Tannins and Related Compounds. LXXIX.[1] Isolation and Characterization of Novel Dimeric and Trimeric Hydrolyzable Tannins, Nuphrins C, D, E and F, from *Nuphar japonicum* DC.

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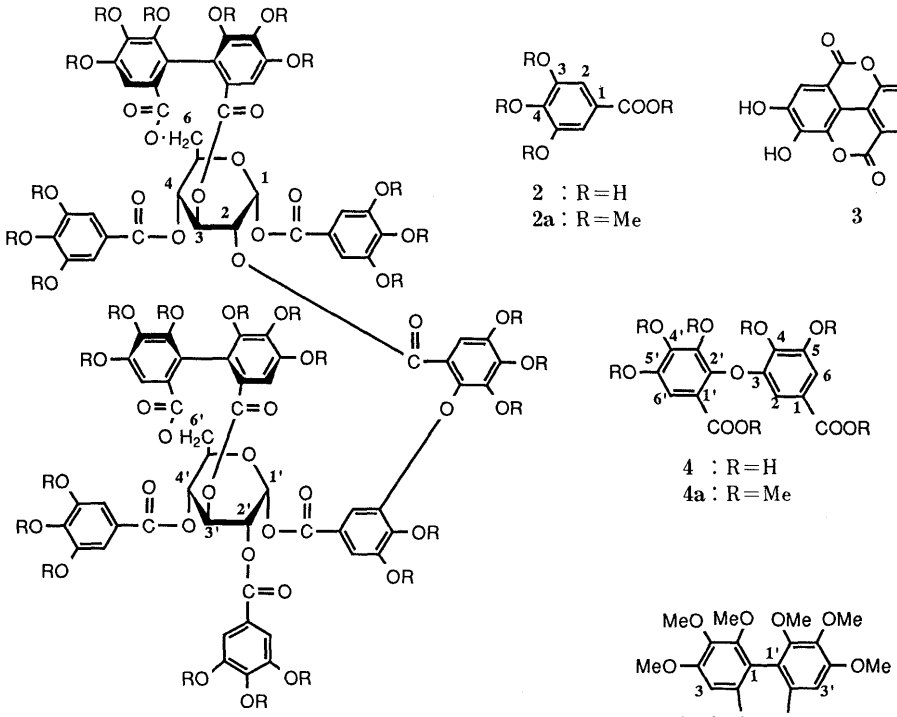
Four novel dimeric and trimeric hydrolyzable tannins, nupharins C (1), D (13), E (15) and F (16), which possess a less favorable α-D-glucopyranose core with an atropisomeric (*R*)- or (*S*)-hexahydroxydiphenoyl group at the 3,6-position, have been isolated from the rhizomes of *Nuphar japonicum* DC. (Nymphaeaceae). The structures of these compounds were established on the basis of spectroscopic and chemical evidence.

Keywords *Nuphar japonicum*; Nymphaeaceae; nupharin C—F; dimeric ellagitannin; trimeric ellagitannin; dehydrodigallic acid; α-D-glucose; *R*-, *S*-hexahydroxydiphenoic acid; atropisomerism; diastereomer

The so-called dimeric hydrolyzable tannins, which possess two glucopyranose cores in the molecule, have been widely found in members of the families Rosaceae,[3)] Fagaceae,[4)] Euphorbiaceae,[5)] Cornaceae,[6)] Nyssaceae,[7)] *etc.* Furthermore, some trimeric and tetrameric hydrolyzable tannins have been isolated from some species of these families.[3)] From their structural features, it has been deduced that these oligomeric hydrolyzable tannins are formed in the plant tissues by oxidative carbon-to-carbon or carbon-to-oxygen coupling(s) of aromatic rings in the corresponding monomeric hydrolyzable tannins.[8)] Recently, we reported the isolation of several hydrolyzable tannins (gallo- and ellagitannins), which contain an unusual α-D-glucopyranose core, from the rhizomes of *Nuphar japonicum* DC. (Nymphaeaceae).[9,10)] Among these tannins, the major ellagitannins, nupharins A (**6**) and B (**7**), were characterized as diastereoisomers differing only in the atropisomerism of the biphenoyl bond of the 3,6-positioned 4,4′,5,5′,6,6′-hexahydroxydiphenoyl ester group.[10)] Our subsequent chemical studies on tannins in this plant have now resulted in the isolation of three dimeric hydrolyzable tannins, named nupharins C (**1**), D (**13**) and E (**15**), and a trimeric hydrolyzable tannin, nupharin F (**16**). We wish to describe herein details of the isolation and structural elucidation of these compounds.

The extraction from the freeze-dried plant materials with aqueous acetone and fractionation by Sephadex LH-20 column chromatography as described in the preceding paper[10)] afforded seven fractions. Among these fractions, fraction 6 was subjected to a combination of column chromatographies over Sephadex LH-20, MCI-gel CHP 20P and Fuji-gel ODS G3 with various elution systems to yield nupharins C (**1**), D (**13**) and E (**15**), while the most polar fraction (fr. 7) yielded nupharin F (**16**) on similar separation.

Nupharin C (**1**) was characterized as an ellagitannin on



1 : R=H
1a : R=Me

2 : R=H
2a : R=Me

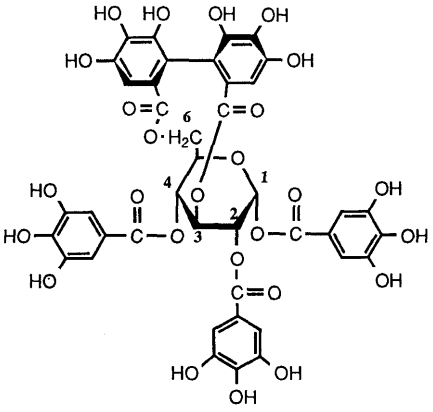
3

4 : R=H
4a : R=Me

5

the basis of its reddish brown coloration with the sodium nitrite–acetic acid reagent.[11] The proton-nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed two lowfield doublets at δ 6.57 (J=2 Hz) and 6.60 (J=3 Hz) arising from anomeric protons, suggesting the presence of two sugar moieties in the molecule. This was supported by the observation of twelve resonances due to sugar carbons in the aliphatic region of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum and also by the prominent $(M+H)^+$ peak at m/z 1875 in the fast atom bombardment mass spectrum (FAB-MS). The ^{1}H-NMR spectrum showed four two-proton singlets at δ 7.12, 7.14, 7.22 and 7.33, each assignable to a galloyl group, five one-proton singlets at δ 6.78, 6.89, 6.93, 7.07 and 7.29 and two *meta*-coupled doublets at δ 6.76 and 7.12 (J=2 Hz) in the aromatic region. Acid hydrolysis with sulfuric acid gave gallic acid (**2**), ellagic acid (**3**), dehydrodigallic acid (**4**)[12] and glucose. Consequently, the above one-proton aromatic signals were attributable to the protons of two hexahydroxydiphenoyl and a dehydrodigalloyl ester group, respectively. Furthermore, methanolysis of the methyl ether (**1a**) with 2% methanolic sodium methoxide afforded methyl trimethoxybenzoate (**2a**), dimethyl penta-*O*-methyldehydrodigallate (**4a**) and dimethyl hexamethoxydiphenoate (**5**). The optically inactive nature of **5** ($[\alpha]_D$ 0°, $CHCl_3$) indicated that **5** consists of an equimolar mixture of (*R*)- and (*S*)-atropisomers,[13] and that the (*R*)- and (*S*)-hexahydroxydiphenoyl groups therefore each exist in the molecule of **1**.

