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STRUCTURES OF ISORUGOSIN E AND HIRTELLIN B, DIMERIC HYDROLYZABLE TANNINS HAVING A TRISGALLOYL GROUP†

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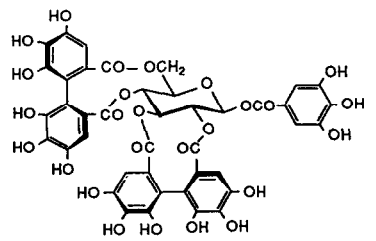
Abstract Two new dimeric hydrolyzable tannins, isorugosin E and hirtellin B, have been respectively isolated from *Liquidambar formosana* (Hamamelidaceae) and *Reaumuria hirtella* (Tamaricaceae), and their structures **7** and **15**, in which two monomers are linked through a trisgalloyl group (valoneoyl or hellinoyl group), have been elucidated based on high resolution NMR techniques. Hirtellin B exhibited a potent host-mediated antitumor activity.

Tannins, a large group of natural polyphenolic compounds, are interesting because of their diverse structural variety and marked biological activities.[1,2,3] The majority of these compounds are classified into two large groups, hydrolyzable tannins (metabolites of gallic acid[3]) and condensed tannins (proanthocyanidins[3]). Monomeric hydrolyzable tannins are complex esters of gallic acid, hexahydroxydiphenic acid and/or their metabolites, with a monosaccharide (mostly D-glucose) or polyalcohol moiety. An example of a monomeric hydrolyzable tannin is casuarictin (**1**).[4]

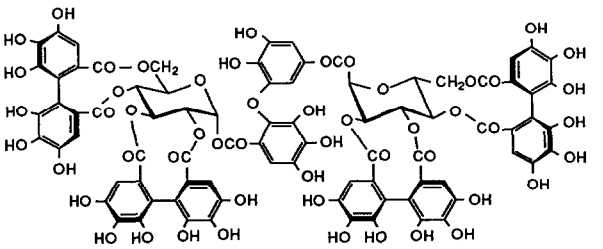
Oligomeric hydrolyzable tannins[5] are condensates of these monomers, and the first oligomer isolated from a plant was agrimoniin (**2**), a dimer from *Agrimonia pilosa*.[6] Tetramers up to m.w. *ca.* 3,800 have been isolated from plants[5] in the past nine years, and the number of isolated oligomers of defined structure is over seventy. It is notable that several of them showed marked biological activities, such as host-mediated antitumor activity[7] (mainly exhibited by oligomers among these polyphenolic compounds tested), inhibition of HIV[8] and herpes simplex virus,[9] and promotion of iodination in human leucocytes.[10]

Biogenetically these hydrolyzable tannin oligomers are regarded as the products of condensation between two monomers, and also of further condensation involving the products of the first condensation and the second condensation, and so on.[5] The hydrolyzable tannin oligomers isolated to date can be classified into four types (see structures on next page) based on the linking group between two smaller hydrolyzable tannin molecules of each oligomer, which is usually a bisgalloyl (dehydrodigalloyl), trisgalloyl (valoneoyl or sanguisorboyl) or tetrakisgalloyl (euphorbinoyl) group.

A fifth type of junction unit, the woodfordinoyl group (tetrakisgalloyl group), has recently been found in



1



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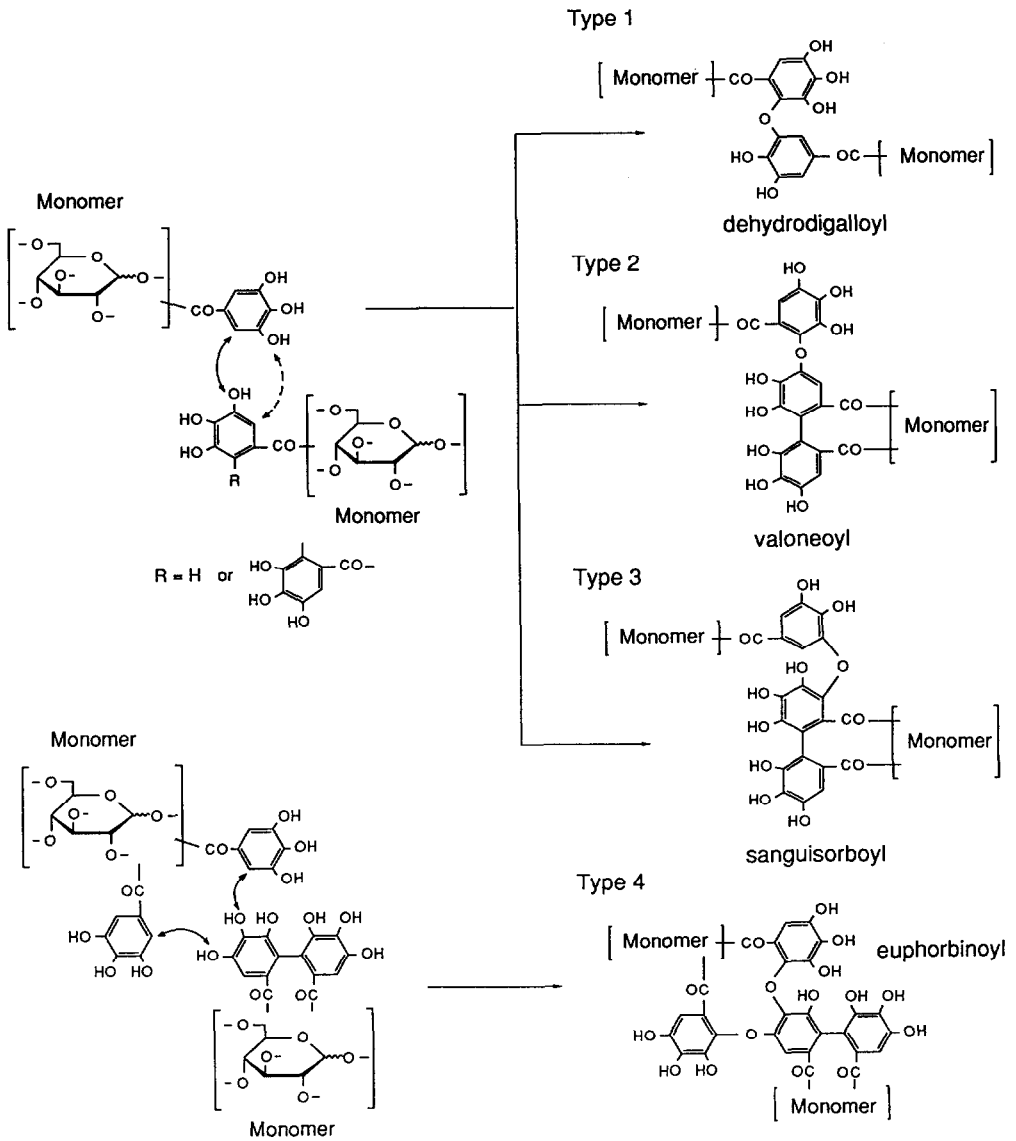
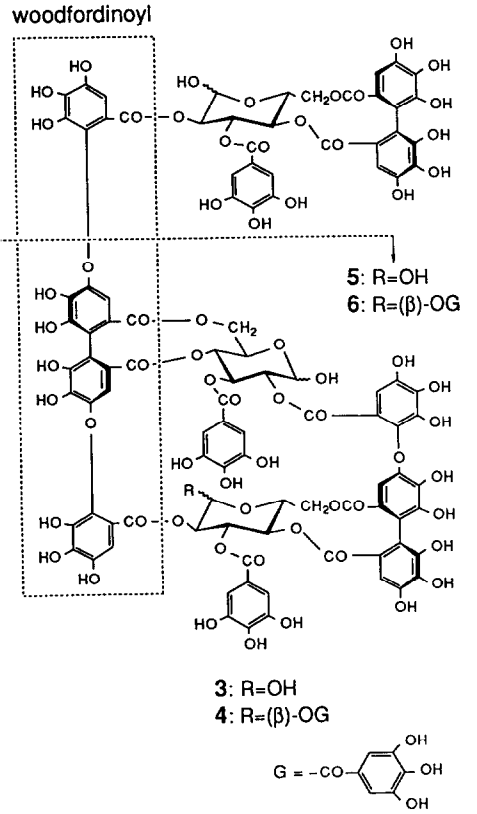

