Communications to the Editor

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NOVEL HYDROLYZABLE TANNINS FROM NUPHAR JAPONICUM DC.

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Two gallotannins (I and II) and an ellagitannin (III) named nupharin A have been isolated from Nuphar japonicum DC. (Nymphaeaceae). On the basis of chemical and spectral evidence, the structures of I, II and III were characterized as 1,2,6-tri-O-galloyl-α-D-glucose, 1,2,3,4,6-penta-O-galloyl-α-D-glucose and 1,2,6-tri-O-galloyl-3,4-(S)-hexahydroxydiphenoyl-α-D-glucose, respectively.

KEYWORDS —— Nuphar japonicum; Nymphaeaceae; 1,2,6-tri-O-galloyl-α-D-glucose; 1,2,3,4,6-penta-O-galloyl-α-D-glucose; nupharin A; hydrolyzable tannins; tannase

Our recent studies on the tannins in crude drugs revealed the presence of hydrolyzable tannins in Nupharis Rhizoma (Nuphar japonicum DC., Nymphaeaceae).[1] In this communication, we report the structures of the tannins isolated from Nuphar japonicum DC. collected at Sapporo in May 1981.

The ethyl acetate soluble portion of the acetone extract was repeatedly chromatographed over Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd., Sweden, solvent: EtOH-H_2O) and Avicel SF (Funakoshi Co. Ltd., Tokyo, solvent: 1% aq. AcOH) to yield light brown amorphous powders of I, $[\alpha]_D$ +120° (acetone, c = 0.74), II, $[\alpha]_D$ +83.5° (acetone, c = 0.15), and III, $[\alpha]_D$ +45.7° (acetone, c = 1.19).

The proton nuclear magnetic resonance (PMR) spectrum of I revealed three signals due to galloyl groups at δ7.12, 7.24 and 7.27 (Table I). The permethylate of I, MS; m/z 790 (M^+), obtained by treatment with dimethyl sulfate (DMS)-K_2CO_3 followed by Kuhn methylation, gave on hydrolysis methyl 3,4-di-O-methylglucoside; thus, C-1, C-2 and C-6 hydroxyl groups of glucose are linked to the galloyl groups. In the PMR spectrum of I, however, the coupling constant (J) of the anomeric proton was 3.7 Hz, and was smaller than that of 1,2,6-tri-O-galloyl-β-D-glucose (IV, 8.0 Hz).[1,2] Furthermore, in the carbon-13 nuclear magnetic resonance (CMR) spectrum, the anomeric carbon signal was observed at a field higher than that of β-anomer (IV, δ93.6).[1] Based on these facts and the large positive $[\alpha]_D$ value,[3] I was determined as 1,2,6-tri-O-galloyl-α-D-glucose.

Compound II contained five galloyl groups as revealed by the PMR and CMR spectra (Table I and II). On acid hydrolysis II yielded D-glucose and gallic acid. Methylation of II with diazomethane afforded a permethylate [II', $[\alpha]_D$ +67.3° (acetone, c = 0.18), MS; m/z 1150 (M^+)], which exhibited no hydroxyl absorption in the infrared (IR) spectrum; thus all glucose hydroxyl groups of II were galloylated.

Table I. PMR Spectral Data (δ Values)[a,b]

	I	IV	II	V	III	IIIa	IIIb	IIIc
glucose								
1-H	6.63 (d,J=3.7)	5.98 (d,J=8.0)	6.77 (d,J=3.7)	6.35 (d,J=8.0)	6.69 (d,J=3.0)	6.41 (d,J=4.0)	5.61 (d,J=4.3)	5.34 (d,J=4.4)
2-H	5.23 (d.d,J=3.7,9.7)	5.25 (t,J=8.0)	5.50 (d.d,J=3.7,10)	5.61 (d.d,J=8.0,9.0)	5.50 (d.d,J=3.0,7.5)	4.30 (d.d,J=4.0,7.5)	4.88 (d.d,J=4.3,8.0)	3.98 (d.d,J=4.4,6.8)
3-H	4.58 (t,J=9.7)	3.83 (t,J=8.0)	6.12 (d.d,J=10,10.5)	6.03 (t,J=9.0)	5.78 (d,J=7.5)	5.47 (d,J=7.5	5.91 (d.d,J=8.0,9.0)	5.46 (d.d,J=6.8,8.8)
4-H	5.33 (d,J=9.7)	4.53 (d,J=8.0)	5.80 (d,J=10,5)	5.66 (t,J=9.0)	5.08 (s)	5.02 (s)	4.60 (d,J=9.0)	4.55 (d,J=8.8)
5-H	3.71 (m)	3.94	4.50 (m)	4.61 (m)	4.39 (br.s)	4.25 (br.s)	4.54 (m)	4.49 (d,J=9.3)
6-H	4.20 (d,J=9.5)	4.08 (m)	4.37 (m)	4.22	4.02 (d,J=13.7)	3.94 (d,J=11.5)	3.86	3.70
6-H'	5.39 (d,J=9.5)	4.67 (d,J=12.0)	4.72 (d,J=11.5)	4.56 (m)	5.30 (d.d,J=3.9,13.7)	5.25 (d.d,J=2.5,11.5)	4.04 (m)	3.95 (m)
galloyl	7.12 7.24 7.27	7.08 7.10 7.16	7.03 7.04 7.11 7.23 7.31	6.98 7.02 7.06 7.12 7.18	6.98 7.17 7.24	7.15 7.33	6.95	—
HHDP	—	—	—	—	7.15 7.26	7.05 7.31	6.79 7.16	6.78 7.18