Comparisons of the ^{1}H- and ^{13}C-NMR spectra of **1** with those of nupharins A (**6**) and B (**7**) revealed the close structural relationship among these tannins. Namely, in the ^{1}H-NMR spectrum of **1**, the chemical shifts and coupling patterns of signals arising from one of two glucose moieties were closely correlated to those of **6**, whereas the remaining signals were almost identical to those of **7** (Table I). Similarly, the chemical shifts of six (δ 65.2, 70.0, 73.4, 75.4, 75.4, 77.9 and 89.6) of twelve sugar signals in the ^{13}C-NMR spectrum of **1** were almost in line with those of **6** and the remaining six signals (δ 59.9, 63.3, 67.7, 69.7, 75.5 and 86.9) corresponded to those of **7** (Table III). These findings suggested that **1** is a dimeric ellagitannin consisting of the moieties of **6** and **7** which are linked *via* the dehydrodigalloyl ester bond. Furthermore, the relatively lowfield shift (+0.3 ppm) of the glucose C-1′ signal as compared with



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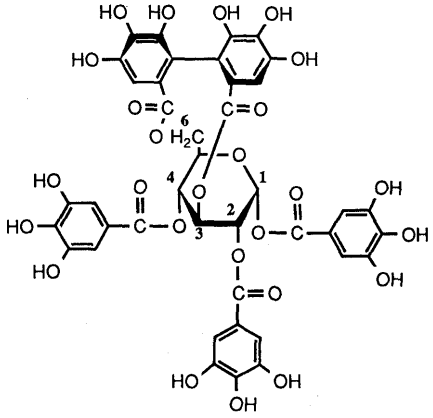

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TABLE I. ^{1}H-NMR Data for Compounds **1**, **6**, **7**, **9** and **10** (δ Values, J Values in Hz)

	1[a]	**6**[b]	**7**[b]	**9**[c]	**10**[c]
Glucose moieties					
H-1	6.57 (d, J=3)	6.69 (d, J=3)		6.34 (d, J=3)	6.59 (d, J=3)
H-2	5.41 (dd, J=3, 8)	5.50 (dd, J=3, 8)		4.27 (dd, J=3, 8)	5.36 (dd, J=3, 8)
H-3	5.56 (d, J=8)	5.71 (d, J=8)		5.79 (d, J=8)	5.44 (d, J=8)
H-4	5.04 (s)	5.10 (s)		4.96 (s)	4.97 (s)
H-5	4.33 (br s)	4.39 (br s)		4.24 (br s)	4.48 (br s)
H-6	5.24 (d, J=12)	5.32 (d, J=13)		5.15 (dd, J=2, 11)	5.22 (d, J=12)
	3.98 (dd, J=2, 12)	3.90 (d, J=13)		4.01 (dd, J=2, 11)	3.90 (d, J=12)
H-1′	6.60 (d, J=2)		6.63 (d, J=2)		
H-2′	5.52 (t-like, J=2)		5.61 (t, J=2)		
H-3′	5.25 (t-like, J=2)		5.26 (t, J=2)		
H-4′	5.56 (d, J=2)		5.71 (d, J=2)		
H-5′	4.86 (t, J=10)		4.8—4.9 (m)		
H-6′	4.78 (t, J=10)		4.8—4.9 (m)		
	4.33 (t, J=10)		4.23 (t, J=10)		
Galloyl-H	7.12, 7.14, 7.22, 7.33	6.96, 7.06, 7.24	7.11 (×2), 7.31	7.09, 7.30	7.08, 7.24
Hexahydroxydiphenoyl-H	6.78, 6.89, 7.07, 7.29	7.15, 7.25	6.81, 6.98	7.14, 7.31	7.10, 7.28
Dehydrodigalloyl-H	6.93 (s)				6.78 (s)
	6.78, 7.12 (d, J=2)				6.89, 7.24 (d, J=2)

a) Measured at 270 MHz in acetone-d_6 with tetramethylsilane (TMS) as an internal standard. *b*) Measured at 100 MHz in acetone-d_6. *c*) Measured at 100 MHz in acetone-d_6+D_2O.

that of **7** implied the dehydrodigalloyl group to be located at this position.

To allocate each acyl group in the glucose moieties, we attempted partial hydrolysis. When heated in water, **1** afforded a complex mixture of products, which were separated by repeated chromatographies over Sephadex LH-20 and various reversed-phase gels to afford five partial hydrolysates, of which three were identified as nupharin B (**7**), 3,6-(*R*)-hexahydroxydiphenoyl-D-glucose (**8**)[14] and nupharin D (**13**) by comparisons of physical and spectroscopic data with those of authentic samples. The generation of **7** could be interpreted in terms of the facile fission of the ether linkage in the dehydrodigalloyl group.[15] The fourth product was characterized as 2-desgalloyl nupharin A (**9**) based on the fact that the ^{1}H-NMR spectra of the product and nupharin A (**6**) showed close similarities in the aromatic and sugar regions, except for the lack of a galloyl singlet and the remarkable upfield shift of the H-2 signal (-1.23 ppm) in the product. The ^{1}H-NMR spectrum of the fifth compound was also closely correlated with that of **6**, except for the appearance of an one-proton aromatic singlet at δ 6.87 and two *meta*-coupled doublets at δ 6.86 and 7.24 ($J=2$ Hz) instead of one galloyl signal. This fact suggested that this compound has a dehydrodigalloyl group in place of a galloyl group. Further support for the structure (**10**) was obtained by FAB-MS analysis, which exhibited a prominent $(M+H)^+$ peak at m/z 1107, consistent with the above observations. The location of the dehydrodigalloyl group was concluded to be at the C-2 position of the glucose moiety on the basis of the lowfield shift ($+0.4$ ppm) of the C-2 signal in the ^{13}C-NMR spectrum as compared with that of **6**. The formation of **10** was consistent with the concomitant isolation of **9** from the reaction mixture. The orientation of the dehydrodigalloyl group in **10** was determined in the following manner. The ^{13}C-NMR spectrum of **10** showed six carboxyl carbon resonances. Among them, the isolated signal appearing at the lowest field (δ 171.0) was assignable to a carboxylic acid carbon of the dehydrodigalloyl moiety. Since this signal appeared as a triplet in the off-resonance spectrum measured in the nuclear Overhauser effect (NOE) mode, indicating the correlation of this carbon and two aromatic protons through three-bond couplings, the orientation of the dehydrodigalloyl group was determined to be as shown in formula **10**.

