

Tannins and Related Polyphenols of Melastomataceous Plants. VII.¹⁾ Nobotanins J and K, Trimeric and Tetrameric Hydrolyzable Tannins from *Heterocentron roseum*

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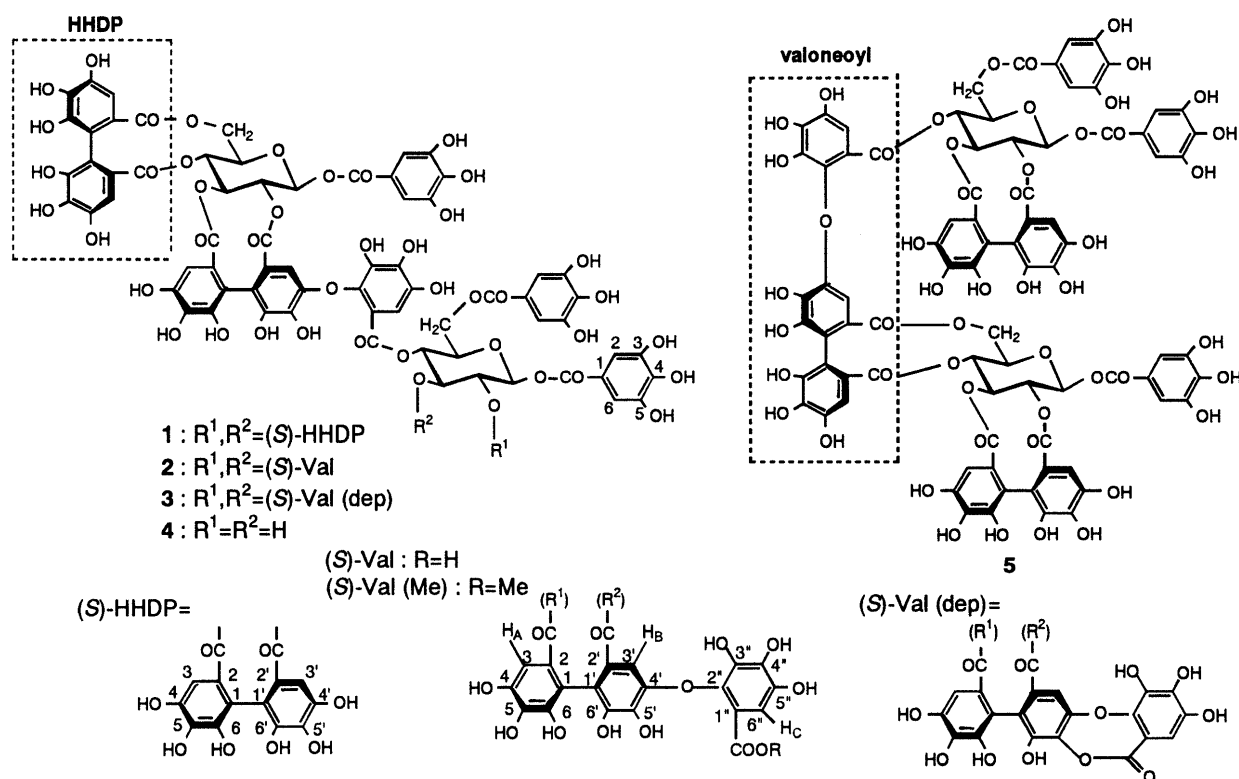
Two new hydrolyzable tannins, nobotanins J and K, were isolated by a combination of column chromatography and centrifugal partition chromatography from the leaf extract of *Heterocentron roseum*, and shown to have trimeric and tetrameric structures composed of combinations of the common monomeric units, casuarictin and pterocarinin C, on the basis of spectral and chemical evidence.

Key words *Heterocentron roseum*; Melastomataceae; tannin; ellagitannin oligomer; nobotanin J; nobotanin K

In a series of studies on tannin constituents of the melastomataceous plants, we have reported the isolation and structure elucidation of novel hydrolyzable tannin oligomers such as nobotanins A–C and E–I from species of various genera, including *Tibouchina semidecandra* COGN.,^{2a,b)} *Heterocentron roseum* A. BR. et BOUCH.,^{2c)} *Medinilla magnifica* LINDLE,^{2d)} *Melastoma malabathricum* L.,^{2e)} and *Bredia tuberculata* (GUIL-LAUM) DIELS.¹⁾ These oligomers have characteristic structures in which the monomeric constituents are linked with each other through a valoneoyl (Val) group produced biogenetically by intermolecular C–O oxidative coupling between a hexahydroxydiphenoyl (HHDP) group of one monomer and a galloyl (Gal) group of another monomer, as exempli-

fied by nobotanins B (1) and F (5).^{2a,b)} Among them, nobotanins H (2) and I (3), the dimers isolated from *H. roseum*,^{2c)} have a Val group (or its depsidone form) in addition to that of the linking unit, which may suggest the occurrence of a related trimer and/or tetramer in the same plant. The polar fraction of the extract of *H. roseum* was thus further examined, leading to the isolation of two new oligomeric hydrolyzable tannins named nobotanins J (9) and K (18), and a known trimer, nobotanin E (6).^{2b)} Here we present a full account of the structure elucidation of the new oligomers.³⁾

The 1-butanol-soluble portion of the aqueous acetone extract of the *H. roseum* leaf was first fractionated by column chromatography over Diaion HP-20 with aqueous