Type 1
Monomer
dehydrodigalloyl
Type 2
valoneoyl
Type 3
sanguisorboyl
Monomer
R = H or
Type 4
euphorbinoyl

oenothein A (**3**)[11] and woodfordin D (**4**),[11] trimeric hydrolyzable tannins in which oenothein B (**5**)[12] or woodfordin C (**6**)[13] (macrocyclic dimers) is bound with a monomer. Although these tannins are classified as macrocyclic oligomers, they are also type-2 oligomers.

The structure determination of these oligomers is principally based on the structures and properties of the monomers composing them, and also the binding among the monomers (structure and location of the junction unit).

In our recent studies on the oligomeric hydrolyzable tannins in plants, we have isolated new dimers having a trisgalloyl group as the junction unit. These oligomers are isorugosin E (**7**) from *Liquidambar formosana* Hance, and hirtellin B (**15**) from *Reaumuria hirtella* Jaub. et Sp.

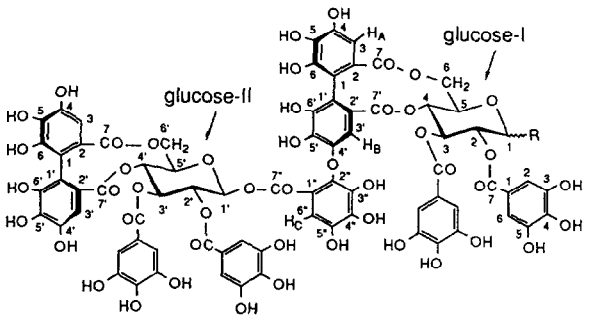
The present article deals with the isolation of these new dimers and elucidation of their structures based on high resolution NMR spectroscopy including ^{1}H-^{13}C long-range shift correlation spectroscopy and chemical methods.



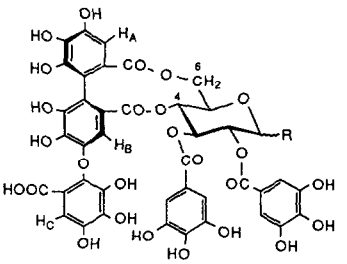
Structure of isorugosin E

Liquidambar formosana (Hamamelidaceae), planted as a roadside tree in Japan, is a medicinal tree in China.[14] We previously reported the isolation and characterization of several hydrolyzable tannins from the leaves of this tree.[14] Among them are isorugosin D (**8**), a dimer, and isorugosins A (**9**) and B (**10**), monomers structurally related to **8**.

