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Oligomeric hydrolyzable tannins from *Monochaetum multiflorum*

José H. Isaza, Hideyuki Ito, Takashi Yoshida*

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan

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Abstract

Four hydrolyzable tannins, nobotanins Q, R, S, and T, were isolated from the aqueous acetone extract of the dried leaves of *Monochaetum multiflorum* (Melastomataceae), a plant indigenous to Colombia. Their dimeric and tetrameric structures were elucidated by spectral and chemical methods. Eight known hydrolyzable tannin monomers and eight ellagitannin oligomers characteristic of melastomataceous plants were also characterized as tannin constituents of the plant.

Keywords: *Monochaetum multiflorum*; Melastomataceae; Tannins; Oligomeric ellagitannin; Nobotanins

1. Introduction

Melastomataceae is a pantropical family with over 166 genera that include about 4300 species (Renner, 1993). Although members of this family are not popularly used for medicinal purposes, several *Melastoma*, *Medinilla,* and *Osbeckia* species, in addition to others, are known to be astringent drugs and are used as remedies for stomach ailments, wounds, hemorrhoids, diarrhea, and dysentery in tropical and subtropical Asian countries (Perry, 1980). During our investigations on tannins and related polyphenols used in traditional medicine, we have found that some genera in this family, such as *Tibouchina* (Yoshida et al., 1991b; Yoshida et al., 1999b), *Medinilla* (Yoshida et al., 1986), *Heterocentron* (Yoshida et al., 1986, 1995), *Melastoma* (Yoshida et al., 1992c,d), and *Bredia* (Yoshida et al., 1994), produce characteristic oligomeric hydrolyzable tannins, nobotanins A through C and E through P, which are likely responsible for their astringency. Nobotanin B, the most abundant and widely distributed dimer in these genera, has been reported to be a specific inhibitor of poly-(ADP-ribose) glycohydrolase, purified from human placenta (Aoki et al., 1993), and also to exhibit anti-HIV activity (Nakashima et al., 1992). Several monomeric hydrolyzable tannins from *Osbeckia* species have been characterized as antioxidant components of the plant (Su et al., 1988).

In our recent study on the polyphenolics of *Monochaetum multiflorum* (Bompl.) Naudin, a shrub endemic to Colombia that has been used as a remedy for infections and skin injuries in parts of that country, we isolated and characterized several new acylated glycosides, including a novel diester (monochaetin) (Isaza et al., 2001) of tetrahydroxy-μ-truxinic acid with two moles of quercetin-3-*O*-galactoside. Further investigation of polar constituents of the plant led to the isolation of additional new polyphenols (**1**–**4**) characterized as oligomeric hydrolyzable tannins, named nobotanins Q (**2**), R (**1**), S (**4**), and T (**3**), and 16 known tannins.

2. Results and discussion

2.1. Isolation of tannins

Dried leaves of *M. multiflorum* collected in Manizales, Colombia, were homogenized in aqueous acetone, and the concentrated homogenate was extracted successively with Et_2O, EtOAc, and 1-butanol. The EtOAc and 1-butanol extracts and the water-soluble fraction were each separately fractionated by column chromatography over polystyrene and/or polyvinyl gel and preparative HPLC to yield the four new polyphenols (**1**–**4**) along with 16 known hydrolyzable tannins. The 16 known tannins were identified by direct comparison of

* Corresponding author. Tel./Fax: +81-86-251-7936.
E-mail address: yoshida@pharm.okayama-u.ac.jp (T. Yoshida).

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their spectral and physical data with those of authentic specimens. Among the known tannins, eight were hydrolyzable tannin monomers: 1,2,6-tri-*O*-galloyl-β-D-glucose; 1,4,6-tri-*O*-galloyl-β-D-glucose; 1,2,4,6-tetra-*O*-galloyl-β-D-glucose (Haddock et al., 1982); nobotanin D (Yoshida et al., 1991a); pterocaryanin C (Yoshida et al., 1991b); isostrictinin, pedunculagin, and casuarictin (Okuda et al., 1983). The other eight were oligomers nobotanins A, B, F (Yoshida et al., 1991b), and O (Yoshida et al., 1999a), and brediatin B (Yoshida et al., 1994) (dimers); nobotanins E (Yoshida et al., 1991b) and J (trimers); and nobotanin K (tetramer) (Yoshida et al., 1995). Nobotanin K was the major tannin in this plant, as in *Heterocentron roseum* (Yoshida et al., 1995).

Upon acid hydrolysis, all of the new compounds **1**–**4** gave the common constituents gallic acid, ellagic acid, and valoneic acid dilactone as polyphenolic acids, suggesting that they were ellagitannins. The retention time of each compound on normal-phase HPLC, which reflects the molecular size of ellagitannins (Okuda et al., 1995), revealed the oligomeric nature of nobotanin R as a dimer and nobotanins Q, S, and T as tetramers. Ellagic acid and valoneic acid dilactone liberated by hydrolysis originated from chiral hexahydroxydiphenoyl (HHDP) and valoneoyl units in the ellagitannin molecules (Yoshida et al., 1992b). The strong positive Cotton effects at 227 and 237 nm in the circular dichroism (CD) spectra of **1**–**4** indicated that their atropisomerisms were all (*S*)-series (see Experimental) (Okuda et al., 1982).

2.2. Structure of dimeric ellagitannin

Nobotanin R (**1**) was isolated as an off-white amorphous powder. Electrospray ionization (ESI)-MS (Yoshida et al., 2000) showed the pseudo-molecular ion peak $[M+NH_4]^+$ at *m*/*z* 1588 corresponding to the molecular formula $C_{68}H_{50}O_{44}$. The ^1H NMR spectrum revealed the presence of three galloyl, an HHDP, and a valoneoyl group (see Fig. 1, and Fig. 1 inset), as evidenced by three 2H singlets at δ 7.23, 7.15, and 7.12, and five 1H-singlets at δ 7.08, 6.71, 6.47, 6.45, and 6.16. The dimeric nature of **1** was indicated by two anomeric proton signals at δ 6.11 [*d*, *J* = 8.5 Hz, glucose (Glc) II-H-1] and 5.66 (*d*, *J* = 8.0 Hz, Glc I-H-1). The other glucose proton signals, which were fully assigned based on ^1H–^1H shift correlation spectroscopy (COSY), indicated the $^4C_1$ conformation of both glucose residues. A large difference between the chemical shifts of the C-6 methylene proton signals on Glc I suggested the presence of a biphenyl moiety with bridged ester linkages between O-4/O-6 (Yoshida et al., 1992b) (Table 1), but there was no bridged ester linkage at O-4/O-6 on the other glucose core (Glc II), based on the close chemical shifts of the C-6 methylene proton signals, as observed in pentagalloyl-β-

