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TETRAMERIC AND PENTAMERIC ELLAGITANNINS FROM *MONOCHAETUM MULTIFLORUM*

José H. Isaza M., Hideyuki Ito, and Takashi Yoshida*

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

Abstract-Two new hydrolyzable tannins, nobotanin S (**1**) and melastoflorin A (**2**), were isolated from the leaves of *Monochaetum multiflorum*, and their structures were characterized as tetrameric and pentameric ellagitannins based on detailed NMR analyses using 2D-NMR techniques and chemical evidence. Melastoflorin A (pentamer) was notably the biggest molecule among numerous hydrolyzable tannins.

Among more than 500 ellagitannins hitherto characterized,[1] oligomeric ellagitannins with a molecular weight over 1500 constitute a unique class of natural polyphenols because of their diverse structures and biological properties including anti-HIV[2] and antitumor[3] activities. Melastomataceae, a pantropical family of flowering plants, is known to be rich in such oligomers.[1] In our continuing study of polyphenols in this plant family, we have isolated a novel pentameric ellagitannin along with a new tetramer from an aqueous acetone extract of the leaves of *Monochaetum multiflorum* (Bompl.) Naudin, a shrub endemic to Colombia. This communication describes the structure elucidation of these new tannins, named nobotanin S (**1**) (tetramer) and melastoflorin A (**2**) (pentamer).

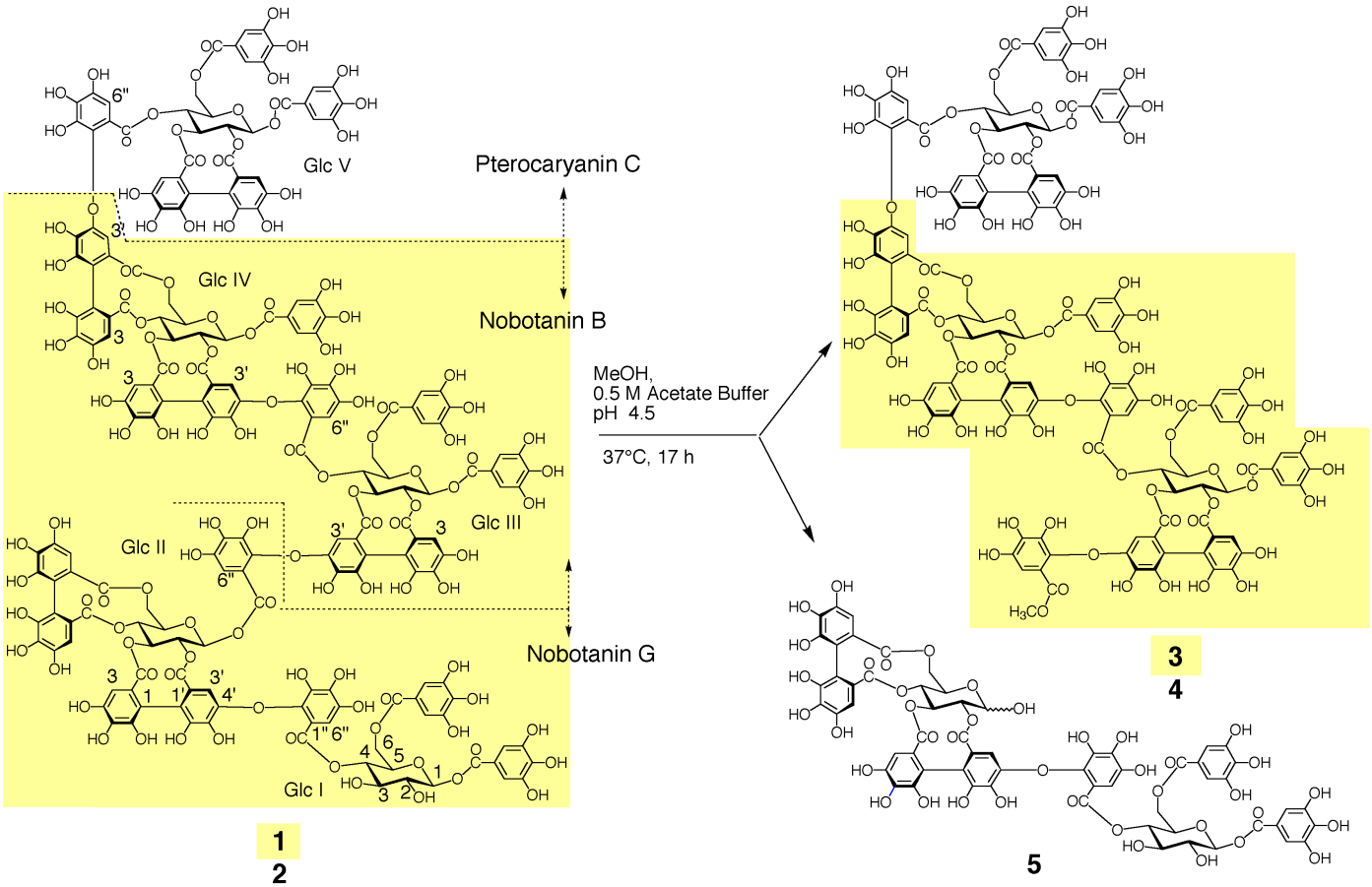
The isolation of the tannins from a water-soluble portion of the extract was achieved by a combination of column chromatography over Dia-ion HP-20, Toyopearl HW-40 (coarse) and/or YMC-GEL (ODS-AQ 120-S50) with aqueous MeOH (20%→60%) and MeOH-acetone-H2O (8:1:1→7:2:1→6:2:2→5:3:2). Both new tannins, which were clearly indicated to be oligomers by their large retention volumes in normal phase HPLC,[4] were characterized as ellagitannins composed of common constituent units [galloyl, hexahydroxydiphenoyl (HHDP), valoneoyl groups and glucose] based on their acid hydrolysis yielding gallic acid, ellagic acid, valoneic acid dilactone and glucose, as the other known nobotanins.[1] Although the glucose proton signals in the ^{1}H-NMR spectra were complicated by overlapping, especially around 5.0~5.2 ppm region, their full assignments as summarized in Table 1 were unambiguously achieved by a combination of ^{1}H-^{1}H COSY, total correlation spectroscopy (TOCSY), ^{1}H *J*-resolved and HMQC spectra.

