Tannins and Related Polyphenols of Melastomataceous Plants. VIII.[1] Nobotanins L, M and N, Trimeric Hydrolyzable Tannins from *Tibouchina semidecandra*

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Three new hydrolyzable tannins, nobotanins L, M and N, were isolated from the water-soluble portion of the leaf extract of *Tibouchina semidecandra*, and their trimeric structures were elucidated from spectral and chemical evidence. Nobotanins L and N exist as equilibrium mixtures of four anomers due to the presence of two unacylated anomeric centers.

Key words *Tibouchina semidecandra*; Melastomataceae; tannin; nobotanin L; nobotanin M; nobotanin N

We previously reported the isolation and structure elucidation of five new hydrolyzable tannins, nobotanins A—E, from *Tibouchina semidecandra* COGN., which were the first ingredients characterized as tannins from Melastomataceae.[2] Melastomataceous plants have proved to be rich sources of oligomeric hydrolyzable tannins in a series of studies on species of the *Heterocentron*,[1,3] *Medinilla*[4] and *Melastoma*[5] genera. The structures of these oligomers are characterized by possessing valoneoyl group(s) attached to O-2/O-3 and/or O-4/O-6 on the glucose core of a monomer and O-4 of the other monomer, as exemplified by nobotanins B and F.[2,3] In a continuation of our studies on the tannins of this family, we have reinvestigated the highly polar constituents of *T. semidecandra*, and isolated three new oligomers from a water-soluble fraction of the plant extract. This paper describes the structural elucidation of these new tannins.

The aqueous acetone extract of *T. semidecandra* leaves was extracted with ether, AcOEt and 1-butanol. Centrifugal partition chromatography (CPC)[6] and subsequent chromatography over polystyrene gel of the aqueous extract remaining after extraction with organic solvents led to the isolation of new hydrolyzable tannins named nobotanins L (**2**), M (**3**), and N (**4**), along with the previously isolated trimer, nobotanin C (**1**).[2b]

All the new tannins were shown to be ellagitannin trimers by the bluish coloration characteristic of ellagitannins following reaction with $NaNO_2$–AcOH reagent[7] and their large retention volumes similar to that of **1** on normal phase HPLC.[8] Upon methylation followed by methanolysis, each of the tannins **2**—**4** yielded methyl tri-*O*-methylgallate (**5**), dimethyl hexamethoxydiphenate (**7**) and trimethyl octa-*O*-methylvaloneate (**6**). The sugar unit liberated on acid hydrolysis of these tannins was identified as glucose by GLC after trimethylsilylation, indicating that these tannins are composed of common constituent units, galloyl, hexahydroxydiphenoyl (HHDP) and valoneoyl groups, and glucose.

Nobotanin L (**2**) was obtained as an off-white amorphous powder and its molecular formula was assigned as $C_{109}H_{76}O_{70}$ based on FAB-MS with a pseudomolecular ion peak at *m/z* 2527 $[M+Na]^+$ and the spectral data described below. The ^{1}H-NMR spectrum of **2** showed a close resemblance to that of nobotanin C (**1**).[2b] The most noticeable difference was that each proton signal appeared as a set of four lines in the spectrum of **2** (Fig. 1), in contrast with the formation of a duplicate signal for each proton in **1**, due to an equilibrium between the α and β-anomers at an unacylated anomeric center of the glucose core. This feature, along with the presence of only one set of acylated anomeric proton sig-

