[Chem. Pharm. Bull. 32( 9 )3461—3470(1984)]

# Tannins and Related Compounds. XXIII.[1] Rhubarb (4): Isolation and Structures of New Classes of Gallotannins

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(Received January 21, 1984)

A chemical examination of hydrolyzable tannins in a rhubarb of high quality (commercial name:馬蹄大黄)has revealed the occurrence of four new acylated sugars, *i.e.*, 2-*O*-cinnamoyl-*β*-D-glucose (I), 2-*O*-cinnamoyl-1,6-di-*O*-galloyl-*β*-D-glucose (II), 2-*O*-*p*-coumaroyl-1-*O*-galloyl-*β*-D-glucose (III) and 1-*O*-galloylfructose (IV), as well as the known compounds 2-*O*-cinnamoyl-1-*O*-galloyl-*β*-D-glucose (VII), (−)-epicatechin 3-*O*-gallate (VIII), 1-*O*-galloyl-*β*-D-glucose (IX) and 1,6-di-*O*-galloyl-*β*-D-glucose (X).

A low-quality rhubarb (commercial name:芋大黄) was found to contain three new gallates, 1-*O*-galloylfructose (IV), 2,6-di-*O*-galloylglucose (V) and 3,5-dihydroxyphenol 1-*O*-*β*-D-(6-*O*-galloyl)-glucopyranoside (VI), together with five known compounds, VIII, X, 6-*O*-galloylglucose (XI), 1,2,6-tri-*O*-galloyl-*β*-D-glucose (XII) and procyanidin B-1 3-*O*-gallate (XIII).

**Keywords**——rhubarb; Polygonaceae; gallotannin; cinnamoylglucose; *p*-coumaroylglucose; galloylfructose; phloroglucinol glucoside gallate; galloylglucose

In previous papers, we reported on the isolation, from a representative of rhubarb (commercial name: 雅黄), of polymeric proanthocyanidin gallates named rhatannins,[2] which have activity to decrease urea-nitrogen concentration in rat serum, as well as several lower-molecular-weight galloyl esters,[2,3] *i.e.*, galloyl proanthocyanidin dimers, galloyl glucoses and gallic acid glucoside gallates. As part of our chemical studies on tannins and related compounds, we have now investigated two varieties of commercial rhubarb (馬蹄大黄: Batei-Daio and 芋大黄: Imo-Daio).

The polar fractions of both aqueous acetone extracts contained complicated mixtures of acylated sugars which could be separated by a combination of Sephadex LH-20, MCI-gel CHP-20P, Sep-pak and Avicel cellulose chromatographies (Charts 1 and 2). Finally, four new acylated sugars (I—IV) were isolated from Batei-Daio, together with four known compounds (VII—X), while three new gallates (IV—VI) and five known compounds (VIII, X—XIII) were obtained from Imo-Daio. The known compounds VII—XIII were identified as 2-*O*-cinnamoyl-1-*O*-galloyl-*β*-D-glucose[4] (VII), (−)-epicatechin 3-*O*-gallate[2] (VIII), 1-*O*-galloyl-*β*-D-glucose[5] (IX), 1,6-di-*O*-galloyl-*β*-D-glucose[3] (X), 6-*O*-galloylglucose[3] (XI), 1,2,6-tri-*O*-galloyl-*β*-D-glucose[2] (XII) and procyanidin B-1 3-*O*-gallate[2] (XIII) by comparisons of their physical and spectral data with those of authentic samples.

Compound I, colorless needles ($H_2O$), mp 156.5—158 °C, $[\alpha]_D$ +49.7° (MeOH), $C_{15}H_{18}O_7$, gave a molecular ion peak at $m/z$ 310 in the field desorption mass spectrum (FD-MS). The carbon-13 nuclear magnetic resonance ($^{13}$C-NMR) spectrum revealed the presence of a sugar moiety and an aromatic ring with a mono-substituted system (Table I). In addition, an ester carbon signal and two olefinic carbon signals were observed. The presence of *trans* olefinic protons was confirmed by proton nuclear magnetic resonance ($^1$H-NMR) signals at $\delta$ 6.62 and 7.68 (each 1H, d, $J$=16 Hz). Alkaline hydrolysis of I with sodium methoxide in methanol afforded glucose and methyl cinnamate. The $^1$H-NMR spectrum of I, measured