The large vicinal coupling constants of the glucose signals clearly indicated that all the glucopyranose cores adopt a $^4C_1$ conformation. The absolute configurations of the chiral biphenyl moieties of the HHDP and valoneoyl units in these tannins were all (*S*) as evidenced by CD spectra showing strong positive Cotton effects around 227 nm ([θ] +6.5 x 10^5 for **1**; [θ] +9.1 x 10^5 for **2**) and 237 nm ([θ] +3.7 x 10^5 for **1**; [θ] +4.7 x 10^5 for **2**).[5]

The tetrameric nature of nobotanin S (**1**), an off-white amorphous powder, $[\alpha]_D$ +49.1˚ (MeOH), was suggested by its retention time on normal phase HPLC similar to that of nobotanin K (tetramer),[6] and verified from a pseudomolecular ion $[M+NH_4]^+$ peak at *m/z* 3458 in the electrospray ionization MS spectrometry (ESI-MS), which corresponds to molecular formula $C_{150}H_{104}O_{96}$. The tetrameric structure of **1** was also confirmed by the ^{1}H- and ^{13}C-NMR spectra (Table 1), which showed well-resolved four anomeric proton and carbon signals of the glucose moieties. The spectrum also exhibited five 2H-singlets (δ_H 7.24, 7.16, 7.11, 7.06, 6.95), and thirteen 1H-singlets [δ_H 7.04, 7.03, 6.98 {valoneoyl (Val) H-6"}, 6.59, 6.56, 6.51, 6.50 (HHDP H-3, 3'), 6.40, 6.37, 6.34 (Val H-3), 6.12, 6.09, 5.95 (Val H-3')] in the aromatic region, indicating the presence of five galloyl, two HHDP and three valoneoyl groups in the molecule.

