In this study, we demonstrate a profile of the chemical constituents of *C. sclerophylla*, and chemical characterization of three new compounds **1** - **3**.

2. Results and Discussion

The fresh leaves were extracted with 80% aqueous acetone, and the extract was subjected to a combination of Sephadex LH-20, Diaion HP20SS, MCI gel CHP 20P, Toyopearl Butyl 650C, and Toyopearl HW F40 column chromatography to afford 65 compounds including three new polyphenols. The known compounds were identified by comparing their physical and spectroscopic data with previously reported data.

Compound **1** was obtained as a brown amorphous powder and gave dark blue coloration with ethanolic $FeCl_3$ reagent. The molecular formula $C_{34}H_{26}O_{23}$ was determined by the $[M+H]^+$ ion peak at *m/z* 803.5751 (Calcd for $C_{34}H_{27}O_{23}$, 803.5746) from HR FABMS. This compound was shown to be an anomer mixture (α:β=3:2) by the appearance of two sets of signals attributable to a hexopyranose moiety in the ^1H and ^{13}C NMR spectra. In the ^1H NMR spectrum, the anomeric proton signals appeared at δ 5.36 (d, *J*=3.9 Hz, H-1, α-anomer) and δ 4.83 (d, *J*=8.1 Hz, H-1, β-anomer). The large coupling constants of the remaining sugar protons ($J_{2,3}$, $J_{3,4}$, and $J_{4,5}$ = 9 – 10 Hz) indicated that this sugar is glucose. Presence of galloyl [α-anomer: δ 7.11 (s), β-anomer: δ 7.12 (s)] and valoneoyl groups

[α-anomer: δ 6.67 (H-3), 6.23 (H-3'), 7.14 (H-6"); β-anomer: δ 6.71 (H-3), 6.24 (H-3'), 7.14 (H-6")] was suggested by comparison of the ^{1}H and ^{13}C NMR signals with those of rugosin A (1,2,3-tri-*O*-galloyl-4,6-(*S*)-valoneoyl-β-D-glucose) (Hatano et al., 1990), and it was confirmed by analysis of the ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) spectrum (Figure 2). Large downfield shifts of the glucose H-2 [α-anomer: δ 4.82 (dd, *J*=9.6, 3.9 Hz), β-anomer: δ 4.98 (dd, *J*=9.6, 8.1 Hz)] and HMBC correlations between the glucose H-2 and galloyl carboxyl carbon indicated that the galloyl group is attached to the glucose C-2. The large chemical differences of the geminal protons at glucose C-6 [Δδ 1.44 (α-anomer) and 1.40 (β-anomer)] indicated that the HHDP moiety of the valoneoyl group is located at the O-4 and O-6 of the glucose core (Gupta et al., 1982; Hatano et al., 1990). This was confirmed by HMBC correlations of H-4 (α-anomer: δ 4.81, β-anomer: 4.87) and H-6 signals (α-anomer: δ 5.06, 3.62; β-anomer: 5.09, 3.69) with valoneoyl-7 (δ 168.4) and valoneoyl-7' (δ 168.3) ester carbonyls (Fig. 2). Discrimination of the C-7 and C-7' was based on the chemical shift difference between the C-1 (δ 115.6) of the valoneoyl ring A and C-1' (δ 117.8) of ring B, which were correlated to H-3 and H-3', respectively (Figure 2). The chemical shift of the valoneoyl C-1 was the same as that of the HHDP esters and the C-1' resonated at lower field. The atropisomerism of the valoneoyl biphenyl bond was determined to be *S* by observation of a positive Cotton effect at 222 nm and negative Cotton effect at 259 nm in the CD spectrum (Okuda et al., 1982). Furthermore, enzymatic hydrolysis of **1** with tannase yielded gallic acid and a hydrolysate, which was identified as 4,6-(*S*)-valoneoyl-D-glucose by comparison of the physical and ^{1}H-NMR data (Hatano et al., 1990; Lee at al., 1992). Based on these spectroscopic and chemical results, the structure of **1** was concluded to be 2-*O*-galloyl- 4,6-*O*- (*S*)-valoneoyl-D-glucose.

