Tannins and Related Compounds. XXI.[1]   Isolation and Characterization of Galloyl and p-Hydroxybenzoyl Esters of Benzophenone and Xanthone C-Glucosides from Mangifera indica L.

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Six new galloyl and p-hydroxybenzoyl esters (2—7) of benzophenone C-glucosides have been isolated, together with a new benzophenone C-glucoside (1), from the leaves of Mangifera indica L. (Anacardiaceae). On the basis of chemical and spectroscopic evidence, the structures of these compounds have been established as maclurin 3-C-β-D-glucoside (1), maclurin 3-C-(6''-O-p-hydroxybenzoyl)-β-D-glucoside (2), maclurin 3-C-(2''-O-galloyl-6''-O-p-hydroxybenzoyl)-β-D-glucoside (3), maclurin 3-C-(2''-O-p-hydroxybenzoyl-6''-O-galloyl)-β-D-glucoside (4), maclurin 3-C-(2'',3'',6''-tri-O-galloyl)-β-D-glucoside (5), iriflophenone 3-C-(2'',6''-di-O-galloyl)-β-D-glucoside (6) and iriflophenone 3-C-(2'',3'',6''-tri-O-galloyl)-β-D-glucoside (7). (−)-Epicatechin 3-O-gallate (9), mangiferin (10), isomangiferin (11) and a new xanthone C-glucoside gallate, mangiferin 6'-O-gallate (8), have also been isolated and their structures have been similarly characterized. Furthermore, the above plant source contained polygalloylglucoses which were characterized on the basis of chemical and high performance liquid chromatographic analyses as a mixture of penta- to undecagalloylglucoses based on a 1,2,3,4,6-penta-O-galloyl-β-D-glucose core.
    Maclurin 3-C-glucoside (1) has been transformed enzymatically to mangiferin (10) and isomangiferin (11), and it has become clear that 1 is a key intermediate in the biosynthesis of 10 and 11.

Keywords——Mangifera indica; Anacardiaceae; galloyl maclurin 3-C-glucoside p-hydroxy-benzoate; galloyl iriflophenone 3-C-glucoside; galloyl mangiferin; benzophenone C-glucoside; biosynthesis; mangiferin; isomangiferin; galloylglucose

Mangiferin, a well-known xanthone C-glucoside, was first obtained more than 70 years ago from Mangifera indica L. (Anacardiaceae),[2] and its structure was established conclusively in the 1960's as 2-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone by several research groups.[3] Its structural isomer, isomangiferin, was isolated from Anemarrhena asphodeloides BUNGE (Liliaceae) in 1970 by Kawasaki et al., and the structure was characterized as 4-C-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone.[4] It has now been demonstrated that these two isomers co-occur in various members of the families Fabaceae,[5] Orchidaceae,[6] Aspleniaceae[7] and Iridaceae,[8] as well as in the above plant sources.[9] The coexistence of mangiferin and isomangiferin, whose structural relation is analogous to that between the C-glycosyl flavonoids, orientin and isoorientin, or vitexin and isovitexin, where C-glycosilation was shown to occur biosynthetically at the chalcone stage,[10] suggests that in the biosynthesis of the xanthone C-glucosides the C-glycosylation occurs at the stage of a benzophenone structure prior to the intramolecular ring closure leading to the formation of the xanthone nucleus. Inoue et al. recently studied the formation of mangiferin in A. asphodeloides by means of feeding experiments, and they proposed a biosynthetic route for mangiferin and related xanthone C-glucosides (Chart 1).[11] In their route, maclurin 3-C-glucoside (1) is postulated to be an intermediate, but this compound has not been isolated from natural sources so far.
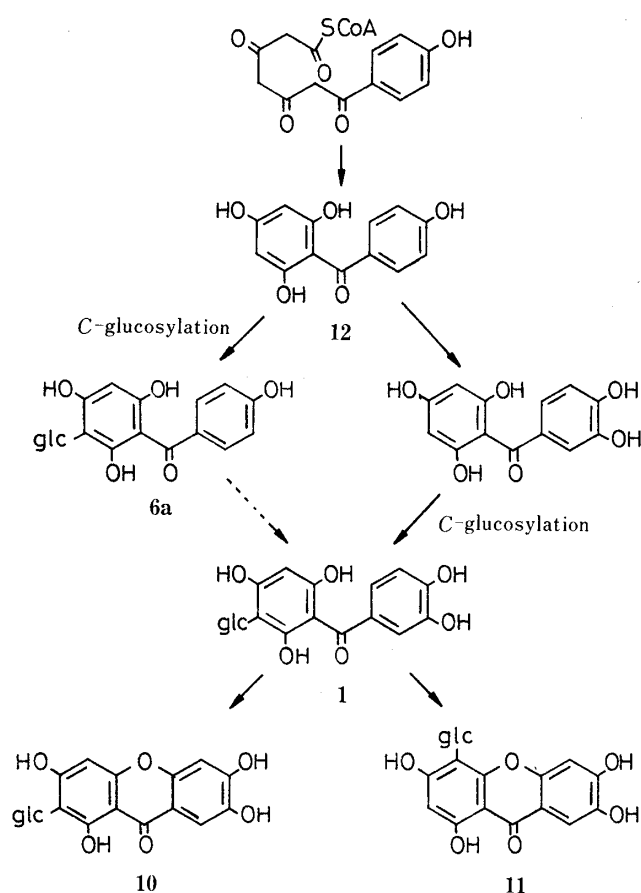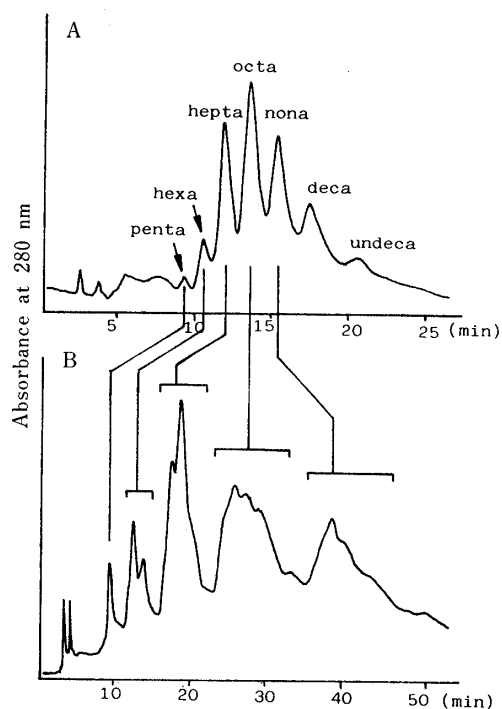
Chart 1

