[Chem. Pharm. Bull. 32(2) 483—489 (1984)]

Tannins and Related Compounds. XVII.[1] Galloylhamameloses from *Castanea crenata* L. and *Sanguisorba officinalis* L.

GEN-ICHIRO NONAKA, KANJI ISHIMARU, TAKASHI TANAKA, and ITSUO NISHIOKA*

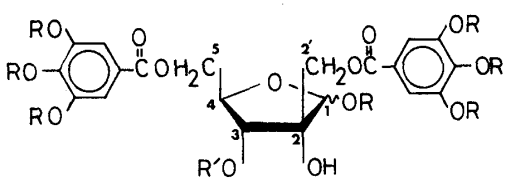
Faculty of Pharmaceutical Sciences, Kyushu University 62, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan

(Received June 10, 1983)

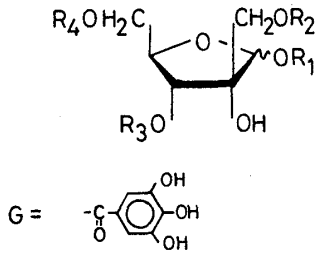
Together with 2′,5-di-*O*-galloylhamamelose (I), which had previously been designated as hamamelitannin, five new galloylhamameloses (II—VI) were isolated from the bark of *Castanea crenata* L. (Fagaceae). On the basis of chemical and spectroscopic evidence, their structures were characterized as 2′,3,5-tri-*O*-galloyl- (II), 5-*O*-galloyl- (III), 1,2′-di-*O*-galloyl- (IV), 1,2′,5-tri-*O*-galloyl- (V) and 1,2′,3,5-tetra-*O*-galloyl-D-hamamelofuranose (VI). In addition, the occurrence of two galloylhamameloses (I and II) in the underground part of *Sanguisorba officinalis* L. (Rosaceae) was demonstrated.

Keywords——*Castanea crenata*; Fagaceae; *Sanguisorba officinalis*; Rosaceae; galloylhamamelose; tannin; deuterium-induced differential isotope shift; ^{13}C-NMR; ^{1}H-NMR

In contrast to the ubiquitous galloylglucoses, hamamelitannin (2′,5-di-*O*-galloylhammelose), first isolated from the bark of *Hamamelis virginiana* L. by Grüttner,[2] and structurally elucidated by Fischer and Freudenberg,[3] and Schmidt,[4] is unique in that it contains a branched-chain sugar, D-hamemelose [2-*C*-(hydroxymethyl)-D-ribose]. So far, hamamelitannin has also been isolated from the bark of *Castanea sativa* MILL.[5] and *Quercus rubra* L.[5] During the course of chemical studies on tannins and related compounds in plants, we have encountered accumulations of several galloylhamameloses including hamamelitannin in the bark of *Castanea crenata* L. (Fagaceae), which has been used in China and Japan as a remedy for erysipelas and as a tanning and dyeing agent. Application of chromatographic techniques similar to those outlined previously[6] resulted in the isolation of six galloylhamameloses (I—VI). In addition, two galloylhamameloses (I and II) have also been isolated from the underground part of *Sanguisorba officinalis* L. (Rosaceae). This paper deals with the isolation and structural characterization of these galloylhamameloses.



I : R, R′=H
Ia : R=Me, R′=H
Ib : R, R′=Me



G =

	R_1	R_2	R_3	R_4
II	H	G	G	G
III	H	H	H	G
IV	G	G	H	H
V	G	G	H	G
VI	G	G	G	G

The aqueous acetone extract of the fresh bark of *Castanea crenata* was extracted with ethyl acetate. Repeated chromatography of the ethyl acetate-soluble portion over Sephadex LH-20 (EtOH–H_2O–acetone, 80% aqueous MeOH, and acetone)[6] and Diaion HP-20 (H_2O–

MeOH)[6d] yielded galloylhamameloses (I—VI). Similar treatment of the underground part of *Sanguisorba officinalis* furnished two galloylhamameloses (I and II).

Compound I, colorless needles, mp 146—147 °C, $[\alpha]_D$ +32.0° (H_2O), $C_{20}H_{20}O_{14} \cdot 1/2H_2O$, was strongly positive to the ferric chloride reagent (a blue coloration). The presence of galloyl groups was confirmed by the appearance of signals ranging between δ 6.84 and 7.02 in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum (Table II) demonstrated the presence of a sugar moiety different from the D-glucose of common gallotannins. In the ^{1}H- and ^{13}C-NMR spectra, duplication of the signals due to the galloyl and sugar moieties implied that the anomeric center was not acylated and hence in solution I exists as an equilibrium mixture of α- and β-forms.

