Phenylethanoid Glycosides from the fresh immature legumes of Golden Trumpet Tree (Tabebuia chrysotricha)

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Abstract

Three phenylethanoid glycosides were isolated from a 1-butanol soluble fraction of a methanol extract of the fresh immature legumes of *Tabebuia chrysotricha*. These glycosides were identified to be 2-(3,4-dihydroxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1 → 3)-(4-*O*-caffeoyl)-β-D-glucopyranoside (acteoside), 2-(3,4-dihydroxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1 → 3)-(4-*O*-caffeoyl)-2-*O*-acetyl-β-D-glucopyranoside (2'-acetylacteoside), and 2-(3,4-dihydroxyphenyl)ethyl *O*-α-L-rhamnopyranosyl-(1→3)-(6-*O*-cafeoyl)-β-D-glucopyranoside (isoacteoside), respectively, by spectroscopic methods and chemical evidences.

Introduction

Golden Trumpet Tree (*Tabebuia chrysotricha,* public name: Ipe, Bignoiaceae) is a small tree with yellow flower found on streets, academic cores and public parks of Okinawa Islands in April and May. It has been reported that lapachol, deoxylapachol and tectoquinone isolated from heartwood of *T. avellanedae*,[1-2] 2-acetylnaphtho[2,3-b]furan-4,9-dione from bark of *T. impetiginosa*,[3] and that these naphthoquinone derivatives have anti-cancer, anti-inflammatory and antitumor activities.[4-7] For *T. chrysotricha*, isolation of lapacol, 5-hydroxy-2(1'-hydroxyethyl) naphtho [2,3-b]furan-4,9 dione and dehydro-α-lapachone from the bark have been reported.[8]

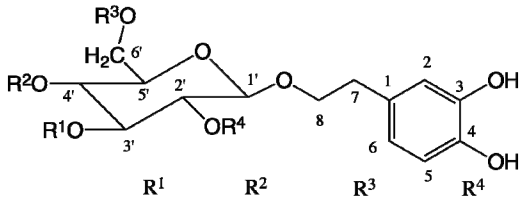
In connection with studies on the useful constituents from the fresh legumes of plants grown subtropical and tropical regions, we examined the constituents from the fresh legumes of *T. chrysotricha* and isolated three phenylethanoid glycosides from the immature ones. Herein, we describe the separation and structural elucidation of these compounds.

[1]

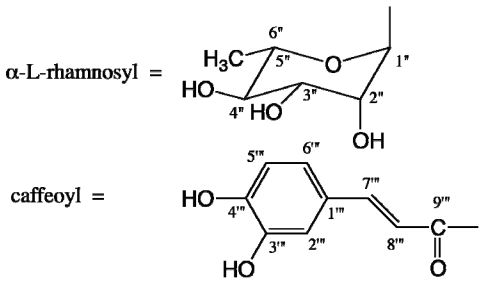
Results and Discussion

1-Butanol (*n*-BuOH)-soluble fraction from a methanol extract of the fresh immature legumes of *T. chrysotricha* was subjected to silica gel chromatography to give three glycosides (**1**, **2**, and **3**).

Compound **1** was obtained as a brown amorphous and has a molecular formula of $C_{29}H_{36}O_{15}$ by observation of a quasi-molecular ion peak at *m*/z 623.1975 $[M–H]^-$ (calcd for $C_{29}H_{35}O_{15}$: 623.1976) in the high resolution electron spray ionization mass spectrum (HR-ESI-MS). Its IR spectrum showed wide bands due to OH at 3600-3000 and due to C-O at 1200-1000 cm^{-1}, indicating that **1** is a glycoside, and bands due to conjugated ester C=O at 1695 and due to aromatic ring at 1597 and 1518 cm^{-1}. Its ^1H and ^{13}C NMR spectra showed signals due to two anomeric protons at δ_H 4.48(d, *J*=8.0 Hz) and 5.24 (d, *J*=1.6 Hz) and due to two anomeric carbon at δ_C 103.0 and 104.2, suggesting that **1** is the glycoside possessing two sugar moieties.



