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Oligomeric hydrolysable tannins from *Tibouchina multiflora*

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Abstract

Two hydrolysable tannins, nobotanin O and nobotanin P, were isolated from the leaf extract of *Tibouchina multiflora* (Melastomataceae) and their dimeric and tetrameric structures elucidated on the basis of spectral data and chemical correlations with nobotanin B and K, respectively. Thirteen known hydrolysable tannins including nobotanins A, B, C and J, which are oligomers characteristic of the Melastomataceae, were also isolated. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Tibouchina multiflora*; Melastomataceae; Tannin; Ellagitannin oligomer; Nobotanin O; Nobotanin P

1. Introduction

In a previous study on the polyphenolic constituents of Melastomataceous plants, we reported the isolation and structure elucidation of unique hydrolysable tannin oligomers including nobotanins A-C and E-K, from *Tibouchina semidecandra* (Yoshida, Haba et al., 1991; Yoshida, Ohwashi et al., 1991), *Heterocentron roseum* (Yoshida et al., 1995; Yoshida, Haba et al., 1992), *Medinilla magnifica* (Yoshida et al., 1986), *Melastoma malabathricum* (Yoshida, Nakata et al., 1992) and *Bredia tuberculata* (Yoshida, Arioka, Fujita, Chen & Okuda, 1994). A chromatographic survey of the tannins in this family revealed that *Tibouchina multiflora* is rich in tannins, particularly in oligomeric hydrolysable tannins. This paper presents the structure elucidation of two new oligomeric hydrolysable tannins named nobotanins O (**1**) and P (**7**) isolated from the leaf extract of *T. multiflora*.

2. Results and discussion

The aq. acetone homogenate of dried leaves was concentrated and extracted successively with Et_2O, AcOEt and *n*-BuOH. Repeated chromatography of the *n*-BuOH extract over polystyrene and/or polyvinyl gel afforded nobotanins O (**1**) and P (**7**), together with eight known compounds, stachyurin (Okuda, Yoshida, Ashida, & Yazaki, 1983), casuarinin (Okuda et al., 1983), medinillin B (Yoshida et al., 1986), nobotanins A, B (**2**) (Yoshida, Haba et al., 1991), C (**9**) (Yoshida, Ohwashi et al., 1991), G (Yoshida, Haba et al., 1992) and J (Yoshida et al., 1995). Similar chromatographic separation of the EtOAc and aq. extracts gave five additional known compounds, pedunculagin (Okuda et al., 1983), casuarictin (Okuda et al., 1983), nobotanins D (Yoshida, Haba et al., 1991), F (Yoshida, Haba et al., 1991) and M (Yoshida, Nakata & Okuda, 1999) as described in Section 3. The tannin composition of *T. multiflora* was shown to be similar to that of *T. semidecandra* and *H. roseum*.

Nobotanin O (**1**), an off-white amorphous powder, has been suggested to be a dimeric hydrolysable tannin based on its positive coloration with $NaNO_2$–AcOH reagent (Bate-Smith, 1972) and a retention volume

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Table 1
^{1}H NMR spectral data of the glucose moieties of **7**, **9** and **10** [500 MHz, Me_2CO-d_6 + D_2O; *J* (Hz) in parentheses]