Methylation of I with dimethyl sulfate and potassium carbonate in dry acetone yielded the heptamethyl ether (Ia) as a major product, along with a small amount of the octamethyl ether (Ib). The ^{1}H-NMR spectrum of Ia showed two singlet signals (δ 7.24 and 7.26) due to 3,4,5-trimethoxybenzoyl groups and an anomeric proton singlet (δ 4.97), together with one aliphatic and six aromatic methoxyl signals (δ 3.52 and δ 3.76—3.96, respectively). Upon ordinary acetylation, Ia formed the mono-acetate (Ic). The ^{1}H-NMR spectrum of Ic showed a lowfield doublet (δ 5.22, J = 6 Hz) ascribable to the proton geminal to the acetoxyl group and a singlet (δ 3.21) which disappeared on addition of D_2O, suggesting the presence of one secondary and one tertiary hydroxyl groups in Ia. ^{13}C-NMR analysis of Ia provided further information on the structure of the sugar moiety; among six sugar–carbon signals, two (δ 63.8 and 66.0) appeared as a triplet, one (δ 76.9) as a singlet and the remainder were observed as a doublet in the proton off-resonance spectrum. These chemical and spectroscopic data suggested that the component sugar was D-hamamelose and that I was hamamelitannin. Comparison of the physical data (melting point and optical rotation) with those described in the literature[5] confirmed this identification.

The locations of the two galloyl groups in hamamelitannin were previously determined by chemical methods, mainly based on a study of periodate oxidation.[5] We have independently established their locations using the deuterium-induced differential isotope shift

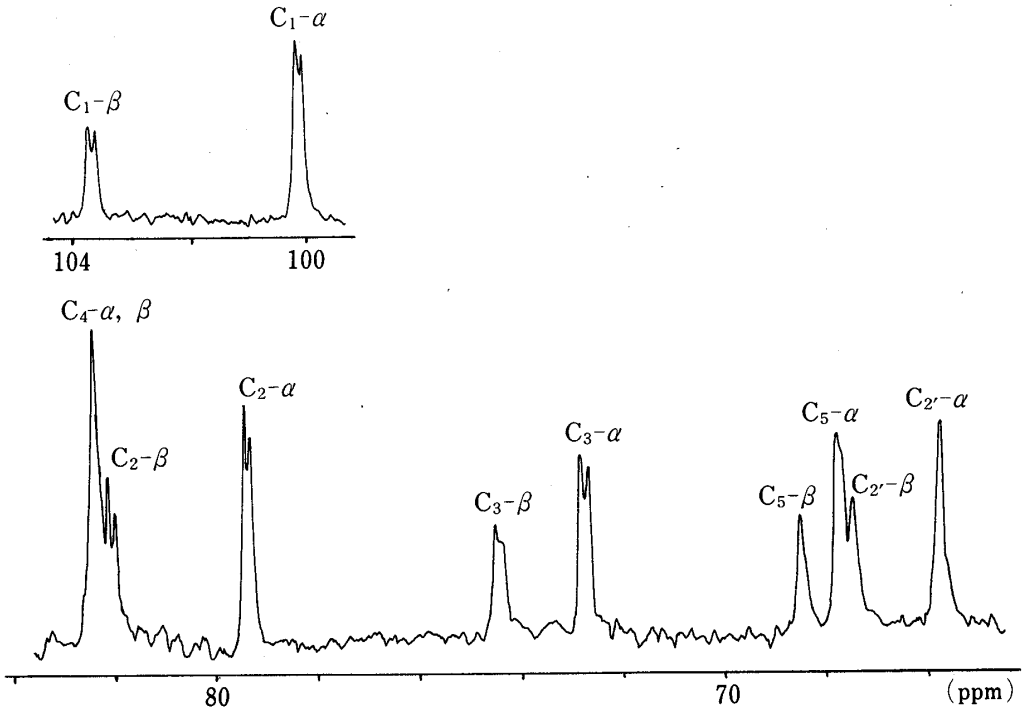


Fig. 1. DIS Spectrum (Proton-Noise Decoupled) of I

TABLE I. Chemical Shifts and DIS Values for I

		Chemical shifts[a]	DIS (ppm)
$C_{2'}$	α	65.7 (t)	—
	β	67.5 (t)	—
C_5	α	67.8 (t)	—
	β	68.5 (t)	—
C_3	α	72.6 (d)	0.176
	β	74.4 (d)	0.170
C_2	α	79.3 (s)	0.117
	β	82.0 (s)	0.117
C_4	α, β	82.4 (d)	—
C_1	α	100.0 (d)	0.117
	β	103.7 (d)	0.149

a) Chemical shifts (in ppm) were obtained in H_2O, relative to internal standard (DSS).