	R1	R2	R3	R4
1	α-L-rhamnosyl	caffeoyl	H	H
2	α-L-rhamnosyl	caffeoyl	H	Ac
3	α-L-rhamnosyl	H	caffeoyl	H



structures

[1] Received: August 14, 2015

The ^{1}H and ^{13}C NMR spectra of **1** showed a carbonyl carbon signal at δ_C 168.30 due to a ester group, signals at δ_H 7.65 (1H, d, *J*=15.9 Hz) and 6.33 (1H, d, *J*=15.9 Hz) due to each *trans*-configuration olefinic proton conjugated with aromatic ring, ABX pattern signals at δ_H 7.11 (1H, d, *J*=2.0 Hz), 6.99 (1H, dd, *J*=8.2, 2.0 Hz), and 6.84 (1H, d, *J*=8.2 Hz) due to aromatic protons, and signals at δ_C 149.8 and 146.8 due to oxygenated aromatic carbons. These spectral data indicated that **1** possesses a caffeoyl (3,4-dihydroxycinnamoyl) moiety as a partial structure.[9-10] Moreover, the ^{1}H and ^{13}C NMR spectra of **1** showed another ABX pattern signals at δ_H 6.76 (1H, d, *J*=2.0 Hz), 6.73 (1H, d, *J*=8.0 Hz), and 6.62 (1H, dd, *J*=8.0, 2.0 Hz) due to aromatic protons, signals at δ_C 147.7 and 146.1 due to oxygenated aromatic carbons, signals at δ_H 4.10 (1H, dd, *J*=7.8, 16.8 Hz), 3.78 (1H, dd, *J*=1.9, 16.8 Hz), and 2.85 (2H, m) due to an ethyloxy group, indicating that **1** possesses (3,4-dihydroxyphenyl)ethyl alcohol moiety as a partial structure.

Hydrolysis of **1** with 5 M HCl gave D-glucose and L-rhamnose as sugars. Assignments of the ^{1}H and ^{13}C NMR data in Table 1 were completely achieved by analyses of 2D-NMR spectra such as homonuclear correlation spectroscopy (COSY), homonuclear Hartmann and Harn spectroscopy (HOHAHA), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) spectra and splitting patterns of signals in the ^{1}H NMR spectrum. The HMBC correlations

Table 1. 1H and 13C NMR data of acteoside (**1**), 2'-acetylacteoside (**2**), and isoacteoside (**3**)