The ^{13}C NMR spectrum of compound **2** showed signals assignable to a methoxyl group (δ 56.2), two benzoyl esters (δ 165.7, 167.0), and β-glucopyranose moiety. A two-proton singlet at δ 7.09 (2H, s) and an ABX-type set of signals [δ 7.56 (d, *J*=1.7 Hz), 6.87 (d, *J*=8.3 Hz), 7.60 (dd, *J*=8.3, 1.7 Hz)] along with a methoxyl signal at δ 3.85 (3H, s) suggested that the acyl groups were galloyl and vanilloyl groups (Harput et al, 2002; Martin et al., 2007). These esters are shown to be located at the glucose C-1 and C-6 hydroxyl groups by the low field shift of the glucose anomeric proton [δ 5.71 (d, *J*=7.6 Hz)] and H-6

[δ 4.55 (dd, *J*=12.1, 2.0 Hz), 4.32 (dd, *J*=12.1, 5.5 Hz)]. Finally, selective hydrolysis of the galloyl group by treatment with tannase yielded gallic acid and 1-*O*-vanilloyl-β-D-glucose (Klick, 1988; Dini et al., 2004). Based on these results, the structure of **2** was determined to be 1-*O*-vanilloyl-β-D-(6-*O*-galloyl)-glucose.

The ^{1}H NMR spectra of compound **3** was closely related to that of chestanin (Ozawa et al., 1978), showing signals attributable to two 3,4,5-trihydroxybenzyl alcohol units, a dehydrodigalloyl moiety, and two glucose residues. Differences included appearance of a signal due to a galloyl group [δ 7.11 (2H, s)] and low field shift of one of the glucose's H-4 in the spectrum of **3** [δ 5.09 (t, *J*=8.6, Hz)]. Enzymatic hydrolysis with tannase **3** yielded gallic acid and chestanin, confirming that **3** is a galloyl ester of chestanin. Connection of these structural components was determined by detailed analysis of the ^{1}H-^{1}H homonuclear correlation spectroscopy (COSY), heteronuclear single-quantum coherence (HSQC) and HMBC spectra of **3** (Figure 3). The HMBC correlations from the aromatic protons of the dehydrodigalloyl group to a glucose anomeric proton through trihydroxybenzyl alcohol units revealed that the signal at δ 5.09 is assignable to the glucose B H-4 (Figure 3). Consequently, **3** was characterized as 4''-*O*-galloylchestanin.

The compounds isolated from *C. sclerophylla* were classified into gallyol and/or HHDP glucoses, phenolic acids, galloyl phenolic glucosides, acylated quinic acids, galloyl shikimic acids, phenylpropanoid-substituted flavan-3-ols, flavonol glycosides, and others. The overall composition was different from those of previously reported *Castanopsis* species, that is, *C. cuspidata* var. *sieboldii*, *C. hystrix*, and *C. fissa.* The most significant difference is the accumulation of dimeric ellagitannin, rugosin E, and chlorogenic acid, which were not major components in other *Castanopsis* species. It is also noteworthy that triterpene HHDP esters, which were major constituents of *C. cuspidata* var. *sieboldii*, *C. hystrix*, and *C. fissa*, were not detected in this plant.

3. Experimental

3.1. General Experimental Procedures

IR spectra were obtained with a JASCO FT/IR-410 spectrophotometer, and UV spectra were obtained using a Jasco V-560 UV/Vis spectrophotometer and optical rotations were

measured with a Jasco DIP-370 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured in acetone-d_6 or CD_3OD at 27ºC using a Varian Unity plus 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) or a JEOL JNM-AL 400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). MS were recorded on a Voyager DE-PRO and a JEOL JMS-700N spectrometer. Column chromatography (CC) was performed using Diaion HP20SS, Sephadex LH-20, Chromatorex ODS, and Toyopearl HW F40 columns with H_2O containing increasing proportion of MeOH (0 – 100 %). TLC was performed on precoated Kieselgel 60 F_{254} plates toluene-ethyl formate-formic acid (1:7:1, v/v) as the solvents, and spots were detected by UV illumination (254 nm) and by spraying with a 2% ethanolic $FeCl_3$ and 10% sulfuric acid reagent, followed by heating.