TABLE II. ^{13}C-NMR Spectral Data for Galloylhamameloses[a]

		I	Ia	Ib	II	III	IV	V
C_1	α	98.3	103.1	103.5	97.9	98.2	101.0	100.6
	β	102.1			102.7	103.0		
C_2	α	77.1	76.9	78.7	78.5	78.5	81.6	80.4
	β	80.1			81.1	80.7		
$C_{2'}$	α	65.2	63.8	63.8	64.8	63.8	67.2	67.0
	β	66.6			66.3	63.8		
C_3	α	72.6	71.8	79.7	73.1	71.5	73.9	74.1
	β	73.8			74.9	72.9		
C_4	α	79.6	80.2	80.2	78.9	79.9	81.8	82.5
	β	80.9			78.9	80.7		
C_5	α	66.6	66.0	66.3	66.7	64.9	63.8	67.0
	β	67.1			67.0	66.6		

a) I, II, III, IV and V were measured in acetone-d_6 + D_2O whereas Ia and Ib were measured in $CDCl_3$.

(DIS) technique[7] which can discriminate a hydroxy-bearing carbon atom from others. The assignments of carbon resonances were made by proton off-resonance decoupling and by comparison with the chemical shifts reported for D-hamamelofuranose.[8] The results are summarized in Table I, and from this it is evident that free hydroxyl groups are present at the C_1-, C_2-, and C_3-atoms, and hence two galloyl groups are located at the $C_{2'}$- and C_5-positions.

In aqueous solution, hamamelitannin (I) was shown by ^{1}H- and ^{13}C-NMR analyses (integrated intensities of the anomeric signals of the α- and β-forms) to be a mixture of the α- and β-hamamelofuranose forms in a ratio *ca.* 2:1.

Compound II, colorless needles, mp 209—210 °C, $[\alpha]_D$ +38.8° (H_2O), $C_{27}H_{24}O_{18}\cdot H_2O$, gave complex ^{1}H- and ^{13}C-NMR patterns analogous to those of hamamelitannin (I). The presence of three galloyl groups in II was deduced from the ^{1}H-NMR signals in the region of δ 7.12—7.20 (6H in total). Enzymatic hydrolysis of II with tannase afforded gallic acid and a component sugar, which was identical with D-hamamelose obtained from I by similar hydrolysis. In the ^{1}H-NMR spectrum of II, the appearance of two doublets at lower field (δ 5.20 and 5.58, J=7 Hz, 1H in total) suggested the presence of a galloyl group at the C_3-position in the hamamelofuranose moiety. This was further supported by ^{13}C-NMR analysis, which showed downfield shifts of the C_3-resonances as compared with those of I (Table II).

The other two galloyl groups were presumed to be attached to the $C_{2'}$- and C_5-hydroxyls since the ^{13}C-NMR chemical shifts of these carbons were close to those observed in I.

In order to confirm the structure of II, the decamethyl ether of 2′,3,5-tri-*O*-galloyl-D-hamamelofuranose was prepared from I as shown in Chart 1. The synthetic sample thus

