Tannins of Tamaricaceous Plants. V.[1] New Dimeric, Trimeric and Tetrameric Ellagitannins from *Reaumuria hirtella*

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A new hydrolyzable tannin dimer [hirtellin G (1)], a trimer [hirtellin T_1 (5)] and a tetramer [hirtellin Q_1 (6)] have been isolated from the 70% acetone extract of the leaves of *Reaumuria hirtella* JAUB. *et* SP. (Tamaricaceae). These tannins were characterized as oligomers composed of two, three or four molecules of tellimagrandin II which are similarly linked with each other through one, two or three dehydrodigalloyl (*m*-GOG) units. Their structures including the orientations of the *m*-GOG units have been elucidated based on chemical degradation and spectroscopic analyses.

Keywords ellagitannin; oligomeric hydrolyzable tannin; *Reaumuria hirtella*; Tamaricaceae; hirtellin T_1; hirtellin Q_1

We previously reported the isolation of new hydrolyzable tannin monomers (remurins A and B) and dimers (hirtellins A—F and tamarixinins A—C)[1,2] having a GOG[3] (dehydrodigalloyl = DHDG = *m*-GOG and/or iso-DHDG = *p*-GOG) group in each molecule, from Tamaricaceous plants, *Reaumuria hirtella* JAUB. *et* SP. and *Tamarix pakistanica* QUAISER (Tamaricaceae). Some of them exhibited significant biological activities, such as promotion of iodination in leucocytes,[4] and host-mediated antitumor activity against sarcoma-180 in mice.[5] Further investigation of the tannin constituents of *R. hirtella* has resulted in the isolation of three additional new oligomeric hydrolyzable tannins, named hirtellin G, hirtellin T_1 and hirtellin Q_1. This paper deals with the isolation and structural elucidation of these new oligomers.

Repeated column chromatographies of the EtOAc-soluble portion of the aqueous acetone homogenate of the dried leaves[2c] over Toyopearl HW-40 and MCI-gel CHP-20P gave hirtellin G (**1**). Hirtellin T_1 (**5**) and hirtellin Q_1 (**6**) were obtained from the *n*-BuOH extract as described in Experimental.

These new compounds showed positive coloration with the $FeCl_3$ and $NaNO_2$–AcOH reagents[6] on a TLC plate, and were characterized as ellagitannin oligomers structurally related to hirtellin A,[2b] as described below.

Structure of Hirtellin G (1) Hirtellin G (**1**) showed the $[M+Na]^+$ ion peak at *m/z* 1595 in the FAB-MS, which is 302 mass units [corresponding to a hexahydroxydiphenyl (HHDP) group] less than that of hirtellin A (**3**). Methylation of **1** with diazomethane followed by methanolysis with sodium methoxide gave methyl tri-*O*-methylgallate (**7**), dimethyl hexamethoxydiphenate (**8**) and dimethyl penta-*O*-methyldehydrodigallate (**9**). The ^{1}H-NMR spectrum of **1** showed two sets of seven-spin system signals due to *C1* glucopyranose as revealed by ^{1}H–^{1}H shift correlation spectroscopy (COSY) (Table I). The presence of a *m*-GOG[3] unit and an HHDP and four galloyl groups as constituent phenolic units in **1** was

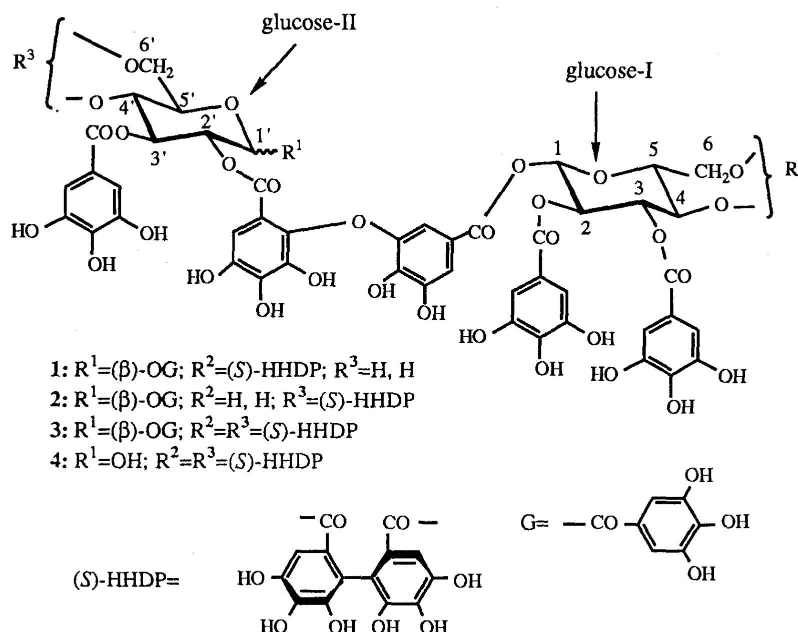

Chart 1

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indicated by three 1H-singlets, two meta-coupled 1H doublets, two 2H-singlets and one 4H-singlet; in the aromatic region from δ 6.39 to 7.25 ppm. Upon ^{1}H-NMR spectral comparison of **1** and **3** (Table I), the chemical shifts and coupling pattern of the proton signals of a glucose core (glucose-I) in these compounds were similar to each other, while the H-4′—6′ signals (δ 3.55—3.86) of the other glucose core (glucose-II) in **1** were shifted significantly upfield ($\Delta\delta$ *ca.* 1.4) relative to those of **3**. These upfield shifts implied the absence of an HHDP group at O-4′ and O-6′ of glucose-II in **1**. Hirtellin G (**1**) was thus assumed to be an analog of hirtellin A (**3**) lacking an HHDP group on the glucose-II. The structure **1** for hirtellin G was chemically supported by partial acid hydrolysis of hirtellin A (**3**), which afforded dimeric hydrolyzates **1** and **2**, besides monomeric hydrolyzates **11**, **14** and **17**.[2b)] Hydrolyzate **2** was characterized as a regioisomer of **1** concerning the position of HHDP group, as follows. The ^{1}H-NMR spectrum of **2** showed the presence of two *C1* glucopyranose cores (Table I), a *m*-GOG unit, an HHDP and four galloyl groups as found in **1**. The chemical shifts of glucose-II proton signals in **2** were almost identical with those of hirtellin A (**3**), whereas the H-4—H-6 signals (δ 3.81—4.02) of glucose-I showed large upfield shifts relative to those of **3**, indicating the absence of an HHDP group at O-4 and O-6 of glucose-I.

The characteristic upfield shift of the anomeric proton of glucose-II in **1** (δ 5.24) relative to that of 1,2,3-tri-*O*-galloyl-β-D-glucose[7)] (δ 6.02) showed that the binding mode (orientation) of the *m*-GOG unit in **1** is the same as that of hirtellin A (**3**).[2b)] Based on these data, the structure of hirtellin G is represented as **1**.