Partial hydrolysis of **1** with tannase afforded compound **11**, together with several structurally unknown hydrolysates. The ^{1}H-NMR spectrum of **11** showed duplicated sugar signals because of the occurrence of α- and β-anomers. The ^{13}C-NMR spectrum exhibited six ester carbon signals, as well as the signals at δ 143.0 and 147.6, indicative of the presence of a dehydrodigalloyl group. In the aliphatic region, six major peaks were closely correlated with those of **6**, whereas the remaining complicated signal pattern was rather consistent with those observed in the

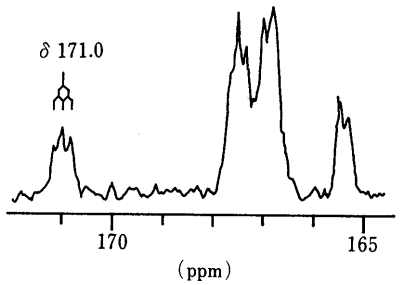
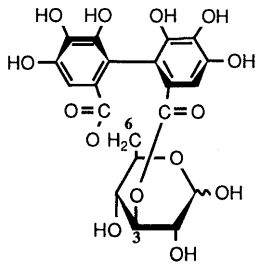


Fig. 1. The Off-Resonance ^{13}C-NMR Spectrum of Compound **10**



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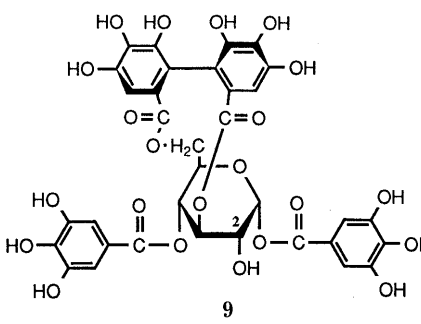

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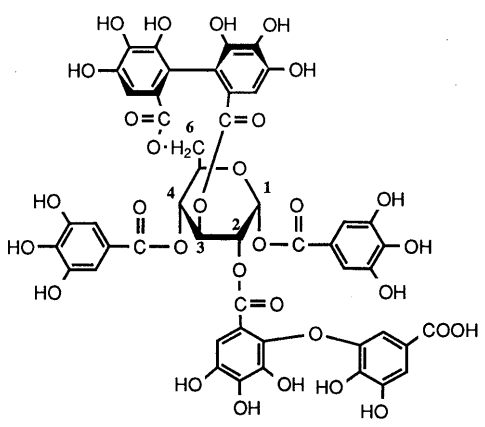

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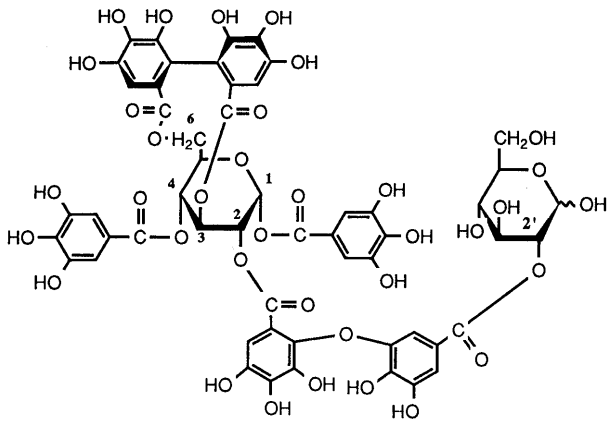

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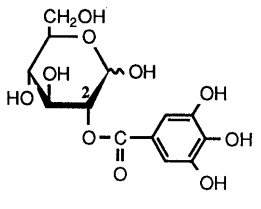


12

spectrum of 2-*O*-galloyl-D-glucose (**12**)[16] (Fig. 2). From these observations, the structure **11** was considered for this compound. On the other hand, the formation of **11** seemed to be inconsistent with the above-mentioned finding that the dehydrodigalloyl group is located at the C-1′ hydroxyl group (in the nupharin B moiety). This discrepancy could however be explained by the facile acyl migration[3,17] of the dehydrodigalloyl group to the C-2 hydroxyl group from the α-position with respect to the anomeric center. Furthermore, since the relatively prominent fragment ion peaks at *m/z* 769 and 1705 in the FAB-MS of **1** were considered to be formed by the cleavage of the galloyl and dehydrodigalloyl groups at the anomeric centers (Chart 1), the location of the dehydrodigalloyl group should be at one of the anomeric positions.

On the basis of these results, the structure of nupharin C was determined to be as shown in formula **1**.

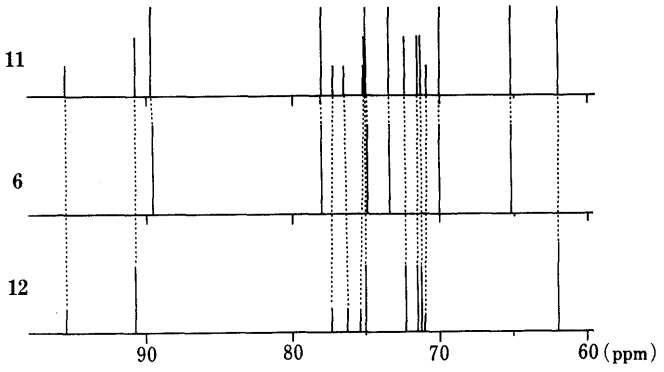


Fig. 2. The ^{13}C-NMR Spectra of Compounds **11**,[a] **6**[b] and **12**[a]
a) Measured at 25.05 MHz in acetone-d_6+D_2O. *b*) Measured in acetone-d_6.

The ^{1}H-NMR spectrum of nupharin D (**13**) showed the presence of four galloyl groups, one hexahydroxydiphenoyl group and one dehydrodigalloyl ester group, together with two sugar moieties (Table II). Acid hydrolysis of **13** gave gallic acid (**2**), ellagic acid (**3**), and dehydrodigallic acid (**4**) as acid components and glucose. In the ^{13}C-NMR spectrum, the chemical shifts of six (δ 59.9, 63.2, 67.6, 69.8, 75.4 and 86.6) of twelve aliphatic carbon signals were similar to those of **7**, and the remaining signals (δ 61.4, 69.8, 71.8, 73.4, 73.9 and 90.3) were found to be closely related to those of 1,2,4-tri-*O*-galloyl-α-D-glucose (**14**).[9] Furthermore, the ^{1}H-NMR spectrum of **13** exhibited sugar proton signals which were almost identical with the combined signal patterns of **7** and **14**. Accordingly, **13** was considered to consist of these hydrolyzable tannin units. This was supported by the FAB-MS of **13** which showed an intense $(M+H)^+$ peak at *m/z* 1573. Further chemical evidence was obtained by partial hydrolysis of **1** with hot water, which afforded **13** as the major hydrolysate. From these results, the structure **13** was assigned to nupharin D.

The FAB-MS of nupharin E (**15**) exhibited a prominent $(M+H)^+$ ion peak at *m/z* 1875, identical with that of **1**. The presence of four galloyl groups, two hexahydroxydiphenoyl groups and one dehydrodigalloyl group in the molecule was shown by similar ^{1}H-NMR analysis (Table II). The appearance of two doublets at δ 6.57 (2H, J=2 Hz) attributable to anomeric protons suggested the occurrence of two sugar moieties. The acid and sugar components were confirmed by acid hydrolysis, which afforded **2**, **3**, **4** and glucose. On the other hand, alkaline methanolysis of the methyl ether (**15a**) yielded **2a**, **4a** and optically active dimethyl hexamethoxydiphenoate (**15b**) ($[\alpha]_D$ +24.6°,