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MeOH. The 40% MeOH eluate, which is rich in oligomeric hydrolyzable tannins, was chromatographed over Toyopearl HW-40 to yield two trimers and a tetramer along with the previously isolated oligomers, 1–3, nobotanin G (4) and 5.^{2c)} One of the trimers was identical with nobotanin E (6) which was first isolated from *T. semidecandra*.^{2b)} The purification of the other crude trimer (nobotanin J) and tetramer (nobotanin K) was unsuccessful; even after repeated column chromatography over Toyopearl HW-40 and/or Sephadex LH-20, they were contaminated by substantial amounts of monomers and dimers, presumably produced by partial hydrolysis during the long-term development required due to their strong

adsorption on the solid support. The purification of these labile oligomers was achieved by centrifugal partition chromatography, which enables quick development in the absence of a solid support.⁴⁾

The new tannin oligomers (9 and 18) showed the typical coloration with $\text{NaNO}_2\text{--AcOH}$,⁵⁾ and were characterized as ellagitannins composed of the common constituent units (Gal, HHDP and Val groups, and glucose) based on their acid hydrolysis, which yielded gallic acid (10), ellagic acid (11), valoneic acid dilactone (12) and glucose.

Nobotanin J (9), an off-white amorphous powder, was shown to be a trimer by its retention volume, which was similar to that of 6 in normal-phase HPLC,⁶⁾ and its ion

