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C-Glycosidic Ellagitannin Metabolites in the Heartwood of Japanese Chestnut Tree (*Castanea crenata* SIEB. *et* ZUCC.)

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HPLC comparison of tannins in the bark, sapwood and heartwood of *Castanea crenata* showed striking differences of composition. The major tannin of the bark is hamamelitannin, whereas vescalagin and castalagin are dominant in the wood. Inner heartwood contained three new *C*-glycosidic ellagitannin metabolites, castacrenins A, B and C. Outer heartwood contained dimeric, trimeric (castaneanin A), tetrameric (castaneanins B and C) and pentameric (castaneanin D) *C*-glycosidic ellagitannins composed of vescalagin and castalagin units. Castacrenins and castaneanins are derived from vescalagin and castalagin by intramolecular and intermolecular dehydration, respectively.

Key words tannin; *Castanea crenata*; heartwood; Fagaceae; polyphenol

Japanese chestnut tree (*Castanea crenata* SIEB. *et* ZUCC., Fagaceae) is a very durable wood, which is used as a building material. The durability is attributable to its extensive heartwood with a high concentration of tannins (about 8%).[1] The outer layer of the wood, *i.e.* sapwood, contains living parenchymal cells and reserve material. In this area, synthesis of phytoalexins and stress metabolites has been observed after wounding, injury or fungal attack.[2] In heartwood, the inner layers of mature trees, all cells are dead and reserve material has been converted into constituents such as tannins and other compounds characteristic of each plant species. Tannins in heartwood are mainly biosynthesized at the sapwood-heartwood transitional region,[3] and they are considered to act as defensive factors against fungi and bacteria.[4] As the tree grows, the parenchymal cells of the inner sapwood die and the heartwood extends. Tannins which are synthesized in parenchymal cells at the sapwood–heartwood transitional region remain where they are synthesized, without active translocation. Although diffusion and penetration of tannins within wood tissue may not be negligible,[5] apparent localization of tannins in parenchymal tissue[6] suggests that the diffusion is restricted. These facts imply that tannins in the inner heartwood have been stored for a long period since having been biosynthesized. We are interested in the chemical conversion of tannins during prolonged storage, and hence examined the phenolic constituents of the heartwood of Japanese chestnut tree. This paper deals with HPLC comparison of the tannin compositions in wood cross sections, and with the isolation and characterization of *C*-glycosidic ellagitannins and related compounds found in heartwood.

Results and Discussion

First, the tannin compositions of the bark (total tannin content, 10%[1]), sapwood (4%), heartwood (8%) of Japanese chestnut tree were compared by HPLC (Fig. 1). In contrast to the bark, which contains hamamelitannin (**1**)[7] as the major constituent, the wood, especially around the sapwood–heartwood transitional region, dominantly accumulated castalagin (**2**) and vescalagin (**3**).[3a,8] Condensed tannins (proanthocyanidins) observed in the bark in addition to **1** are completely absent in the wood. In the center of the heartwood, five characteristic compounds were detected, two of which were identified as castalin (**4**) and vescalin (**5**).[9] The remaining three, designated as castacrenins A (**6**), B (**7**) and C (**8**), were isolated from the inner heartwood by extraction with aqueous acetone followed by successive chromatographies on Sephadex LH-20, MCI-gel CHP20P and Cosmosil 75C_{18}-OPN. Besides **2** and **3**, there are some minor compounds which mainly exist in the outer heartwood and decrease in the inner heartwood (Fig. 1, **9** and **11**). Six compounds **9**—**14** showing a similar distribution were isolated from the aqueous acetone extract of outer heart-

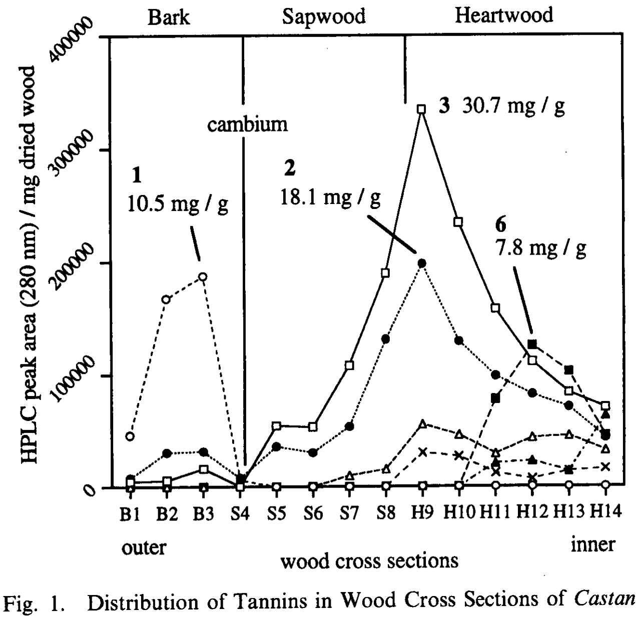

Fig. 1. Distribution of Tannins in Wood Cross Sections of *Castanea crenata*

B, bark; S, sapwood; H, heartwood; S4: cambium; H14: center of wood; (○), **1**; (●), **2**; (□), **3**; (▲), **4**; (■), **6**; (△), **9**; (×), **11**.

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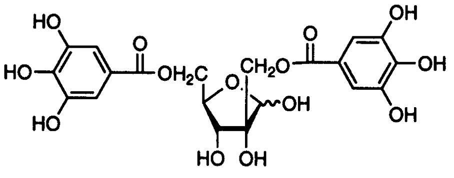
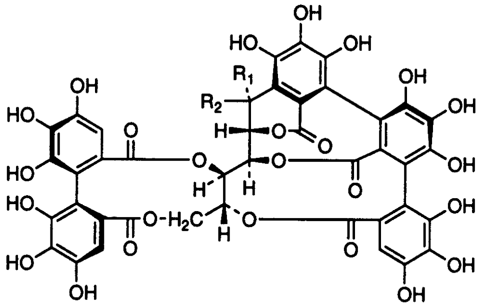
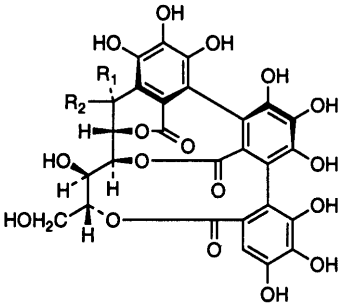


1



2 : $R_1 = OH$, $R_2 = H$
3 : $R_1 = H$, $R_2 = OH$



4 : $R_1 = OH$, $R_2 = H$
5 : $R_1 = H$, $R_2 = OH$

wood by column chromatographies over Sephadex LH-20, MCI-gel CHP20P and TSK-gel Toyopearl HW-40F, and finally by preparative HPLC.

Castacrenin A (**6**) was isolated as a yellow powder and showed the $[M-H]^-$ ion peak at m/z 613 in the FAB-MS (negative ion mode), *i.e.*, 18 mass unit less than those of **4** and **5**. The ^{1}H-NMR spectrum (Table 1) showed an aromatic singlet at δ 7.61 and seven aliphatic signals due to the five oxygen-bearing methines (H-1—H-5) and one methylene (H-6) of a polyalcohol moiety. The ^{13}C-NMR spectrum was related to that of **4**, indicating the presence of three pyrogallol-type aromatic rings and three carboxyl carbons besides the six aliphatic carbons of the polyalcohol moiety. The carboxyl carbon signals (δ 159.0, 160.8 and 162.9) appeared at significantly higher field compared with those of **4** (δ 167.8, 166.1 and 165.0), and their chemical shifts were similar to those of the δ-lactones of ellagic acid derivatives (δ 158—160).[10] In addition, the negative FAB-MS exhibited a fragment ion peak at m/z 301, which coincided with the $[M-H]^-$ peak of ellagic acid. These spectroscopic observations suggested that two of the three pyrogallol rings form a bislactone similar to ellagic acid. The remaining pyrogallol ring was revealed to be attached to the C-1 of the polyalcohol moiety by a C–H long-range shift correlation spectroscopic (COSY) experiment, in which H-1 correlated with C-1′, C-5′ and C-6′ of the pyrogallol ring. Furthermore, the chemical shift of H-2 (δ 4.79) suggested that the remaining carboxyl carbon was linked to the C-2 position through an ester linkage, analogously with the structure of **4**. From these spectral observations the presence of a flavogallonyl moiety in the form of a bislactone was inferred.