3.2. Plant Material

The leaves of *Castanopsis sclerophylla* were collected at Guangxi Institute of Botany, Guangxi, China, in August 2009, and identified by Prof. Yan Liu. The voucher specimen (CS20090720) was deposited in the Herbarium of Guangxi Institute of Botany, China.

3.3. Extraction and Isolation The fresh leaves of *C. sclerophylla* (8.60 kg) were extracted 3 times with acetone-H_2O (8:2, v/v) at room temperature. The extract (570 g) was partitioned between water and Et_2O. The Et_2O layer (5.3 g) was separated by silica gel CC with $CHCl_3$-MeOH-H_2O to give 3,4-dihydroxybenzoic acid (27 mg) and 2,5-dihydroxybenzoic acid (19 mg). The aqueous layer was subjected to Sephadex LH-20 CC with MeOH in H_2O (0—100%) and then 60% acetone to give nine fractions: fr. 1 (202.7 g), 2 (16.2 g), 3 (11.3 g), 4 (45.3 g), 5 (73.2 g), 6 (20.1 g), 7 (10.3 g), and 8 (99.4 g). The fractions were separately subjected to CC of Diaion HP20SS (H_2O-MeOH), Sephadex LH-20, Toyopearl HW F40, and Chromatorex ODS. Separation of Fr. 2 yielded 2,6-dimethoxy-1,4-benzenediol (5 mg) and junipetrioloside A (13 mg), and gentisic acid 5-*O*-β-D-glucoside (20 mg). Fr. 3 gave 2,3-(*S*)-HHDP-D-glucose (28 mg), chlorogenic acid (4.2 g), and 4-*O*-galloylquinic acid (48 mg). From Fr. 4 chlorogenic acid (30.5 g), 5-feruloylquinic acid (2.9 g), and chlorogenic acid butyl ester (1.1 g) were obtained. CC of Fr. 5 yielded sclerophynin A (42 mg), sclerophynin B (12 mg), apocynin D (22 mg),

rhinchoin Ia (7 mg), (4*R*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-b:3,4-b']dipyran-2-one (156 mg), (4*S*,8*R*,9*S*)-4,8-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-5,9-dihydroxy-2*H*,8*H*-benzo[1,2-b:3,4-b']dipyran-2-one (164 mg), (2*R*,3*S*,10*S*)-2,10-bis(3,4-dihydroxyphenyl)-3,4,9,10-tetrahydro-3,5-dihydroxy-2*H*,8*H*-benzo[1,2-b:3,4-b']dipyran-8-one (32 mg), 2,3-di-*O*-galloyl-D-glucose (365 mg), hippomanin A (98 mg), coriarin F (31 mg), castalagin (99 mg), vescalagin (5 mg), dehydrodigallic acid (300 mg), 3-*O*-galloyl-(-)-shikimic acid (14 mg), 5-*O*-galloyl-(-)-shikimic acid (14 mg), gemin D (3 mg), (-)-(2*R*,3*S*)-2, 3-dihydroxy-3-(3,4-dihydroxyphenyl)-propionic acid (5 mg), gentisic acid 3-*O*-β-D-(6′-*O*-galloy)-glucopyranoside (28 mg), gallic acid 4-*O*-β-D-(6′-*O*-galloy)-glucopyranoside (11 mg), gentisic acid 5-*O*-β-D-(6'-*O*-galloyl)-glucopyranoside (59 mg), 6-*O*-galloylarbutin (30 mg), cretanin (213 mg), chesnation (516 mg), mallophenol A (61 mg), chestanin (4.8 g), chlorogenic acid (8.7 g), caffeic acid (23 mg), 5-*O*-coumaroylquinic acid (63 mg), 5-*O*-caffeoylshikimic acid (8 mg), quercetin-3-*O*-β-D-glucuropyranoside (295 mg), quercetin-3-*O*-β-D-glucopyranoside (17 mg), resedin C (3 mg), quercitrin (184 mg), astragalin (11 mg), isorhamnetin-3-*O*-β-D-glucoside (69 mg), scopoletin (9 mg), and new compounds **1** (57 mg) and **2** (7 mg). Separation of Fr. 6 gave ellagic acid (30 mg), 1,2,3-tri-*O*-galloyl-β-D-glucose (350 mg), isochestanin (18 mg), crenatin (10 mg), reynoutrin (52 mg), quercetin-3-*O*-β-L-arabinoside (35 mg), quercetin-3-*O*-(6″-*E*-*p*-coumaroyl)-glucoside (45 mg), quercetin (21 mg), tiliroside (10 mg), kaempferol-3-*O*-(2″-*E*-*p*-coumaroyl)-glucoside (11 mg) and **3** (530 mg). From Fr. 7, 1-desgalloyl eugeniin (2.1 g) and pedunculagin (20 mg) were isolated. Fr. 8 afforded rugosin E (42.6 g), galic acid (107 mg), 1,2,3,6-tetra-*O*-galloyl-β-D-glucose (29 mg), 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (41 mg), and eugeniin (820 mg).

