[Chem. Pharm. Bull.
33(1) 96—101 (1985)]

Tannins and Related Compounds. XXV.[1] A New Class of Gallotannins Possessing a (−)-Shikimic Acid Core from *Castanopsis cuspidata* var. *sieboldii* NAKAI. (1)

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(Received May 1, 1984)

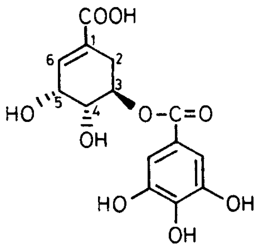
A homologous series of (−)-shikimic acid gallates (I—V) has been isolated, together with 1,6-di-*O*-galloyl-β-D-glucopyranoside (VI), 5-*O*-galloyl-D-hamamelose (VII), 2′,5-di-*O*-galloyl-D-hamamelose (VIII) and 2′,3,5-tri-*O*-galloyl-D-hamamelose (IX), from the leaves of *Castanopsis cuspidata* var. *sieboldii* NAKAI. On the basis of spectroscopic analysis, enzymatic hydrolysis and methanolysis, their structures have been established as 3-*O*-gallate (I), 3-*O*-digallate (II), 3-*O*-trigallate (III), 3,5-di-*O*-gallate (IV) and 3,4-di-*O*-gallate (V) of (−)-shikimic acid.

Keywords——*Castanopsis cuspidata* var. *sieboldii*; Fagaceae; gallotannin; (−)-shikimic acid gallate; galloylglucose; galloylhamamelose; tannase hydrolysis; methanolysis

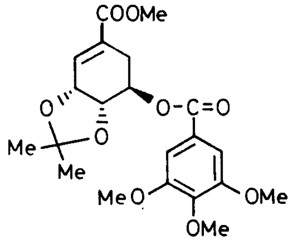
Recent chemical examinations of vegetable tannins have revealed that widely distributed gallotannins (excluding simple esters of gallic acid) are based upon not only D-glucose,[2] D-hamamelose[3] and 1,5-anhydro-D-glucitol[4] cores, but also a variety of polyol cores such as proto-quercitol,[5] quinic acid,[1,6] methyl-β-D-glucopyranoside,[7] a simple phenolic glucoside (salidroside),[8] xanthone C-glucosides (mangiferin and isomangiferin)[9] and benzophenone C-glucosides.[9] Our continuing search for new classes of gallotannins having other polyol cores has now resulted in the isolation of five galloyl esters with shikimic acid (I—V), together with a galloylglucose (VI) and galloylhamameloses (VII—IX), from the leaves of *Castanopsis cuspidata* var. *sieboldii* NAKAI (Fagaceae) (Japanese name: Sudazii), and in this paper we wish to describe the structural characterization of these compounds.

The 80% aqueous acetone extract of the fresh leaves was subjected to a combination of adsorption and partition (Sephadex LH-20 and MCI-gel CHP-20P) chromatography with various solvent systems as described in previous papers[10] to give compounds I—IX. Among these compounds, VI—IX were identified as 1,6-di-*O*-galloyl-β-D-glucopyranoside,[2c] 5-*O*-galloyl-D-hamamelose,[3c] 2′,5-di-*O*-galloyl-D-hamamelose (hamamelitannin)[3] and 2′,3,5-tri-*O*-galloyl-D-hamamelose,[3c] respectively, by comparisons of their physical and spectral data with those of authentic samples.

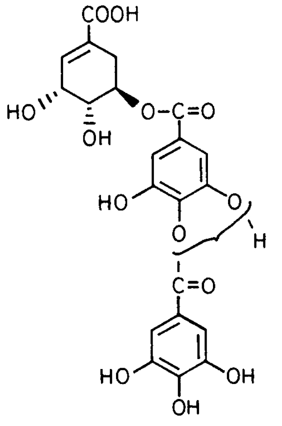
Compound I was obtained in a good yield (*ca.* 2% from the fresh leaves) as a white powder, mp 255 °C (dec.), $[\alpha]_D$ −111.7° (acetone). The molecular formula, $C_{14}H_{14}O_9 \cdot 3/2H_2O$, was confirmed by elemental analysis and field desorption mass spectrometry (FD-MS). The presence of a galloyl group in I was indicated by a positive ferric chloride test (a blue coloration) and by the appearance of a two-proton singlet signal at δ 7.12 in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum. The ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectrum showed, in addition to signals due to a galloyl group, the following carbon signals; a carboxylic acid (δ 168.9, s), a tri-substituted double bond (δ 129.3, s and 138.9, d), a methylene (δ 28.1, t) and three methines carrying an oxygen function (δ 66.8, 68.8 and 71.2, each d). On enzymatic hydrolysis with tannase, I yielded gallic acid and a crystalline hydrolysate (Ia), mp 186—187 °C, $[\alpha]_D$ −163.2° (MeOH), which was identified as (−)-