	7		**9**		**10**
	α-Anomer	β-Anomer	α-Anomer	β-Anomer	
Glc-I					
H-1	5.45 *d* (3.5)	5.07 *d* (8.5)	5.46 *d* (3.5)	5.07 *d* (8)	
H-2	5.02 *dd* (3.5, 10)	4.81 *dd* (8.5, 9.5)	5.02 *dd* (3.5, 9.5)	4.80 *dd* (8, 9.5)	
H-3	5.58 *t* (10)	5.18 ~ 5.09[a]	5.58 *t* (9.5)	5.14	
H-4	5.50 *t* (10)	5.47 *t* (9.5)	5.50 *t* (9.5)	5.47 *t* (9.5)	
H-5	4.37 *br d* (10)	3.78 ~ 3.73[b]	4.38 *br d* (9.5)	3.80	
H-6	4.13 *dd* (3, 13)	4.20 *d* (3, 12)	4.14 *dd* (3.5, 12)	4.20 *dd* (3.5, 12)	
	4.39 *br d* (13)	4.57 *d* (12)	4.38 *br d* (12)	4.59 *br d* (12)	
Glc-II					
H-1′	5.97 *d* (8.5)	5.98 *d* (8.5)	5.98 *d* (8.5)	5.99 *d* (8.5)	
H-2′	5.18 ~ 5.09[a]	5.18 ~ 5.09[a]	5.16 *dd* (8.5, 9.5)	5.15 *dd* (8.5, 9.5)	
H-3′	5.81 *t* (9.5)	5.84 *t* (10)	5.80 *t* (9.5)	5.84 *t* (9.5)	
H-4′	5.18 ~ 5.09[a]	5.18 ~ 5.09[a]	5.18 *t* (10)	5.16 *t* (10)	
H-5′	4.57 *m*		4.58 *m*		
H-6′	5.18 ~ 5.09[a,c]		5.12[e]		
	3.78 ~ 3.73[b]		3.78[e]		
Glc-III					
H-1″	6.13 *d* (8.5)	6.14 *d* (8.5)	6.10 *d* (8)	6.14 *d* (8)	
H-2″	5.31 *br t* (10)	5.18 ~ 5.09[a]	5.30 *dd* (8, 9.5)	5.17 *dd* (8, 9.5)	
H-3″	5.31 *t* (10)		5.32 *t* (9.5)		
H-4″	5.72 *t* (10)		5.68 *t* (9.5)		
H-5″	3.40 ~ 3.30[e]		3.37 *br d* (9.5)		
H-6″	4.88 *br d* (12.5)		4.87 *br d* (13)		
	3.78 ~ 3.73[b]		3.79 *m*		
Glc-IV					
H-1‴	6.05 *d* (8.5)				6.18 *d* (8.5)
H-2‴	5.18 ~ 5.09[a]				5.16 *dd* (8.5, 10)
H-3‴	5.34 *t* (10)				5.41 *t* (10)
H-4‴	5.18 ~ 5.09[a]				5.13 *t* (10)
H-5‴	4.36 *dd* (6.5, 10)				4.47 *dd* (6.5, 10)
H-6‴	5.25 *dd* (6.5, 13)[c]				5.31 *dd* (6.5, 13)
	3.76 *br d* (13)				3.85 *d* (13)

[a,b] Overlapped with each other; [c]these values may be interchanged; [d]overlapped with HOD; [e]coupling constants are not clear because of overlapping with other signals.

similar to that of other dimers in normal phase HPLC. The FABMS spectrum showed the $[M+H]^+$ and $[M+Na]^+$ ion peaks at *m/z* 1571 and 1593, respectively, corresponding to the molecular formula $C_{68}H_{50}O_{44}$. The ^{1}H NMR spectrum of **1** exhibited three 2H-singlets (δ 7.26, 7.07 and 6.94) due to three galloyl units and five 1H-singlets (δ 7.12, 6.71, 6.47, 6.46 and 6.09) ascribable to a hexahydroxydiphenoyl (HHDP) and a valoneoyl group. The presence of these units in **1** was confirmed by methylation and subsequent methanolysis yielding methyl tri-*O*-methylgallate (**4**), dimethyl hexamethoxydiphenate (**5**) and trimethyl octa-*O*-methylvaloneate (**6**). The atropisomerism of the chiral HHDP and valoneoyl groups in **1** was shown to be in the (*S*)-series by the strong positive Cotton effect at 239 nm and the negative Cotton effect at 264 nm in the CD spectrum (Okuda et al., 1982). The sugar proton signals assigned by the ^{1}H-^{1}H COSY technique were characteristic of a C1 glucopyranose. The spectrum also showed a double-doublet at δ 3.47 that is characteristic of the H-5 of glucose-II in nobotanin B (**2**) and its analogues (Yoshida, Haba et al., 1992). Actually, the aliphatic proton signals of **1** were very similar to those of **2** except for remarkable upfield shifts of the H-4 and H-6 signals of the glucose core-I [δ 5.41 (H-4) in **2** → δ 3.82 in **1**; δ 5.33 (H-6) in **2** → δ 3.76 in **1**]. These spectral features indicate that the hydroxyl groups at C-4 and C-6 in the glucose core-I are unacylated as depicted in formula **1**. Structure **1** proposed for nobotanin O was confirmed by partial hydrolysis of **2** with hot water producing two compounds, one of which was identical with nobotanin O. The other compound was assigned as **3** on the basis of the ^{1}H NMR spectral analysis and identified with nobotanin G (Yoshida, Haba et al., 1992). Based on these data, the structure of nobotanin O was determined to be **1**.

