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GEMINS D, E AND F, ELLAGITANNINS FROM *GEUM JAPONICUM**

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Key Word Index—*Geum japonicum*, Rosaceae, *Camellia japonica*, Theaceae, tannin, ellagitannin, gemin D, gemin E, gemin F, *C*-glucosidic tannin

Abstract—Three new ellagitannins, gemin D, E and F were isolated from the leaves of *Geum japonicum* The structures of gemin D and F were established as 3-*O*-galloyl-4,6-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose and 6-*O*-caffeoyl-2,3-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose, respectively Gemin E is a novel *C*-glucosidic ellagitanin having a dehydrohexahydroxydiphenoyl group in the molecule Gemin D was also isolated from the flower buds of *Camellia japonica*

INTRODUCTION

We previously reported the isolation of dimeric hydrolysable tannins, gemin A, B and C, and related monomeric tannins, potentillin, tellimagrandin I, tellimagrandin II, pedunculagin, 1,2,3-tri-*O*-galloyl-β-D-glucose, and 1-*O*-galloyl-2,3-*O*-[(*S*)-hexahydroxydiphenoyl]-α-D-glucose, from *Geum japonicum* which has been used as a diuretic and an astringent in Japan and China [1–3] Further investigation of the leaf extract of this plant has resulted in the isolation of three additional new hydrolysable tannins, named gemin D (**1**), E (**2**) and F (**3**), together with four known hydrolysable tannins Gemin D, along with pedunculagin and tellimagrandin I, were also isolated from the flower buds of *Camellia japonica* (Theaceae) which have been used as a haemostatic and an astringent in Japan and China In this paper, we report isolation and structural elucidation of these new tannins

RESULTS AND DISCUSSION

An aqueous acetone homogenate of the fresh leaves of *G japonicum* was fractionated by droplet counter-current chromotography and Sephadex LH-20 chromatography to yield gemin D (**1**), E (**2**) and F (**3**), along with casuarictin [4], casuarinin [4], geraniin [5], and praecoxin D [6], which were identified by direct comparisons with authentic samples The isolation of tannins from *C japonica* was carried out in a similar way

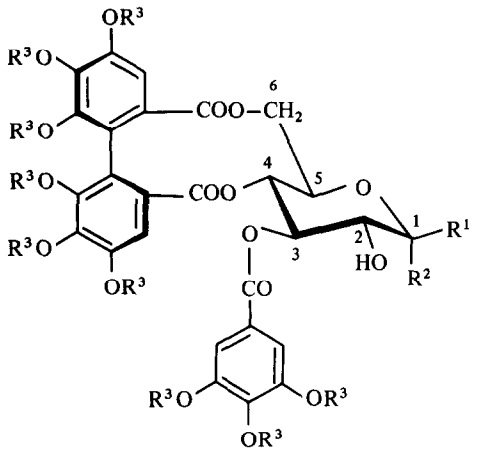
Gemin D (**1**), an off-white amorphous powder, $C_{27}H_{22}O_{18}$ $2H_2O$, showed the positive $FeCl_3$ reaction, and the characteristic colour of ellagitannins upon spraying the $NaNO_2$–HOAc reagent [7] on thin-layer chromatogram The TLC (cellulose) and HPLC (normal phase) analyses suggested its homogeneity, whereas the reversed-phase HPLC gave two peaks which changed to a single peak of different retention time upon the treatment with $NaBH_4$ This behaviour on HPLC is analogous to that of some ellagitannins which have a free hydroxyl group at the anomeric centre of the sugar moiety [8] The ^{1}H NMR spectrum of **1** showed the dual peak for each proton, and the absence of an anomeric proton signal in the region of δ 6 0–6 5 where a proton on the acylated anomeric centre is expected to appear These data coupled with appearance of two anomeric carbon signals of small peak height at δ 99 2 and 94 5 in the ^{13}C NMR spectrum indicate that the anomeric hydroxyl group in **1** is free Methylation of **1** with dimethyl sulphate and potassium carbonate in dry acetone furnished the α-anomer (**1a**) and β-anomer (**1b**) of deca-*O*-methylgemin D (M^+ *m/z* 774), which were separated by prep TLC Their ^{1}H NMR spectra exhibited the two 1-H singlets assignable to a hexahydroxydiphenoyl group and a 2-H singlet due to a galloyl group Upon methanolysis, **1a** yielded methyl tri-*O*-methylgallate, dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate and methyl α-D-glucoside The extensive decoupling experiments for **1** as well as **1a** and **1b** showed that H-2–H-5 of glucose have all axial configuration, which indicates the $^4C_1$ conformation of the glucopyranose ring Comparison of the ^{1}H NMR spectrum of **1** with that of gemin A (**4**) [1] indicates that the hydroxyl groups at C-3, C-4 and C-6 of glucose in **1** are acylated (Table 1) Selective degalloylation of **1** with tannase provided 4,6-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose and gallic acid Therefore, the structure of gemin D was established as 3-*O*-galloyl-4,6-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose (**1**)