On methylation with CH_2N_2, **6** yielded a heptamethyl ether (**6a**) (EI-MS m/z: 712 $[M^+]$). Acetylation of **6a** afforded a triacetate **6b** [FAB-MS (positive ion mode) m/z: 839 $[M+H]^+$], the ^{1}H-NMR spectrum of which showed large low field shifts of H-3 ($\Delta\delta$ 1.10), H-5 ($\Delta\delta$ 1.25) and H-6 ($\Delta\delta$ 0.78 and 0.86), indicating the presence of free hydroxyl groups at these positions of **6**. Taking the molecular weight into account, these results implied an ether linkage between C-1 and C-4. From these observations, the plane structure of castacrenin A was deduced to be **6**. The relative stereochemistry of H-1 and H-2 was concluded to be *cis* on the basis of the small J value ($J_{1,2} = 3$ Hz) and the result of computer-assisted molecular modeling (CAChe system). The configuration of the remaining methines and the atropisomerism of the biphenyl bond were determined by derivation of **6** from **4** by heating in diluted HCl. Hence, the structure of castacrenin A was established to be as shown in formula **6**.

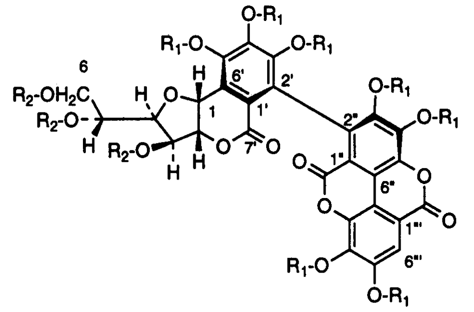
Castacrenin B (**7**) has the same molecular weight as that of **6** [FAB-MS (negative ion mode) m/z: 613 $[M-H]^-$]. The ^{1}H- and ^{13}C-NMR spectra were also related to those of **6**, suggesting the presence of a bislactone form of the flavogallonyl moiety and a string of six oxygen-bearing aliphatic carbons. Methylation with CH_2N_2, followed by acetylation, afforded a triacetylheptamethyl ether **7a** (FAB-MS (positive ion mode) m/z: 839 $[M+H]^+$), indicating the occurrence of seven phenolic and three alcoholic hydroxyl groups in **7**. The most striking difference between **7** and **6** was the larger J values of aliphatic protons of **7** (Table 1). In the heteronuclear multiple bond coherence (HMBC) spectrum, H-1 of the polyalcohol moiety was correlated with C-5 (δ 76.7) as well as C-2 (δ 78.8), C-3 (δ 73.5) and C-1′ (δ 116.0). This observation suggested that **7** has a tetrahydropyran ring structure similar to that of bergenin.[11] This was supported by the large low-field shifts of H-3 ($\Delta\delta$ 1.3), H-4 ($\Delta\delta$ 1.44) and H-6 ($\Delta\delta$ 0.65 and 0.58) in **7a** compared with those of **7**. The relative configuration between H-1 and H-2 was concluded to be *cis* from the smaller J value (6 Hz) between H-1 and H-2 compared with that of bergenin ($J_{1,2} = 10$ Hz), and from the difference of the chemical shifts of C-1 and C-5 [**7**: 68.4 (C-1), 76.7 (C-5); bergenin: 73.4 (C-1), 82.3 (C-5)].[11] Furthermore, **7** was also obtained by treatment of **4** with diluted HCl, along with **6**, confirming the structure of castacrenin B (**7**).

Castacrenin C (**8**) showed the $[M-H]^-$ peak at m/z 613 in the FAB-MS (negative ion mode) indicating the same molecular weight as those of **6** and **7**. The ^{13}C-NMR spectrum was also related to those of **6** and **7**, showing the signals arising from a flavogallonyl moiety; however, instead of six aliphatic carbons of **6** and **7**, the signals due to a polarized double bond (δ 98.4 and 155.0) appeared along with four aliphatic carbon signals (δ 72.7, 72.0, 70.0 and 64.5). In the ^{1}H-NMR spectrum, the olefinic methine proton signal was observed at δ 7.08 as a doublet ($J = 1$ Hz) which coupled with the methine proton (δ 4.76) at the allylic position. Furthermore, **8** was also generated as a minor product on acid treatment of **4**. Taking the molecular weight into account, these observations suggested that the structure is represented by the formula **8**. The allylic carbon (C-3) readily underwent intramolecular substitution by attack of the hydroxyl group at C-6 to yield **8a**, the structure of which was deduced from the FAB-MS [m/z: 595 $(M-H)^-$] and the large low-field

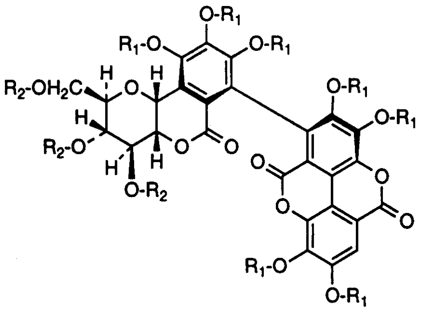
Table 1. ^{1}H-NMR Data for Castalin (**4**), Castacrenins A (**6**), B (**7**) and C (**8**) and **8a** (in Acetone-d_6 + D_2O)

	4	**6**	**7**	**8**	**8a**
H-1	5.57 (d, 5)[a]	5.51 (d, 3)	5.71 (d, 6)	7.08 (d, 1)	6.96 (s)
H-2	5.14 (dd, 1, 5)	4.79 (dd, 1, 3)	4.39 (dd, 6, 8)		
H-3	4.92 (dd, 1, 7)	4.59 (dd, 1, 3)	4.02 (t, 8)	4.76 (t, 1)	4.46 (d, 5.5)
H-4	3.96 (t, 7)	4.15 (dd, 3, 9)	3.50 (t, 8)	[b]	4.31 (m)
H-5	5.11 (m)	4.09 (ddd, 3, 5, 9)	3.59[b]	[b]	4.31 (m)
H-6	3.889 (dd, 3, 12)	3.87 (dd, 3, 11)	4.01 (dd, 3, 12)	[b]	4.14 (dd, 5, 9)
	3.806 (dd, 6, 12)	3.79 (dd, 5, 11)	3.77 (dd, 7, 12)	[b]	3.80 (dd, 3, 9)
H-6‴	6.75 (s)	7.61 (s)	7.62 (s)	7.64 (s)	7.62 (s)

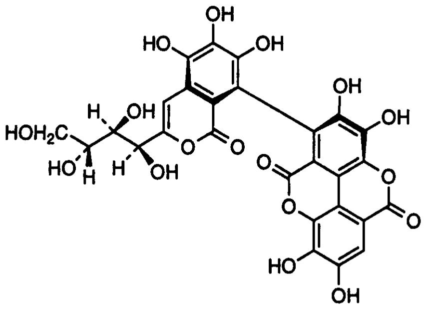
[a] J values in parentheses are expressed in Hz. [b] Overlapped with the large HOD signal at δ 3.89.