Nobotanin P (**7**), a light brown amorphous powder,

had an ion peak at m/z 3591 $[M+H]^+$ in the ESIMS, and gave a large retention volume on normal-phase HPLC, similar to that of nobotanin K (**8**) (Yoshida et al., 1995) and other tetramers. Methylation of **7** followed by methanolysis yielded **4**, **5** and **6** as in the case of **1**. The CD spectrum of **7** showed a positive Cotton effect at 228 and 239 nm with large amplitudes, indicating the *S*-configuration for each of biphenyl moieties of the HHDP and valoneoyl groups in the molecule. Although the ^{1}H NMR spectrum of **7** was complicated owing to the formation of a mixture of α- and β-anomers, the presence of four galloyl, three HHDP and three valoneoyl groups was indicated by four 2H-singlets and fifteen 1H-singlets appearing as duplicated signals in the aromatic region. The tetrameric nature of **7** was clearly indicated by three pairs of acylated anomeric proton signals at δ 6.13, 6.14 (each *d*, J = 8.5 Hz, 1H), 6.05 (*d*, J = 8.5 Hz, 1H) and 5.98, 5.97 (each *d*, J = 8.5 Hz, 1H), and the fourth pair of signals due to an unacylated anomeric proton at δ 5.45 (*d*, J = 3.5 Hz) and 5.07 (*d*, J = 8.5 Hz). As seen in Table 1, the signals of three sugar moieties (glucose-I, II and III) are virtually identical with those of nobotanin C (**9**), and those of the fourth sugar residue (glucose-IV) are similar to those of casuarictin (**10**). An analogous similarity was also observed upon comparing the ^{13}C-NMR spectrum of **7** with those of **9** and **10** (Yoshida, Ohwashi et al., 1991). Therefore, nobotanin P was a degalloylated congener of nobotanin K (**8**), which was consistent with the molecular ion species in ESIMS.

Although chemical conversion from **8** to **7** by the selective degalloylation at C-1 of **8** with tannase was not successful, these two were chemically correlated as follows. Mild methanolysis of **8** gave the trimeric methanolysate (**11**) (Yoshida et al., 1995), which when treated with tannase provided the monodegalloyl derivative (**12**). The structure of **12** was based on its ^{1}H NMR spectrum which showed close resemblance with that of **9** except for the presence of extra signals due to an aromatic 1H-singlet and a methoxyl group. This compound was also obtained by mild methanolysis of **7** in MeOH-buffer solution. Consequently, the structure of nobotanin P was established as **7**, and is the second example of a tetrameric ellagitannin found in the Melastomataceae.

3. Experimental

3.1. General

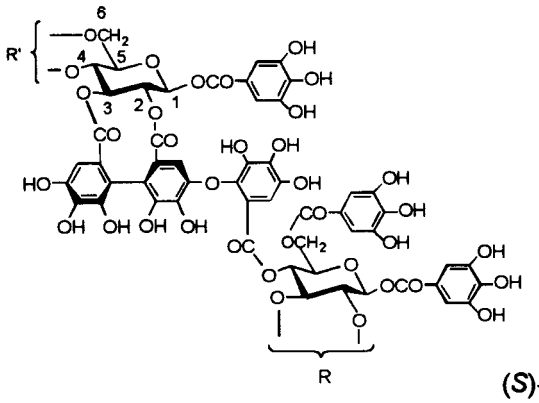
^{1}H (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded in $(CD_3)_2CO+D_2O$. FABMS were recorded on a VG 70-SE mass spectrometer and ESIMS with a Micromass AutoSpec OA-Tof mass spectrometer [solvent was MeOH–H_2O (1:1) + 0.1% $AcONH_4$]. Optical rotations were obtained on a JASCO DIP-1000 digital polarimeter and CD spectra on a JASCO J-720W spectropolarimeter. Chromatographic conditions were the same as described in a previous paper (Yoshida et al., 1995).

