Tannins and Related Compounds. LXXV.[1] Isolation and Characterization of Novel Diastereoisomeric Ellagitannins, Nupharins A and B, and Their Homologues from *Nuphar japonicum* DC.

Makoto ISHIMATSU,[a] Takashi TANAKA,[a] Gen-ichiro NONAKA,[a] Itsuo NISHIOKA,*,[a] Makoto NISHIZAWA[b,2] and Takashi YAMAGISHI[b]

Faculty of Pharmaceutical Sciences, Kyushu University,[a] 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan and Hokkaido Institute of Public Health,[b] N-19, W-12, Kita-ku, Sapporo 060, Japan. *Received July 9, 1988*

The diastereoisomeric ellagitannins, nupharins A (1) and B (11), and related compounds (8, 10, and 15), have been isolated from the rhizomes of *Nuphar japonicum* DC. (Nymphaeaceae). On the basis of physicochemical evidence, their structures were characterized as 1,2,4-tri-*O*-galloyl-(1) and 1,2-di-*O*-galloyl-3,6-(*S*)-hexahydroxydiphenoyl-α-D-glucopyranoses (8), 2-*O*-galloyl-3,6-(*S*)-hexahydroxydiphenoyl-D-glucose (10), and 1,2,4-tri-*O*-galloyl- (11) and 1,2-di-*O*-galloyl-(*R*)-hexahydroxydiphenoyl-α-D-glucopyranoses (15).

Keywords *Nuphar japonicum*; Nymphaeaceae; diastereoisomer; ellagitannin; *R*-, *S*-hexahydroxydiphenoic acid; atrop-isomerism; nupharin A; nupharin B; α-D-glucose

In one of the previous papers of this series, we reported on the isolation and characterization of several gallotannins possessing an α-glucopyranose core from the crude drug, Senkotsu, the rhizome of *Nuphar japonicum* DC. (Nymphaeaceae).[3] In addition, the presence of an ellagitannin based on a similar α-glucose core was demonstrated.[4] Our continuing chemical work on tannins in this plant material has now resulted in the isolation of two major ellagitannins named nupharins A (**1**) and B (**11**), together with three accompanying ellagitannins (**8**, **10** and **15**). We wish to report herein the isolation and structural elucidation of these compounds.

The aqueous acetone extract of the freeze-dried rhizomes of *Nuphar japonicum* was chromatographed on Sephadex LH-20 with water containing increasing amounts of ethanol and then with ethanol containing increasing amounts of a mixture of water–acetone (1 : 1) to yield seven fractions (frs. 1—7). Fractions 1 and 3 consisted of 6-*O*-galloyl-D-glucose and 1,2,4-tri-*O*-galloyl-α-D-glucopyranose,[3] respectively. Fraction 2 was repeatedly chromatographed on Sephadex LH-20 and MCI-gel CHP 20P to afford compound **10**. On similar separation, fr. 4 gave compounds **1** (nupharin A), **8** and **15**, together with 2,3,4,6-tetra-*O*-galloyl-D-glucopyranose,[3] while fr. 5 yielded compound **11** (nupharin B) and 1,2,3,4,6-penta-*O*-galloyl-α-D-glucopyranose.[3]

Nupharin A (**1**) gave, with the ferric chloride and sodium nitrite-acetic acid reagents,[5] dark blue and reddish brown colorations, respectively, which are characteristic of ellagitannins. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed three two-proton singlets at δ 6.96, 7.06 and 7.24, each assignable to a galloyl group, and two one-proton singlets at δ 7.15 and 7.25 suggestive of the presence of a hexahydroxydiphenoyl ester group in the molecule. The observation of five carboxyl carbon resonances in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum was consistent with the presence of three galloyl and one hexahydroxydiphenoyl ester groups. Acid hydrolysis of **1** with sulfuric acid gave gallic acid, ellagic acid and D-glucose, thus confirming the constitution.

The lowfield shifts of all of the glucose signals [δ 6.69 (d, J = 3 Hz), 5.50 (dd, J = 3, 8 Hz), 5.71 (d, J = 8 Hz), 5.10 (s), 4.39 (br s), 5.32 (d, J = 13 Hz) and 4.03 (d, J = 13 Hz)] in the ^{1}H-NMR spectrum of **1** suggested that the hydroxyl groups were exhaustively esterified. In addition, the unusual coupling patterns of these signals implied that the conformation of the glucopyranose ring is not a common $^4C_1$ form, but a skew boat form.

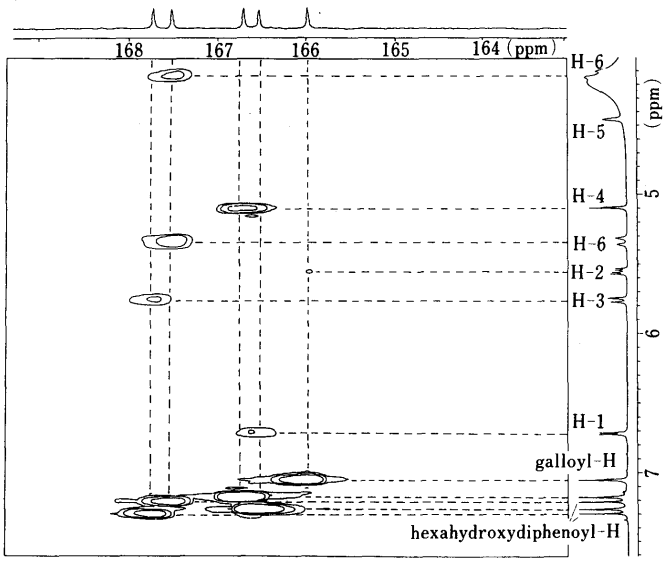


Fig. 1. ^{1}H–^{13}C Long-Range Shift Correlation Spectrum of **1** in Acetone-d_6 (J_{CH} = 10 Hz)