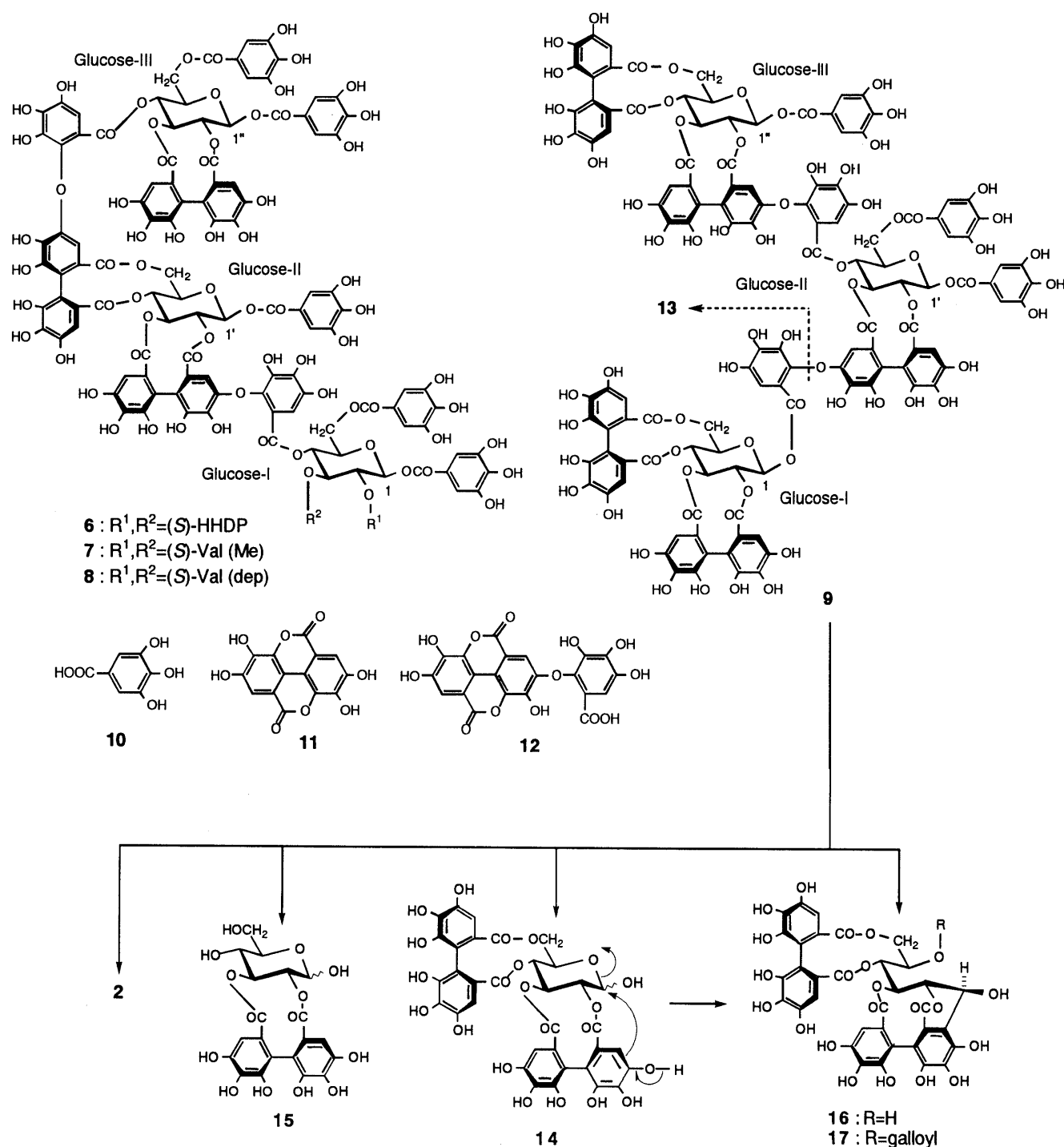


Chart 2

peak at m/z 2797, attributable to $[M+Na]^+$, in the fast-atom bombardment mass spectrum (FAB-MS). These data, along with the following nuclear magnetic resonance (NMR) spectral data, indicated its molecular formula to be $C_{123}H_{82}O_{76}$. The 1H -NMR spectrum of nobotanin J (**9**) exhibited three 2H singlets (δ 7.30, 7.10 and 6.70) due to three Gal groups, and twelve 1H-singlets (δ 7.23, 7.13, 6.67, 6.64, 6.54, 6.51, 6.41, 6.39, 6.35, 6.29, 6.11 and 6.03) ascribable to three HHDP and two Val groups. Three anomeric proton signals were observed at δ 6.21, 6.08 and 5.97 (each d, $J=9$ Hz), indicating the presence of three glucose residues in the molecule. The signals of each glucose core were unambiguously assigned by 1H - 1H shift correlation spectroscopy (COSY) as summarized in Table 1. The large vicinal coupling constants of the sugar proton signals clearly indicate that all the glucopyranose cores adopt the 4C_1 conformation. A comparison of these glucose signals with those of the co-existing known tannins revealed that the resonances of two glucose cores (glucose-II and III) were closely similar to those of nobotanin B (**1**), while the signals of glucose-I resembled those of casuarictin (**13**)⁷⁾ (Table 1). These observations imply that nobotanin J is a trimer possessing nobotanin B and casuarictin moieties which are linked with each other through a Val group biogenetically produced by oxidative coupling^{2b)} between an HHDP group of **1** or **13** and a Gal group of **13** or **1**. This structural feature of **9**

was further supported by a ^{13}C -NMR spectral comparison with **1** and **13** (Table 2).

Partial hydrolysis of nobotanin J (**9**) in a boiling-water bath yielded five products which were isolable by column chromatography over MCI-gel CHP-20P. Among them, the major two were identified as nobotanin H (**2**)^{2c)} and pedunculagin (**14**),⁷⁾ thus confirming the locations of all acyl groups on the glucose cores in **9**, including the orientation of the Val groups. The other two hydrolysates were characterized as nobotanin G (**4**)^{2c)} and 2,3-O-hexahydroxydiphenoylglucose (**15**).⁸⁾ The 1H -NMR spectrum of the fifth hydrolysate (**16**) exhibited three aromatic proton signals at δ 6.79, 6.52 and 6.41 (each 1H, s), and aliphatic proton signals characteristic of an open-chain glucose residue, which were very similar to those of stachyurin (**17**),⁷⁾ a C-glucosidic ellagitannin, except for a remarkable upfield shift of H-5 (δ 5.36 for **17** $\rightarrow$ δ 4.06 for **16**). Based on these data along with the FAB-MS data (m/z 807 $[M+Na]^+$), the hydrolysate was presumed to be a degalloylstachyurin (**16**). This assumption was substantiated by enzymatic degalloylation of **17** with tannase, yielding **16**. Compound **16** was regarded as a by-product produced by an intramolecular phenol-aldehyde condensation of the major hydrolysate **14**. Based on the above spectral and chemical evidence, the structure of nobotanin J was represented by **9**.

Nobotanin K (**18**), $[\alpha]_D +66^\circ$ (MeOH), was obtained

Table 1. 1H -NMR Data for the Glucose Moieties of Nobotanins B (**1**), J (**9**), K (**18**) and Monomeric Units, Casuarictin (**13**) and Pterocaryanin C (**19**) [500 MHz, Acetone- d_6 + D_2O ; J (Hz) in Parenthesis]

	1	9^{a)}	13	18^{a)}	19
Glucose-I					
H-1		6.21 d (9)	6.22 d (9)	6.07 d (8)	
H-2		5.11 t (9)	5.18 t (9)	5.10 dd (8, 10)	
H-3		5.36 dd (9, 10)	5.45 dd (9, 10)	5.36 t (10)	
H-4		5.17 t (10)	5.17 t (10)	5.10 t (10)	
H-5		4.40 dd (7, 10)	4.50 dd (7, 10)	4.39 dd (6, 10)	
H-6		3.87 d (13)	3.88 d (13)	3.70 br d (13)	
		5.33 dd (7, 13)	5.37 dd (7, 13)	5.10 ^{b)}	
Glucose-II					
H-1'	6.02 d (8.5)	5.97 d (9)		5.96 d (8)	
H-2'	5.18 dd (8.5, 10)	5.13 dd (9, 10)		5.13 dd (8, 10)	
H-3'	5.41 t (10)	5.30 t (10)		5.29 t (10)	
H-4'	5.83 t (10)	5.70 t (10)		5.70 t (10)	
H-5'	3.45 br d (10)	3.34 d (10)		3.34 d (10)	
H-6'	4.92 br d (13)	3.83 d (13)		3.85 br d (12)	
	3.91 br d (13)	4.88 d (13)		4.87 br d (12)	
Glucose-III					
H-1''	6.20 d (8.5)	6.08 d (9)		6.18 d (8)	
H-2''	5.10 dd (8.5, 9)	5.10 t (9)		5.09 dd (8, 10)	
H-3''	5.82 dd (9, 10)	5.85 dd (9, 10)		5.82 t (10)	
H-4''	5.17 t (10)	5.11 t (10)		5.10 t (10)	
H-5''	4.67 dd (6, 10)	4.68 dd (6, 10)		4.60 dd (6, 10)	
H-6''	5.33 dd (6, 13.5)	5.29 dd (6, 13)		5.28 dd (6, 13)	
	3.92 d (13.5)	3.92 d (13)		3.82 br d (13)	
Glucose-IV					
H-1'''				6.13 d (8)	6.19 d (8)
H-2'''				5.10 dd (8, 10)	5.14 dd (8, 10)
H-3'''				5.35 t (10)	5.44 t (10)
H-4'''				5.55 t (10)	5.58 t (10)
H-5'''				4.07 dd (3, 10)	4.17 dd (3, 10)
H-6'''				4.25 dd (3, 13)	4.24 dd (3, 13)
				4.46 d (13)	4.46 brd (13)

a) Measured at 400 MHz. b) Overlapped by the H-4 and H-4'' signals.