Fig. 1. High-Performance Liquid Chromato-grams of Galloylglucoses in Leaves of *M. indica*

A (normal-phase), column, Zorbax SIL (4.6 mm i.d. × 250 mm); solvent, *n*-hexane–methanol–tetra-hydrofuran–formic acid (50 : 30 : 10 : 1) (oxalic acid 150 mg/l); flow rate, 1 ml/min.

B (reverse-phase), column, TSK gel-ODS 120A (4.0 mm i.d. × 300 mm); solvent, acetonitrile–water (22 : 78) (50 mM NaH_2PO_4); flow rate, 0.75 ml/min.

In the course of chemical studies on tannins and related compounds, we have encountered in the leaves of *M. indica* a large accumulation of maclurin 3-*C*-glucoside (**1**) and related compounds (**2**—**8**), together with (−)-epicatechin 3-*O*-gallate (**9**) and polygalloyl-glucoses. This paper deals with the isolation and structure elucidation of these compounds and also describes the enzymatic transformation of **1** to mangiferin (**10**) and isomangiferin (**11**).

The aqueous acetone extract of the fresh leaves of *M. indica* was partitioned between ethyl acetate and water. From the water-soluble portion, compounds **1**, **10** and **11** were isolated by MCI-gel chromatography. The ethyl acetate-soluble portion was repeatedly chromatographed as described in the experimental section to afford compounds **2**—**8**, along with compound **9** and polygalloylglucoses. Compounds **9**, **10** and **11** were identified by direct comparisons with authentic samples as (−)-epicatechin 3-*O*-gallate,[12] mangiferin and isomangiferin, respectively. The polygalloylglucose fraction was characterized on the basis of hydrolytic studies and analyses by means of normal- and reverse-phase high-performance liquid chromatography (HPLC) (Fig. 1) as a mixture of penta- to undecagalloylglucoses which have a 1,2,3,4,6-penta-*O*-galloyl-*β*-D-glucose core.[13]

Compound **1**, a yellow amorphous powder, $[\alpha]_D$ +33.2° (MeOH), $C_{19}H_{20}O_{11}\cdot H_2O$, whose constitution was confirmed by the fast atom bombardment mass spectrum (FAB-MS: m/z 425 [M+H]$^+$), gave positive color tests with the ferric chloride (a dark blue) and Gibbs reagents.[14] It showed ultraviolet (UV) maxima at 296 and 326 nm (log ε: 3.93 and 4.03, respectively) and infrared (IR) bands at 3400, 1628 and 1605 cm^{-1}. The ^{13}C-nuclear magnetic

fresh leaves of *Mangifera indica* L. (9 kg)

    90% aq. acetone
    evaporated *in vacuo*

H_2O solution

    filtered

residue
(chlorophyll *etc.*)   filtrate

            $EtOAc-H_2O$

EtOAc layer   aq. layer

            filtered

    yellow crystals   filtrate

            MCI gel
            $(H_2O-MeOH)$

    compd. **10**
    (17 g)

           compd. **1**   compd. **11**
           (2 g)   (40 mg)

Sephadex LH-20
$(EtOH-H_2O-acetone)$

fr. 1   fr. 2   fr. 3   fr. 4

1) MCI gel
  (10—50% MeOH)

2) Sephadex LH-20
  (80% MeOH, EtOH)

compds. **2** (35 mg), **3** (33 mg),
  4 (670 mg), **6** (1.4 g), **8** (15 mg),
  9 (80 mg)