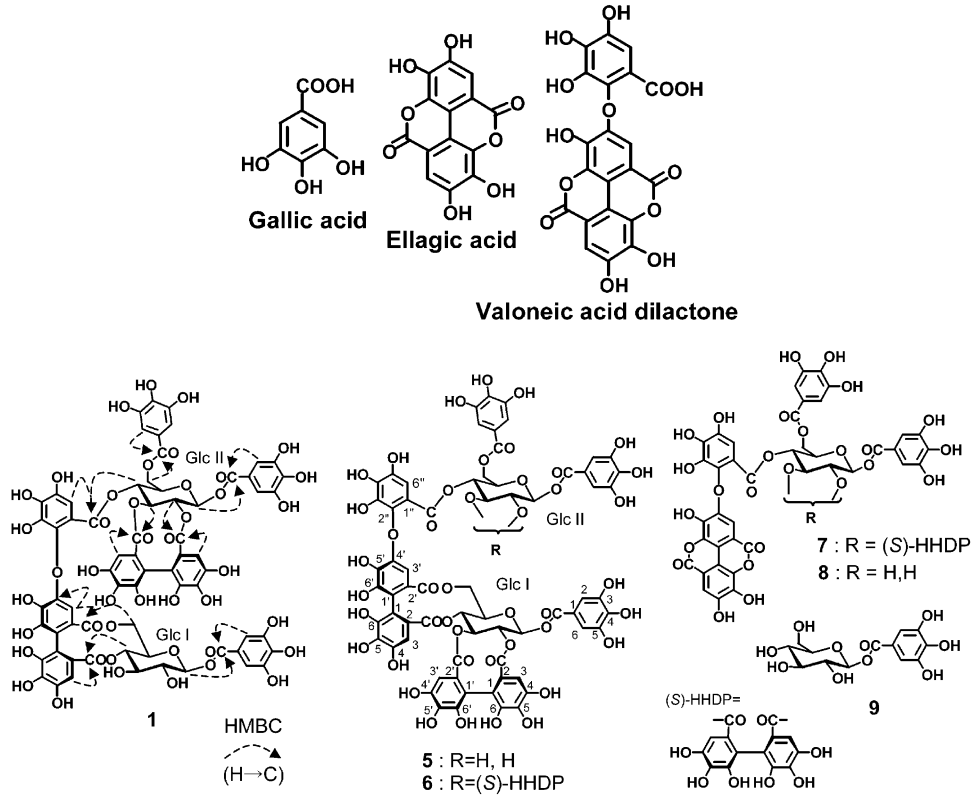

Fig. 1. Structures of acid hydrolysates of new tannins and **1**–**18** including selected HMBC correlations of **1**.

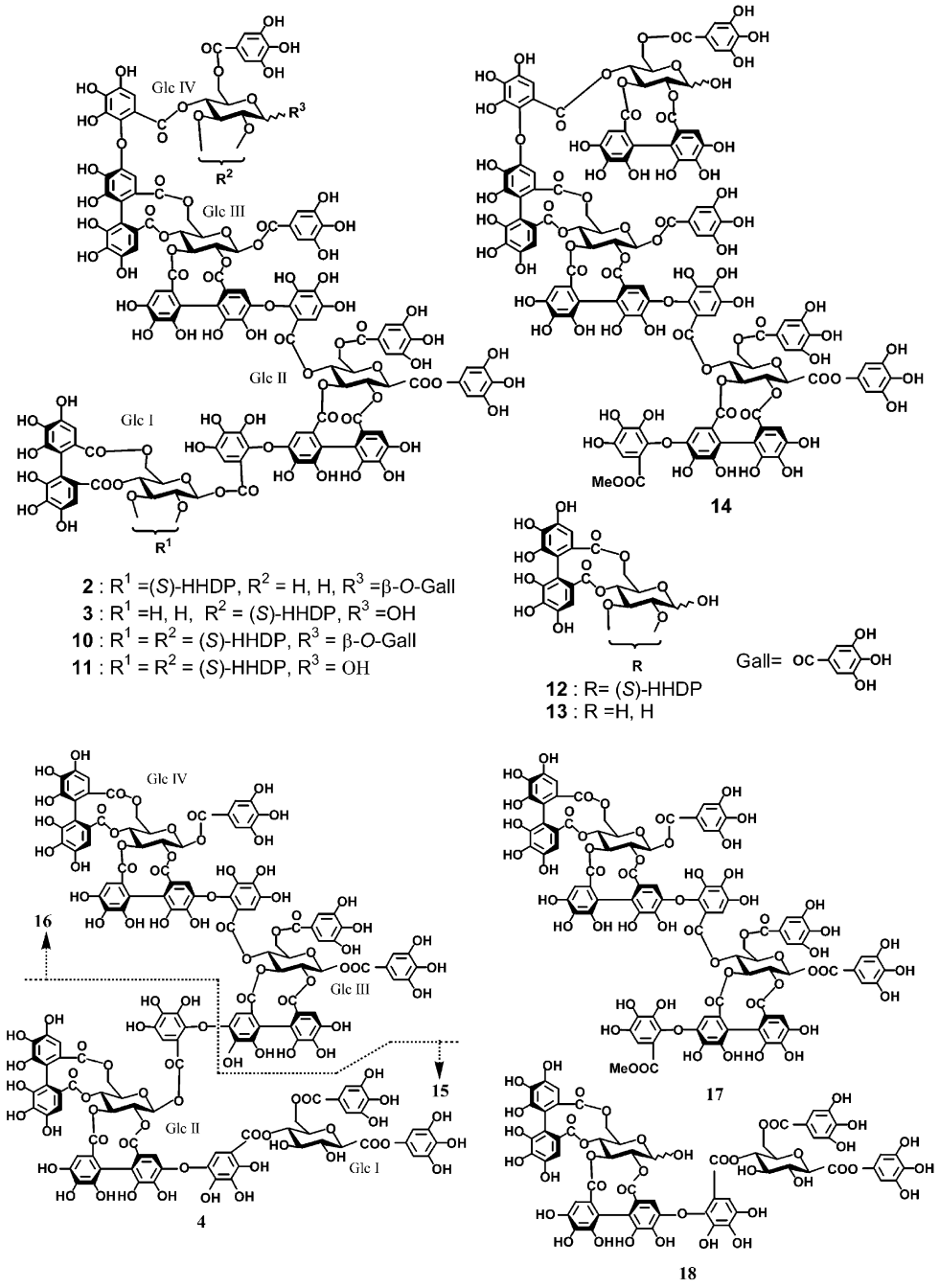

Fig. 1 (*continued*).