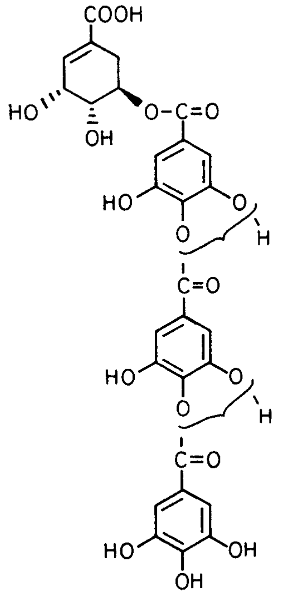
I



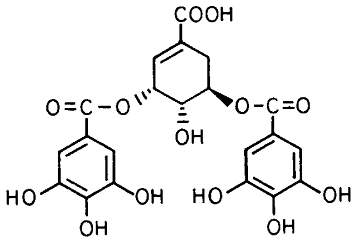
Ib



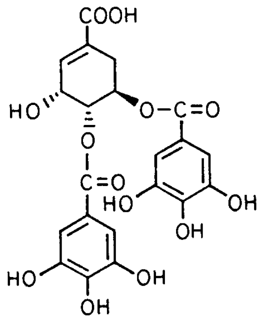
II



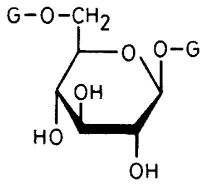
III



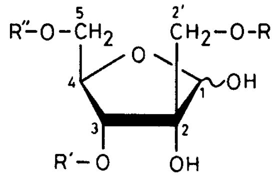
IV



V

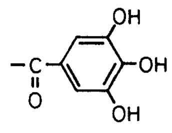


VI



VII : R=R'=H R''=G
VIII : R'=H R=R''=G
IX : R=R'=R''=G

G :



shikimic acid by direct comparison of the physical and spectral data with those of an authentic sample.[11] Examination of the ^{1}H-NMR spectrum of I suggested that the galloyl group is located at the C_3-position of the shikimic acid moiety; of three signals attributed to methine protons, one was shifted considerably downfield (δ 5.38, m). The assignment of this signal to C_3-H followed from the fact that the upper-field methine signals appearing at δ 4.07 as a double doublet (J=4, 7 Hz) and at δ 4.56 as a triplet (J=4 Hz) were assignable to C_4-H and C_5-H, respectively. The location of the galloyl group at the C_3-position of the shikimic acid moiety was supported by the facile formation of the tetramethyl 4,5-isopropylidene derivative

TABLE I. ^{1}H-NMR Spectral Data for Compounds I—V (in Acetone-d_6)

Compound	H-2	H-3	H-4	H-5	H-6	Galloyl-H
I	2.42 (dd, $J=6$, 18 Hz) 2.88 (dd, $J=6$, 18 Hz)	5.38 (m)	4.07 (dd, $J=4$, 7 Hz)	4.56 (t, $J=4$ Hz)	6.94 (d, $J=4$ Hz)	7.12 (2H, s)
II	2.45 (dd, $J=6$, 18 Hz) 2.91 (dd, $J=6$, 18 Hz)	5.41 (m)	4.07 (dd, $J=4$, 7 Hz)	4.55 (t, $J=4$ Hz)	6.90 (d, $J=4$ Hz)	7.19 (1/2H, s), 7.27 (2H, s), 7.38, 7.46 (each 3/4H, d, $J=2$ Hz)
III	2.45 (dd, $J=6$, 18 Hz) 2.91 (dd, $J=6$, 18 Hz)	5.42 (m)	4.06 (dd, $J=4$, 7 Hz)	4.54 (t, $J=4$ Hz)	6.89 (d, $J=4$ Hz)	7.17 (2/3H, s), 7.29 (2H, s), 7.33 (2/3H, s), 7.41, 7.46, 7.52, 7.59 (each 2/3H, d, $J=2$ Hz)
IV	2.55 (dd, $J=6$, 18 Hz) 3.04 (dd, $J=6$, 18 Hz)	5.49 (m)	4.42 (dd, $J=4$, 7 Hz)	5.86 (t, $J=4$ Hz)	6.94 (d, $J=4$ Hz)	7.17, 7.22 (each 2H, s)
V	2.56 (dd, $J=6$, 18 Hz) 3.01 (dd, $J=6$, 18 Hz)	5.63 (m)	5.42 (dd, $J=4$, 7 Hz)	4.83 (t, $J=4$ Hz)	7.00 (d, $J=4$ Hz)	7.12 (4H, s)
Ia[a)]	2.22 (dd, $J=6$, 18 Hz) 2.76 (dd, $J=6$, 18 Hz)	4.05 (m)	3.76 (dd, $J=4$, 7 Hz)	4.49 (t, $J=4$ Hz)	6.84 (d, $J=4$ Hz)	