a) Measured in acetone-d_6 at 100MHz with TMS as an internal standard.
s: singlet, br s: broad singlet, d: doublet, d.d: double doublet, t: triplet, m: multiplet.
b) J-Values are expressed in Hz.

Table II. CMR Spectral Data (δValues)[a]

	I	IV	II	II'	V	III
glucose						
C-1	90.3	93.6	90.3	91.1	93.3	89.5
C-2	72.0	73.9	70.8	72.0	71.7	70.2
C-3	73.5[b]	75.5	71.4[c]	71.5	73.3	73.7
C-4	70.1	71.7	68.9	70.1	69.2	75.1
C-5	74.1[b]	76.0	71.5[c]	72.0	73.9	78.0
C-6	61.8	63.9	62.5	63.8	62.8	65.2
carbonyl						
galloyl	165.0 166.3 166.4	165.0 166.0 166.7	164.8 165.5 165.9 166.1 166.3	164.5 165.3 165.8 165.9 166.3	164.9 165.5 165.6 166.8 166.3	165.3 166.0 166.2
HHDP	—	—	—	—	—	167.1 167.3

a) Measured in acetone-d_6 at 25.05MHz with TMS as an internal standard.
b,c) Assignment may be interchangeable.

In the PMR spectrum of II, the coupling constant (J) of the anomeric proton was 3.7 Hz analogous to that of I. In the CMR spectrum, the anomeric carbon signal was also observed at δ90.3, which was higher than that of 1,2,3,4,6-penta-O-galloyl-β-D-glucose (V, δ93.3).[4] Therefore, II was determined as 1,2,3,

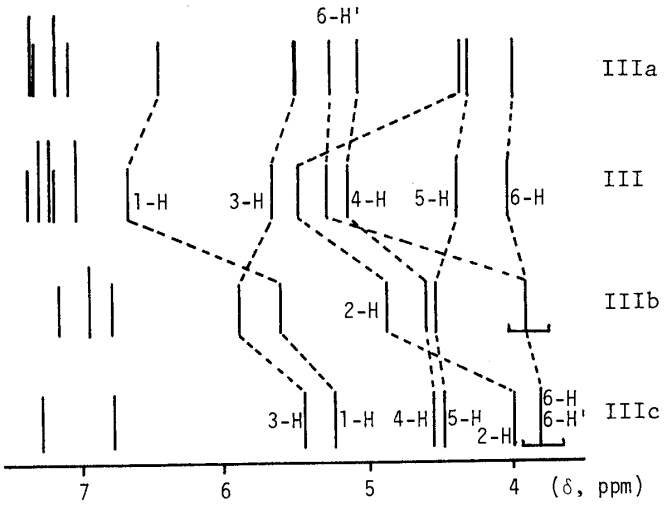
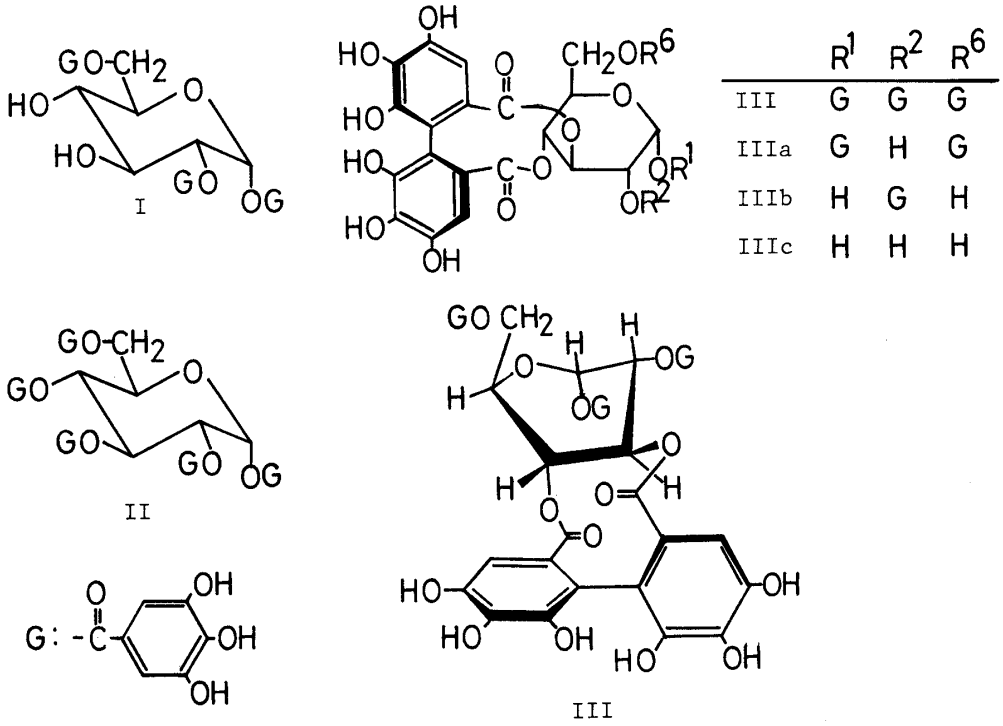


