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TWO NEW NAPHTHALENYL GLUCOSIDES AND A NEW PHENYLBUTYRIC ACID GLUCOSIDE FROM THE FRUIT OF *JUGLANS MANDSHURICA*

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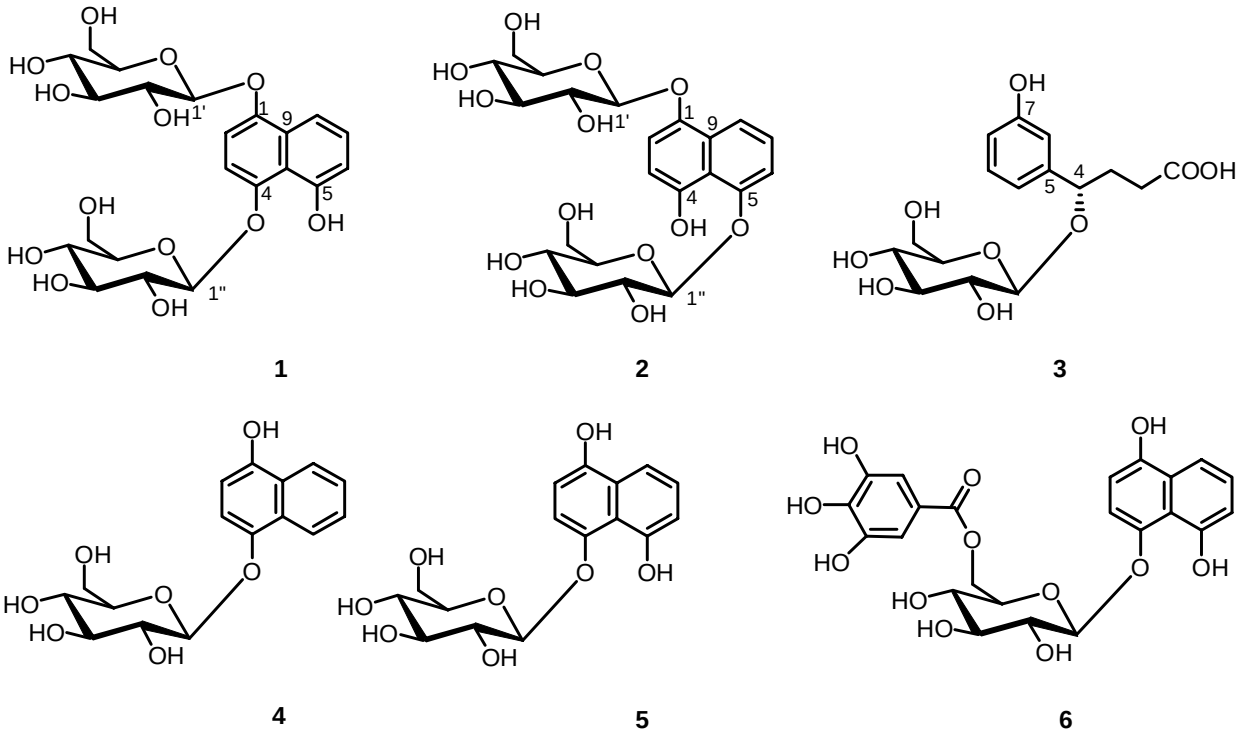
Abstract – Two new naphthalenyl glucosides, juglanosides F – G (**1** – **2**) and a new phenylbutyric acid glucoside, juglanoside H (**3**), along with three known naphthalenyl glucosides (**4** – **6**) were isolated from the fresh rejuvenated fruit of *Juglans mandshurica*. The structures of the new compounds were elucidated as 1,4,5-trihydroxynaphthalene 1,4-di-*O*-β-D-glucopyranoside (**1**), 1,4,5-trihydroxy-naphthalene 1,5-di-*O*-β-D-glucopyranoside (**2**), (*R*)-4-hydroxy-4-(3-hydroxy-phenyl)butyric acid 4-*O*-β-D-glucopyranoside (**3**), on the basis of spectroscopic analysis and chemical evidence.