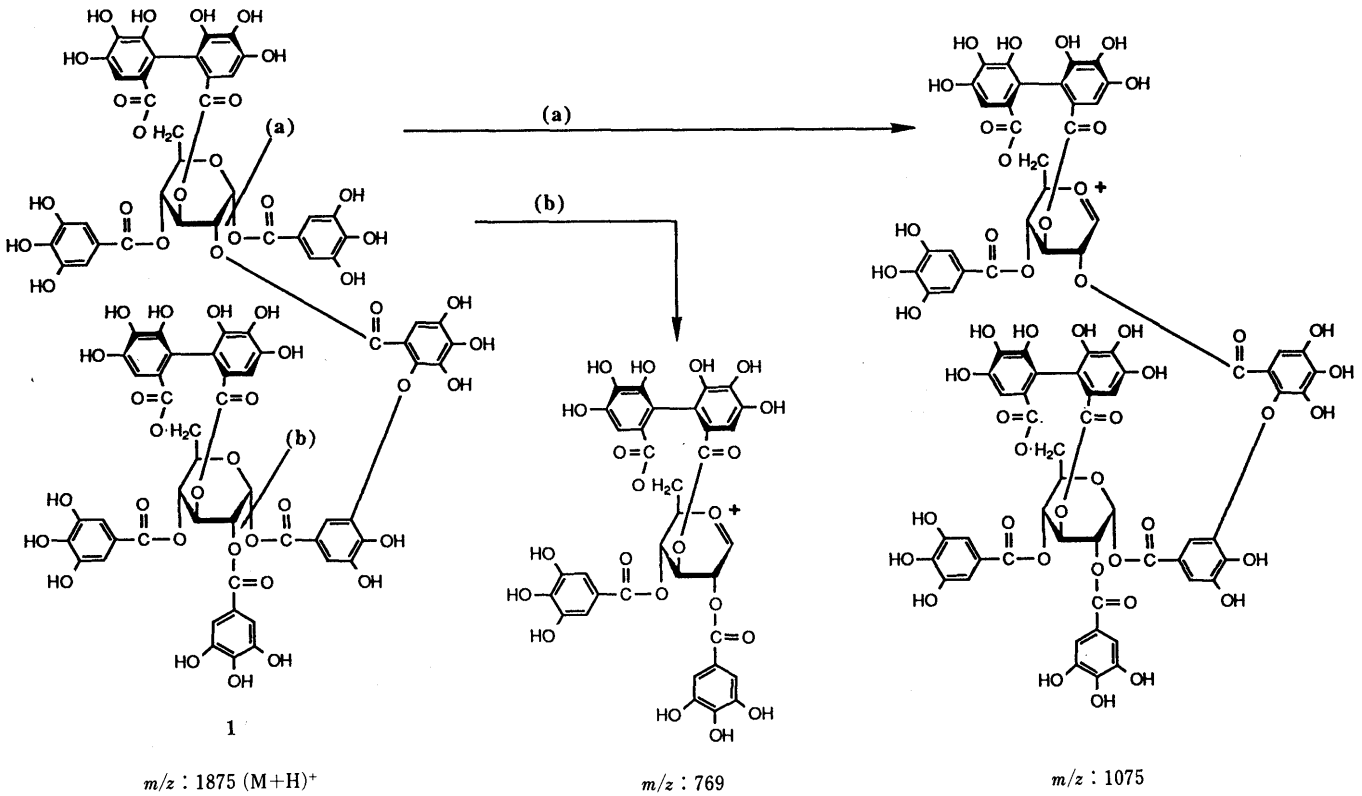
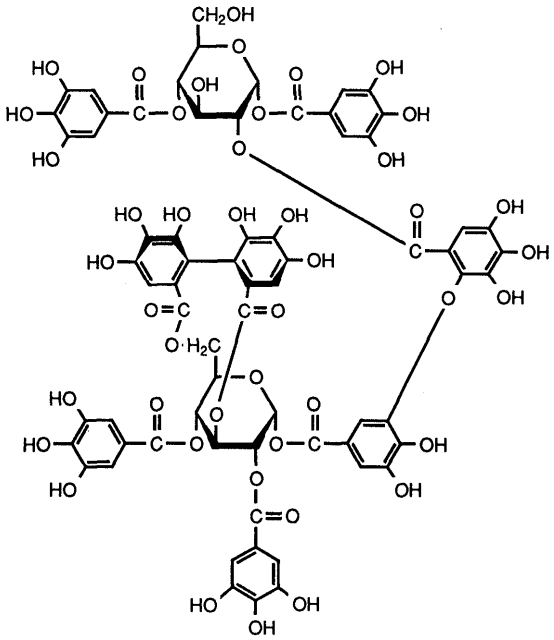


Chart 1. The FAB-MS Fragmentation of **1**



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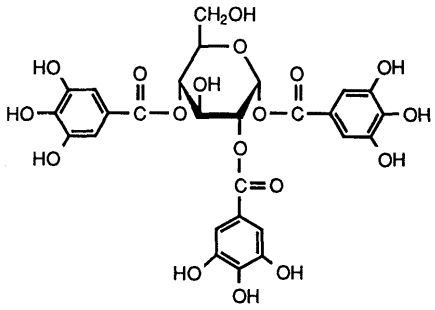

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TABLE II. ^{1}H-NMR Data for Compounds **13**—**16** (δ Values, J Values in Hz)[a]

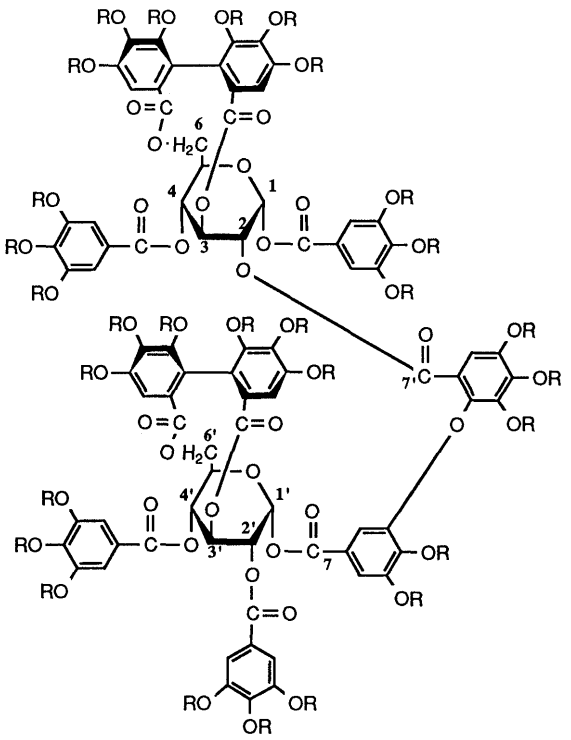
	14[b]	**13**	**15**	**16**
Glucose moieties				
H-1	6.59 (d, J=4)	6.49 (d, J=4)	6.57 (d, J=2)	6.55 (d, J=3)
H-2	5.19 (dd, J=4, 9)	5.53 (dd, J=4, 10)	5.38 (t-like, J=2)	5.43 (dd, J=3, 8)
H-3	4.59 (t, J=9)	4.47 (t, J=10)	5.16 (t-like, J=2)	5.61 (d, J=8)
H-4	5.29 (t, J=9)	5.25 (t, J=10)	5.64 (d, J=2)	5.02 (s)
H-5	4.33 (br s)	4.10 (dt, J=10, 2)	4.7—4.9 (m)	4.28—4.40 (m)
H-6	3.66 (2H, d, J=2)	3.62 (2H, br s)	4.7—4.9 (m)	5.22 (d, J=11)
			4.30 (t, J=10)	4.01 (dd, J=2, 11)
H-1′		6.56 (d, J=2)	6.57 (d, J=2)	6.52 (d, J=2)
H-2′		5.50 (t-like, J=2)	5.48 (t-like, J=2)	5.37 (t-like, J=2)
H-3′		5.21 (t-like, J=2)	5.21 (t-like, J=2)	5.21 (t-like, J=2)
H-4′		5.67 (d, J=2)	5.66 (d, J=2)	5.62 (d, J=2)
H-5′		4.83 (dd, J=8, 10)	4.7—4.9 (m)	4.7—4.9 (m)
H-6′		4.78 (t, J=10)	4.7—4.9 (m)	4.7—4.9 (m)
		4.30 (dd, J=8, 10)	4.30 (t, J=10)	4.28—4.40 (m)
H-1″				6.60 (d, J=2)
H-2″				5.48 (t-like, J=2)
H-3″				5.24 (t-like, J=2)
H-4″				5.66 (d, J=2)
H-5″				4.7—4.9 (m)
H-6″				4.7—4.9 (m)
				4.28—4.40 (m)
Galloyl-H	7.10, 7.19, 72.0	7.11, 7.19 (×2), 7.30	7.09, 7.10, 7.11, 7.29	7.08, 7.10, 7.14, 7.21, 7.31
Hexahydroxydiphenoyl-H		6.77, 6.91	6.76, 6.78, 6.90, 6.98	6.80, 6.81, 6.90, 6.98, 7.09, 7.27
Dehydrodigalloyl-H		7.07 (s)	7.45 (s)	6.94, 7.44 (each s)
		6.80, 7.10 (d, J=2)	6.88, 7.13 (d, J=2)	6.71, 6.87, 7.16, 7.22 (d, J=2)