3.4. 2-*O*-Galloyl-*O*-4,6-(*S*)-valoneoyl-D-glucose (**1**)

Brown amorphous powder; $[\alpha]_D$ +17.6 (*c* 0.11, MeOH); UV λ_{max} (MeOH) nm (log ε): 264 (4.59), 217 (5.04); IR ν_{max} cm^{-1}: 3350, 1702, 1560, 1214; CD (MeOH) [θ] (nm):

+1.0×10^6 (222), -3.0×10^5 (259), +2.5×10^5 (283); ^{1}H-NMR (500 MHz, acetone-d_6): δ 3.62 (1H, dd, *J*=13, 2 Hz, α-H-6a), 3.69 (1H, dd, *J*=12, 1 Hz, β-H-6a), 3.94 (1H, t, *J*=10 Hz, α-H-3), 3.95 (1H, m, β-H-5), 4.19 (1H, t, *J*=10 Hz, β-H-3), 4.41 (1H, br dd, *J*=10, 7 Hz, α-H-5), 4.81 (1H, t, *J*=10 Hz, α-H-4), 4.82 (1H, dd, *J*=10, 4 Hz, α-H-2), 4.83 (1H, d, *J*=8 Hz, β-H-1), 4.87 (1H, t, *J*=10 Hz, β-H-4), 4.98 (1H, dd, *J*=10, 8 β- H-2), 5.06 (1H, dd, *J*=13, 7 Hz, α-H-6b), 5.09 (1H, dd, *J*=13, 7 Hz, β-H-6b), 5.36 (1H, d, *J*=4 Hz, α-H-1), 6.23 [1H, s, α-valoneoyl (Val) H-3'], 6.24 (1H, s, β-Val H-3'), 6.67 (1H, s, α-Val H-3), 6.71 (1H, s, β-Val H-3), 7.11 (1H, s, β- Val H-6"), 7.12 (1H, s, α-Val H-6"), 7.14 (4H, s, α,β-galloyl H-2, 6); ^{13}C-NMR (125 MHz, acetone-d_6): δ 63.9 (2C) (α, β-6), 67.1 (α-5), 70.4 (β-5), 72.0 (β-4), 72.1 (β-3), 73.3 (α-4), 73.4 (α-3), 75.2 (α-2), 76.7 (β-2), 91.1 (α-1), 96.7 (β-1), 105.6 (2C) (α, β-3'), 107.9 (2C) (α,β-3), 109.8 (2C) (α-galloyl-2,6), 109.9 (4C) (β-galloyl-2,6; α,β-Val-6"), 115.6 (α-Val-1), 115.7 (α-Val-1"), 116.1 (2C) (β-Val-1,1"), 117.8 (2C) (α,β-Val-1'), 121.1 (α-galloyl-1), 121.5 (β-galloyl-1), 125.9 (2C) (α-Val-2,2'), 126.2 (2C) (β-Val-2,2'), 136.5 (2C) (α,β-Val-5), 137.0 (2C) (α,β-Val-5'), 137.3 (2C) (α,β-Val-5'), 139.7 (2C) (α,β-Val-4"), 140.1 (2C) (α,β-Val-3"), 144.4 (2C) (α,β-Val-6), 144.9 (2C) (α,β-Val-6'), 145.1 (2C) (α,β-Val-4), 145.8 (4C) (α,β-galloyl-3,5), 146.9 (2C) (α,β-Val-4'), 166.3 (α-Val-7"), 166.7 (2C), (α,β-galloyl-7), 167.3 (β-Val-7"), 168.3 (2C) (α,β-galloyl-7'), 168.4 (2C) (α,β-Val-7); MALDI-TOF-MS *m/z*: 825 $[M+Na]^+$; HR-FAB-MS *m/z*: 803.0941 $[M+H]^+$ (Calcd for $C_{34}H_{27}O_{23}$, 803.0943).