D-glucose. These spectral features suggested that nobotanin R (**1**) is an isomer of brediatin B (**5**) (Yoshida et al., 1994) with free hydroxyl groups at different locations on the glucose cores. The presence of free hydroxyl groups at O-2/O-3 in Glc I was evidenced by the remarkable upfield shifts of the H-2 and H-3 signals, shifts of 1.34 and 1.71 ppm, respectively, as compared with the corresponding signals in Glc II. The location of the valoneoyl group in **1** was substantiated by HMBC measurements. The signal at δ 7.08 due to the valoneoyl H-6″ showed connectivity to the glucose-II H-4 (δ 5.70) through the respective three-bond couplings with a common ester carbonyl carbon resonance at δ 165.0. Similarly, the valoneoyl H-3 (δ 6.71) was correlated with a carbonyl carbon (δ 168.4), which also showed a correlation with the glucose-I H-4 (δ 4.91). The location of a galloyl group at each anomeric position, as well as the HHDP group at glucose II O-2/O-3, was supported by evidence from the HMBC correlations, as illustrated in Fig. 1. Thus structure **1** was assigned to nobotanin R. Upon partial hydrolysis in hot water, **1** yielded three hydrolysates: **7**, **8**, and β-glucogallin (**9**) (Jachymczyk et

al., 1964). The hydrolysates **7** and **8** were identified as the products previously obtained by a similar treatment of nobotanin F (**6**) (Yoshida et al., 1991a). A re-examination of the partial hydrolysis of **6** yielded nobotanin R (**1**), as well as brediatin B (**5**), **7**, **8**, and **9**, providing further evidence for the structure of **1**. Based on these data, the structure of nobotanin R was depicted as **1**.

2.3. Structures of tetrameric ellagitannins

Nobotanin Q (**2**) was isolated as an off-white amorphous powder (Fig. 1). Its ESIMS gave an $[M+NH_4]^+$ ion peak at m/z 3458 corresponding to $C_{150}H_{104}O_{96}$, which was consistent with the ^1H and ^{13}C NMR spectral data. The presence of five galloyl groups in **2** was indicated by the ^1H NMR spectrum exhibiting five 2H singlets (δ 7.27, 7.19, 7.17, 7.10, and 6.98). Among thirteen 1H-singlets in the aromatic proton region, three 1H-singlets at the lowest field (δ 7.21, 7.15 and 7.12) were characteristic of a proton due to a galloyl part (Val H-6″) of each valoneoyl unit. Ten 1H-singlets [δ 6.66, 6.55, 6.51, 6.48, 6.41, 6.35, 6.28 (Val H-3, HHDP H-3, 3′), 6.20, 6.08, 6.02 (Val H-3′)] plus the above three thus accounted for the presence of two HHDP and three valoneoyl groups. The presence of four glucose residues (Glc I–IV) was indicated by four well-resolved anomeric carbon and proton signals at δ_C 92.0, δ_H 6.07 ($J=8.5$ Hz, Glc I-H-1), δ_C 92.4, δ_H 5.96 ($J=8.5$ Hz, Glc II-H-1), δ_C 92.2, δ_H 6.20 ($J=8.5$ Hz, Glc III-H-1), and δ_C 95.4, δ_H 5.78 ($J=8$ Hz, Glc IV-H-1). The coupling patterns of the other sugar proton signals assigned by ^1H–^1H COSY were characteristic of β-D-glucopyranose adopting a $^4C_1$ conformation, as shown in Table 2. These proton signals and the ^{13}C resonances of the glucose residues that were assigned by HMQC were almost superimposable on those of nobotanin K (**10**) (Yoshida et al., 1995), except for those due to glucose core IV. The H-2 and H-3 of Glc IV shifted remarkably, by 1.53–1.84 ppm, to a higher field than did the corresponding signals of **10**, suggesting the presence of two free hydroxyl groups at C-2/C-3 of Glc IV (Table 2). Based on these data, nobotanin Q (**2**) was deduced to be a des-HHDP derivative of **10**, as shown in its formula (Fig. 1). The locations of the two HHDP groups on glucose core I in **2** were chemically confirmed by partial hydrolysis, which yielded pedunculagin (**12**) (Okuda et al., 1983) and **8**.

Table 1
^1H and ^{13}C NMR spectroscopic data for the glucose moieties of nobotanin R (**1**) and brediatin B (**5**) [500 MHz for ^1H, 126 MHz for ^{13}C, $(CD_3)_2CO+D_2O$, coupling constants (J in Hz) in parentheses]

Position	**1**		**5**
	δ_H	δ_C	δ_H
Glc I			
1	5.66 *d* (8)	98.8	6.15 *d* (8.5)
2	3.81 *dd* (8, 9.5)	73.9	5.17 *dd* (8.5, 10)
3	3.75 *t* (9.5)	75.7	5.41 *t* (10)
4	4.91 *t* (9.5)	72.7	5.11 *t* (10)
5	4.05 *dd* (7, 9.5)	72.9	4.43 *dd* (6, 10)
6	5.07 *dd* (7, 13)	63.6	5.17 *dd* (6, 12.5)
	3.62 *br d* (13)		3.79 *d* (12.5)
Glc II			
1	6.11 *d* (8.5)	92.1	5.75 *d* (8.5)
2	5.15 *dd* (8.5, 10)	75.2	3.57 *dd* (8.5, 10)
3	5.46 *t* (10)	77.7	3.43 *t* (10)
4	5.70 *t* (10)	67.5	5.20 *t* (10)
5	3.93 *dd* (2.5, 10)	73.7	3.89 *ddd* (1.5, 8, 10)
6	4.69 *br d* (13)	62.9	4.49 *dd* (1.5, 13.5)
	4.03 *dd* (2.5, 13)		4.19 *dd* (8, 13.5)

Nobotanin T (**3**) was isolated as a light brown powder. Its molecular formula was determined as $C_{143}H_{100}O_{92}$ by ESIMS, which showed a doubly charged pseudo-molecular ion peak at m/z 1662 $[M+2NH_4]^{2+}$, and by ^1H and ^{13}C NMR spectral data analysis. Most of the proton signals are duplicated in the ^1H NMR spectrum of **3**, due to the presence of a mixture of α and β anomers (1:2). The presence of four galloyl, three valoneoyl, and two HHDP groups in **3** was suggested by respective paired-signals in the aromatic proton region (see Experimental). The four glucose residues were indicated by well-resolved anomeric carbon and proton signals at δ_C 95.4, δ_H 5.55 ($J=8.5$ Hz, Glc I-H-1), δ_C 92.2, δ_H 6.01, 6.00 (each *d*, $J=8.5$ Hz, 1H in total, Glc II-H-1), δ_C 92.2, δ_H 6.12, 6.11 (each *d*, $J=8.5$ Hz, 1H in total, Glc III-H-1), and δ_C 91.2, δ_H 5.44 ($J=3.5$ Hz, Glc IV-H-1α), δ_C 94.7, δ_H 5.06 ($J=8.5$ Hz, Glc IV-H-1β). The other sugar protons, which were assigned by COSY and TOCSY, were characteristic of β-D-glucopyranose with a $^4C_1$ conformation, as shown in Table 2. Glucose proton signals, except for those of glucose core I, were similar to the corresponding signals of nobotanin P (**11**) (Yoshida et al., 1999a) in both ^1H and ^{13}C NMR spectra, indicating that this tannin is a des-HHDP derivative of nobotanin P (**11**). This assumption was supported by mild methanolysis of **3** which gave 4,6-(*S*)-HHDP-glucopyranose (**13**) (Okuda et al., 1983) and the methanolysis product **14**. The methanolysate **14** was identical with a product that was also obtained along with **12** by treatment of **11** in a similar way. Consequently, the structure of nobotanin T was established as **3**.