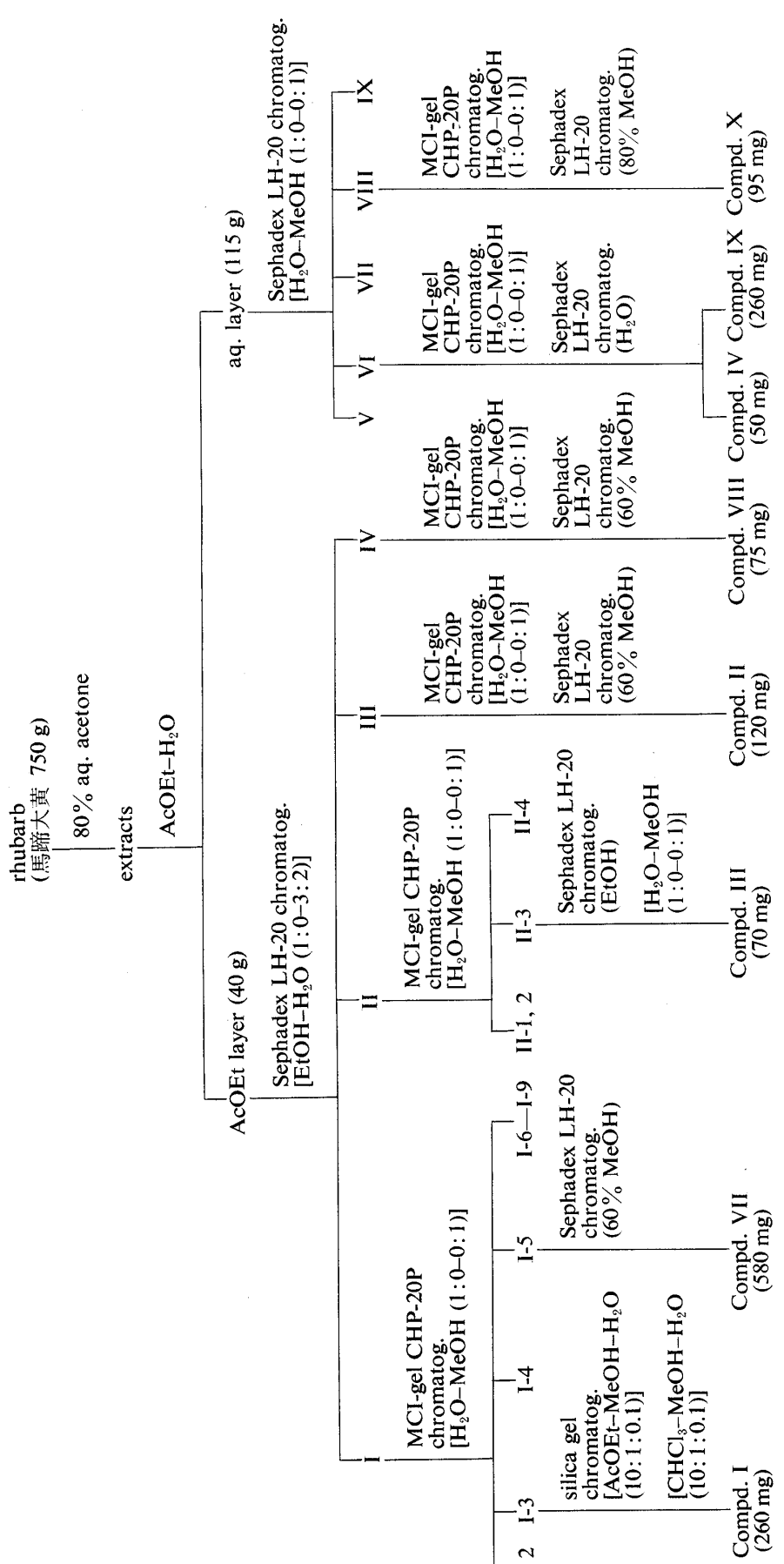



Chart 1

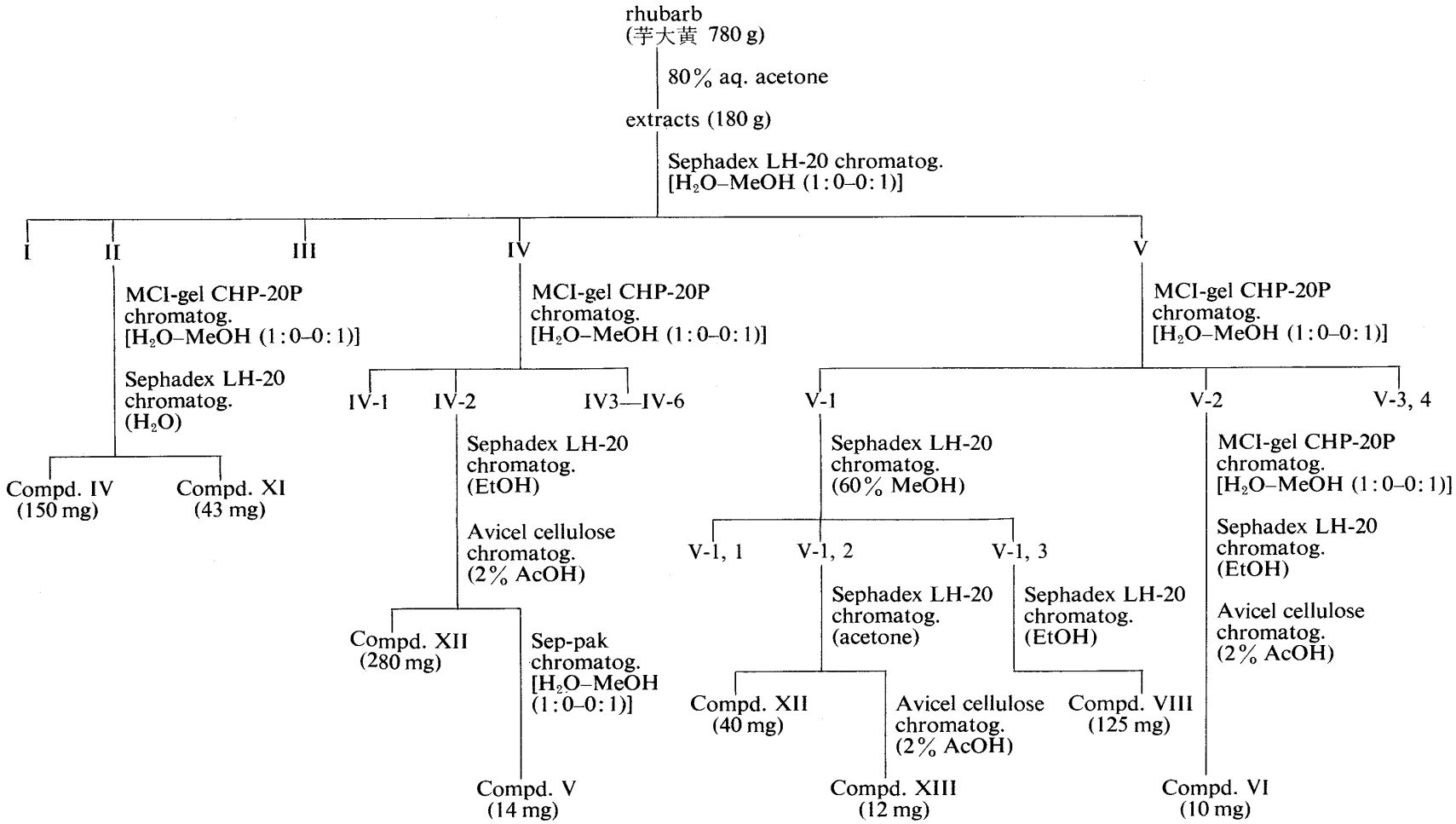



Chart 2

TABLE I. $^{13}$C-NMR Data for Compounds I, II, III and VII ($\delta$ Values)