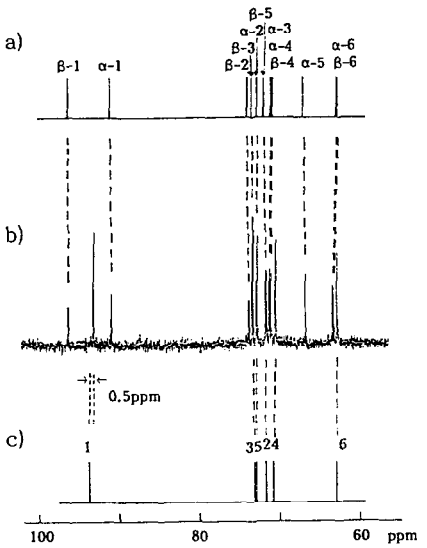
A new dimer, isorugosin E (**7**) has now been isolated as a light-brown amorphous powder from the *n*-BuOH soluble portion of the 70% acetone extract of the fresh leaves. Its molecular formula was determined to be $C_{75}H_{54}O_{48}$, based on a $(M+Na)^+$ ion peak (*m/z* 1745) in its FABMS and microanalytical data. The ^{1}H-NMR spectrum of **7** showed duplication of each signal which is characteristic of anomer mixture formation. The presence of a valoneoyl group [δ7.22, 7.17 (1H; H_C); 6.66, 6.65 (1H; H_A); 6.21, 6.14 (1H; H_B)], a hexahydroxydiphenoyl (HHDP) group [δ6.63, 6.62 (1H); 6.46, 6.46 (1H)], four galloyl groups [δ7.03, 7.01 (2H in total); 7.01, 7.01 (2H); 6.95, 6.95 (2H); 6.90, 6.87 (2H)] and two $^{4}C_1$ glucopyranose cores (see Experimental) was also exhibited. The chemical shifts of the anomeric protons [α-anomer, δ6.07 (d, *J*=8.5 Hz, H-1') and 5.49 (d, *J*=3.5 Hz, H-1); β-anomer, δ6.09 (d, *J*=8.5 Hz, H-1') and 5.00 (d, *J*=8Hz, H-1)] indicated that one of the two anomeric centers in **7** is acylated and the other is unacylated. The coupling constant of the H-1' signal indicates the presence of a β-oriented acyloxy group at the anomeric center of glucose core-II in the structure of **7**. The large differences in the ^{1}H chemical shifts between the methylene

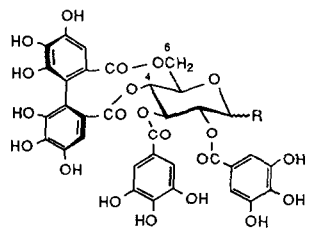


7: R=OH
8: R=(β)-OG

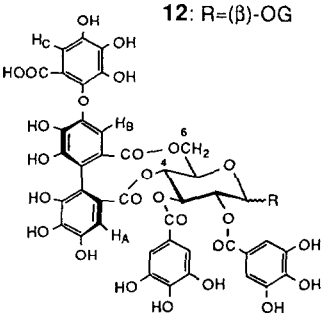


9: R=(β)-OG
10: R=OH





11: R=OH
12: R=(β)-OG



13: R=(β)-OG
14: R=OH

Fig. 1. Comparisons of the ^{13}C Chemical Shifts of Glucose Carbons of a) **11**,[16b] b) **7** and c) **12**[16a]

β-1 means C-1 of the glucose core of the β-anomer.

protons on each of the two $^4C_1$ glucopyranose cores [α-anomer, Δδ1.32 (glucose-II) and 1.51 (glucose-I); β-anomer, Δδ1.32 (glucose-II) and 1.50 (glucose-I)] are characteristic of the ellagitannins having an HHDP group or an HHDP moiety of the valoneoyl group at O-4/O-6 of the $^4C_1$ glucopyranose.[15] Therefore, the four galloyl groups and the galloyl moiety of the valoneoyl group in **7** are at O-2, O-3, O-1', O-2' and O-3' of the glucose cores.

The ^{13}C-NMR spectrum of **7** also showed the presence of a valoneoyl, an HHDP and four galloyl groups, and two glucose cores (see Experimental). The chemical shifts of the glucose carbon signals of **7** are almost the same as those of tellimagrandin I (**11**) and tellimagrandin II (**12**)[16] (Fig. 1), to support the above mentioned locations of the acyl groups on the two glucose cores. However, the signal of the acylated anomeric carbon (C-1') of **7** shifts slightly upfield (0.5 ppm) relative to that of **12**, indicating the location of the galloyl moiety of the valoneoyl group in **7** at O-1'. The orientation of the valoneoyl group in **7** is the

same as in isorugosin B (**10**),[14] since the chemical shifts of the valoneoyl H_A of **7** (δ6.66 and 6.65) are the same as the corresponding signals of **10** (δ6.66 and 6.65), but are different from those of rugosin B (**14**)[14,17] (δ6.48 and 6.46) which is an isomer of **10** concerning the orientation of the valoneoyl group.

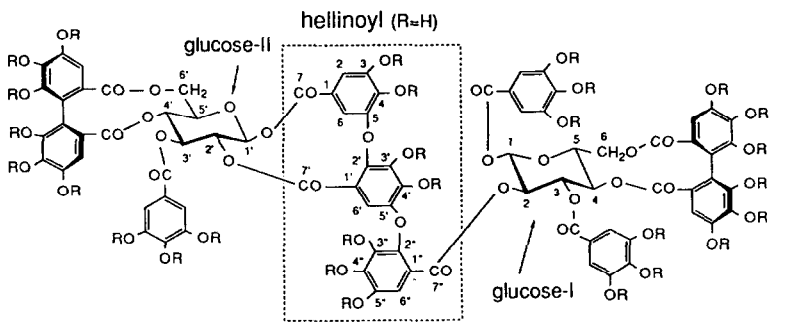
The CD spectrum of **7** exhibited a positive Cotton effect in the short-wavelength region ($[\theta]_{222}$ +1.9 x 10^5), indicating that the absolute configurations of the valoneoyl and HHDP groups are both *S*.[18]

The structure **7** thus assigned for isorugosin E was substantiated by the treatment of **7** in a buffer solution (pH 5.8) producing **11** and **10**, and also by the partial hydrolysis of isorugosin D (**8**)[14] with tannase yielding **7**.