Structure of Hirtellin T_1 (5) Hirtellin T_1 (**5**) was established as a trimer composed of hirtellin A (**3**) and tellimagrandin II (**10**), which are linked with each other forming a *m*-GOG unit, as follows. The trimeric nature of hirtellin T_1 was shown by stronger adsorption on Toyopearl HW-40 gel as compared with **3** and other dimers, and by its retention time, which was similar to that of trimers,[8)] in normal-phase HPLC. Methylation of **5** with dimethyl sulfate and potassium carbonate in dry acetone afforded the permethylated derivative **5a**. Upon methanolysis with sodium methoxide, **5a** gave **7**, **8** and **9**, which were identical with those obtained by similar treatment of hirtellin A (**3**).[2b)] The sugar liberated upon complete acid hydrolysis of **5** was identified as glucose by GLC after trimethylsilylation. The ^{1}H and ^{13}C-NMR spectra of **5** indicated the presence of three glucose residues in the molecule. The chemical shifts and coupling patterns of the glucose proton signals, which were assigned as shown in Table II by ^{1}H–^{1}H COSY, suggested that all the three glucose cores adopt the *C1* conformation, and are fully acylated. The ^{1}H-NMR spectrum also displayed five 2H singlets at δ 7.02, 7.00, 6.95, 6.95 and 6.91, eight 1H singlets and two pairs of 1H doublets ($J=2$ Hz) in the aromatic region. These data, together with the fifteen ester carbonyl carbon signals (δ 163.71—168.31) in the ^{13}C-NMR spectrum, indicated that **5** is a trimeric ellagitannin composed of five galloyl, two *m*-GOG and three HHDP groups, and three glucose cores, and that its molecular formula corresponds to $C_{123}H_{86}O_{78}$. The presence of the HHDP group at O-4 and O-6 of each glucopyranose core was evident from the large difference among the chemical shifts of each C-6 methylene proton[9)] ($\Delta\delta$ *ca.* 1.4 ppm), as also seen in **3**. The absolute configuration at the HHDP groups of **5** was determined to be the same as that of hirtellin A (**3**)[2b)] (*S*-configuration) on the basis of the levorotatory optical activity of **8**, and the strong positive Cotton effect at 235 nm ($[\theta]+34.7\times10^{4}$) in the CD spectrum.[10)] Based on these data, coupled with the fact that the chemical shifts of glucose carbon signals in the ^{13}C-NMR spectrum of **5** are closely similar to those of **10**[11)] and **3** (Table III), hirtellin T_1 (**5**) was regarded as a trimer, biogenetically produced by intermolecular C-O oxidative coupling between **10** and **3** forming a *m*-GOG unit. The orientation of the two *m*-GOG units, which link the three glucose moieties in **5**, was deduced from the following observations. The hydrolyzable tannins possessing a *m*-GOG unit at O-2 of *C1* glucopyranose generally show a remarkable upfield shift of the anomeric proton due to the anisotropic effect of the aromatic ring of the *m*-GOG unit.[2b)] This characteristic anomaly is useful for determining the position of the *m*-GOG unit. In the ^{1}H-NMR spectrum of **5**, the proton signals of a glucose core (glucose-I) were in agreement with those of **10** or of glucose-I in **3** (Table I), while those of the other two glucoses (II and III), including the characteristic upfield shifts (δ 5.77, 5.50) of the anomeric protons (H-1′, H-1″) were closely similar to those of glucose-II of **3**. Hirtellin T_1 (**5**) was thus concluded to have two *m*-GOG units with the same binding mode as that of **3**, *i.e.*, the *m*-GOG units bind three glucoses in the following sequence; O-1—O-2′ and O-1′—O-2″. Further chemical evidence in support of this linking mode was obtained by partial degradation of **5** in hot water, which afforded **11**, **14** and **17** together with a new compound **18**, as major hydrolyzates (Chart 5). The ^{1}H-NMR spectrum of the hydrolyzate **18** showed two sets of fully acylated glucose proton signals, of which one (H-1—6) was similar to that of remurin A (**14**).[2b)] The other set of signals (H-1′—6′) was similar to that of glucose-II of hirtellin A (**3**)[2b)] (Table I). The presence of two *m*-GOG, two HHDP and three galloyl groups in **18** was shown by three 2H singlets, six 1H singlets and two pairs of 1H doublets ($J=2$ Hz) in the aromatic region (δ 6.40—7.19). The significant upfield shifts of the anomeric protons H-1 (δ 5.51—5.55) and H-1′ (δ 5.38) in **18** are consistent with the presence of the *m*-GOG unit at O-2 of each glucose. This hydrolyzate was thus characterized as a dimer represented by formula **18**. Based on these data, the structure **5** was assigned to hirtellin T_1.

Structure of Hirtellin Q_1 (6) Hirtellin Q_1 (**6**) showed a longer retention time than the trimer **5** in normal-phase HPLC,[8)] suggesting its tetrameric nature. Methylation of **6** with diazomethane followed by methanolysis with sodium methoxide gave **7**, **8** and **9**, which are the same compounds as those obtained from hirtellin A (**3**) and hirtellin T_1 (**5**). The sugar component liberated upon acid hydrolysis was identified as glucose. The ^{1}H-NMR spectrum (Table II) of **6** showed the presence of six galloyl, four HHDP groups and three *m*-GOG units, as well as four glucose cores, which were supported by the ^{13}C-NMR

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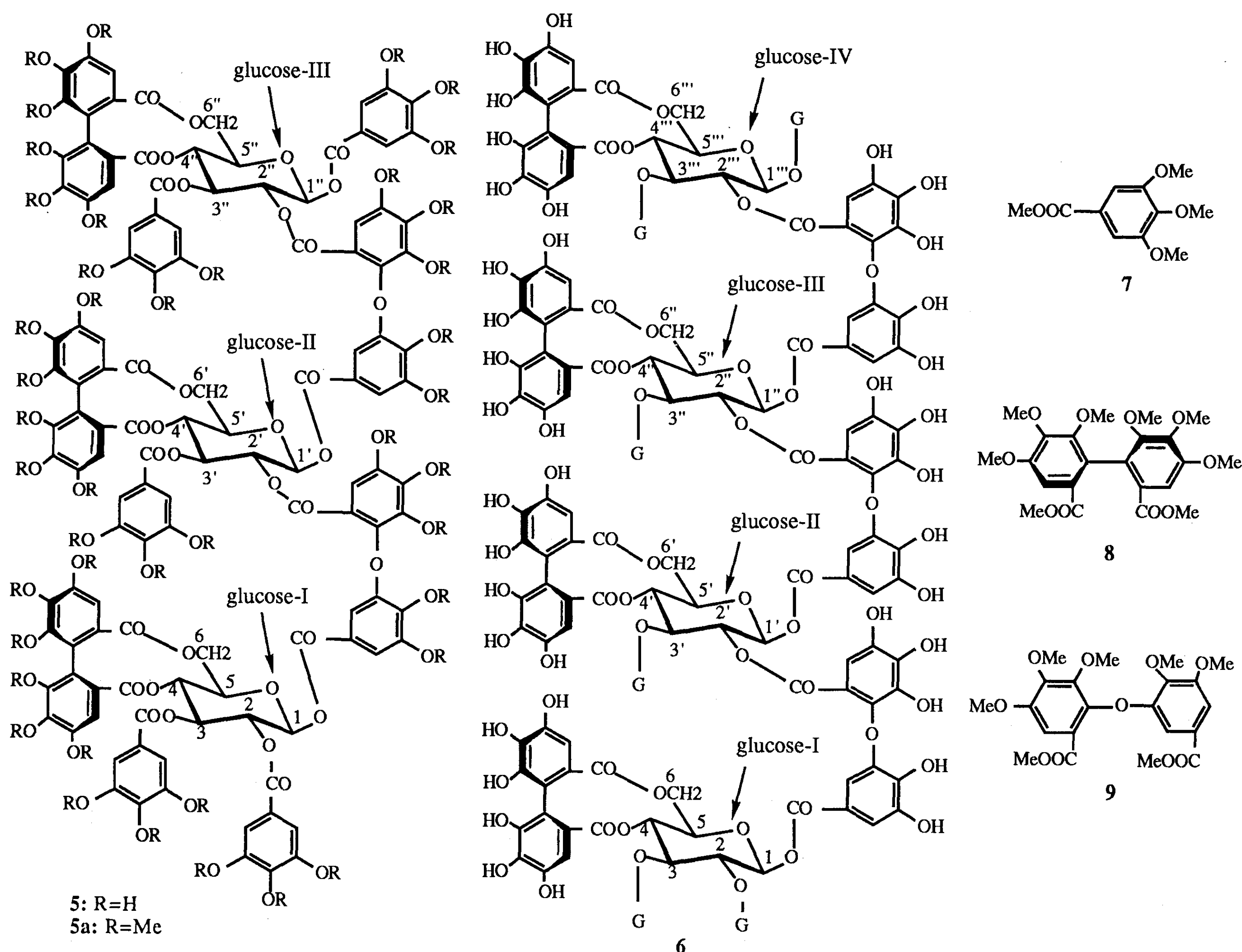