| | I[a] | II[b] | III[b] | VII[a] |
|---|---|---|---|---|
| Glucose | | | | |
| 1 | 94.2 | 93.5 | 93.6 | 92.0 |
| 2 | 75.2[c] | 73.8[c] | 73.7[c] | 72.8[c] |
| 3 | 74.0[c] | 74.9[c] | 74.9[c] | 73.5[c] |
| 4 | 70.1 | 71.0 | 70.8 | 69.4 |
| 5 | 76.6 | 75.9 | 78.4 | 77.7 |
| 6 | 60.8 | 64.1 | 61.8 | 60.1 |
| Cinnamoyl | | | | |
| 1 | 133.9 | 134.8 | | 133.6 |
| 2 | 128.1 (2C) | 129.0 (2C) | | 128.2 (2C) |
| 3 | 128.9 (2C) | 129.8 (2C) | | 128.9 (2C) |
| 4 | 130.3 | 131.5 | | 130.5 |
| –CH=CH– | 118.3 | 118.1 | | 117.9 |
| | 144.3 | 146.6 | | 145.1 |
| –COO– | 165.3 | 165.7 | | 164.3 |
| *p*-Coumaroyl | | | | |
| 1 | | | 126.3 | |
| 2 | | | 131.3 (2C) | |
| 3 | | | 116.7 (2C) | |
| 4 | | | 160.8 | |
| –CH=CH– | | | 114.4 | |
| | | | 146.1 | |
| –COO– | | | 165.9 | |
| Galloyl | | | | |
| 1 | | 119.6, 121.0 | 119.7 | 117.1 |
| 2 | | 109.9 (2C), 110.2 (2C) | 110.2 (2C) | 108.8 (2C) |
| 3 | | 146.0 (2C), 146.1 (2C) | 146.8 (2C) | 145.3 (2C) |
| 4 | | 139.1, 139.8 | 139.9 | 138.9 |
| –COO– | | 167.2 (2C) | 167.8 | 165.3 |

*a*) Measured in DMSO-$d_6$+$D_2O$.
*b*) Measured in acetone-$d_6$+$D_2O$.
*c*) Assignments with the superscript *c*) may be interchanged in each column.

immediately after dissolution in pyridine-$d_5$, showed an anomeric doublet at $\delta$ 5.46 (d, $J$= 8 Hz) indicative of $\beta$-configuration of the glucose moiety. However, on measurement after 24 h, a new $\alpha$-anomeric proton signal was also seen at $\delta$ 6.15 (d, $J$=4 Hz). These observations indicated that the anomeric center does not carry the cinnamoyl group, and that the anomeric carbon in I adopts $\beta$-configuration in the crystalline form. The location of the cinnamoyl group was presumed to be at the C-2 position from the observation of a pair of lowfield signals at $\delta$ 5.63 (dd, $J$=4, 8 Hz) and $\delta$ 5.84 (t, $J$=8 Hz) assignable to the C-2 protons in the $\alpha$- and $\beta$-glucosyl moieties, respectively. In order to establish the structure of I definitively, 2-*O*-cinnamoylglucose was prepared from 2-*O*-cinnamoyl-1-*O*-galloyl-$\beta$-D-glucose (VII) by enzymatic hydrolysis with tannase. Spectral comparison revealed that I was identical with the synthetic sample thus obtained. Consequently, the structure of this compound was established as 2-*O*-cinnamoylglucose (I).

Compound II, an off-white amorphous powder, $[\alpha]_D$ −95.2° (acetone), $C_{29}H_{26}O_{15}\cdot 3/2H_2O$, showed a blue coloration with ferric chloride reagent. The FD-MS exhibited peaks at $m/z$ 653 and 637 due to $[M+K]^+$ and $[M+Na]^+$, respectively, together with prominent peaks at $m/z$ 170 and 148 suggestive of the presence of galloyl and cinnamoyl group. The $^1$H-NMR spectrum was very similar to that of 1,2,6-tri-*O*-galloyl-$\beta$-D-glucose (XII), with lowfield sugar signals at $\delta$ 5.93 (d, $J$=8 Hz, $C_1$–H), 5.24 (t, $J$=8 Hz, $C_2$–H), 4.67 (dd, $J$=2, 12 Hz, $C_6$–

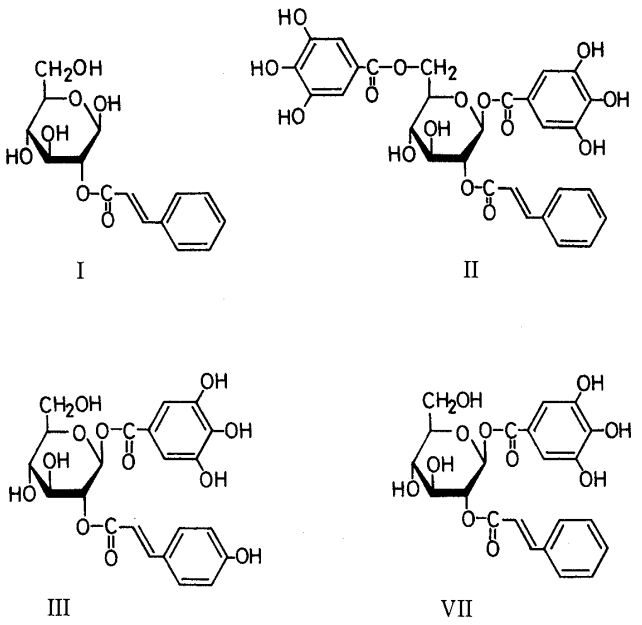



Fig. 1

H), and 4.42 (dd, $J=4$, 12 Hz, $C_6$–H), but indicated the presence of a cinnamoyl group ($\delta$ 6.51, 7.70, each 1H, d, $J=16$ Hz; 7.30—7.72, 5H, m) and two galloyl groups ($\delta$ 7.10, 7.15, each 2H, s). The position of the cinnamoyl group in the glucose residue was confirmed to be at the C-2 position since compound I was formed by tannase hydrolysis of II. On the basis of the evidence described above, the structure of this compound was characterized as 2-*O*-cinnamoyl-1,6-di-*O*-galloyl-$\beta$-D-glucose (II).

Compound III, colorless needles ($H_2O$), mp 181—183 °C, $[\alpha]_D$ −124.5° (acetone), $C_{22}H_{22}O_{12}\cdot H_2O$, was positive to the ferric chloride reagent (a blue coloration). The FD-MS, with a peak at $m/z$ 501 $[M+Na]^+$, suggested the presence of a galloyl group ($m/z$ 170). The $^1$H-NMR spectrum closely resembled that of 2-*O*-cinnamoyl-1-*O*-galloyl-$\beta$-D-glucose (VII), except for $A_2B_2$-type aromatic signals at $\delta$ 6.84 and 7.47 (each 2H, d, $J=8$ Hz). Enzymatic hydrolysis of III with tannase furnished gallic acid and a hydrolysate (IIIa), colorless granules, mp 204—206 °C, $[\alpha]_D$ +30.3° (MeOH). Subsequent alkaline hydrolysis of IIIa with sodium methoxide in methanol yielded glucose and methyl *p*-coumaroate. Since the sugar proton signals in the $^1$H-NMR spectrum of IIIa were similar to those of I, showing the presence of a mixture of $\alpha$- and $\beta$-glucosyl moieties, it was concluded that the galloyl and *p*-coumaroyl groups were located at the C-1 and C-2 positions, respectively, in the glucose moiety. Accordingly, the structure of III was assigned as 2-*O*-*p*-coumaroyl-1-*O*-galloyl-$\beta$-D-glucose.

