Communications to the Editor

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GEMIN B AND C, DIMERIC ELLAGITANNINS FROM *GEUM JAPONICUM*

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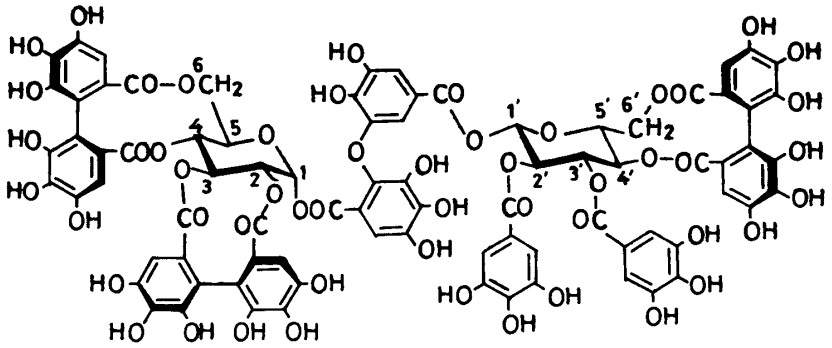
Two new dimeric ellagitannins, gemin B (**2**) and C (**3**), and a monomeric tannin, gemin D (**4**), have been isolated from *Geum japonicum*. Their structures were elucidated on the basis of NMR analysis and partial hydrolysis. Gemin D was isolated also from *Camellia japonica*.

KEYWORDS —— *Geum japonicum*; Rosaceae; gemin B; gemin C; gemin D; dimeric ellagitannin; *Camellia japonica*; Theaceae; ^{1}H- and ^{13}C-NMR; tannase

Gemin A (**1**), a novel dimeric ellagitannin from *Geum japonicum* (Rosaceae) has been reported recently.[1] Further examination of the leaf extract of this plant has led to isolation of three additional new tannins, named gemin B (**2**), C (**3**) and D (**4**), along with tellimagrandin I[2,3] and 1-*O*-galloyl-2,3-*O*-[(*S*)-hexahydroxydiphenoyl]-α-D-glucose.[4] Two of these, gemin D and tellimagrandin I were also isolated, along with pedunculagin,[3] from the flower buds of *Camellia japonica* (Theaceae).

These tannins were isolated from the *n*-butanol soluble portion of the crude extract by combined droplet counter-current and Sephadex LH-20 gel chromatography.

Both gemin B (**2**), $[\alpha]_D$ +67° (c=1.4, EtOH), and gemin C (**3**), $[\alpha]_D$ +133° (c=1.1, EtOH), were obtained as off-white amorphous powders and were analysed as $C_{68}H_{50}O_{44}\cdot 8H_2O$. Comparison of the ^{1}H- and ^{13}C-NMR spectra showed close similarity between these tannins. The presence of a dehydrodigalloyl (DHDG),[1] two hexahydroxydiphenoyl (HHDP) and two galloyl groups in each tannin was indicated by the ^{1}H-NMR resonances: **2**, δ 7.25 (1H, s), 7.28 and 6.85 (1H each, d, *J*=2 Hz)(DHDG); 6.73, 6.65, 6.51 and 6.50 (1H each, s)(HHDP x 2); 7.03 and 6.99 (2H each, s)(galloyl x 2); **3**, δ 7.33 (1H, s), 7.24 and 6.81 (1H each, d, *J*=2 Hz)(DHDG); 6.66, 6.61, 6.52 and 6.35 (1H each, s)(HHDP x 2); 7.05 and 7.00 (2H each, s)(galloyl x 2). Two anomeric protons of the coupling constants 3.5 and 8 Hz, besides the other twelve sugar protons (Table), were also exhibited by **2** and **3**. The presence of two monomeric hydrolyzable tannin units in these tannins was also indicated in the ^{13}C-NMR spectra by eight ester carbonyl carbon signals [**2**, δ 171.5, 170.1, 169.5, 169.1, 167.5, 166.7, 166.4 and 164.1; **3**, δ 169.6, 168.5 (2C), 168.0, 166.7, 166.1, 165.4 and 163.2]. Acid hydrolysis of these tannins gave glucose, and the glucose protons in the ^{1}H-NMR



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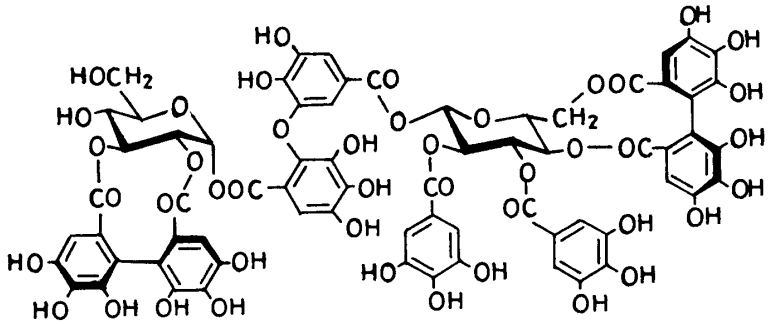