Chart 2

spectrum exhibiting four sets of glucose carbon signals closely similar to those of hirtellin A (**3**) and hirtellin T_1 (**5**) (Table III), and twenty ester carbonyl carbon signals at δ 163.71—168.31. Moreover, the sugar proton signals in the ^{1}H-NMR spectrum of **6** were in agreement with the sum of those of **5** and glucose-II of **3** (Tables I and II). The orientations of the *m*-GOG units in **6** were assumed to be the same as those in **3** or **5** on the basis of characteristic upfield shifts ($\Delta\delta$ 0.37—0.76) of the three anomeric protons (H-1′—H-1‴). The CD spectrum of **6** showed a strong positive Cotton effect at 233 nm ($[\theta]+47.2\times10^4$), of the same sign as that of **10**[9] but with an amplitude about 4 times larger, indicating the presence of four (*S*)-HHDP groups in the molecule.[10] The partial hydrolysis of **6** with hot water gave seven monomeric (**11**, **12**, **13**, **14**, **15**, **16** and **17**), two dimeric (**4** and **18**) and a trimeric (**19**) hydrolyzates (Chart 5). The monomeric hydrolyzates, except for **16**, were identified by comparison with authentic samples. Hydrolyzate **16** exhibited, in the aromatic region of the ^{1}H-NMR spectrum, two 2H singlets, two 1H doublets ($J=2$ Hz) and one 1H singlet attributable to two galloyl groups and a *m*-GOG unit. Upon the ^{1}H-NMR spectral comparison of the glucose signals between **16** and **14**, the shifts of H-1—3 signals were analogous, whereas the H-4—6 signals in the former were shifted markedly upfield from those of the latter. Therefore, **16** was considered as an analog of remurin A (**14**) lacking the HHDP group, and this correlation was confirmed by partial degradation of **14** in hot water, yielding **16**. Hydrolyzate **4** showed in the FAB-MS the $(M+Na)^+$ ion peak at *m*/*z* 1745, which is 152 (galloyl group) mass units lower than that of hirtellin A (**3**). The presence of three galloyl groups in **4** was shown by the ^{1}H-NMR signals at δ 6.99, 6.98, 6.95 and 6.94 (each s, 5H in total), although these signals were complicated by duplication of each proton signal due to the formation of a mixture of α- and β-anomers. A series of singlets (5H in total) at δ 6.45—6.64 and δ 7.09—7.08, and two pairs of *meta*-coupled doublets at δ 7.23, 7.21, 6.68, and 6.67 (each d, $J=2$ Hz, 2H in total) are attributable to two HHDP groups and a *m*-GOG unit. The chemical shifts of the anomeric protons of **4** [δ 6.11, 6.10 (α- and β-anomer), 5.36 (α-anomer) and 4.45 (β-anomer)] (Table I) clearly indicate that one pair of the anomeric centers is acylated and the other is free. As the signals of the fully acylated glucose core (glucose-I) in **4** are closely similar to the corresponding signals of **3**, the hydrolyzate **4** is regarded as a degalloylated congener of **3**, and this structure was confirmed by enzymatic hydrolysis of **3** with tannase, yielding compound **4**. The other dimeric hydrolyzate **18**

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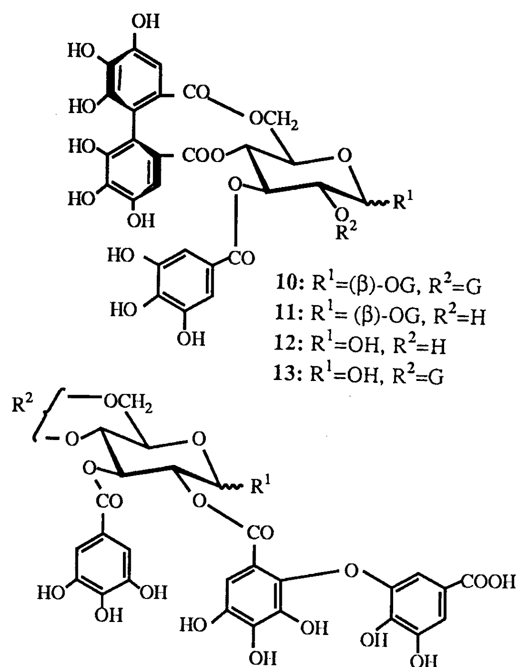


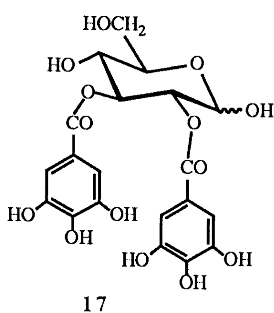
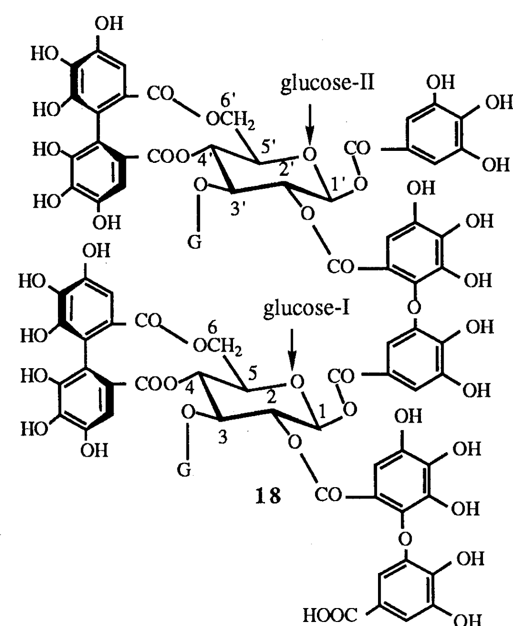