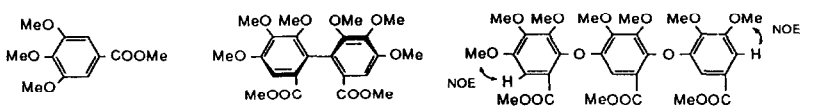
It is notable that isorugosin-type hydrolyzable tannins have been found only in *L. formosana*, although their isomers concerning the orientation of the valoneoyl group, rugosins A (**13**), B (**14**) (monomers), and D (dimers) have been found in plants of various families (Rosaceae, Coriariaceae, Euphorbiaceae and even Hamamelidaceae).[5,14,19]

Structure of hirtellin B

Hirtellin B (**15**), which has a new acyl group (a trisgalloyl group named hellinoyl group) as the sixth type of junction unit, has been isolated from the leaves of *Reaumuria hirtella* (Tamaricaceae) which is a bushy shrub at a salt-rich desert in Egypt.[20] Methylation of **15** with dimethyl sulfate and potassium carbonate in acetone afforded an octacosamethyl derivative (**15a**), which yielded upon methanolysis with sodium methoxide, methyl tri-*O*-methylgallate (**16**), dimethyl hexamethoxydiphenate (**17**) and a decamethyl derivative (**18**) [FABMS *m/z* 669 $(M+Na)^+$]. The parent acid (hellinic acid) of **18** was defined as a gallic acid trimer, in which three galloyl groups are linked to each other through ethereal bonds at 5-2' and 5'-2", as follows. The ^{1}H-NMR spectrum of **18** exhibited two 1H-singlets (δ6.86 and 7.32), two *meta*-coupled doublets (*J*=2 Hz) (δ6.78 and 7.30) and ten methoxyl signals. The ^{13}C-NMR spectrum showed the presence of twenty-one sp^2 carbons, among which three are ester carbonyl carbons (δ165.11, 165.50 and 166.52), and eleven carbons



15: R=H
15a: R=Me



16 17 18

bear ether oxygen. The ^{1}H-^{13}C long-range 2D NMR spectrum revealed that the ethereal carbon signals at δ142.09 and 142.23 show no cross peak with the methoxyl proton signals, and are correlated with the proton signals at δ6.86 and 7.32 through a three-bond coupling. The chemical shifts of these carbon signals are similar to that (δ142.5) of C-2" in a methylated valoneic acid,[21] and indicate that **18** has two subunits analogous to the valoneoyl C-ring (C-1"~C-7"), as shown in the formula **18**. This structural feature was substantiated by the NOESY spectrum of **18** which exhibited clear cross peaks among two low field signals [δ7.32 (s) and 7.30 (s, *J*=2 Hz)] and the methoxyl signals (δ3.94 and 3.92).

The ^{1}H-NMR spectrum of hirtellin B (**15**) exhibited four 2H-singlets (δ7.06, 6.92, 6.83 and 6.72), four 1H-singlets (δ7.59, 6.62, 6.54 and 6.45), and two *meta*-coupled doublets (*J*=2 Hz) (δ6.82 and 5.75). The 2H-singlet at δ6.72 was found to be an overlapped signal of nonequivalent two protons by HETCOR in which this signal was correlated with two carbons at δ108.35 and 108.86. Therefore, hirtellin B (**15**) is a dimer composed of three galloyl, two HHDP and a hellinoyl group. The presence of two $^{4}C_{1}$ glucopyranose cores in **15** was indicated by the coupling pattern of two sets of aliphatic seven-spin systems which were assigned by ^{1}H-^{1}H COSY. The chemical shifts of the C-6 (6') methylene protons of each glucose core [δ5.35 (5.37) and 3.86 (4.13)] are similar to those of isorugosin E (**7**) mentioned above, indicating the locations of two HHDP groups to be at O-4(O-4')/O-6(O-6') on each glucose core. The glucose carbon signals in the ^{13}C-NMR spectrum of **15** are closely similar to those of tellimagrandin II (**12**) (Table 1), suggesting that biogenetically hirtellin B is produced from two tellimagrandin II molecules forming a hellinoyl group. This structural feature is consistent with the FABMS data [*m/z* 1895 $(M+Na)^{+}$], which indicates the molecular formula $C_{82}H_{56}O_{52}$. It is also in accord with the CD spectrum showing a strong positive Cotton effect at 233 nm ([θ] +3.6 x 10^{5}), which indicates the presence of two (*S*)-HHDP groups in the molecule.[18]

Table 1. ^{13}C-NMR data for the glucose moieties of **15** and **12**.

	C-1 (C-1')	C-2 (C-2')	C-3 (C-3')	C-4 (C-4')	C-5 (C-5')	C-6 (C-6')
15	93.36 (93.68)	71.40 (70.58)	73.03 (74.00)	70.94 (70.67)	72.78 (72.40)	63.11 (62.92)
12[a)]	93.8	71.8	73.3	70.8	73.1	63.1

a) Data taken from ref. 16a.

The locations of two HHDP groups on the glucose cores were confirmed by the ^{1}H-^{13}C long-range COSY measured with J_{CH}=9 Hz (Fig. 2), in which the connectivities among H-4(4')/H-6(6') of glucoses and the HHDP protons were shown through the ester carbonyl carbons by three-bond couplings. Similarly, three galloyl groups in **15** were determined to be at O-1, O-3 and O-3'. The lowest singlet (δ7.59) ascribable to H-6" of the hellinoyl group was correlated with the H-2 signal of glucose through a common ester carbonyl carbon signal at δ163.97. The other singlet (δ6.72) of the hellinoyl group was similarly correlated with the H-2' signal of the glucose-II. Therefore, the hellinoyl group in **15** was determined to be at O-1', O-2' and O-2 of the glucose cores, leading to the gross structure (**15**) for hirtellin B. Reflecting this unique structure (**15**), hirtellin B has a different feature from other hydrolyzable tannins in the ^{1}H-NMR spectrum. It shows an unusually large upfield shift of the anomeric proton signal [H-1'; δ5.60 (d, *J*=8.5 Hz)] compared with that (δ6.20) of **12**. A large upfield shift of one [δ5.75 (d, *J*=2 Hz)] of the *meta*-coupled doublets in the hellinoyl group relative to that (δ6.94) of the dehydrodigalloyl group in **2** is also notable. These anomalies can be respectively interpreted in terms of a shielding effect of the hellinoyl A-ring and the ester carbonyl group of B-