Table 2. ^{13}C -NMR Data for the Glucose Moieties of Nobotanins B (1), J (9) and K (18), and Their Monomeric Units [Casuarictin (13) and Pterocaryanin C (19)] [126 MHz, Acetone- d_6 + D_2O]

	1	9 ^{a)}	13	18 ^{a)}	19
Glucose-I					
C-1		92.0	92.4	92.3	
C-2		75.8	76.0	76.6 ^{b)}	
C-3		77.2	77.3	77.1 ^{c)}	
C-4		69.0	69.3	68.9 ^{d)}	
C-5		73.5	73.5	73.5	
C-6		63.1	63.1	63.2 ^{e)}	
Glucose-II					
C-1'	92.3	92.2		91.9	
C-2'	75.4	75.2		75.1	
C-3'	78.1	77.9		77.9	
C-4'	66.9	66.6		66.5	
C-5'	73.9	73.8		73.8	
C-6'	63.4	63.6		63.6 ^{e)}	
Glucose-III					
C-1''	92.3	92.3		92.2	
C-2''	76.8	76.7		75.8 ^{b)}	
C-3''	77.0	76.9		76.8	
C-4''	69.6	69.6		69.5 ^{d)}	
C-5''	73.4	73.3		73.2	
C-6''	63.6	63.3		62.5 ^{e)}	
Glucose-IV					
C-1'''				92.1	91.9
C-2'''				75.1	75.3
C-3'''				77.5 ^{c)}	77.4
C-4'''				67.7	67.8
C-5'''				73.9	73.9
C-6'''				63.0 ^{e)}	62.7

a) Measured at 100 MHz. b—e) Exchangeable.

as an off-white amorphous powder. Among the oligomers of *H. roseum*, it was most strongly adsorbed on the solid support upon chromatography over vinyl polymer gel, and was eluted finally with 70% aqueous acetone. Its retention volume in normal-phase HPLC was also larger than those of the trimers, **6** and **9**, suggesting it to be at least a tetramer.^{4a,6)} Four anomeric proton signals were clearly seen at δ 5.96, 6.07, 6.13 and 6.18 (each d, $J=8$ Hz) in the ^1H -NMR spectrum of **18**. The other sugar proton signals, which were assigned with the aid of the ^1H - ^1H COSY spectrum, indicated the presence of four $^4\text{C}_1$ glucopyranose residues (Table 1). The spectrum also showed five 2H-singlets due to the galloyl groups and fifteen 1H-singlets in the aromatic region (see Experimental). Among the aromatic 1H-singlets, three at the highest field (δ 6.02, 6.08 and 6.20) and three at the lowest region (δ 7.21, 7.12 and 7.03) were attributable to the Val H_B and H_C , respectively,⁹⁾ suggesting the presence of three Val groups. The other nine 1H-singlets at δ 6.30–6.66 were assigned to three Val H_A and the protons of three HHDP groups. The ^1H - and ^{13}C -NMR signals of the three glucose moieties (glucose-I, II and III) in **18** were closely similar to those of nobotanin J (**9**). The fourth monomeric unit in **18** was suggested to be pterocaryanin C (**19**)^{2b,e)} on the basis of spectral similarity (Tables 1 and 2). As the glucose-I moiety in nobotanin J (**9**) is accessible to hydrolysis as mentioned earlier, partial hydrolysis of **18** was attempted in order to confirm the terminal monomeric unit in **18**. As expected, pedunculagin (**14**) and two trimeric hydrolysates (**7** and **8**) were obtained upon mild

methanolysis of **18**. The ^1H -NMR spectra of **7** and **8** showed close similarity to that of nobotanin E (**6**) (Table 3), except for the presence of an extra aromatic proton signal in the former two. The ^1H -NMR spectrum of **7** exhibited, in addition to five 2H-singlets due to the Gal groups, eleven aromatic 1H-singlets, whose chemical shifts indicated the presence of an HHDP and three Val groups. Compound **7** was thus regarded as a trimer with a structure related to that of nobotanin E (**6**), in which an HHDP group in the latter is replaced by a Val group (monomethyl ester) in the former. The hydrolysate (**8**) was characterized by a remarkable downfield shift of one of the Val H_B signals at δ 6.08–6.19 in **7** to a region of δ 7.13–6.97 in the ^1H -NMR spectrum, which is characteristic of that observed between a Val group and its depsidone-form.^{2c,9)} On the other hand, partial hydrolysis of **18** in hot water afforded two monomeric hydrolysates, along with gallic acid (**10**) and ellagic acid (**11**). One of them was identified as **16**. The ^1H -NMR spectrum of the other hydrolysate indicated the presence of an HHDP, two Gal groups and a dilactonized Val group attached to the $^4\text{C}_1$ glucopyranose core, and was identical with that of the hydrolysate (**20**) obtained by similar hydrolysis of nobotanins B (**1**), E (**6**) and F (**5**).^{2a,b)} The production of **16** (or **14**) and **20** from **18** provided definite evidence for the terminal monomeric units of nobotanin K.