a) Measured in D$_2$O.

TABLE II. ^{13}C-NMR Spectral Data for Compounds I—V: (−)-Shikimic Acid Moiety (in Acetone-d_6 + D$_2$O)

Compound	C-1	C-2	C-3	C-4	C-5	C-6	COOH
I	129.3	28.1	71.2	68.8	66.8	138.9	168.9
II	129.3	28.3	71.4	69.2	66.9	139.1	168.0
III	129.4	28.3	71.4	69.2	66.9	—[a)]	168.1
IV	131.8	28.4	71.1	67.2	70.0	134.7	168.2
V	129.7	29.5	68.2	71.8	65.4	138.5	167.7
Ia[b)]	132.3	32.9	68.4	73.7	69.1	139.9	172.7

a) Overlapped with galloyl C-4 signals. *b*) Measured in D$_2$O.

(Ib) upon treatment with dimethyl sulfate and potassium carbonate in dry acetone. On the basis of these chemical and spectroscopic data, I was concluded to be 3-*O*-galloyl-(−)-shikimic acid.

Compounds II, an off-white amorphous powder, $[\alpha]_D$ −72.8° (acetone), and III, an off-white amorphous powder, $[\alpha]_D$ −47.7° (acetone), were strongly positive to the ferric chloride reagent. Upon tannase hydrolysis, II and III yielded (−)-shikimic acid and gallic acid in molar ratios of 1 : 2 and 1 : 3, respectively. In the ^{1}H-NMR spectra of II and III, the chemical shifts of the three methine protons of the shikimic acid ring were virtually identical to those of compound I (Table I), thus indicating the presence of the gallic acid ester at the C$_3$-position. This conclusion was further confirmed by conversion of these compounds to I on methanolysis with methanolic acetate buffer (pH 5.4).[2a,2b,12)] The fast atom bombardment mass spectra (FAB-MS) of II and III showed $(M+H)^+$ ion peaks at m/z 479 and 631, respectively. These findings combined with the elemental analysis data indicated that II and III are a shikimic acid digallate and a trigallate, where the galloyl groups are linked through depside bonds.

Depsidically linked galloyl groups have been reported to occur in solution as an equilibrium mixture of *m*- and *p*-depside forms owing to the facile migration between the *m*- and *p*-hydroxyl group of proximal galloyl groups.[2a)] Inspection of the ^{13}C-NMR spectra revealed that these compounds actually exist, in each case, as a mixture of *m*- and *p*-isomers

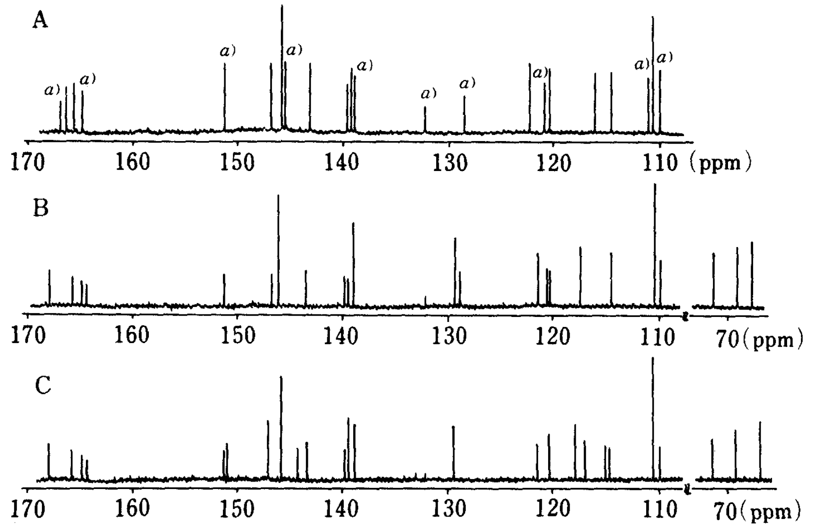


Fig. 1. ^{13}C-NMR Spectra of (A) Methyl Digallate, (B) Compound II and (C) Compound III

a) Signal arising from methyl *p*-digallate.