a) Measured at 270 MHz in acetone-d_6 with TMS as an internal standard. b) Measured at 100 MHz in acetone-d_6.

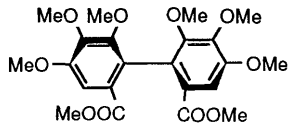
$CHCl_3$).[12] The positive sign of the specific optical rotation of **15b** indicated that the chirality of the two hexahydroxydiphenoyl groups is *R*.

The ^{13}C-NMR spectrum (Table III) of **15** exhibited five two-carbon peaks at δ 59.9, 63.2, 69.6, 75.3 and 86.6 due to glucose C-6, C-4, C-3, C-5 and C-1 carbons and two one-carbon peaks at δ 67.3 and 67.6 assignable to the C-2 carbons. The chemical shifts of these signals were found to be in good agreement with those in **7**, except for the upfield shift (−0.4 ppm) of one of the C-2 signals. This observation indicated that **15** is a dimeric ellagitannin which consists of two molecules of **7**. This was further supported by analysis of the ^{1}H-NMR spectrum of **15**, which was strikingly similar to that of **7** in the sugar region. The upfield shift of the C-2 signal suggested that the dehydrodigalloyl group is located at this position.

To confirm the location of the dehydrodigalloyl group in the molecule, a long-range selective proton-decoupling (LSPD) experiment was performed. The ^{13}C-NMR spectrum of **15** showed ten ester carbonyl signals, of which



15 : R=H
15a : R=Me



15b

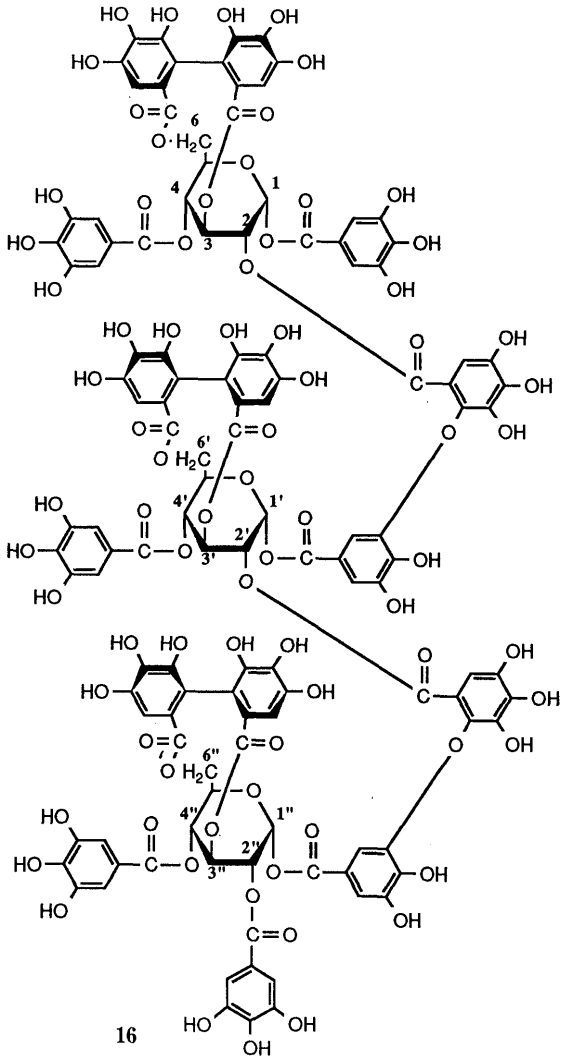


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TABLE III. ^{13}C-NMR Data for Compound **1**, **6**, **7**, **10**, **13**, **14**, **15** and **16** (δ Values)

	Glucose					
	C-1	C-2	C-3	C-4	C-5	C-6
Monomers						
14[a]	90.4	72.1	73.4	70.3	74.3	61.9
6[a]	89.5	70.0	73.5	74.9	77.9	65.1
10[b]	89.6	70.4	73.5	74.8	77.9	65.0
7[a]	86.6	67.7	70.1	63.3	75.4	60.2
Dimers						
1[a]	89.6	70.0	73.4	75.4	77.9	65.2
	86.9	67.7	69.6	63.3	75.4	59.9
13[b]	90.3	71.8	73.4	69.8	73.9	61.4
	86.6	67.6	69.8	63.2	75.4	59.9
15[b]	86.6	67.3	69.6	63.2	75.3	59.9
	(2C)	67.7	(2C)	(2C)	(2C)	(2C)
Trimers						
16[b]	89.8	69.9	73.4	75.3	77.9	65.3
	86.7	67.3	69.7	63.2	75.3	59.9
	(2C)	67.7	(2C)	(2C)	(2C)	(2C)

a) Measured at 25.05 MHz in acetone-d_6 with TMS as an internal standard. *b*) Measured in acetone-d_6 + D_2O.

the signals at δ 164.2 and 165.3 were unambiguously assigned to C-7 and C-7′[18] esters of the dehydrodigalloyl group, respectively, by means of ^{1}H–^{13}C long-range shift correlation spectroscopy[19]; the signal at δ 164.2 was shown to be coupled with the aromatic singlet at δ 7.45 in the dehydrodigalloyl moiety, while the signal at δ 165.3 was coupled with the two *meta*-coupled doublets at δ 6.88 and 7.13. In the non-decoupling mode, the C-7 signal (δ 164.2) was observed as a triplet, and this signal was changed into a doublet when irradiated at δ 5.38 (H-2). Therefore, the C-7 ester could be concluded to be located at the glucose C-2 position. On the other hand, the C-7′ signal (δ 165.3) was changed from a multiplet into a sharp triplet on irradiation at δ 6.57 (H-1 and H-1′), concomitantly with a change of a signal at δ 165.5 due to a galloyl ester carbon located at the glucose C-1 position. Accordingly, the location of the C-7′ ester was shown to be at the glucose C-1′ position. Based on these findings, the structure of nupharin E was established to be as shown in **15**.