1) Sephadex LH-20
  (80% MeOH)

2) MCI gel
  (20—60% MeOH)

compds. **5** (300 mg),
  7 (97 mg)

galloylglucoses

Chart 2

9   10   11

Chart 3

resonance (^{13}C-NMR) spectrum exhibited six aliphatic carbon signals [δ 60.1 (t, C_6), 69.3 (d, C_2), 71.9 (d, C_4), 74.6 (d, C_1), 77.9 (d, C_3), 80.7 (d, C_5)][15] due to a sugar moiety. The appearance of the anomeric carbon signal at higher field suggested that **1** possessed a *C*-glycosidic nature, and the sugar was confirmed to be glucose by ferric chloride degradation.[16]

The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** exhibited ABX-type aromatic signals [δ 6.74 (d, $J=8$ Hz), 7.07 (dd, $J=8$, 2 Hz), 7.15 (d, $J=2$ Hz)] and an aromatic singlet signal (δ 5.96). These observations coupled with the ^{13}C-NMR spectral data (Table I) suggested that **1** contained two aromatic rings, one with a catechol- and the other with a phloroglucinol-type substitution system. On treatment with 2 N hydrochloric acid, **1** gave protocatechuic acid as a sole isolable product, and it should be noted that glucose was readily liberated during this reaction in spite of its *C*-glycosidic nature. The ^{13}C-NMR spectrum of **1** showed a signal at δ 194.8, characteristic of the carbonyl group of a benzophenone derivative.

TABLE I. ^{13}C-NMR Spectral Data for Compounds 1—7 and 6a (δ Values)

	$1^{a)}$	$2^{b)}$	$3^{b)}$	$4^{b)}$	$5^{b)}$	$6^{b)}$	$6a^{b)}$	$7^{b)}$
Aglycone								
1	103.3	104.0	102.6	102.4	102.1	102.2	104.1	102.1
2	156.1[c)	160.3[d)	160.6[e)	160.9[f)	160.9	160.5[g)	160.2[h)	161.1[i)
3	107.5	106.4	105.6	106.3	106.5	106.5	106.7	106.2
4	158.2[c)	162.1[d)	162.8[e)	162.5[f)	160.9	161.9[g)	161.8[h)	161.8[i)
5	94.7	96.7	96.2	96.3	96.2	95.9	96.4	96.2
6	156.4[c)	160.8[d)	161.4[e)	160.9[f)	160.9	160.5[g)	160.2[h)	161.1[i)
1'	130.9	133.3	133.4	133.3	133.3	132.4	132.5	132.9
2'	116.0	117.1	117.1	117.0	116.8	132.4	132.5	132.4
3'	144.4	144.9	144.9	144.9	145.0	115.2	115.2	115.2
4'	149.8	150.2	150.1	150.1	150.2	161.9[g)	162.1[h)	161.8[i)
5'	114.7	115.1	115.1	115.0	115.1	115.2	115.2	115.2
6'	122.5	123.6	123.7	123.6	123.8	132.4	132.5	132.4
–CO–	194.8	197.7	197.7	197.8	197.6	197.6	197.6	197.8
Glucose								
1	74.6	76.5	74.4	74.4	74.2	74.0	76.0	74.2
2	69.3	70.7	71.2	71.3	69.6	71.2	70.4	69.7
3	77.9	78.8	76.9	76.9	77.6	76.7	78.9	77.6
4	71.9	74.0	73.8	73.9	71.4	73.6	73.5	71.4
5	80.7	79.3	79.7	79.8	79.9	79.6	81.6	79.9
6	60.1	63.9	64.0	64.2	64.2	64.4	61.5	64.2
Galloyl								
1			121.6	121.3	121.3	121.2		121.3
2			110.2	109.9	110.0	109.9		110.0
3			145.7	146.0	146.0	145.9		145.6
4			138.7	138.9	138.8	138.7		138.8
p-OH benzoyl								
1		121.7	122.0	122.0				
2		132.4	132.5	132.6				
3		116.1	116.1	115.7				
4		162.7[d)	162.8[e)	162.5[f)				
–COO–		166.7	166.1	166.0	166.0	166.4		165.6
			166.6	166.8	166.5	167.3		164.4
					166.9			166.8

a) Measured in DMSO-d_6.   b) Measured in acetone-d_6.
Assignments with the superscripts c—i) may be interchanged in each column.
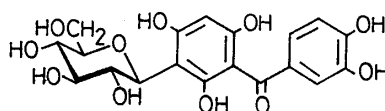
1

Chart 4

On the basis of the chemical and spectroscopic evidence described above, the structure of 1 was presumed to be maclurin 3-C-β-D-glucoside (1), which had not previously been found in nature. This was confirmed by successful conversion of 1 to mangiferin (10) and isomangiferin (11) by treatment with potassium ferricyanide in alkaline solution.[17)