(Fig. 1). From these observations, II and III were characterized as 3-*O*-digalloyl-(−)-shikimic acid and 3-*O*-trigalloyl-(−)-shikimic acid, respectively.

Compounds IV, pale brown granules, mp 237—238 °C, $[\alpha]_D$ −168.3 ° (acetone), and V, pale brown granules, mp 268—270 °C, $[\alpha]_D$ −183.1 ° (acetone), afforded (−)-shikimic acid and gallic acid on tannase hydrolysis, and showed, in the FD-MS, the same $(M+H)^+$ ion peak at *m*/*z* 479, consistent with a digalloyl shikimic acid structure. The ^1H-NMR spectrum of IV exhibited two galloyl singlets at δ 7.22 (2H, s) and 7.17 (2H, s), and two lowfield methine signals at δ 5.86 (t, $J=4$ Hz) and 5.49 (m) assignable to C_5- and C_3-H, respectively, of the shikimic acid moiety. The signal due to C_4-H appeared at δ 4.42 as a double doublet ($J=4$, 7 Hz). Thus, compound IV was concluded to be 3,5-di-*O*-galloyl-(−)-shikimic acid. On the other hand, in the ^1H-NMR spectrum of V, signals attributed to C_3-H and C_4-H were shifted downfield (δ 5.63, m, and 5.42, dd, $J=4$, 7 Hz, respectively), indicating V to be 3,4-di-*O*-galloyl-(−)-shikimic acid.

The leaves of *Castanopsis cuspidata* var. *siebolidii* contain other gallotannins based on a variety of polyol cores, whose isolation and structure elucidation will be reported elsewhere.

Experimental

All 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. Infrared (IR) spectra were recorded on a JASCO DS-301 spectrometer. EI-, FD- and FAB-MS were taken with JEOL JMS D-300 and DX-300 instruments equipped with a direct inlet system. ^1H- and ^{13}C-NMR spectra were recorded on JEOL PS-100 and JEOL FX-100 spectrometers, respectively. Chemical shifts are reported in δ (ppm) relative to tetramethylsilane (TMS) as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; dd, double doublet. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), MCI-gel CHP-20P (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 thick, Merck) with benzene–ethyl formate–formic acid (1 : 7 : 1) and precoated cellulose F_{254} plates (0.10 mm thick, Merck) with 2% acetic acid, and spots were detected by spraying 2% ethanolic ferric chloride or by spraying 10% sulfuric acid followed by heating.

Isolation of Compounds I—IX——The fresh leaves (3.1 kg) of *Castanopsis cuspidata* var. *sieboldii*, collected at Mt. Seburi, Fukuoka Prefecture, were extracted three times at room temperature with 80% aqueous acetone (20 l each). The extract was concentrated under reduced pressure, and the resulting precipitates consisting mainly of chlorophylls and waxes were removed by filtration. The filtrate was subjected to Sephadex LH-20 chromatography. Elution with H_2O containing increasing amounts of MeOH furnished six fractions; frs. 1 (22 g), 2 (73.2 g), 3 (41 g), 4 (19.6 g), 5 (15 g) and 6 (4.5 g). Fr. 1 was rechromatographed over Sephadex LH-20 with EtOH to yield 5-*O*-galloyl-D-hamamelose (VII)[3c] (329 mg). Fr. 2 was further purified by chromatography over MCI-gel CHP-20P with H_2O–MeOH (7 : 3), and the product was crystallized from H_2O to give compound I (52 g). Rechromatography of fr. 3 over MCI-gel CHP-20P with H_2O–MeOH (7 : 3) and subsequent purification by Sephadex LH-20 chromatography with EtOH yielded 1,6-di-*O*-galloyl-β-D-glucopyranoside (VI)[2c] (215 mg) and 2′,5-di-*O*-galloyl-D-hamamelose (VIII)[3] (203 mg). Fr. 5 containing digallates was separated by repeated chromatography over Sephadex LH-20 with H_2O–MeOH (4 : 1), MCI-gel CHP-20P with H_2O–MeOH (7 : 3) and cellulose with 2% acetic acid to afford compounds II (104 mg), IV (658 mg) and V (2.6 g). Repeated chromatography of fr. 6 over Sephadex LH-20 with a variety of solvent system, H_2O-MeOH (4 : 1), (3 : 2) and EtOH, gave 2′,3,5-tri-*O*-galloyl-D-hamamelose (IX)[3c] (150 mg) and compound III (118 mg).