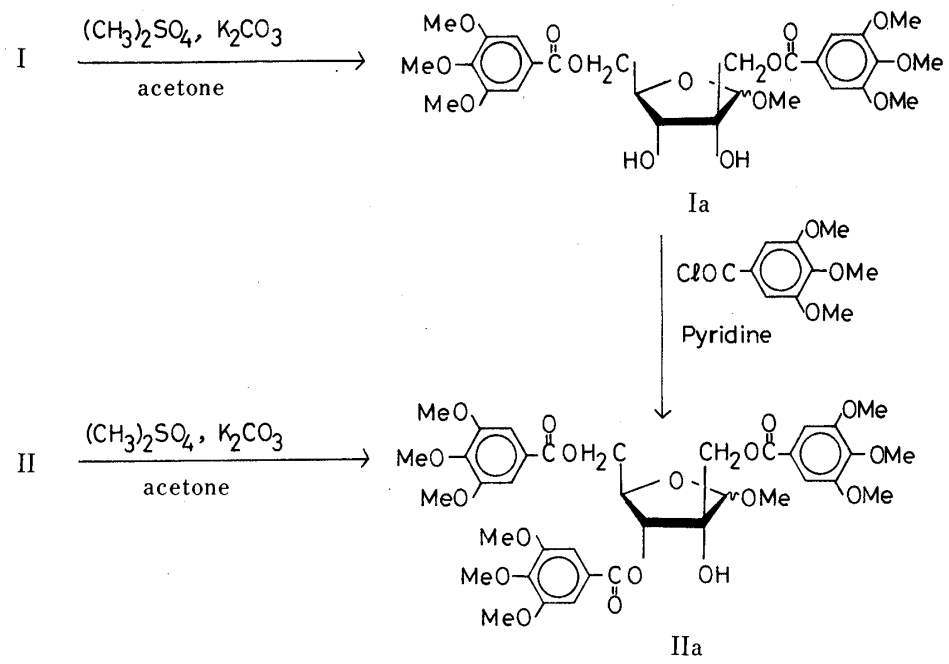


Chart 1

obtained was identical with the methylate (IIa) derived from II. On the basis of these results, the structure of II was concluded to be 2′,3,5-tri-*O*-galloyl-D-hamamelofuranose.

Compound III, a white amorphous powder, $[\alpha]_D$ +19.9° (H_2O), $C_{13}H_{16}O_{10}$, was shown by ^1H- and ^{13}C-NMR analyses (Table II) to be an equilibrium mixture of α- and β-hamamelofuranose froms, suggesting that the anomeric center was not acylated. On enzymatic hydrolysis with tannase, III furnished gallic acid and D-hamamelose in approximately equimolar amounts. The ^{13}C-NMR spectrum of III indicated the location of the galloyl group to be at the C_5-position in the hamamelofuranose moiety; the signal (δ 63.8) attributable to the $C_{2'}$-atom was shifted upfield as compared with those of I [δ 65.2 (α) and 66.6 (β)], while the chemical shifts for the C_5-atom were almost the same as those of I (Table II). This evidence is consistent with the observation of highfield singlet signals (δ 3.56 and 3.76) assignable to the $C_{2'}$-methylene protons in the ^1H-NMR spectrum. Consequently, the structure of III was characterized as 5-*O*-galloyl-D-hamamelofuranose.

Compound IV, colorless needles, mp 216 °C, $[\alpha]_D$ −26.1° (acetone–H_2O), $C_{20}H_{20}O_{14}\cdot H_2O$, and compound V, colorless needles, mp 230 °C, $[\alpha]_D$ −60.0° (acetone–H_2O), $C_{27}H_{24}O_{18}\cdot 3/2H_2O$, contained two and three galloyl groups, respectively, as shown by ^1H-NMR analysis (δ 7.12 and 7.14 in IV: δ 7.08, 7.10 and 7.12 in V, each 2H). In both cases, D-hamamelose was detected upon enzymatic hydrolysis with tannase. In the ^1H-NMR spectra of IV and V, a characteristic downfield shift of a singlet signal (δ 6.24 in IV; δ 6.44 in V) due to the anomeric proton indicated the presence of a galloyl group at this position, and the absence of a lowfield doublet for the C_3-proton in the hamamelofuranose structure implied that the C_3-hydroxyl was not acylated. The locations of the remaining galloyl groups in IV and V were determined by comparison of their ^{13}C-NMR spectra with that of I. Thus, in the spectrum of IV, the C_5-signal was observed at higher field (δ 63.8) than those of I (δ 66.6 and 67.1), whereas the C_4-signal was shifted downfield (δ 81.8), indicating the absence of the galloyl

group at the C_5-position in IV. Since the chemical shift for the $C_{2'}$-atom was similar to those of I (Table II), the galloyl group was considered to be located at the $C_{2'}$-position. The spectrum of V was analogous to that of IV except for the appearance of the C_5-signal at low field (δ 67.2), thus indicating the presence of galloyl groups at the $C_{2'}$ and C_5-hydroxyls in the hamamelofuranose moiety.

Based upon the findings described above, the structures of IV and V were determined to be 1,2′-di-*O*-galloyl- and 1,2′,5-tri-*O*-galloyl-D-hamamelose, respectively. In both cases, the configuration of the anomeric center still remains to be determined.

Compound VI, colorless needles, mp >290 °C, $[\alpha]_D$ −47.7° (acetone–H_2O), $C_{34}H_{28}O_{22} \cdot 3/2H_2O$, was obtained in a very small amount, but it gave a ^{1}H-NMR spectrum which permitted a direct and unequivocal assignment of the structure. Thus, the spectrum exhibited an anomeric proton singlet (δ 6.60), the C_3-proton doublet (δ 5.65, J=8 Hz) and lowfield methylene signals (δ 4.2—4.9) corresponding to the $C_{2'}$- and C_5-protons, as well as four well-defined galloyl signals (δ 7.08, 7.16, 7.24 and 7.28). The chemical shifts for these sugar–proton signals, except for the C_3-proton doublet, were similar to those observed in V. From these spectroscopic findings, the structure of VI was concluded to be 1,2′,3,5-tetra-*O*-galloyl-D-hamamelofuranose.