Compound 2, a yellow amorphous powder, $[\alpha]_D$ −50.0 ° (MeOH), $C_{26}H_{24}O_{13} \cdot H_2O$, gave a ^{1}H-NMR spectrum similar to that of 1 except for additional A_2B_2-type aromatic signals [δ 6.88 (2H, d, $J = 9$ Hz), 7.86 (2H, d, $J = 9$ Hz)]. The ^{13}C-NMR spectrum revealed the presence
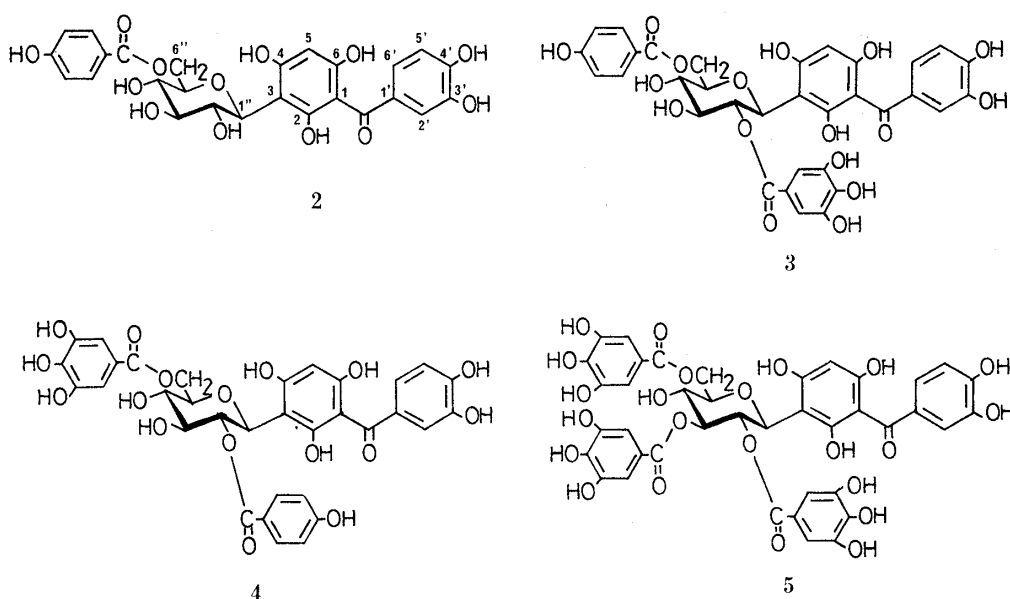
Chart 5

of an ester carbonyl group (δ 166.7) and a p-substituted benzene ring [δ 116.1 (2C), 121.7, 132.4 (2C), 162.7]. On enzymatic hydrolysis with crude hesperidinase,[18] 2 gave p-hydroxybenzoic acid and compound 1. The p-hydroxybenzoyl group in 2 was considered to be at the C_6-position in the sugar moiety on the basis of ^1H- and ^{13}C-NMR analyses, which showed downfield shifts of the glucose C_6-methylene signals [δ 4.50 (dd, $J=13$, 4 Hz), 4.70 (dd, $J=13$, 2 Hz); δ 63.9] as compared with those of 1. Consequently, compound 2 was characterized as maclurin 3-C-(6''-O-p-hydroxybenzoyl)-β-D-glucoside (2).

Compound 3, a yellow amorphous powder, [α]$_D$ $-181.3°$ (MeOH), $C_{33}H_{28}O_{17} \cdot 2H_2O$, showed signals due to a galloyl group [δ 7.07 (2H, s)] and a p-hydroxybenzoyl group [δ 6.88 (2H, d, $J=9$ Hz), 7.88 (2H, d, $J=9$ Hz)] in the ^1H-NMR spectrum. Enzymatic hydrolysis of 3 with tannase afforded gallic acid and a hydrolysate, the latter being shown to be identical with compound 2 by comparison of the spectral data. In the ^1H-NMR spectrum of 3, a signal which was coupled with the anomeric proton signal and was hence assignable to the sugar C_2-proton was shifted downfield [δ 5.54 (t, $J=10$ Hz)] as compared with that of 2, indicating that the additional galloyl group was attached to this position. On the basis of these results, the structure of compound 3 was established as maclurin 3-C-(2''-O-galloyl-6''-O-p-hydroxybenzoyl)-β-D-glucoside (3).

Compound 4, pale yellow needles (H_2O), mp 193—194 °C, [α]$_D$ $-143.8°$ (MeOH), $C_{33}H_{28}O_{17} \cdot 2H_2O$, gave a greenish-blue color similar to that of 3 with ferric chloride reagent. The occurrence of galloyl and p-hydroxybenzoyl groups was easily deduced from the ^1H- and ^{13}C-NMR spectra, which were closely related to those of 3. Enzymatic hydrolysis of 4 with crude hesperidinase[18] gave gallic acid, p-hydroxybenzoic acid and compound 1. In the ^1H-NMR spectrum of 4, signals due to the sugar C_2- and C_6-protons [δ 5.54 (t, $J=10$ Hz) and δ 4.64 (2H, br s), respectively] were observed at lower field than in 1, indicating that these positions were acylated.