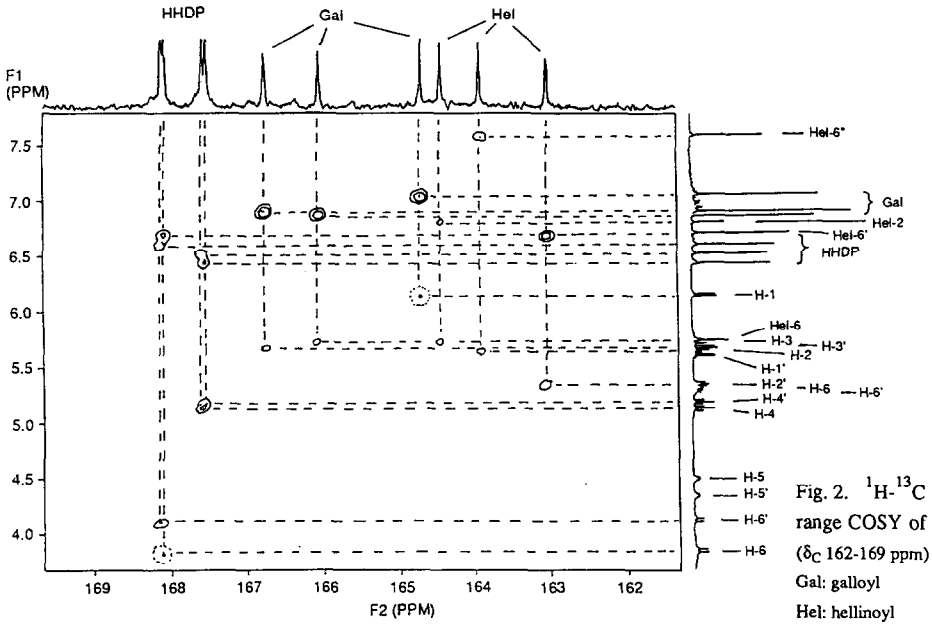
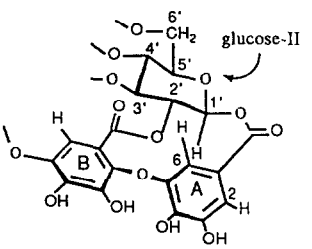


Fig. 2. ^{1}H-^{13}C Long-range COSY of **15** (δ_C 162-169 ppm)
Gal: galloyl
Hel: hellinoyl

ring, as illustrated by the formula **15'**.

Hirtellin B (**15**) exhibited a remarkable host-mediated antitumor activity against sarcoma-180 in mice. Upon intraperitoneal administration of **15** (10 mg/kg) to female ddY mice (6 mice/group) 4 days before inoculation of tumor cells (1 x 10^5/mouse), the number of 60 day survivors was 3 in a group, and the mean life-span of the others was prolonged up to 114 % relative to that of the control group.[22] This potent activity was comparable to those of oenothein B (**5**) and coriariin A.[7]



15'

It is also noteworthy that hirtellin B is the first example of an oligomeric hydrolyzable tannin isolated from the family Tamaricaceae, although the oligomers of various structures have been found in many plant families,[5,14] such as Rosaceae, Euphorbiaceae, Coriariaceae, Cornaceae, Oenotheraceae, Hamamelidaceae and Lythraceae.

Experimental

Optical rotations were measured on a JASCO DIP-4 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were obtained on a Varian VXR-500 (500 MHz for ^{1}H and 125.7 MHz for ^{13}C) spectrometer each with an exclusive probe. Chemical shifts are given in δ values (ppm) relative to TMS. Samples were dissolved, unless otherwise stated, in acetone-d_6 + D_2O which is suitable for the most of hydrolyzable tannins and related polyphenols. Varian's standard programs were used for COSY, NOESY, HETCOR and ^{1}H-^{13}C long-range COSY. NOESY was measured with mixing time at 0.5 sec, 32 scans, and 256 x 256 K, taking *ca.* 5 hr for 5 mg sample / 0.6 ml solvent. HETCOR and ^{1}H-^{13}C long-range COSY were obtained with average J_{CH} values 140 Hz, and 5, 7, 9 or 10 Hz for overnight runs, respectively, on *ca.* 0.05 mM solutions. FABMS were recorded on a VG 70-SE mass spectrometer at 8 kV (accelerating voltage), and the samples (2-3 mg)

were dissolved in 3 drops of acetone or acetone-H_2O. 3-Nitrobenzyl alcohol was used as a matrix agent, and a small amount of NaCl was added for positive ion detection. For high-performance liquid chromatography (HPLC), a Shimadzu Model LC-6A liquid chromatograph equipped with a Shimadzu SPD-6A UV detector (280 nm) was used. Normal-phase HPLC was carried out on a Superspher Si60 cartridge column (4 mm I.D. x 250 mm) using *n*-hexane-MeOH-THF-HCOOH (47:39:13:1) containing oxalic acid (450 mg/l) as the eluent, and reversed-phase HPLC on a LiChrospher RP-18 cartridge column (4 mm I.D. x 250 mm; Merck) in an oven at 40°C, using solvents A (0.01M KH_2PO_4-0.01M H_3PO_4-MeOH, 17:17:6), solvent B (0.01M KH_2PO_4-0.01M H_3PO_4-MeCN, 87:87:26), or solvent C (0.01M KH_2PO_4-0.01M H_3PO_4-EtOH-EtOAc, 42.5:42.5:15:1). Flow rate was set at 1.0 ml/min. Analytical and preparative thin-layer chromatography (TLC) were performed using Kieselgel 60 PF_{254} (Merck), and visualized under UV irradiation. Toyopearl HW-40 (Toso, Japan), Dia-ion HP-20 and MCI-gel CHP-20P (Mitsubishi Kasei Co. Ltd., Japan) were used for column chromatography. Ligroin refers to light petroleum, bp 75-120°C. Solvents were removed under reduced pressure below 40°C.