Gemin E (**2**), a pale yellow amorphous powder, showed a characteristic colour of *C*-glucosidic ellagitannins such as casuarinin (**5**) and stachyurin (**6**) [4], by the reaction with the $NaNO_2$–HOAc reagent The ^{1}H NMR spectrum of **2** is analogous to that of **6** in the most part including the number of aromatic protons The main difference of **2** from **6** is that each signal in the former accompanies a small peak to form a paired-signal in the ratio of *ca* 4 1, and the former shows an extra singlet at δ 5 44 which is

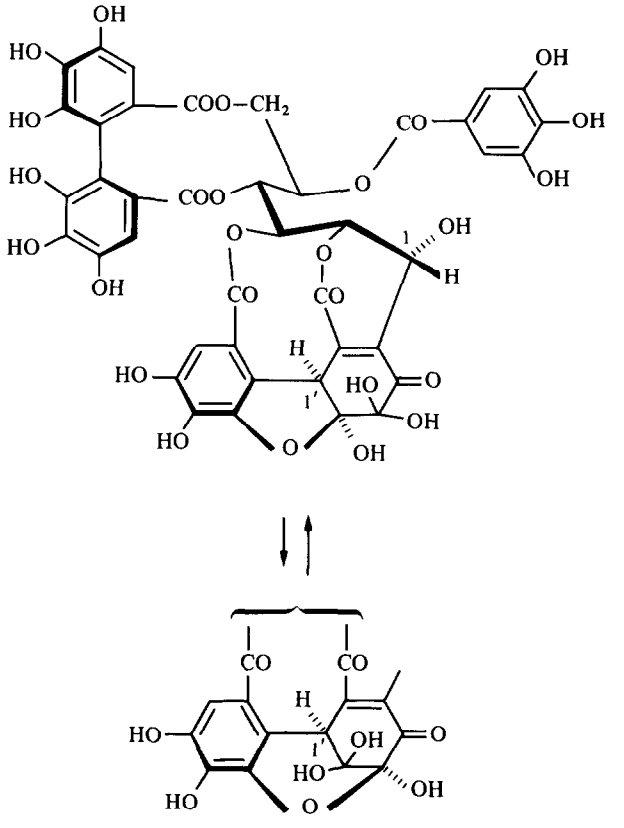
*Part 3 in the series of "Tannins of Rosaceous Medicinal Plants" For Part 2, see ref [3]

Table 1 ^{1}H NMR spectral data of the glucose moieties in **1**, **3** and **4** (200 MHz, Me_2CO-d_6, TMS as int standard, J in Hz)

		1	**3**	**4**
α-Glucose	H-1	5 28 *d* (J = 3 5)	5 41 *d* (J = 3 5)	6 56 *d* (J = 4)
	H-2	3 84 *dd* (J = 3 5, 10)	4 95 *dd* (J = 3 5, 9 5)	5 38 *dd* (J = 4, 10)
	H-3	5 51 *t* (J = 10)	5 41 *t* (J = 9 5)	5 54 *t* (J = 10)
	H-4	4 95 *t* (J = 10)	3 89 *t* (J = 9 5)	5 19 *t* (J = 10)
	H-5	4 58 *ddd* (J = 1, 7, 10)	4 22 *ddd* (J = 2 5, 5, 9 5)	4 52 *m*
	H-6	5 26 *dd* (J = 7, 13)	4 42 *dd* (J = 5, 12)	5 31 *dd* (J = 7, 14)
		3 74 *dd* (J = 1, 13)	4 51 *dd* (J = 2 5, 12)	3 69 *d* (J = 14)
β-Glucose	H-1′	4 75 *d* (J = 8)	5 05 *d* (J = 8)	6 17 *d* (J = 8)
	H-2′	3 58 *dd* (J = 8, 9)	4 75 *dd* (J = 8, 10)	5 59 *dd* (J = 8, 10)
	H-3′	5 33 *dd* (J = 9, 10)	5 07 *t* (J = 10)	5 85 *t* (J = 10)
	H-4′	4 98 *t* (J = 10)	3 87 *t* (J = 10)	5 22 *t* (J = 10)
	H-5′	4 13 *ddd* (J = 1, 6, 10)	3 81 *m*	4 52 *m*
	H-6′	5 24 *dd* (J = 6, 13)	4 55 *dd* (J = 2, 12)	5 24 *dd* (J = 6, 14)
		3 82 *dd* (J = 1, 13)	4 38 *dd* (J = 5, 12)	3 79 *d* (J = 14)