Nobotanin S (**4**) showed the pseudo-molecular ion peak $[M+NH_4]^+$ at m/z 3458 in ESIMS, corresponding to the molecular formula $C_{150}H_{104}O_{96}$, which was the same as that of nobotanin Q (**2**). The composition of the polyphenolic acyl groups of **4** was the same as that of **2**, as indicated by five 2H-singlets, due to five galloyl groups, and thirteen 1H-singlets, due to three valoneoyl and two HHDP groups. Both ^1H and ^{13}C NMR spectra of **4** were assigned by ^1H–^1H COSY and HMQC, as summarized in Table 3. A comparison of these NMR data with those of the known nobotanin-series tannins

revealed that the signals of two glucose cores (Glc I and II) were close to those of nobotanin G (**15**) (Yoshida et al., 1992a) and that the signals of the other two (Glc III and IV) resembled those of nobotanin B (**16**) (Yoshida et al., 1991b). This observation implied that nobotanin S is a tetramer biogenetically formed by intermolecular oxidative coupling (Okuda et al., 1990) between **15** and **16** to form a valoneoyl group. The binding modes of the three valoneoyl and other units in **4** were established by the three-bond correlations (glucose proton-ester carbonyl carbon-aromatic proton) in HMBC. The assignment of the H-3′ signal in each valoneoyl group, which was a key step in analyzing its binding mode, was based on its correlation with one of three downfield shifted oxygen-bearing carbons (Val C-4′) at around δ 146. The structure **4** was substantiated as that of nobotanin S by a mild methanolysis yielding malabathrin D (**17**) (Yoshida et al., 1992d) of known structure and a dimeric methanolysate (**18**). The ESIMS [m/z 1436 $(M+NH_4)^+$] and ^{1}H NMR spectra of **18** were consistent with the proposed structure (see Experimental).

The present study provides additional evidence that nobotanins are oligomers characteristically produced by melastomataceous plants and are composed of a combination of monomeric units of pterocaryanin C (1,4,6-*O*-trigalloyl-2,3-*O*-(*S*)-HHDP-β-D-glucose) and casuarictin (1-*O*-galloyl-2,3/4,6-*O*-bisHHDP-β-D-glucose) (or their analogues) (Yoshida et al., 1991b).

Table 2
^{1}H and ^{13}C NMR data for the glucose moieties of nobotanins Q (**2**), R (**3**), K (**10**) and P (**11**) [500 MHz for ^{1}H, 126 MHz for ^{13}C, $(CD_3)_2CO + D_2O$, coupling constants (J in Hz) in parentheses]

Position	**2**		**10**	**3**				**11**	
	δ_H	δ_C	δ_H	δ_H		δ_C		δ_H	
				α-anomer	β-anomer	α-anomer	β-anomer	α-anomer	β-anomer
Glc I									
1	6.07 *d* (8.5)	92.0	6.07 *d* (8)	5.55 *d* (8.5)		95.4		6.05 *d* (8.5)	
2	5.10[a]	76.8	5.10 *dd* (8, 10)	3.55 *t* (8.5)		74.0		5.09–5.18	
3	5.35 *dd* (9, 10)	77.1	5.36 *t* (10)	3.73[c]		75.4		5.34 *t* (10)	
4	5.19 *t* (10)	69.0	5.10 *t* (10)[b]	4.82 *t* (10)		72.2		5.09–5.18	
5	4.39 *br d* (10)	73.5	4.39 *dd* (6, 10)	4.02 *dd* (6.5, 10)		72.9		4.36 *dd* (6.5, 10) 5.25 *dd* (6.5, 13)	
6	5.10[a] 3.89 *br d* (13)	63.2	5.10 *dd* (6, 13) 3.70 *br d* (13)	5.12 *dd* (7, 13) 3.71 *d* (13)		63.5		3.76 *br d* (13)	
Glc II									
1	5.96 *d* (8.5)	92.4	5.96 *d* (8)	6.01 *d* (8.5)	6.00 *d* (8.5)	92.2		6.13 *d* (8.5)	6.14 *d* (8.5)
2	5.12 *dd* (8.5, 10)	75.1	5.13 *dd* (8, 10)	5.18[d]	5.17[d]	75.1	75.0	5.31 *br t* (10)	5.09–5.18
3	5.28 *t* (10)	77.9	5.29 *t* (10)	5.32 *t* (9.5)	5.70 *t* (10)	77.7		5.31 *t* (10)	
4	5.69 *t* (10)	66.6	5.70 *t* (10)	5.71 *t* (9.5)	5.70 *t* (10)	66.6		5.72 *t* (10)	
5	3.35 *br d* (10)	73.8	3.34 *d* (10)	3.40 *d* (10)		73.6		3.30–3.40	
6	4.86 *br d* (13.5) 3.83 *br d* (13.5)	63.6	4.87 *br d* (12) 3.85 *br d* (12)	4.84 *br d* (12.5) 3.77 *br d* (12.5)		63.3		4.88 *br d* (12.5) 3.73–3.78	
Glc III									
1	6.20 *d* (8.5)	92.2	6.18 *d* (8)	6.12 *d* (8.5)	6.11 *d* (8.5)	92.2		5.97 *d* (8.5)	5.98 *d* (8.5)
2	5.08 *dd* (8.5, 10)	75.8	5.09 *dd* (8, 10)	5.25 *dd* (8.5, 9)	5.17[d]	76.4		5.09–5.18	
3	5.82 *t* (10)	76.8	5.82 *t* (10)	5.80 *t* (9)	5.78 *t* (9)	76.9		5.81 *t* (9.5)	5.84 *t* (10)
4	5.14 *t* (10)	69.8	5.10 *t* (10)[b]	5.16[d]		69.5		5.09–5.18	
5	4.64 *dd* (6, 10)	73.2	4.60 *dd* (6, 10)	4.54 *br d* (10)		72.8	82.7	4.57 *m*	
6	5.29 *dd* (6, 13.5) 3.83 *br d* (13.5)	63.0	5.28 *dd* (6, 13) 3.82 *br d* (13)	5.15 *dd* (7, 12.5) 3.78 *br d* (12.5)		63.5		5.09–5.18 3.73–3.78	
Glc IV									
1	5.78 *d* (8)	95.4	6.13 *d* (8)	5.44 *d* (3.5)	5.06 *d* (8)	91.2	94.7	5.45 *d* (3.5)	5.07 *d* (8.5)
2	3.57 *dd* (8, 9)	74.3	5.10 *dd* (8, 10)	5.02 *dd* (3.5, 9.5)	4.81 *dd* (8, 10)	75.0	77.2	5.02 *dd* (3.5, 10)	4.81 *dd* (8.5, 9.5)
3	3.51 *br t* (10)	75.2	5.35 *t* (10)	5.57 *t* (9.5)	5.16[d]	75.4	77.7	5.58 *t* (10)	5.09–5.19
4	5.19 *t* (10)	71.3	5.55 *t* (10)	5.47 *t* (9.5)	5.48 *t* (10)	68.5	68.1	5.50 *t* (10)	5.47 *t* (9.5)
5	3.92 *ddd* (1.5, 4.5, 10)	73.2	4.07 *dd* (3, 10)	4.37 *dd* (3, 9.5)	3.73[c]	68.2	72.6	4.37 *br d* (10)	3.73–3.78
6	4.42 *br d* (12) 4.22 *dd* (4.5, 12)	63.7	4.46 *d* (13) 4.25 *dd* (3, 13)	4.43 *br d* (12) 4.12 *dd* (3, 12)	4.55 *m* 4.20 *dd* (3.5, 12)	62.9		4.38 *br d* (13) 4.13 *dd* (3, 13)	4.57 *br d* (12) 4.20 *dd* (3, 12)