3.5. Tannase hydrolysis of 1

An aqueous solution of **1** (14 mg) was incubated with tannase at room temperature for 2 h. The reaction mixture was concentrated to dryness under reduced pressure. The EtOH-soluble portion was applied to a column of Sephadex LH-20 with EtOH to give gallic acid (2 mg) and 4,6-(*S*)-valoneoyl-D-glucose (8 mg).

3.6. 6-*O*-Galloyl-1-*O*-vanilloyl-β-D-glucose (2)

Brown amorphous powder, $[\alpha]_D$ +37.5 (*c* 0.10, MeOH); UV λ_{max} (MeOH) nm (log ε): 269 (4.20), 217 (4.60); IR ν_{max} cm^{-1}: 3356, 1693, 1606, 1214; ^{1}H-NMR (400 MHz, acetone-d_6): δ 3.51-3.59 (3H, m, glc-H-2,3,4), 3.78 (1H, m, glc-H-1), 3.85 (3H, s, -OCH_3),

4.32 (1H, dd, *J*=12.1, 5.5 Hz, glc-H-6a), 4.55 (1H, dd, *J*=12.1, 2.0 Hz, glc-H-6b), 5.71 (1H, d, *J*=7.6 Hz, glc-H-1), 6.87 [1H, d, *J*=8.3 Hz, vanilloyl(Van)-H-5], 7.09 (2H, s, galloyl-H-2,6), 7.56 (1H, d, *J*=1.7 Hz, Van-H-2), 7.60 (1H, dd, *J*=8.3, 1.7 Hz, Van-H-6); ^{13}C-NMR (100 MHz, acetone-d_6): δ 56.2 (Van-OCH_3), 64.2 (glc-6), 70.7 (glc-4), 73.4 (glc-2), 75.7 (glc-5), 77.3 (glc-3), 95.6 (glc-1), 109.7 (2C) (galloyl-2,6), 113.6 (Van-2), 115.6 (Van-5), 121.2 (2C) (Van-1, galloyl-1), 125.2 (Van-6), 138.9 (galloyl-4), 145.9 (2C) (galloyl-3,5), 148.2 (Van-3), 152.8 (Van-4), 165.7 (galloyl-7), 167.0 (Van-7); MALDI-TOF-MS *m/z*: 505 $[M+Na]^+$; HR-FAB-MS *m/z*: 483.1140 $[M+H]^+$ (Calcd for $C_{21}H_{23}O_{13}$, 483.1139).

3.7. Tannase hydrolysis of 2

An aqueous solution of **2** (3 mg) was incubated with tannase at room temperature for 2 h. The reaction mixture was applied to MCI gel CHP 20P (0—100% MeOH) to yield gallic acid (0.8 mg) and 1-*O*-vanilloyl-β-D-glucose (1.2 mg).

3.8. 4''-*O*-Galloylchestanin (3)