Fig. PMR Chemical Shifts of III, IIIa, IIIb and IIIc



	R^1	R^2	R^6
III	G	G	G
IIIa	G	H	G
IIIb	H	G	H
IIIc	H	H	H

Chart

4,6-penta-O-galloyl-α-D-glucose. The structure of II was further confirmed by comparing the spectral data of II' with those of an authentic sample synthesized from trimethoxybenzoyl chloride and D-glucose.

Compound III, named nupharin A, afforded D-glucose, gallic acid and ellagic acid on acid hydrolysis, and the PMR spectrum showed the presence of three galloyl groups and one hexahydroxydiphenoyl (HHDP) group. Methylation of nupharin A with DMS-K_2CO_3 gave a permethylate which revealed the M^+ ion peak at m/z 1148 in the mass spectrum and no hydroxyl absorption band in the IR spectrum; thus all glucose hydroxyl groups were esterified.

On partial hydrolysis with tannase, nupharin A furnished IIIa, IIIb, IIIc and gallic acid. The PMR and CMR spectra indicated that IIIa, IIIb and IIIc were mono-, di- and tri-desgalloyl nupharin A, respectively. As shown in the Fig., the signals due to 2-H in IIIa, 1-H and 6-H in IIIb, and 1-H, 2-H and 6-H in IIIc were shifted upfield as compared with those of nupharin A. The measurement of the deuterium-induced differential isotope shifts (DIS)[6] in IIIc also supported the presence of free hydroxyl groups at the C-1, C-2 and C-6 positions in the glucose residue (δ62.6, Δ0.12ppm, C-6; δ72.3, Δ0.12, C-2; δ92.4, Δ0.13, C-1); thus it is concluded the HHDP group is attached to the C-3 and C-4 positions in the glucose moiety.

The HHDP group of nupharin A was hydrolyzed selectively in boiling water to yield I as a main product. From this fact, the C-1 position of glucose in nupharin A was determined to have an α-configuration.

Since the circular dichroism spectra of nupharin A and the methylated hexahydroxydiphenic acid obtained by methanolysis of nupharin A permethylate showed a negative Cotton effect around 260 nm ($[\theta]_{265} = -6.6\times10^4$ for nupharin A), it was concluded that the atropisomerism of the HHDP group in nupharin A is an S-configuration.[7] Based on these data, nupharin A was characterized as 1,2,6-tri-O-galloyl-3,4-(S)-HHDP-α-D-glucose. The conformation of the glucose moiety in nupharin A has been presumed to be an intermediate between the B2 and 1B conformations on the basis of the coupling constants of glucose protons in the PMR spectrum.[8]

To our knowledge, this is the first report on the isolation of hydrolyzable tannins which have a galloyl group attached to α-hydroxyl group at the C-1 position of glucose.

REFERENCES

1) M. Nishizawa, T. Yamagishi, G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, Abstracts of Paper, 23rd Symposium of the Chemistry of Natural Products, Nagoya, October 1980, p. 514.
2) G. Nonaka, Y. Matsumoto, I. Nishioka, M. Nishizawa, and T. Yamagishi, Chem. Pharm. Bull., 29, 1184 (1981).
3) E. Fischer and M. Bergmann, Chem. Ber., 52, 829 (1919).
4) M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, Chem. Pharm. Bull., 28, 2850 (1980).
5) E. Haslam, R. D. Haworth, K. Jones, and H. J. Rogers, J. Chem. Soc., 1961, 1829.
6) P. E. Peffer, K. M. Valentine, and F. W. Parrich, J. Amer. Chem. Soc., 101, 1265 (1979).
7) Y. Ikeya, H. Taguchi, I. Yoshioka, and H. Kobayashi, Chem. Pharm. Bull., 27, 1383 (1979).
8) J. C. Jochins, G. Taigel, and O. T. Schmidt, Ann. Chem., 717, 169 (1968).

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