It is interesting from the viewpoint of sugar metabolism that D-hamamelose has hitherto been found to occur exclusively as its gallates in Hamamelidaceous, Fagaceous and Rosaceous plants, whereas D-apiose [3-*C*-(hydroxymethyl)-D-glycero-tetrose], one of two plant-originating sugars having a branched chain, is found widely in a variety of plants, either in glycosides or in polysaccharides.[9]

The bark of *Castanea crenata* L. and the underground part of *Sanguisorba officinalis* L.[1,10] contain, together with condensed tannins, other hydrolyzable tannins (ellagitannins, gallotannins, *etc.*) based on a glucose core, and the isolation and structures of these tannins will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Field-desorption (FD) and electron-impact (EI) mass spectra (MS) were recorded on JEOL DX-300 and JEOL D-300 spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL PS-100 and JEOL FX-100 spectrometers, respectively, with tetramethylsilane as an internal standard, and chemical shift values are given in δ (ppm). Column chromatography was carried out with Sephadex LH-20 (25—100 μ; Pharmacia Fine Chemicals), Diaion HP-20AG (75—150 μ; Mitsubishi Chemical Industries Ltd.), Avicel micro-crystalline cellulose (Funakoshi) and Kieselgel 60 (70—230 mesh; Merck). Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm; Merck) and precoated Avicel SF cellulose plates (Funakoshi), and spots were located by ultraviolet illumination, and with $FeCl_3$, 10% H_2SO_4 and aniline-hydrogen phthalate spray reagents. Analytical gas-liquid chromatography (GLC) for sugars was performed on a Shimadzu gas chromatograph, model GC-4BMPF, using 1.5% OV-17 and 1.5% SE-30 columns.

Isolation of Compounds I—VI from *Castanea crenata* L.——The fresh bark (2.13 kg) of *Castanea crenata*, collected in the Kasuya experimental forest of Kyushu University, Fukuoka Prefecture, was extracted three times with 80% aqueous acetone. The extracts were concentrated under reduced pressure (*ca.* 40 °C), and the resulting aqueous solution was partitioned with AcOEt. The organic layer was separated and evaporated under reduced pressure to give a dark brown gum (57.2 g), which was chromatographed over Sephadex LH-20. Elution with a solvent system of EtOH–H_2O–acetone[6] (monitoring by silica gel and cellulose TLC) gave eight fractions (fractions I—VIII). Fraction I (8.8 g) was subjected to Diaion HP-20 AG chromatography using an H_2O–MeOH system[6d] to yield compound III (63 mg). Fraction II (12.2 g) was purified by chromatography over Sephadex LH-20 with EtOH, and the product was crystallized from H_2O to furnish compound I (*ca.* 5 g). Repeated chromatography of fraction III (4.6 g) over Sephadex LH-20 using 80 and 60% aqueous MeOH and over Diaion HP-20 AG using 30% aqueous MeOH afforded compounds II (603 mg) and V (166 mg). Fraction IV (1.2 g) was repeatedly chromatographed over Sephadex LH-20 and Diaion HP-20 AG using an H_2O–MeOH solvent system to give compound VI (14 mg). Fractions V—VIII contained other gallo- and ellagitannins with higher molecular weights. The aqueous layer, after removal of the organic layer, was mixed with Celite 545, and the solvent was evaporated off under reduced pressure

(*ca.* 40 °C), yielding a brown powder (net weight: 323 g), which was packed in a glass column. Elution with acetone and evaporation of the solvent from the eluate gave a brown gum (140 g). Chromatography of this gum over Sephadex LH-20 using H_2O with increasing amounts of EtOH yielded seven fractions, among which fraction II (42 g) yielded, after repeated chromatography over Sephadex LH-20 (acetone–H_2O, H_2O–MeOH, EtOH, *etc.*), additional crops of compounds I (200 mg), III (51 mg) and IV (155 mg).