1 R^1, R^2 = H, OH, R^3 = H
1a R^1 = H, R^2 = OMe, R^3 = Me
1b R^1 = OMe, R^2 = H, R^3 = Me



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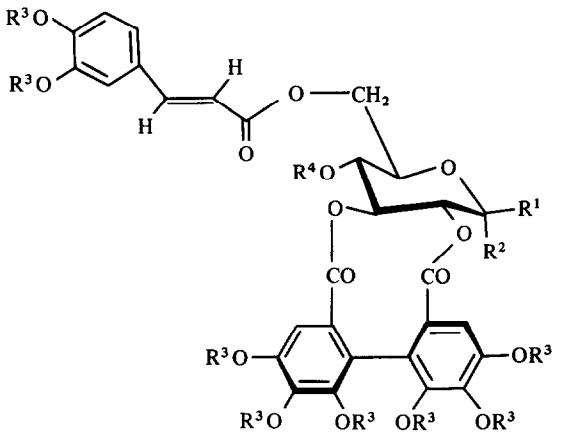
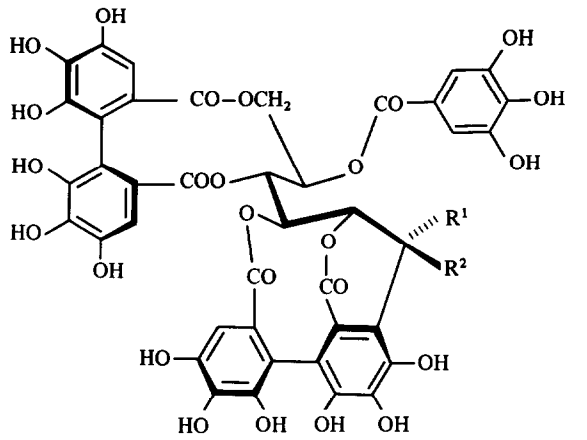
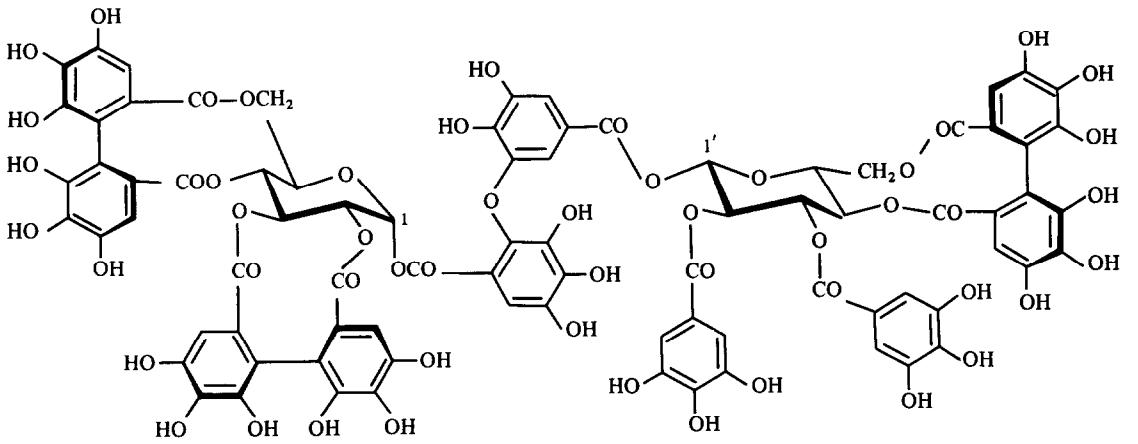
attributable to a methine proton of dehydrohexahydroxydiphenoyl (DHHDP) group [5] The presence of DHHDP group in **2** was also supported by the reaction with 2,4-dinitrophenylhydrazine Thus the appearance of dual peaks in the ^{1}H NMR spectrum of **2** is accounted for by the formation of an equilibrium mixture of the five- and six-membered hemiacetals in the DHHDP moiety like that of mallotusinic acid [9] and geranin in an aq soln [5] An allylic coupling (J = *ca* 2 Hz) between a methine proton and a vinyl proton on the α-carbon of α,β-unsaturated ketone system has been usually observed in the ^{1}H NMR spectrum of the five-membered hemiacetal structure of the DHHDP group, while sharp singlets have been shown by these protons in the six-membered



3 R^1, R^2 = H, OH, R^3 = R^4 = H
3a R^1 = R^4 = H, R^2 = OMe, R^3 = Me
3b R^1 = OMe, R^2 = R^4 = H, R^3 = Me
3c R^1 = H, R^2 = OMe, R^3 = R^4 = Me