INTRODUCTION

The fresh rejuvenated fruit of *Juglans mandshurica* (Juglandaceae), commonly called “Qing Long Yi”, has been employed as folk medicine for treatment of cancer and dermatosis and as anodyne to relieve aches in China.[1] Previously, we reported the isolation and structural elucidation of five new α-tetralonyl glucopyranosides from the fruits.[2] In continuation of our study on the fruit, two new naphthalenyl glucosides, named juglanosides F – G (**1** – **2**) and one new phenylbutyric acid glucoside, named juglanoside H (**3**), together with three known naphthalenyl glucosides (**4** – **6**) were isolated. The known compounds were identified as 4-hydroxy-1-naphthalenyl β-D-glucopyranoside (**4**),[3] 1,4,8-trihydroxynaphthalene 1-*O*-β-D-glucopyranoside (**5**),[4] 1,4,8-trihydroxynaphthalene 1-*O*-β-D-[6'-*O*-(3'',4'',5''-trihydroxybenzoyl)]glucopyranoside (**6**)[5] by comparison of spectral and chemical data with

those reported in literature. In this paper, we describe the isolation and structural elucidation of the new compounds on the basis of spectroscopic analysis.



1 **2** **3**

4 **5** **6**

RESULTS AND DISCUSSION

Compound (**1**) was obtained as an amorphous yellow powder. The molecular formula was established as $C_{22}H_{28}O_{13}$ by HR-FAB-MS analysis. On acid hydrolysis, **1** afforded D-glucose as a component sugar, which was identified by GLC analysis of its trimethylsilyl thiazolidine derivative.[6] The ^{1}H-NMR spectrum (Table 1) of **1** showed signals assignable to five aromatic protons [δ 7.12 (1H, d, *J* = 8.5 Hz), 7,27 (1H, d, *J* = 8.5 Hz), 6.86 (1H, dd, *J* = 7.7, 1.1 Hz), 7.32 (1H, dd, *J* = 8.4, 7.7 Hz), 7.88 (1H, dd, *J* = 8.4, 1.1 Hz)], twelve sugar protons between δ 3.44 to 3.93, and two anomeric protons [δ 5.01 (1H, d, *J* = 7.7 Hz) and 5.04 (1H, d, *J* = 7.7 Hz)]. The ^{13}C-NMR spectrum (Table 1), in combination with distortionless enhancement by polarization transfer (DEPT) and ^{1}H-detected multiple quantum coherence (HMQC) experiments, showed 10 carbon signals in the range δ_C 111-155 ascribable to a naphthalene ring, which bore five directly attached hydrogen atoms. Inspection of the ^{1}H-^{1}H correlation spectroscopy (COSY) spectrum indicated a vicinal relation between signals H-2 and H-3; H-6, H-7 and H-8, confirmed by their coupling constants. The signal pattern of those protons indicated **1** to be a trisubstituted naphthalene derivative with two substituents on one ring and the third one on the other. Comparison of the ^{1}H-NMR spectrum of **1** with that of **5** [4], the chemical shift of H-2 of **1** at δ 7.12 was shifted downfield by 0.41 ppm, and H-8 at δ 7.88 was shifted upfield by 0.31 ppm, respectively, suggesting one β-D-glucopyranose is the same as that of **5** and the other one is located at C-1. This was further confirmed

by the heteronuclear multiple-bond correlations (HMBC) experiment, where long-range correlations were observed between signals at δ_H 5.01(Glc-H-1') and δ_C 150.6 (C-1), δ_H 5.04 (Glc-H-1") and δ_C 150.9 (C-4). The β-anomeric configuration for glucopyranoses was determined from their large $^3J_{H1,H2}$ coupling constant values (J = 7.7 Hz). Based on the aforementioned evidence, the structure of **1** was elucidated to be 1,4,5-trihydroxynaphthalene 1,4-di-*O*-β-D-glucopyranoside.

Table 1. ^{1}H-(500 MHz) and ^{13}C-NMR (125 MHz) spectral data for **1 – 3** in CD_3OD.