Isolation of Compounds I and II from *Sanguisorba officinalis* L.——The commercially available undergound parts of *Sanguisorba officinalis* were extracted with 80% aqueous acetone at room temperature. The acetone was removed by evaporation under reduced pressure (*ca.* 40 °C), and the aqueous solution was partitioned with AcOEt. The AcOEt layer, after removal of the solvent by evaporation, was treated with acetone. The acetone-soluble portion thus obtained was subjected to Sephadex LH-20 chromatography. Elution with acetone furnished four fractions (fractions I—IV). Fraction III (25.2 g) was rechromatographed over Sephadex LH-20 using EtOH and over Diaion HP-20 using H_2O with increasing amounts of MeOH to give compound I (2.7 g). Further chromatography of fraction IV over Sephadex LH-20 (EtOH), cellulose (2% acetic acid) and Diaion HP-20 AG (H_2O–MeOH) gave compound II (160 mg).

Compound I (I)——Colorless needles (H_2O), mp 146—147 °C, $[\alpha]_D^{23}$ +32.0° ($c=1.1$, H_2O), *Anal.* Calcd for $C_{20}H_{20}O_{14}\cdot 1/2H_2O$: C, 48.69; H, 4.29. Found: C, 48.39; H, 4.35. FD-MS *m/z*: 507 $(M+Na)^+$, 485 $(M+H)^+$, 484 $(M)^+$, 467 $(M-OH)^+$. ^{1}H-NMR (acetone-d_6): 3.9—4.6 (6H, m, $C_{2',3,4,5}$–H), 5.32, 5.38 (1H in total, each s, anomeric H), 7.14—7.17 (4H in total, galloyl H). ^{1}H-NMR (D_2O): 4.1—5.2 (6H, m, $C_{2',3,4,5}$–H), 5.43 (1H, br s, anomeric H), 6.84—7.02 (4H in total, galloyl H).

Methylation of I——A mixture of I (290 mg), dimethyl sulfate (2 ml) and anhydrous K_2CO_3 (4.0 g) in dry acetone (30 ml) was heated under reflux for 3 h. After removal of inorganic salts, the filtrate was concentrated to a syrup, which was subjected to silica gel chromatography. Elution with benzene–acetone (9 : 1) afforded the octamethyl ether (Ib) (26 mg), colorless needles (MeOH), mp 178—179 °C, $[\alpha]_D^{27}$ +61.3° ($c=0.5$, acetone), *Anal.* Calcd for $C_{28}H_{36}O_{14}$: C, 56.37; H, 6.08. Found: C, 56.30; H, 6.17. ^{1}H-NMR ($CDCl_3$): 3.17 (1H, s, C_2–OH, disappeared on addition of D_2O), 3.49, 3.55 (each 3H, s, aliphatic OMe), 3.80—4.08 (19H, C_3–H, 6 × aromatic OMe), 4.25 (1H, m, C_4–H), 4.19—4.79 (4H, m, $C_{2',5}$–H), 4.76 (1H, s, anomeric H), 7.22 (4H, s, galloyl H). Subsequent elution with benzene–acetone (17 : 3) gave the heptamethyl ether (Ia) (130 mg), colorless needles (MeOH), mp 111—113 °C, $[\alpha]_D^{27}$ +36.9° ($c=0.6$, acetone), *Anal.* Calcd for $C_{27}H_{34}O_{14}\cdot 1/2H_2O$: C, 54.82; H, 5.96. Found: C, 54.64; H, 6.10. EI-MS *m/z*: 582 $(M)^+$, 551 $(M-OMe)^+$, 370, 212, 195. ^{1}H-NMR ($CDCl_3$): 2.96 (1H, d, $J=7$ Hz, C_3–OH, disappeared on addition of D_2O), 3.52 (3H, s, aliphatic OMe), 3.69 (1H, s, C_2–OH, disappeared on addition of D_2O), 3.76—3.96 (19H, C_3–H, aromatic OMe), 4.15 (1H, m, C_4–H), 4.23—4.76 (4H, m, $C_{2',5}$–H), 4.97 (1H, s, anomeric H), 7.24, 7.26 (each 2H, s, galloyl H). I(a)-Monoacetate (Ic): prepared by ordinary acetylation with acetic anhydride and pyridine, a white powder, EI-MS *m/z*: 625 $(M)^+$, 212, 195. ^{1}H-NMR ($CDCl_3$): 2.13 (3H, s, $COCH_3$), 3.21 (1H, s, C_2–OH, disappeared on addition of D_2O), 3.53 (3H, s, aliphatic OMe), 3.83—3.89 (18H, aromatic OMe), 4.19—4.65 (5H, m, $C_{2',4,5}$–H), 4.84 (1H, s, anomeric H), 5.22 (1H, d, $J=6$ Hz, C_3–H), 7.22, 7.30 (each 2H, s, galloyl H).