(–)-Shikimic Acid 3-*O*-Gallate (I)——A white powder (H_2O), mp 255 °C (dec.), $[\alpha]_D^{24}$ $-111.7°$ ($c=0.97$, acetone). *Anal.* Calcd for $C_{14}H_{14}O_9 \cdot 3/2H_2O$: C, 47.59; H, 4.85. Found: C, 47.76; H, 5.01. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1695 (C=C–COOH, Ar-COO–). FD-MS *m/z*: 327 $(M+H)^+$.

Hydrolysis of I with Tannase——An aqueous solution if I (100 mg) was incubated with tannase at 37 °C for 3 h. The reaction mixture was concentrated to dryness under reduced pressure, and the residue was treated with EtOH. The EtOH-soluble portion was chromatographed over Sephadex LH-20. Elution with EtOH yielded gallic acid (40 mg) and crude Ia, of which the latter was purified by chromatography over cellulose with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2→7 : 3 : 0.3) to furnish Ia as colorless needles (43 mg), mp 186—187 °C, $[\alpha]_D^{24}$ $-163.2°$ ($c=0.96$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3480, 3380 (OH), 1675, 1640 (C=C–COOH); this product was identified as (–)-shikimic acid by direct comparison with an authentic sample.[11]

Methylation of I——A mixture of I (8.0 g), dimethyl sulfate (25 ml) and anhydrous potassium carbonate (40 g) in dry acetone (250 ml) was refluxed for 2.5 h with stirring. The inorganic salts were removed by filtration, and the filtrate was concentrated to dryness under reduced pressure, and the residue was subjected to silica gel chromatography. Elution with benzene–acetone (4 : 1) gave Ib as a white amorphous powder (6.5 g), $[\alpha]_D^{24}$ $-75.2°$ ($c=1.1$, $CHCl_3$). *Anal.* Calcd for $C_{21}H_{26}O_9$: C, 59.71; H, 6.20. Found C, 59.68; H, 6.15. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (–COO–). ^1H-NMR ($CDCl_3$): 1.42, 1.46 (each 3H, s, isopropylidene Me), 2.50 (1H, dd, $J=6$, 18 Hz, C_2-H), 2.92 (1H, dd, $J=5$, 18 Hz, C_2-H), 3.78 (3H, s, –COOMe), 3.89, 3.91 (9H in total, each s, aromatic OMe), 4.39 (1H, t, $J=6$ Hz, C_4-H), 4.82 (1H, dd, $J=4$, 6 Hz, C_5-H), 5.42 (1H, m, C_3-H), 6.96 (1H, d-like, $J=4$ Hz, C_6-H), 7.25 (2H, s, galloyl H).

(–)-Shikimic Acid 3-*O*-Digallate (II)——An off-white amorphous powder, $[\alpha]_D^{23}$ $-72.8°$ ($c=1.1$, acetone). *Anal.* Calcd for $C_{21}H_{18}O_{13} \cdot 5/2H_2O$: C, 48.19; H, 4.43. Found: C, 48.17; H, 4.44. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1700 (–C=C–COOH, Ar-COO–). FAB-MS *m/z*: 479 $(M+H)^+$.

Methanolysis of II with Methanolic Acetate Buffer——A solution of II (60 mg) in a mixture of MeOH (6 ml) and 0.1 M acetate buffer (6 ml, pH 5.4) was refluxed for 1.5 h. The solution was concentrated to dryness under reduced pressure, and the residue was applied to a Sephadex LH-20 column. Elution with EtOH yielded gallic acid (24 mg) and shikimic acid 3-*O*-gallate (I) (20 mg).

(–)-Shikimic Acid 3-*O*-Trigallate (III)——An off-white amorphous powder, $[\alpha]_D^{23}$ $-47.7°$ ($c=1.1$, acetone). *Anal.* Calcd for $C_{28}H_{22}O_{17} \cdot 3H_2O$: C, 49.13; H, 4.12. Found: C, 48.71; H, 4.19. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1700 (–C=C–COOH, Ar-COO–). FAB-MS *m/z*: 631 $(M+H)^+$.