White amorphous powder, $[\alpha]_D$ -22.5 (*c* 0.19, MeOH); UV λ_{max} (MeOH) nm (log ε): 275 (5.02), 207 (4.48); IR ν_{max} cm^{-1}: 3358, 2356, 1692, 1613, 1195; ^{1}H-NMR (500 MHz, acetone-d_6): δ 3.51-3.58 (4H, m, glc-A-H-2,3,4; glc-B-H-3), 3.62 (1H, dd, *J*=12.0, 5.5 Hz, glc-B-H-6a), 3.68 (1H, d, *J*=12.0 Hz, glc-B-H-6b), 3.69 (1H, m, glc-A-H-5), 3.70 (1H, dd, *J*=11.8, 4.6 Hz, glc-A-H-6a), 3.84 (1H, m, glc-B-H-5), 3.90 (1H, d, *J*=11.8 Hz, glc-A-H-6b), 3.91 (1H, t, *J*=8.8 Hz, glc-B-H-2), 4.58 (1H, d, *J*=7.6 Hz, glc-B-H-1), 4.72 (1H, d, *J*=8.0 Hz, glc-A-H-1), 4.94 [2H, s, trihydroxybenzyl alcohol (THBA) B-H-7], 5.08 (2H, s, THBA A-H-7), 5.09 (1H, t, *J*=8.6 glc-B-H-4), 6.33 (2H, s, THBA B-H-2,6), 6.44 (2H, s, THBA A-H-2,6), 6.79 [1H, d, *J*=1.8, dehydrodigalloyl(DDG)-H-2], 7.11 (2H, s, galloyl-2,6), 7.13 (1H, s, DDG-H-6'), 7.24 (1H, d, *J*=1.8 Hz, DDG-H-6); ^{13}C-NMR (125 MHz, acetone-d_6): δ 61.8 (glc-A-6), 61.9 (glc-B-6), 66.2 (THBA A-7), 66.2 (THBA B-7), 70.6 (glc-A-4), 71.7 (glc-B-4), 74.5 (glc-A-2), 74.7 (glc-A-5), 75.1 (glc-B-2), 76.5 (glc-B-5), 77.3 (glc-B-3), 78.3 (glc-A-3), 107.5 (glc-A-1), 107.6 (2C) (glc-B-1, DDG-2), 107.9 (2C) (THBA A-2,6), 108.0 (2C) (THBA B-2,6), 109.6 (DDG-6'), 110.1 (2C) (galloyl-2,6), 111.9 (DDG-6), 115.1

(DDG-1'), 121.1 (DDG-1), 121.2 (galloyl-1), 133.9 (THBA A-4), 134.0 (THBA B-4), 135.7 (2C) (THBA A-1, THBA B-1), 137.0 (DDG-2'), 139.1 (galloyl-4), 139.9 (DDG-4, DDG-4'), 140.4 (DDG-3'), 143.3 (DDG-5'), 146.0 (2C) (galloyl-3,5), 146.4 (DDG-5), 148.0 (DDG-3), 150.9 (2C) (THBA B-3,5), 151.0 (2C) (THBA A-3,5), 165.0 (DDG-7'), 166.3 (2C), (DDG-7, galloyl-7); MALDI-TOF-MS *m/z*: 1113 $[M+Na]^+$; HR-FAB-MS *m/z*: 1091.2159 $[M+H]^+$ (Calcd for $C_{47}H_{47}O_{30}$, 1091.2153).

3.9. Tannase hydrolysis of 3

An aqueous solution of **3** (33 mg) was incubated with tannase at room temperature for 2 h. The reaction mixture was filtrated and the filtrate concentrated to dryness under reduced pressure. The EtOH-soluble portion was applied to a column of Sephadex LH-20 with EtOH to give gallic acid (3 mg) and chestanin (20 mg).