Nupharin F (**16**) was strongly adsorbed on Sephadex LH-20 gels and was eluted with a mixture of acetone and water. This chromatographic behavior suggested that **16** possesses a larger molecular weight than those of dimeric ellagitannins. The coloration with the sodium nitrite–acetic acid reagent and the results of acid hydrolysis were similar to those of **1**, **13** and **15**. The ^{1}H-NMR spectrum of **16** clearly showed three anomeric proton signals at δ 6.52 (d, J=2 Hz), 6.55 (d, J=3 Hz) and 6.60 (d, J=2 Hz), the chemical shifts of which indicated that the sugar C-1

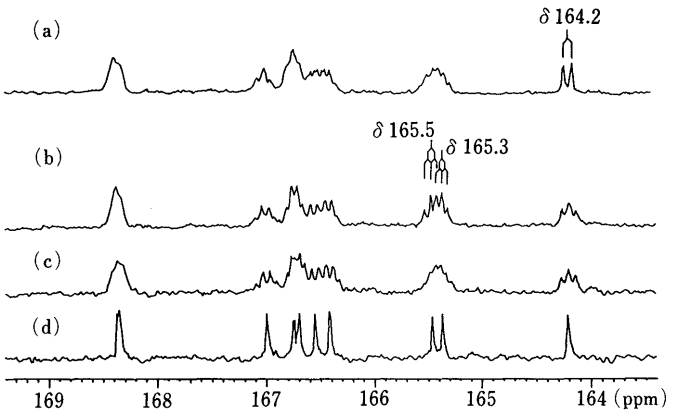

Fig. 3. Long-Range Selective Proton-Decoupling (LSPD) Experiment in **15**

a) Irradiated at δ 5.38. b) Irradiated at δ 6.57. c) Non-decoupling. d) Decoupling.

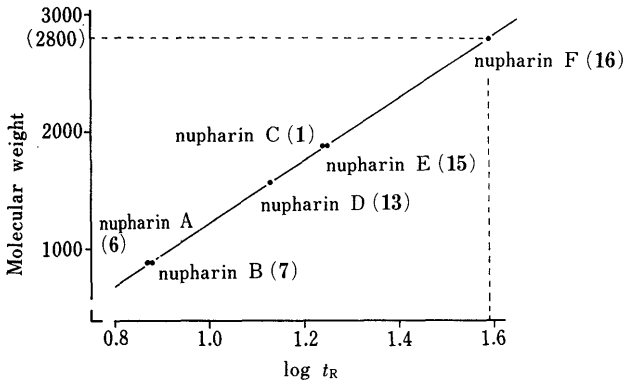

Fig. 4. Correlation between Retention Times and Moleculalr Weights of Nupharins A—F

positions were all acylated. The ^{13}C-NMR spectrum exhibited eighteen aliphatic resonances corresponding to three sugar moieties. In the ^{1}H-NMR spectrum, the appearance of five two proton singlets at δ 7.08, 7.10, 7.14, 7.21 and 7.31 suggested the presence of five galloyl groups, and the remaining aromatic resonances consisting of two pairs of *meta*-coupled doublets and eight one-proton singlets implied the occurrence of two dehydrodigalloyl and three hexahydroxydiphenoyl groups. These findings suggested that **16** is a trimeric ellagitannin having a molecular weight of 2810. Conformation of the molecular weight was provided by normal-phase high-performance liquid chromatographic (HPLC) analyses[3] as follows. When the retention times of nupharins A—E (**6**, **7**, **1**, **13** and **15**) were plotted on a semilogarithmic scale against their molecular weights, a straight line was obtained as shown in Fig. 4. Based on the retention time, the molecular weight of **16** was shown to be approximately 2800, in good agreement with the above calculation.

In the ^{13}C-NMR spectrum of **16**, signals arising from one of the three sugar moieties were in good agreement with those of **6**, whereas the remaining signals were analogous to those of **15**. Furthermore, the hexahydroxydiphenoyl C-1 and C-1′ resonances at δ 115.8 and 116.5 were characteristic of a 3,6-(*S*)-hexahydroxydiphenoyl-α-glucose moiety as found in the case of **6**, and the two-carbon signals at δ 117.8 and 118.2 were analogous to those of **7** having a 3,6-(*R*)-hexahydroxydiphenoylglucose moiety. These findings indicated that **16** is a trimeric ellagitannin consisting of one molecule of **6** and two of **7**. In addition, the ^{1}H-NMR signals of **16** were closely correlated with the combined signal patterns of nupharins A (**6**) and E (**15**) in the sugar region, and the chemical shifts of the dehydrodigalloyl protons were also in good agreement with those of **1** and **15**. From these observations, it was concluded that the locations of the two dehydrodigalloyl groups were similar to those in **1** and **15**. On the basis of these results, the structure **16** was assigned to the trimeric tannin, nupharin F.

TABLE IV. Relention Times and Calculated Molecular Weights of Nupharins A—F (**6**, **7**, **13**, **15** and **16**)

Nupharin	t_R (min)	log t_R	Molecular weight
A (**6**)	7.5	0.87	938
B (**7**)	7.6	0.88	938
C (**1**)	17.0	1.24	1874
D (**13**)	13.4	1.12	1572
E (**15**)	17.8	1.25	1874
F (**16**)	38.6	1.59	(2810)

Column, Zorbax SIL (4.6 i.d. × 250 mm); mobile phase, *n*-hexane–methanol–tetrahydrofuran–formic acid (50 : 30 : 10 : 1) containing oxalic acid (500 mg/l); flow rate, 1.2 ml/min; temperature, 25 °C.

Nupharins C—F represent the first examples of dimeric and trimeric hydrolyzable tannins which possess only α-glucopyranose cores. Their structural features suggested that these tannins were derived from monomeric hydrolyzable tannin units, **6**, **7** and **14**, by intermolecular oxidative coupling (s) between the galloyl groups located at the C-2 position of the upper unit glucose and the other galloyl group at the α-anomeric position of the lower unit. It is interesting from the viewpoint of plant physiology that the tannins in *N. japonicum* consist of less favorable α-glucopyranose core(s) with atropisomeric (*R*)- and (*S*)-hexahydroxydiphenoyl groups at the 3,6-positions.

Experimental

The instruments and chromatographic conditions used throughout this work were the same as described in the preceding paper,[10] except that HPLC was coducted with a Toyo Soda CCPM solvent delivery system and a UV-8000 spectrophotometer.

Isolation of Nupharins C(1), D(13), E(15) and F(16) Fraction 6 (19.0 g), which was previously obtained from the the 80% aqueous acetone extract by Sephadex LH-20 chromatography,[10] was chromatographed over Sephadex LH-20 with a solvent system of ethanol–water–acetone[20] to provide two fractions. The first fraction (630 mg) was rechromatographed over MCI-gel CHP 20P with water containing increasing amounts of methanol to yield **13** (140 mg). The second fraction (14.3 g) was subjected to repeated chromatographies over MCI-gel CHP 20P and Fuji-gel ODS G3 with a mixture of water and methanol (2 : 8—4 : 6) to yield **1** (7.14 g) and **15** (1.23 g). On similar separation, fr. 7 (14.7 g) yielded **16** (171 mg).