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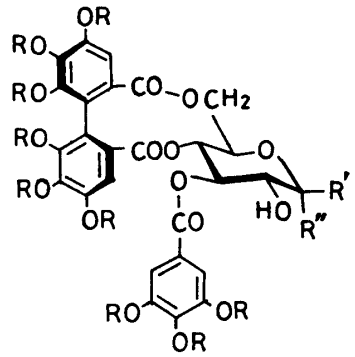
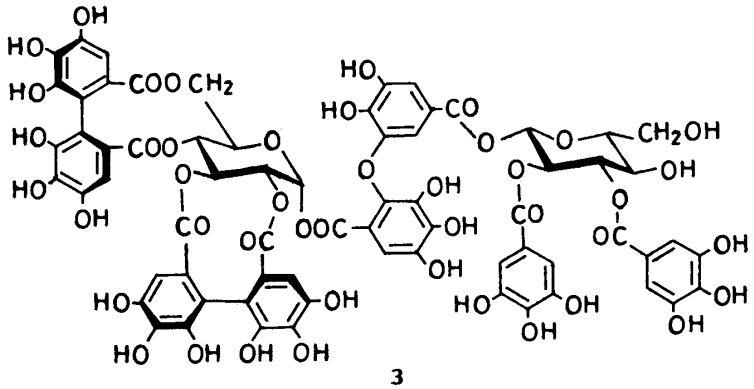


4 R=H, R',R"=H,OH
6 R=Me, R'=H, R"=OMe
7 R=Me, R'=OMe, R"=H



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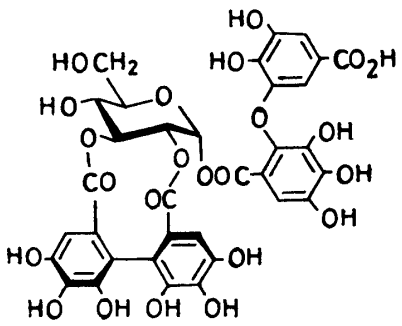

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spectra of **2** and **3** were confirmed by spin-spin decoupling experiments as summarized in the Table. The two glucose cores were shown to form α- and β-anomers of the *C1* conformation. These data show that **2** and **3** are dimeric ellagitannins closely related to gemin A (**1**). Free hydroxyl groups at C-4 and C-6 in **2** are indicated by the upfield shifts of H-4 and H-6 from those of **1**, and the other protons of essentially the same shifts between **1** and **2** (Table). The location of the hydroxyl groups at C-4' and C-6' in **3** was analogously determined. Partial hydrolysis of **2** and **3** with tannase yielded 1-*O*-dehydrodigalloyl-2,3-*O*-hexahydroxydiphenoyl-α-D-glucose (**5**),[5] $[\alpha]_D$ +56° (c=1.0, EtOH), which is also a product of the analogous treatment of **1**, to

establish the location of two aromatic groups on a glucose core. The location of the second hexahydroxydiphenoyl group in these tannins is regarded as O-4'~O-6' for **2**, and O-4~O-6 for **3**, based on the difference of the chemical shifts between the C-6 methylene protons, which is analogous to that exhibited generally[2,6] by the compounds in which one of the two ester linkages between a hexahydroxydiphenoyl group and glucose is formed at O-6 of the sugar. The *S*-configuration of the hexahydroxydiphenoyl groups in **2** and **3** was indicated by the strong positive Cotton effect around 235 nm in the CD spectra (**2**, $[\theta]_{236}$ +25.8 x 10^4; **3**, $[\theta]_{235}$ +20.4 x 10^4 in MeOH) which is analogous to that of pedunculagin.[7] The structures **2** and **3** were therefore assigned to gemin B and C, respectively.

Gemin D (**4**), $C_{27}H_{22}O_{18}\cdot 2H_2O$, $[\alpha]_D$ +40° (c=0.9, acetone), UV λ_{max}^{MeOH} nm (log ε) 221 (4.58), 265 (4.26), forms an off-white amorphous powder. Upon methylation with dimethyl sulfate and potassium carbonate in dry acetone, **4** afforded the α-anomer (**6**) and the β-anomer (**7**) of deca-*O*-methylgemin D (M^+ *m/z* 774), whose ^{1}H-NMR spectra (acetone-d_6) showed the presence of a galloyl and a hexahydroxydiphenoyl group [**6**, δ 7.29 (2H, s), 6.92 and 6.71 (1H each, s); **7**, δ 7.29 (2H, s), 6.93 and 6.69 (1H each, s)]. This result, coupled with absence of an anomeric proton signal in the region of δ 6.0-6.5 in the ^{1}H-NMR spectrum of **4**, indicates that the anomeric hydroxyl group in **4** is free. The formation of a mixture of α- and β-anomers was indicated by the double signals of the glucose protons as in the Table, as well as those of the galloyl [δ 7.01 and 7.02 (2H in total)] and hexahydroxydiphenoyl protons [δ 6.64, 6.63 (1H in total), and 6.43 and 6.41 (1H in total)].