Compound IV, an off-white amorphous powder, $[\alpha]_D$ −8.5° (acetone), $C_{13}H_{16}O_{10}\cdot 1/2H_2O$, showed a peak at $m/z$ 355 due to $[M+Na]^+$ in the FD-MS. The molecular formula and the molecular weight were consistent with a monogalloyl hexose. On tannase hydrolysis, IV afforded gallic acid and a sugar. Analysis of the sugar by means of gas-liquid chromatography (GLC) and thin-layer chromatography (TLC) showed it to be fructose. From the observation that the $^1$H-NMR spectrum of IV exhibited no significant downfield shift of sugar methine protons, the galloyl group was assumed to be located at the primary hydroxyl group in the fructose moiety. The $^{13}$C-NMR spectrum of IV showed eighteen sugar signals analogous to those of fructose (Fig. 1), suggesting that IV exists in solution as an equilibrium mixture of $\beta$-pyranose, $\beta$-furanose and $\alpha$-furanose forms. The downfield shift of the C-1 methylene carbon as well as the upfield shift of the neighboring C-2 atom, indicated the occurrence of the galloyl group at the C-2 position. From these chemical

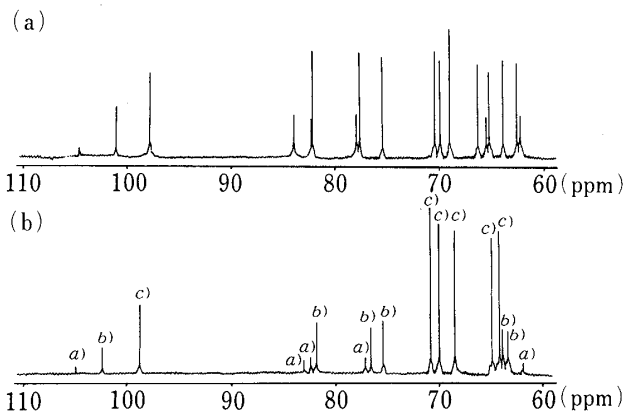
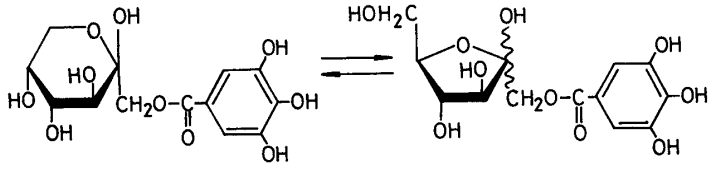



Fig. 2. $^{13}$C-NMR Spectra of Compound IV (*a*) and D-Fructose (*b*) (in Acetone-$d_6$+$D_2O$)

*a*) Signals arising from α-D-fructofuranose.
*b*) Signals arising from β-D-fructofuranose.
*c*) Signals arising from β-D-fructopyranose.



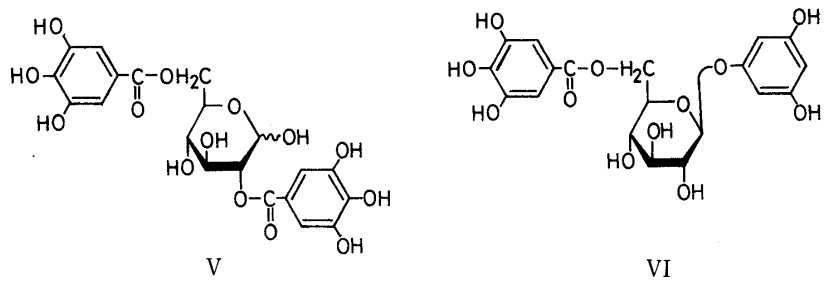
IV

Fig. 3

and spectroscopic data, the structure of IV was concluded to be 1-*O*-galloylfructose.

Compound V, colorless needles ($H_2O$), mp 184—186 °C, $[\alpha]_D$ −18.5° (acetone), $C_{20}H_{20}O_{14}\cdot 2H_2O$, contained two galloyl groups as revealed by a four-proton singlet at $\delta$ 7.10 in the $^1$H-NMR spectrum. On enzymatic hydrolysis with tannase, V afforded glucose and gallic acid. The $^1$H- and $^{13}$C-NMR spectra of V showed duplicated sugar signal patterns, indicating that V occurs as a mixture of α- and β-forms. The locations of the galloyl groups were determined spectroscopically as follows. In the $^{13}$C-NMR spectrum, the glucose C-6 carbon signal appeared at lower field ($\delta$ 64.5) than that of glucose, the chemical shift being in good agreement with those observed in 6-acylated glucoses (II, XI, XII and XIII). From this observation, one galloyl group was concluded to be linked at the C-6 position. The remaining galloyl group was shown to be located at the C-2 position by analysis of the $^1$H-NMR spectrum, which displayed perturbed lowfield signals ($\delta$ 5.70—5.98) assignable by means of spin-decoupling techniques[6] to the C-2 proton. On the basis of these observations, compound V was concluded to be 2,6-di-*O*-galloylglucose.

Compound VI, colorless needles ($H_2O$), mp 168—169 °C, $[\alpha]_D$ −41.2° (MeOH), $C_{19}H_{22}O_{13}\cdot H_2O$, exhibited, in the $^1$H- and $^{13}$C-NMR spectra, anomeric signals ($\delta$ 4.96, d, $J$= 8 Hz; $\delta$ 101.3) consistent with a glycosidic nature. The presence of a galloyl group in VI was shown by $^1$H- and $^{13}$C-NMR signals [$\delta$ 7.14, 2H, s; $\delta$ 109.9 (2C), 121.4, 139.0, 146.0 (2C), 167.3]. Furthermore, the appearance of a *meta*-coupled aromatic proton signal at $\delta$ 6.04 (t, $J$=2 Hz) and a two-proton doublet at $\delta$ 6.14 ($J$=2 Hz) in the $^1$H-NMR spectrum suggested the occurrence of a phloroglucinol moiety in VI. Enzymatic hydrolysis of VI with tannase yielded gallic acid and a hydrolysate (VIa), colorless needles (AcOEt–EtOH), mp 236—239 °C, which was identified as 3,5-dihydroxyphenol 1-*O*-β-D-glucopyranoside.[7] The location of the galloyl group in VI was concluded to be at the C-6 position in the glucose moiety, since



V VI

Fig. 4

two-proton signals ($\delta$ 4.35, dd, $J=4$, 12 Hz; $\delta$ 4.59, dd, $J=2$, 12 Hz), which were assignable to the C-6 methylene protons on the basis of the large coupling constant, were shifted downfield. Based on these observations, the structure of VI was concluded to be 3,5-dihydroxyphenol 1-*O*-$\beta$-D-(6-*O*-galloyl)-glucopyranoside.