Isolation of isorugosin E (7) from Liquidambar formosana

Fresh leaves (5.4 kg) of *L. formosana*, collected in April 1987, from a tree planted at the Okayama University campus were homogenized in 70% acetone (21 l). Concentrated filtrate (3 l) from the homogenate was extracted with CH_2Cl_2 (3 l), EtOAc (3 l x 3) and *n*-BuOH (3 l x 3), successively. A portion (18.9 g) of the *n*-BuOH extract (89.6 g) was chromatographed over Dia-ion HP-20 with increasing concentrations of MeOH in water, and the eluate with 40% MeOH was chromatographed over Toyopearl HW-40 with 70% EtOH-70% acetone (10:0→9:1→8:2). A fraction containing **7** obtained from the eluate with 70% EtOH-70% acetone (9:1) was further purified by column chromatography on MCI-gel CHP-20P with 40% MeOH to give **7** (32 mg).

Isorugosin E (7)

A light-brown amorphous powder, $[\alpha]_D$ +63° (*c*=0.5, MeOH). *Anal.* Calcd for $C_{75}H_{54}O_{48}\cdot 9H_2O$: C, 47.78; H, 3.84. Found: C, 47.84; H, 3.87. FABMS: *m/z* 1745 $(M+Na)^+$. UV λ_{max} (MeOH) nm (log ε): 217 (5.12), 273 (4.76). CD (MeOH): $[\theta]_{222}$ +1.9 x 10^5, $[\theta]_{240}$ +1.4 x 10^5, $[\theta]_{259}$ -7.3 x 10^4, $[\theta]_{284}$ +7.3 x 10^4. ^{1}H-NMR: Aromatic protons, see text. Glucose protons, δ5.49 (d, *J*=3.5 Hz, H-1), 5.02, (dd, *J*=3.5, 10 Hz, H-2), 5.65 (t, *J*=10 Hz, H-3), 5.01 (t, *J*=10 Hz, H-4), 4.51 (ddd, *J*=1, 6.5, 10 Hz, H-5), 5.25 (dd, *J*=6.5, 13 Hz, H-6), 3.74 (dd, *J*=1, 13 Hz, H-6), 6.07 (d, *J*=8.5 Hz, H-1'), 5.55 (dd, *J*=8.5, 10 Hz, H-2'), 5.76 (t, *J*=10 Hz, H-3'), 5.17 (t, *J*=10 Hz, H-4'), 4.46 (ddd, *J*=1, 6.5, 10 Hz, H-5'), 5.14 (dd, *J*=6.5, 13.5 Hz, H-6'), 3.82 (dd, *J*=1, 13.5 Hz, H-6') (α-anomer); δ5.00 (d, *J*=8 Hz, H-1), 5.15 (dd, *J*=8, 10 Hz, H-2), 5.28 (t, *J*=10 Hz, H-3), 4.96 (t, *J*=10 Hz, H-4), 4.00 (dd, *J*=6.5, 10 Hz, H-5), 5.28 (dd, *J*=6.5, 13 Hz, H-6), 3.78 (d, *J*=13 Hz, H-6), 6.09 (d, *J*=8.5 Hz, H-1'), 5.58 (dd, *J*=8.5, 10 Hz, H-2'), 5.78 (t, *J*=10 Hz, H-3'), 5.18 (t, *J*=10 Hz, H-4'), 4.48 (ddd, *J*=1, 6.5, 10 Hz, H-5'), 5.12 (dd, *J*=6.5, 13.5 Hz, H-6'), 3.80 (dd, *J*=1, 13.5 Hz, H-6') (β-anomer). ^{13}C-NMR: δ62.98 (C-6', α- and β-anomers), 63.47, 63.60 (C-6, α- and β-anomers), 66.90 (C-5, α-anomer), 70.55 (C-4', α- and β-anomers), 71.15, 71.27, 71.33 (C-4, α- and β-anomers; C-3, α-anomer), 71.68, 71.73, 71.77 (C-2', α- and β-anomers; C-5, β-anomer), 72.82 (C-5', α- and β-anomers), 72.93 (C-2, α-anomer), 73.39 (C-3', α- and β-anomers), 73.44 (C-3, β-anomer), 73.85 (C-2, β-anomer), 91.03 (C-1, α-anomer), 93.24 (C-1', α- and β-anomers), 96.36 (C-1, β-anomer), 105.00, 105.38 [valoneoyl (Val) C-3'], 107.77 (HHDP C-3, Val C-3), 108.11, 108.18 (HHDP C-3'), 109.8-110.2 [Val C-6", galloyl (Gal) C-2 and C-6], 112.61, 112.64 (Val C-1"), 115.63, 115.80, 115.85 (HHDP C-1 and C-1', Val C-1), 117.64, 117.74 (Val C-1'), 119.82, 119.91, 120.12, 120.33, 120.39, 120.48, 120.75 (Gal C-1), 125.48, 125.54, 125.60, 125.90, 125.95, 126.22, 126.24 (Val C-2 and C-2', HHDP C-2 and C-2'), 136.28, 136.31, 136.50 (HHDP C-5 and C-5', Val C-5), 136.98, 137.17 (Val C-5'), 137.82, 137.96 (Val C-2"), 138.82, 138.88, 138.99, 139.17, 139.48 (Gal C-4), 140.54, 140.58 (Val C-3"), 141.06 (Val C-4"), 143.07, 143.16 (Val C-5"), 144.25, 144.28 (HHDP C-6 and C-6'), 144.52, 144.58 (Val C-6 and C-6'), 145.14, 145.18, 145.41, 145.49, 145.75, 145.84, 145.90 (HHDP C-4 and C-4', Val C-4, Gal C-3 and C-5), 146.35, 146.45 (Val C-4'), 162.62, 162.74 (Val C-7"), 165.98, 166.11, 166.28, 166.45, 166.56 (Gal C-7), 167.62, 167.70, 167.73, 168.19, 168.30, 168.47 (HHDP C-7 and C-7', Val C-7 and C-7').