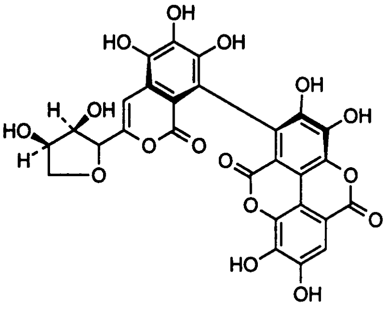
6 : $R_1 = R_2 = H$
6a : $R_1 = Me$, $R_2 = H$
6b : $R_1 = Me$, $R_2 = Ac$



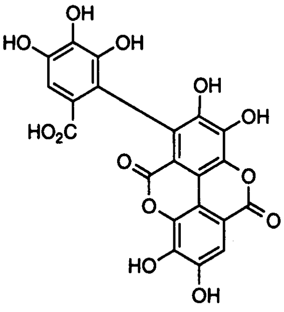
7 : $R_1 = R_2 = H$
7a : $R_1 = Me$, $R_2 = Ac$



8



8a



8b

shifts of C-6 and C-3 in the ^{13}C-NMR spectrum. Prolonged heating of **4** in aqueous acidic solution afforded **8a** as the major product together with flavogallonic acid bislactone (**8b**).[12] Considering their structural relationship, castacrenins A (**6**), B (**7**) and C (**8**) originate biogenetically from **2**—**5**; however, **8a** was not isolated from the wood.

Compounds **9** and **10** were identified as ellagitannin dimers, roburins D and A,[13] respectively, by comparison of ^{1}H- and ^{13}C-NMR data with reported values. The remaining compounds **11**—**14** were found to be new compounds and were designated as castaneanins A (**11**), B (**12**), C (**13**) and D (**14**). Their molecular weights were considered to be significantly higher than those of **2**, **3**, **9** and **10** on the basis of their lower *Rf* value on silica gel and cellulose TLC. It is known that a plot of the molecular weights (M.W.) of a series of structurally related oligomeric ellagitannins *versus* their t_R in normal-phase HPLC on a semilongarithmic scale gives a straight line.[14] Since ^{1}H-NMR spectral comparison showed that all of compounds **9**—**14** are structurally related, *vide infra*, the plot obtained from **2**, **3**, **9** and **10** was extrapolated to estimate the molecular weight of **11**—**14** (Fig. 2). The results suggested that these compounds are trimeric (**11**, M.W.: 2766), tetrameric (**12** and **13**, M.W.: 3682) and pentameric (**14**, M.W.: 4598) *C*-glycosidic ellagitannins. Their molecular weights were finally confirmed by FAB-MS in the negative ion mode.

The ^{1}H-NMR spectra of compounds **11**—**14** were closely related to those of **9** and **10** showing aromatic singlets as well as the signals arising from *C*-glycosidically linked open-chain glucoses. The chemical shifts of the glucose moieties indicated that their hydroxyl groups were fully acylated (Table 2). The coupling constants and ^{1}H–^{1}H COSY analyses indicated that these compounds consist of castalagin and vescalagin units, which differ in the configuration at C-1 of the polyalcohol moiety. The appearance of doublet signals around δ 5.72 (J=5 Hz, H-1) and around δ 5.05 (J=7 or 8 Hz, H-3) in the spectra of **11**, **12** and **14** implied the presence of a castalagin unit at the upper terminal, which is analogous to that of **9**. On the other hand, the doublet signal at δ 4.66 (J=7 or 8 Hz, H-3) is characteristic of the vescalagin unit at the upper terminal of compounds **10** and **13**. In the spectrum of **9**, the signal of one of the four methylene protons (H-6a, b and H-6′a, b) was greatly shifted to higher field (about δ 2.8) compared to those of **2** and **3**. This change in chemical shift is attributable to anisotropic shielding caused by stacking of the aromatic ring of the lower vescalagin unit. Similarly, two of the six methylene protons in **11**, three of the eight in **12** and **13**, and four of the ten in **14** resonated at upper field (Table 2), suggesting that the mode of inter-unit linkage of these compounds is analogous to those of **9** and **10**. This was further supported by the following ^{13}C-NMR spectral comparison: among 18 aliphatic carbon signals in the spectrum of **11** two were observed at δ 40.0 and 40.4, which chemical shifts are similar to that of C-1′ (δ 40.5) of **9**. Similarly, **12** showed three signals at δ 40.32, 40.34 and 40.7, **13** showed three signals at δ 40.3, 40.5 and 40.7, and **14** exhibited four signals at δ 40.4, 40.5, 40.7 and 40.8. These ^{1}H- and ^{13}C-NMR spectral observations suggested that the C-1 carbon of the lower vescalagin unit was linked to the hexahydroxydiphenoyl group of the upper unit through a C–C bond.

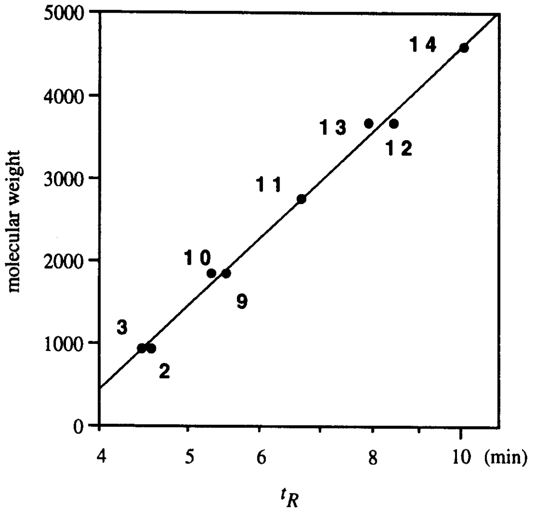
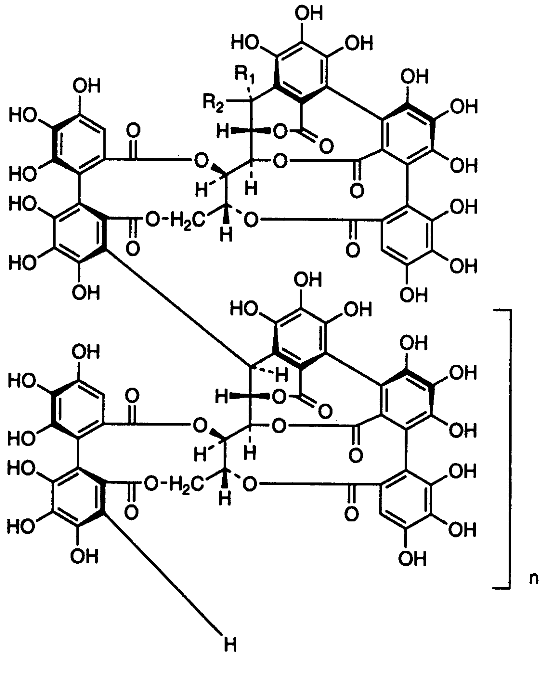


Fig. 2. Correlation between Retention Times of **2**, **3**, **9**—**14** on Normal-Phase HPLC and Their Individual Molecular Weights

The isolation yields of these compounds were so low, due to the difficulty of purification, that each pure compound could not be examined chemically. After methylation of a fraction containing the mixture of these oligomeric ellagitannins, the products were hydrolyzed and treated with diazomethane to afford dimethyl (*S*)-hexamethoxydiphenate (**15**).[15] The remaining products were acetylated and separated to yield **16**. This product showed the $[M+H]^+$ peak at *m*/*z* 1349 in the FAB-MS (positive ion mode), and signals due to eighteen methoxyl and three acetyl groups along with two aromatic singlets in the ^{1}H-NMR spectrum. In addition, the appearance of eight carboxyl carbons and a methine carbon at δ 43.6 (C-1) in the ^{13}C-NMR spectrum suggested that the structure of this product was represented by the formula **16**, which is in agreement with the structure of the middle units of the oligomers. On the basis of this spectroscopic and chemical evidence, the structures of castaneanins A—D were presumed to be as shown by formulae **11**—**14**, respectively.