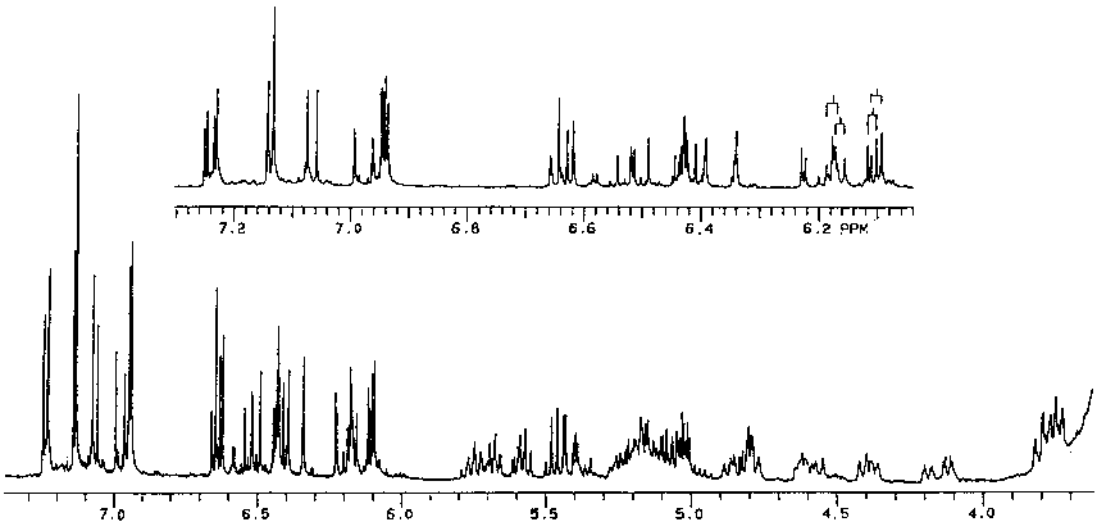

Fig. 1. ^{1}H-NMR Spectrum of Nobotanin L (**2**) (500 MHz, Acetone-d_6+D_2O)

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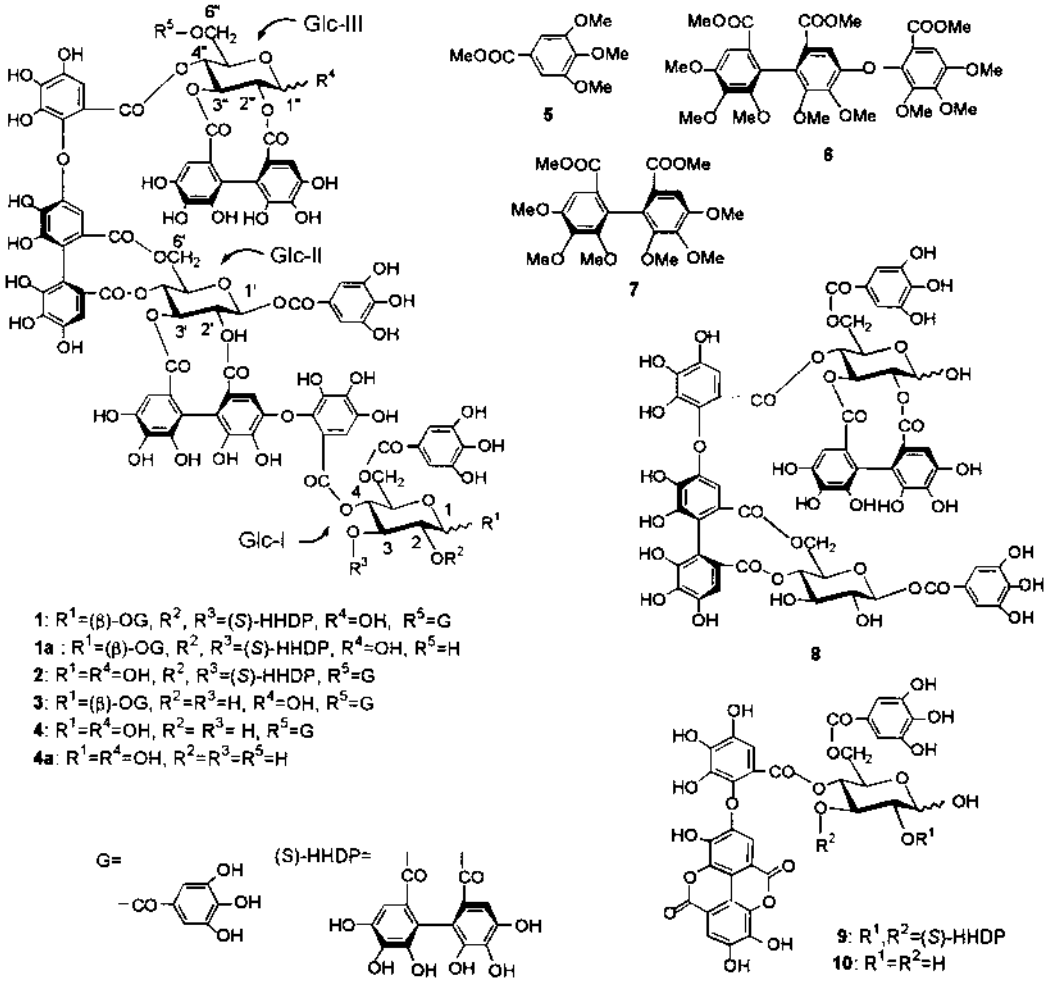

Chart 1

nals at the low field region (δ 6.11, 6.10, 6.18, 6.17, each d, J=8.0 Hz), suggested that two of the three glucose residues in **2** have unacylated anomeric centers, forming an equilibrium mixture of four anomers.