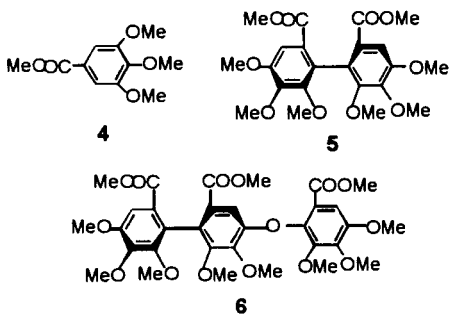
3.2. Plant materials

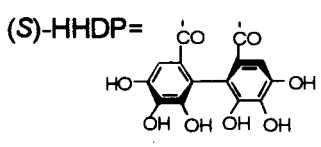
The leaves of *T. multiflora* were collected in Pereira, Colombia in December 1995 and identified by one of the authors (S. S. Renner). A voucher specimen is deposited in the Missouri Botanical Garden.

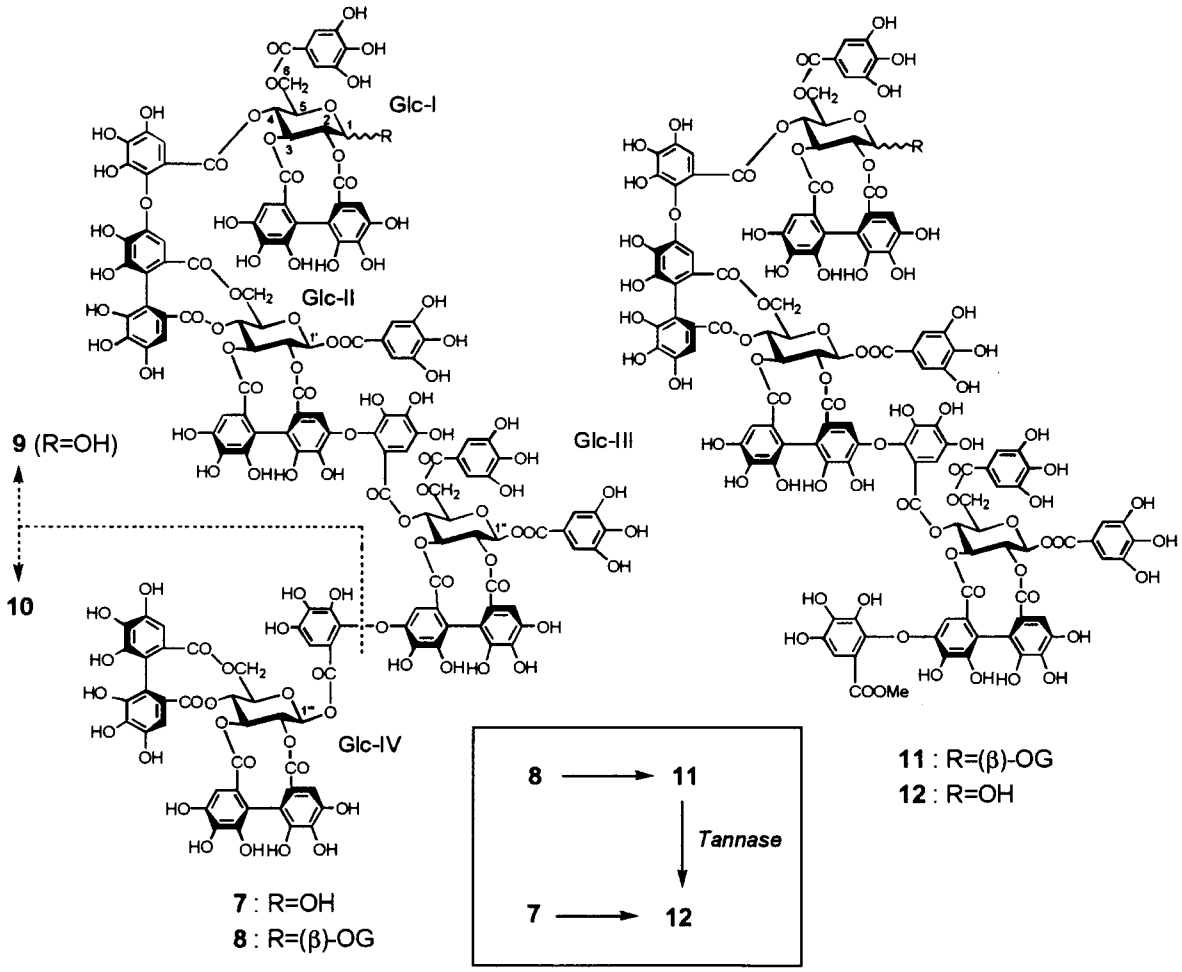
3.3. Extraction and isolation

The dried leaves (200 g) of *T. multiflora* were homogenized in Me_2CO–H_2O (7:3) (1 L × 3). The filtered homogenate was concentrated and extracted with Et_2O, EtOAc and *n*-BuOH, successively. The *n*-BuOH extract (3.3 g) was chromatographed over Toyopearl HW-40 (fine) (2.2 cm ID × 40 cm) with MeOH–H_2O (5:5 → 6:4 → 7:3) → MeOH–H_2O–Me_2CO









(7:2:1 → 6:2:2 → 5:3:2) → Me_2CO–H_2O (7:3) in a stepwise gradient mode. The eluate with MeOH–H_2O (6:4) yielded stachyurin (2.4 mg) and casuarinin (52 mg). The eluate with MeOH–H_2O–Me_2CO (8:1:1) gave nobotanin A (22 mg) and nobotanin G (47 mg). The eluate with MeOH–H_2O–Me_2CO (7:2:1) yielded nobotanin B (**2**) (11 mg) and nobotanin C (**9**) (110 mg). The eluates with MeOH–H_2O–Me_2CO (6:2:2) and (5:3:2) yielded nobotanin J (42 mg) and nobotanin P (**7**) (31 mg), respectively. The fractions showing similar HPLC pattern in the eluate with MeOH–H_2O (7:3) were combined and further purified by CC over MCI-gel CHP-20P with aq. MeOH to afford medinillin B (14 mg) and nobotanin O (**1**) (3.9 mg). The AcOEt extract (1.0 g) was similarly fractionated by the repeated CC over Toyopearl HW-40 (fine) and MCI-gel CHP-20P to yield