Partial hydrolysis of 4 with tannase afforded gallic acid and a hydrolysate (4a), a yellow amorphous powder, [α]$_D$ $-140.6°$ (MeOH), $C_{26}H_{24}O_{13} \cdot H_2O$. In the ^1H-NMR spectrum of 4a, a singlet signal due to the galloyl group had disappeared and the sugar C_6-proton signal was shifted to higher field as compared with that of 4, whereas the signals due to the benzoyl group were seen and there was a downfield shift of the sugar C_2-proton signal. These results indicated that the benzoyl and galloyl groups were attached to the C_2''- and C_6''-positions,

respectively. Therefore, compound **4** was characterized as maclurin 3-*C*-(2''-*O*-*p*-hydroxybenzoyl-6''-*O*-galloyl)-β-D-glucoside (**4**).

Compound **5**, a yellow amorphous powder, $[\alpha]_D$ $-48.8\,°$ (MeOH), $C_{40}H_{32}O_{23} \cdot H_2O$, was strongly positive (a dark blue color) to the ferric chloride reagent. The ^{1}H-NMR spectrum showed the occurrence of three galloyl groups in the molecule [δ 6.96, 7.05, 7.16 (each 2H, s)]. Treatment of **5** with tannase afforded gallic acid and compound **1**. The galloyl groups were determined to be at the C_2-, C_3- and C_6-positions in the glucosyl moiety by ^{1}H- and ^{13}C-NMR analyses; the chemical shifts of the $C_{2''}$- [δ 5.62 (t, $J = 8$ Hz)] and $C_{6''}$-protons [δ 4.68 (2H, br s)] were similar to those observed in **3** and **4**, suggesting that these positions were acylated. The $C_{3''}$-carbon signal appeared at 0.7 ppm downfield as compared with those in **3** and **4**, while the neighboring $C_{2''}$ and $C_{4''}$ signals were shifted upfield (-1.7 ppm and -2.5 ppm, respectively), suggesting that the additional galloyl group was attached to the $C_{3''}$-position. Thus, the structure of **5** was confirmed to be maclurin 3-*C*-(2'',3'',6''-tri-*O*-galloyl)-β-D-glucoside (**5**).

Compound **6**, a yellow amorphous powder, $[\alpha]_D$ $-135.0\,°$ (MeOH), $C_{33}H_{28}O_{18} \cdot 2H_2O$, gave a ^{1}H-NMR spectrum which showed two two-proton singlet signals (δ 7.05, 7.13) ascribable to galloyl groups. Treatment of **6** with tannase afforded gallic acid and a hydrolysate (**6a**), a yellow amorphous powder, $[\alpha]_D$ $+45.8\,°$ (MeOH), $C_{19}H_{20}O_{10} \cdot 1/4H_2O$. Compound **6a** gave a reddish-brown color with ferric chloride reagent, and exhibited an $[M+H]^+$ peak at m/z 409 in the FAB-MS. The ^{1}H-NMR spectrum of **6a** showed A_2B_2-type aromatic signals [δ 6.84 (2H, d, $J = 9$ Hz), 7.64 (2H, d, $J = 9$ Hz)], suggesting the occurrence of a *p*-hydroxyphenyl moiety. An aromatic singlet signal (δ 6.02) and an anomeric proton signal [δ 4.94 (d, $J = 10$ Hz)] closely resembled those of **1**. Furthermore, in the ^{13}C-NMR spectrum (Table I), the chemical shifts of six aliphatic carbon signals due to a *C*-glucosyl moiety, aromatic signals arising from a phloroglucinol ring and a carbonyl signal were similar to those observed in **1**. These spectroscopic data indicated that **6a** was a 3-*C*-glucoside of 2,4,6,4'-tetrahydroxyl benzophenone, that is, iriflophenone (**12**), which had previously been isolated from *Iris florentina* L.[19] Since the ^{1}H- and ^{13}C-NMR spectra of **6** displayed sugar signals which were almost identical with those of **3** and **4**, it is apparent that the two galloyl groups were attached to the sugar C_2- and C_6-positions. Consequently, the structure of **6** was established as iriflophenone 3-*C*-(2'',6''-di-*O*-galloyl)-β-glucoside (**6**).
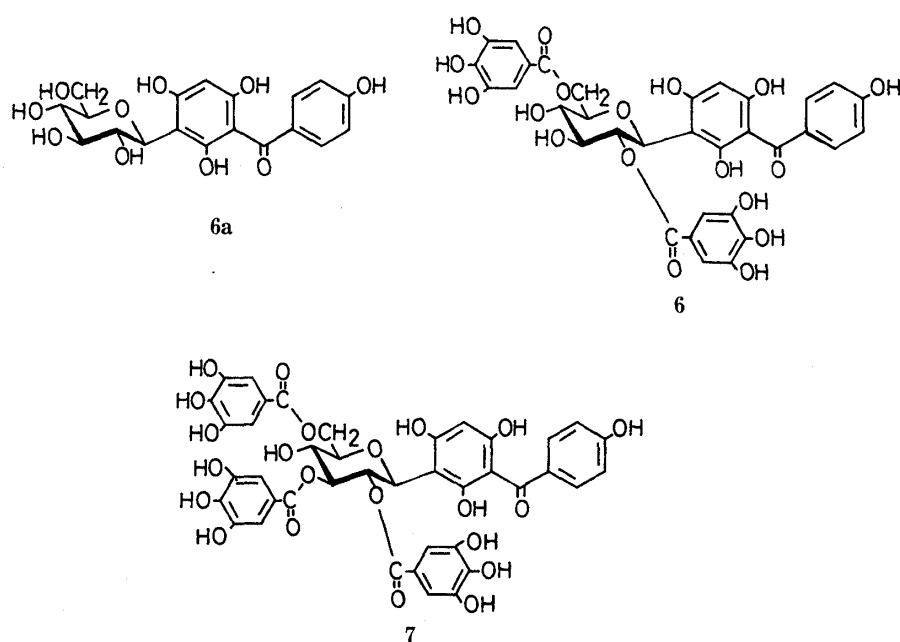