Although the spectrum of **2** was extremely complicated, the ^{1}H–^{1}H shift correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY) revealed that a glucose core having the acylated anomeric center is fully acylated and has an HHDP or biphenyl part of the valoneoyl group at O-4/O-6 as shown by the large difference in chemical shifts between the C-6 methylene proton signals (δ 5.18 and 3.75).[9] Actually, the chemical shifts of the glucose (Glc-II) signals, which could be distinguished from those of the other glucose cores by sequential correlations from H-1′ in the ^{1}H–^{1}H COSY, were virtually identical with those of the corresponding signals of nobotanin C (**1**).[2b] Nobotanin L (**2**) was, thus, deduced to be a congener of **1** in which the galloyl group at O-1 of **1** is replaced by a hydroxyl group. The aromatic proton signals at δ 6.1—7.3 in the ^{1}H NMR spectrum of **2** are consistent with the presence of three galloyl, two valoneoyl and two HHDP groups (see Experimental).

Enzymatic hydrolysis of nobotanin C (**1**) with tannase afforded, in addition to gallic acid and unreacted **1**, two degalloylated derivatives, one of which was identified as nobotanin L (**2**), establishing their structural relationships. The ^{1}H-NMR spectrum of the other hydrolysate (**1a**), which showed a duplicate signal for each proton, was very similar to that of nobotanin C (**1**), except for the absence of a pair of galloyl signals and upfield shifts of the C-6″ methylene protons (δ 3.4—3.9) of the glucose-III residue. Hence, this hydrolysate was formulated as **1a**.

The (*S*)-configurations of the chiral HHDP and the valoneoyl groups in **2** were evident from the CD spectrum showing strong positive Cotton effects at 226 and 238 nm.[10] Based on the above evidence, the structure of nobotanin L was determined to be **2**.

Nobotanin M (**3**) had an $[M+Na]^+$ ion peak at *m/z* 2377 in FAB-MS which corresponds to the molecular formula $C_{102}H_{74}O_{66}$. The ^{1}H-NMR spectrum of **3**, in which each proton signal is duplicated owing to the presence of a mixture of α- and β-anomers, indicated the presence of four galloyl [δ 7.20, 7.19 (each s, 2H in total), 7.15, 7.14 (each s, 2H in total), 7.11, 7.10 (each s, 2H in total), 6.97, 6.96 (each s, 2H in total)], an HHDP and two valoneoyl groups (δ 6.17—7.10, eight pairs of singlets; see Experimental section). The absolute configurations of the chiral HHDP and valoneoyl groups were shown to be the *S*-configuration from the CD spectrum (positive Cotton effects at 224 and 237 nm).[10] In the aliphatic proton region, two pairs of anomeric proton signals were observed at δ 6.08, 6.09 (each d, J=8.5 Hz, 1H in total), and 5.67, 5.68 (each d, J=8.0 Hz, 1H in total), and a pair for the third anomeric proton signal appeared at δ 5.44

Table 1. ^{1}H-NMR Data[a] of Glucose Moieties of Nobotanins C (**1**) and M (**3**) (500 MHz, Acetone-d_6+D_2O, J in Hz)