Chart 3



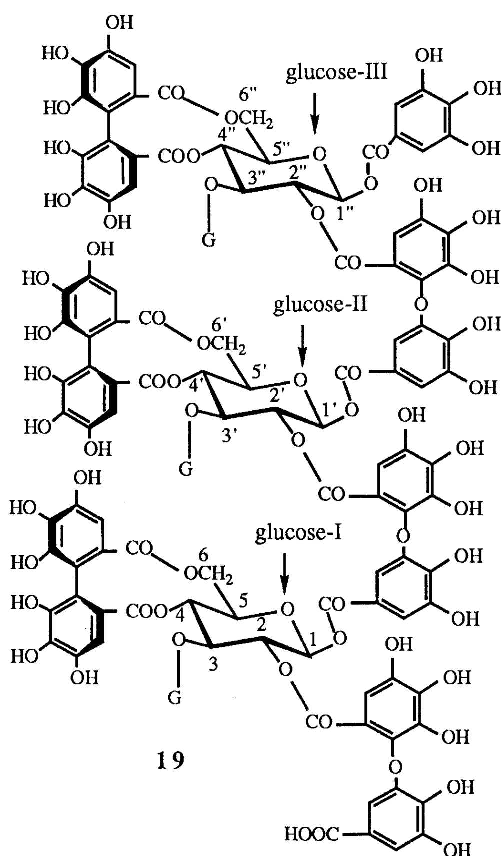


Chart 4

was identical with the product obtained by partial hydrolysis of **5**, mentioned above. Compound **19** was suggested to be a trimeric hydrolyzate on the basis of its behavior on normal-phase HPLC, and the following ^{1}H-NMR spectral data. The presence of three *m*-GOG units, three HHDP and four galloyl groups was shown by four 2H singlets, nine 1H singlets and three pairs of 1H doublets ($J=2$ Hz) in the aromatic region (δ 6.45—7.40). Three sets of fully acylated glucose proton signals were exhibited in the aliphatic region, and their chemical shifts and coupling patterns were almost identical with those of remurin A (**14**). The characteristic upfield shifts of the three anomeric protons, H-1 (δ 5.45—5.54), H-1′ (δ 5.81) and H-1″ (δ 5.31), indicated the presence of three *m*-GOG units at O-2 of each glucose core. Compound **19** was thus characterized as a trimer produced by removal of the terminal tellimagrandin I moiety from **6**. The tetrameric ellagitannin structure of hirtellin Q_1, as represented by **6**, was thus established. Hirtellin Q_1 (**6**) is regarded as a tetramer biogenetically producible by C–O oxidative coupling between the galloyl groups at glucose O-1″ of **5** and O-2 of **10**.

It is noteworthy that the dimers (hirtellins and tamarixinins)[1,2] previously isolated from Tamaricaceous plants, and the oligomers presented in this paper, have common structural features, with the *m*-GOG and/or *p*-GOG units[3] between monomeric constituents at O-1 of a *C1* glucopyranose core and O-2 of the other *C1* glucopyranose. These oligomers may be useful as chemo-

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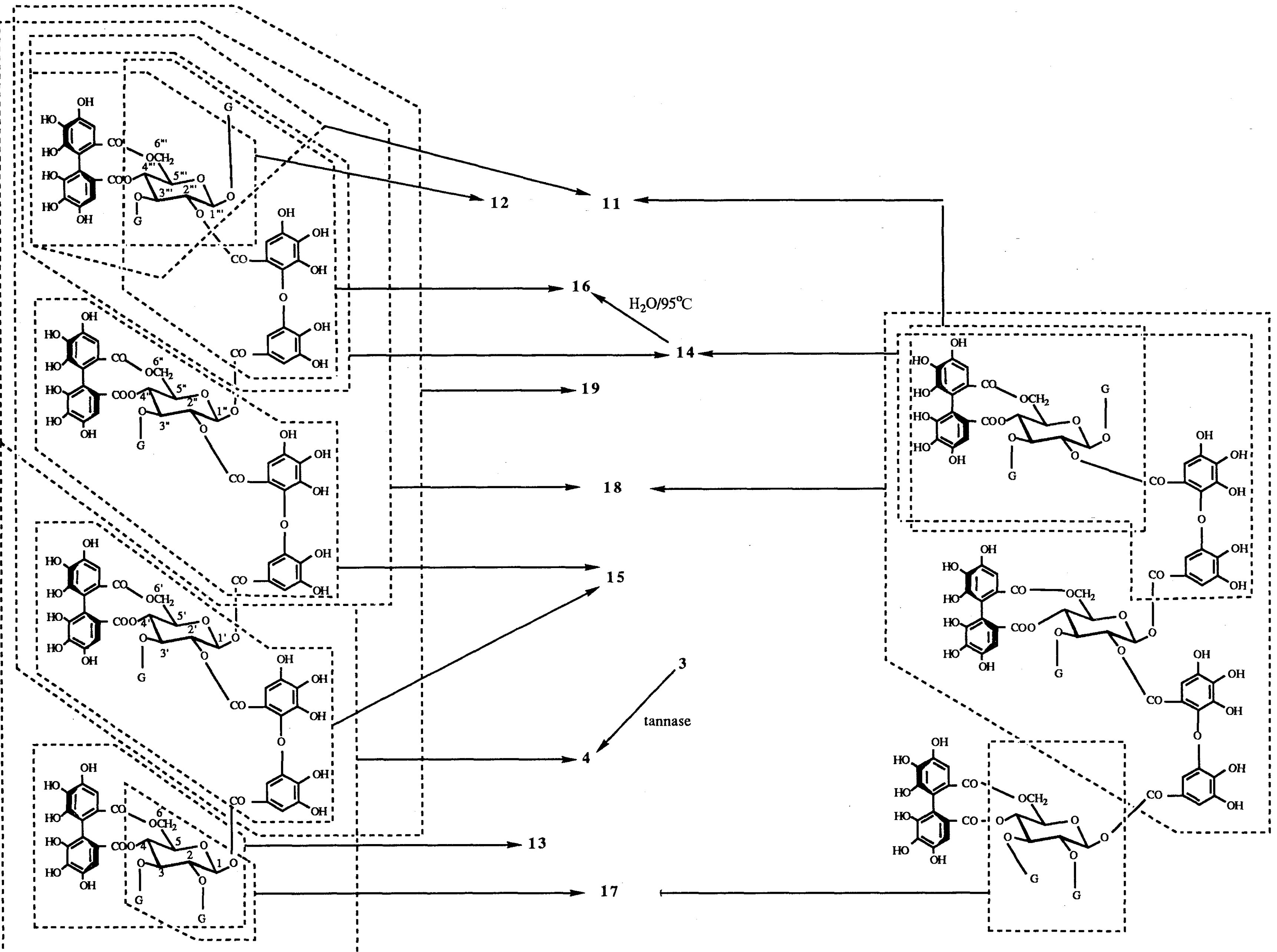

Chart 5

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TABLE I. ^{1}H-NMR Spectral Data for Glucose Moieties of **1**, **2**, **3**, **4**, **10**, **14** and **18** (500 MHz, Acetone-d_6+D_2O, J in Hz)