Partial hydrolysis of 7

A solution of **7** (1 mg) in 0.03M phosphate buffer (pH 5.8) (1 ml) was kept at 37°C for 6 h, and then the reaction mixture was analyzed by normal-phase and reversed-phase HPLC, to show the formation of **11** [normal-phase HPLC, R_t 6.9 and 7.5 min (anomer mixture); reversed-phase HPLC, R_t 5.4 and 12.6 min (solvent A), R_t 4.2 and 5.8 min (solvent B)] and **10** [normal-phase HPLC, R_t 7.5 and 8.0 min; reversed-phase HPLC, R_t 4.2 and 13.4 min (solvent A), R_t 3.7 and 5.2 min (solvent B)]. The formation of 2,3-di-*O*-galloyl-D-glucose [normal-phase HPLC, R_t 5.6 and 5.9 min; reversed-phase HPLC, R_t 3.1 and 4.0 min (solvent A), R_t 2.7 and 3.0 min (solvent B)] was also observed.

Treatment of isorugosin D (8) with tannase

Tannase was added to an aqueous solution (2.5 ml) of isorugosin D (**8**)[14] (5 mg), and the mixture was kept at 37°C for 1 h. After the enzyme reaction was stopped by adding 10% HCl to pH 1, the solution was subjected to column chromatography over MCI-gel CHP-20P with increasing concentrations of MeOH in water. The eluate with 30% MeOH afforded **7** (1.3 mg).

Isolation of hirtellin B (15) from Reaumuria hirtella

The dried leaves (3.3 kg) of *R. hirtella*, collected at Sinai peninsula, Egypt, in March 1989 (identified by Prof. N. El-Hadidi, Faculty of Science, Cairo University, and a voucher specimen is deposited in the Herbarium of Faculty of Pharmaceutical Sciences, Okayama University), were homogenized in 70% acetone (7 l x 3). The homogenate was filtered, and the filtrate was concentrated to *ca.* 5 l, and kept overnight. The insoluble lipoid portion was removed by decantation, and then the solution was further concentrated to *ca.* 2.5 l, from which brown astringent viscous materials were precipitated. The insoluble materials were collected by centrifugation, washed with water and suspended in MeOH. The MeOH soluble portion gave dark brown residue (348.8 g) after removal of the solvent. The washings and the supernatant from the centrifugation were combined and extracted with Et_2O, EtOAc and *n*-BuOH (presaturated with water), successively. A part (8 g) of the EtOAc extract (65.7 g) was subjected to column chromatography over Toyopearl HW-40 (2.2 cm I.D. x 55 cm) developing with MeOH-H_2O (8:2)→MeOH-acetone-H_2O (7:2:1)→MeOH-acetone-H_2O (6:2:2) in a stepwise gradient mode. The residue obtained from the eluate with MeOH-acetone-H_2O (7:2:1) was further purified by column chromatography over MCI-gel CHP-20P using increasing amount of MeOH in water. The 30% MeOH eluate gave hirtellin B (**15**) (392 mg). A further crop (350 mg) of hirtellin B was also obtained by column chromatography of a part (20 g) of the *n*-BuOH extract (131 g) over Dia-ion HP-20 [H_2O→aqueous MeOH (10→30→50→60%)→MeOH] followed by chromatography over MCI-gel CHP-20P using the same solvent system.

Hirtellin B (15)

An off-white amorphous powder, $[\alpha]_D$ +116° (*c*=1.0, MeOH). *Anal.* Calcd for $C_{82}H_{56}O_{52}\cdot 12H_2O$: C, 47.14; H, 3.86. Found: C, 46.99; H, 3.53. UV λ_{max} (MeOH) nm (log ε): 218 (5.14), 275 (4.80). FABMS *m/z*: 1895 $(M+Na)^+$. CD (MeOH): $[\theta]_{233}$ +3.6 x 10^5, $[\theta]_{260}$ -6.6 x 10^4, $[\theta]_{280}$ +5.7 x 10^4, $[\theta]_{320}$ -0.9 x 10^4. ^{1}H-NMR: δ6.16 (d, *J*=8 Hz, H-1), 5.66 (dd, *J*=8, 10 Hz, H-2), 5.74 (t, *J*=10 Hz, H-3), 5.14 (t, *J*=10 Hz, H-4), 4.51 (dd, *J*=6, 10 Hz, H-5), 5.35 (dd, *J*=6, 13 Hz, H-6), 3.86 (d, *J*=13 Hz, H-6), 5.60 (d, *J*=8.5 Hz, H-1'), 5.36 (dd, *J*=8.5, 10 Hz, H-2'), 5.69 (t, *J*=10 Hz, H-3'), 5.19 (t, *J*=10 Hz, H-4'), 4.35 (ddd, *J*=1.5, 7, 10 Hz, H-5'), 5.31 (dd, *J*=7, 13 Hz, H-6'), 4.13 (dd, *J*=1.5, 13 Hz, H-6'). Aromatic protons, see text. ^{13}C-NMR: δ107.17 [hellinoyl (Hel) C-6], 107.75, 107.82, 107.90, 108.86 (HHDP C-3 and C-3'), 108.35 (Hel C-6'), 110.05 (2C), 110.13 (2C), 110.45 (2C) (Gal C-2 and C-6), 111.31, 114.71, 119.43 (Hel C-1, C-1' and C-1"), 111.81 (Hel C-2), 115.56, 115.64, 115.67 (HHDP C-1 and C-1'), 119.23 (Hel C-6"), 120.03, 120.05, 120.16 (2C) (Gal C-1), 125.70, 125.84, 126.21, 126.24 (HHDP C-2 and C-2'), 136.27, 136.30, 136.40 (2C) (HHDP C-5 and C-5'), 138.87, 139.10, 139.56 (Gal C-4), 139.18 (Hel C-4'), 139.71 (Hel C-4), 139.75 (Hel C-3'), 141.85 (Hel C-5'), 142.25 (Hel C-2'), 140.42, 143.17, 144.20 (Hel C-2", C-4" and C-5"), 144.24, 144.29 (3C) (HHDP C-6 and C-6'), 145.08 (4C) (HHDP C-4 and C-4'), 145.42 (2C), 145.62 (2C), 145.71 (2C) (Gal C-3 and C-5), 146.85 (Hel C-3), 147.51 (Hel C-5), 163.09 (Hel C-7'), 163.97 (Hel C-7"), 164.49 (Hel C-7), 164.76, 166.09, 166.80 (Gal C-7), 167.57, 167.62, 168.13, 168.16 (HHDP C-7 and C-7'), glucose carbons, see Table 1.