[a] Overlapped with each other.
[b] Overlapped with each other.
[c] Overlapped with each other.
[d] Overlapped with each other.

Table 3
^{1}H and ^{13}C NMR data for the glucose moieties of nobotanins S (**4**), G (**15**), and B (**16**) [500 MHz for ^{1}H, 126 MHz for ^{13}C, $(CD_3)_2CO + D_2O$, coupling constants (*J* in Hz) in parentheses]

Position	4		15	16
	δ_H	δ_C	δ_H	δ_H
Glc I				
1	5.67 *d* (7.5)	95.4	5.70 *d* (8)	
2	3.64 *dd* (7.5, 10)	73.6	3.64 *dd* (8, 10)	
3	3.71 *t* (10)	74.8	3.79 *t* (10)	
4	5.36 *t* (10)	70.9	5.43 *t* (10)	
5	3.52 *br d* (10)[a]	73.0	3.40 *br d* (10)	
6	4.64 *d* (12)	63.3	4.75 *br d* (13)	
	3.88[a]		3.83 *dd* (2, 13)	
Glc II				
1	5.88 *d* (8)	91.7	6.12 *d* (8.5)	
2	5.04 *dd* (8, 9)	76.3	5.08 *t* (8.5)	
3	5.51 *dd* (9, 10)	76.9	5.70 *dd* (8.5, 10)	
4	4.97 *t* (10)	69.2	5.12 *t* (10)	
5	4.33 *dd* (6.5, 10)	72.8	4.56 *dd* (6, 10)	
6	5.11 *dd* (6.5, 13.5)	63.0	5.28 *dd* (6, 13)	
	3.65[b]		3.86 *d* (13)	
Glc III				
1	6.00 *d* (8)	92.0		6.02 *d* (8.5)
2	5.17 *dd* (8, 10)	75.1		5.18 *dd* (8.5, 10)
3	5.34 *t* (10)	77.6		5.41 *t* (10)
4	5.73 *t* (10)	66.6		5.83 *t* (10)
5	3.39 *br d* (10)	73.6		3.45 *br d* (10)
6	4.89 *d* (12.5)	63.1		4.92 *br d* (13)
	3.68[b]			3.91 *dd* (2, 13)
Glc IV				
1	6.10 *d* (8.5)	92.1		6.20 *d* (8.5)
2	5.08 *dd* (8.5, 10)	76.7		5.10 *dd* (8.5, 9)
3	5.79 *t* (10)	76.6		5.82 *dd* (9, 10)
4	5.09 *t* (10)	69.4		5.17 *t* (10)
5	4.57 *dd* (6.5, 10)	73.0		4.67 *dd* (6, 10)
6	5.24 *dd* (6.5, 13)	63.2		5.33 *dd* (6, 13.5)
	3.88[a]			3.92 *d* (13.5)

[a] Overlapped with each other.
[b] Overlapped with each other.

3. Experimental

3.1. General experimental procedures

^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded in $(CD_3)_2CO + D_2O$ on a Varian VXR-500 spectrometer; ESIMS were recorded on a Micromass AutoSpec OA-Tof mass spectrometer using 1% NH_4OAc in 50% aq. MeOH as solvent (flow rate 20 μl/min). Optical rotations were measured on a JASCO DIP-1000 polarimeter; CD spectra were measured on a JASCO J-720W spectropolarimeter. Normal-phase HPLC was conducted on a YMC Pack Sil A-003 column (5 μm, 4.6 mm i.d.×250 mm) at a flow rate of 1.5 ml/min with detection at 280 nm. The following solvent systems were used: NP-1, *n*-hexane–MeOH–THF–HCOOH (55:33:11:1) containing oxalic acid (450 mg/l); NP-2, *n*-hexane–MeOH–THF–HCOOH (60:45:15:1) containing oxalic acid (500 mg/1.2 l); and/or NP-3, *n*-hexane–MeOH–THF–HCOOH (47:39:13:1) containing oxalic acid (450 mg/l). Reversed-phase HPLC was conducted on a J'sphere ODS M-80 column (4.6 mm i.d.×250 mm, 4 μm, 80 Å) at a flow rate of 1.0 ml/min and a temperature of 40° C with detection at 280 nm. The solvent systems used were: RP-1, 0.01 M H_3PO_4–0.01 M KH_2PO_4–EtOH–EtOAc (42.5:42.5:10:5), and/or RP-2, 0.01 M H_3PO_4–0.01 M KH_2PO_4–MeCN (42.5:42.5:15). Preparative reversed-phase HPLC was carried out on a J'sphere ODS M-80 column (10 mm i.d.×250 mm, 4 μm, 80 Å) with mobile phase RP-2 at a flow rate of 3.0 ml/min and a temperature of 40° C with an MCPD detector at 240–400 nm. Preparative normal-phase HPLC was performed on an Inertsil 100-5 column (10 mm i.d.×250 mm) with mobile phase NP-3 at a flow rate of 4.0 ml/min at room temperature.

3.2. Plant material

M. multiflorum (Bompl) Naudin was collected near Manizales City, Caldas, Colombia. Dr. Gustavo Lozano C. identified the plant, and a voucher specimen (No. 361696) was deposited at Herbario Nacional de Colombia.

3.3. Extraction and isolation

Dried leaves (400 g) of *M. multiflorum* were homogenized in 70% aqueous acetone, filtered, and concentrated in vacuo. The concentrated solution was extracted successively with Et_2O, EtOAc, and 1-butanol to yield respective Et_2O (4.6 g), EtOAc (13.4 g), and 1-butanol (33.8 g) extracts and an aqueous residue (54.3 g). The EtOAc extract (10.2 g) was fractionated on a Toyopearl HW-40C column (2.2 cm i.d.×45 cm) to give four fractions, 40% MeOH in H_2O (3.9 g), 70% MeOH in H_2O (2.2 g), $(CH_3)_2CO$–MeOH–H_2O (20:50:30) (3.4 g), and $(CH_3)_2CO$–H_2O (70:30) (0.7 g).