nobotanins A (22 mg), B (**2**) (36 mg), D (1.8 mg), F (5.6 mg), G (5.2 mg), casuarinin (18 mg) and casuarictin (16 mg). The aq. extract (5.6 g) was subjected to CC over Dia-ion HP-20 (3.3 cm ID × 35 cm) with aq. MeOH. The eluate (1.0 g) with MeOH–H_2O (4:6) was further purified by CC over Toyopearl HW-40 and MCI-gel CHP-20P with aq. MeOH to give nobotanin C (**9**) (21 mg), G (9.7 mg), M (9.1 mg), pedunculagin (35 mg), stachyurin (21 mg) and casuarinin (4 mg). Known tannins were identified by direct comparison of their HPLC and NMR spectra with those of authentic specimens.

3.4. Nobotanin O (*1*)

An off-white amorphous powder. $[\alpha]_D + 33°$ (MeOH; *c* 1.0). FABMS *m/z*: 1571 $[M+H]^+$, 1593 $[M+Na]^+$. ESIMS *m/z*: 1588 $[M+NH_4]^+$. UV λ_{max} MeOH nm (log ϵ): 219 (5.03), 274 (4.69). CD (MeOH): $[\theta]_{228} + 3.7 \times 10^5$, $[\theta]_{239} + 2.7 \times 10^5$, $[\theta]_{264} - 1.1 \times 10^5$, $[\theta]_{286} + 4.0 \times 10^4$, $[\theta]_{312} + 3.8 \times 10^4$. ^1H NMR: δ 7.26, 7.07, 6.94 (each 2H, *s*, galloyl-H), 7.12, 6.71, 6.47, 6.46, 6.09 (each 1H, *s*, HHDP and valoneoyl-H), 6.25 (*d*, *J* = 8 Hz, H-1), 4.93 (*dd*, *J* = 8, 9.5 Hz, H-2), 5.52 (*t*, *J* = 9.5 Hz, H-3), 3.82 (*t*, *J* = 9.5, H-4), 4.02 (*m*, H-5), 3.93 (*d*, *J* = 12.5 Hz, H-6), 3.76 (*dd*, *J* = 6, 12.5 Hz, H-6), 5.97 (*d*, *J* = 8.5 Hz, H-1′), 5.15 (*d*, *J* = 8.5, 10 Hz, H-2′), 5.35 (*t*, *J* = 10 Hz, H-3′), 5.81 (*t*, *J* = 10 Hz, H-4′), 3.47 (*br d*, *J* = 10, H-5′), 4.86 (*br d*, *J* = 13, H-6′), 3.90 (*d*, *J* = 13 Hz, H-6′) (glucose protons).