	1		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		150.6		147.5		177.7
2	7.12 d (8.5)	111.0	7.19 d (8.3)	113.9	2.36 m (2H)	30.9
3	7.27 d (8.5)	112.0	6.73 d (8.3)	110.8	2.14 m	33.2
					2.04 m	
4		150.9		150.2	4.72 t (6.3)	82.1
5		154.8		155.7		145.2
6	6.86 dd (7.7, 1.1)	112.5	7.39 dd (7.8, 1.6)	112.7	6.85 d (2.1)	114.8
7	7.32 dd (8.4, 7.7)	128.0	7.35 dd (8.1, 7.8)	126.7		158.3
8	7.88 dd (8.4, 1.1)	114.8	8.11 dd (8.1, 1.6)	119.2	6.70 dd (7.8, 2.1)	115.5
9		130.2		130.2	7.13 t (7.8)	130.2
10		117.6		117.3	6.84 dd (7.8, 2.1)	119.1
1'	5.01 d (7.7)	103.1	4.90 d (7.8)	104.0	4.41 d (7.8)	104.0
2'	3.62 dd (9.0, 7.7)	75.1	3.59 dd (8.7, 7.8)	75.2	3.24 dd, (8.9, 7.8)	75.5
3'	3.51 dd (9.0, 8.8)	78.2	3.48*	78.2	3.35 dd (9.2, 8.9)	78.2
4'	3.45*	71.4	3.46*	71.4	3.26 t (9.2)	71.6
5'	3.44 m	78.2	3.42 m	78.2	3.15 ddd (9.2, 5.7, 2.3)	77.7
6'	3.71 dd (11.7, 5.8)	62.6	3.72 dd (12.2, 5.1)	62.6	3.54 dd (11.9, 5.7)	62.8
	3.89 br.d (11.7)		3.90 dd (12.2, 2.0)		3.69 dd (11.9, 2.3)	
1"	5.04 d (7.7)	105.0	5.10 d (7.8)	104.6		
2"	3.57 dd (9.0, 7.7)	75.1	3.57 dd (9.2, 7.8)	75.2		
3"	3.50 dd (9.0, 8.5)	78.2	3.49*	78.2		
4"	3.45*	71.4	3.43*	71.4		
5"	3.46 m	78.7	3.52 m	78.8		
6"	3.74 dd (12.2, 5.2)	62.6	3.75 dd (12.2, 5.8)	62.6		
	3.93 dd (12.2, 1.8)		3.95 dd (12.2, 2.1)			

* Overlapped signals.

Compound (**2**) was isolated as an amorphous powder. Its molecular formula, $C_{22}H_{28}O_{13}$, which is the same as that of **1**, was determined by HR-FAB-MS. On acid hydrolysis, **2** gave D-glucose as the component sugar. Besides the signals due to two sets of the β-glucopyranose moiety, the ^{1}H-NMR spectrum of **2** also showed signals assignable to five aromatic protons [δ 7.19 (1H, d, J = 8.3 Hz), 6.73 (1H, d, J = 8.3 Hz), 7.39 (1H, dd, J = 7.8, 1.6 Hz), 7.35 (1H, dd, J = 8.1, 7.8 Hz), 8.11 (1H, dd, J = 8.1,

1.6 Hz)], which were extremely similar to those of **2**. Comparison of the ^{1}H-NMR spectrum of **2** with that of **1** suggested a different glycosylation position. Namely, the chemical shift of H-3 of **2** at δ 7.27 was shifted upfield by 0.54 ppm, and H-6 at δ 6.86 was shifted downfield by 0.53 ppm, respectively, suggesting one β-D-glucopyranose is located at C-5 and the other one is the same as that of **1**. This was further confirmed by the HMBC experiment, where long-range correlations were observed between signals at δ_H 4.90 (Glc-H-1') and δ_C 147.5 (C-1), δ_H 5.10 (Glc-H-1") and δ_C 155.7 (C-5). Thus, the structure of compound (**2**) was determined to be 1,4,5-trihydroxynaphthalene 1,5-di-*O*-β-D-glucopyranoside.