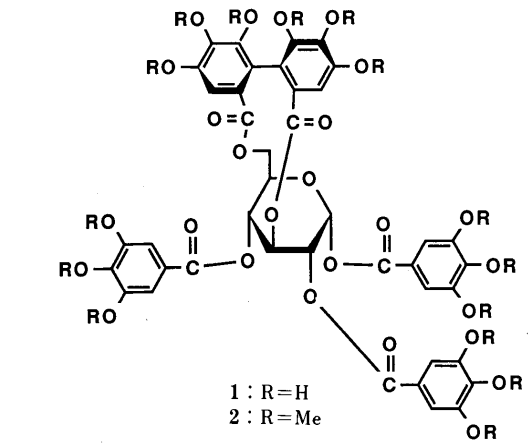
Methylation of **1** with dimethyl sulfate and anhydrous potassium carbonate in dry acetone gave the pentadeca-methyl ether (**2**), which showed a molecular ion peak at m/z 1148 in the field-desorption mass spectrum (FD-MS). Hydrolysis of **2** in an alkaline solution, followed by methylation with diazomethane, afforded methyl 3,4,5-trimethoxybenzoate (**3**) and dimethyl 4,4′,5,5′,6,6′-hexamethoxydiphenoate (**4**). The specific optical rotation [−27.4° ($CHCl_3$)] of **4** confirmed the chirality of the biphenyl bond to be in the *S*-series.[6]

The ^{1}H–^{13}C long-range shift correlation spectrum (Fig. 1)[7] of **1** showed the correlation of the glucose C-6 methylene protons at δ 5.32 and 4.03 with the carboxyl carbon at δ 167.4 through a three-bond coupling. Furthermore, a three-bond coupling was observed between this carboxyl signal and one of the hexahydroxydiphenoyl signals at δ 7.15. Similarly, the glucose C-3 proton signal at δ 5.71 was

TABLE I. ^{1}H-NMR Data for Compounds **1**, **8**, **10**, **5**, **11** and **15** (δ Values, J Values in Hz)[a]

	1	8	10	5[b]	11	15
Glucose						
C_1-H	6.69 (d, J=3)	6.67 (d, J=3)	5.65 (d, J=3)	5.22 (d, J=3)	6.63 (d, J=2)	6.56 (d, J=2)
C_2-H	5.50 (dd, J=3, 8)	5.49 (dd, J=3, 9)	4.90 (dd, J=3, 8)	3.94 (dd, J=3, 8)	5.61 (t, J=2)	5.40 (t, J=2)
C_3-H	5.71 (d, J=8)	5.50 (d, J=9)	5.92 (t, J=8)	5.44 (dd, J=8, 9)	5.26 (t, J=2)	5.03 (t, J=2)
C_4-H	5.10 (s)	3.83 (s)	4.62 (d, J=8)	4.55 (d, J=9)	5.71 (d, J=2)	4.35 (d, J=2)
C_5-H	4.39 (br)	4.35 (br)	3.74 (dd, J=4, 10)	3.80 (dd, J=4, 10)	4.80—4.90 (m)	4.70—4.80 (m)
C_6-H	5.32 (d, J=13)	5.10 (d, J=13)	4.56 (dd, J=4, 10)	4.51 (dd, J=4, 10)	4.80—4.90 (m)	4.70—4.80 (m)
	4.03 (d, J=13)	3.90 (d, J=13)	4.00 (t, J=10)	3.80 (t, J=10)	4.23 (d, J=13)	4.26 (d, J=13)
Galloyl-H	6.96, 7.06, 7.24	6.90, 7.24	6.98		7.11 (×2), 7.31	7.11, 7.30
Hexahydroxy-diphenoyl-H	7.15, 7.25	7.07, 7.32	6.81, 7.17	6.81, 7.15	6.81, 6.93	6.90, 6.94

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



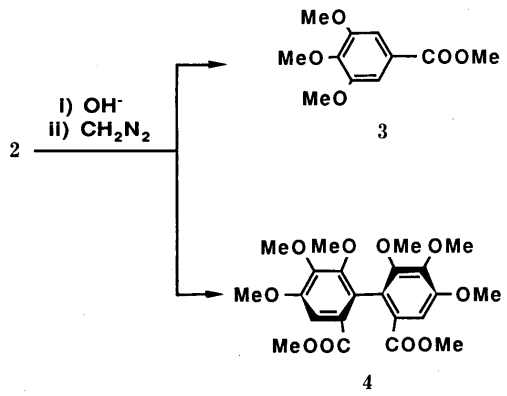


Chart 1

shown to be correlated with the carboxyl signal at δ 167.7, which also gave a cross peak with the hexahydroxydiphenoyl signal at δ 7.25. These facts suggested that the hexahydroxydiphenoyl group is located at the 3,6-positions of the glucose moiety.

To confirm the location of each acyl group chemically, we attempted partial hydrolysis of **1**. When treated with tannase, **1** yielded, among other products, gallic acid and a hydrolysate (**5**). The ^{1}H-NMR spectrum of **5** showed the presence of one hexahydroxydiphenoyl group in the molecule. Compound **5** was subsequently methylated with diazomethane to give the hexamethyl ether (**6**) [negative fast atom bombardment mass spectrum (FAB-MS) m/z: 565 $(M-H)^-$]. Further methylation of **6** by the Kuhn method, followed by alkaline hydrolysis and methanolic acid treatment, yielded methyl 2,4-di-*O*-methylglucopyranoside. On the other hand, when heated in water, **1** afforded ellagic acid and a partial hydrolysate which was found to be identical with 1,2,4-tri-*O*-galloyl-α-D-glucopyranose (**7**) by direct comparisons of the physical and spectral data with those of an authentic sample[3] formerly isolated from the same material.