Castacrenins and castaneanins apparently originate from **2** and **3** which are the dominant metabolites of the sapwood–heartwood transitional part. Castacrenins are formed by intramolecular dehydration of **4** or **5**, generated by hydrolysis of **2** and **3**. These compounds are partially responsible for the yellow color of the inner heartwood of the Japanese chestnut tree. In contrast, castaneanins are derived by intermolecular dehydrative condensation of **2** and **3**. Although all the cells are dead in heartwood, the possible participation of residual enzyme molecules in the biosynthesis of these compounds can not be ruled out. However, generation of the dimer **10** by non-enzymatic condensation of two molecules of **3** was reported,[16] suggesting that castaneanins are formed non-enzymatically. In European oak heartwood, ellagitannins are polymerized into darker polymers insoluble in cold water.[17] The presence of similar insoluble phenolic substances has also been observed in the heartwood of Japanese chestnut tree, although their relation to castaneanins, all of which



Dimer
9 : R_1=OH, R_2=H, n=1
10 : R_1=H, R_2=OH, n=1

Trimer
11 : R_1=OH, R_2=H, n=2

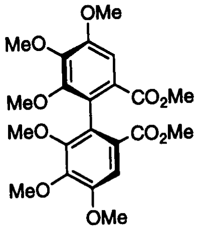
Tetramer
12 : R_1=OH, R_2=H, n=3
13 : R_1=H, R_2=OH, n=3

Pentamer
14 : R_1=OH, R_2=H, n=4

Table 2. ^{1}H-NMR Spectral Data for **2**, **3**, **9**, **10**, **11**, **12**, **13** and **14** (270 and 400 MHz, in Acetone-d_6 + D_2O)

	2	**3**	**9**	**10**	**11**	**12**	**13**	**14**
H-1	5.72 (d, 5)	4.92 (d, 2)	5.71 (d, 5)	4.95 (d, 2)	5.72 (d, 5)	5.73 (d, 5)	4.97 (br s)	5.72 (d, 5)
H-1′			4.89 (d, 1)	4.89 (d, 1)	4.97 (br s)	4.98$^{a)}$ (br s)	4.95 (br s)	4.97$^{a)}$ (br s)
H-1″					4.89 (d, 1)	4.96$^{a)}$ (br s)	4.95 (br s)	4.95$^{a)}$ (br s)
H-1‴						4.90 (br s)	4.90 (br s)	4.95$^{a)}$ (br s)
H-1⁗								4.90 (br s)
H-2	5.06 (dd, 1, 5)	5.30 (dd, 2, 2)	5.00 (d, 5)	5.24 (br s)	5.02 (br d, 5)	5.03 (d, 6)	5.14$^{a)}$ (br s)	5.03 (d, 5)
H-2′			5.23 (br s)	5.24 (br s)	5.10 (br s)	5.14$^{b)}$ (br s)	5.14$^{a)}$ (br s)	5.11$^{b)}$ (br s)
H-2″					5.26 (br s)	5.11$^{b)}$ (br s)	5.12$^{a)}$ (br s)	5.14$^{b)}$ (br s)
H-2‴						5.27 (br s)	5.27 (br s)	5.14$^{b)}$ (br s)
H-2⁗								5.27 (br s)
H-3	5.04 (dd, 1, 7)	4.59 (dd, 2, 7)	5.03 (d, 8)	4.66 (d, 8)	5.04 (d, 7)	5.05 (d, 8)	4.66 (d, 7)	5.05 (d, 7)
H-3′			4.79 (d, 7)	4.80 (d, 8)	4.87 (d, 8)	4.88 (d, 7)	4.88 (d, 7)	4.87 (d, 7)
H-3″					4.81 (d, 8)	4.88 (d, 7)	4.88 (d, 7)	4.87 (d, 7)
H-3‴						4.83 (d, 8)	4.82 (d, 7)	4.87 (d, 7)
H-3⁗								4.82 (d, 7)
H-4	5.25 (dd, 7, 8)	5.23 (dd, 7, 8)	5.21 (dd, 7, 8)	5.19 (t, 7)	5.22 (t, 7)	5.22 (t, 7)	5.20 (t, 7)	5.22 (t, 7)
H-4′			5.30 (t, 7)	5.30 (t, 7)	5.26 (t, 7)	5.25 (t, 7)	5.25 (t, 7)	5.25 (t, 7)
H-4″					5.29 (t, 7)	5.25 (t, 7)	5.25 (t, 7)	5.25 (t, 7)
H-4‴						5.29 (t, 7)	5.29 (t, 7)	5.25 (t, 7)
H-4⁗								5.29 (t, 7)
H-5	5.61 (dd, 2, 8)	5.66 (dd, 3, 13)	5.42 (dd, 2, 7)	5.46 (br d, 7)	5.43 (br d, 7)	5.44 (br d, 7)	5.47 (br d, 7)	5.44 (br d, 7)
H-5′			5.53 (br d, 7)	5.53 (br d, 7)	5.36 (br d, 7)	5.37 (br d, 7)	5.37 (br d, 7)	5.37 (br d, 7)
H-5″					5.54 (br d, 7)	5.37 (br d, 7)	5.37 (br d, 7)	5.37 (br d, 7)
H-5‴						5.55 (br d, 7)	5.54 (br d, 7)	5.37 (br d, 7)
H-5⁗								5.55 (br d, 7)
H-6a	5.09 (dd, 2, 13)	5.08 (dd, 3, 13)	4.37 (dd, 2, 13)	4.38 (dd, 3, 13)	4.38 (br d, 11)	4.39 (br d, 11)	4.39 (br d, 11)	4.39 (br d, 11)
H-6b	4.02 (d, 13)	4.08 (d, 13)	2.85 (d, 13)	2.86 (d, 13)	2.83 (d, 12)	2.85 (d, 12)	2.88 (d, 11)	2.85 (d, 12)
H-6′a			4.76 (br d, 12)	4.76 (br d, 12)	4.23 (br d, 11)	4.24 (br d, 12)	4.23 (br d, 12)	4.24 (br d, 12)
H-6′b			4.03 (d, 12)	4.04 (d, 12)	2.86 (d, 12)	2.88$^{c)}$ (d, 12)	2.88$^{b)}$ (d, 12)	2.88$^{c)}$ (d, 12)
H-6″a					4.75 (br d, 11)	4.24 (br d, 12)	4.23 (br d, 12)	4.24 (br d, 12)
H-6″b					4.06 (d, 12)	2.85$^{c)}$ (d, 12)	2.85$^{b)}$ (d, 12)	2.85$^{c)}$ (d, 12)
H-6‴a						4.75 (br d, 11)	4.75 (br d, 12)	4.24 (br d, 12)
H-6‴b						4.08 (d, 12)	4.07 (d, 12)	2.85$^{c)}$ (d, 12)
H-6⁗a								4.76 (br d, 12)
H-6⁗b								4.07 (d, 12)
Arom. H	6.67	6.67	6.62	6.62	6.63	6.65	6.64	6.64
	6.81	6.82	6.72	6.73	6.73	6.75	6.74	6.74
	6.82	6.82	6.79	6.84	6.79	6.81	6.86	6.81
			7.36	7.36	7.37 (2H)	7.36	7.36	7.37
			7.40	7.39	7.44	7.39	7.38	7.38
					7.45	7.41	7.40	7.39
						7.450	7.44	7.40
						7.451	7.45	7.44
						7.46	7.46	7.45
								7.46 (2H)

a—c) Assignments may be interchanged in each column.