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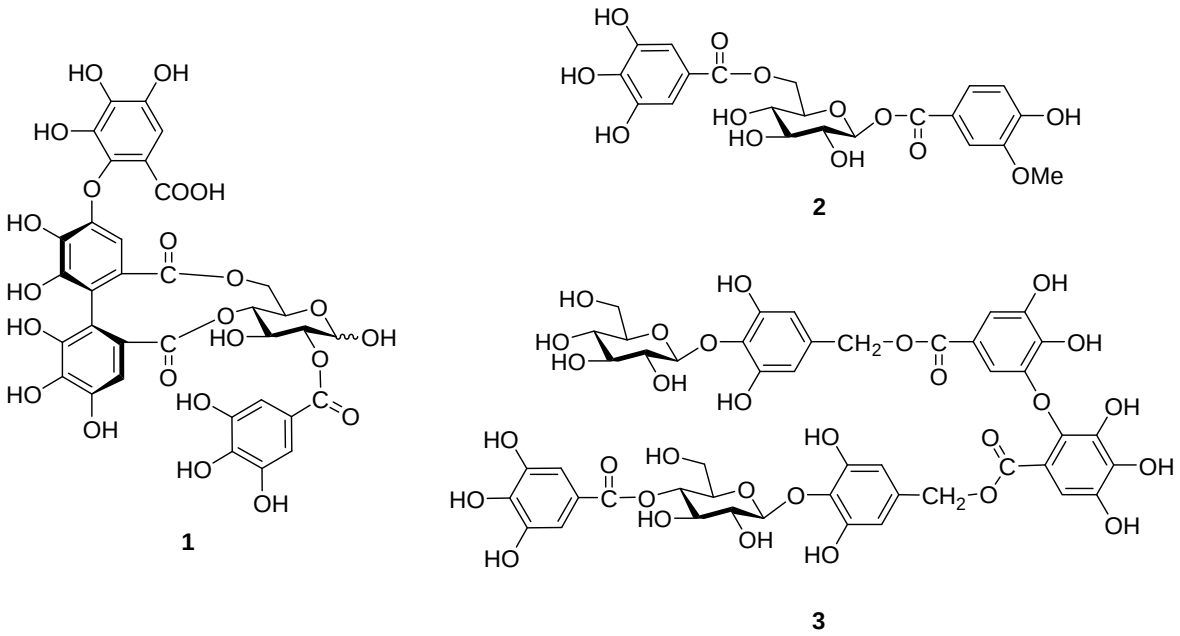

Figure 1. Structures of **1- 3**

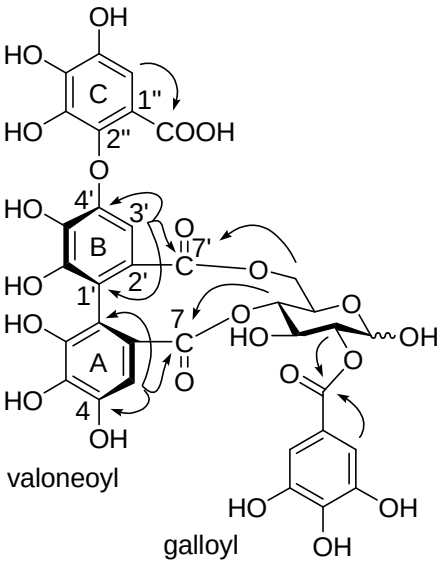

Figure 2. Selected HMBC correlations (H to C) of **1**

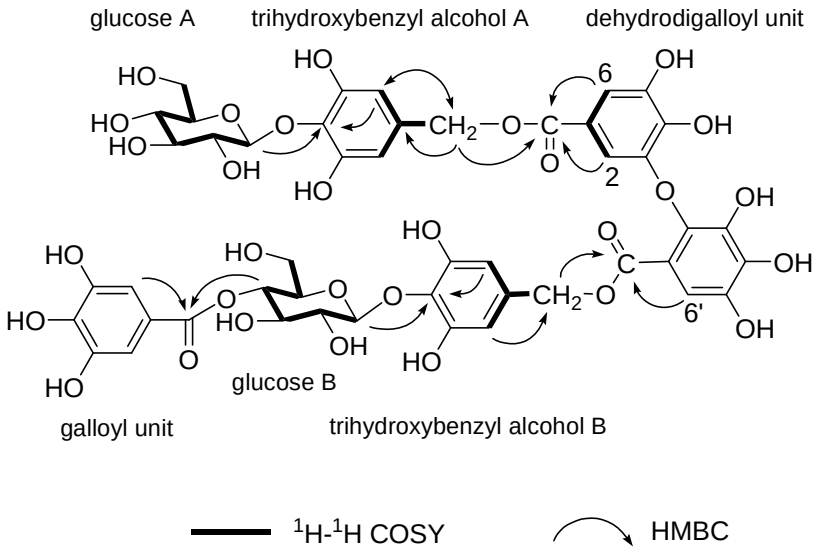

Figure 3. Selected ^{1}H-^{1}H COSY and HMBC correlations (H to C) of **3**