Protons		**1**		**3**	
		α-Anomer	β-Anomer	α-Anomer	β-Anomer
Glc-I	H-1	6.10 d (8)	6.14 d (8)	5.67 d (8)	5.68 d (8)
	H-2	5.30 dd (8, 9.5)	5.17 dd (8, 9.5)	3.64 dd (8, 9.5)	
	H-3	5.32 t (9.5)		3.74 t (9.5)	
	H-4	5.68 t (9.5)		5.39 t (9.5)	
	H-5	3.37 br d (9.5)		3.43 br d (9.5)	
	H-6	4.87 br d (13)		4.72 br d (13)	
		3.79 m		3.85 br d (13)	
Glc-II	H-1′	5.98 d (8.5)	5.99 d (8.5)	6.08 d (8.5)	6.09 d (8.5)
	H-2′	5.16 dd (8.5, 9.5)	5.15 dd (8.5, 9.5)	5.27 br t (10)	5.17 dd (8.5, 10)
	H-3′	5.80 t (9.5)	5.84 t (9.5)	5.64 t (10)	5.66 t (10)
	H-4′	5.18 t (9.5)	5.16 t (9.5)	5.15 t (10)	5.08 t (10)
	H-5′	4.58 m		4.50 m	4.48 m
	H-6′	5.12[b]		5.13[b]	
		3.78[b]		3.74[b]	
Glc-III	H-1″	5.46 d (3.5)	5.07 d (8)	5.44 d (3.5)	5.06 d (8)
	H-2″	5.02 dd (3.5, 9.5)	4.80 dd (8, 9.5)	5.02 dd (3.5, 9.5)	4.80 dd (8, 9.5)
	H-3″	5.58 t (9.5)	5.14[b]	5.58 t (9.5)	5.16 t (9.5)
	H-4″	5.50 t (9.5)	5.47 t (9.5)	5.48 t (9.5)	5.47 t (9.5)
	H-5″	4.38 br d (9.5)	3.80[b]	4.37 m	3.78 m
	H-6″	4.35 d (12)	4.59 br d (12)	4.40 br d (13)	4.57 br d (13)
		4.14 br d (3.5, 12)	4.20 dd (3.5, 12)	4.11 dd (3, 13)	4.18 dd (3, 13)

a) Assigned by ^{1}H–^{1}H COSY and TOCSY. *b*) Coupling constant is not clear due to overlapping with other signals.

(d, J=3.5 Hz) and 5.06 (d, J=8.0 Hz), indicating that two glucose cores have β-oriented acyloxy groups at anomeric centers and the other has a hydroxyl group allowing it to form α- and β-anomers. The remaining proton signals of each glucose core, which were assigned by correlations with the above anomeric protons in the ^{1}H–^{1}H COSY, were almost superimposable on the corresponding signals of **1** except for significant upfield shifts of the H-2 and H-3 in **3**, as summarized in Table 1. These observations imply that nobotanin M is a trimer lacking an HHDP group at O-2/O-3 in **1**. This structural feature was further supported by a ^{13}C-NMR spectral comparison of **3** and **1**.

Partial hydrolysis of nobotanin M (**3**) in boiling water yielded four hydrolysates (**8**, **9**, **10**, **11**) as well as gallic and ellagic acids. The hydrolysates **8**—**10** were identified by HPLC and NMR comparisons with the compounds obtained from **3** following similar treatment.[2b] Compound **11** was identified as 6-*O*-galloylglucose. Accordingly, nobotanin M has the structure represented by **3**.

Nobotanin N (**4**) is a degalloylated congener of nobotanin M as was evident from the $[M+Na]^+$ ion peak at *m/z* 2225 in FAB-MS, consistent with the molecular formula $C_{95}H_{70}O_{62}$ [152 msu ($C_7H_4O_4$) smaller than **3**]. Its ^{1}H-NMR spectrum exhibited a set of four lines for each proton, as seen in the spectrum of **2**, indicating that it existed as four equilibrated anomers. The presence of an HHDP, two valoneoyl, and three galloyl groups was implied by the aromatic proton signals described in the Experimental section. One of the three glucose residues in **4** was shown to be fully acylated by the ^{1}H–^{1}H COSY spectrum, *i.e.*, a sequentially coupled seven-spin system starting from an anomeric proton signal (H-1′) at δ 6.0—6.15 (d, J=8.0 Hz) includes C-6 methylene proton signals (H-6′) at δ 5.18 and 3.75 that are characteristic of ellagitannins having an HHDP or valoneoyl biphenyl moiety at O-4/O-6 of the C1 glucopyranose residue.[7] To addition, partial hydrolysis of **4** in boiling water afforded the hydrolysates, **8**—**10**.[2b] These data were consistent with the structure **4** for nobotanin N. The structural relationships between **3** and **4** were further confirmed by degalloylation with tannase which gave a common derivative (**4a**) from each of them.