6a

6

7

Chart 6

Compound **7**, a yellow amorphous powder, $[\alpha]_D$ $-47.4°$ (MeOH), $C_{40}H_{32}O_{22} \cdot 1/2H_2O$, gave a ^{1}H-NMR spectrum almost identical with that of **5** except for A_2B_2-type aromatic proton signals [δ 6.81 (2H, d, $J=9$ Hz), 7.52 (2H, d, $J=9$ Hz)]. On enzymatic hydrolysis with tannase, **7** afforded gallic acid and **6a**. The galloyl groups were concluded to be at the $C_{2''}$-, $C_{3''}$- and $C_{6''}$-positions based on the observation of ^{1}H- and ^{13}C-NMR signals analogous to those observed in **5**. These results permitted the assignment of the structure **7** for this compound.

Compound **8**, a yellow granular solid, $[\alpha]_D$ $-35.2°$ (MeOH), $C_{26}H_{22}O_{15} \cdot 1/2H_2O$, was closely related to mangiferin (**10**), showing in the ^{1}H-NMR spectrum an anomeric proton signal [δ 5.01 (d, $J=10$ Hz)] and three aromatic singlet signals (δ 6.40, 6.91, 7.51). The appearance of a two-proton singlet at δ 7.13 indicated the presence of a galloyl group in **8**. On
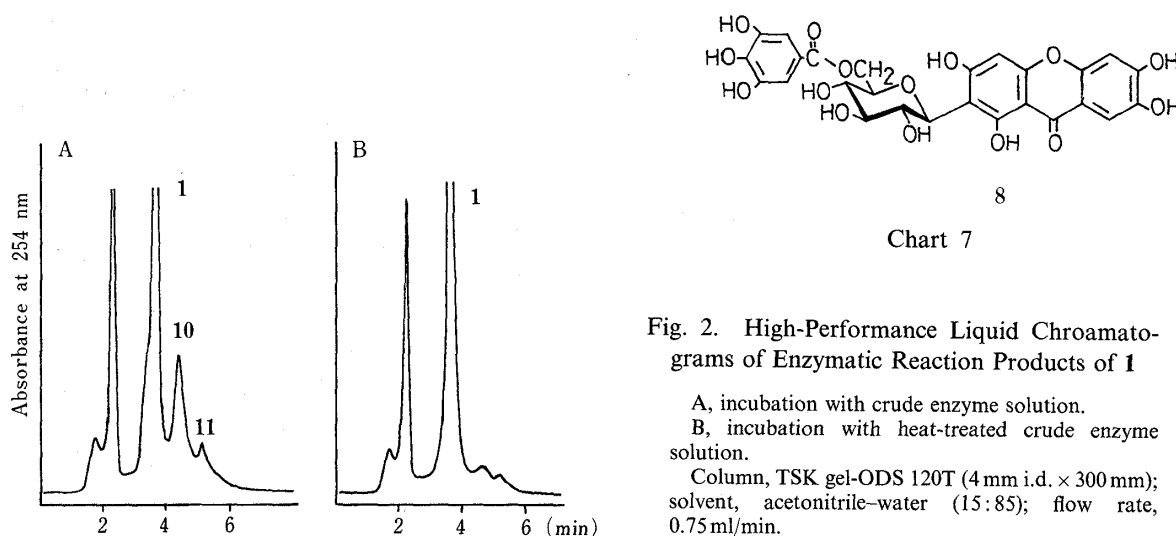
Chart 7

Fig. 2. High-Performance Liquid Chroamatograms of Enzymatic Reaction Products of **1**

A, incubation with crude enzyme solution.
B, incubation with heat-treated crude enzyme solution.
Column, TSK gel-ODS 120T (4 mm i.d. × 300 mm); solvent, acetonitrile–water (15:85); flow rate, 0.75 ml/min.

fresh leaves of *M. indica* (26 g)

1) Waring blender
   10 mM KH_2PO_4 (100 ml)
2) filtration
3) centrifugation
   (7000 rpm)

precipitates   supernatant

1) acetone
   (-20°C, 400 ml)
2) filtration

precipitates   filtrate
(67 mg)