The above results imply that hydrolyzable tannins in rhubarb are composed of not only common gallotannins consisting exclusively of gallic acid and glucose, but also new classes of gallotannins possessing a fructose core, a glucoside moiety and a (hydroxy)cinnamoyl residue.

## Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. IR spectra were obtained with a JASCO DS-301 spectrometer. The EI- and FD-MS were obtained with JEOL D-300 and JEOL DX-300 spectrometers, respectively. The $^1$H- and $^{13}$C-NMR spectra were taken with JEOL PS-100 and FX-100 spectrometers, respectively, using tetramethylsilane as an internal standard; chemical shifts are given in $\delta$ (ppm). Column chromatography was carried out with Sephadex LH-20 (25—100 $\mu$, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP-20P (75—150 $\mu$, Mitsubishi Chemical Industries, Ltd.), Kieselgel 60 (70—230 mesh, Merck), Avicel microcrystalline cellulose (Funakishi) and Sep-pak (Waters Associates, Inc.). 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 by spraying 10% $H_2SO_4$, $FeCl_3$ and aniline–hydrogen phthalate reagents. Analytical GLC for sugar was performed over 1.5% OV-17 and 1.5% SE-30 with nitrogen as the carrier gas.

**Isolation from Batei-Daio**——The pulverized rhubarb (commercial name: 馬蹄大黄 750 g) was extracted with 80% aqueous acetone three times at room temperature. The acetone was removed by evaporation under reduced pressure (*ca.* 40 °C), and the aqueous solution was shaken three times with AcOEt. The AcOEt layer (40 g) and the aqueous layer (115 g) were separately chromatographed as shown in Chart 1 to give four fractions (fractions I—IV) and five fractions (fractions V—IX), respectively. Fractions I (23.8 g) and II (8 g) were rechromatographed over MCI-gel CHP-20P (solvent: $H_2O$–MeOH) to afford nine (I-1—I-9) and five (II-1—II-5) fractions, respectively. Fractions I-6—I-9, II-4 and II-5, which contained relatively lower-molecular-weight phenolics, were not examined further. Fraction II-2 consisted of a large amount of gallic acid. Fraction I-3 was repeatedly chromatographed over silica gel (solvent: AcOEt–MeOH–$H_2O$, $CHCl_3$–MeOH–$H_2O$) to afford compound I (260 mg). Fraction I-5 was further chromatographed over Sephadex LH-20 (solvent: 60% MeOH) to give compound VII (580 mg). Fraction II-3 was subjected to chromatography over Sephadex LH-20 (solvent: EtOH, $H_2O$–MeOH) to furnish compound III (70 mg). Fractions III (4 g) and IV (3.3 g) were repeatedly chromatographed over MCI-gel CHP-20P (solvent: $H_2O$–MeOH) and Sephadex LH-20 (solvent: 60% MeOH) to give compounds II (120 mg) and VIII (75 mg), respectively. Repeated chromatography of fraction VI (5.6 g) over MCI-gel CHP-20P (solvent: $H_2O$–MeOH) and Sephadex LH-20 (solvent: $H_2O$) afforded compounds IV (50 mg) and IX (260 mg), while purification of fraction VII (10 g) by chromatography over MCI-gel CHP-20P (solvent: $H_2O$–MeOH) and Sephadex LH-20 (solvent: 80% MeOH) furnished compound X (95 mg).

**Isolation from Imo-Daio**——The pulverized rhubarb (commercial name: 芋大黄 780 g) was extracted three times with 80% aqueous acetone at room temperature. The aqueous acetone extracts, after removal of the solvent by evaporation, were chromatographed over Sephadex LH-20 using $H_2O$ containing increasing amounts of MeOH (1 : 0–0 : 1) to afford five fractions (fractions I—V). Fraction II (2 g), consisting of a mixture of monogallates, was further chromatographed over MCI-gel CHP-20P (solvent: $H_2O$–MeOH) and Sephadex LH-20 (solvent: $H_2O$) to afford compounds IV (150 mg) and XI (43 mg). Fraction III contained gallic acid. Fraction IV (62 g) was rechromatographed over MCI-gel CHP-20P (solvent: $H_2O$–MeOH) to give six fractions (fractions IV-1—IV-6). Fractions IV-3—IV-6 consisted of a large amount of stilbene glycosides, which exhibited bluish-purple fluorescent

spots on silica gel TLC under ultraviolet (UV) irradiation. Fraction IV-2 was repeatedly chromatographed over Sephadex LH-20 (solvent: EtOH), Avicel cellulose (solvent: 2% AcOH) and Sep-pak (solvent: $H_2O$–MeOH) to afford compounds V (14 mg) and XII (280 mg). Fraction V (85 g), contaminated by stilbene glycosides and their gallates, was chromatographed over MCI-gel CHP-20P (solvent: $H_2O$–MeOH) to give four fractions (fractions V-1—V-4). Fractions V-1 and V-2 were repeatedly chromatographed over Sephadex LH-20 (solvent: acetone, EtOH, 60% MeOH), MCI-gel CHP-20P (solvent: $H_2O$–MeOH) and Avicel cellulose (solvent: 2% AcOH) to afford compounds VI (10 mg), VIII (125 mg), XII (40 mg) and XIII (12 mg).