Experimental

^{1}H- (500 MHz) and ^{13}C-NMR (126 MHz) were measured in acetone-d_6+D_2O and chemical shifts are given in δ values, relative to tetramethylsilane (TMS). The instruments for FAB-MS, optical polarimetry, and the chromatographic conditions including CPC used throughout this study were the same as those described in our previous paper.[1]

Plant Materials The leaves of cultivated *T. semidecandra* were collected from the green house of the Medicinal Plants Garden, Faculty of Pharmaceutical Sciences, Okayama University in October. A voucher specimen was deposited in the same Herbarium.

Isolation of Tannins Fresh leaves (380 g) of *T. semidecandra* were homogenized in 70% aqueous Me_2CO and the concentrated homogenate was extracted with Et_2O, AcOEt and 1-butanol. The remaining aqueous layer was concentrated and chromatographed over Amberlite XAD-2 (3.3 i.d.×45 cm) with H_2O and MeOH (each 700 ml). The MeOH eluate (3.8 g) was fractionated by CPC with 1-butanol–PrOH–H_2O (4 : 1 : 5) in ascending mode. The fraction rich in oligomers, as judged by normal phase HPLC, was submitted to further chromatography over MCI-Gel CHP-20P (1.1 i.d.×28 cm) with 20% (150 ml) and 30% MeOH (150 ml). The 30% MeOH eluate gave nobotanins C (**1**, 49 mg), L (**2**, 27 mg), M (**3**, 40 mg), and N (**4**, 15 mg).

Nobotanin L (2) An off-white amorphous powder, $[\alpha]_D$ +68° (c=0.7, MeOH), *Anal.* Calcd for $C_{109}H_{76}O_{70}\cdot 19H_2O$: C, 45.96; H, 4.01. Found: C, 46.02; H, 4.23. FAB-MS: *m/z* 2527 $[M+Na]^+$. CD (c=0.01, MeOH): $[\theta]_{226}$ +3.55×10^5, $[\theta]_{238}$ +2.33×10^5, $[\theta]_{261}$ −1.37×10^5, $[\theta]_{282}$ +5.10×10^4, $[\theta]_{312}$ −3.33×10^4. ^{1}H-NMR δ: 7.26, 7.25, 7.24, 7.23 (each s, 2H in total), 7.15, 7.14 (each s, 2H in total), 6.95, 6.94 (each s, 2H in total) [galloyl (Gal)], 7.08, 7.07 (each s, 1H in total), 7.00, 6.97, 6.96 (each s, 1H in total), 6.67, 6.66, 6.65 (each s, 1H in total), 6.63, 6.62 (each s, 1H in total), 6.55, 6.52, 6.51, 6.49 (each s, 1H in total), 6.45, 6.44, 6.43 (each s, 1H in total), 6.42, 6.41 (each s, 1H in total), 6.40, 6.39, 6.35, 6.34 (each s, 1H in total), 6.24, 6.23 (each s, 1H in total), 6.19, 6.18 (each s, 1H in total) (HHDP and valoneoyl), most of the glucose signals as follows were assigned by ^{1}H–^{1}H COSY and TOCSY but the coupling constants for most of the signals were obscured due to overlapping; δ 4.82 (d, J=8.5 Hz, Glc-I, β-anomer H-1), 5.05 (Glc-I, β-anomer H-2), 5.60 (Glc-I, β-anomer H-3), 5.68 (Glc-I, β-anomer H-4), 3.15 (Glc-I, β-anomer H-5), 4.78 (Glc-I, β-anomer H-6), 3.75 (Glc-I, β-anomer H-6), 6.11, 6.10, 6.18, 6.17 (each d, J=8 Hz, H-1′), 5.17, 5.18, 5.26 (dd, J=8, 10 Hz, H-2′), 5.76, 5.75, 5.73 (each t, J=10 Hz, H-3′),

5.11 (t, J=10 Hz, H-4′), 4.63 (m, H-5′), 5.18, 3.75 (m, H-6′), 5.44, 5.41 (each d, J=3.5 Hz), 5.07, 5.06 (each d, J=8 Hz) (H-1″), 5.03, 5.02, 4.83 (H-2″), 5.60, 5.59, 5.16 (each t, J=10 Hz, H-3″), 5.46, 5.48 (each t, J=10 Hz, H-4″), 4.37, 3.81 (m, H-5″), 4.39, 4.57, 4.14, 4.19 (H-6″), the other sugar protons were obscured by overlapping).