5 mM phosphate buffer
(pH 7.0) (10 ml)

crude enzyme solution (10 ml)

(1 ml)   (1 ml)

compd. **1**   1) heated (100°C, 1 h)
(5 mg/0.5 ml
H_2O)   2) compd. **1**
                                   (5 mg/0.5 ml H_2O)

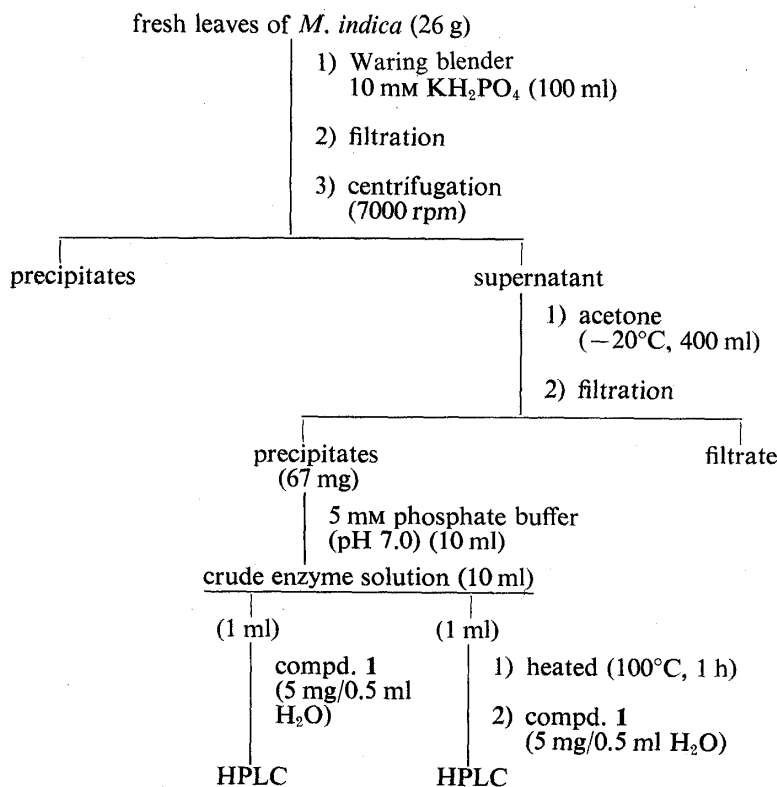HPLC   HPLC

Chart 8

enzymatic hydrolysis with tannase, **8** afforded gallic acid and **10**. The downfield shift of the sugar C_6-methylene proton signals [δ 4.56 (2H, br s)] in the ^{1}H-NMR spectrum of **8** demonstrated that the galloyl group was located at this position. Thus, the structure of compound **8** was established as mangiferin 6'-O-gallate (**8**).

In biosynthetic studies of the C-glycosylation of mangiferin (**10**), Inoue *et al.* fed radioactive 1,3,6,7-tetrahydroxyxanthone and maclurin to *Anemarrhena asphodeloides*, and found that maclurin was efficiently incorporated into mangiferin (**10**) and isomangiferin (**11**) without randomization, but 1,3,6,7-tetrahydroxyxanthone, the aglycone of **10** and **11**, was essentially not incorporated. They speculated, therefore, that **10** and **11** may be biosynthesized *via* maclurin 3-C-glucoside (**1**).[11b]

In order to confirm this speculation, an enzymatic transformation of maclurin 3-C-glucoside (**1**) to mangiferin (**10**) and isomangiferin (**11**) was attempted. Incubation of **1** with a crude enzyme preparation obtained from fresh leaves of *M. indica* as illustrated in Chart 8 gave rise to products which were shown to be identical with **10** and **11** by HPLC analysis (Fig. 2).

As described above, maclurin 3-C-glucoside (**1**) appears to be a key intermediate in the biosynthesis of mangiferin and isomangiferin, and the presence of **1** and its galloyl and *p*-hydroxybenzoyl esters in the leaves of *M. indica* is of interest from the viewpoint of plant physiology. Furthermore, the occurrence of iriflophenone 3-C-glucoside (**6a**) suggests that C-glycosylation may also take place at the iriflophenone stage, and it remains to be determined whether **6a** acts as an intermediate in the biosynthesis of **10** and **11**.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-4 digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL FX-100 spectrometer (consisting in part of a JEOL PS-100 spectrometer for ^{1}H-NMR) using tetramethylsilane as an internal standard, and chemical shifts are given on a δ (ppm) scale. FAB-MS was taken with a JEOL JMS DX-300 instrument with a FAB-06 ion source. IR and UV spectra were obtained with JASCO IR-G and Shimadzu MPS-2000 spectrometers, respectively. Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60 F_{254} plates (0.20 mm, Merck) with benzene–ethyl formate–formic acid (1:7:1) and precoated cellulose F_{254} plates (0.10 mm, Merck) with 2% acetic acid; spots were visualized by spraying the plates with 2% ethanolic ferric chloride, 5% H_2SO_4 and aniline–hydrogen phthalate reagents. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co., Ltd.), and MCI gel CHP-20P (75—150 μ, Mitsubishi Chemical Industries Ltd.). HPLC was conducted on a Toyo Soda apparatus equipped with an SP 8700 solvent delivery system and a UV-8 spectrophotometer.