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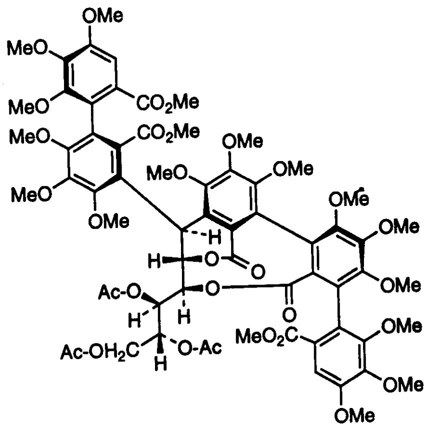

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are soluble in cold water, is not clear yet. The heartwood constituents of Japanese chestnut tree are very complex, and the overall metabolism of tannins in wood is beyond the scope of this report; however, the increase of castacrenins, **4** and **5**, along with ellagic acid seems to be insufficient to explain the steep decrease of **2** and **3** in the inner heartwood (Fig. 1). This observation would be consistent with copolymerization of ellagitannins with cell-wall components in this region.[3a)]

Experimental

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. Analytical HPLC was performed on a Tosoh apparatus equipped with a CCPM solvent delivery system, UV-8000 spectrometer (280 nm) and a Cosmosil 5C$_{18}$-AR (Nacalai Tesque Inc.) column (4.6 i.d. × 250 mm) (mobile phase, acetonitrile–50 mM phosphoric acid, gradient elution from 0→15% (60 min)→30% (30 min) acetonitrile; flow rate, 0.8 ml/min). Preparative HPLC was performed on TSKgel ODS-80T$_M$ (21.5 i.d. × 300 mm) with H_2O–MeOH. Column chromatographies were performed with Sephadex LH-20 (25—100 μm, Pharmacia Fine Chemical Co. Ltd.), MCI-gel CHP 20P (75—150 μm, Mitsubishi Chemical Industries, Ltd.), Cosmosil 75C$_{18}$-OPN (42—105 μm, Nacalai Tesque Inc.), TSK-gel Toyopearl HW-40F (30—60 μm, Tosoh Co.) and Silica gel 60 (Merck). Thin layer chromatographies were performed on precoated Silica gel 60 F$_{254}$ plates (0.2 mm thick, Merck) with benzene–ethyl formate–formic acid (1 : 7 : 1 or 1 : 5 : 2, v/v) and precoated cellulose (0.1 mm thick, Merck) with 2% AcOH, and spots were detected by ultraviolet (UV) illumination and by spraying 2% ethanolic ferric chloride reagent or 5% H_2SO_4, followed by heating. Negative and positive FAB-MS were recorded on JEOL JMX DX-303, JEOL HX100/JMA 3500 and JEOL SX 100/JMA 3500 spectrometers with glycerol, glycerol-HMPA[18)] or *m*-nitrobenzyl alcohol as a matrix. ^{1}H- and ^{13}C-NMR spectra were obtained with Varian Unity plus 500, JEOL JNM-GX400, Varian Gemini 300 and JEOL GX-270 spectrometers operating at 500, 400, 300 and 270 MHz for ^{1}H, and 125, 100, 75 and 67.8 MHz for ^{13}C, respectively; chemical shifts are reported in parts per million on the δ scale from internal tetramethylsilane, and coupling constants are given in hertz.

HPLC Analysis of Tannins in Wood Cross Sections Samples (50—60 mg) cut out of bark (3 pieces), sapwood (5 pieces) and heartwood (6 pieces) were dried and separately extracted with 70% aqueous acetone (3 ml) twice. After concentration, each extract was passed through a Sep-pak C$_{18}$ cartridge (Waters) with 60% MeOH and analyzed by reversed-phase HPLC. Peak area was obtained by using an integrator connected to a UV detector. Concentration (mg/g dried wood) of **1**, **2**, **3**, and **6** was estimated on the basis of calibration curves obtained by using standard compounds. The distribution of castacrenins B (**7**) and C (**8**) was similar to that of **6**. Compounds **10**, **12**, **13** and **14** showed similar distribution to **9** and **11**. The distribution of vescalagin (**5**) was similar to that of **4**. Concentrations of gallic acid and ellagic acid were higher in inner heatwood than in outer heartwood.

Isolation of Tannins from Inner Heartwood Inner heartwood (250 g) of Japanese chestnut tree (*Castanea crenata* SIEB. *et* ZUCC. cv. GINYOSE, about 17 years old, collected in June in Yamaguchi prefecture, Japan) was chipped into small pieces and extracted with acetone–H_2O (8 : 2, v/v) three times at room temperature. The extract was concentrated under reduced pressure and subjected to Sephadex LH-20 column (4 i.d. × 37 cm) chromatography with H_2O containing increasing proportions of MeOH. The fractions eluted with 40—60% MeOH were collected and further separated on MCI-gel CHP20P (3.5 i.d. × 32 cm) and Cosmosil 75C$_{18}$-OPN (3.5 i.d. × 32 cm) using H_2O–MeOH as a solvent to yield **8** (53.9 mg as a yellow powder from water, mother liquor 50 mg), **6** (93.9 mg as a yellow powder from water, mother liquor 47 mg) and **7** (230 mg as a yellow amorphous powder), together with vescalagin and castalagin, which were not subjected to further purification.

Castacrenin A (6) A yellow powder (H_2O), mp 234—238° (dec.), $[\alpha]_D^{26}$ $-47.2°$ ($c=0.8$, MeOH). FAB-MS (negative ion mode) *m/z*: 613 $(M-H)^-$. *Anal.* Calcd for $C_{27}H_{18}O_{17} \cdot 1/2H_2O$: C, 52.02; H, 3.07. Found: C, 51.89; H, 3.10. ^{1}H-NMR (300 MHz, acetone-d_6+D_2O): Table 1. ^{13}C-NMR (67.8 MHz, acetone-d_6+D_2O) δ: 64.8 (C-6), 68.6 (C-1), 71.0 (C-5), 76.0 (C-3), 82.4 (C-4), 84.8 (C-2), 109.1 (2C, C-2‴, C-6″), 111.2 (C-6‴), 113.9, 114.0 (C-1″, C-1‴), 114.8 (C-6′), 117.4 (C-1′), 119.8, 124.6 (C-2′, C-2″), 136.6, 137.4 (C-4′, C-4″), 139.3 (C-3‴), 139.7, 139.9, 143.9, 145.2, 146.1 (C-3′, C-3″, C-4‴, C-5′, C-5″), 148.7 (C-5‴), 159.0 (C-7″), 160.8 (C-7‴), 162.9 (C-7′).

Methylation of 6 A crude sample of **6** (227 mg) was dissolved in methanol (3 ml) and treated with CH_2N_2–Et_2O. The solution was concentrated and separated by silica gel chromatography with benzene–EtOH (9 : 1) to give **6a** (31 mg) as a yellow amorphous powder, $[\alpha]_D^{28}$ $-65.3°$ ($c=1.0$, $CHCl_3$), FAB-MS (positive ion mode) *m/z*: 712 (M^+). *Anal.* Calcd for $C_{34}H_{32}O_{17} \cdot 1/2H_2O$: C, 56.59; H, 4.61. Found: C, 56.27, H, 4.85. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 3.64, 3.69, 4.01, 4.04, 4.06, 4.16, 4.25 (each 3H, s, OCH_3), 3.80 (2H, m, H-6), 4.69 (1H, br s, H-3), 4.88 (1H, d, $J=3$ Hz, H-2), 5.51 (1H, d, $J=3$ Hz, H-1), 7.72 (1H, s, H-6‴).