From these findings, the hexahydroxydiphenoyl group was concluded to be located at the 3,6-positions in the glucose moiety. Thus, nupharin A was characterized as 1,2,4-tri-*O*-galloyl-3,6-(*S*)-hexahydroxydiphenoyl-α-D-glucopyranose (**1**).[8]

The ^{1}H-NMR spectrum of compound **8** showed the presence of two galloyl groups [δ 6.90 and 7.24 (each 2H, s)] and one hexahydroxydiphenoyl group [δ7.07 and 7.32 (each 1H, s)]. The chemical shifts and coupling patterns of the glucose signals were similar to those found in **1**, except for the remarkable upfield shift [δ 3.83 (s)] of the glucose C-4 signals. The lack of one galloyl group was consistent with

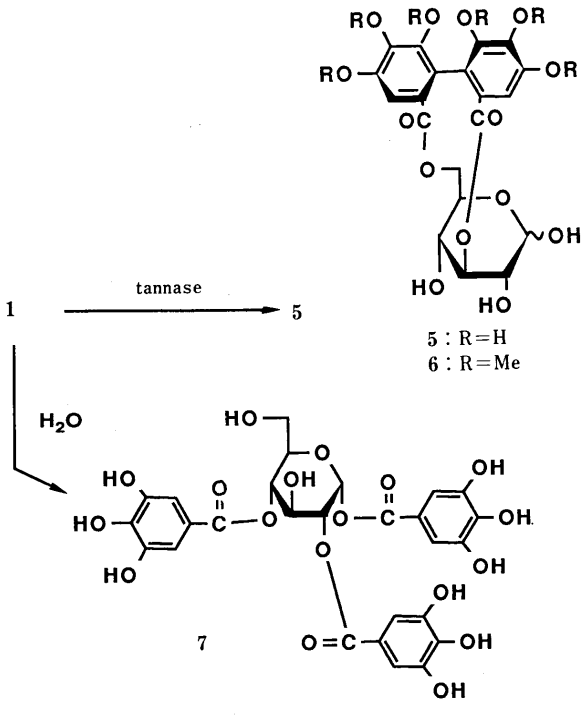


Chart 2

the FAB-MS, which exhibited the prominent $[M+H]^+$ peak at *m/z* 787, corresponding to one galloyl group less than in **1**.

When heated in water, **8** yielded a partial hydrolysate (**9**). The ^{1}H-NMR spectum of **9** showed two galloyl singlets [δ 7.08 and 7.15 (each 2H, s)] and the absence of the hexahydroxydiphenoyl group. Since the anomeric doublet [δ 6.48 (d, J=3 Hz)] and a signal [δ 5.05 (dd, J=3, 7 Hz)] coupled with this anomeric signal were observed at lower field, **9** was concluded to be 1,2-di-*O*-galloyl-α-D-glucose. On the other hand, enzymatic hydrolysis of **8** with tannase afforded two hydrolysates, together with gallic acid. By comparisons of their physical and spectral data, the hydrolysates were identified as compound **10** (*vide infra*) and compound **5**.

Based on these results, **8** was characterized as 1,2-di-*O*-galloyl-3,6-(*S*)-hexahydroxydiphenoyl-α-D-glucopyranose.

The FAB-MS [*m/z*: 635 $(M+H)^+$ and 647 $(M+Na)^+$] of compound **10** corresponded to the loss of two galloyl groups from the molecule of compound **1**. The ^{1}H-NMR spectrum agreed with this mass spectral observation, showing signals due to one galloyl group [δ 6.98 (2H, s)] and one hexahydroxydiphenoyl ester group [δ 6.81 and 7.17 (each 1H, s)].

The ^{1}H–^{13}C long-range shifts correlation spectrum showed the correlation of the glucose C-3 proton [δ 5.92 (t, J=8 Hz)] and the glucose C-6 [δ 4.56 (dd, J=4, 10 Hz)] signals with the hexahydroxydiphenoyl signals through the carboxyl carbon signals [δ 168.4 (2C)]. These facts clearly indicated the location of the hexahydroxydiphenoyl group to be at the 3,6-positions of the glucose moiety. The position of the galloyl group was determined to be at the 2-position by the lowfield shift of the corresponding signal [δ 4.90 (dd, J=3,8 Hz)].