5 R^1 = H, R^2 = OH
6 R^1 = OH, R^2 = H



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hemiacetal counterpart [5] Therefore, the sharp singlets of all signals in the aromatic region of **2** indicate that the α-carbon of α,β-unsaturated ketone system has no proton, and is thus participating in the formation of *C*-glucosidic linkage The position and orientation of DHHDP group in **2** was thus presumed to be as in the formula Examination of stereomodel suggests that the five-membered hemiacetal should be predominant over the six-membered counterpart which has severe strain in the *C*-glucosidic structure

The coupling constants observed for the glucose protons whose assignments were confirmed by the decoupling experiments, show close similarity to those of **6** rather than to those of **5** [4], indicating that the configuration of hydroxyl group at C-1 is α (Table 2)

The absolute configurations at both hexahydroxydiphenoyl group and C-1′ of the DHHDP group in **2** are shown to be *S*, by the CD spectrum ($[\theta]_{234}$ + 97 000, $[\theta]_{260}$ − 37 000 and $[\theta]_{335}$ + 2500), as the empirical rules [10, 11] indicate that the sign and amplitude of the Cotton effect around 235 nm reflect the absolute configuration and number of hexahydroxydiphenoyl group in the molecule, and the Cotton effect around 350 nm due to n–π^* transition of α,β-unsaturated ketone is associated with the configuration at C-1′ of the DHHDP group Based on these data, the most plausible structure of gemin E is represented by **2**

Gemin F (**3**), a light brown amorphous powder, was found, like gemin D, to be an ellagitannin possessing a free hydroxyl group at the anomeric centre of sugar portion, as revealed by its colouration with $NaNO_2$–HOAc, dual signal for each proton in the ^{1}H NMR spectrum, and similarity of the HPLC (normal and reversed-phases) pattern to that of **1** This characterization was further substantiated by the presence of signals at δ 92 0 and 95 4 in the ^{13}C NMR spectrum of **3**, which are attributable to the α- and β-anomeric carbons The ^{1}H NMR spectrum of **3** exhibited, in addition to the signals due to a hexahydroxydiphenoyl group, an AB system (2H) of a large coupling constant (J = 16 Hz) and an ABX system of three protons in a 1,3,4-trisubstituted phenyl ring, which are characteristic of a *trans*-caffeoyl group (Table 1) The presence of a

Table 2 ^{1}H NMR spectral data* of the glucose moieties in **2**, **5** and **6** (200 MHz, Me_2CO-d_6, TMS as int standard, J in Hz)

	2†	5	6
H-1	5 18 *br s*	5 64 *d* (J = 5)	4 93 *d* (J = 2)
H-2	4 58 *br s*	4 67 *dd* (J = 5, 2)	4 86 *t* (J = 2)
H-3	5 61 *d* (J = 2)		4 98 *t* (J = 2)
H-4	5 76 *dd* (J = 2, 10)	5 45–5 33	5 62 *dd* (J = 2, 9)
H-5	5 55 *dd* (J = 4, 10)		5 36 *dd* (J = 9, 3)
H-6	4 99 *dd* (J = 4, 13)	4 18 *dd* (J = 3, 13)	4 84 *dd* (J = 3, 13)
	3 94 *d* (J = 13)	4 06 *d* (J = 13)	4 02 *d* (J = 13)

*Assignments were confirmed by spin–spin decoupling experiments
†Data are of the predominant component in the equilibrium mixture (4 1)

caffeoyl group in **3** was also supported by the UV absorptions at 220, 230, 290 and 328 nm, and by the ^{13}C NMR spectrum (see Experimental) Upon methylation with dimethyl sulphate and potassium carbonate in acetone, **3** yielded nona-*O*-methyl-α-gemin F (**3a**), M^+ *m/z* 770, and its β-isomer (**3b**) Methanolysis of **3b** gave dimethyl (*S*)-4,4′,5,5′,6,6′-hexamethoxydiphenate, methyl caffeate and methyl β-D-glucoside These components are regarded as at O-2, O-3 and O-6 of glucose in **3** based on the comparison of its ^{1}H NMR spectrum with that of **4** as done for **1** (Table 1), as H-1 and H-4 in the α- and β-anomers of **3** are shifted significantly upfield from the corresponding signals of **4** This assignment was confirmed by production of methyl 4-*O*-methyl-α-D-glucoside upon methanolysis of the permethylated α-anomer (**3c**), M^+ *m/z* 784, which was prepared by the treatment of **3a** with BF_3–$CHCl_3$ in CH_2Cl_2 at −5°