Nupharin C (1) An off-white amorphous powder, $[\alpha]_D^{24}$ +19.9° (c = 1.2, acetone). *Anal.* Calcd for $C_{82}H_{58}O_{52}\cdot 2H_2O$: C, 51.77; H, 3.21. Found: C, 52.11; H, 3.27. FAB-MS (methanol–glycerol) *m/z*: 1875 $(M+H)^+$, 1705, 769. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6)δ: 108.5, 108.6, 109.7 (2C) (DHDG[21] C-2, 6′, *R*-HHDP[21] C-3, 3′), 110.5 (4C), 110.7 (2C), 110.0 (2C) (galloyl C-2, 6), 109.7, 112.4, 112.6 (DHDG C-6, *S*-HHDP C-3, 3′), 113.7 (DHDG C-1′), 115.9, 116.6 (*R*-HHDP C-1, 1′), 117.5, 118.1 (*S*-HHDP C-1, 1′), 119.5 (DHDG C-1), 120.2 (4C) (galloyl C-1), 120.2, 124.6, 124.7, 124.8 (HHDP C-2, 2′), 136.8, 137.1, 137.8 138.8 (DHDG C-4′, HHDP C-5, 5′), 139.5, 139.9, 140.5, 140.7, 141.0 (galloyl C-4, DHDG C-2′, 3′, 4′), 143.0 (DHDG C-4′), 144.2, 144.4, 144.6, 144.9, 145.0, 145.8, 146.0 (galloyl C-3, 5, DHDG C-5, HHDP C-4, 4′, 6, 6′), 147.8 (DHDG C-3), 163.8, 165.3, 166.4 (2C), 166.5, 166.8, 167.3, 167.5, 168.3 (–COO–).

Methylation of 1, Followed by Alkaline Methanolysis A mixture of **1** (480 mg), dimethyl sulfate (5 ml) and anhydrous potassium carbonate

(5.5 g) in dry acetone (30 ml) was heated under reflux with stirring for 3.5 h. After removal of the inorganic salts by filtration, the filtrate was concentrated to a syrup, which was chromatographed over silica gel. Elution with benzene–acetone (8 : 1) gave the nonacosamethyl ether (**1a**) (234 mg), a white amorphous powder, $[\alpha]_D^{23}$ +29.4° (c = 1.0, $CHCl_3$). A solution of **1a** (109 mg) in 2% methanolic sodium methoxide (5 ml) was left standing overnight at room temperature. The reaction mixture was neutralized with Amberlite IR-120B (H^+ form) resin. The filtrate was concentrated *in vacuo* and the residue was separated by silica gel chromatography with benzene–acetone to yield methyl trimethoxybenzoate (**2a**) (30.7 mg), colorless needles, mp 81 °C, dimethyl penta-*O*-methyldehydrodigallate (**4a**) (12.6 mg), colorless needles, mp 118—119 °C, and dimethyl hexamethoxydiphenoate (**5**) (19.2 mg), colorless syrup, $[\alpha]_D^{24}$ 0° (c = 1.0, $CHCl_3$).

Partial Hydrolysis of 1 with Hot Water A solution of **1** (1.4 g) in water (40 ml) was heated at 90 °C for 70 h. After cooling, the resulting precipitates were filtered off. Thin layer chromatography (TLC) examination of the precipitates showed the presence of gallic acid and ellagic acid. The filtrate was subjected to a combination of repeated column chromatographies over Sephadex LH-20, MCI-gel CHP 20P and Bondapak C_{18}/Porasil B with various solvent systems to afford five hydrolysates, **7** (82 mg), **8** (42 mg), **9** (28 mg), **10** (52 mg) and **13** (205 mg). **7**: Nupharin B. A white powder (H_2O), mp 258—260 °C, $[\alpha]_D^{25}$ +39.2° (c = 0.8, acetone). ^{1}H-NMR: Table I. **8**: 3,6-(*R*)-Hexahydroxydiphenoyl-D-glucose. An off-white amorphous powder, $[\alpha]_D^{23}$ −20.3° (c = 1.0, acetone). ^{1}H-NMR (100 MHz, acetone-d_6) δ: 6.76, 6.77, 6.78, 6.79 (2H in total, s, HHDP-H). **9**: 1,4-di-*O*-galloyl-3,6-(*S*)-hexahydroxydiphenoyl-α-D-glucose. An off-white amorphous powder, $[\alpha]_D^{25}$ −32.0° (c = 0.6, acetone). *Anal.* Calcd for $C_{34}H_{26}O_{22}\cdot 3H_2O$: C, 48.58; H, 3.85. Found: C, 49.44; H, 3.87. ^{1}H-NMR: Table I. **10**: 1,4-Di-*O*-galloyl-3,6-(*S*)-hexahydroxydiphenoyl-2-*O*-dehydrodigalloyl-α-D-glucose. An off-white amorphous powder, $[\alpha]_D^{24}$ −34.7° (c = 0.8, acetone). FAB-MS m/z: 1107 $(M+H)^+$. *Anal.* Calcd for $C_{48}H_{34}O_{31}\cdot 6H_2O$: C, 47.45; H, 3.82. Found: C, 47.36; H, 3.93. ^{1}H-NMR: Table I. ^{13}C-NMR (acetone-d_6 + D_2O) δ: 108.9 (2C) (DHDG C-2,6′), 109.9, 112.3, 112.8 (DHDG C-6, HHDP C-3,3′), 110.0, 110.9 (galloyl C-2,6), 115.0 (DHDG C-1′), 118.0 (2C) (HHDP C-1,1′), 119.8 (2C) (galloyl C-1), 120.1, 124.0, 124.5 (DHDG C-1, HHDP C-2,2′), 137.5, 137.7, 138.7 (2C) (DHDG C-3′,4′, HHDP C-5,5′), 139.5, 139.7, 140.0, 140.4 (galloyl C-4, DHDG C-2′, 4), 142.9 (DHDG C-5′), 144.0, 144.4, 144.6, 144.9, 145.7 (4C), 146.0 (galloyl C-4,6, DHDG C-5, HHDP C-4, 4′, 6, 6′), 147.3 (DHDG C-3), 165.3, 166.7 (2C), 167.3, 167.5 (–COO–), 171.0 (COOH).

Enzymatic Hydrolysis of 1 with Tannase A solution of **1** (480 mg) in water (20 ml) was incubated with tannase at 37 °C for 24 h. The reaction mixture was concentrated and was treated with ethanol. The ethanol-soluble portion was subjected to a combination of chromatographies over Sephadex LH-20 with ethanol and MCI-gel CHP 20P with a mixture of water and methanol to yield **11** (48 mg), together with a complex mixture of hydrolysates. **11**, an off-white amorphous powder, $[\alpha]_D^{25}$ +9.5° (c = 1.0, acetone). FAB-MS m/z: 1291 $(M+Na)^+$. *Anal.* Calcd for $C_{54}H_{44}O_{36}\cdot 7H_2O$: C, 46.49; H, 4.19. Found: C, 46.44; H, 4.20. ^{1}H-NMR (100 MHz, acetone-d_6 + D_2O) δ: 3.6—5.6 (m, glc.-H), 6.57 (1H, d, J = 2 Hz, anomeric-H), 6.85, 6.87 (1H in total, d, J = 2 Hz, DHDG-H), 7.00 (1H, s, DHDG-H), 7.09, 7.25 (each 1H, s, HHDP-H), 7.12, 7.18 (each 2H, s, galloyl-H). ^{13}C-NMR (acetone-d_6 + D_2O) δ: 62.0 (glc. C-6′), 65.2 (glc. C-6), 70.0 (glc. C-2), 70.9, 71.2, 71.5, 72.5, 75.2, 75.3, 76.5, 77.2 (glc. C-2′, 3′, 4′; 5′), 73.4 (glc. C-3), 75.1 (glc. C-4), 77.9 (glc. C-5), 89.6 (glc. C-1), 90.7 (glc. C-1′α), 95.5 (glc. C-1′β), 108.4 (DHDG C-2), 109.7 (2C), 112.4 (2C) (DHDG C-6, 6′, HHDP-3, 3′), 110.4 (2C), 111.1 (2C) (galloyl C-2, 6), 114.0 (DHDG C-1′), 117.6, 118.2 (HHDP C-1, 1′), 119.9, 120.1, 120.1, 120.8 (galloyl C-1, DHDG C-1), 121.4, 124.5 (HHDP C-2, 2′), 137.8, 138.8, 138.8 (DHDG C-3′, 4′, HHDP C-5, 5′), 139.5, 140.0, 140.2, 140.7 (galloyl C-4, DHDG C-2′, 4), 143.0 (DHDG C-5′), 144.1, 144.5 (2C), 145.0, 145.5 (2C), 145.8 (2C), 145.9 (galloyl C-3,5, DHDG C-5, HHDP C-4,4′, 6,6′), 147.6 (DHDG C-3), 164.2, 166.6, 166.9, 167.1, 167.4, 167.6 (–COO–).