Determination of Constituent Units of Nobotanins L (2), M (3) and N (4) A mixure of **2** (or **3**, **4**) (5 mg), Me_2SO_4 (5 ml) and K_2CO_3 (20 mg) in Me_2CO (5 ml) was stirred overnight at room temperature and then heated under reflux for 5 h. After removal of inorganic material by centrifugation, the supernatant was evaporated *in vacuo*. The residue was subjected to prep. TLC (silica-gel, $CHCl_3$–Me_2CO 10 : 1) to yield **5**, **6** and **7** which were identified with authentic samples by direct comparison of EI-MS, TLC and HPLC [normal phase; hexane–AcOEt (2 : 1)] data.

An aqueous solution of **2** (or **3**, **4**) (1 mg) in 5% H_2SO_4 (1 ml) was heated in a boiling-water bath for 4 h. After neutralization with Amberlite IR-45 ion-exchange resin (OH^- form), the filtrate was extracted with EtOAc. The aqueous layer was evaporated to dryness *in vacuo*. The trimethylsilyl derivative of the residue was analyzed by GLC (1% OV-1, column temp., 170 °C) indicating α- and β-anomers of glucose.

Partial Hydrolysis of Nobotanin C (1) A solution of nobotanin C (**1**) (60 mg) in H_2O (40 ml) was incubated with tannase (0.3 ml) prepared from *Aspergillus niger*[11] at 37 °C for 17 h. The reaction mixture was then concentrated and chromatographed over MCI gel CHP-20P with H_2O and aqueous MeOH to yield gallic acid (2.3 mg) and two degalloylated derivatives (**2**; 3 mg, **1a**; 9 mg) besides unreacted **1** (7 mg). Compound **2** was identified as natural nobotanin L by cochromatography on normal- and reversed-phase HPLC and ^{1}H-NMR spectral comparison.

Compound (**1a**): An off-white amorphous powder, $[\alpha]_D$ +64° (c=1.0, MeOH), FAB-MS *m/z*: 2527 $[M+Na]^+$. ^{1}H-NMR δ: 7.25, 7.24 (each s, 2H in total), 7.09, 7.08 (each s, 2H in total), 6.97, 6.96 (each s, 2H in total), 6.97, 6.96 (each s, 2H in total) (Gal), 7.11 (s, 1H), 7.00 (s, 1H), 6.69, 6.65 (each s, 1H in total), 6.53, 6.52 (each s, 1H in total), 6.47 (s, 1H), 6.46 (s, 1H), 6.45 (s, 1H), 6.43, 6.40 (each s, 1H in total), 6.17, 6.15 (each s, 1H in total), 6.12, 6.11 (each s, 1H in total) (HHDP, Val), glucose α-anomer; δ 6.14 (d, J=8 Hz, H-1), 5.15 (H-2), 5.41 (t, J=10 Hz, H-3), 5.81 (t, J=10 Hz, H-4), 3.49 (br d, J=10 Hz, H-5), 6.01 (d, J=8.5 Hz, H-1′), 5.14, 5.78 (H-2′, H-3′), 5.18 (t, J=10 Hz, H-4′), 4.58 (m, H-5′), 5.10, 3.78 (H-6′, coupling constants are not clear due to overlapping), 5.36 (d, J=3.5 Hz, H-1″), 4.97 (dd, J=3.5, 9.5 Hz, H-2″), 5.55 (t, J=9.5 Hz, H-3″), 5.38 (t, J=9.5 Hz, H-4″), 4.01 (m, H-5″): β-anomer; δ 6.15 (d, J=8 Hz, H-1), 5.07 (H-2), 5.40 (t, J=10 Hz, H-3), 5.81 (t, J=10 Hz, H-4), 3.49 (br d, J=10 Hz, H-5), 4.87 (br d, J=13 Hz, H-6), 3.85 (br d, J=13 Hz, H-6), 6.02 (d, J=8.5 Hz, H-1′), 5.14, 5.78 (H-2′, H-3′), 5.18 (t, J=10 Hz, H-4′), 4.58 (m, H-5′), 5.07, 3.78 (H-6′), 5.05 (d, J=8 Hz, H-1″), 4.76 (dd, J=8, 9.5 Hz, H-2″), 5.14 (t, J=9.5 Hz, H-3″), 5.79 (t, J=9.5 Hz, H-4″), 3.54 (m, H-5″), Glc-I α-anomer H-6 and Glc-III (α- and β-anomer) H-6″ signals overlapped with the H_2O signal (δ 3.4—3.9).