		1 in CD_3OD		2 in DMSO-d_6	2 in CD_3OD		3 in CD_3OD	
		^{1}H (400 MHz)	^{13}C (100 MHz)	^{1}H (500 MHz)	^{1}H (400 MHz)	^{13}C (100 MHz)	^{1}H (400 MHz)	^{13}C (100 MHz)
aglycone								
	1		131.6			130.3		131.4
	2	6.76 d (2.0)	117.2	6.58 d (1.6)	6.67 d (1.8)	115.8	6.58 d (2.0)	117.1
	3		146.1			144.6		146.1
	4		147.7			143.2		144.7
	5	6.73 d (8.0)	116.4	6.62 d (8.0)	6.70 d (8.0)	114.9	6.54 d (8.0)	116.4
	6	6.62 dd (2.0, 8.0)	121.3	6.43 dd (1.6, 8.0)	6.54 dd (1.8, 8.0)	119.9	6.44 dd (2.0, 8.0)	121.3
	7	2.85 m	36.6	2.61 t (7.1)	2.72 m	34.9	2.69 dd (7.8, 8.0)	36.7
	8	4.10 dd (7.8, 16.8)	72.3	3.88 m	4.09 dt (6.4, 9.6)	70.4	3.85 dd (7.8, 15.0)	72.4
		3.78 dd (1.9, 16.8)		3.55 m	3.55 m**		3.61 m	
glucose								
	1'	4.48 d (8.0)	104.2	4.63 d (8.0)	4.43 d (8.1)	100.3	4.23 d (9.8)	104.4
	2'	3.45 dd (8.0, 9.2)	76.2	4.80 dd (8.0, 9.4)	4.80*	73.7	3.30*	75.7
	3'	3.87 *t* (9.2)	81.7	3.98 t (9.4)	3.90 t (9.5)	79.1	3.43 t (9.8)	84.0
	4'	4..97 *t* (9.2)	70.7	4.82 t (9.4)	4.90 t (9.5)	69.2	3.31 t (9.8)	70.4
	5'	3.58 *m*	76.0	3.60 m	3.30-3.61**	74.7	3,45 m	75.4
	6'	3.67 dd (12.2, 2.0)	62.4	3.29 dd (6.0, 9.4)	3.30-3.61**	60.7	4.40 dd (2.0, 12.1)	64.6
		3.60 dd (12.2, 6.4)		3.22 dd (3.0, 9.4)			4.26 dd (5.8, 12.1)	
rhamnose								
	1''	5.24 d (1.6)	103.0	4.62 br s	4.69 br s	101.9	5.10 d (1.0)	102.7
	2''	3.98 dd (1.6, 3.4)	72.4	3.41 br s	3.44 br s	71.2	3.85 m	72.4
	3''	3.64 dd (9.5, 3.4)	70.4	3.16 d (9.4)	3.30-3.61**	70.4	3.61 m	72.3
	4''	3.35 t (9.5)	73.9	3.09 t (9.4)	3.28 t (9.6)	72.2	3.31 dd (2.0, 9.7)	74.0
	5''	3.66 m	72.6	3.35 m	3.30-3.61**	69.4	3.91 m	70.1
	6''	1.15 d (6.4)	18.5	0.94 d (6.2)	1.09 d (6.2)	17.1	1.15 d (6.2)	17.9
caffeoyl								
	1'''		127.7			126.2		127.7
	2'''	7.11 d (2.0)	115.3	7.04 br s	7.08 d (1.6)	113.8	6.94 d (2.0)	115.1
	3'''		146.8			145.4		146.8
	4'''		149.8			146.8		149.7
	5'''	6.84 d (8.2)	116.6	6.74 d (8.0)	6.81 d (8.2)	115.1	6.68 d (8.2)	116.6
	6'''	6.99 dd (2.0, 8.2)	123.2	6.77 d (8.0)	6.97 dd (1.6, 8.2)	121.8	6.79 dd (2.0, 8.2)	123.2
	7'''	6.33 d (15.9)	148.0	7.47 d (15.8)	7.62 d (15.8)	145.5	7.46 d (15.9)	147.3
	8'''	7.65 d (15.9)	114.8	6.19 d (15.8)	6.30 d (15.8)	113.1	6.19 d (15.9)	114.9
	9'''		168.3			166.7		169.2
acetyl				2.00 s	2.01 s	19.5		
						170.1		

* overlapped with solvent peak.
** overlapped with other peaks.

are shown in Fig 1. In the HMBC spectrum, correlation between a doublet (*J*=8.0 Hz) at δ_H 4.48 due to an anomeric proton of glucose and a signal at δ_C 72.3 due to an oxygenated methylene carbon of (3,4-dihydroxyphenyl) ethyl alcohol moiety was observed, indicating that the glucose was attached to the (3,4-dihydroxyphenyl)ethyl alcohol moiety in β-configration as *O*-glycoside bond.[11-15] Therefore, **1** possesses a (3,4-dihydroxyphenyl) ethyl 1-*O*-β-D-glucopyranoside as a partial structure. Moreover, observation of correlation between a triplet (*J*=9.2 Hz) at δ_H 4.97 due to a methine proton at C-4 of glucose and a carbonyl carbon at δ_C 168.3 due to caffeoyl moiety revealed that the caffeoyl moiety was attached to C-4 hydroxy group of glucose as ester bond. The observation of correlation between a doublet (J=1.2 Hz) at δ_H 5.24 due to an anomeric proton of rhamnose and a carbon signal at δ_C 81.7 due to C-3 of glucose suggested that rhamnosyl moiety was attached to C-3 of glucose in α-configuration as *O*-glycoside bond. [11-15]