H	1	2	3	4		10	14	18
				α-Anomer	β-Anomer			
Glucose-I								
1	6.08 d (J=8)	5.93 d (J=8)	6.07 d (J=8)	6.11, 6.10 each d (J=8)		6.20 d (J=8)	5.49—5.54 Overlapped	5.51—5.55 Overlapped
2	5.59 dd (J=8, 10)	5.36 dd (J=8, 10)	5.58 dd (J=8, 10)	5.55, 5.54 each dd (J=8, 10)		5.58 dd (J=8, 9.5)		
3	5.76 t (J=10)	5.54 t (J=10)	5.77 t (J=10)	5.79 t (J=10)		5.75 t (J=9.5)		
4	5.22 t (J=10)	3.91 t (J=10)	5.22 t (J=10)	5.17 t (J=10)		5.14 t (J=9.5)	5.14 t (J=10)	5.14 t (J=10)
5	4.50 dd (J=6.5, 10)	3.81 ddd (J=2, 6.5, 10)	4.53 dd (J=6, 10)	4.48 dd (J=6.5, 10)		4.41 dd (J=5.5, 9.5)	4.22 dd (J=6.5, 10)	4.23 dd (J=6.5, 10)
6	5.33 dd (J=6.5, 13.5)	3.85 dd (J=6.5, 12)	5.33 dd (J=6, 13)	5.29 dd (J=6.5, 13.5)		5.33 dd (J=5.5, 13.5)	5.31 dd (J=6.5, 13.5)	5.30 dd (J=6.5, 13.5)
	4.00 d (J=13.5)	4.02 dd (J=2, 12)	3.98 d (J=13)	3.85, 3.84 each d (J=13.5)		3.80 d (J=13.5)	3.81 d (J=13.5)	3.82 d (J=13.5)
Glucose-II								
1′	5.24 d (J=8)	5.45 d (J=8.5)	5.35 d (J=8)	5.36 d (J=4)	4.45 d (J=8)			5.38 d (J=8.5)
2′	5.30 dd (J=8, 10)	5.52 dd (J=8.5, 10)	5.55 dd (J=8, 10)	4.99 dd (J=4, 10)	5.14 dd (J=8, 10)			5.44 dd (J=8.5, 10)
3′	5.42 t (J=10)	5.63 t (J=10)	5.64 t (J=10)	5.81 t (J=10)	5.53 t (J=10)			5.67 t (J=10)
4′	3.87 t (J=10)	5.13 t (J=10)	5.13 t (J=10)	5.06 t (J=10)	5.05 t (J=10)			5.14 t (J=10)
5′	3.52, overlapped	4.25 ddd (J=1, 6.5, 10)	4.22 dd (J=6.5, 10)	4.60 dd (J=6.5, 10)	4.05 dd (J=6.5, 10)			4.24 dd (J=6.5, 10)
6′	3.74 dd (J=4.5, 12.5)	5.29 dd (J=6.5, 13.5)	5.29 dd (J=6.5, 13)	5.32 dd (J=6.5, 13)	5.25 dd (J=6.5, 13.5)			5.28 dd (J=6.5, 13)
	3.86 dd (J=2, 12.5)	3.81, overlapped	3.82 d (J=13)	3.73 d (J=13)	3.80 d (J=13.5)			3.92 d (J=13)

taxonomic markers of Tamaricaceous plants.

Experimental

General The material (*R. hirtella*), instruments and chromatographic methods in this work were the same as those described in the preceding paper.[2c] Preparative HPLC (R) was carried out on YMC A312 (ODS) (6 mm i.d. × 150 mm) and YMC A324 (ODS) (10 mm i.d. × 300 mm) (Yamamura Kagaku, Japan) at 40 °C, using 0.01 M KH_2PO_4–0.01 M H_3PO_4–EtOH–EtOAc (41.5 : 41.5 : 12 : 5) as a mobile phase at a flow rate of 1.3 ml/min (detection, Union-Giken MCPD-350 at 280 or 300 nm). Normal-phase HPLC was carried out on a Superspher Si 60 cartridge column (4 mm i.d. × 125 mm) (Merck) at room temperature using the following solvent systems: (A) *n*-hexane–EtOAc (2 : 1); (B) *n*-hexane–MeOH–THF–HCOOH (60 : 45 : 15 : 1) and oxalic acid (450 mg/l), at a flow rate of 1.0 ml/min (detection: Shimadzu SPD-6A UV detector at 280 nm).

Isolation of Tannins An EtOAc-soluble portion (28.0 g) obtained from the 70% acetone extract of *R. hirtella* leaves was chromatographed over Toyopearl HW-40 (coarse) using 70% MeOH → MeOH–H_2O–acetone (7 : 2 : 1 → 6 : 2 : 2) as the eluant, as described in the previous paper.[2c] The earlier fractions eluted with MeOH–H_2O–acetone (6 : 2 : 2) gave a mixture of two minor dimeric tannins which were separated by rechromatography over MCI-gel CHP-20P with H_2O containing increasing amounts of MeOH to yield hirtellin F[2c] and a new tannin, hirtellin G (**1**) (12.1 mg).

A part (35 g) of the *n*-BuOH extract was subjected to column chromatography over Toyopearl HW-40 (coarse),[1c] using aqueous MeOH (70% → 80% MeOH) and MeOH–H_2O–acetone (7 : 2 : 1 → 6 : 2 : 2 → 5 : 2 : 3) as eluants to afford remurin B (**15**), and hirtellins D, E, B and A. A mixture of trimers (4.3 g) and a crude tetramer (1.4 g) were obtained from the MeOH–H_2O–acetone (5 : 2 : 3) eluate. A part of the former (660 mg) was subjected to repeated column chromatography over MCI-gel CHP-20P and/or preparative HPLC (R) to yield hirtellin T_1 (**5**) (250 mg) as the major trimer. The crude tetramer was also purified by column chromatography over MCI-gel CHP-20P followed by preparative HPLC (R) to afford hirtellin Q_1 (**6**) (166 mg).

Hirtellin G (1) An off-white amorphous powder, FAB-MS m/z: 1595 $(M+Na)^+$, $[\alpha]_D$ +57° (c=1, MeOH). UV λ_{max}^{MeOH} nm (log ε): 216 (5.11), 276 (4.75). CD (MeOH) [θ] (nm): $+16\times10^4$ (228), -5.9×10^4 (264), $+2.5\times10^4$ (302). ^{1}H-NMR (acetone-d_6+D_2O): δ 7.05, 6.95 (2H each, s, Gal × 2), 6.97 (4H, s, Gal × 2), 6.67, 6.48 (1H each, s, HHDP), 7.25, 6.39 (1H each, d, J=2 Hz, *m*-GOG), 7.03 (1H, s, *m*-GOG); glucose protons, see Table I.

Methylation of 1 Followed by Methanolysis A solution of **1** (1.3 mg) in MeOH (0.5 ml) was methylated with ethereal CH_2N_2 at room temperature for 5 h. The residue after removal of the solvent was directly methanolyzed with 1% NaOMe (50 μl) in MeOH (0.2 ml) overnight at room temperature. The reaction mixture was worked up in a similar way to that described for methanolysis of hirtellin D,[1c] to give **7**, **8** and **9**, which were shown to be identical with authentic samples by TLC and normal-phase HPLC (solvent A). The identity of **9** was further confirmed by EI-MS spectral comparison (M^+, m/z 436) with authentic material.

Partial Hydrolysis of Hirtellin A (3) An aqueous solution of **3** (100 mg) was heated at 95 °C, and the reaction mixture was submitted to column chromatography over MCI-gel CHP 20P, developing with water containing increasing amounts of MeOH. The fractions eluted with 30% and 40% MeOH gave **1** (1.5 mg) and **2** (1.1 mg), respectively. The product **1** was identified as hirtellin G by co-chromatography with the natural product using normal-phase HPLC (solvent B) and reversed-phase HPLC.[2c]

2: An off-white amorphous powder, ^{1}H-NMR (acetone-d_6+D_2O): δ 7.04, 7.00 [2H each, s, galloyl (Gal) × 2], 6.94 (4H, s, Gal × 2), 6.59, 6.48 (1H each, s, HHDP), 7.14, 6.44 (1H each, d, J=2 Hz, *m*-GOG), 7.04 (1H, s, *m*-GOG); glucose protons, see Table I.