The circular dichroism (CD) spectrum of nobotanin K (**18**) showed positive Cotton effects at 227 and 235 nm, which have larger amplitudes than those of **6** and **9**, indicating the (*S*)-configuration for each of the HHDP and Val groups in the molecule.¹⁰⁾ Based on these data, the structure of nobotanin K was determined to be **18**.

Although nobotanin J (**9**) has also been found in *Melastoma malabathricum*^{2e)} and *M. normale*,¹¹⁾ nobotanin K (**18**) has not yet been found in any other species. It is also noticeable that nobotanin K exhibited a significant host-mediated antitumor activity¹²⁾ and a potent inhibitory effect on poly(ADP-ribose)glycohydrolase purified from mouse mammary tumor 34I cells.¹³⁾

Experimental

The instruments for FAB-MS and optical polarimetry, and the chromatographic conditions used throughout this study were the same as those described in the previous paper.^{1,2a)} NMR spectra were taken on Varian VXR-500 (500 MHz for ^1H and 126 MHz for ^{13}C) and Bruker AM-400 (400 MHz for ^1H and 100 MHz for ^{13}C) instruments. Centrifugal partition chromatography (CPC) was performed using a Model L-90 CPC apparatus (Sanki Engineering, Nagaokakyo, Kyoto, Japan) which consists of a centrifuge with twelve column cartridges, each containing a polyfluoroethylene resin block (150 × 40 × 40 mm) and being connected by fine resin tubes. The sample solution and the solvent [1-butanol–1-propanol–water (4 : 1 : 5, v/v/v); upper layer as the mobile phase for the normal phase development] were pumped into the columns (flow rate 3 ml/min), which were rotating at 700 rpm, with a Model CPC-LBP-II pump (Sanki).

Isolation of Tannins The 40% MeOH eluate (3.6 g) of Diaion HP-20 column chromatography of the 1-butanol-soluble portion (8.1 g), which was obtained from the aqueous acetone extract of the fresh leaves of *H. roseum* (2.1 kg), was rechromatographed over Toyopearl HW-40 using MeOH– H_2O –acetone (7 : 2 : 1 → 6 : 2 : 2) and 70% aqueous acetone.^{2c)} The eluate with MeOH– H_2O –acetone (6 : 2 : 2) gave crude nobotanin J (**9**) (1.12 g) after elution of nobotanin B (**1**).^{2c)} Crude nobotanin K (**18**) (653 mg) was obtained from the 70% aqueous acetone eluate. The 60% MeOH eluate (1 g) of Diaion HP-20 column chromatography was similarly subjected to rechromatography over Toyopearl HW-40 using