The *S*-configuration of the hexahydroxydiphenoyl group in **3** is evident from its CD spectrum which displays a strong positive Cotton effect at 235 nm ([θ] + 87 300), and a negative one at 263 nm ([θ] − 29 700) [10] This assignment, combined with the $^4C_1$ conformation of glucose in **3**, indicate that the hexahydroxydiphenoyl group in **3** must be located at O-2 and O-3, since the *S*-hexahydroxydiphenoyl group can be only at either O-2–O-3 or O-4–O-6 of glucose having the $^4C_1$ conformation [12] Therefore the structure of gemin F was established as 6-*O*-*trans*-caffeoyl-2,3-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose (**3**)

Upon purification of gemin F by column chromatography on Sephadex LH-20, the *cis*-caffeoyl congener which was contaminated by a small amount of **3** was also isolated However, this compound may be an artefact from **3**, since the *cis–trans* isomerization of the caffeoyl and coumaroyl esters often occurs on Sephadex in daylight [13]

Although caffeic acid has been known to exist as glucose esters, and more frequently as D-quinic acid esters in plants [14], gemin F is the first example of an ellagitannin containing caffeoyl group

EXPERIMENTAL

^{1}H NMR and ^{13}C NMR spectra were measured at 200 MHz and 50 1 MHz, respectively with TMS as int standard EI-MS were recorded by using a direct inlet system at 70 eV TLC was carried out on Avicel and on Kieselgel PF_{254} plates (0 3 mm for analytical and 0 5 mm for prep TLC) using the following solvents (A) 7% HOAc, (B) *n*-BuOH–H_2O–HOAc (4 1 5), (C) C_6H_6–EtOH (15 1), (D) petrol–CH_2Cl_2–Me_2CO (6 3 1), (E) C_6H_6–Me_2CO (6 1) Spots were visualized by UV irradiation (254 nm) or by spraying $FeCl_3$ or $NaNO_2$–HOAc reagents Petrol refers to that fraction boiling in the range 85–120°

Isolation of tannins from G japonicum Fresh leaves (1 6 kg) of *G japonicum* were homogenized in 50% aq Me_2CO (4 3 1 × 3), and filtered through Celite-545 The homogenate was concd and extracted with Et_2O and EtOAc The EtOAc layer was evapd to give a dark brown residue (13 g) Aq layer was further extracted with *n*-BuOH, and *n*-BuOH was evapd to give a hygroscopic brown residue (20 g)

A portion (4 g) of the EtOAc extract was subjected to droplet counter-current chromatography (100 glass tubes, 3 2 mm × 120 cm) by ascending method, using *n*-BuOH–PrOH–H_2O (4 1 5) Every fifth fraction (12 g each) was monitored by the UV absorption at 280 nm, TLC (cellulose, solvent A) and HPLC (normal- and reversed-phases) Fractions 20–40 (454 mg) were combined and further purified by CC on Sephadex LH-20 using EtOH as eluant to yield gemin F (**3**) (32 mg), geranin [5] (35 mg) and tellimagrandin II [4] (27 mg) The *cis*-caffeoyl isomer (10 mg) of **3** was obtained from the fractions eluting before **3** Fractions 41–45 (346 mg) were similarly subjected to CC over Sephadex LH-20 eluting with EtOH to give praecoxin D [6] (14 mg), in addition to 1,2,3-tri-*O*-galloyl-β-D-glucose, tellimagrandin I [4], casuarictin [4] and potentillin Fractions 66–85 (227 mg) gave, after Sephadex LH-20 chromatography (EtOH), gemin E (**2**) (30 mg) and casuarinin (**5**) [4] (75 mg)

A portion (3 g) of the *n*-BuOH extract was fractionated by droplet counter-current chromatography in a way similar to that of the EtOAc extract The crude tannin obtained from fractions 91–140, was further purified by CC over Sephadex LH-20 to yield gemin D (**1**) (20 mg)

Isolation of tannins from C japonica Fresh flower buds (3 45 kg) of *C japonica* collected in March, was homogenized in 50% aq Me_2CO (13 1) After filtration through Celite-545, the homogenate was concd, and the resulting aq soln was extracted with Et_2O, and then continuously with EtOAc The aq soln was further extracted with *n*-BuOH, and the combined *n*-BuOH layers were evapd to yield a dark brown residue (18 6 g) A portion (5 g) of the *n*-BuOH extract was chromatographed over Sephadex LH-20 using EtOH–H_2O (4 1) as eluant to give gemin D (**1**) (65 mg), tellimagrandin II (30 mg) and pedunculagin [4] (20 mg)

Gemin D (**1**) An off-white, amorphous powder, R_fs (A, B) on TLC (cellulose), 0 68, 0 33 $[\alpha]_D^{20}$ + 40° (Me_2CO, *c* 0 9) (Found C, 50 42, 4 59 $C_{27}H_{22}O_{18}$ $2H_2O$ requires C, 50 39, H, 4 60%) UV λ_{max}^{MeOH} nm (log ε) 221 (4 58), 265 (4 26) IR ν_{max}^{KBr} cm^{-1} 3400,