A comparison of the glucose signals in the ^{1}H- and ^{13}C-NMR spectra of **1** with those of known tannins revealed that the resonances of the glucose cores were closely similar to those of nobotanin B[7] [glucose (Glc) III and IV] and nobotanin G[8] (Glc I and II), respectively. These spectral features implied that nobotanin S (**1**) is a tetramer, in which the above two dimers are linked to each other through a valoneoyl unit biogenetically formed by an oxidative coupling between an HHDP group of nobotanin B or G and a galloyl group of nobotanin G or B. The location and orientation of the valoneoyl group in **1** were clearly established from the following three-bond correlations between the aromatic proton—ester carbonyl carbon—glucose proton signals in the HMBC spectrum: δ_H 5.95 (Val H-3')—δ_C 169.2—δ_H 5.34 (GlcIII H-3) ; δ_H 6.40 (Val H-3) —δ_C 169.0 —δ_H 5.17 (GlcIII H-2) ; δ_H 6.98 (Val H-6")—δ_C 161.9—δ_H 5.88 (GlcII H-1). Assignment of the Val H-3' signal was based on a correlation with one of three downfield-shifted oxygen-bearing carbons (Val C-4') at δ_C146.7. The locations of the other two valoneoyl and two HHDP groups were also confirmed in a similar way. Based on these data, the structure of nobotanin S was established as **1**, which was chemically substantiated by mild methanolysis of **1** yielding malabathrin D (**3**)[9] and a dimeric metanolysate (**5**)[10].

The ^{1}H- and ^{13}C-NMR spectra of melastoflorin A (**2**) [an off-white amorphous powder, $[\alpha]_D$ +65.2˚ (MeOH), ESI-MS *m/z* 2206 $(M+2NH_4)^{2+}$], exhibited five anomeric signals at δ_H 5.67—6.11 and δ_C 91.7—95.4, which indicated its pentameric nature. The remaining glucose proton signals shown in Table 1 were also consistent with the presence of five $^4C_1$ glucopyranose residues. The ^{1}H-NMR spectrum of **2** also indicated the presence of seven galloyl, two HHDP and four valoneoyl groups as revealed by seven 2H-singlets [δ_H 7.26, 7.18, 7.13, 7.12 x 2, 7.08, 6.92 (galloyl H-2, 6)], and sixteen 1H-singlets [δ_H 7.076, 7.05, 7.03, 6.99 (Val H-6"), 6.58, 6.48, 6.433, 6.428 (HHDP H-3,3'), 6.51, 6.42, 6.41, 6.34 (Val H-3), 6.18, 6.14, 6.09, 5.96 (Val H-3')] in the



aromatic region. Among the glucose signals in the ^{1}H- and ^{13}C-NMR spectra of **2** (Table 1), those due to four glucose cores (Glc I~IV) could almost be superimposed on the corresponding resonances of nobotanin S (**1**), while the remaining glucose (Glc V) signals closely resembled those of a monomic ellagitannin, pterocaryanin C.[6] From these findings, melastoflorin A (**2**) was deduced to be a pentamer composed of **1** and pterocarianin C. The binding mode between the tetramer and monomer (i.e., location and orientation of the newly formed valoneoyl group) was clearly established by HMBC, which showed the correlations of δ_H 6.18 (Val H-3')—δ_C 168.9—δ_H 5.06 and 3.66 (Glc IV H-6) ; δ_H 6.51 (Val H-3)—δ_C168.2—δ_H 5.09 (Glc IV H-4); δ_H 7.05 (Val H-6")—δ_C 165.1—δ_H 5.51 (Glc V H-4). The other correlations confirming the connectivities of each acyl unit on the glucose residues (Glc I-IV) were also observed as in the case for **1**. Mild methanolysis of **2** afforded **5** and a trimeric methyl ester (**4**), which was identified as a product previously prepared from nobotanin K.[7] Based on these chemical and spectral evidence, the structure (**2**) was assigned to melastoflorin A.