The 40% MeOH in the fraction was applied to a Toyopearl HW-40C column (2.2 cm i.d.×45 cm) eluted with aqueous MeOH (20%→40% MeOH) in a step-wise gradient mode. Fractions with similar HPLC patterns were combined and further purified by a combination of chromatography on a YMC ODS AQ120 S-50 column (1.1 cm i.d.×45 cm), MCI-Gel CHP-20P, and/or preparative HPLC. This procedure gave 1,2,6-tri-*O*-galloyl-β-D-glucopyranose (16 mg), 1,4,6-tri-*O*-galloyl-β-D-glucopyranose (5.2 mg), isostrictinin (60 mg), and valoneic acid dilactone (80 mg).

The 70% MeOH in H_2O fraction was further separated on Toyopearl HW-40C and eluted with aqueous MeOH (20–70% MeOH) followed by MCI-gel CHP-20P (1.1 cm i.d.×45 cm) or YMC ODS AQ-120 (1.1 cm i.d.×45 cm) column chromatography to yield nobotanin D (180 mg), pedunculagin (**12**) (183 mg), and nobotanin A (18 mg).

The $(CH_3)_2CO$–MeOH–H_2O (20:50:30) eluate was similarly purified by successive chromatography over Toyopearl HW-40C, MCI-Gel CHP-20P, and YMC ODS columns. This method provided pterocaryanin C (178 mg), casuarictin (178 mg), 1,2,4,6-tetra-*O*-galloyl-β-D-glucopyranose (20 mg), nobotanin A (30 mg), nobotanin B (157 mg), nobotanin F (**6**) (214 mg), brediatin B (**5**) (82 mg), nobotanin E (280 mg), nobotanin J (142 mg), and nobotanin K (**10**) (770 mg). The eluate with $(CH_3)_2CO$–H_2O (70:30) contained only **10** (700 mg).

The 1-butanol extract (6.4 g) was fractionated by a combination of chromatographic steps over a Toyopearl HW-40C column (2.2 cm i.d.×45 cm) eluted with aqueous MeOH (20–70% MeOH) and H_2O–MeOH–$(CH_3)_2CO$ (30:70:0–30:30:40), MCI-gel CHP20P column (1.1 cm i.d.× 45 cm) eluted with aqueous MeOH (0–100% MeOH), and YMC-Gel ODS AQ-120 eluted with aqueous MeOH (5–60% MeOH), to yield **12** (310 mg), nobotanin O (20.3 mg), nobotanin R (**1**) (119 mg), nobotanin B (**16**) (114.7 mg), nobotanin F (**6**) (205.3 mg), nobotanin E (272 mg), **10** (52.9 mg), and a mixture of nobotanins J and Q (**2**) (73 mg). The mixture of nobotanins J and Q was separated by preparative normal-phase HPLC (RP-3) to yield pure nobotanins J (10 mg) and **2** (6 mg).

The water soluble portion (54 g) was separated by chromatography on Diaion HP-20 (8×70 cm) to give the following eluates: H_2O (35 g), MeOH–H_2O (1:4) (4.14 g), MeOH–H_2O (4:6) (6.63 g), MeOH–H_2O (6:4) (7.20 g), MeOH (0.66 g), and $(CH_3)_2CO$–H_2O (7:3) (0.15 g). The MeOH-H_2O (4:6) eluate was divided into two portions (2.7 g and 3.7 g) and separated respectively by repeated chromatography on Toyopearl HW 40C (2.2×45 cm) with a step-wise gradient elution (50–70% MeOH; in H_2O, then MeOH–$(CH_3)_2CO$–H_2O 80:10:10, 70:20:10, 60:20:20, 50:30:20, 40:30:30, 30:40:30, 20:50:30, 10:60:30 and 0:70:30) then on YMC ODS AQ 120, S-50, (1.1×45 cm) eluted with a step-wise gradient of aqueous MeOH (5–50% MeOH) to yield nobotanins S (**4**) (73 mg) and T (**3**) (54 mg).

3.4. Acid hydrolysis of nobotanins Q (2), R (1), S (4), and T (3)

A solution of each compound (1∼2 mg) in 5% H_2SO_4 (1.0 ml) was heated at 100 °C for 8 h. After cooling, the reaction mixture was adsorbed onto an ODS Mega Bond-Elut cartridge (1 g). After the cartridge was washed with H_2O, the product was eluted with MeOH, and the concentrated solution was analyzed by reversed-phase HPLC (RP-2) to detect gallic acid (t_R 3.27 min), valneic acid dilactone (t_R 7.28 min), and ellagic acid (t_R 16.50 min), which were identified by co-chromatography with authentic samples.

3.5. Nobotanin R (1)

Off-white amorphous powder; $[\alpha]_D^{27}$ +86.5° (MeOH; *c* 1.0); ESIMS *m/z* 1588 $[M+NH_4]^+$; UV (MeOH) λ_{max} nm (log ϵ): 217 (5.29), 274 (4.91); CD (MeOH) [θ] (nm): $+3.32\times10^5$ (227), $+2.36\times10^5$ (238), -7.98×10^4 (261), $+4.52\times10^4$ (282), -1.67×10^4 (310); for ^{1}H NMR see Table 1 and text; ^{13}C NMR δ: 104.4 (Val C-3′), 107.3, 107.4 (HHDP C-3,3′), 107.9 (Val C-3) 109.9 (Val C-6″), 110.1 (2C), 110.2 (4C) [galloyl (Gall) C-2,6], 114.4 (3C, HHDP C-1, C-1′, Val C-1″), 114.7 (Val C-1′), 115.7 (Val C-1), 119.5, 120.4, 121.1 (Gall C-1), 125.7 (Val C-2′), 125.8 (2C, HHDP C2, C-2′), 126.5 (Val C-2), 136.1 (2C), 136,3 (2C), 136.6 (Val C-2″, 3,3′, HHDP C-5,5′), 139.2 (2C), 139.4 (2C), 140.0 (3C) (Gall C-3,5, Val C-3″), 140.4 (Val C-4″), 143.5 (Val C-5″), 144.4 (2C) (HHDP C-6,6′), 145.0, 145.1 (HHDP C-4,4′), 145.9 (4C) (Gall C-4, Val C-4,6,6′), 146.1, 146.6 (Gall C-4), 146.9 (Val V-4′), 165.0 (Val 7″), 165.1, 165.6, 166.8 (Gall C-7), 168.0 (Val C-7′), 168.4 (Val C-7), 168.7 (HHDP C-7′), 169.6 (HHDP C-7); for sugar moieties, see Table 1.