**Compound I**——Colorless needles ($H_2O$), mp 156.5—158 °C, $[\alpha]_D^{20}$ +49.7° ($c$=0.87, MeOH). *Anal.* Calcd for $C_{15}H_{18}O_7$: C, 58.06; H, 5.85. Found: C, 57.65; H, 5.84. FD-MS ($m/z$): 310 $[M]^+$, 148. $^1$H-NMR (DMSO-$d_6$): 3.12—3.94 (5H, m, $C_{3-6}$–H), 4.52—4.70 (2H, m, $C_{1,2}$–H), 6.62, 7.68 (each 1H, d, $J$=16 Hz, olefinic-H), 7.36—7.78 (5H, m, arom.-H). (pyridine-$d_5$ + $D_2O$; after 24 h): 4.02—5.20 (5H, m, $C_{3-6}$–H), 5.46 (1/2H, d, $J$=8 Hz, β-$C_1$–H), 5.63 (1/2H, dd, $J$=4, 8 Hz, α-$C_2$–H), 5.84 (1/2H, t, $J$=8 Hz, β-$C_2$–H), 6.15 (1/2H, d, $J$=4 Hz, α-$C_1$–H), 6.72, 6.76 (1H in total, each d, $J$=16 Hz, olefinic-H), 7.16—7.34 (5H, m, arom.-H), 7.92 (1H, d, $J$=16 Hz, olefinic-H). $^{13}$C-NMR: Table I.

**Alkaline Hydrolysis of I**——A solution of I (5 mg) in 2% NaOMe–MeOH (1 ml) was kept standing at room temperature for 1 h. The reaction mixture was neutralized with Dowex 50WX ($H^+$ form), and the hydrolysates were analyzed by TLC [solvent: $CHCl_3$–MeOH–$H_2O$ (8:2:0.2)]. Spots (*Rf*: 0.06 and 0.78) corresponding to glucose and methyl cinnamate, respectively, were detected.

**Enzymatic Hydrolysis of VII**——An aqueous solution of VII (50 mg) was incubated with tannase at 37 °C for 30 min. The solution was concentrated to dryness under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was chromatographed over Sephadex LH-20 (solvent: EtOH) to afford gallic acid and compound I (21 mg), colorless needles ($H_2O$), mp 156—159 °C, $[\alpha]_D^{19}$ +47.7° ($c$=0.36, MeOH). $^1$H-NMR (DMSO-$d_6$): 3.06—4.10 (5H, m, $C_{3-6}$–H), 4.55—4.65 (2H, m, $C_{1,2}$–H), 6.63, 7.63 (each 1H, d, $J$=16 Hz, olefinic-H), 7.36—7.80 (5H, m, arom.-H).

**Compound II**——An off-white amorphous powder, $[\alpha]_D^{24}$ −95.2° ($c$=1.29, acetone). *Anal.* Calcd for $C_{29}H_{26}O_{15}\cdot 3/2H_2O$: C, 54.29; H, 4.56. Found: C, 54.11; H, 4.55. FD-MS ($m/z$): 653 $[M+K]^+$, 637 $[M+Na]^+$, 170, 148. $^1$H-NMR (acetone-$d_6$ + $D_2O$): 3.56—4.08 (3H, m, $C_{3-5}$–H), 4.42 (1H, dd, $J$=4, 12 Hz, $C_6$–H), 4.67 (1H, dd, $J$=2, 12 Hz, $C_6$–H), 5.24 (1H, t, $J$=8 Hz, $C_2$–H), 5.93 (1H, d, $J$=8 Hz, $C_1$–H), 6.51, 7.70 (each 1H, d, $J$=16 Hz, olefinic-H). 7.10, 7.15 (each 2H, s, galloyl-H), 7.30—7.48 (5H, m, arom.-H). $^{13}$C-NMR: Table I.

**Enzymatic Hydrolysis of II**——An aqueous solution of II (50 mg) was shaken with tannase at room temperature for 15 min. The reaction mixture was treated in the same way as described above to afford gallic acid and a hydrolysate (12 mg), colorless needles ($H_2O$), mp 159—160 °C, $[\alpha]_D^{24}$ +50.9° ($c$=0.29, MeOH), $^1$H-NMR (DMSO-$d_6$): 3.10—3.95 (5H, m, $C_{3-6}$–H), 4.55—4.70 (2H, m, $C_{1,2}$–H), 6.62, 7.64 (each 1H, d, $J$=16 Hz, olefinic-H), 7.32—7.84 (5H, m, arom.-H). This product was shown to be identical with compound I by direct comparison of physical and spectral data.

**Compound III**——Colorless needles ($H_2O$), mp 181—183 °C, $[\alpha]_D^{24}$ −124.5° ($c$=1.33, acetone). *Anal.* Calcd for $C_{22}H_{22}O_{12}\cdot H_2O$: C, 53.23; H, 4.87. Found: C, 53.38; H, 4.94. FD-MS ($m/z$): 501 $[M+Na]^+$, 308 $[M-galloyl]^+$, 170. $^1$H-NMR (acetone-$d_6$ + $D_2O$): 3.55—4.08 (5H, m, $C_{3-6}$–H), 5.14 (1H, t, $J$=8 Hz, $C_2$–H), 5.86 (1H, d, $J$=8 Hz, $C_1$–H), 6.29, 7.61 (each 1H, d, $J$=16 Hz, olefinic-H), 6.84, 7.47 (each 2H, d, $J$=8 Hz, $C_{2',6'}$ and $C_{3',5'}$–H), 7.10 (2H, s, galloyl-H). $^{13}$C-NMR: Table I.

**Enzymatic Hydrolysis of III**——An aqueous solution of III (60 mg) was treated with tannase at room temperature for 15 min. Work-up in the same way as for II gave gallic acid and a hydrolysate (IIIa) (20 mg), colorless granules ($H_2O$), mp 204—206 °C, $[\alpha]_D^{25}$ +30.3° ($c$=0.32, MeOH), $^1$H-NMR (DMSO-$d_6$ + $D_2O$): 3.12—3.88 (5H, m, $C_{3-6}$–H), 3.46—3.64 (3/2H, β-$C_1$–H and $C_2$–H), 5.14 (1/2H, d, $J$=4 Hz, α-$C_1$–H), 6.36, 6.40 (1H in total, each d, $J$=16 Hz, olefinic-H), 6.76, 6.82 (2H in total, each d, $J$=8 Hz, $C_{3',5'}$–H), 7.64—7.76 (3H, m, olefinic-H and $C_{2',6'}$–H).

**Alkaline Hydrolysis of IIIa**——A solution of IIIa (18 mg) in 2% NaOMe–MeOH (5 ml) was left standing at room temperature for 3 h. The reaction mixture was treated as described for I to give a mixture of products, which were separated by chromatography over Sephadex LH-20 (solvent: EtOH) to give a sugar and methyl *p*-coumaroate, colorless needles (dil. MeOH), mp 140—143 °C, IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3380, 1680, 1640, 1600, 1580, 1510. The sugar was shown to be identical with glucose by TLC examination [solvent: *n*-BuOH–pyridine–$H_2O$ (6:4:3), *Rf*: 0.32].