Nobotanin M (3) An off-white amorphous powder, $[\alpha]_D$ +76° (c=1.0, MeOH), *Anal.* Calcd for $C_{102}H_{74}O_{66}\cdot 20H_2O$: C, 45.10; H, 4.20. Found: C, 45.12; H, 4.23. FAB-MS *m/z*: 2377 $[M+Na]^+$, CD (c=0.01, MeOH): $[\theta]_{224}$ $+2.71\times10^5$, $[\theta]_{237}$ $+1.47\times10^5$, $[\theta]_{260}$ -1.00×10^5, $[\theta]_{281}$ $+5.35\times10^4$, $[\theta]_{313}$ 2.51×10^4. ^{1}H-NMR δ: 7.20, 7.19 (each s, 2H in total), 7.15, 7.14 (each s, 2H in total), 7.11, 7.10 (each s, 2H in total), 6.97, 6.96 (each s, 2H in total) (Gal), 7.05 (s, 1H), 6.99, 6.95 (each s, 1H in total), 6.66, 6.63 (each s, 1H in total), 6.53, 6.50 (each s, 1H in total), 6.45, 6.42 (each s, 1H in total), 6.40, 6.34 (each s, 1H In total), 6.22, 6.17 (each s, 1H in total), 6.13, 6.12 (each s, 1H in total) (HHDP, valoneoyl), glucose protons, see Table 1. ^{13}C-NMR δ: 95.6 (1C) (C-1), 73.7 (1C) (C-2), 75.0 (1C) (C-3), 70.8 (1C) (C-4), 73.0 (1C) (C-5), 63.5, 63.2, 63.0, 62.9 (each 1C, C-6, C-6′, C-6″), 92.1 (1C) (C-1′), 76.4, 76.3 (C-2′), 77.2 (1C) (C-3′), 69.4 (1C) (C-4′), 72.9, 72.8 (C-5′), 91.2, 94.7 (C-1″), 75.0, 77.7 (C-2″), 75.5, 77.1 (C-3″), 68.5, 68.2 (C-4″), 68.1, 72.7 (C-5″).

Partial Hydrolysis of Nobotanin M (3) An aqueous solution (20 ml) of **3** (19 mg) was heated in a boiling-water bath for 26 h. After concentration, the reaction products were separated by column chromatography over Sephadex LH-20 using EtOH and EtOH–MeOH (9 : 1→8 : 2→7 : 3→5 : 5→3 : 7). The EtOH eluate gave gallic acid (2.5 mg), ellagic acid (0.5 mg), 6-*O*-galloylglucose (**11**) (1 mg) and the hydrolysate (**10**) (1.5 mg). The hydrolysates (**9**) (1 mg) and (**8**) (1 mg) were obtained from the EtOH–MeOH solutions, (9 : 1) and (5 : 5), respectively. The identification of these compounds was confirmed by direct comparisons of their HPLC and NMR data with those of authentic specimens obtained from nobotanin C (**1**).[2b]