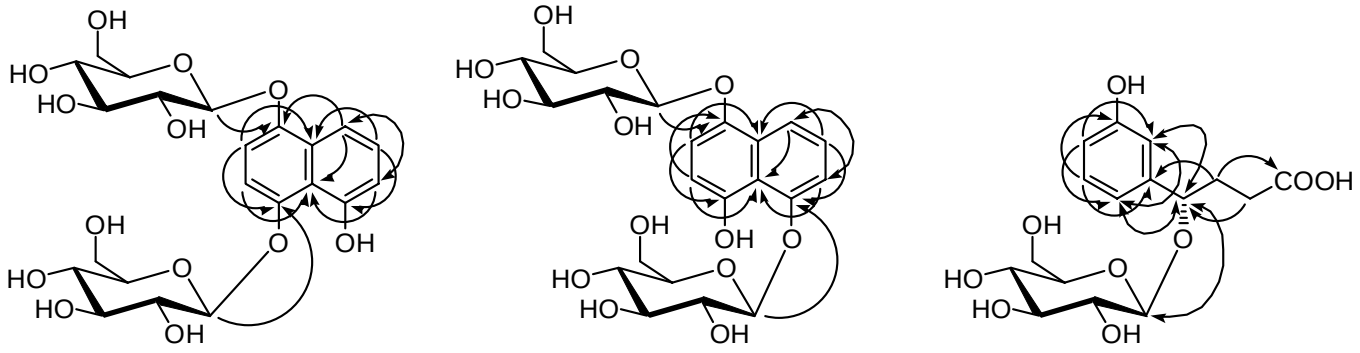
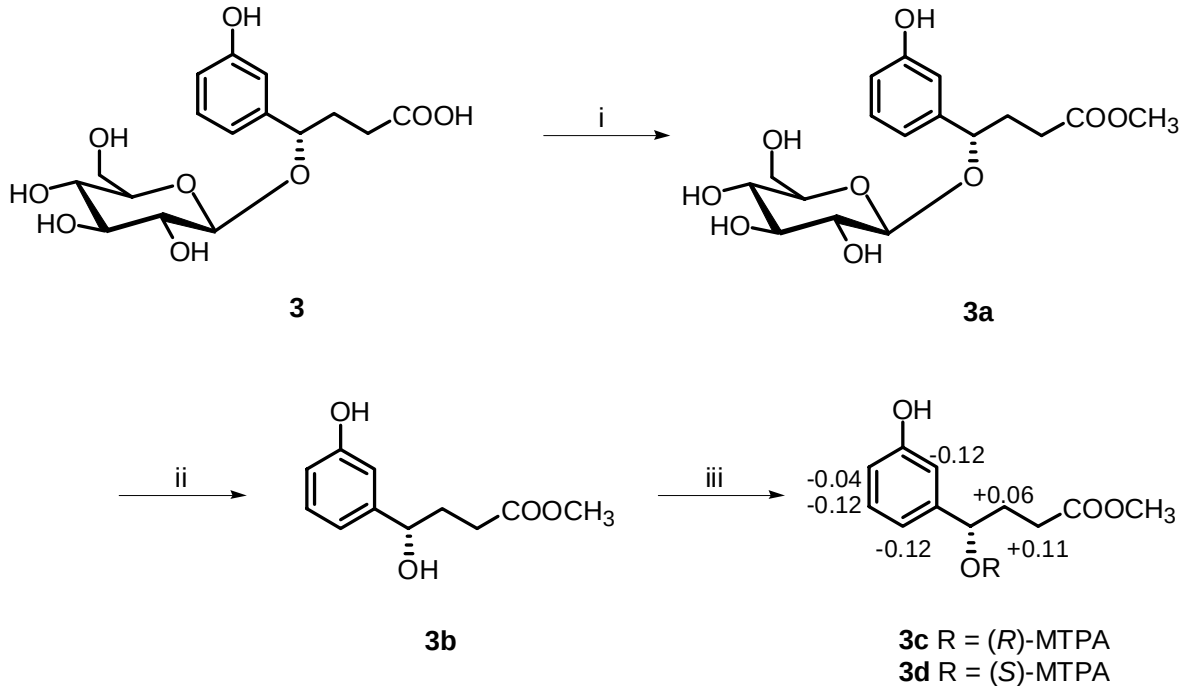


Figure 1 Key HMBC correlations of **1** – **3**.