**Compound IV**——An off-white amorphous powder, $[\alpha]_D^{24}$ −8.5° ($c$=1.23, acetone). *Anal.* Calcd for $C_{13}H_{16}O_{10}\cdot 1/2H_2O$: C, 45.75; H, 5.02. Found: C, 45.43; H, 5.22. FD-MS ($m/z$): 355 $[M+Na]^+$, $^1$H-NMR (acetone-$d_6$): 3.56—4.40 (7H, m, sugar-H), 7.14 (2H, s, galloyl-H). $^{13}$C-NMR (acetone-$d_6$ + $D_2O$): 62.5 (t, α-fur.-$C_6$), 62.8 (t, β-fur.-$C_6$), 64.3 (t, β-pyr.-$C_6$), 65.5 (t, β-fur.-$C_1$), 66.6 (t, β-pyr.-$C_1$), 69.4 (d, β-pyr.-$C_3$), 70.2 (d, β-pyr.-$C_5$), 70.8 (d, β-pyr.-$C_4$), 75.7 (d, β-fur.-$C_4$), 77.9 (d, β-fur.-$C_3$), 78.1 (d, α-fur.-$C_4$), 82.5 (d, α-fur.-$C_5$), 82.7 (d, β-fur.-$C_5$), 84.1 (d, α-fur.-$C_3$), 98.0 (s, β-pyr.-$C_2$), 101.3 (s, β-fur.-$C_2$), 105.1 (s, α-fur.-$C_2$), 109.5, 110.0 (2C in total) (each d, galloyl $C_{2,6}$), 121.0, 121.1 (2C in total) (each s, galloyl $C_1$), 139.1 (s, galloyl $C_4$), 145.9 (2C) (s, galloyl $C_{3,5}$), 167.1, 176.3 (1C in total) (s, –COO–).

**Enzymatic Hydrolysis of IV**——An aqueous solution of IV (10 mg) was treated with tannase at room temperature for 30 min. The reaction mixture was worked up in the same way as described above to furnish gallic acid and a sugar. Analysis of the sugar by means of GLC showed it to be identical with fructose [$t_R$, 6.5 (major), 9.5

(minor): column, 1.5% OV-17; column temp., 150 °C; flow rate, 50 ml/min. $t_R$, 6.9 (major), 10.5 (minor); column, 1.5% SE-30; column temp., 165 °C; flow rate, 40 ml/min).

**Compound V**——Colorless needles ($H_2O$), mp 184—186 °C, $[\alpha]_D^{25}$ +18.5° ($c=0.62$, acetone). *Anal.* Calcd for $C_{20}H_{20}O_{14}\cdot 2H_2O$: C, 46.15; H, 4.64. Found: C, 45.81; H, 4.70. FD-MS (*m/z*): 467 $[M-OH]^+$, 170. $^1$H-NMR (acetone-$d_6$ + $D_2O$): 3.56—3.88 (3H, m, $C_{3-5}$–H), 4.36 (1H, dd, $J=4$, 12 Hz, $C_6$–H), 4.61 (1H, br d, $J=12$ Hz, $C_6$–H), 4.88—4.96 (2H, m, $C_{1,2}$–H), 7.10 (4H, s, galloyl-H). (pyridine-$d_5$): 4.20—4.40 (3H, m, $C_{3-5}$–H), 5.01 (1H, dd, $J=4$, 12 Hz, $C_6$–H), 5.20 (1H, br d, $J=12$ Hz, $C_6$–H), 5.41 (4/5H, d, $J=8$ Hz, β-$C_1$–H), 5.70—5.98 (1H, m, $C_2$–H), 6.08 (1/5H, d, $J=4$ Hz, α-$C_1$–H), 7.87, 7.99 (each 2H, s, galloyl-H). $^{13}$C-NMR (acetone-$d_6$ + $D_2O$): 64.5 ($C_6$), 70.3 (α-$C_4$), 71.5 (β-$C_4$), 71.7 (α-$C_3$), 73.0 (α-$C_5$), 75.0 (β-$C_3$), 75.4 (α-$C_2$), 75.6 (β-$C_2$), 90.9 (α-$C_1$), 96.1 (β-$C_1$), 109.9, 110.1 (4C in total (galloyl $C_{2,6}$), 121.3, 121.6 (2C in total) (galloyl $C_1$), 139.1 (2C) (galloyl $C_4$), 146.0 (4C) (galloyl $C_{3,5}$), 167.4 (2C) (–COO–).

**Enzymatic Hydrolysis of V**——An aqueous solution of V (5 mg) was shaken with tannase at room temperature for 30 min. The solvent was evaporated off under reduced pressure, and the residue was treated with MeOH. The MeOH-soluble portion was directly analyzed by TLC [solvent: $CHCl_3$–MeOH–$H_2O$ (7 : 3 : 0.5)]. The *Rf* values (0.28 and 0.21) were consistent with those of gallic acid and glucose, respectively.

**Compound VI**——Colorless needles ($H_2O$), mp 168—169 °C, $[\alpha]_D^{25}$ −41.2° ($c=0.59$, MeOH). *Anal.* Calcd for $C_{19}H_{22}O_{13}\cdot H_2O$: C, 49.78; H, 4.84. Found: C, 49.63; H, 4.80. FD-MS (*m/z*): 441 $[M+H]^+$, 315, 170, 126. $^1$H-NMR (acetone-$d_6$ + $D_2O$): 3.40—4.02 (4H, m, $C_{2'-5'}$–H), 4.35 (1H, dd, $J=4$, 12 Hz, $C_6$–H), 4.59 (1H, dd, $J=2$, 12 Hz, $C_6$–H), 4.96 (1H, d, $J=8$ Hz, anom.-H), 6.04 (1H, t, $J=2$ Hz, $C_4$–H), 6.14 (2H, d, $J=2$ Hz, $C_{2,6}$–H), 7.14 (2H, s, galloyl-H). $^{13}$C-NMR (acetone-$d_6$ + $D_2O$): 64.6 ($C_{6'}$), 70.4 ($C_{4'}$), 74.2 ($C_{2'}$), 74.6 ($C_{5'}$), 77.3 ($C_{3'}$), 96.5 ($C_4$), 97.9 (2C) ($C_{2,6}$), 101.3 ($C_{1'}$), 109.9 (2C) (galloyl $C_{2,6}$), 121.4 (galloyl $C_1$), 139.0 (galloyl $C_4$), 146.0 (2C) (galloyl $C_{3,5}$), 159.5 (2C) ($C_{3,5}$), 160.3 ($C_1$), 167.3 (–COO–).