Hirtellin T_1 (5) An off-white amorphous powder, $[\alpha]_D$ +51° (c=1.0, MeOH). *Anal.* Calcd for $C_{123}H_{86}O_{78}\cdot13H_2O$: C, 48.21; H, 3.66. Found: C, 48.27; H, 3.86. UV λ_{max}^{MeOH} nm (log ε): 223 (5.37), 275 (5.08). CD (MeOH) [θ] (nm): $+35\times10^4$ (235), -8.4×10^4 (260), $+8.4\times10^4$ (285), -0.8×10^4 (333). Normal phase HPLC (solvent B), t_R 17.07 min. ^{1}H-NMR (acetone-d_6+D_2O): δ 7.02, 7.00, 6.91 (2H each, s, Gal × 3), 6.67, 6.65, 6.62, 6.49, 6.46, 6.45 (1H each, s, HHDP × 3), 7.25, 7.23, 6.54, 6.45 (1H each, d, J=2 Hz), 7.07, 7.05 (1H each, s) (*m*-GOG × 2); glucose protons, see Table II. ^{13}C-NMR (acetone-d_6+D_2O): δ 107.22 (*m*-GOG C-2), 107.79 (4C) (HHDP C-3, 3′, *m*-GOG C-2), 107.96, 108.19, 108.23 (HHDP C-3, C-3′), 110.08 (6C), 110.20 (4C) (Gal C-2, C-6), 110.40 (2C) (*m*-GOG C-6′), 112.60 (*m*-GOG C-6), 113.30 (2C) (*m*-GOG C-1, C-6), 113.52 (*m*-GOG C-1), 115.54 (2C), 115.58, 115.69, 115.73, 115.77 (HHDP C-1, C-1′), 119.55, 119.60 (*m*-GOG C-1′), 119.89, 120.12 (2C), 120.21, 120.29 (Gal C-1), 125.60, 125.71 (2C), 126.26, 126.35 (2C) (HHDP C-2, C-2′), 136.30 (4C), 136.53 (3C), 136.96 (HHDP C-5, C-5′, *m*-GOG C-2′), 139.10 (2C), 139.28 (2C), 139.50 (Gal C-4), 140.00, 140.21

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TABLE II. ^{1}H-NMR Spectral Data[a] for Glucose Moieties of **5**, **6** and **19** (500 MHz, Acetone-d_6+D_2O, J in Hz)

H	5	6	19
Glucose-I			
1	6.12 d (J=8.5)	6.12 d (J=8)	5.45—5.54 Overlapped
2	5.53 dd (J=8.5, 10)	5.53 dd (J=8, 10)	
3	5.78 t (J=10)	5.77 t (J=10)	
4	5.18 t (J=10)	5.19 t (J=10)	5.15 t (J=10)
5	4.48 dd (J=6.5, 10)	4.50 dd (J=7, 10)	4.26 dd (J=6.5, 10)
6	5.30 dd (J=6.5, 13.5)	5.32 dd (J=7, 13)	5.29 dd (J=6.5, 12.5)
	3.94 d (J=13.5)	3.93 d (J=13)	3.83 d (J=12.5)
Glucose-II			
1′	5.77 d (J=8.5)	5.83 d (J=7.5)	5.81 d (J=8)
2′	5.51 dd (J=8.5, 10)	5.51 dd (J=7.5, 10)	5.45—5.54
3′	5.63 t (J=10)	5.63 t (J=10)	5.45—5.54
4′	5.13 t (J=10)	5.13 t (J=10)	5.10 t (J=10)
5′	4.32 ddd (J=7.5, 10)	4.33 dd (J=7, 10)	4.34 dd (J=6.5, 10)
6′	5.29 dd (J=7.5, 13.5)	5.27 dd (J=7, 13)	5.24 dd (J=6.5, 13)
	3.83 dd (J=13.5)	3.94 d (J=13)	3.83 d (J=13)
Glucose-III			
1″	5.50 d (J=8)	5.75 d (J=7.5)	5.31 d (J=8)
2″	5.47 dd (J=8, 10)	5.54 dd (J=7.5, 10)	5.45—5.54
3″	5.52 t (J=10)	5.46 t (J=10)	5.63 t (J=10)
4″	5.14 t (J=10)	5.13 t (J=10)	5.14 t (J=10)
5″	4.25 dd (J=6.5, 10)	4.26 dd (J=7, 10)	4.17 dd (J=6.5, 10)
6″	5.30 dd (J=6.5, 13)	5.30 dd (J=7, 13)	5.31 dd (J=6.5, 13)
	3.89 d (J=13)	3.84 d (J=13)	3.93 d (J=13)
Glucose-IV			
1‴		5.44 d (J=8)	
2‴		5.45 dd (J=8, 10)	
3‴		5.52 t (J=10)	
4‴		5.09 t (J=10)	
5‴		4.29 dd (J=7, 10)	
6‴		5.25 dd (J=7, 13)	
		3.85 d (J=13)	

a) Assignments of **5**, **6** and **19** were based on the COSY, and correspondences with known compounds **1**, **10** and **14**

(2C), 140.30, 140.60 (2C) (*m*-GOG C-4, C-3′, C-4′), 143.30, 143.50 (*m*-GOG C-5′), 144.26 (3C), 144.34 (3C) (HHDP C-6, C-6′), 145.10 (6C) (HHDP C-4, C-4′), 145.70 (6C), 145.82 (4C) (Gal C-3), 147.68 (2C), 147.76 (2C) (*m*-GOG C-3, C-5), 163.71, 164.20, 164.42 (2C), 164.71, 165.63, 166.39, 166.41, 166.63, 167.55, 167.63, 167.65, 168.09, 168.13. 168.31 (ester carbonyl carbons); glucose carbons, see Table III.

Acid Hydrolysis of Hirtellin T_1 (5) A solution of **5** (2 mg) in 1 N H_2SO_4 (1 ml) in a sealed ampule was heated on a boiling-water bath for 5 h. After cooling, the solution was extracted with EtOAc. The aqueous layer was neutralized with Amberlite IR-410 (OH-form) and evaporated to give a syrup. The GLC analysis after trimethylsilylation of the syrup showed the sugar component to be glucose.

Methylation of 5 A mixture of **5** (100 mg), anhydrous K_2CO_3 (700 mg) and dimethyl sulfate (1 ml) in dry acetone (20 ml) was stirred overnight at room temperature, and refluxed for 8 h. After removal of the inorganic material by filtration, the filtrate was concentrated *in vacuo*, and subjected to preparative TLC (Kieselgel PF_{254}, benzene–acetone 5 : 1) to give the permethylated derivative of hirtellin T_1 (**5a**) (60.8 mg).