Compound (**3**) was isolated as an amorphous powder. Its molecular formula, $C_{16}H_{22}O_9$, was determined by HR-FAB-MS. On acid hydrolysis, **3** gave D-glucose as the component sugar. The ^{1}H-NMR spectrum displayed a group of signals for a 1,3-disubstituted aromatic system at δ 6.85 (1H, d, *J* = 2.1 Hz), 6.70 (1H, dd, *J* = 7.8, 2.1 Hz), 7.13 (1H, t, *J* = 7.8 Hz) and 6.84 (1H, dd, *J* = 7.8, 2.1 Hz). In addition, a set of signals due to two methylenes at δ 2.14 (1H, m), 2.04 (1H, m), 2.36 (2H, m) and an oxymethine at δ 4.72 (1H, t, *J* = 6.3 Hz), together with a ^{13}C-NMR resonance at δ 177.7 indicated the presence of a 4-hydroxybutyric acid group. The placement of the 4-hydroxybutyric acid group and the sugar unit at C-5 and C-4 was confirmed by analysis of the HMBC spectrum which showed the following important correlations: H-10, H-6/C-4; H-1'/C-4. Therefore, the plane structure of **3** was determined as 4-hydroxy-4-(3-hydroxyphenyl)butyric acid 4-*O*-β-D-glucopyranoside.

The stereochemistry of **3** was established by chemical transformations and application of the modified Mosher's method.[7] Present in **3** was an asymmetric center at position C-4. Since it possesses a secondary hydroxy group, assignment of the absolute configuration at C-4 was attempted by conversion to Mosher's esters, and by application of Mosher's rules.[7] **3a**, obtained by methylation of **3** with boron trifluroride (BF_3)/ anhydrous methanol, [8] was hydrolyzed with naringinase [9] and yielded **3b**. The structures of **3a** and **3b** were confirmed by analysis of MS and NMR spectral data, followed by converting **3b** into the two stereometric Mosher esters (**3c** and **3d**) by reaction with the *S* and *R* enantiomers of

α-methoxy-α-(trifluoromethyl)phenyl acetyl chloride (MTPA-Cl), respectively (Scheme 1). The differences in ^{1}H-NMR chemical shifts between the diastereoisomers (**3c**) and (**3d**) are illustrated in Scheme 1. Based on Mosher's theory, these data suggested the configuration at C-4 to be *R*. Thus, compound (**3**) was established as (*R*)-4-hydroxy-4-(3'-hydroxyphenyl)butyric acid 4-*O*-β-D-glucopyranoside.



3 **3a** **3b**

3c R = (*R*)-MTPA
3d R = (*S*)-MTPA

i) boron trifluroride/ anhydrous methanol; ii) naringinase, 40°C; iii) (*S*)- or (*R*)-MTPA-Cl, pyridine (dehydrated)

Scheme 1

EXPERIMENTAL

General Experimental Procedures. The UV spectra were obtained with a Shimadzu UV-160 spectrophotometer, whereas the IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5-dm cell. The ESI-MSs were taken on an LCQ mass analyzer. The HR-FAB-MS was taken on a JEOL JMS-700 Mstation spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL ECP-500 spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). For HPLC, a JASCO PU-2080 HPLC system, equipped with a Shodex RI-101 Differential Refractometer detector, was used. Reversed-phase column chromatography (CC) was accomplished with RP-C_{18} silica gel (Chromatorex DM1020T ODS, Fuji Silysia Chemical Ltd.). Silica gel CC was carried out using Kieselgel 60 (E. Merck). TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck). GLC was carried out on a Perkin Elmer Clarus 500 GC-MS instrument.

Plant Material. The fresh rejuvenated fruit of *Juglans mandshurica* was collected in August 2002 at a mountain area of Wuchang, Heilongjiang Province, China. A voucher specimen (QLY 2002008) is preserved at the department of Chinese traditional medicine, Heilongjiang Provincial Institute for Drug Control.