Hydrolysis of I with Tannase——An aqueous solution (5 ml) of I (330 mg) was incubated with tannase (30 mg) at 37 °C for 1.5 h. Gallic acid was detected on TLC [solvent: benzene–ethyl formate–formic acid (2 : 7 : 1), *Rf* 0.8] of the reaction mixture as a sole poduct positive to the ferric chloride reagent. After removal of the solvent by evaporation, the residue was treated with EtOH. The EtOH-soluble portion was passed through a column of Amberlite IRA-400 (OH^- form), and the eluate was purified by Sephadex LH-20 (MeOH) and silica gel [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)] chromatography to furnish D-hamamelose (70 mg), a colorless syrup, $[\alpha]_D^{19}$ −16.3° ($c=0.57$, MeOH, after 24 h). ^{13}C-NMR ($CDCl_3$): 62.2, 62.6, 64.0, 64.4, 64.9, 66.9 (t, $C_{2',5}$), 67.4, 69.2, 69.9, 70.9, 71.3, 72.5 (d, $C_{3,4}$), 76.1, 76.5, 78.9, 81.5 (s, C_2), 83.1, 84.1 (d, C_4), 95.9, 96.7, 98.5, 102.9 (d, anomeric C).

Measurement of DIS of I——The DIS spectrum was obtained in coaxial NMR tubes (4 mm i.d. and 10 mm i.d.).[7] The inner tube contained a solution of I (50 mg) in H_2O containing 3-(trimethylsilyl)-propanesulfonic acid sodium salt (DSS) as the internal standard, and the outer tube contained an equal amount of I in D_2O plus DSS.

Compound II (II)——Colorless needles (H_2O), mp 209—210 °C, $[\alpha]_D^{23}$ +38.8° [$c=0.79$, H_2O–acetone (4 : 1)], $[\alpha]_D^{23}$ +16.3° ($c=0.65$, acetone), *Anal.* Calcd for $C_{27}H_{24}O_{18}\cdot H_2O$: C, 49.56; H, 4.00. Found: C, 49.47; H, 4.05. ^{1}H-NMR (acetone-d_6): 4.24—4.70 (5H, m, $C_{2',4,5}$–H), 5.20, 5.58 (1H in total, each d, $J=7$ Hz, C_3–H), 5.43, 5.49 (1H in total, each s, anomeric H), 7.12—7.19 (6H in total, galloyl H).

Methylation of II——A mixture of II (77 mg), dimethyl sulfate (1 ml) and anhydrous K_2CO_3 (2 g) was heated with stirring for 2.5 h. Work-up as described above yielded the decamethyl ether (IIa) (51 mg), a white amorphous powder, $[\alpha]_D^{20}$ +22.5° ($c=0.32$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 3.25 (1H, s, C_2–OH, disappeared on addition of D_2O), 3.57 (3H, s, aliphatic OMe), 3.80—3.92 (27H, aromatic OMe), 4.38—4.80 (5H, m, $C_{2',4,5}$–H), 4.90 (1H, s, anomeric H), 5.39 (1H, d, $J=5$ Hz, C_3–H), 7.21, 7.28, 7.34 (each 2H, s, galloyl H).

Preparation of II-Decamethyl Ether from Ia——A solution of trimethoxybenzoyl chloride in dry benzene was added to a solution of Ia (42 mg) in pyridine (0.5 ml). The mixture was kept at room temperature overnight, washed with 3% Na_2CO_3 and then with H_2O, and dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded a syrup, which was subjected to silica gel chromatography using benzene–acetone (9 : 1) to give a white amorphous powder (21 mg). This compound was shown to be identical with the octamethyl ether of II by physical and spectroscopic

comparisons.

Compound III (III)——A white amorphous powder, $[\alpha]_D^{19}$ +19.9° (c=1.0, H_2O), *Anal.* Calcd for $C_{13}H_{16}O_{10}$: C, 46.99; H, 4.85. Found: C, 46.80; H, 5.26. ^{1}H-NMR (acetone-d_6): 5.20, 5.28 (1H in total, each s, anomeric H), 7.12, 7.16 (2H in total, galloyl H).

Compound IV (IV)——Colorless needles (H_2O), mp 216 °C, $[\alpha]_D^{27}$ −26.1° [c=1.0, acetone–H_2O (3:7)], *Anal.* Calcd for $C_{20}H_{20}O_{14}\cdot H_2O$: C, 47.81; H, 4.58. Found: C, 48.06; H, 4.39. ^{1}H-NMR (acetone-d_6+D_2O): 3.76—4.64 (6H, m, $C_{2',3,4,5}$–H), 6.24 (1H, s, anomeric H), 7.12, 7.14 (each 2H, s, galloyl H).