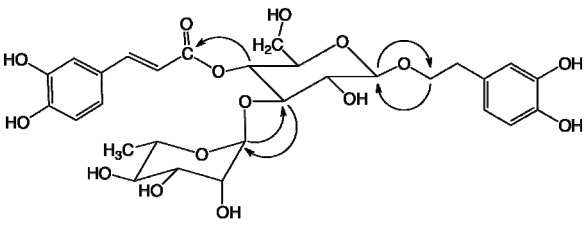


Fig. 1. Selected HMBC correlations of **1**.

Thus, **1** was elucidated to be 2-(3,4-dihydroxyphenyl) ethyl *O*-α-L-rhamnopyranosyl-(1→3)-(4-*O*-caffeoyl)-β-D-glucopyranoside (**1**, acteoside).[13,15-17]

Compound **2** was obtained as a brown amorphous and has a molecular formula of $C_{31}H_{38}O_{16}$ by observation of quasi-molecular ion peak at *m*/z 665.2112 $[M-H]^-$ (calcd for $C_{31}H_{37}O_{16}$: 665.2082) in its HR-ESI-MS and the peak was more 42 mass units than that of **1**. Its IR spectrum showed wide bands due to OH at 3600-3000 and due to C-O at 1200-1000 cm^{-1}, indicating that **2** was a glycoside, and a wide band due to ester C=O at 1615 and a band due to aromatic ring at 1521 cm^{-1}. Its ^1H NMR spectrum showed the presence of two sugar moieties [δ_H 4.69 (1H, br s, anomeric H) and 4.43(1H, d, *J*=8.1 Hz)], a caffeoyl moiety [δ_H 7.62 (1H, d, *J*=15.8 Hz), 7.08 (1H, d, *J*=1.6 Hz), 6.97 (1H, dd, *J*=1.6, 8.2 Hz), 6.81 (1H, d, *J*=8.2 Hz), and 6.30 (1H, d, *J*=15.8 Hz)], and (3,4-dihydroxyphenyl)ethyl alcohol moiety [δ_H 6.70 (1H, d, *J*=8.0 Hz), 6.67 (1H, d, *J*=1.8 Hz), 6.54 (1H, dd, *J*=1.8, 8.0 Hz), 4.09 (1H, dt, *J*=9.6, 6.4 Hz), 3.55 (1H, m), and 2.72 (2H, m)] as same as **1** and a singlet at δ_H 2.01 due to acetyl group was newly observed by comparison with that of **1** as shown in Table 1. These facts indicated that **2** was an acetyl derivative of **1**. The ^{13}C NMR spectrum of **2** was similar to that of **1**, except for signals due to one acetyl group, which also supported that **2** was an acetyl derivative of **1**. Hydrolysis of **2** with 5 M HCl gave D-glucose and L-rhamnose as sugars. In the ^1H NMR spectrum, a signal due to H-2 of glucose at δ_H 4.80 for **2** was a downfield shift of 1.35 ppm by comparison with that at δ_H 3.45 for **1**. The HMBC correlations are shown in Fig 2. The HMBC spectrum of **2** revealed a correlation between the signal due to H-2 of glucose and a carbonyl carbon signal due to acetyl group. These results indicated that the acetyloxy group was attached to C-2 of glucose.

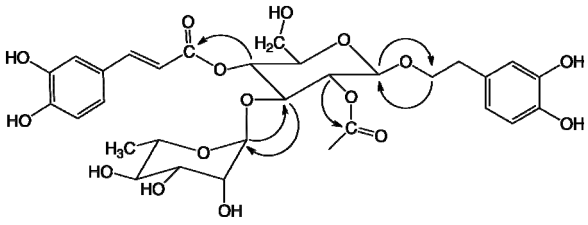


Fig. 2. Selected HMBC correlations of **2**.