3.5. Partial hydrolysis of nobotanin B (2)

An aq. soln (2 ml) of **2** (17 mg) was heated at 100°C for 18 h. The reaction mixture was subjected to CC over Toyopearl HW-40 (fine) developing with 60% aq. MeOH and 70% aq. MeOH. The eluate with the 60% aq. MeOH gave the hydrolysate (2.9 mg) which was identical with nobotanin O in all respects. The 70% aq. MeOH eluate yielded **3** (nobotanin G; 0.5 mg) (Yoshida, Haba et al., 1992: Yoshida, Nakata et al., 1992).

3.6. Methylation of 1 followed by methanolysis

A mixture of **1** (0.5 mg), Me_2SO_4 (0.01 ml) and K_2CO_3 (50 mg) in Me_2CO (1.5 ml) was stirred overnight at room temp. and then refluxed for 3 h. After removal of the inorganic material by centrifugation, the supernatant was evaporated to dryness in vacuo. The reaction mixt. was then directly methanolyzed with 1% NaOMe (0.1 ml) in MeOH (1 ml) at room temp. for 6 h. After acidification with a few drops of HOAc, the solvent was removed in vacuo. The residue was re-dissolved in MeOH and analysed by normal-phase HPLC (*n*–hexane–AcOEt 9:1) which demonstrated the production of **4**, **5** and **6**.

3.7. Nobotanin P (7)

A light brown amorphous powder. $[\alpha]_D + 36^\circ$ (MeOH; *c* 1.1). FABMS *m/z*: 3591 $[M+H]^+$, 3613 $[M+Na]^+$. ESIMS *m/z*: 3591 $[M+H]^+$. UV λ_{max} MeOH nm (log ϵ): 220 (5.13), 271 (5.02). CD (MeOH): $[\theta]_{227} + 1.0 \times 10^6$, $[\theta]_{240} + 6.7 \times 10^5$, $[\theta]_{262} - 3.3 \times 10^5$, $[\theta]_{284} + 1.5 \times 10^5$, $[\theta]_{312} + 6.0 \times 10^4$. ^{1}H NMR: δ 7.27, 7.26 (each *s*, 2H), 7.14 (2H, s), 7.09 (2H, *s*), 6.98, 6.95 (each *s*, 2H) (galloyl-H), 7.17, 7.15 (each s, 1H), 7.08 (1H, *s*), 6.96, 6.94 (each *s*, 1H), 6.68, 6.63 (each *s*, 1H), 6.64 (1H, *s*), 6.50 (1H, *s*), 6.48, 6.46 (each *s*, 1H), 6.43, 6.40 (each *s*, 1H), 6.41 (1H, *s*), 6.35 (1H, *s*), 6.35, 6.34 (each *s*, 1H), 6.34 (1H, *s*), 6.23, 6.17 (each *s*, 1H), 6.11, 6.10 (each *s*, 1H), 6.02 (1H, *s*) (HHDP and valoneoyl groups), glucose protons, see Table 1. ^{13}C NMR: δ 91.3 (C-1; α-anomer), 94.8 (C-1; β-anomer), 75.1 (C-2; α), 77.8 (C-2; β), 75.5 (C-3; α), 77.6 (C-3; β), 68.6 (C-4; α), 68.1 (C-4; β), 68.2 (C-5; α), 72.7 (C-5; β), 63.1 (C-6; α, β), 92.3 (C-1′; α, β), 76.5 (C-2′; α, β), 77.3 (C-3′; α, β), 69.6 (C-4′; α, β), 73.7 (C-5′; α), 73.1 (C-5′; β), 63.5 (C-6′; α, β), 92.2 (C-1″; α, β), 75.8 (C-2″; α), 75.4 (C-2″; β), 77.8 (C-3″; α, β), 66.6 (C-4″; α, β), 73.8 (C-5″; α, β), 63.5 (C-6″; α, β), 91.8 (C-1‴; α, β), 75.8 (C-2‴; α, β), 77.1 (C-3‴; α, β), 69.0 (C-4‴; α, β), 73.4 (C-5‴; α, β), 63.0 (C-6‴; α, β).