Although approximately 200 oligomeric ellagitannins have been found in nature to date, most are dimers, and the numbers of trimers~tetramers are limited.[1] Although a sole pentamer, castaneanin D, from heartwood of the Japanese Chestnut tree, has been reported, [11] it is a condensate through C-C bond formations among five moles of a *C*-glucosidic tannin, castalagin. Melastoflorin A is thus the first example of a pentameric hydrolyzable tannin composed of different monomeric units with $^{4}C_{1}$ glucopyranose. The present study should prompt a further search for this class of higher oligomers in nature.

Table 1. ^{1}H-NMR [a] and ^{13}C-NMR [b] Data for the Glucose Moieties of Nobotanin S (**1**) and Melastoflorin A (**2**)

		1		**2**	
		δ_H	δ_C	δ_H	δ_C
Glc I	1	5.67 d (7.5)	95.4	5.67 d (8.0)	95.4
	2	3.64 dd (7.5, 10)	73.6	3.64 dd (8.0, 10)	73.6
	3	3.71 t (10)	74.8	3.70 t (10)	74.8
	4	5.36 t (10)	70.9	5.36 t (10)	70.8
	5	3.52 br d (10)	73.0	3.53 br d (10)	72.8
	6	4.64 d (12)	63.3	4.65 d (12.5)	63.3
		3.88 [c]		3.88 br d (12.5)	
Glc II	1	5.88 d (8.0)	91.7	5.89 d (8.5)	91.7
	2	5.04 dd (8.0, 9.0)	76.3	5.09 [d]	76.3
	3	5.51 dd (9.0, 10)	76.9	5.54 br t (10)	76.9
	4	4.97 t (10)	69.2	4.97 t (10)	69.2
	5	4.33 dd (6.5, 10)	72.8	4.38 dd (6.5, 10)	72.7
	6	5.11 dd (6.5, 13.5)	63.0	5.08 [d]	63.0
		3.65 [c]		3.67 br d (12.5)	
Glc III	1	6.00 d (8.0)	92.0	5.99 d (8.5)	91.9
	2	5.17 dd (8.0, 10)	75.1	5.17 dd (8.5, 10)	75.1
	3	5.34 t (10)	77.6	5.32 t (10)	77.6
	4	5.73 t (10)	66.6	5.73 t (10)	66.6
	5	3.39 br d (10)	73.6	3.38 [c]	73.7
	6	4.89 d (12.5)	63.1	4.89 d (12.5)	63.1
		3.68 [c]		3.69 dd (4.0, 12.5)	
Glc IV	1	6.10 d (8.5)	92.1	6.10 d (8.5)	92.0 [e]
	2	5.08 dd (8.5, 10)	76.7	5.07 dd (8.5, 10)	76.7
	3	5.79 t (10)	76.6	5.79 t (10)	76.4
	4	5.09 t (10)	69.4	5.09 t (10)	69.4
	5	4.57 dd (6.5, 10)	73.0	4.54 dd (6.0, 10)	72.9
	6	5.24 dd (6.5, 13)	63.2	5.06 dd (6.0, 12.5)	63.2
		3.88 [c]		3.66 br d (12.5)	
Glc V	1			6.11 d (8.5)	92.0 [e]
	2			5.11 dd (8.5, 10)	75.0
	3			5.30 t (10)	77.4
	4			5.51 t (10)	67.7
	5			4.04 br d (10)	73.1
	6			4.47 d (12.5)	62.5
				4.24 dd (4.0, 12.5)	

[a] 500 MHz in acetone-d_6 + D_2O (J in Hz).
[b] 126 MHz in acetone-d_6 + D_2O. Assignments were made by means of HMQC.
[c] Overlapped with HDO.
[d], [e] Overlapped.

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