Acetylation of 6a The methyl derivative **6a** (12 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml). The product was purified by silica gel chromatography (benzene–acetone, 10 : 1) to yield the acetate **6b** (12.3 mg) as a yellow amorphous powder, $[\alpha]_D^{28}$ $-42.1°$ ($c=1.0$, $CHCl_3$), FAB-MS (positive ion mode) *m/z*: 839 $([M+H]^+)$. *Anal.* Calcd for $C_{40}H_{38}O_{20} \cdot H_2O$: C, 56.08; H, 4.71. Found: C, 56.04, H, 4.74. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 2.01, 2.08, 2.12 (each 3H, s, $COCH_3$), 3.66 (×2), 4.02, 4.05, 4.08, 4.18, 4.24 (each 3H, s, OCH_3), 4.59 (1H, dd, $J=4$, 9 Hz, H-4), 4.65 (1H, dd, $J=3$, 12 Hz, H-6), 4.84 (1H, dd, $J=1$, 3 Hz, H-2), 5.34 (1H, ddd, $J=3$, 5, 9 Hz, H-5), 5.44 (1H, d, $J=3$ Hz, H-1), 5.69 (1H, dd, $J=1$, 4 Hz, H-3), 7.72 (1H, s, H-6‴).

Castacrenin B (7) A yellow amorphous powder, $[\alpha]_D^{26}$ $+46.8°$ ($c=0.8$, MeOH), FAB-MS (negative ion mode) *m/z*: 613 $(M-H)^-$. *Anal.* Calcd for $C_{27}H_{18}O_{17} \cdot 1/2H_2O$: C, 52.02; H, 3.07. Found: C, 51.68, H, 3.21. ^{1}H-NMR (300 MHz, acetone-d_6+D_2O): Table 1. ^{13}C-NMR (67.8 MHz, acetone-d_6+D_2O) δ: 62.2 (C-6), 68.4 (C-1), 70.6 (C-4), 73.5 (C-3), 76.7 (C-5), 78.8 (C-2), 107.7, 109.0 (C-2‴, C-6″), 111.3 (C-6‴), 113.8 (2C, C-1″, C-1‴), 115.3 (C-6′), 116.0 (C-1′), 119.9, 125.2 (C-2′. C-2″), 136.7, 137.1 (C-4′, C-4″), 139.4 (C-3‴), 139.8 (2C), 142.5, 144.8, 146.1 (C-3′, C-3″, C-4‴, C-5′, C-5″), 148.5 (C-5‴), 159.6 (C-7″), 160.6 (C-7‴), 163.0 (C-7′).

Methylation Followed by Acetylation of 7 A crude sample of **7** (100 mg) was methylated and then acetylated in a manner similar to that described for **6** to yield **7a** (23 mg) as a yellow amorphous powder, $[\alpha]_D^{28}$ $-93.0°$ ($c=0.5$, $CHCl_3$), FAB-MS (positive ion mode) *m/z*: 839 $([M+H]^+)$. *Anal.* Calcd for $C_{40}H_{38}O_{20}$: C, 57.28; H, 4.57. Found: C, 57.77, H, 4.81. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.06, 2.13, 2.15 (each 3H, s, $COCH_3$), 3.67, 3.71, 4.01, 4.05, 4.06, 4.16, 4.24 (each 3H, s, OCH_3), 4.18 (1H, m, H-5), 4.42 (1H, dd, $J=5$, 12 Hz, H-6), 4.43 (1H, br t, $J=3$ Hz, H-2), 4.59 (1H, dd, $J=7$, 12 Hz, H-6), 4.94 (1H, t, $J=4$ Hz, H-4), 5.32 (2H, m, H-1, H-3), 7.71 (1H, s, H-6‴).

Castacrenin C (8) A yellow powder (H_2O), mp 240—243° (dec.), $[\alpha]_D^{26}$ $-7.6°$ ($c=0.9$, MeOH). FAB-MS (negative ion mode) *m/z*: 613 $(M-H)^-$. *Anal.* Calcd for $C_{27}H_{18}O_{17} \cdot 1/2H_2O$: C, 52.02; H, 3.07. Found: C, 52.07; H, 3.08. ^{1}H-NMR (300 MHz, acetone-d_6+D_2O): Table 1. ^{13}C-NMR (67.8 MHz, acetone-d_6+D_2O) δ: 64.5 (C-6), 70.0, 72.0, 72.7 (C-3, C-4, C-5), 98.4 (C-1), 108.9, 109.2 (C-2‴, C-6″), 111.1 (C-6‴), 111.2, 113.6, 113.9, 117.4, 123.0, 124.4 (C-1′, C-1″, C-1‴, C-2′, C-2″, C-6′), 136.8, 137.3 (C-4′, C-4″), 139.1, 139.2, 139.7, 140.2, 144.3, 146.7 (C-3′, C-3″, C-3‴, C-4‴, C-5′, C-5″), 148.5 (C-5‴), 155.0 (C-2), 158.8 (C-7″), 160.3 (C-7‴), 161.8 (C-7′).

Derivation of Castacrenins from Castalin (4) A solution of **4** (500 mg) in 1 M HCl (10 ml) was heated at 80 °C for 8 h. The mixture was directly applied to a column of MCI gel CHP20P. Elution with H_2O afforded vescalin (**5**, 77.3 mg), with recovery of **4** (277 mg). Further elution of the column with H_2O containing increasing amounts of MeOH afforded **8** (9 mg), **6** (8 mg), **7** (20 mg) and **8a** (13 mg). **8a**: a yellow amorphous powder, $[\alpha]_D^{28}$ 0° ($c=0.4$, MeOH). FAB-MS (negative ion mode) *m/z*: 595 $(M-H)^-$. *Anal.* Calcd for $C_{27}H_{16}O_{16} \cdot 2H_2O$: C, 51.28; H, 3.19. Found: C, 51.67; H, 3.42. ^{1}H-NMR (300 MHz, acetone-d_6+D_2O): Table 1. ^{13}C-NMR (67.8 MHz, acetone-d_6+D_2O) δ: 71.5, 73.5, 74.9 (C-4, C-5, C-6), 81.9 (C-3), 100.6 (C-1), 108.5, 108.9 (C-2‴, C-6″), 111.0 (C-6‴), 111.6, 113.5, 113.8, 117.8, 122.1, 124.3 (C-1′, C-1″, C-1‴, C-2′, C-2″, C-6′), 136.6, 137.1 (C-4′, C-4″), 139.1, 139.5, 139.8, 140.4, 144.8, 146.7 (C-3′, C-3″, C-3‴, C-4‴, C-5′, C-5″), 148.4 (C-5‴), 151.2 (C-2), 158.9 (C-7″), 160.7 (C-7‴), 161.8 (C-7′). A similar reaction of **4** (500 mg) under reflux for 6 h yielded **8a** (135 mg) and flavogallonic acid bislactone (**4a**, 27 mg). **4a**: a yellow powder. ^{1}H-NMR (300 MHz, acetone-d_6+D_2O) δ: 7.62, 7.33 (each 1H, s).

Derivation of 8a from Castacrenin C (8) A solution of **8** (1 mg) in 1 ml of 1 M HCl–MeOH (1 : 1) was heated at 90 °C and examined by HPLC. After heating for 3 h, 75% of **8** was converted to **8a** (estimated from peak area).