Methylation of hirtellin B (15)

A mixture of **15** (100 mg), anhydrous K_2CO_3 (1 g) and dimethyl sulfate (1 ml) in dry acetone (25 ml) was stirred overnight at room temperature, and refluxed for 6 h. After removal of the inorganic materials by filtration, the filtrate was concentrated and submitted to preparative TLC (benzene-acetone 5:1) to yield the octacosamethyl derivative (**15a**) (86 mg) as a white amorphous powder. FABMS: *m/z* 2287 $(M+Na)^+$. ^{1}H-NMR: δ7.66 (1H, s, Hel), 7.28, 7.19, 7.05 (2H each, s, Gal), 6.99 (1H, s, Hel), 6.95, 6.00 (1H each, d, *J*=2 Hz, Hel), 6.92, 6.88, 6.81, 6.79 (HHDP), 6.31 (d, *J*=8 Hz, H-1), 5.81 (m, H-2 and H-3), 5.27 (t, *J*=10 Hz, H-4), 4.51 (ddd, *J*=2, 7, 10 Hz, H-5), 5.36 (dd, *J*=7, 13 Hz, H-6), 4.11 (dd, *J*=2, 13 Hz, H-6), 5.80 (d, *J*=8.5 Hz, H-1'), 5.44 (dd, *J*=8.5, 10 Hz, H-2'), 5.84 (t, *J*=10 Hz, H-3'), 5.32 (t, *J*=10 Hz, H-4'), 4.67 (dd, *J*=6, 10 Hz, H-5'), 5.32 (dd, *J*=6, 13 Hz, H-6'), 3.96 (overlapped by OMe signals, H-6'), 4.01, 3.98, 3.91, 3.88, 3.87, 3.85, 3.81, 3.78, 3.74, 3.72, 3.69, 3.66, 3.65, 3.64, 3.60, 3.53 (each 3H, s, OMe), 3.80, 3.83, 3.82, 3.84, 3.86, 3.89 (each 6H, s, OMe). ^{13}C-NMR: δ56.19-56.51, 60.56-61.60 (OMe), 93.53 (C-1), 72.22 (C-2), 75.43 (C-3), 71.18 (C-4), 72.39 (C-5), 63.70 (C-6), 93.47 (C-1'), 71.49 (C-2'), 73.86 (C-3'), 71.21 (C-4'), 72.57 (C-5'), 63.58 (C-6'), 106.41, 106.53, 106.68, 107.21, 107.76 (2C), 107.90 (2C), 108.06, 108.20 (2C), 109.41, 109.60, 114.68, 120.66, 121.60 (2C), 122.81, 122.88, 123.00, 123.28, 123.62, 123.99, 124.60, 124.64, 129.13, 129.43, 129.45, 129.63, 143.55, 143.60, 144.02, 144.83, 159.99 (2C), 145.19, 146.05, 147.24, 148.45, 148.49, 148.57, 149.05, 149.54, 150.07,

153.20 (4C), 153.36 (2C), 153.80 (2C), 153.92 (4C), 154.06 (3C), 154.52, 162,86, 163.29, 163.95, 164.13, 165.89, 166.72, 167.43, 167.52, 167.83, 167.94.

*Methanolysis of methylated hirtellin B (**15a**)*

A mixture of **15a** (85 mg) and 1% NaOMe (1 ml) in absolute MeOH (1 ml) was left standing overnight at room temperature. After neutralization with AcOH, the reaction mixture was evaporated under N_2, and treated with an excess of ethereal CH_2N_2 for 3 h. The residue obtained after removal of the solvent was submitted to preparative TLC (ligroin-CH_2Cl_2-acetone 6:4:1) to give methyl tri-*O*-methylgallate (**16**) (15 mg), dimethyl hexamethoxydiphenate (**17**) (26 mg), $[\alpha]_D$ -34° (*c*=1.0, acetone), and trimethyl hepta-*O*-methylhellinate (**18**) (18 mg) [white amorphous powder, FABMS: *m/z* 669 $(M+Na)^+$. ^{1}H-NMR (acetone-d_6): δ7.32 (1H, s, H-6"), 6.86 (1H, s, H-6'), 7.30 (1H, d, *J*=2 Hz, H-2), 6.78 (1H, d, *J*=2 Hz, H-6), 3.58, 3.76 (6H), 3.77, 3.79, 3.92, 3.93, 3.935, 3.94, 4.07 (OMe). ^{13}C-NMR: δ52.34 (2C), 52.47, 56.53, 56.57, 60.88, 61.29, 61.58 (2C), 61.67 (OMe), 107.83 (C-2), 109.23 (C-6), 109.52 (C-6"), 111.69 (C-6'), 120.25 (C-1"), 120.46 (C-1), 125.66 (C-1'), 142.23 (C-2"), 143.09 (C-2'), 147.79 (C-4'), 148.11 (C-4"), 150.89, 153.30 (C-5, C-5'), 142.92, 147.79, 148.19, 151.69, 154.56 (C-3, C-4, C-3', C-3", C-5")].

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References and Notes

†Tannins of Hamamelidaceous Plants. Part V. For Part IV, see ref. 19. This paper also constitutes Part I in the series of Tannins of Tamaricaceous Plants.

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22. Details of the antitumor activity of **15** will be reported elsewhere.