1710, 1600, 1500, 1430, 1210, 1020 CD (MeOH). $[\theta]_{234}$ + 66 000, $[\theta]_{261}$ − 18 400, $[\theta]_{281}$ + 5000 ^{13}C NMR (Me_2CO-d_6) δ 169 6, 169 5, 169 0, 168 8, 168 2 (ester C=O), 99 1, 94 4, 74 7, 72 2, 71 9, 71 8, 71 6, 71 5, 67 7, 64 3 (glucose). ^{1}H NMR (Me_2CO-d_6) δ 7 01 and 7 02 (2H in total, each *s*), 6 64 and 6 63 (1H in total, each, *s*), 6 43 and 6 41 (1H in total, each, *s*), glucose moiety, see Table 1

Methylation of **1** Gemin D (**1**) (30 mg) was methylated with Me_2SO_4 (70 μl) and K_2CO_3 (150 mg) in dry Me_2CO (2 ml) under reflux for 1 hr Removal of the solvent after filtration gave a syrupy residue which was purified by prep TLC (silica gel, solvent C) to give α- (4 5 mg) and β-anomers (1 3 mg) of deca-*O*-methylgemin D *Deca-O-methyl-α-gemin D* (**1a**) An off-white, amorphous solid, R_f 0 40 on TLC (silica gel, solvent C) MS *m/z* 774 $[M]^+$, 404, 212, 195 ^{1}H NMR (Me_2CO-d_6) δ 7 29 (2H, *s*, galloyl), 6 92, 6 71 (1H each, *s*, HHDP*), 4 87 (1H, *d*, *J* = 3 5 Hz, H-1), 5 49 (1H, *t*, *J* = 10 Hz, H-3), 5 25 (1H, *dd*, *J* = 6, 13 Hz, H-6), 5 02 (1H, *t*, *J* = 10 Hz, H-4), 4 40 (1H, *dd*, *J* = 3 5, 10 Hz, H-2), 3 90 (H-6', overlapped by OMe signal), 3 95–3 51 (OMe × 10) *Deca-O-methyl-β-gemin D* (**1b**) An off-white, amorphous solid, R_f 0 30 on TLC (silica gel, solvent C) MS *m/z* 774 $[M]^+$ ^{1}H NMR (Me_2CO-d_6) δ 7 29 (2H, *s*, galloyl), 6 93, 6 69 (1H each, *s*, HHDP), 5 38 (1H, *dd*, *J* = 9, 10 Hz, H-3), 5 25 (1H, *dd*, *J* = 6, 13 Hz, H-6), 5 03 (1H, *t*, *J* = 10 Hz, H-4), 4 88 (1H, *d*, *J* = 5 Hz, C_2–OH), 4 50 (1H, *d*, *J* = 8 Hz, H-1), 4 21 (1H, *ddd*, *J* = 1, 6, 10 Hz, H-5), 3 95 (1H, *dd*, *J* = 3, 13 Hz, H-6'), 3 75 (H-2, overlapped by OMe signal), 3 95–3 49 (OMe × 10)

Methanolysis of **1a** A mixture of **1a** (6 mg) and 1% NaOMe (0 1 ml) in absolute MeOH (1 ml) was left overnight at room temp The reaction mixture was neutralized with HOAc and the solvent was evapd with flushing nitrogen stream at room temp The residue was treated with CH_2N_2 and was partitioned between CH_2Cl_2 and H_2O The CH_2Cl_2 layer was evapd and purified by prep TLC (silica gel, solvent D) to afford methyl tri-*O*-methylgallate, MS *m/z* 226 $[M]^+$, and dimethyl (*S*)-4,4',5,5',6,6'-hexamethoxydiphenate, MS *m/z* 450 $[M]^+$, $[\alpha]_D$ − 37° (EtOH, *c* 0 6), which were identified with authentic samples by $[\alpha]_D$ and mass spectra The aq layer gave methyl α-D-glucoside which was identified by GLC of TMSi ether

Enzymatic hydrolysis of **1** A soln of **1** (35 mg) in H_2O (2 ml) was incubated with tannase for 5 days at 37° The residue after evapn was purified by CC over Sephadex LH-20 eluting with EtOH to give gallic acid (7 mg) and 4,6-*O*-[(*S*)-hexahydroxydiphenoyl]-D-glucose (13 mg), $[\alpha]_D^{20}$ + 41° (EtOH, *c* 1 0), which were identified with authentic specimens by TLC (cellulose, solvent A) and HPLC (reversed-phase)