3.6. Partial hydrolysis of 1

An aqueous solution (1 ml) of **1** (20 mg) was heated at 100 °C for 6 h. After cooling, the solution was applied to a Mega Bond-Elut C18 cartridge (1 g) elution with in a step-wise gradient [$H_2O\rightarrow$aq. MeOH (2.5%→40% MeOH)]. The isolated products were identified as **7** (1 mg), **8** (0.5 mg), and β-glucogallin (1 mg) by direct comparisons of their ^{1}H NMR spectra and HPLC elution profiles with those of products prepared from **6**, as described below.

3.7. Partial hydrolysis of nobotanin F (6)

An aqueous solution (5 ml) of **6** (107.5 mg) was heated at 80 °C for 13 h. After cooling, the solution was directly applied to a YMC-ODS AQ-120 S-50 column (1.1 cm i.d.×45 cm) eluted with aqueous MeOH in a step-wise gradient (5%→60% MeOH) to give gallic acid (2.2 mg), isostrictinin (2.1 mg), nobotanin A (11.4 mg), brediatin B (**5**) (3.0 mg), nobotanin R (**1**) (2.2 mg), recovered nobotanin F (**6**) (10.8 mg), **7** (7.2 mg), and **8** (1 mg).

3.8. Nobotanin Q (2)

Off-white amorphous powder; $[\alpha]_D^{20}$ +36.6° (MeOH; *c* 1.0); ESIMS *m/z* 3458 $[M+NH_4]^+$; UV λ_{max} (MeOH) nm (log ϵ): 216 (5.29), 273 (4.93); CD (MeOH) [θ] (nm): $+4.44\times10^5$ (227), $+2.5\times10^5$ (237), -1.5×10^5 (263), $+7.4\times10^4$ (283), -2.83×10^4 (307); For ^{1}H NMR data, see Table 2 and text; ^{13}C NMR δ: 102.4, 104.4, 104.6

(Val C-3′), 106.8, 107.2 (2C), 107.6 (2C), 107.8, 108.3 (Val C-3, HHDP C-3,3′), 110.1 (2C), 110.2 (2C), 110.3 (3C), 110.4 (5C), 110.6 (Gall C-2,6 and Val C-6″), 113.0, 114.1, 114.5, 114.8, 115.2, 115.7, 116.0, 117.5 (HHDP C-1,1′ and Val C-1,1′,1″), 119.8, 120.1, 120.8, 121.3, 121.7 (Gall C-1), 125.2, 125.5, 125.6, 125.7, 125.9, 126.1 (2C), 126.1, 126.2, 126.3, 126.5 (Val C-2,2′, HHDP C-2,2′), 135.3, 136.1, 138.8 (Val C-2″), 136.4 (2C), 136.5 (4C), 137.0 (HHDP C-5,5′ and Val C-5,5′), 139.3, 139.4, 139.6, 139.8, 139.9 (2C each, Gall C-3,5), 140.5, 140.6, 140.7, 141.5 143.0, 143.1, 143.5 (Val C-3″,4″,5″), 144.2, 144.3, 144.5, 144.6, 144.8, 144.9 (Val C-6,6′), 145.0 (2C), 145.1, 145.2 (HHDP C-4,4′,6,6′), 145.9, 146.0, 146.1, 146.2 (Gall C-4, Val C-3), 146.6 (2C), 147.3 (Val C-4′), 163.1 (Val C-7″), 164.1, 164.3, 164.6, 164.9, 165.5, 166.6, 167.4, 167.8, 167.9, 168.0, 168.2, 168.4, 168.5, 168.6, 169.2 (2C), 169.3, 169.6 (ester carbons); for sugar moieties, see Table 2.

3.9. Nobotanin T (3)

Off-white amorphous powder; $[\alpha]_D^{27}$ +45.0° (MeOH, *c* 1.0); ESIMS *m*/*z* 1662 $[M+2NH_4]^{2+}$; UV (MeOH) λ_{max} nm (log ϵ): 217 (5.41), 271 (5.05); CD (MeOH) [θ] (nm): $+6.1\times10^5$ (227), $+3.1\times10^5$ (239), -1.8×10^4 (263), $+7.9\times10^4$ (283), -3.9×10^4 (310); ^{1}H NMR δ: galloyl group, 7.25, 7.24, (each *s*, 2H in total), 7.14, 7.13, (each *s*, 2H in total), 7.09, 7.08, (each *s*, 2H in total), 6.95, 6.94, (each *s*, 2H in total), valoneoyl and HHDP groups, 7.20, 7.19 (each *s*, 1H in total, Val H-6″), 7.06, 7.03, (each s, 1H in total, Val H-6″), 6.99, 6.96 (each s, 1H in total, Val H-6″); 6.22, 6.17, (each *s*, 1H in total), 6.11, 6.10, (each *s*, 1H in total), 5.99, 5.98, (each *s*, 1H in total); 6.46, 6.45, (each *s*, 1H in total), 6.44, 6.43, (each *s*, 1H in total), 6.41, 6.36, (each *s*, 1H in total), 6.70, 6.67, (each *s*, 1H in total), 6.64, 6.59×3, (each s, 4H in total), 6.51, 6.49 (each s, 1H in total), glucose protons, see Table 2; ^{13}C NMR δ: 102.7, 104.3, 104.6 (Val C-3′), 106.7, 106.9, 107.4 (2C), 107.6, 107.8, 108.1 (Val C-3, HHDP C-3,3′), 110.0, 110.1 (5C), 110.3 (2C), 110.6, 110.7 (Gall C-2,6 and Val C-6″), 113.1, 113.8, 114.3 (Val C-1″), 114.3, 114.7, 114.8, 115.2, 115.6, 115.8, 115.9, 116.1, 116.7, 117.3 (Val C-1′, HHDP C-1,1′), 119.3, 119.5, 120.8, 121.2 (Gall C-1), 125.3, 125.6, 125.7, 125.8, 125.9, 126.0 (2C), 126.3, 126.4, 127.0 (Val C-2,2′, HHDP C-2, 2′), 135.7, 136.1 (Val C-2″), 136.4 (5C), 136.6 (4C), 136.9 (Val C-5, 5′, HHDP C-5, 5′), 137.5 (Val C-2″), 138.9, 139.3, 139.7, 139.8 (2C each, Gall C-3,5), 139.9, 140.0, 140.1, 140.5, 140.8, 141.0, 142.8, 143.0, 143.5 (Val C-2″,4″,5″), 144.2 (2C), 144.3, 144.6, 144.8, 144.9 (Val C-6,6′), 145.0 (6C), 145.3 (2C) (HHDP C-4,4′,6,6′), 145.6, 145.7, 145.8, 145.9 (2C), 146.1 (2C) (Val C-4′, Gall C-4), 146.7, 146.9, 147.3 (Val C-4′), 163.8 (Val C-7″), 164.6, 164.9 (2C), (165.1, 165.3, 1C in total), (166.8, 166.9, 1C in total), 167.3, 167.9, 168.2, 168.4 (3C), 168.5, (168.8, 168.9, 1C in total), (169.0, 169.1, 1C in total), 169.4, 169.7 (ester carbons); for sugar moieties, see Table 2.