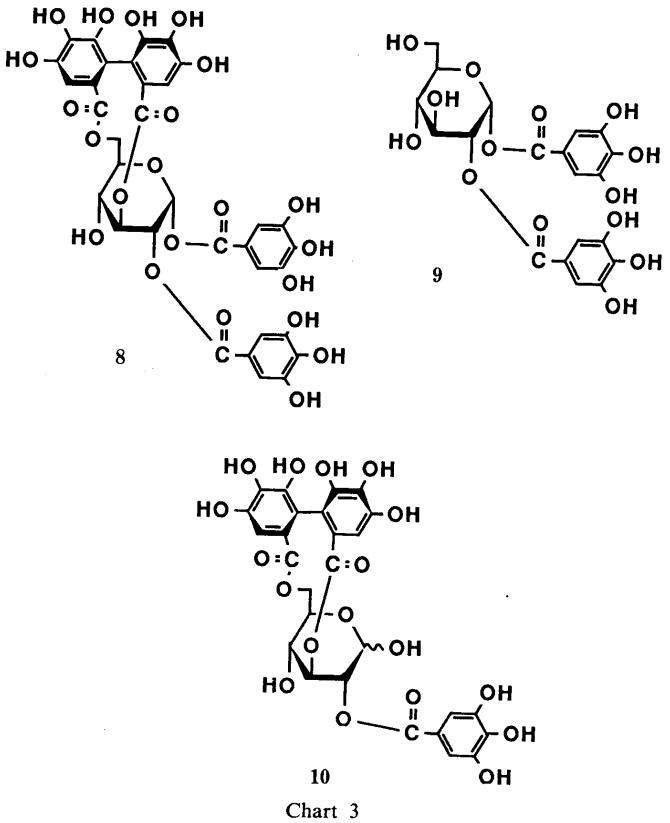


Chart 3

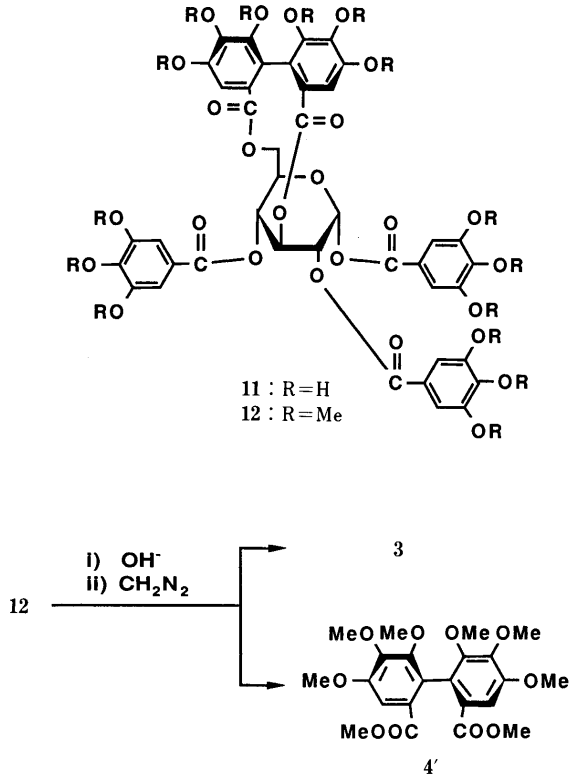


Chart 4

Final structural confirmation was obtained by partial hydrolysis of **1** with tannase, which furnished compound **10**, together with gallic acid and compound **5**, thus permitting the assignment of the structure (**10**) for this compound.

Nupharin B (**11**) has the same molecular weight as nupharin A. The ^{1}H-NMR spectrum, which showed three galloyl singlets [δ 7.11 (4H, s) and 7.31 (2H, s)] and two one-proton singlets (δ 6.81 and 6.93 (ascribable to the hexahydroxydiphenoyl ester group, was consistent with the appearance of five carboxyl carbon resonances (δ 165.0, 165.9, 166.2, 166.6 and 167.9) in the ^{13}C-NMR spectrum. The component sugar was identified as glucose, which was obtained, together with gallic acid and ellagic acid, by acid hydrolysis. The lowfield shifts of all of the glucose proton signals indicated the sugar hydroxyl groups to be fully acylated, while their small coupling constants showed that the glucopyranose ring adopts a $^1C_4$ or an intermediate skew boat conformation.

On methylation in the same way as for **1**, **11** yielded the pentadecamethyl ether (**12**) [FD-MS *m/z*: 1148 $(M)^+$], which was subjected to alkaline hydrolysis and then treated with diazomethane to give **3** and dimethyl hexamethoxydiphenoate (**4′**). The positive sign of the specific optical rotation [+24.6° ($CHCl_3$)] of 4′ established unequivocally the atropisomerism of the biphenyl bond to be in the *R*-series, which is opposite to the cases of compounds **1**, **8** and **10**.

In order to locate the galloyl and hexahydroxydiphenoyl ester groups in **11**, the following partial hydrolyses were attempted. When heated with hydrochloric acid, **11** yielded 1,2,4-tri-*O*-galloyl-α-D-glucose (**7**) as the major hydrolysate. On the other hand, enzymatic hydrolysis of **11** with tannase

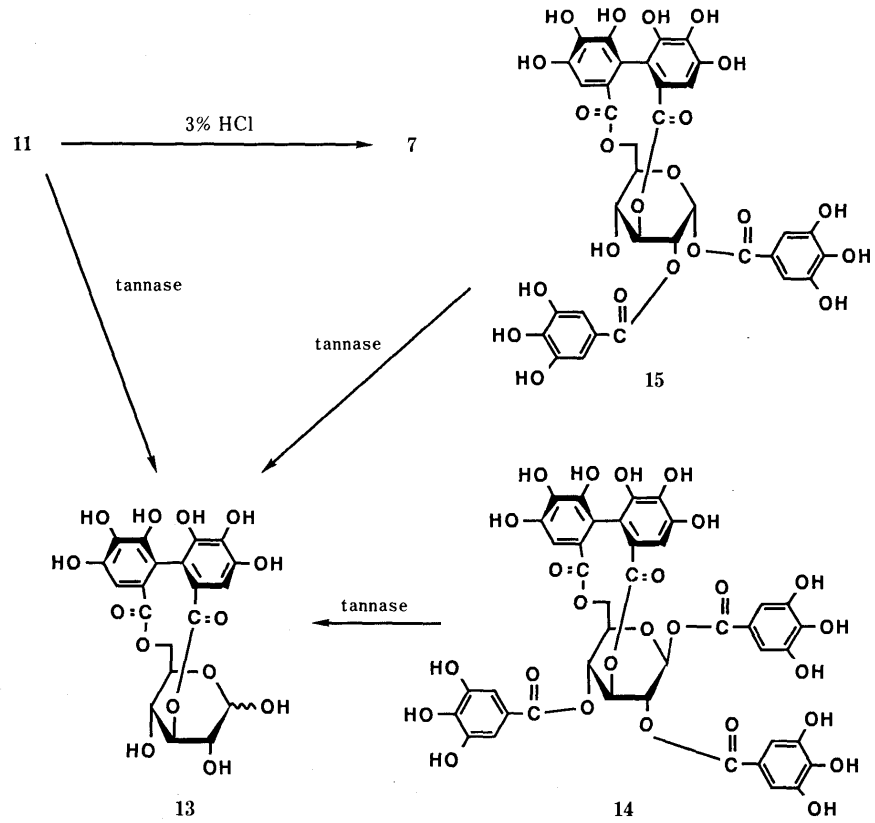


Chart 5

furnished 3,6-(*R*)-hexahydroxydiphenoyl-D-glucose (**13**), which was identified by comparison with an authentic sample obtained previously by similar hydrolysis of punicafolin (**14**).[9] Thus, nupharin B was characterized as 1,2,4-tri-*O*-galloyl-3,6-(*R*)-hexahydroxydiphenoyl-α-D-glucopyranose (**11**).