Thus, **2** was elucidated to be 2-(3,4- dihydroxyphenyl) ethyl *O*-α-L-rhamnopyranosyl-(1→3)- (4-*O*-caffeoyl-2-*O*-acetyl)-β-D-glucopyranoside (**2**, 2'-acetylacteoside).[17-18]

Compound **3** was obtained as a brown amorphous and has a molecular formula of $C_{29}H_{36}O_{15}$ by observation of quasi-molecular ion peak at *m*/z 623.1979$[M-H]^-$ (calcd for $C_{29}H_{35}O_{15}$: 623.1976) in HR-ESI-MS. Its IR spectrum showed wide bands due to OH at 3600-3000 and due to C-O at 1200-1000 cm^{-1}, indicating that **3** was a glycoside, and bands due to conjugated ester C=O at 1680 and due to aromatic ring at 1600 and 1520 cm^{-1}. Its ^1H and ^{13}C NMR spectra showed signals at δ_H 4.23(d, J=9.8 Hz) and 5.10 (d, J=1.0 Hz) due to two anomeric protons and at δ_C 104.4 and 102.7 due to two anomeric carbons, suggesting that **3** was the glycoside possessing two sugar moieties.

The ^{1}H and ^{13}C NMR spectra of **3** showed the presence of (3,4-dihyodroxyphenyl)ethyl alcohol [ABX pattern signals at δ_H 6.58 (1H, d, *J*=2.0 Hz), 6.54 (1H, d, *J*=8.0 Hz), and 6.44 (1H, dd, *J*=8.0, 2.0 Hz) due to aromatic protons, signals at δ_C 146.1 and 144.7 due to oxygenated aromatic carbons, signals at δ_H 3.85 (1H, dd, *J*=7.8, 15.0 Hz), 3.61 (1H, m) and 2.69 (2H, dd, *J*= 7.8, 8.0 Hz) due to an oxyethyl group] and caffeoyl moieties [δ_H 7.46 (1H, d, *J*=15.9 Hz) and 6.19 (1H, d, *J*=15.9 Hz) due to each *trans*-configuration olefinic proton conjugated with aromatic ring, ABX pattern signals at δ_H 6.94 (1H, *d*, *J*=2.0 Hz), 6.79 (1H, *dd*, *J*=8.2, 2.0 Hz), and 6.68 (1H, *d*, *J*=8.2 Hz) due to aromatic protons, a carbonyl carbon signal at δ_C 169.2 due to a ester group, and signals at δ_C 149.7 and 146.8 due to oxygenated aromatic carbon] as the same partial structure as **1** and **2** as shown in Table 1. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **1**, except for signals due to H-6 of glucose moiety for **3** and due to H-4 of that for **1**.

Hydrolysis of **3** with 5 M HCl gave D-glucose and L-rhamnose as sugars. Assignments of the ^{1}H and ^{13}C NMR data in Table 1 were completely achieved by analyses of 2D-NMR spectra such as COSY, HOHAHA, HSQC, and HMBC spectra and splitting pattern of signals in the ^{1}H NMR spectrum. In the ^{1}H NMR spectrum, two sets of doublet of doublets due to H-6 of glucose moiety at δ_H 4.40 and 4.26 for **3** was downfield shifts of 0.73 and 0.66 ppm by comparison with those at δ_H 3.67 and 3.60 for **1**, respectively. A triplet at δ_H 3.31 due to H-4 of glucose moiety for **3** was a upfield shift of 1.65 ppm by comparison with that at δ_H 4.97 for **1**. The HMBC correlations are shown in Fig 3. The HMBC spectrum of **3** revealed a correlation between the doublet of doublets due to H-6 of glucose moiety at δ_H 4.26 and a carbonyl carbon signal due to caffeoyl moiety at δ_C 169.2. These results indicated that the caffeoyl group was bonded to C-6 of glucose. In the HMBC spectrum, correlation between a doublet (*J*=9.8 Hz) due to an anomeric proton of glucose at δ_H 4.23 and a signal due to an oxygenated methylene carbon of 2-(3,4-dihydroxyphenyl)ethyl alcohol moiety at δ_C 72.4 was observed, indicating that the glucose was attached to C-8 of 2-(3,4-dihydroxyphenyl)ethyl alcohol moiety in β-configuration as *O*-glycoside bond. Therefore, **3** possesses a 2-(3,4-dihydroxyphenyl)ethyl 1-*O*-β-D-glucopyranoside as a partial structure. The observation of correlation between a doublet (*J*=1.0 Hz) due to an anomeric proton of rhamnose at δ_H 5.10 and a carbon signal due to C-3 of glucose at δ_C 84.0 suggested that the rhamnose was bonded to C-3 of glucose in α-configration as *O*-glycoside bond.