Nupharin D (13) A white powder (H_2O), mp 241—243 °C, $[\alpha]_D^{25}$ +65.3° (c = 1.0, acetone). FAB-MS (methanol–glycerol) m/z: 1573 $(M+H)^+$, 765. *Anal.* Calcd for $C_{68}H_{52}O_{44}\cdot 3H_2O$: C, 50.20; H, 3.37. Found: C, 50.19; H, 3.41. ^{1}H-NMR: Table II. ^{13}C-NMR acetone-d_6 + D_2O) δ: 108.4 (2C), 109.5, 109.7 (DHDG C-2,6′, HHDP C-3,3′), 110.3 (6C), 110.7 (2C) (galloyl C-2,6), 112.1 (DHDG C-6), 114.0 (DHDG C-1′), 115.9, 116.5 (HHDP C-1,1′), 119.6 (DHDG C-1), 120.1 (2C), 120.3, 120.6 (galloyl C-1), 124.8, 124.8 (HHDP C-2,2′), 136.7, 137.1, 137.5 (DHDG C-4, HHDP C-5,5′), 139.4 (2C), 139.7, 139.8, 140.0, 140.5 (2C) (galloyl C-4, DHDG C-2′,3′,4′), 143.8 (DHDG C-5′), 144.8, 145.0, 145.3, 145.5, 145.9, 145.5, 145.9, 146.1 (galloyl C-3,5, DHDG C-5, HHDP C-4, 4′, 6, 6′), 148.0 (DHDG C-3), 164.4, 164.9, 165.6, 166.3, 166.5, 166.8, 167.0, 168.2 (–COO–).

Nupharin E (15) An off-white amorphous powder, $[\alpha]_D^{23}$ +45.3° (c = 1.3, acetone). FAB-MS (methanol–glycerol) m/z: 1875 $(M+H)^+$, 1705, 765. *Anal.* Calcd for $C_{82}H_{58}O_{52}\cdot 7H_2O$: C, 49.21; H, 3.66. Found: C, 49.12; H, 3.86. ^{1}H-NMR: Table II. ^{13}C-NMR (acetone-d_6 + D_2O) δ: 108.6 (3C), 109.7 (2C), 109.9 (DHDG C-2,6′, HHDP C-3,3′), 110.7 (6C), 110.9 (2C) (galloyl C-2,6), 112.5 (DHDG C-6), 114.3 (DHDG C-1′), 116.1 (2C), 116.7 (2C) (HHDP C-1,1′), 119.9 (DHDG C-1), 120.3, 120.6, 120.8, 120.9 (galloyl C-1), 125.1 (2C), 125.4 (2C) (HHDP C-2,2′), 136.9 (2C), 137.3 (2C) (HHDP C-5,5′), 137.9 (DHDG C-4′), 139.2 (2C), 140.0 (2C), 140.6, 140.8 (galloyl C-4, DHDG C-3′, 4), 141.3 (DHDG C-2′), 143.4 (DHDG C-5′), 145.1 (2C), 145.3, 145.6 (HHDP C-4, 4′, 6, 6′), 146.1, 146.2 (galloyl C-3,5, DHDG C-5), 148.4 (DHDG C-3), 164.2, 165.3, 165.5, 166.4, 166.7 (3C), 167.0, 168.4 (2C) (–COO–).

Methylation of 15, Followed by Alkaline Methanolysis A mixture of **15** (280 mg), dimethyl sulfate (2.5 ml) and anhydrous potassium carbonate (3.0 g) in dry acetone (20 ml) was heated under reflux for 3.5 h. The reaction mixture was worked up as described for **1** to yield the nonacosamethyl ether (**15a**) (182 mg), a white amorphous powder, $[\alpha]_D^{27}$ +34.6° (c = 0.6, $CHCl_3$). A solution of **15a** (110 mg) in 2% methanolic sodium methoxide (5 ml) was left standing for 10 h at room temperature. The reaction mixture was treated in the same way as described for **1a** to afford **2a** (32.4 mg), **4a** (14.4 mg) and (*R*)-dimethyl hexamethoxydiphenoate (**15b**) (28.2 mg), colorless syrup, $[\alpha]_D^{24}$ +24.6° (c = 0.8, $CHCl_3$).

Nupharin F (16) An off-white amorphous powder, $[\alpha]_D^{24}$ +40.3° (c = 0.9, acetone). *Anal.* Calcd for $C_{123}H_{86}O_{78}\cdot 9H_2O$: C, 49.70; H, 3.52. Found: C, 49.80; H, 3.56. ^{1}H-NMR: Table II. ^{13}C-NMR (acetone-d_6 + D_2O) δ: 108.5, 108.5, 109.7 (DHDG C-2,6′, *R*-HHDP C-3,3′), 110.0, 110.5 (galloyl C-1), 109.7, 112.4 (DHDG C-6, *S*-HHDP C-3,3′), 113.7, 113.8 (DHDG C-1′), 115.8 (2C), 116.5 (2C) (*R*-HHDP C-1,1′), 117.4, 118.2 (*S*-HHDP C-1,1′), 119.5 (2C) (DHDG C-1), 120.2, 120.4 (galloyl C-1), 124.8, 124.9 (HHDP C-2,2′), 136.7, 137.2, 137.5, 138.8 (DHDG C-4′, HHDP C-5,5′), 139.4, 140.0, 140.5, 141.0 (galloyl C-4, DHDG C-2′, 3′, 4), 143.0 (2C) (DHDG C-5′), 144.2, 144.6, 144.9, 145.3, 145.6, 145.8 (galloyl C-3,5, DHDG C-5, HHDP C-4, 4′, 6, 6′), 148.0 (2C) (DHDG C-3), 163.6, 164.0, 165.0, 165.5 (2C), 166.2, 166.4 (3C), 166.8 (2C), 167.2, 167.4, 168.1 (2C) (–COO–).

Acid Hydrolyses with Sulfuric Acid A solution of each sample (**1**, **13**, **15** or **16**) (5—20 mg) in 1 N sulfuric acid (2—5 ml) was heated on a water bath at 90 °C for 12—24 h. After cooling, the solution was extracted with ethyl acetate (2—5 ml). TLC examination of the organic solution showed the presence of gallic acid (**2**), ellagic acid (**3**) and dehydrodigallic acid (**4**). The aqueous layer was neutralized with barium carbonate and then filtered. The filtrate was analyzed by TLC to detect the spot corresponding to glucose.

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