The digalloyl and monohexahydroxydiphenoyl constitution of compound **15** was confirmed by FAB-MS [*m/z*: 787 $(M+H)^+$] and ^{1}H-NMR spectroscopy [δ 7.30 and 7.11 (each 2H, s), 6.94 and 6.90 (each 1H, s)]. The ^{1}H-NMR signal patterns in the aliphatic region were similar to those found in **11**, but differed only in the upfield shift [δ 4.35, (d, $J=2$ Hz)] of the methine signal. The assignment of this upfield signal could be achieved by decoupling techniques. Namely, irradiation at the frequency of the anomeric proton at δ 6.56 caused a change of a triplet ($J=2$ Hz) at δ 5.40 into a doublet. Next, when this triplet was irradiated in turn, a triplet ($J=2$ Hz) at δ 5.03 was sharpened. Accordingly, the triplet at δ 5.03 was assignable to the C-3 proton. Since the upfield doublet at δ 4.35 was changed into a singlet when irradiated at the frequency of this C-3 proton signal, the doublet was assigned to the C-4 proton. Based on these findings, the glucose C-4 position was concluded to be free of an acyl group.

The location of the hexahydroxydiphenoyl ester group was established to be at the 3,6-positions, since **15** yielded **13** on tannase hydrolysis. Furthermore, the mode of the anomeric linkage was concluded to be α on the basis of a coupling pattern analogous to that of **11**. Consequently, **15** was concluded to be 1,2-di-*O*-galloyl-(*R*)-hexahydroxydiphenoyl-α-D-glucopyranose (**15**).

Although ellagitannins, which have the 3,6-positioned (*R*)-hexahydroxydiphenoyl group, and widely distributed in the plant kingdom, especially in the members of the families Euphorbiaceae,[10] Geraniaceae,[11] Punicaceae,[9] Combretaceae,[12] Elaeocarpaceae,[13] Cercidiphyllaceae,[11] *etc.*, nupharin A (**1**) and its congeners **8** and **10** represent the first ellagitannins possessing the enantiomeric (*S*)-hexahydroxydiphenoyl group at the glucose 3,6-positions. Taking the specificity of enzymes into account, the coexistence of *R*- and *S*-hexahydroxydiphenoyl esters in the same plant tissues is rather unusual. Moreover, it is interesting from the viewpoint of plant physiology that all the hydrolyzable tannins (both gallo- and ellagitannins) isolated from *N. japonicum* contain the less common α-D-glucopyranose core.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. FD- and FAB-MS were taken with a JEOL JMS DX-300 instrument. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL PS-100, JEOL FX-100 and JEOL GX-270 spectrometers, with tetramethylsilane as an internal standard, and chemical shifts are given in δ (ppm). Column chromatography was performed with Kieselgel 60 (70—230 mesh, Merck), Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.) and MCI-gel CHP-20P (75—150 μ, Mitsubishi Chemical Industries, Ltd.). Thin layer chromatography (TLC) was conducted on precoated Kieselgel 60 F_{254} plates (Merck, 0.2 mm thick) and precoated cellulose plates (Funakoshi). Analytical gas-liquid chromatography (GLC) was conducted with a Shimadzu gas chromatograph, a model GC-4BMPF instrument, over 2% neopentylglycol succinate polyester.

Isolation of Ellagitannins The freeze-dried rhizomes (4.3 kg) of *N. japonicum*, collected in Hokkaido, Japan, were extracted three times with 80% aqueous acetone. Concentration of the extracts under reduced pressure afforded dark brown precipitates, which were removed by filtration.

The filtrate was chromatographed on a Sephadex LH-20 column. Elution with water containing increasing amounts of ethanol and then with ethanol containing increasing amounts of a mixture of water–acetone (1 : 1) yielded seven fractions; fr. 1 (9.5 g), fr. 2 (4.5 g), fr. 3 (4.5 g), fr. 4 (18.7 g), fr. 5 (17.8 g), fr. 6 (19.0 g) and fr. 7 (14.6 g). Fractions 1 and 3 yielded 6-*O*-galloyl-D-glucose[3] and 1,2,4-tri-*O*-galloyl-α-D-glucose,[3] respectively, by repeated chromatography over Sephadex LH-20 and MCI-gel CHP 20P. Fraction 2 was repeatedly chromatographed on Sephadex LH-20 with ethanol and MCI-gel CHP-20P with water containing increasing amounts of methanol to give compound **10** (184 mg). On similar chromatographic separation, fr. 4 gave compounds **1** (10.4 g), **8** (184 mg) and **15** (28.4 mg), together with 2,3,4,6-tetra-*O*-galloyl-D-glucose,[3] while fr. 5 yielded compound **11** (11.3 g) and 1,2,3,4,6-penta-*O*-galloyl-α-D-glucose.[3]

Nupharin A (1) A white powder (H_2O), mp 243—245 °C (dec.). $[\alpha]_D^{24}$ −51.4° ($c=1.2$, acetone). *Anal.* Calcd for $C_{41}H_{30}O_{26}\cdot 3/2H_2O$: C, 50.99; H, 3.45. Found: C, 51.08; H, 3.48. ^{13}C-NMR (acetone-d_6): 65.1 (C-6), 70.0 (C-2), 73.5 (C-3), 74.9 (C-4), 77.9 (C-5), 89.5 (C-1), 110.0, 110.4, 110.8 (galloyl C-2,6), 109.4, 112.5 (HHDP[14] C-3,3′), 117.8, 118.2 (HHDP C-1,1′), 120.0, 120.2 (galloyl C-1), 120.4, 124.8 (HHDP C-2,2′), 137.1, 138.8 (HHDP C-5,5′), 139.1, 139.8, 140.1 (galloyl C-4), 144.5, 144.8, 145.2, 145.8, 146.0 (galloyl C-3,5, HHDP C-4,4′,6,6′), 165.9, 166.4, 166.6, 167.4, 167.7 (–COO–).