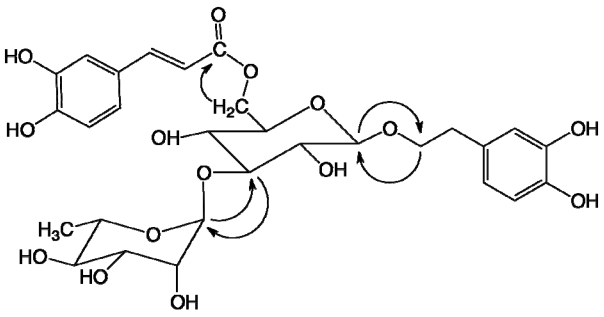


Fig. 3. Selected HMBC correlations of 3.

Thus, **3** was elucidated to be 2-(3,4- dihydroxyphenyl) ethyl *O*-α-L-rhamnopyranosyl-(1→3)-(6-*O*-cafeoyl)-β-D-glucopyranoside (**3**, isoacteoside).[16-18]

J. Schlauer and co-workers researched plant genera in which acteoside (**1**) has been detected in plant belonging to Bignoniaceae and there is no report on detection of acteoside (**1**) in *Tabebuia* plants. Recently, H. Otsuka and co-workers reported isolation of 23 compounds from the branches of *T. chrysotricha* and there is no compound related to **1**−**3**.[19] Therefore, this is the first report of isolations of **1**−**3** in *Tabebuia* genus.[16]

Experimental

Analytical and preparative TLC was carried out on Merck 60 F_{254} silica gel plate (thickness: 0.25 mm) and on the plates (thickness: 0.5 and 2.0 mm), respectively. Column chromatography (C.C.) and flash-column chromatography (F.C.C.) were carried out with Kieselgel 60 F_{254} (Merck). ^{1}H-, ^{13}C-, and two-dimensional NMR spectra were acquired on a Bruker Avance III 400 (^{1}H: 400 MHz, ^{13}C: 100 MHz) and a Burker Avance III 500 (^{1}H: 500 MHz, ^{13}C: 12 MHz) spectrometers in CD_3OD for **1-4**. The symbols s, d, t, q, dd, and ddd denote singlet, doublet, triplet, quartet, doublet of doublets, and double doublet of doublets, respectively. HR-ESI-MS was obtained on a JEOL JMS-T100LP mass spectrometer.