Nobotanin N (4) An off-white amorphous powder, $[\alpha]_D$ +67° (c=1.0, MeOH), *Anal.* Calcd for $C_{95}H_{70}O_{62}\cdot 23H_2O$: C, 43.58; H, 4.43. Found: C, 43.70; H, 4.43. FAB-MS: *m/z* 2225 $[M+Na]^+$. CD (c=0.01, MeOH): $[\theta]_{225}$ $+2.35\times10^5$, $[\theta]_{235}$ $+1.95\times10^5$, $[\theta]_{261}$ -9.56×10^4, $[\theta]_{283}$ $+2.39\times10^4$, $[\theta]_{310}$ 2.59×10^4. ^{1}H-NMR δ: 7.22, 7.21, 7.20, 7.19 (each s, 2H in total), 7.14, 7.13 (each s, 2H in total), 6.96, 6.95, 6.94 (each s, 2H in total) (Gal), 7.04 (s, 1H), 6.99, 6.96 (each s, 1H in total), 6.66, 6.62 (each s, 1H in total), 6.56, 6.53, 6.52, 6.50 (each s, 1H in total), 6.44, 6.43, 6.42, 6.41 (each s, 1H in total), 6.39, 6.34 (each s, 1H in total), 6.23 (s, 1H), 6.18, 6.17 (each s, 1H in total) (HHDP, valoneoyl), 4.44, 4.43 (each d, J=8 Hz, H-1), 5.65 (H-4), 4.78 (H-6), 3.77 (H-6), 3.2—3.6 (H-2, 3, 5, overlapped with DHO signal), 6.11, 6.13, 6.14, 6.15 (each d, J=8.5 Hz, H-1′), 5.26, 5.24, 5.20, 5.19 (H-2′), 5.64, 5.62, 5.59 (each t, J=10 Hz, H-3′), 5.17, 5.16, 5.12 (H-4′), 4.53 (m, H-5′), 5.18, 3.75 (H-6′), 5.43 (d, J=4 Hz), 5.05 (d, J=8.5 Hz) (H-1″), 5.02 (dd, J=4, 10 Hz), 4.80 (dd, J=8.5, 10 Hz) (H-2″), 5.57, 5.15 (each t, J=10 Hz, H-3″), 5.46, 5.48 (each t, J=10 Hz, H-4″), 4.36, 3.73 (m, H-5″), 4.50, 4.63 (m, H-6″), 4.11, 4.10, 4.17 (m, H-6″).

Partial Hydrolysis of Nobotanin N (4) An aqueous solution (1 ml) of **4** (1 mg) was refluxed on a hot plate for 2 h, and the reaction mixture was analyzed by normal- and reversed-phase HPLC showing the presence of **8**—**10**.

Enzymatic Hydrolysis of Nobotanin M (3) and N (4) An aqueous solution (5 ml) of **3** (7 mg) was incubated with tannase[11] at 37 °C for 4 h. The concentrated reaction mixture was chromatographed over MCI-Gel CHP-20P with H_2O and aq. MeOH to give gallic acid (2 mg) from the H_2O eluate and **4a** (2.3 mg) from the 40% MeOH eluate. **4a**: an off-white amorphous powder, $[\alpha]_D$ +60° (c=0.5, MeOH), ^{1}H-NMR: δ 6.15—7.20 (each s, 8H in total). FAB-MS *m/z*: 2103 $(M+Na)^+$. Compound **4a** was also detected on HPLC (normal and reversed-phases) of the reaction mixture obtained by similar treatment of **4**.

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References and Notes

1) Part VII: Yoshida T., Haba K., Arata R., Nakata F., Shingu T. Okuda T., *Chem. Pharm. Bull*., **43**, 1101—1106 (1995).
2) *a*) Yoshida T., Haba K., Ohbayashi H., Ishihara K, Ohwashi W., Okano, Y., Shingu T. Okuda T., *Chem. Pharm Bull*., **39**, 2233—2240 (1991); *b*) Yoshida T., Ohwashi W., Haba K., Ishihara K., Okano Y., Shingu T. Okuda T., *ibid*, **39**, 2264—2270 (1991).
3) Yoshida T., Haba K., Nakata F., Okano, Y., Shingu T. Okuda T., *Chem. Pharm. Bull.*, **40**, 66—71 (1992).
4) Yoshida T., Ikeda Y., Ohbayashi H., Ishihara K., Ohwashi W., Shingu T. Okuda T., *Chem. Pharm. Bull*., **34**, 2676—2679 (1986).
5) Yoshida T., Nakata F., Hosotani K., Nitta A. Okuda T., *Phytochemistry*, **31**, 2829—2833 (1992).
6) Yoshida T., Hatano T., Okuda T., *J. Chromatogr.*, **467**, 139—147 (1989).
7) Bate-Smith E. C., *Phytochemistry*, **11**, 1153—1156 (1972).
8) Okuda T., Yoshida T., Hatano T., *J. Nat. Prod*., **9**, 1—31 (1989).
9) Wilkins C. K., Bohm B. A., *Phytochemistry*, **15**, 211—214 (1976).
10) Okuda T., Yoshida T., Hatano T., Koga T., Toh N. Kuriyama K., *Tetrahedron Lett.*, **23**, 3937—3940 (1982).
11) Yoshida T., Tanaka K., Chen X.-M., Okuda T., *Chem. Pharm. Bull*., **37**, 920—924 (1989).