Isolation of Tannins from Outer Heartwood The outer part of the heartwood (6 kg, fresh weight) of Japanese chestnut tree (about 40 years old) was chipped and extracted with acetone–H_2O (7 : 3, v/v) four times at room temperature. After removal of acetone by evaporation, the resulting precipitates were filtered off, and the filtrate was subjected to column chromatography over Sephadex LH-20 (12 i.d. × 60 cm) with H_2O containing increasing proportions of MeOH to afford eight fractions (frs.): frs. **1** (*ca.* 300 g), **2** (35.1 g), **3** (30 g), **4** (155 g), **5** (113 g), **6** (40 g), **7** (25 g), and **8** (10 g). Fraction 2 was chromatographed on Sephadex LH-20 (5 i.d. × 50 cm) with 20% MeOH and then on TSK-gel Toyopearl HW-40F with 40% MeOH to yield **4** (2.4 g). Fraction 4 was passed through MCI gel CHP 20P (60% MeOH) to remove non-polar substances, and then separated by Sephadex LH-20 chromatography (20→80% MeOH, stepwise gradient elution) to give vescalagin (33 g) and castalagin (1.1 g). Fraction 5 was also passed through MCI-gel CHP 20P (60% MeOH) and subjected to Sephadex LH-20 chromatography (20→80% MeOH) to give two fractions: frs. 5-1 (22 g) and 5-2 (50 g). A portion (6.6 g) of the first fraction was applied to a column of TSK-gel Toyopearl HW-40F (4.0 i.d. × 25 cm) with MeOH–H_2O–acetone

(60:20:20→50:25:25→40:30:30 and then 0:1:1 v/v/v) solvent systems, and the fractions obtained were further purified by preparative HPLC to yield **9** (36 mg), **10** (9 mg), **11** (36 mg), **12** (46 mg), **13** (10 mg) and **14** (40 mg). Fraction 5-2 was crystallized from water to give castalagin (14 g). Fraction 8 was separated by repeated chromatography on MCI-gel CHP 20P, Sephadex LH-20 and Cosmosil 75C_{18}-OPN with H_2O–MeOH to give 1,2,3,6-tetra-*O*-galloyl-*β*-D-glucopyranose (120 mg).

Castaneanin A (11) A tan amorphous powder, $[\alpha]_D^{25}$ +9.3° (c=0.9, MeOH). *Anal.* Calcd for $C_{123}H_{74}O_{76} \cdot 3H_2O$: C, 52.35; H, 2.86. Found: C, 52.45; H, 3.10. FAB-MS (negative ion mode) m/z: 2765 $(M-H)^-$. ^{13}C-NMR (acetone-d_6+D_2O, 67.8 MHz) δ: 40.0, 40.4 (C-1′, 1″), 65.7(2C), 65.8 (C-6, 6′, 6″), 66.3 (C-3), 67.2 (C-1), 69.3 (C-4), 70.37, 70.43, 71.2, 71.5, 71.7 (2C), 71.8, (C-5, 3′, 3″, 4′, 4″, 5′, 5″), 74.5 (C-2), 77.9, 78.8 (C-2′, 2″), 107.1, 108.5 (2C), 110.0, 110.2, 110.46, 110.53 (aromatic CH), 113.4—117.2 (23C), 121.8—127.8 (15C), 134.9—138.3 (15C), 143.4—146.9 (30C), 164.6, 165.4, 165.46, 165.53, 165.6 (2C), 166.5, 166.9, 167.2, 167.3, 168.2, 168.3, 168.8, 168.9, 169.5 (COO).

Castaneanin B (12) A tan amorphous powder, $[\alpha]_D^{25}$ +24.5° (c=0.9, MeOH). *Anal.* Calcd for $C_{164}H_{98}O_{101} \cdot 2H_2O$: C, 52.94; H, 2.76. Found: C, 52.91; H, 3.04. FAB-MS (negative ion mode) m/z: 3681 $(M-H)^-$. ^{13}C-NMR (acetone-d_6+D_2O, 67.8 MHz) δ: 40.32, 40.34, 40.7 (C-1′, 1″, 1‴), 66.0 (4C) (C-6, 6′, 6″, 6‴), 66.7 (C-3), 67.5 (C-1), 69.6 (C-4), 70.7 (3C) (C-4′, 4″, 4‴), 71.6 (C-5), 71.9 (2C), 72.0 (2C), 72.2 (2C) (C-3′, 3″, 3‴, 5′, 5″, 5‴), 74.8 (C-2), 78.3, 78.9, 79.1 (C-2′, 2″, 2‴), 107.5, 108.9 (2C), 110.5 (3C), 110.8, 110.9(2C) (aromatic CH), 113.8—117.6 (31C), 122.2—128.2 (20C), 135.3—138.8 (20C), 143.6—147.2 (40C), 165.0, 165.7 (2C), 165.77, 165.83, 165.86, 165.94, 166.0 (2C), 166.9, 167.2 (2C), 167.5, 167.6, 168.58, 168.63, 169.10, 169.14, 169.2, 169.9 (COO).

Castaneanin C (13) A tan amorphous powder, $[\alpha]_D^{25}$ +49.3° (c=0.8, MeOH). *Anal.* Calcd for $C_{164}H_{98}O_{101} \cdot H_2O$: C, 53.20; H, 2.72. Found: C, 53.14; H, 3.09. FAB-MS (negative ion mode) m/z: 3682.9, theoretical ion distribution for $C_{164}H_{97}O_{101}$ m/z: 3681.2454, theoretical base peak 3683.2520. ^{13}C-NMR (acetone-d_6+D_2O, 67.8 MHz) δ: 40.3, 40.5, 40.7 (C-1′, 1″, 1‴), 65.6, 63.1 (4C) (C-6, 6′, 6″, 6‴), 69.7, 70.4 (3C), 71.6, 71.9 (3C), 72.0 (2C), 72.3 (2C) (C-3, 3′, 3″, 3‴, 4, 4′, 4″, 4‴, 5, 5′, 5″, 5‴), 78.3, 78.9 (2C), 79.1 (C-2, 2′, 2″, 2‴), 107.5, 108.9 (2C), 110.5 (3C), 110.9 (3C) (aromatic CH), 113.8—118.1 (31C), 123.7—128.4 (20C), 135.3—138.8 (20C), 143.6—147.9 (40C), 165.7 (2C), 165.8, 165.9 (3C), 166.0 (2C), 166.7, 167.2 (2C), 167.5, 167.6, 168.6 (2C), 168.7, 169.15 (2C), 169.23, 169.9 (COO).

Castaneanin D (14) A tan amorphous powder, $[\alpha]_D^{25}$ +43.2° (c=0.8, MeOH). *Anal.* Calcd for $C_{205}H_{122}O_{126} \cdot 2H_2O$: C, 53.10; H, 2.74. Found: C, 53.11; H, 3.04. FAB-MS (negative ion mode) m/z: 4600.2, theoretical ion distribution for $C_{205}H_{121}O_{126}$ m/z: 4597.3061, theoretical base peak 4599.3127. ^{13}C-NMR (acetone-d_6+D_2O, 67.8 MHz) δ: 40.4, 40.5, 40.7, 40.8 (C-1′, 1″, 1‴, 1⁗), 66.1 (5C) (C-6, 6′, 6″, 6‴, 6⁗), 66.7 (C-3), 67.6 (C-1), 69.6 (C-4), 70.8 (4C) (C-4′, 4″, 4‴, 4⁗), 71.6 (C-5), 71.9 (4C), 72.1 (2C), 72.3 (2C) (C-3′, 3″, 3‴, 3⁗, 5′, 5″, 5‴, 5⁗), 74.8 (C-2), 78.3, 78.88, 78.93, 79.08 (C-2′, 2″, 2‴, 2⁗), 107.6, 109.0, 110.5 (3C), 110.7, 110.8, 110.9 (3C), 111.0 (aromatic CH), 113.7—117.7 (39C), 122.3—128.2 (25C), 135.3—139.3 (25C), 143.6—147.2 (50C), 164.8, 165.65 (2C), 165.71, 165.76, 165.80 (3C), 165.9, 166.0, 166.9, 167.2 (3C), 167.5, 167.6, 168.5, 168.6 (3C), 169.09, 169.14 (2C), 169.2, 169.8 (COO).