Acid Hydrolysis of 1 A solution of **1** (110 mg) in 1 N sulfuric acid (5 ml) was heated at 90°C for 12 h. After cooling, the solution was extracted with ethyl acetate. TLC examination of the ethyl acetate layer showed the presence of gallic acid and ellagic acid. The aqueous layer was neutralized with barium carbonate and the inorganic precipitates were filtered off. The filtrate was concentrated under reduced pressure, and the residue was chromatographed on a Sephadex LH-20 column with ethanol to yield D-glucose (14 mg), $[\alpha]_D^{27}$ +50.2° ($c=0.5$, H_2O), *Rf* 0.39 [cellulose TLC, solvent, *n*-butanol–pyridine–water (6 : 4 : 3); detection, aniline–hydrogen-phthalate reagent].

Methylation of 1 A mixture of **1** (240 mg), dimethyl sulfate (2 ml) and anhydrous potassium carbonate (2.5 g) in dry acetone (20 ml) was heated under reflux for 2 h with stirring. 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 (7 : 1) gave the pentadecamethyl ether (**2**) (201 mg) as a white powder (MeOH), $[\alpha]_D^{28}$ −59.8° ($c=0.6$, $CHCl_3$). *Anal.* Calcd for $C_{56}H_{60}O_{26}$: C, 58.53; H, 5.26. Found: C, 58.86; H, 5.34, FD-MS *m/z*: 1148 $(M)^+$. ^{1}H-NMR ($CDCl_3$) δ: 3.5—4.1 (OCH_3), 4.20 (1H, dd, $J=3$, 12 Hz, H-6), 4.50 (1H, br, H-5), 5.17 (1H, s, H-4), 5.34 (1H, d, $J=12$ Hz, H-6), 5.54 (1H, dd, $J=3$, 8 Hz, H-2), 5.68 (1H, d, $J=8$ Hz, H-3), 6.75 (1H, d, $J=3$ Hz, H-1), 7.01, 7.13, 7.20 (each 2H, s, trimethoxybenzoyl-H), 7.34, 7.37 (each 2H, s, HMDP[14]-H).

Methanolysis of 2 **2** (100 mg) was treated with a mixture of 10% aqueous NaOH (3 ml) and methanol (3 ml) under reflux for 1 h. The reaction mixture was concentrated under reduced pressure. The aqueous solution was acidified with 10% HCl (5 ml), and extracted with ether. The organic layer was washed with water, dried (Na_2SO_4) and concentrated. The residue was treated with ethereal diazomethane for 1 h. After concentration, the products were separated by silica gel chromatography. Elution with benzene–acetone (19 : 1) yielded **3** (45 mg) as colorless needles, mp 81 °C and **4** (29 mg) as a colorless syrup, $[\alpha]_D^{27}$ −27.4° ($c=1.1$, $CHCl_3$).

Enzymatic Hydrolysis of 1 with Tannase A solution of **1** (112 mg) in water (30 ml) was incubated with tannase (10 mg) at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* and the residue was treated with ethanol. The ethanol-soluble portion was applied to a Sephadex LH-20 column with ethanol to give gallic acid (44 mg), **10** (24 mg) and **5** (35 mg) as a white amorphous powder, $[\alpha]_D^{28}$ +154.3° ($c=0.8$, MeOH). Negative FAB-MS *m/z*: 481$(M-H)^-$. *Anal.* Calcd for $C_{20}H_{18}O_{14}\cdot 2H_2O$: C, 46.33; H, 4.28. Found: C, 46.70; H, 4.30. ^{13}C-NMR (acetone-d_6+D_2O): 61.8 (C-6), 65.3 (C-5), 75.1 (C-3), 78.8 (C-2), 79.8 (C-4), 95.2 (C-1), 107.7, 111.9 (HHDP C-3,3′), 116.6, 117.7 (HHDP C-1,1′), 120.6, 126.6 (HHDP C-2,2′), 136.5, 138.2 (HHDP C-5,5′), 144.5, 144.7, 145.0 (HHDP C-4,4′,6,6′), 168.7, 168.8 (–COO–).

Methylation of 5 A solution of **5** (40 mg) in methanol (5 ml) was methylated with ethereal diazomethane with ice-cooling for 1 h. After removal of the solvent by evaporation, the residue was chromatographed over silica gel with benzene–acetone to afford the hexamethyl ether (**6**) (32 mg) as a white amorphous powder, $[\alpha]_D^{24}$ +120.4° ($c=0.5$, $CHCl_3$). Negative FAB-MS *m/z*: 565 $(M-H)^-$. ^{1}H-NMR ($CDCl_3+D_2O$): 3.62, 3.67, 3.78, 3.91, 3.93, 3.97 (each 3H, s, OCH_3), 3.83 (1H, dd, $J=4$, 7 Hz, H-2), 3.99 (2H, m, H-5,6), 4.69 (1H, dd, $J=4$, 10 Hz, H-6), 4.70 (1H, d, $J=9$ Hz, H-4), 5.35 (1H, d, $J=4$ Hz, H-1), 5.41 (1H, dd, $J=7$, 9 Hz, H-3), 6.98, 7.12 (each 1H, s, HMDP-H).