Gemin E (**2**) A pale yellow, hygroscopic amorphous powder, R_fs (A, B) on TLC (cellulose) 0 50, 0 41 $[\alpha]_D^{20}$ + 2 3° (Me_2CO, *c* 1 2) UV λ_{max}^{MeOH} nm (log ε) 221 (4 72), 270 (4 32) CD (MeOH) $[\theta]_{234}$ + 97 000, $[\theta]_{260}$ − 37 000, $[\theta]_{335}$ + 2500 ^{1}H NMR (Me_2CO-d_6) δ 7 17 (2H, *s*), 6 96 and 6 90 (each *s*, 1H in total), 6 60 and 6 59 (each *s*, 1H in total), 6 42 and 6 41 (each *s*, 1H in total), glucose moiety, see Table 2

Gemin F (**3**) A pale yellow, amorphous powder HPLC (reversed phase) showed two peaks of t_R 10 8 and 14 4 min which were changed to a single peak of t_R 6 6 min after reduction with $NaBH_4$ R_fs (A, B) on TLC (cellulose) 0 35, 0 41 $[\alpha]_D^{18}$ + 43° (Me_2CO, *c* 1 0) (Found C, 52 17, H, 4 54 $C_{29}H_{24}O_{17}$ 3/2H_2O requires C, 51 87, H, 4 05%) UV λ_{max}^{MeOH} nm (log ε) 220 (4 53), 230 sh (4 46), 290 (4 17), 328 (4 14) IR ν_{max}^{KBr} cm^{-1} 3400, 1740, 1690, 1600, 1510, 1440, 1350, 1380, 1170 CD (MeOH) $[\theta]_{235}$ + 87 300, $[\theta]_{263}$ − 29 700 ^{13}C NMR (Me_2CO-d_6) δ 170 7, 170 5, 170 3, 170 2, 168 7 (ester C=O), 127 8, 115 8, 145 9, 150 0, 149 6, 117 1, 123 4, 142 0, 115 3 (*trans*-caffeoyl), 95 4, 92 0, 78 6, 78 3, 78 2, 75 8, 71 1, 69 1, 68 9, 64 4, 64 3 (glucose) ^{1}H NMR (Me_2CO-d_6) δ 7 58 (4/7H, *d*, *J* = 16 Hz), 7 59 (3/7H, *d*, *J* = 16 Hz), 7 19 (4/7H, *d*, *J* = 2 Hz), 7 20 (3/7H, *d*, *J* = 2 Hz), 6 87 (4/7H, *d*, *J* = 8 Hz), 6 89 (3/7H, *d*, *J* = 8 Hz), 6 70 (4/7H, *s*), 6 69 (3/7H, *s*), 6 60 (1H, *s*), 6 36 (4/7H, *d*, *J* = 16 Hz), 6 37 (3/7H, *d*, *J* = 16 Hz)

The *cis*-caffeoyl isomer was not fully characterized because of contamination by **3** in the ratio of *ca* 8 1 as revealed by the ^{1}H NMR spectrum The signals due to the *cis*-isomer (α-anomer· β-anomer, *ca* 4 3) δ 7 75, 7 73 (1H in total, each *d*, *J* = 2 Hz), 7 16 (1H, *dd*, *J* = 2, 8 Hz), 6 82, 6 83 (1H in total, each *d*, *J* = 8 Hz), 6 84, 6 83 (1H in total, each *d*, *J* = 13 Hz), 5 82 (1H, *d*, *J* = 13 Hz) (*cis*-caffeoyl), 6 70, 6 69 (1H in total, each *s*), 6 59 (1H, *s*) (HHDP), 5 42 (*d*, *J* = 3 5 Hz, H-1 of α-glucose), 4 90 (*d*, *J* = 8 Hz, H-1 of β-glucose)