5a: A yellowish-white amorphous powder. ^{1}H-NMR (acetone-d_6): δ 7.28 (1H, d, J=2 Hz), 7.26 (2H, s), 7.22 (3H, s), 7.19 (4H, s), 7.18 (2H overlapped signals), 7.16 (2H, s), 6.94 (1H, s), 6.90 (2H, s), 6.80 (1H, s), 6.76 (1H, s), 6.75 (3H, br d), 6.26 [1H, d, J=8.5 Hz, glucose (Glc) H-1], 5.62 (1H, t, J=8.5 Hz, Glc H-2), 5.87 (1H, t, J=10 Hz, Glc H-3), 5.30 (1H, t, J=10 Hz, Glc H-4 or H-4″), 4.61 (1H, dd, J=6.5, 10 Hz, Glc H-5), 5.31 (1H, dd, J=6.5, 13.5 Hz, Glc H-6), the other H-6 is overlapped by OMe signals, 6.21 (1H, d, J=7 Hz, Glc H-1′), 5.66 (1H, t, J=7 Hz, Glc H-2′), 5.82 (1H, t, J=10 Hz, Glc H-3′), 5.44 (1H, t, J=10 Hz, Glc H-4′), 4.47 (1H, m, Glc H-5′), 5.21 (1H, dd, J=6, 13.5 Hz, Glc H-6′), the other H-6′ is overlapped by OMe signals, 6.10 (1H, d, J=7 Hz, Glc H-1″), 5.58 (1H, t, J=7 Hz, Glc H-2″), 5.62 (1H, t, J=9.5 Hz, Glc H-3″), 5.29 (1H, t, J=10 Hz, Glc H-4″ or H-4), 4.29 (1H, dd, J=6.5, 10 Hz, Glc H-5″), 5.17 (1H, dd, J=6.5, 13.5 Hz, Glc H-6′), the other H-6″ is overlapped by OMe signals, 3.97 (6H, s, OMe × 2), 3.90, 3.88 (each 3H, s, OMe × 2), 3.85, 3.84 (18H each, s, OMe × 12), 3.83, 3.81, 3.80, 3.79 (3H each, s, OMe × 4), 3.75 (15H, s, OMe × 5), 3.73 (6H, s, OMe × 2), 3.72—3.71 (15H, overlapped signals, OMe × 5), 3.69 (3H, s, OMe × 1), 3.67, 3.65 (6H each, s, OMe × 4), 3.64, 3.63 (3H each, s, OMe × 2), 3.62 (6H, s, OMe × 2), 3.53, 3.51 (each 3H, s, OMe × 2).

TABLE III. ^{13}C-NMR Data for the Glucose Moieties of **10**, **3**, **5** and **6**

	C-1 C-1′ C-1″ C-1‴	C-2 C-2′ C-2″ C-2‴	C-3 C-3′ C-3″ C-3‴	C-4 C-4′ C-4″ C-4‴	C-5 C-5′ C-5″ C-5‴	C-6 C-6′ C-6″ C-6‴
10	93.8	71.8	73.3	70.8	73.1	63.1
3	93.5 (2C)	71.5	73.4	70.3	72.9 (2C)	62.9
		71.8	73.2	70.6		63.1
5	93.5	71.4	73.2	70.4	73.0 (3C)	63.0 (3C)
	93.6	71.5	73.3	70.5		
	93.7	71.7	73.4	70.6		
6	93.5 (2C)	71.7	73.3 (3C)	70.5 (3C)	72.9 (4C)	63.1 (2C)
	93.7	71.5	73.5	70.6		63.0 (2C)
	93.6	71.4 (2C)				

Methanolysis of 5a A solution of **5a** (20 mg) in 1% NaOMe (0.5 ml) in absolute MeOH (1 ml) was left standing overnight at room temperature. After neutralization with AcOH, the solvent was evaporated off under an N_2 stream, and the residue was treated with an excess of ethereal CH_2N_2 for 6 h. The residue obtained after removal of the solvent was subjected to preparative TLC (Kieselgel PF_{254}, benzene–acetone, 15 : 1) to yield **7** (3 mg), **8** (5 mg), $[\alpha]_D$ −34° (c=1, acetone) and **9** (4 mg), which were identified by co-chromatography and by EI-MS and ^{1}H-NMR spectral comparison with authentic samples.

Partial Hydrolysis of Hirtellin T_1 (5) A solution of **5** (150 mg) in H_2O (50 ml) was heated at 80 °C for 17 h. After cooling, the precipitate formed was removed by centrifugation, and the concentrated supernatant was subjected to column chromatography over MCI-gel CHP-20P (1.1 cm i.d. × 20 cm) with H_2O → 10% MeOH → 15% MeOH → 20% MeOH → 30% MeOH → 40% MeOH in a stepwise gradient mode. The H_2O eluate gave gallic acid (3.6 mg) and the 10% MeOH eluate gave 2,3-di-*O*-galloyl-D-glucose (**17**) (2.9 mg). Mixture eluted by 30% MeOH was subjected to further chromatographic separation on MCI-gel CHP-20P column and preparative HPLC (R) to afford 1,3-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucose (**11**) (5.1 mg) and remurin A (**14**) (3 mg). Their identities were confirmed by co-chromatography (normal-phase and reversed-phase HPLC) and by ^{1}H-NMR spectral comparison with authentic samples. The fraction eluted with 40% MeOH gave a crude dimeric hydrolyzate and unreacted hirtellin T_1 (37.2 mg). Further purification of the crude material by preparative HPLC (R) afforded the hydrolyzate **18** (2.1 mg).

Hydrolyzate **18**: An off-white amorphous powder. ^{1}H-NMR (acetone-d_6+D_2O): δ 7.00, 6.96, 6.92 (2H each, s, Gal × 3), 6.65, 6.61, 6.49, 6.48 (1H each, s, HHDP × 2), 7.19, 7.12, 6.44, 6.40 (1H each, d, J=2 Hz), 7.09, 7.07 (1H each, s) (*m*-GOG × 2); glucose protons, see Table I.

Hirtellin Q_1 (6) An off-white amorphous powder, $[\alpha]_D$ +60° (c=1.0, MeOH). *Anal.* Calcd for $C_{164}H_{114}O_{104}\cdot 21H_2O$: C, 47.69; H, 3.81. Found: C, 47.40; H, 3.64. UV λ_{max}^{MeOH} nm (log ε): 217 (5.39), 275 (5.17). CD (MeOH) [θ] (nm): +47.2 × 10^{4} (235), −9.7 × 10^{4} (262) and +12.7 × 10^{4} (283) and normal-phase HPLC (solvent B), t_R 22.23 (N2). ^{1}H-NMR (acetone-d_6+D_2O): δ 7.02, 6.99, 6.96, 6.95, 6.91, 6.90 (2H each, s, Gal × 6), 6.64, 6.47 (2H each, s), 6.67, 6.61, 6.51, 6.48 (1H each, s)

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(HHDP × 4), 7.25 (2H, overlapped d, $J=2$ Hz), 7.24, 6.51, 6.50, 6.44 (1H each, d, $J=2$ Hz), 7.9, 7.07, 7.06 (1H each, s) (*m*-GOG × 3); glucose protons, see Table II. ^{13}C-NMR (acetone-d_6 + D_2O): δ 107.16, 107.68 (2C) (*m*-GOG C-2), 107.76 (5C), 108.06, 108.20 (2C) (HHDP C-3, C-3′), 110.02 (4C), 110.14 (8C) (Gal C-2, C-6), 110.31, 110.36 (2C) (*m*-GOG C-6′), 112.69, 112.99, 113.16 (*m*-GOG C-6), 113.20, 113.33 (2C) (*m*-GOG C-1), 115.52, 115.55 (2C), 115.59, 115.78 (2C), 115.84 (2C) (HHDP C-1, C-1′), 119.41 (3C) (*m*-GOG C-1′), 119.78, 119,89, 119.96 (2C), 120.07 (2C) (Gal C-1), 125.43, 125.50, 125.52, 125.54, 126.10, 126.16 (3C) (HHDP C-2, C-2′), 136.22 (2C), 136.26 (2C), 136.44, 136.49, 136.54 (3C), 136.90, 136.93 (HHDP C-5, C-5′, *m*-GOG C-2′), 139.07, 139.14, 139.17, 139.28, 139.35, 139.66 (Gal C-4), 139.88, 140.15 (2C), 140.18 (2C), 140.26, 140.54, 140.58, 140.67 (*m*-GOG C-4, C-3′, C-4′), 143.20, 143.22, 143.40 (*m*-GOG C-5′), 144.17, 144.21 (4C), 144.26, 144.31, 144.34 (HHDP C-6, C-6′), 145.02, 145.07 (2C), 145.09 (3C), 145.18 (HHDP C-4, C-4′), 145.54 (2C), 145.59 (2C), 145.68 (2C), 145.72 (2C), 145.80 (4C) (Gal C-3), 145.86, 145.93, 146.05, 147.57, 147.70, 147.77 (*m*-GOG C-3, C-5), 163.82, 163.93, 164.40, 164.50, 164.59, 164.65, 164.72, 165.79, 166.44, 166.56 (2C), 166.73, 167.62 (2C), 167.70, 167.75, 168.24 (2C), 168.37, 168.46 (ester carbonyl carbons); glucose carbons, see Table III.