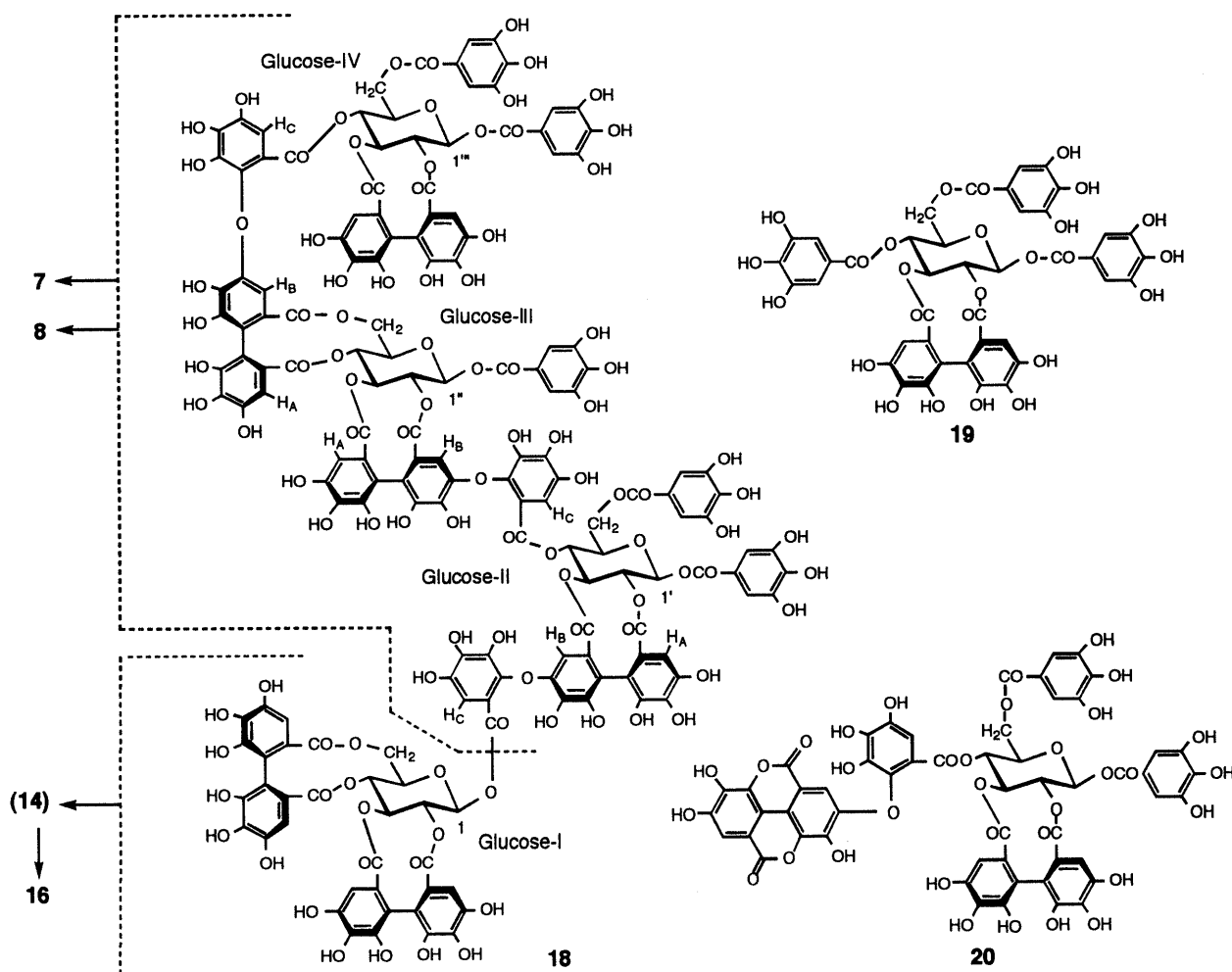


Chart 3

Table 3. ^1H -NMR Data for the Glucose Moieties of Nobotanin E (6) and the Hydrolysate (7) (500 MHz, Acetone- d_6 + D_2O ; J in Hz)

	6	7
Glucose-I		
H-1	6.01 d ($J=8.5$)	5.99 d ($J=8.5$)
H-2	5.18 dd ($J=8.5, 10$)	5.14 dd ($J=8.5, 10$)
H-3	5.40 dd ($J=10$)	5.30 t ($J=10$)
H-4	5.83 t ($J=10$)	5.70 t ($J=10$)
H-5	3.45 br d ($J=10$)	3.37 dd ($J=1, 10$)
H-6	4.91 br d ($J=13$)	4.82 br d ($J=13$)
	3.91 br d ($J=13$)	3.82 dd ($J=1, 13$)
Glucose-II		
H-1'	6.14 d ($J=8.5$)	6.14 d ($J=8.5$)
H-2'	5.08 dd ($J=8.5, 10$)	5.07 dd ($J=8.5, 10$)
H-3'	5.80 t ($J=10$)	5.80 t ($J=10$)
H-4'	5.10 t ($J=10$)	5.07 t ($J=10$)
H-5'	4.60 dd ($J=6, 10$)	4.57 dd ($J=6, 10$)
H-6'	5.09 dd ($J=6, 13$)	5.07 dd ($J=6, 13$)
	3.70 d ($J=13$)	3.68 d ($J=13$)
Glucose-III		
H-1''	6.18 d ($J=8.5$)	6.11 d ($J=8.5$)
H-2''	5.12 dd ($J=8.5, 10$)	5.10 dd ($J=8.5, 10$)
H-3''	5.37 t ($J=10$)	5.32 t ($J=10$)
H-4''	5.56 t ($J=10$)	5.53 t ($J=10$)
H-5''	4.09 ddd ($J=2, 4, 10$)	4.04 ddd ($J=1, 4, 10$)
H-6''	4.47 br d ($J=13$)	4.44 dd ($J=1, 13$)
	4.28 dd ($J=4, 13$)	4.25 dd ($J=4, 13$)

the same solvent system^{2c}) to give crude nobotanins J (9) (13.7 mg) and K (18) (255 mg), in addition to pure 9 (13.3 mg). The final purification of the crude nobotanin J was achieved by centrifugal partition chromatography to give 9 (154 mg), as previously reported.⁴⁾ A part (410 mg) of the crude nobotanin K was similarly purified to afford 18 (112 mg).⁴⁾

Nobotanin J (9) An off-white amorphous powder, $[\alpha]_D^{25} + 51^\circ$ ($c=1.0$, MeOH), *Anal.* Calcd for $\text{C}_{123}\text{H}_{82}\text{O}_{76} \cdot 12\text{H}_2\text{O}$: C, 48.48; H, 3.77. Found: C, 48.54; H, 3.60. FAB-MS m/z : 2797 ($\text{M} + \text{Na}$)⁺. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 200 (5.66), 246 (5.36). CD ($c=0.01$, MeOH) $[\theta]$ (nm): $+50.8 \times 10^4$ (225), -20.8×10^4 (261), $+4.3 \times 10^4$ (283), -3.7×10^4 (308). ^1H -NMR: aromatic protons, see text; glucose protons, see Table 1. ^{13}C -NMR (100 MHz, acetone- d_6 + D_2O) δ : 102.60, 104.56, 106.69, 107.25, 107.69 (3C), 107.99, 108.11, 108.42 (HHDP C-3, C-3', Val C-3, C-3'), 110.65, 111.41 (Val C-6''), 110.34 (4C), 110.44 (2C) (Gal C-2, C-6), 112.99, 114.02, 114.44, 114.48, 114.57, 114.92, 115.37, 115.77, 115.95, 115.98, 116.05, 116.68 (HHDP C-1, C-1', Val C-1, C-1', C-1''), 119.77, 120.07, 121.30 (Gal C-1), 125.52, 125.60, 125.71, 125.75, 125.87, 126.00, 126.21, 126.43, 126.53 (HHDP C-2, C-2', Val C-2, C-2'), 135.77 (2C), 136.21, 136.40, 136.43 (2C), 136.50, 136.53, 136.57, 136.66, 136.95, 137.41 (HHDP C-5, C-5', Val C-5, C-5', C-2''), 139.23, 139.63, 139.87 (Gal C-4), 136.69, 140.49, 140.55, 141.47 (Val C-3'', C-4''), 143.08, 143.12 (Val C-5''), 144.09, 144.19, 144.41, 144.43, 144.45, 144.75, 144.83, 145.03 (2C), 145.13, 145.17 (2C), 145.92 (3C) (Gal C-3, C-5, HHDP C-4, C-4', C-6, C-6', Val C-4, C-6, C-6'), 146.18, 146.66 (Val C-4'), 163.16, 164.08, 164.32, 164.56, 167.29, 167.70, 167.72, 167.85, 168.15, 168.28, 168.56, 169.10 (2C), 169.29, 169.56 (ester carbonyl), glucose carbons, see Table 2.