Permethylation of 6, Followed by Methanolysis **6** (29 mg) was stirred with methyl iodide (1 ml) and silver oxide (0.5 g) in dimethylformamide (1 ml) at room temperature for 4 h. The reaction mixture, after removal of the inorganic salts by filtration, was evaporated *in vacuo* to give an oily residue, which was passed through a silica gel column with benzene–acetone (8 : 1). The crude permethyl derivative thus obtained was heated with 5% methanolic NaOH (5 ml) under reflux for 30 min. The reaction mixture was neutralized with Amberlite IR-120B resins and concentrated to dryness. The residue was heated in 1 N methanolic HCl (5 ml) under reflux for 1 h. The solution was neutralized with Amberlite MB-3 resins and concentrated. The product was shown to be identical with methyl 2,4-di-*O*-methylglucopyranoside by GLC examination (t_R 4.60 min; column temperature, 160 °C; flow rate 60 ml/min).

Partial Hydrolysis of 1 A solution of **1** (210 mg) in water (10 ml) was heated at 70 °C for 20 h. The resulting precipitates were filtered off and identified as ellagic acid by TLC examination. The filtrate was chromatographed over Sephadex LH-20. Elution with ethanol yielded 1,2,4-tri-*O*-galloyl-α-D-glucopyranose (**7**) (33 mg) as a white powder (H_2O), mp 207 °C. $[\alpha]_D^{29}$ +73.4° ($c=1.0$, acetone). ^{1}H-NMR (acetone-d_6): 3.66 (2H, br d, $J=4$ Hz, H-6), 4.18 (1H, dd, $J=4$, 9 Hz, H-5), 4.57 (1H, t, $J=9$ Hz, H-3), 5.19 (1H, dd, $J=4$, 9 Hz, H-2), 5.29 (1H, t, $J=9$ Hz, H-4), 6.59 (1H, d, $J=4$ Hz, H-1), 7.10, 7.19, 7.20 (each 2H, s, galloyl-H). Further elution with ethanol afforded unreacted **1** (100 mg).

Compound 8 A white powder (H_2O), mp 218—220 °C. $[\alpha]_D^{27}$ +154.4° ($c=0.8$, acetone). *Anal.* Calcd for $C_{34}H_{26}O_{22}\cdot 2H_2O$: C, 50.37; H, 3.38. Found: C, 50.32; H, 3.48. ^{13}C-NMR (acetone-d_6): 65.2 (C-6), 70.4 (C-2), 73.2 (C-3), 76.5 (C-4), 80.3 (C-5), 89.1 (C-1), 109.2, 112.7 (HHDP C-3,3′), 109.9, 110.5, (galloyl C-2,6), 117.1, 118.2 (HHDP C-1,1′), 120.2 (2C) (galloyl C-1), 120.7, 125.1 (HHDP C-2,2′), 137.0, 138.9 (HHDP C-5,5′), 138.9, 139.4 (galloyl C-4), 144.5, 144.7, 145.1, 145.9 (galloyl C-3,5, HHDP C-4,4′,6,6′), 166.1, 166.4, 167.7, 168.1 (–COO–).

Partial Hydrolysis of 8 A solution of **8** (10 mg) in water (2 ml) was heated at 90 °C for 6 h. The reaction mixture was separated by chromatography over MCI-gel CHP 20P with water containing increasing amounts of methanol to yield **9** (4.4 mg) as a white amorphous powder, $[\alpha]_D^{24}$ +47.6° ($c=0.3$, acetone). *Anal.* Calcd for $C_{20}H_{20}O_{14}\cdot 2H_2O$: C, 46.15; H, 4.68. Found: C, 46.62; H, 4.66.^{1}H-NMR (acetone-d_6+D_2O): 3.6—3.8 (4H in total, m, H-4,5,6,6′), 4.15 (1H, t, $J=9$ Hz, H-3), 5.05 (1H, dd, $J=3$ Hz, H-2), 6.48 (1H, d, $J=3$ Hz, H-1), 7.08, 7.15 (each 2H, s, galloyl-H).

Enzymatic Hydrolysis of 8 with Tannase A solution of **8** (43 mg) in water–methanol (3 : 1) (4 ml) was shaken with tannase (3 mg) at room temperature for 2 h. The reaction mixture was worked-up as described for **1** to give gallic acid, **5** (14 mg) and **10** (12 mg).

Compound 10 A white amorphour powder, $[\alpha]_D^{24}$ +91.4° ($c=1.0$, MeOH). FAB-MS *m/z*: 635 $(M+H)^+$, 657 $(M+Na)^+$. *Anal.* Calcd for $C_{27}H_{22}O_{18}\cdot H_2O$: C, 48.34; H, 3.90. Found: C, 48.28; H, 3.73. ^{13}C-NMR (acetone-d_6): 61.8 (C-6), 65.3 (C-5), 75.1 (C-3), 77.5 (C-2), 78.1 (C-4), 93.6 (C-1), 107.5, 117.5 (HHDP C-3,3′), 110.4 (galloyl C-2,5), 115.9, 117.8 (HHDP C-1,1′), 120.5 (galloyl C-1), 120.6, 126.5 (HHDP C-2,2′), 136.4, 139.1 (HHDP C-5,5′), 144.4, 144.7, 145.1, 145.1 (galloyl C-3,5, HHDP C-4,4′, C-6,6′), 166.7, 168.4 (2C) (–COO–).