Evaluation of the Molecular Weight by Normal-Phase HPLC HPLC was performed on a Cosmosil 5SL (Nacarai Tesque) column (4.6 i.d. × 250 mm) [mobile phase, A : B = 32.5 : 67.5 v/v. A, hexane, B, MeOH–tetrahydrofuran : formic acid (30 : 10 : 1, v/v/v, containing oxalic acid 0.5 g/l); flow rate 1.0 ml/min; detection 280 nm]. Retention times (t_R) are average values of three determinations. t_R 4.45 for **3**, t_R 4.56 for **2**, t_R 5.51 for **9**, t_R 5.31 for **10**, t_R 6.67 for **11**, t_R 8.43 for **12**, t_R 7.91 for **13**, t_R 10.07 for **14**.

Methylation Followed by Hydrolysis of Oligomeric Ellagitannin Fraction Fraction 6 obtained from the outer heartwood (*vide supra*) was chromatographed over TSK-gel Toyopearl HW-40F and the fractions showing similar HPLC and cellulose TLC behavior to fraction 5-2 were collected (7.7 g). The combined fraction (1.5 g) was methylated with $(CH_3)_2SO_4$ (5 ml) and K_2CO_3 (5 g) in acetone–MeOH (5 : 1 v/v, 60 ml) under reflux for 5 h. The inorganic precipitates were removed by filtration and the filtrate was subjected to chromatography on silica gel (40 g) with benzene–acetone (19 : 1) and then benzene–EtOH (9 : 1—4 : 1). The fraction (1.7 g) eluted with benzene–EtOH was heated (80 °C) with 3% NaOH in MeOH–H_2O (6 : 1, 35 ml) for 3 h. The solution was concentrated, acidified with HCl and extracted with EtOAc. The organic layer was dried over Na_2SO_4, concentrated and treated with CH_2N_2–Et_2O at 0 °C. After removal of the solvent by evaporation, the residue was separated by silica gel chromatography (benzene–acetone, 19 : 1) to yield dimethyl (*S*)-hexamethoxydiphenate (**15**) as a colorless syrup [311 mg, $[\alpha]_D^{25}$ −26.7° (c=0.8, $CHCl_3$)]. Further elution of the column with benzene–acetone (7 : 3) afforded a mixture of products (503 mg), of which a portion (68 mg) was treated with Ac_2O–pyridine. The products were separated by silica gel chromatography (benzene–acetone) to yield **16** (38 mg) as a white amorphous powder, $[\alpha]_D^{25}$ −10.0° (c=0.9, $CHCl_3$). FAB-MS (positive ion mode) m/z: 1349 $[M+H]^+$, 1317 $[M-MeOH+H]^+$, 1269 $[M-AcOH+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.49 (1H, s), 7.28 (1H, s), 5.24 (dd, J=1, 10 Hz, H-4), 4.74 (1H, dd, J=1, 2 Hz, H-2), 4.57 (1H, t, J=2 Hz, H-3), 4.14 (1H, dd, J=3, 12 Hz, H-6), 3.97 (overlapped, H-6), 3.82 (1H, d, J=1 Hz, H-1), 3.74 (overlapped, H-5), 4.18, 4.06, 3.99, 3.98, 3.96, 3.93, 3.91, 3.86, 3.77, 3.76, 3.75, 3.68, 3.63, 3.61, 3.56, 3.54, 3.51, 3.38 (each 3H, s, OCH_3), 2.20, 1.99, 1.78 (each 3H, s, $COCH_3$). ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 170.5, 170.3, 168.7, 168.4, 167.3, 167.2, 166.6, 164.3 (COO), 154.1—145.5 (15C), 129.9—122.7 (13C), 109.8, 109.2 (aromatic CH), 79.6, 72.7, 70.2, 68.4 (CH), 61.2—51.8 (CH_3), 43.6 (C-1), 20.6 (CH_3).

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References and Notes

1) Total tannin content was estimated by the hide powder method according to the procedure described in Japanese Industrial Standards.
2) Kemp M. S., Burden R. S., *Phytochemistry*, **25**, 1261—1269 (1986).
3) *a*) Peng S., Scalbert A., Monties B., *Phytochemistry*, **30**, 775—778 (1991); *b*) Higuchi T., *Wood Research*, **59/60**, 180—199 (1976).
4) Scalbert A., *Phytochemistry*, **30**, 3875—3883 (1991); Zucker W. V., *The American Naturalist*, **121**, 335—365 (1983).
5) Streit W., Fengel D., *Holzforschung*, **48**, 361—367 (1994).
6) Masson G., Puech J. L., Moutounet M., *Phytochemistry*, **37**, 1245—1249 (1994).
7) Fischer E., Freudenberg K., *Chem. Ber.*, **45**, 2709—2729 (1912); Nonaka G., Ishimaru K., Tanaka T., Nishioka I., *Chem. Pharm. Bull.*, **32**, 483—489 (1984).
8) Mayer W, Gabler W., Riester A., Korger H., *Justus Liebigs Ann. Chem.*, **707**, 177—181 (1967); Mayer W., Seits H., Jochims J. C., *ibid.*, **721**, 186—193 (1969); Mayer W., Seitz H., Jochims J. C., Schauerte K., Schilling G., *ibid.*, **751**, 60—68 (1971).
9) Mayer W., Einwiller A., Jochims J. C., *Justus Liebigs Ann. Chem.*, **707**, 182—189 (1967); Mayer W., Kuhlmann F., Schilling G., *ibid.*, **747**, 51—59 (1971).
10) Nonaka G., Nakayama S., Nishioka I., *Chem. Pharm. Bull.*, **37**, 2030—2036 (1989); Khac D. D., Tran-Van S., Campos A. M., Lallemand J. Y., Fetizon M., *Phytochemistry*, **29**, 251—256 (1990).
11) Saijo R., Nonaka G., Nishioka I., *Phytochemistry*, **29**, 267—270 (1990).
12) Grimshaw J., Haworth R. D., *J. Chem. Soc.*, **1956**, 4225—4232; Tanaka T., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **34**, 1039—1049 (1986).
13) Hervé du Penhoat C. L. M., Michon V. M. F., Peng S., Viriot C., Scalbert A., Gage D., *J. Chem. Soc., Perkin Trans. 1*, **1991**, 1653—1660; Nakayama S., Tanaka T., Nonaka G., Nishioka I., Abstracts of Papers, 16th International Symposium on the Chemistry of Natural Products, Kyoto, May 1998, p. 209; Lin T., Tanaka T., Nonaka G., Nishioka I., Young T., *Chem. Pharm. Bull.*, **39**, 1144—1147 (1991).
14) Tanaka T., Nonaka G., Nishioka I., *J. Chem. Res.*, (*S*), **1985**, 176—177; *ibid.*, (*M*), **1985**, 2001—2029.
15) Ikeya G., Taguchi H., Yoshida I., Kobayashi H., *Chem. Pharm. Bull.*, **27**, 1383—1394 (1979).
16) Viriot C., Scalbert A., Hervé du Penhoat C. L. M., Moutounet M., *Phytochemistry*, **36**, 1253—1260 (1994).
17) Klumpers J., Scalbert A., Janin G., *Phytochemistry*, **36**, 1249—1252 (1994).
18) Isobe R., Tanaka T., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **37**, 1748—1750 (1989).