Nobotanin K (18) An off-white amorphous powder, $[\alpha]_D^{25} + 66^\circ$ ($c=0.5$, MeOH), *Anal.* Calcd for $\text{C}_{164}\text{H}_{110}\text{O}_{104} \cdot 12\text{H}_2\text{O}$: C, 49.98; H, 3.51. Found: C, 49.74; H, 3.93. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 219 (5.43), 270 (5.12). CD (MeOH) $[\theta]$ (nm): $+52.4 \times 10^4$ (224), $+37.9 \times 10^4$ (234), -18.3×10^4 (260), $+7.6 \times 10^4$ (280), -3.2×10^4 (308). ^1H -NMR δ : 7.29, 7.17, 7.15, 7.10, 6.96 (2H each, s, Gal), 7.21, 7.12, 7.03, 6.66, 6.51, 6.49,

6.45, 6.44, 6.42, 6.41, 6.35, 6.30, 6.20, 6.08, 6.02 (each 1H, s, HHDP and Val), glucose protons, see Table 1. ^{13}C -NMR (100 MHz, acetone- d_6 + D_2O) δ : 102.4, 104.4, 104.7, 106.6, 107.1, 107.2, 107.6 (3C), 107.8, 108.2 (HHDP C-3, C-3', Val C-3, C-3'), 110.1 (2C), 110.2 (2C), 110.3 (6C) (Gal C-2, C-6), 109.8, 110.5, 111.4 (Val C-6"), 112.9, 114.0, 114.1, 114.4, 114.5 (2C), 114.7, 114.8, 115.2, 115.4, 115.6, 115.7, 115.9, 116.7, 117.1 (HHDP C-1, C-1', Val C-1, C-1', C-1"), 119.7, 119.8, 120.0, 121.2, 121.3 (Gal C-1), 125.1, 125.4, 125.6, 125.7, 125.8 (2C), 125.9, 126.0, 126.1, 126.2, 126.3, 126.4 (HHDP C-2, C-2', Val C-2, C-2'), 135.2, 135.3, 135.9, 136.0, 136.3, 136.4 (5C), 136.5, 136.6, 136.8, 136.9, 137.3 (HHDP C-5, C-5', Val C-5, C-5', C-2"), 139.0, 139.2, 139.6, 139.7 (3C), 140.1, 140.4, 140.5, 140.6, 141.4 (Gal C-4, Val C-3", C-4"), 143.0 (2C), 143.6 (Val C-5"), 144.1, 144.2, 144.3 (2C), 144.5 (2C), 144.7 (2C), 144.8, 144.9, 145.0, 145.3 (HHDP C-4, C-4', C-6, C-6', Val C-4, C-6, C-6'), 145.0 (2C), 145.1 (2C), 145.8 (2C), 145.9 (2C), 146.1 (2C) (Gal C-3, C-5), 146.5, 146.6, 146.8 (Val C-4'), 163.0, 164.0, 164.3, 164.5, 164.9, 165.0, 166.4, 167.3, 167.5, 167.7, 167.8, 167.9, 168.3, 168.5, 168.6, 169.1 (2C), 169.3, 169.5 (2C) (ester carbonyl), glucose carbons, see Table 2.

Acid Hydrolysis of Nobotanins J (9) and K (18) A solution of **9** (3 mg) in 5% sulfuric acid (0.8 ml) was heated in a boiling-water bath for 10 h. After cooling, the reaction mixture was extracted with AcOEt. The aqueous layer was neutralized with ion exchange resin (Amberlite IRA-410, OH form), filtered and evaporated to dryness. The sugar component was identified as glucose by gas liquid chromatography (GLC) [2.5% OV-1, column temperature 200°C] after trimethylsilylation. The AcOEt-soluble portion was evaporated, and analyzed by reversed-phase HPLC to detect gallic acid (t_R 3.10 min), valoneic acid dilactone (t_R 7.87 min) and ellagic acid (t_R 11.48 min). A mixture of these polyphenolic products was methylated with an excess of ethereal CH_2N_2 . The residue was subjected to preparative TLC (SiO_2 , benzene-acetone 15:1) to yield the respective methylated derivatives, which were identified by co-chromatography (TLC) with authentic samples and MS spectral comparisons.

Nobotanin K (**18**) was similarly hydrolyzed to give the same products as described above.

Partial Hydrolysis of Nobotanin J (9) An aqueous solution (50 ml) of **9** (106 mg) was heated on a water-bath at 70°C for 2 h. The concentrated solution was submitted to column chromatography over MCI-gel CHP-20P developing with H_2O and aqueous MeOH (10% $\rightarrow$ 20% $\rightarrow$ 25% $\rightarrow$ 30% $\rightarrow$ 35% $\rightarrow$ 40%). The eluate from H_2O gave 2,3-*O*-(*S*)-hexahydroxydiphenoylglucose (1.8 mg). The 10% MeOH eluate yielded **16** (3 mg). The major products, **14** (18 mg) and **2** (10 mg) were obtained from the 20% MeOH and 35% MeOH eluates, respectively. The 30% MeOH eluate gave nobotanin G (**4**) (2 mg).