Nupharin B (11) Colorless needles (H_2O), mp 258 °C. $[\alpha]_D^{24}$ +38.5° ($c=0.8$, acetone). *Anal.* Calcd for $C_{41}H_{30}O_{26}\cdot 9/2H_2O$: C, 40.29; H, 3.86. Found: C, 48.27; H, 3.88. ^{13}C-NMR (acetone-d_6): 60.2 (C-6), 63.3 (C-4), 67.7 (C-2), 70.1 (C-3), 75.4 (C-5), 86.6 (C-1), 108.9, 109.8 (HHDP C-3,3′), 110.5 (2C), 110.7 (galloyl C-2,6), 115.4, 116.4, (HHDP C-1,1′), 120.3, 120.7, 120.8 (galloyl C-1), 125.2, 125.4 (HHDP C-2,2′), 137.1, 136.7 (HHDP C-5,5′), 139.3, 139.7 (2C) (galloyl C-4), 145.1, 144.9 145.5, 145.8, 145.9 (galloyl C-3,5, HHDP C-4,4′, 6,6′), 165.0, 165.9, 166.2, 166.6, 167.9 (–COO–).

Acid Hydrolysis of 11 A solution of **11** (20 mg) in 1 N H_2SO_4 (5 ml) was heated at 90 °C for 24 h. After cooling, the solution was extracted with ethyl acetate (5 ml). TLC examination of the organic layer showed the presence of gallic acid and ellagic acid. The aqueous layer was neutralized with barium carbonate and analyzed by cellulose TLC. A spot corresponding to glucose was detected.

Methylation of 11 A mixture of **11** (584 mg), dimethyl sulfate (5 ml) and anhydrous potassium carbonate (6.0 g) in dry acetone (50 ml) was heated under reflux for 2.5 h. The reaction mixture was treated in the same way as described for **1** to give the pentadecamethyl ether (**12**) (444 mg) as a white amorphous powder, $[\alpha]_D^{24}$ +27.4° ($c=1.2$, $CHCl_3$). *Anal.* Calcd for $C_{56}H_{60}O_{26}$: C, 58.53; H, 5.26. Found: C, 58.48; H, 5.14. ^{1}H-NMR ($CDCl_3$): 3.48—4.00 (OCH_3), 4.51 (1H, dd, $J=8$, 10 Hz, H-6), 4.84 (1H, t,

$J=8$ Hz, H-5), 5.12 (1H, t, $J=10$ Hz, H-6), 5.39 (1H, t, $J=2$ Hz, H-3), 5.52 (1H, t, $J=3$ Hz, H-2), 5.66 (1H, br s, H-4), 6.62 (1H, d, $J=2$ Hz, H-1), 6.96, 7.05 (each 2H, s, HMDP-H), 7.08, 7.12, 7.36 (each 2H, s, trimethoxybenzoyl-H).

Methanolysis of 12 **12** (100 mg) was treated in the same way as described for **2** to yield **3** (41 mg) and **4'** (28 mg), $[\alpha]_D^{23}$ +24.6° ($c=1.2$, $CHCl_3$).

Partial Hydrolysis of 11 A solution of **11** (290 mg) in 3% HCl (50 ml) was heated under reflux for 30 min. After cooling, the reaction mixture was directly applied to an MCI-gel CHP 20P column. Elution with 30% aqueous methanol yielded **7** (43 mg) and then elution with 40% aqueous methanol yielded unreacted **11** (50 mg).

Enzymatic Hydrolysis of 11 A solution of **11** (460 mg) in water (10 ml) was shaken with tannase (6 mg) at room temperature for 1 h. The reaction mixture was worked-up as for **1** to yield gallic acid (150 mg) and 3,6-(*R*)-hexahydroxydiphenoyl-D-glucose (**13**) (143 mg) as a white amorphous powder, $[\alpha]_D^{23}$ −20.4° ($c=1.0$, MeOH).

Compound 15 A white powder (H_2O), mp 187—190 °C. $[\alpha]_D^{23}$ +11.8° ($c=0.4$, acetone). *Anal.* Calcd for $C_{34}H_{26}O_{22}\cdot 2H_2O$: C, 50.37; H, 3.38. Found: C, 50.36; H, 3.52. FAB-MS *m/z*: 787 $(M+H)^+$.

Enzymatic Hydrolysis of 15 with Tannase A solution (1 ml) of **15** (3 mg) was incubated with tannase at room temperature for 10 min. The reaction mixture was directly analyzed by TLC examination, and spots corresponding to gallic acid and **13** were detected.

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References and Notes

1) Part LXXIV: T.-H. Hwang, Y. Kashiwada, G. Nonaka and I. Nishioka, *Phytochemistry*, "in press."
2) Present address: *Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan.*
3) G. Nonaka, I. Ishimatsu, T. Tanaka, I. Nishioka, M. Nishizawa and T. Yamagishi, *Chem. Pharm. Bull.*, **35**, 3127 (1987).
4) M. Nishizawa, T. Yamagishi, G. Nonaka, I. Nlishioka and H. Bando, *Chem. Pharm. Bull.*, **30**, 1094 (1982).
5) E. C. Bate-Smith, *Phytochemistry*, **11**, 1153 (1972).
6) Y. Ikeya, H. Taguchi, I. Yoshioka and H. Kobayashi, *Chem. Pharm. Bull.*, **27**, 1383 (1979).
7) G. Nonaka, K. Ishimaru, K. Mihashi, Y. Iwase, M. Ageta and I. Nishioka, *Chem. Pharm. Bull.*, **36**, 857 (1988).
8) The structure of nupharin A was formerly reported to be 1,2,6-tri-*O*-galloyl-3,4-(*S*)-hexahydroxydiphenoyl-α-D-glucose.[4)]
9) T. Tanaka, G. Nonaka and I. Nishioka, *Phytochemistry*, **24**, 2075 (1985).
10) R. Saijo, G. Nonaka, I. Nishioka and T.-H. Hwang, Abstracts of Papers, 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April, 1987, p. 356.
11) E. A. Haddock, R. K. Gupta and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2535.
12) T. Schmidt, *Forsher. Chem. Org. Naturst.*, **13**, 570 (1965).
13) T. Tanaka, G. Nonaka, I. Nishioka, K. Miyahara and T. Kawasaki, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 360.
14) HHDP = hexahydroxydiphenoyl, HMDP = hexamethoxydiphenoyl.