Table. Chemical Shifts of the Glucose Protons of **1**, **2**, **3** and **4** in acetone-d_6 [200 MHz, δ(ppm) from TMS, *J*(Hz)]

	1	**2**[a]	**3**	**4**[b]
α-glucose				
H-1	6.56 (d,*J*=4)	6.42 (d,*J*=3.5)	6.53 (d,*J*=3.5)	5.28 (d,*J*=3.5)
H-2	5.38 (dd,*J*=4,10)	5.13 (dd,*J*=3.5,10)	5.35 (dd,*J*=3.5,10)	3.84 (dd,*J*=3.5,10)
H-3	5.54 (t,*J*=10)	5.44 (t,*J*=10)	5.49 (t,*J*=10)	5.51 (t,*J*=10)
H-4	5.19 (t,*J*=10)	4.03 (t,*J*=10)	5.14 (t,*J*=10)	4.95 (t,*J*=10)
H-5	4.52 (m)	3.80[d]	4.45 (dd,*J*=6,10)	4.58 (dd,*J*=7,10)
H-6	5.31 (dd,*J*=7,14)[c]		5.18 (dd,*J*=6,13)	5.26 (dd,*J*=7,13)
	3.69 (d,*J*=14)		3.61 (d,*J*=13)	3.74 (d,*J*=13)
β-glucose				
H-1'	6.17 (d,*J*=8)	6.15 (d,*J*=8)	6.01 (d,*J*=8)	4.75 (d,*J*=8)
H-2'	5.59 (dd,*J*=8,10)	5.60 (dd,*J*=8,9)	5.35 (dd,*J*=8,10)	3.58 (dd,*J*=8,10)
H-3'	5.85 (t,*J*=10)	5.83 (t,*J*=9)	5.56 (t,*J*=10)	5.33 (t,*J*=10)
H-4'	5.22 (t,*J*=10)	5.21 (t,*J*=9)	3.98 (t,*J*=10)	4.98 (t,*J*=10)
H-5'	4.52 (m)	4.52 (m)	3.80 (m)	4.13 (dd,*J*=6,10)
H-6'	5.24 (dd,*J*=6,14)[c]	5.28 (dd,*J*=6,13)		5.24 (dd,*J*=6,13)
	3.79 (d,*J*=14)	~3.80[d]		3.82 (d,*J*=13)

a) Measured in acetone-d_6-D_2O.
b) Mixture of α- and β-anomers in a ratio of 6:5.
c) Assignments for H-6 and H-6' may be reversed.
d) Overlapped by the signal of DHO.

The ester linkages on glucose were shown to be at C-3, -4 and -6 by comparison of the ^{1}H-NMR resonances with those of **1** (Table). Partial hydrolysis of **4** with tannase afforded 4,6-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose, $[\alpha]_D$ +41° (EtOH) and gallic acid. The structure of gemin D was thus assigned as 3-*O*-galloyl-4,6-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose (**4**).

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3) T. Okuda, T. Yoshida and M. Ashida, Heterocycles, 16, 1681 (1981).
4) Physico-chemical data are as follows: $[\alpha]_D$ +99.4° (c=1.6, acetone); UV λ_{max}^{MeOH} nm (log ε) 222 (4.66), 265 (4.29); ^{1}H-NMR (90 MHz, acetone-d_6-D_2O) δ 7.21 (2H, s, galloyl), 6.73, 6.48 (1H each, s, HHDP), 6.52 (1H, d, *J*=3.5 Hz, gluc H-1), 5.48 (1H, t, *J*=9 Hz, gluc H-3), 5.18 (1H, dd, *J*=3.5, 9 Hz, gluc H-2), 4.05-3.83 (4H, m, gluc H-4, 5 and 6). These data are coincident with those for sanguiin H-4, presented by T. Tanaka, G. Nonaka and I. Nishioka, at the 102nd Annual Meeting of the Pharmaceutical Society of Japan (April, 1982, Osaka).
5) ^{1}H-NMR (200 MHz, acetone-d_6) δ 6.69, 6.49 (1H each, s, HHDP), 7.26 (1H, s), 7.27 6.85 (1H each, d, *J*=2 Hz)(DHDG), 6.41 (1H, d, *J*=4 Hz, H-1), 5.10 (1H, dd, *J*=4, 10 Hz, H-2), 5.41 (1H, t, *J*=10 Hz, H-3), 4.00 (1H, t, *J*=10 Hz, H-4), 3.73 (3H, m, H-5 and 6).
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