**Enzymatic Hydrolysis of VI**——An aqueous solution of VI (9 mg) was treated with tannase at room temperature for 30 min. Work-up in the same way as described for VII gave gallic acid and an aglycone (3 mg), colorless needles (AcOEt–EtOH), mp 236—239 °C, $^1$H-NMR (DMSO-$d_6$): 3.20—3.80 (7H, sugar-H), 4.68 (1H, d, $J=7$ Hz, anom-H), 5.98 (3H, s, $C_{2,4,6}$–H). The latter product was found to be identical with 3,5-dihydroxyphenol 1-*O*-β-D-glucopyranoside (VIa) by direct comparisons.

**Compound VII**——Colorless needles (dil. MeOH), mp 190—193 °C, $[\alpha]_D^{19}$ −125.2° ($c=1.00$, MeOH). $^1$H-NMR (DMSO-$d_6$): 3.30—4.10 (5H, m, $C_{3-6}$–H), 4.97 (1H, t, $J=8$ Hz, $C_2$–H), 5.83 (1H, d, $J=8$ Hz, $C_1$–H), 6.59, 7.65 (each 1H, d, $J=16$ Hz, olefinic-H), 6.94 (2H, s, galloyl-H), 7.30—7.78 (5H, m, arom.-H). $^{13}$C-NMR: Table I.

**Compound VIII**——Colorless needles ($H_2O$), mp 224—226 °C, $[\alpha]_D^{24}$ −136.2° ($c=1.20$, acetone). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3400—3500, 1675, 1610, 1520. $^1$H-NMR (acetone-$d_6$ + $D_2O$): 2.98 (2H, m, $C_4$–H), 5.12 (1H, s, $C_2$–H), 5.50 (1H, br s, $C_3$–H), 6.02, 6.06 (each 1H, d, $J=2$ Hz, $C_{6,8}$–H), 6.76 (1H, d, $J=8$ Hz, $C_{5'}$–H), 6.90 (1H, dd, $J=2$, 8 Hz, $C_{6'}$–H), 7.02 (2H, s, galloyl-H), 7.08 (1H, d, $J=2$ Hz, $C_{2'}$–H).

**Compound IX**——Colorless needles ($H_2O$), mp 226—227 °C, $[\alpha]_D^{24}$ −18.5° ($c=0.93$, $H_2O$). $^1$H-NMR (acetone-$d_6$ + $D_2O$): 3.44—4.28 (6H, m, $C_{2-6}$–H), 5.88 (1H, d, $J=8$ Hz, $C_1$–H), 7.19 (2H, s, galloyl-H).

**Compound X**——Colorless needles ($H_2O$), mp 201—202 °C, $[\alpha]_D^{24}$ −25.8 °C ($c=0.95$, acetone), $^1$H-NMR (acetone-$d_6$): 3.50—3.90 (4H, m, $C_{2-5}$–H), 4.38 (1H, dd, $J=4$, 12 Hz, $C_6$–H), 4.57 (1H, dd, $J=2$, 12 Hz, $C_6$–H), 5.75 (1H, d, $J=8$ Hz, $C_1$–H), 7.12, 7.16 (each 2H, s, galloyl-H).

**Compound XI**——Colorless needles ($H_2O$), mp 135—137 °C, $[\alpha]_D^{16}$ +23.7° ($c=0.90$, $H_2O$), $^1$H-NMR (acetone-$d_6$ + $D_2O$): 3.2—3.5 (7H, m, sugar-H), 7.14 (2H, s, galloyl-H). $^{13}$C-NMR (acetone-$d_6$ + $D_2O$): 64.6 ($C_6$), 70.4 (α-$C_4$), 71.1 (β-$C_4$), 71.3 (α-$C_2$), 73.2 (α-$C_5$), 74.3 (α-$C_3$), 74.8 (β-$C_2$), 75.6 (β-$C_5$), 77.3 (β-$C_3$), 93.4 (α-$C_1$), 97.7 (β-$C_1$), 109.9 (2C) (galloyl $C_{2,6}$), 121.3 (galloyl $C_1$), 139.4 (galloyl $C_4$), 146.0 (2C) (galloyl $C_{3,5}$), 167.5 (–COO–).

**Compound XII**——Colorless needles ($H_2O$), mp 207—209 °C, $[\alpha]_D^{16}$ −81.4° ($c=0.69$, acetone). $^1$H-NMR (acetone-$d_6$ + $D_2O$): 3.6—4.1 (3H, m, $C_{3-5}$–H), 4.42 (1H, dd, $J=4$, 12 Hz, $C_6$–H), 4.64 (1H, br d, $J=12$ Hz, $C_6$–H), 5.26 (1H, t, $J=8$ Hz, $C_2$–H), 5.97 (1H, d, $J=8$ Hz, $C_1$–H), 7.07, 7.09, 7.14 (each 2H, s, galloyl-H).

**Compound XIII**——An off-white amorphous powder, $[\alpha]_D^{16}$ −18.6° ($c=0.50$, acetone). $^1$H-NMR (acetone-$d_6$): 2.55 (1H, dd, $J=8$, 16 Hz, $C_{4'}$–H), 2.90 (1H, dd, $J=6$, 16 Hz, $C_{4'}$–H), 4.03 (1H, m, $C_{3'}$–H), 4.42 (1H, d, $J=6$ Hz, $C_{2'}$–H), 4.62 (1H, d, $J=2$ Hz, $C_4$–H), 5.32 (1H, m, $C_3$–H), 5.47 (1H, br s, $C_2$–H), 5.96, 6.81 (each 1H, d, $J=2$ Hz, $C_{6,8}$–H), 5.98 (1H, s, $C_{6'}$–H), 6.60—6.95 (6H, m, B, B′-ring), 6.97 (2H, s, galloyl-H).

**Acknowledgement** The authors wish to thank Dr. H. Okazaki of Sankyo Co., Ltd. for providing tannase. Thanks are also due to Mr. Y. Tanaka, Miss K. Soeda and K. Isobe for the measurements of EI-MS, FD-MS, $^1$H- and $^{13}$C-NMR spectra, and to the staff of the Central Analysis Room of this university for elemental analysis.

## References and Notes

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