3.10. Methanolysis of 2 and 3

A solution of **2** (1 mg/ml) in MeOH containing 0.1 ml of 0.5 M acetate buffer (pH 4.5) was incubated at 37 °C for 24 h. The reaction product was analyzed by normal-phase (RP-1 and RP-3) and reversed-phase (RP-2) HPLC. Nobotanin Q (**2**) released pedunculagin (**12**) (a mixture of α- and β-anomers) (NP-1 t_R 8.4, 8.6 min, RP-2 t_R 3.5, 4.0 min) and a methanolysate which appeared to be a trimer by normal-phase HPLC (NP-3 t_R 10.1 min). Nobotanin T (**3**) was similarly treated to produce 4,6-hexahydroxydiphenoyl-β-D-glucose (**13**) (a mixture of α- and β-anomers) (NP-1 t_R 5.6, 5.7 min, RP-2 t_R 2.7 min) and the trimeric methyl ester (**14**) (a mixture of α- and β-anomers) (NP-3 t_R 11.9 min, RP-2 t_R 16.7, 18.5 min), which was identified by co-chromatography with authentic samples prepared by a similar methanolysis of nobotanin P (**11**).

3.11. Nobotanin S (4)

Off-white amorphous powder; $[\alpha]_D^{27}$ +49.1° (MeOH; *c* 1.0); ESIMS *m*/*z* 3458 $[M+NH_4]^+$; UV (MeOH) λmax nm (log ϵ): 217 (5.49), 271 (5.16); CD (MeOH) [θ] (nm): $+6.5\times10^5$ (227), $+3.7\times10^5$ (238), -2.1×10^5 (260), $+7.4\times10^4$ (282), -3.9×10^4 (311); ^{1}H NMR δ: 7.24, 7.16, 7.11, 7.06, 6.95 (each 2H, *s*, Gall), 7.04, 7.03, 6.98 (each 1H, *s*, Val H-6″), 6.59, 6.56, 6.51, 6.50 (each 1H, *s*, HHDP H-3, 3′), 6.40, 6.37, 6.34 (each 1H, *s*, Val H-3), 6.12, 6.09, 5.95 (each 1H, *s*, Val H-3′), glucose protons, see Table 3; ^{13}C NMR δ: 103.1, 102.5, 104.2 (Val C-3′), 106.9, 106.7 (2C), 107.5 (2C), 107.6, 107.8, (Val C-3, HHDP C-3, 3′), 110.0 (2C), 110.1 (6C), 110.2 (4C), 110.5 (Gall C-2,6 and Val C-6″), 111.7, 113.6, 114.6 (Val C-1″), 114.1 (2C), 114.2, 115.6, 115.7, 115.9, 116.1, 116.2, 116.5, 116.7 (Val C-1′, HHDP C-1,1′), 119.2, 119.4, 120.0, 120.8, 122.0 (Gall C-1), 125.2, 125.3 (2C), 125.6, 125.7, 125.8, 125.9 (2C), 126.0 (2C), (Val C-2,2′, HHDP C-2, 2′), 135.6 (2C), 138.2 (Val C-2″), 135.8, 136.0, 136.1, 136.2 (2c), 136.3, 136.4, 136.5 (2C), 136.9 (Val C-5, 5′, HHDP C-5, 5′), 135.6 (2C), 138.2 (Val C-2″), 145.8 (2C), 145.9 (6C), 146.0 (2C) (Gall C-3,5), 135.6 (2C), 138.2, 140.6, 140.7, 141.4, 142.7, 143.0, 143.2 (Val C-2″,4″,5″), 144.3 (2C), 144.4, 144.5, 144.7 (2C), 144.8 (2C), 144.9 (2C) (Val C-6, 6′), 145.0 (2C), 145.1, 145.2, 144.8 (2C), 144.9 (2C) (HHDP C-4,4′,6,6′), 139.0, 139.2, 139.5, 139.8, 139.9 (Gall C-4), 145.0, 145.2 (2C), 146.6, 146.7, 147.1 (Val C-4, 4′), 140.6, 140.7, 142.7 (Val C-4″), 161.9, 164.6, 165.3 (Val C-7″), 164.8, 164.9, 165.9, 167.2 (2C) (Gall H-7), 168.1, 168.3, 168.4, 169.1 (HHDP, C-7, C-7′), 168.6, 168.9, 169.0, 169.2, 169.5, 169.7 (Val C-7, 7′); for sugar moieties, see Table 3.

3.12. Methanolysis of 4

A solution of **4** (10 mg) in MeOH (3 ml) containing 0.5 M acetate buffer (pH 4.5) (0.3 ml) was incubated at 37 °C for 17 h. After removal of the solvent, the residue was dissolved in H_2O and applied to a Mega Bond-Elut cartridge (1 g), which was eluted with H_2O and H_2O–MeOH (1:1). The MeOH–H_2O (1:1) eluate gave the methanolysates **17** (2.3 mg) and **18** (1.9 mg). Identity of **17** as malabathrin D was confirmed by direct comparison of its ^{1}H NMR and HPLC data with those of an authentic sample. **18**: ESIMS *m/z* 1436 $[M+NH_4]^+$ ($C_{61}H_{46}O_{40}$); ^{1}H NMR δ: 5.67 (*d*, *J* = 8.5 Hz, Glc I H-1), 3.65 (*dd*, *J* = 8.5, 9.5 Hz, Glc I H-2), 3.75 (*t*, *J* = 9.5, Glc I H-3), 5.42 (*dd*, *J* = 9.5, 10 Hz, Glc I H-4), 3.43 (*m*, Glc I H-5), 4.70 (*br d*, *J* = 13 Hz, Glc I H-6), 3.80 (*d*, *J* = 13 Hz, Glc I H-6), 5.28 (*d*, *J* = 3.5 Hz, Glc II H-1α), 4.97 (*d*, *J* = 8.5, Glc II H-1β), 4.6 (*dd*, *J* = 3.5, 9.5 Hz, Glc I H-2α), 4.79 (*dd*, *J* = 8.5, 9.0 Hz, Glc II H-2β), 5.49 (*t*, *J* = 9.5 Hz, Glc II H-3α), 5.46 (*t*, *J* = 9.5 Hz, Glc II H-3β),. 5.02 (*t*, *J* = 9.5 Hz, Glc II H-4α), 5.03 (*t*, *J* = 9.5 Hz, Glc II H-4β), 4.28 (*dd*, *J* = 9.5, 6.5 Hz, Glc II H-5α, β), 5.24(*dd*, *J* = 13.5, 6.5 Hz, Glc II H-6α, β), 3.83 (*d*, *J* = 13.5 Hz, Glc II H-6α, β).

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