Methylation of **3** A mixture of **3** (25 mg), K_2CO_3 (125 mg) and Me_2CO_3 (70 μl) in dry Me_2CO was stirred for 12 hr at room temp, and then refluxed for 2 hr Usual work-up gave a syrupy residue which was purified by prep TLC (silica gel, solvent D) developed three times, to yield nona-*O*-methyl-α-gemin F (**3a**) (7 5 mg) and its β-anomer (**3b**) (6 5 mg) *Nona-O-methyl-α-gemin F* (**3a**) A white amorphous solid, R_f 0 31 on TLC (silica gel, solvent C) $[\alpha]_D^{20}$ + 35 5° ($CHCl_3$, *c* 1 1) UV λ_{max}^{MeOH} nm (log ε) 213 (4 71), 295 (4 20), 320 (4 18) MS *m/z* 770 $[M]^+$, 404, 208, 191 ^{1}H NMR ($CDCl_3$) δ 7 70 (1H, *d*, *J* = 16 Hz), 6 88 (1H, *d*, *J* = 8 Hz), 7 13 (1H, *dd*, *J* = 2, 8 Hz), 7 08 (1H, *d*, *J* = 2 Hz), 6 37 (1H, *d*, *J* = 16 Hz) (*trans*-caffeoyl), 6 77, 6 79 (1H each, *s*, HHDP), 4 99 (1H, *d*, *J* = 3 5 Hz, H-1), 5 15 (1H, *dd*, *J* = 3 5, 10 Hz, H-2), 5 48 (1H, *t*, *J* = 10 Hz, H-3), 4 86 (1H, *dd*, *J* = 3, 12 Hz, H-6), 4 35 (1H, *dd*, *J* = 2, 12 Hz, H-6'), 3 12 (1H, *d*, *J* = 4 Hz, C_4-OH), 3 90 (H-4 and H-5, overlapped by OMe signals), 3 90–3 49 (OMe × 9) *Nona-O-methyl-β-gemin F* (**3b**) An off-white, amorphous powder, R_f 0 27 (C) on TLC (silica gel) $[\alpha]_D^{20}$ − 14 4° ($CHCl_3$, *c* 0 56) UV λ_{max}^{MeOH} nm (log ε) 208 (4 63), 290 (4 10), 320 (4 06) MS *m/z* 770 $[M]^+$, 404, 208, 191 ^{1}H NMR ($CDCl_3$) δ 7 71 (1H, *d*, *J* = 16 Hz), 6 39 (1H, *d*, *J* = 16 Hz), 6 89 (1H, *d*, *J* = 8 Hz), 7 31 (1H, *dd*, *J* = 2, 8 Hz), 7 08 (1H, *d*, *J* = 2 Hz) (*trans*-caffeoyl), 6 77, 6 80 (1H each, *s*, HHDP), 4 64 (1H, *d*, *J* = 8 Hz, H-1), 4 97 (1H, *dd*, *J* = 8, 9 5 Hz, H-2), 5 20 (1H, *t*, *J* = 9 5 Hz, H-3), 3 83 (1H, *dt*, *J* = 3 5, 9 5 Hz, H-4), 3 13 (1H, *d*, *J* = 3 5 Hz, C_4-OH), 4 87 (1H, *dd*, *J* = 3, 12 Hz, H-6), 4 38 (1H, *dd*, *J* = 2, 12 Hz, H-6'), 4 07 (1H, *m*, H-5), 3 90–3 60 (OMe × 9)

Permethylation of **3a** *with* BF_3–CH_2N_2 To a soln of **3a** (9 mg) in dry $CHCl_3$ (2 ml) was added BF_3–etherate (5 μl) and an excess of CH_2N_2 at − 5° After 4 hr, white solid (polymethylene) was filtered off, and the filtrate was evapd and purified by prep TLC (silica gel, solvent E) to yield deca-*O*-methyl-α-gemin F (**3c**) (5 mg), $[\alpha]_D^{18}$ − 45 5° ($CHCl_3$, *c* 0 6) UV λ_{max}^{MeOH} nm (log ε) 205 (4 82), 225 (4 19), 300 (3 64) MS *m/z* 784 $[M]^+$ ^{1}H NMR ($CDCl_3$) δ 7 68 (1H, *d*, *J* = 16 Hz), 6 38 (1H, *d*, *J* = 16 Hz), 6 88 (1H, *d*, *J* = 8 Hz), 7 13 (1H, *dd*, *J* = 2, 8 Hz), 7 11 (1H, *diff s*) (*trans*-caffeoyl), 6 78, 6 69 (1H each, *s*, HHDP), 4 98 (1H, *d*, *J* = 3 5 Hz, H-1), 5 19 (1H, *dd*, *J* = 3 5, 10 Hz, H-2), 5 58 (1H, *t*, *J* = 10 Hz, H-3), *ca* 3 90 (H-5, overlapped by OMe), 4 51 (2H, *br s*, H-6, 6'), 3 47–3 95 (OMe × 10)

Methanolysis of **3c** A mixture of **3c** (2 mg) and 1% NaOMe (50 μl) in absolute MeOH (0 2 ml) was left overnight at room temp After neutralization with HOAc, the solvent was evapd with nitrogen stream at room temp A portion of the residue was methylated with CH_2N_2 and the products were identified as methyl di-*O*-methyl-caffeate and dimethyl (*S*)-4,4',5,5',6,6'-hexamethoxydiphenate by co-chromatography with authentic samples on TLC (silica gel, solvent E) Another portion of the residue was trimethylsilylated, and subjected to GLC and GC-MS analyses on which the methylated sugar was identified as methyl 4-*O*-methyl-α-D-glucoside

*HHDP hexahydroxydiphenoyl

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