Extraction and isolation. Fresh immature legumes (weight: 6.6 kg) of *T. chrysotricha* collected at campus of University of the Ryukyus, Okinawa-prefecture in April were ground in a blender and immersed in MeOH for ca. 2 weeks. After filtration, the residue was re-extracted with MeOH at 60 ˚C for 8 hours 6 times. The MeOH soln combined were concentrated *in vacuo* into dryness and was suspended with water. The suspension was successively partitioned with chloroform ($CHCl_3$), ethyl acetate (EtOAc), and 1-butanol (*n*-BuOH) to give $CHCl_3$- (8.968 g, 0.14%), EtOAc- (5.595 g, 0.09%), *n*-BuOH- (48.359 g, 0.73%), and H_2O-soluble fractions (24.627 g, 0.37%), respectively. An aliquot of the *n*-BuOH -soluble fraction (2.882 g) was subjected to F.C.C. on silica gel with solvent system of $CHCl_3$ increasing MeOH as solvent ratio of 10:0, 9:1, 4:1, 7:3, 3:2, 1:1, and 0:10 to give fractions A−G. As fraction D (2.00 g) showed a positive DPPH-radical scavenging activity, this fraction was re-chromatographed on a silica gel column with solvent system of EtOAc-MeOH-H_2O-formic acid (HCOOH) such as 50:1:0.1:0.1, 20:1:0.1:0.1, and MeOH to give fractions D1-D4. The fraction D-3 was subjected to reversed phase chromatography on Cosmosil C-18 with solvent system of H_2O-MeOH-HCOOH (1:1:0.006 v/v) to give fractions D3-1−D3-6. The fraction D3-1 was concentrated to give **1** (102 mg). The fraction D3-2 (0.27 g) was subject to reversed phase C.C. on Cosmosil C-18 with H_2O-MeOH-HCOOH (7:3:0.003) and then preparative TLC on silica gel with $CHCl_3$-MeOH-HCOOH (4:1:0.1) to give **2** (9 mg). The *n*-BuOH -soluble fraction (6.230 g) was newly subject to chromatography by the same manner described above to give fraction D3' (5.214 g) corresponding to the fraction D3. The fraction D3' (0.439 g) was subject to preparative TLC on silica gel with solvent system of $CHCl_3$-MeOH-H_2O (4:1:0.05) to give **3** (6 mg).

***Acteocide* (1).** Brown amorphous, $[\alpha]_D^{21}$ – 73.6 (*c* 1.0, MeOH). HR-ESI-MS: *m/z* 623.1975 $[M–H]^–$ (Calcd for $C_{29}H_{35}O_{15}$: 623.1976). IR ν cm^{-1}: 3600-3000 (OH), 1695 (conjugated C=O), 1597 and 1518 (aromatic ring) and 1200-1000 (C-O) ; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Table 1. These physical and spectral data coincided with those in references. [13,15-17,20)]

2'-acetyl acteoside (2). Brown amorphous, $[\alpha]_D^{25}$ − 117.6 (*c* 0.5 , MeOH). HR-ESI-MS: *m/z* 665.2112 $[M−H]^–$ (Calcd for $C_{31}H_{37}O_{16}$: 665.2082). IR ν cm^{-1}: 3600-3000 (OH), 1615 (C=O), 1521 (aromatic ring) and 1200-1000 (C-O) ; ^1H (CD_3OD, 500 and 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Table 1. These physical and spectral data coincided with those in references. [17-18,20)]

Isoacteoside (3). Brown amorphous, $[\alpha]_D^{21}$ + 43.0 (*c* 0.45, MeOH). HR-ESI-MS: *m/z* 623.1979 [M□H]$^–$ (Calcd for $C_{29}H_{35}O_{15}$: 623.1976). IR ν cm^{-1}: 3600-3000 (OH), 1680 (conjugated C=O), 1200-1000 (C-O), and 1600 and 1520 (aromatic ring); ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz): Table 1. These spectral data coincided with those in references. [16-18)]

***Acid hydrolysis of* 1, 2, *and* 3**. Acid hydrolysis was performed by modifying method described in references.[14, 22-23)] Typical procedure was descried as follows: **1** (10 mg) dissolved in 1.0 mL of 5 M HCl was heated at 80˚C for 4 hr. After cooling, reaction mixture was passed through anion exchange resin to neutralize and elute was subjected to chromatography by a reversed phase column as solvent with H_2O → MeOH. The fraction eluted with H_2O was subjected to HPLC analysis. L-rhamnose and D-glucose were detected at retention times of 11.7 and 18.9 min, respectively. Compounds **2** and **3** were hydrolyzed by the same manner as **1** to give L-rhamnose and D-glucose, respectively. HPLC conditions were as follows: HPLC column, Polyamine II, 4.6 mm i.d. × 300 mm (YMC co. Ltd); solvent, CH_3CN-H_2O (3:1v/v); flow rate, 1 mL/min.; detection, optical rotation (JASCO OR-990). L-Rhamnose and D-glucose were detected as a negative optical peak and as a positive optical peak, respectively.

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