Compound V (V)——Colorless needles (H_2O), mp 230 °C, $[\alpha]_D^{20}$ −60.0° [c=0.9, acetone–H_2O (1:1)], *Anal.* Calcd for $C_{27}H_{24}O_{18}\cdot 3/2H_2O$: C, 48.87; H, 4.10. Found: C, 48.73; H, 4.28. ^{1}H-NMR (acetone-d_6): 4.20—4.70 (6H, m, $C_{2',3,4,5}$–H), 6.44 (1H, s, anomeric H), 7.08, 7.10, 7.12 (each 2H, s, galloyl H).

Compound VI (VI)——Colorless needles (H_2O), mp >290 °C, $[\alpha]_D^{22}$ −47.7° [c=0.45, acetone–H_2O (1:1)], *Anal.* Calcd for $C_{34}H_{28}O_{22}\cdot 3/2H_2O$: C, 50.07; H, 3.83. Found: C, 50.51; H, 4.19. ^{1}H-NMR (acetone-d_6): 4.20—4.90 (5H, m, $C_{2',4,5}$–H), 5.65 (1H, d, J=8 Hz, C_3–H), 6.60 (1H, s, anomeric H), 7.08, 7.16, 7.24, 7.28 (each 2H, s, galloyl H).

Hydrolysis of II—VI with Tannase——An aqueous solution (0.5 ml) of II—VI (3 mg in each case) was shaken with tannase at 37 °C for 1 h. The reaction mixture was concentrated to dryness under reduced pressure, and the residue was treated with EtOH. The EtOH-soluble portion was trimethylsilylated with trimethylsilyl imidazole (20 μl) and pyridine (20 μl), and analyzed by GLC [(A) column 1.5% SE-30; column temp. 170 °C; flow rate 60 ml/min. (B) column 1.5% OV-17; column temp. 170 °C; flow rate 60 ml/min]. The chromatogram showed two major peaks [(A) t_R 5.6, 7.4 min (B) t_R 3.5, 5.2 min], which were identical with those of D-hamamelose obtained by hydrolysis of I.

Acknowledgement The authors are grateful to Dr. H. Okazaki of Sankyo Co., Ltd. for the supply of tannase. They are also indebted to Mr. Y. Tanaka, Miss K. Soeda and Mr. I. Maetani for the measurements of FD-MS, EI-MS, ^{1}H- and ^{13}C-NMR spectra, and to the staff of the Central Analysis Room of this university for elemental analyses.

References and Notes

1) Part XVI: T. Tanaka, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 117 (1984).
2) F. Grüttner, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **236**, 278 (1898).
3) E. Fischer and K. Freudenberg, *Chem. Ber.*, **45**, 2712 (1912); *idem, ibid.*, **46**, 3289 (1913); K. Freudenberg and F. Blümmel, *Justus Liebigs Ann. Chem.*, **440**, 45 (1924).
4) O. T. Schmidt, *Justus Liebigs Ann. Chem.*, **476**, 250 (1929); O. T. Schmidt and K. Heintz, *ibid.*, **515**, 77 (1935).
5) W. Mayer, W. Kunz, and F. Loebich, *Justus Liebigs Ann. Chem.*, **688**, 232 (1965).
6) *a*) G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981); *b*) M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *ibid.*, **28**, 2850 (1980); *c*) *Idem, J. Chem. Soc., Perkin Trans. 1*, **1982**, 2963; *d*) G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 4268 (1982).
7) P. E. Pfeffer, K. Valentine, and F. W. Parrish, *J. Am. Chem. Soc.*, **101**, 1265 (1979); P. E. Pfeffer, F. W. Parrish, and J. Unruh, *Carbohydr. Res.*, **84**, 13 (1980); K. M. Valentine, L. W. Doner, and P. E. Pfeffer, *ibid.*, **96**, 293 (1981).
8) G. Schilling and A. Keller, *Justus Liebigs Ann. Chem.*, **1977**, 1475.
9) J. S. D. Bacon, *Biochem. J.*, **89**, 103P (1963); R. B. Duff and K. M. Knight, *ibid.*, **88**, 33P (1963).
10) G. Nonaka, T. Tanaka, and I. Nishioka, *J. Chem. Soc.; Perkin Trans. 1*, **1982**, 1067; G. Nonaka, T. Tanaka, M. Nita, and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 2255 (1982); T. Tanaka, G. Nonaka, and I. Nishioka, *Phytochemistry*, accepted.