Extraction and Isolation. The fraction A (542 mg) and fraction D (226 mg) obtained from Lobar RP-C_{18} column as mentioned in the previous paper [2] were loaded on a silica gel column and eluted with $CHCl_3$-MeOH-H_2O (60:30:2). Further purification was achieved by repeated RP-HPLC (20 × 150 mm, Senshupak pegasil ODS), affording **1** (44 mg), **2** (6 mg) and **3** (15 mg). The EtOAc extract (7 g) [2] was chromatographed on a silica gel column eluted using a stepwise gradient of $CHCl_3$, MeOH and H_2O to give five fractions (Fr. A-E). Repeated column chromatography of fraction E (1.07 g) on Sephadex LH-20, eluted with MeOH gave 6 fractions. Fraction 1 (425 mg), fraction 2 (265 mg) and fraction 6 (50 mg) was further purified by repeated RP-HPLC and Sephadex LH-20 to give **4** (7 mg), **5** (69 mg) and **6** (27 mg).

Juglanoside F (**1**): amorphous powder, $[\alpha]_D^{25}$ –104° (*c* = 1.1, MeOH). UV (MeOH) λ_{max} (log ε): 337.4 (3.9), 322.2 (3.9), 304.8 (4.0), 224.2 (4.9). IR (KBr) ν_{max}: 3414, 1387, 1253, 1069. ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1. ESI-MS (positive): *m/z* 523.1 $[M+Na]^+$. HR-FAB-MS *m/z* 523.1431 (Calcd for $C_{22}H_{28}O_{13}$ $[M+Na]^+$ 523.1428).

Juglanoside G (**2**): amorphous yellow powder, $[\alpha]_D^{25}$ –86° (*c* = 0.6, MeOH). UV (MeOH) λ_{max} (log ε): 307.2 (4.1), 217.4 (4.8). IR (KBr) ν_{max}: 3426, 1589, 1386, 1073. ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1. ESI-MS (positive): *m/z* 523.3 $[M+Na]^+$. HR-FAB-MS *m/z* 523.1437 (Calcd for $C_{22}H_{28}O_{13}$ $[M+Na]^+$ 523.1428).

Juglanoside H (**3**): amorphous powder, $[\alpha]_D^{25}$ –21° (*c* = 0.5, MeOH). UV (MeOH) λ_{max} (log ε): 275.0 (3.3). IR (KBr) ν_{max}: 3640, 3425, 1729, 1711, 1570, 1397, 1271, 1073. ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1. ESI-MS (positive): *m/z* 381.3 $[M+Na]^+$. HR-FAB-MS *m/z* 381.1169 (Calcd for $C_{16}H_{22}O_9$ $[M+Na]^+$ 381.1161).

Acid Hydrolysis and Determination of the Absolute Configuration of Sugars in 1 – 3 Each solution of **1** – **3** (each 1 mg), in 1 M HCl (dioxane-H_2O, 1:1, 200 μL) was heated at 100°C for 1 h under an Ar atmosphere. After dioxane was removed, the solution was extracted with EtOAc (1 mL × 3) to remove the aglycon. The aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column, concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (1 mL), to which 0.1 M L-cysteine methyl ester hydrochloride in pyridine (2 mL) was added. The mixture was kept at 60°C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.2 mL)

for 2 h. The mixture was partitioned between hexane and H_2O (0.3 mL each) and the hexane extract was analyzed by GLC under the following conditions: capillary column, EQUITY^{TM}-1 (30 m × 0.25 mm × 0.25 μm, Supelco), column temperature, 230°C; injection temperature, 250°C; carrier N_2 gas. In the acid hydrolysate of **1** – **3**, D-glucose was confirmed by comparison of the retention times of their derivatives with those of D-glucose and L-glucose derivatives prepared in a similar way, which showed retention times of 11.79 and 11.33 min, respectively.

Methylation of 3 The sample (5 mg) was dissolved in 150 μL boron trifluoride (BF_3)/anhydrous methanol, and was stirred at 60°C for 20 min. The reaction mixture was evaporated to dryness and the residue was chromotographed on silica gel eluted with $CHCl_3$-MeOH (90:10) to give **3a** (4 mg).