Methanolysis of III with Methanolic Acetate Buffer——A solution of II (30 mg) in a mixture of MeOH (3 ml) and 0.1 M acetate buffer (3 ml, pH 5.4) was heated under reflux for 1.5 h. The reaction mixture was worked up as described above to yield gallic acid (18 mg) and compound I (9 mg).

(–)-Shikimic Acid 3,5-Di-*O*-Gallate (IV)——Pale brown granules (H_2O), mp 237—238 °C, $[\alpha]_D^{24}$ $-168.3°$ ($c=1.0$, acetone). *Anal.* Calcd for $C_{21}H_{18}O_{13} \cdot 5/2H_2O$: C, 48.19; H, 4.43. Found: C, 48.39; H, 4.53. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1700 (–C=C–COOH, Ar-COO–). FD-MS *m/z*: 479 $(M+H)^+$.

Hydrolysis of IV with Tannase——An aqueous solution (2 ml) of IV (120 mg) was shaken with tannase at 37 °C for 3 h. Work-up as described before yielded gallic acid (78 mg) and (–)-shikimic acid (37 mg).

(–)-Shikimic Acid 3,4-Di-*O*-Gallate (V)——Pale brown granules (H_2O), mp 268—270 °C, $[\alpha]_D^{24}$ $-183.1°$ ($c=1.0$, acetone). *Anal.* Calcd for $C_{21}H_{18}O_{13} \cdot 3H_2O$: C, 47.37; H, 4.54. Found: C, 47.37; H, 4.42. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1700 (–C=C–COOH, Ar-COO–). FD-MS *m/z*: 479 $(M+H)^+$.

Hydrolysis of V with Tannase——An aqueous solution (1 ml) of V (40 mg) was incubated with tannase at 37 °C for 1 h. Work-up as described above yielded gallic acid (28 mg) and (–)-shikimic acid (11 mg).

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. K. Isobe for ^1H-NMR, ^{13}C-NMR, EI-MS, FD-MS and FAB-MS measurements, and to the staff of the Central Analysis Room of this university for elemental analyses.

References and Notes

1) Part XXIV: H. Nishimura, G. Nonaka, and I. Nishioka, *Phytochemistry*, **23**, 2621 (1984).

2) *a*) M. Nishizawa, T. Yamagishi, G. Nonaka, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2963; *b*) *Idem, ibid.*, **1983**, 961; *c*) G. Nonaka and I. Nishioka, *Chem. Pharm. Bull.*, **31**, 1652 (1983); *d*) G. Britton, P. W. Crabtree, E. Haslam, and J. E. Stangroom, *J. Chem. Soc.* (*C*), **1966**, 783; *e*) E. Haslam, *ibid.*, **1967**, 1734; *f*) E. A. Haddock, R. K. Gupta, S. M. K. Al-Shafi, and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2515.

3) *a*) W. Mayer, W. Kunz, and F. Loebich, *Justus Liebigs Ann. Chem.*, **688**, 232 (1965); *b*) O. T. Schmidt and K. Heintz, *ibid.*, **515**, 77 (1935); *c*) G. Nonaka, K. Ishimaru, T. Tanaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 483 (1984).

4) K. Bock, N. F. LaCour, S. R. Jensen, and B. J. Nielsen, *Phytochemistry*, **19**, 2033 (1980).

5) H. Nishimura, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 1741 (1984).

6) E. Haslam, R. D. Haworth, and P. C. Keen, *J. Chem. Soc.* (*C*), **1962**, 3814.

7) T. Tanaka, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 117 (1984).

8) G. Nonaka, H. Nishimura, and I. Nishioka, *Chem. Pharm. Bull.*, **30**, 2061 (1982); H. Nishimura, G. Nonaka, and I. Nishioka, *ibid.*, **32**, 1735 (1984).

9) T. Tanaka, T. Sueyasu, G. Nonaka, and I. Nishioka, *Chem. Pharm. Bull.*, **32**, 2676 (1984).

10) G. Nonaka, I. Nishioka, T. Nagasawa, and H. Oura, *Chem. Pharm. Bull.*, **29**, 2862 (1981); G. Nonaka and I. Nishioka, *ibid.*, **30**, 4268 (1982); G. Nonaka, S. Morimoto, and I. Nishioka, *J. Chem. Soc., Perkin Trans. 1*, **1983**, 2139.

11) A commercial sample of (−)-shikimic acid was used for the identification.

12) R. Armitage, E. Haslam, R. D. Haworth, and T. Searle, *J. Chem. Soc.* (*C*), **1962**, 3808.