Acid Hydrolysis of Hirtellin Q_1 (6) A solution of **6** (1 mg) in 2 N HCl (1 ml) in a sealed ampule was heated on a boiling-water bath for 10 h. After cooling, the solution was extracted with EtOAc. HCl in the aqueous layer was removed by adding Ag_2O, followed by filtration. The TLC analysis of the concentrated filtrate showed the presence of glucose. TLC: Kieselgel PF_{254}, *n*-BuOH–AcOH–H_2O (4 : 1 : 2); detection: 2-amino-biphenylhydrogen oxalate reagent.

Methylation of Hirtellin Q_1 (6) Followed by Methanolysis A solution of **6** (4.5 mg) in MeOH (1 ml) was methylated with ethereal CH_2N_2 overnight at room temperature. The residue after removal of the solvent was treated with 1% NaOMe (0.15 ml) in MeOH (1 ml), and the reaction mixture was purified by preparative TLC to give **7** (0.7 mg) (EI-MS: M^+, *m/z* 226), **8** (0.9 mg) (EI-MS: M^+, *m/z* 450) and **9** (0.8 mg) (EI-MS: M^+, *m/z* 436) which were identified by co-chromatography with authentic samples.

Partial Hydrolysis of Hirtellin Q_1 (6) A solution of **6** (140 mg) in H_2O (50 ml) was heated at 80 °C for 15 h. After cooling, the precipitate was collected by centrifugation and identified as ellagic acid. The concentrated supernatant was subjected to column chromatography over Toyopearl HW-40 (fine) (1.1 cm i.d. × 40 cm) with 70% MeOH → MeOH–H_2O–acetone [(7 : 2 : 1) → (6 : 2 : 2) → (5 : 2 : 3)] in a stepwise gradient mode. The 70% MeOH eluate afforded gallic acid (2 mg), 1,3-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl-β-D-glucose (**11**), gemin D (**12**) (1.3 mg), tellimagrandin I (**13**) (4.2 mg), remurin B (**15**) (1 mg), compound **16** (1.1 mg) and 2,3-di-*O*-galloyl-D-glucose (**17**) (1.8 mg). The fractions eluted with MeOH–H_2O–acetone (7 : 2 : 1) afforded remurin A (**14**) (4.4 mg) in addition to a mixture of two dimers, which were separated by preparative HPLC (R) to give compounds **4** (1.2 mg) and **18** (3.2 mg). The fraction eluted with MeOH–H_2O–acetone (6 : 2 : 2) gave a crude trimeric hydrolyzate which was purified by preparative HPLC to give compound **19** (2.5 mg) and unreacted hirtellin Q_1 (53.7 mg).

Compound **4**: An off-white amorphous powder, FAB-MS: *m/z* 1745 $(M+Na)^+$. ^{1}H-NMR (acetone-d_6 + D_2O): δ 6.99, 6.98, 6.95, 6.94 (each, s, 6H in total, Gal), 6.64, 6.63, 6.62, 6.46, 6.45 (each, s, 4H in total, HHDP), 7.23, 7.21, 6.68, 6.67 (each, d, $J=2$ Hz, 2H in total, *m*-GOG), 7.09, 7.08 (each, s, 1H in total, *m*-GOG), 6.11, 6.11 (each, d, $J=8$ Hz, 1H in total, Glc H-1), 5.55, 5.54 (each, dd, $J=8$, 10 Hz, 1H in total, Glc H-2), 5.79 (1H, t, $J=10$ Hz, Glc H-3), 5.17 (1H, t, $J=10$ Hz, Glc H-4), 4.48 (1H, dd, $J=6.5$, 10 Hz, Glc H-5), 5.29 (1H, dd, $J=6.5$, 13.5 Hz, Glc H-6), 3.85, 3.84 (each, d, $J=13.5$ Hz, 1H in total, Glc H-6). α- and β-anomer; 5.36 (d, $J=4$ Hz, Glc H-1′), 4.99 (dd, $J=4$, 10 Hz, Glc H-2′), 5.81 (t, $J=10$ Hz, Glc H-3′), 5.06 (t, $J=10$ Hz, Glc H-4′), 4.60 (dd, $J=6.5$, 10 Hz, Glc H-5′), 5.23 (dd, $J=6.5$, 13.5 Hz, Glc H-6′), 3.73 (dd, $J=13$ Hz, Glc H-6′) (α-anomer); 4.45 (d, $J=8$ Hz, Glc H-1′), 5.14 (dd, $J=8$, 10 Hz, Glc H-2′), 5.53 (t, $J=10$ Hz, Glc H-3′), 5.05 (t, $J=10$ Hz, Glc H-4′), 4.05 (dd, $J=6.5$, 10 Hz, Glc H-5′), 5.25 (dd, $J=6.5$, 13.5 Hz, Glc H-6′), 3.80 (d, $J=13.5$ Hz, Glc H-6′) (β-anomer).

Compound **16**: An off-white amorphous powder. ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.06, 6.94 (2H each, s, Gal), 7.21, 6.48 (1H each, d, $J=2$ Hz, *m*-GOG), 6.92 (1H, s, *m*-GOG), 5.35 (1H, d, $J=8$ Hz, Glc H-1), 5.32 (1H, dd, $J=8$, 9 Hz, Glc H-2), 5.41 (1H, t, $J=9$ Hz, Glc H-3), 3.89 (1H, t, $J=10$ Hz, Glc H-4), 3.57 (1H, ddd, $J=2.5$, 4.5, 10 Hz, Glc H-5), 3.73 (1H, dd, $J=4.5$, 12.5 Hz, Glc H-6), 3.84 (1H, dd, $J=2.5$, 12.5 Hz, Glc H-6).

Compound **19**: An off-white powder, ^{1}H-NMR (acetone-d_6 + D_2O): δ 7.02, 6.95, 6.93, 6.91 (each 2H, s, Gal), 6.65, 6.64, 6.62, 6.50, 6.48, 6.47 (each 1H, s, HHDP), 7.40, 7.21, 7.09, 6.48, 6.453, 6.450 (1H each, d, $J=2$ Hz, *m*-GOG), 7.11, 7.06, 6.95 (each 1H, s, *m*-GOG); glucose protons, see Table II.

Degalloylation of Hirtellin A (3) to Compound 4 A solution of hirtellin A (**3**) (21 mg) in H_2O (2 ml) was incubated with tannase (15 drops) at 37 °C for 12.5 h. The reaction was terminated by acidification with two drops of 1 N HCl, and the reaction mixture was applied to a BondElut RP-18 cartridge. Elution was conducted with H_2O (2 ml × 5) and then MeOH. The H_2O eluate afforded gallic acid (1.8 mg), and the MeOH eluate gave compound **4** (18 mg), which was identified as the degalloylated derivative of hirtellin A[2b] by co-chromarography on HPLC and ^{1}H-NMR spectral comparison with an authentic sample.

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