Methyl 4-hydroxy-4-(3'-hydroxyphenyl)-butyrate 4-*O*-β-D-glucopyranoside (**3a**): ^{1}H-NMR (CD_3OD, 500 MHz): δ 2.09 (2H, m, H-3), 2.05 (1H, m, H-2), 2.13 (1H, m, H-2), 3.14 (1H, m, H-5'), 3.23 (1H, dd, *J* = 8.9, 7.5 Hz, H-2'), 3.25 (1H, t, *J* = 9.2 Hz, H-4'), 3.34 (1H, dd, *J* = 9.2, 8.9 Hz, H-3'), 3.54 (1H, dd, *J* = 11.9, 5.4 Hz, H-6'), 3.62 (3H, s, CH_3), 3.68 (1H, dd, *J* = 11.9, 2.5 Hz, H-6'), 4.39 (1H, d, *J* = 7.5 Hz, H-1'),4.73 (1H, t, *J* = 6.3 Hz, H-4), 6.67 (1H, ddd, *J* = 7.8, 2.1, 1.2 Hz, H-8), 6.82 (1H, br d, *J* = 7.8 Hz, H-10), 6.83 (1H, br. s, H-6), 7.12 (1H, t, *J* = 7.8 Hz, H-9). ESI-MS (positive): *m/z* 395.1 ($[M+Na]^+$).

Enzymatic hydrolysis of 3a A solution of **3a** (4 mg) in 0.1 M acetate buffer (pH 4.0, 1.0 mL) was treated with naringinase (Sigma Chemical Co., 3 mg) and then the reaction mixture was stirred at 40°C for 4 h. The reaction mixture was extracted with EtOAC (1 mL × 3) and the organic layer was evaporated to dryness to give **3b** in 40% yields. ^{1}H-NMR ($CDCl_3$): δ 2.06 (2H, q, *J* = 6.8 Hz, H-3), 2.44 (2H, t, *J* = 6.8 Hz H-2), 3.68 (3H, s, CH_3), 4.73 (1H, t, *J* = 6.8 Hz, H-4), 6.74 (1H, dd, *J* = 7.8, 2.5 Hz, H-8), 6.86 (1H, brd, *J* = 2.5 Hz, H-6), 6.89 (1H, br d, *J* = 7.8 Hz, H-10), 7.21 (1H, t, *J* = 7.8 Hz, H-9). ESI-MS (positive): *m/z* 233.3 ($[M+Na]^+$).

(*R*)-MTPA Ester of 3b *S* (+)-MTPA chloride (10 μL) was added to a solution of **3b** (0.6 mg) in pyridine (0.1 mL). After stirring at rt for 4 h, the mixture was evaporated to dryness and purified with preparative HPLC with 70% MeOH to give an (*R*)-MTPA ester (**3c**, 0.5 mg). (**3c**) ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.17 (2H, m, H-3), 2.26 (2H, m, H-2), 6.03 (1H, dd, *J* = 7.8, 5.6 Hz, H-4), 7.10 (1H, d, *J* = 2.2 Hz, H-6), 7.13 (1H, dd, *J* = 7.7, 2.2 Hz, H-8), 7.27 (1H, br d, *J* = 7.7 Hz, H-10), 7.45 (1H, t, *J* = 7.7 Hz, H-9).

(*S*)-MTPA Ester of 3b *R* (–)-MTPA chloride (10 μL) was added to a solution of **3b** (0.6 mg) in pyridine (0.1 mL). Work-up as described above gave an (*S*)-MTPA ester (**3d**, 0.4 mg). (**3d**) ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.23 (2H, m, H-3), 2.37 (2H, t, *J* = 7.3 Hz, H-2), 5.94 (1H, dd, *J* = 7.8, 5.6 Hz, H-4), 6.98 (1H, dd, *J* = 2.0, 1.6 Hz, H-6), 7.09 (1H, dd, *J* = 7.7, 2.0 Hz, H-8), 7.15 (1H, br d, *J* = 7.7 Hz, H-10), 7.33 (1H, t, *J* = 7.7 Hz, H-9).

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