Hydrolysate (16): A light brown amorphous powder. FAB-MS m/z : 807 ($\text{M} + \text{Na}$) $^+$. ^1H -NMR (500 MHz, acetone- d_6 + D_2O) δ : 6.79, 6.52, 6.41 (1H each, s) (HHDP), 4.87 [1H, d, $J=2$ Hz, glucose (Glc) H-1], 4.85 (1H, t, $J=2$ Hz, Glc H-2), 4.95 (1H, t, $J=2$ Hz, Glc H-3), 5.22 (1H, dd, $J=2, 8$ Hz, Glc H-4), 4.06 (1H, dd, $J=3.5, 8$ Hz, Glc H-5), 4.71 (1H, dd, $J=3.5, 12$ Hz, H-6), 3.81 (1H, d, $J=12$ Hz, Glc H-6).

This compound was identified as the degalloylstachyurin which was obtained upon treatment of an aqueous solution of stachyurin (**17**) with tannase at 37°C for 2 h.

Methanolysis of Nobotanin K (18) A solution of **18** (60 mg) in absolute MeOH (60 ml) was left standing at 37°C for a week. After evaporation of the solvent, the reaction mixture was chromatographed over MCI-gel CHP 20P with aqueous MeOH (10% $\rightarrow$ 20% $\rightarrow$ 30% $\rightarrow$ 40% MeOH) to yield pedunculagin (**14**) (7 mg), and trimeric hydrolysates **7** (18 mg) and **8** (6 mg).

Hydrolysate (7): A light brown amorphous powder, $[\alpha]_D^{21} (c=0.5, \text{MeOH})$. ^1H -NMR (500 MHz, acetone- d_6 + D_2O) δ : 7.27, 7.15, 7.13, 7.09, 6.94 (2H each, s, Gal), 7.11, 7.09, 7.04, 6.48, 6.47, 6.44, 6.43, 6.41, 6.19, 6.10, 6.08 (1H each, s, HHDP and Val), 3.63 (3H, s, OMe), glucose protons, see Table 3.

Hydrolysate (8): A light brown amorphous powder, $[\alpha]_D^{34} (c=0.5, \text{MeOH})$. ^1H -NMR (500 MHz, acetone- d_6 + D_2O) δ : 7.32, 7.19, 7.17, 7.13, 7.00 (each 2H, s, Gal), 7.13, 7.08, 7.05, 6.97 (Val- $\text{H}_C \times 3$, Val- H_B), 6.52,

6.51, 6.48, 6.47, 6.44 (HHDP and Val- H_A), 6.23, 6.12 (Val- H_B), 6.05 (1H, d, $J=8.5$ Hz, H-1), 5.21 (1H, dd, $J=8.5, 10$ Hz, H-2), 5.37 (1H, t, $J=10$ Hz, H-3), 5.80 (1H, t, $J=10$ Hz, H-4), 3.47 (1H, br d, $J=10$ Hz, H-5), 4.92 (1H, br d, $J=13$ Hz, H-6), 3.89 (1H, br d, $J=13$ Hz, H-6), 6.15 (1H, d, $J=8.5$ Hz, H-1'), 5.11—5.15 (4H, overlapped signal, H-2', H-4', H-6', H-2"), 5.86 (1H, t, $J=10$ Hz, H-3'), 4.59 (1H, dd, $J=6, 10$ Hz, H-5'), 3.71 (1H, d, $J=13$ Hz, H-6'), 6.21 (1H, d, $J=8.5$ Hz, H-1"), 5.46 (1H, t, $J=10$ Hz, H-3"), 5.57 (1H, t, $J=10$ Hz, H-4"), 4.08 (1H, br d, $J=10$ Hz, H-5"), 4.50 (1H, br d, $J=13$ Hz, H-6"), 4.28 (1H, dd, $J=4, 13$ Hz, H-6").

Partial Hydrolysis of Nobotanin K (18) An aqueous solution (80 ml) of **18** (80 mg) was heated in a boiling-water bath for 8 h. The reaction mixture was concentrated, and the residue was submitted to column chromatography over Sephadex LH-20 with EtOH to give gallic acid, ellagic acid and the hydrolysates **16** (5 mg) and **20** (2.9 mg).

Hydrolysate (20): An off-white amorphous powder. FAB-MS m/z : 1261 ($\text{M} + \text{Na}$) $^+$. ^1H -NMR (400 MHz, acetone- d_6) δ : 7.10, 6.70 (2H each, s, Gal), 6.43, 6.39 (1H each, s, HHDP), 7.54, 7.19, 7.16 (1H each, s, dilactonized Val), 6.18 (1H, d, $J=8.5$ Hz, Glc H-1), 5.08 (1H, dd, $J=8.5, 9.5$ Hz, Glc H-2), 5.41 (1H, dd, $J=9.5, 10$ Hz, Glc H-3), 5.54 (1H, t, $J=10$ Hz, Glc H-4), 4.27 (1H, m, Glc H-5), 4.33 (1H, br d, $J=13$ Hz, Glc H-6), 4.09 (1H, dd, $J=4, 13$ Hz, Glc H-6). These data were identical with those reported.^{2a)}

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