3.8. Methanolysis of 7

A soln of **7** (15 mg) in MeOH (3 ml) containing 0.5 M acetate buffer (0.3 ml, pH 4.6) (0.3 ml) was left standing at 37°C for 1 day. After removal of the solvent, the reaction mixture was applied to a Sep-pak cartridge (Waters), and eluted with H_2O and aq. MeOH (10% → 20% → 30% → 40% → 50%) to yield pedunculagin (1.5 mg) and the trimeric methanolysate (**12**) (8.7 mg).

3.8.1. Compound 12

An off-white amorphous powder, $[\alpha]_D + 34^\circ$ (MeOH; *c* 1.0), UV λ_{max} MeOH nm (log ϵ): 220 (5.34), 270 (5.03), ^{1}H NMR: δ 7.27, 7.25 (each *s*, 2H), 7.15, 7.14 (each *s*, 2H) 7.09, 7.08 (each *s*, 2H), 6.95 (2H, *s*) (galloyl-H), 7.10, 7.09 (each *s*, 1H), 7.09 (1H, *s*), 7.08, 7.07 (each *s*, 1H), 6.98, 6.97 (each *s*, 1H), 6.67, 6.63 (each *s*, 1H), 6.50, 6.48 (each *s*, 1H), 6.46 (1H, *s*), 6.45, 6.43 (each *s*, 1H), 6.40, 6.34 (each *s*, 1H), 6.23, 6.17 (each *s*, 1H), 6.09, 6.08 (each *s*, 1H) (HHDP and valoneoyl groups), 3.63 (3H, *s*) [OMe], 5.45 [*d, J* = 3.5 Hz, H-1 (α)], 5.07 [*d, J* = 8 Hz, H-1 (β)], 5.02 [*dd, J* = 3.5, 9.5 Hz, H-2 (α)], 4.80 [*dd, J* = 8, 9.5 Hz, H-2 (β)], 5.58 [*t, J* = 9.5, H-3 (α)], 5.49 [*t, J* = 9.5 Hz, H-4 (α)], 5,47 [*t, J* = 9.5 Hz, H-4 (β)], 4.38 [*m*, H-5 (α)], 4.39 [*br d, J* = 12 Hz, H-6 (α)], 4.57 [*br d, J* = 12 Hz, H-6 (β)], 4.13 [*dd, J* = 3, 12 Hz, H-6 (α)], 4.20 [*dd, J* = 3, 12 Hz, H-6 (β)], 5.98 [*d, J* = 8.5 Hz, H-1′ (α)], 5.99 [*d, J* = 8.5 Hz, H-1′ (β)], 5.79 [*t, J* = 10 Hz, H-3′ (α)], 5.82 [*t, J* = 10 Hz, H-3′ (β)], 4.56 [*m*, H-5' (α, β)], 6.12 [*d,J* = 8 Hz, H-1″ (α)], 6.14 [*d, J* = 8 Hz, H-1″ (β)], 5.32 [*dd, J* = 8, 10 Hz, H-2″ (α)], 5.31 [*t, J* = 10 Hz, H-3″ (α, β)], 5.71 [*t, J* = 10 HZ, H-4″ (α, β)], 3.39 [*br d, J* = 10 Hz, H-5″ (α, β)], 4.85 [*br d, J* = 13 Hz, H-6″ (α, β)], 5.17 ~ 5.10 [H-3 (β), H-2′ (β), H-4′ (β), H-6′ (α, β), H-2‴ (β), overlapped with each other], 3.82 ~ 3.72 [H-5 (β), H-6′ (α, β), H-6″ (α, β), overlapped with each other].

3.9. Enzymatic hydrolysis of 11

An aq. soln of **11** (0.6 mg/1 ml), which was obtained by methanolysis of **8** (Yoshida et al., 1995), was incubated at 37°C with tannase prepared from *Aspergillus niger* (Yoshida, Tanaka, Chen & Okuda, 1989), and the progress of the reaction was monitored by HPLC. The formation of **12** together with gallic acid was observed accompanied by the disappearance of the starting material, and the product (**12**) was identified by co-HPLC with the sample obtained from **7**.

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