Isolation——The fresh leaves (9 kg) of *M. indica*, collected in April at Ibusuki City, Kagoshima Prefecture, were extracted three times with acetone–H_2O (9:1). The aqueous solution, after removal of the acetone from the extract by evaporation under reduced pressure, was partitioned with EtOAc. The aqueous layer was concentrated and left to stand overnight. The resulting precipitates were collected by filtration and crystallized from H_2O–MeOH to give compound **10** (17 g), pale yellow needles, $[\alpha]_D^{26}$ +34.0° (c = 0.1, pyridine), mp 250 °C (dec.), ^{1}H-NMR (DMSO-d_6) δ: 4.62 (1H, d, J = 10 Hz, H-1'). 6.38 (1H, s, H-4), 6.87 (1H, s, H-5), 7.38 (1H, s, H-8). This product was identified as mangiferin by comparison of the ^{1}H-NMR and IR spectra with those of an authentic sample and by mixed melting point (mmp) determination. A part (30 g) of the mother liquor was chromatographed over MCI gel with a mixture of H_2O–MeOH (1:0—1:1) to furnish compounds **1** (2 g) and **11** (40 mg). **11**, pale yellow needles (H_2O), mp 255 °C (dec.), $[\alpha]_D^{26}$ +5.6° (c = 0.2, pyridine), IR ν_{max}^{KBr} cm^{-1}: 1670, ^{1}H-NMR (DMSO-d_6) δ: 4.70 (1H, d, J = 10 Hz, H-1'), 6.23 (1H, s, H-2), 6.84 (1H, s, H-5), 7.73 (1H, s, H-8), was shown to be identical with isomangiferin by ^{1}H-NMR comparison.[4,8] The above EtOAc-soluble portion (94 g) was subjected to column chromatography on Sephadex LH-20 with an EtOH–H_2O–acetone solvent system (10:0:0—9:1:0—8:2:0—7:3:0—6:4:0—54:36:10—48:32:20—0:1:1)[20] to give four fractions; fr. 1 (9 g), 2 (26 g), 3 (9 g) and 4 (35 g). Fraction 1 was negative to the $FeCl_3$ reagent and was not examined further. Fraction 2 was chromatographed repeatedly over MCI gel with increasing concentrations of MeOH in H_2O, and over Sephadex LH-20 (80% MeOH, EtOH) to afford compounds **2** (35 mg), **3** (33 mg), **4** (670 mg), **6** (1.4 g), **8** (15 mg) and **9** (80 mg). Compound **9**, colorless needles (H_2O), mp 262—263 °C, $[\alpha]_D^{24}$ −180.3° (c = 1.0, MeOH), ^{1}H-NMR (acetone-d_6) δ: 2.70—3.26 (2H, m, H-4), 5.14 (1H, br s, H-2), 5.56 (1H, m, H-3), 7.24 (2H, s, galloyl-H), was shown to be identical with (−)-epicatechin 3-O-gallate by mmp and ^{1}H-

NMR comparison. Similarly, repeated chromatography of fraction 3 afforded compounds 5 (300 mg) and 7 (97 mg). Fraction 4 was considered to be composed of galloylglucoses because it gave only gallic acid and glucose on tannase hydrolysis. Methanolysis of fraction 4 with a mixture (9:1) of MeOH and acetate buffer (pH 5.5) at 60 °C for 4 h afforded 1,2,3,4,6-penta-O-galloyl-β-D-glucose (TLC: Kieselgel, Rf 0.26; cellulose, Rf 0.1) and methyl gallate (TLC: Kieselgel, Rf 0.87; cellulose, Rf 0.54).

Compound 1 (1)——A yellow amorphous powder, $[\alpha]_D^{25}$ +33.2° (c=0.9, MeOH). *Anal.* Calcd for $C_{19}H_{20}O_{11} \cdot H_2O$: C, 51.59; H, 5.01. Found: C, 51.99; H, 5.18. UV λ_{max}^{MeOH} nm (log ε): 296 (3.93), 326 (4.03). IR ν_{max}^{KBr} cm^{-1}: 3400, 1605. FAB-MS m/z: 425 ([M+H]$^+$). ^{1}H-NMR (DMSO-d_6) δ: 3.10—3.80 (m, sugar-H), 4.61 (1H, d, J=10 Hz, H-1''), 5.96 (1H, s, H-5), 6.74 (1H, d, J=8 Hz, H-5'), 7.07 (1H, dd, J=8, 2 Hz, H-6'), 7.15 (1H, d, J=2 Hz, H-2'). ^{13}C-NMR: Table I.

Degradation of 1 with FeCl₃——A mixture of 1 (10 mg) and $FeCl_3 \cdot 6H_2O$ (50 mg) in H_2O (2 ml) was refluxed for 6 h. The reaction mixture was passed through an Amberlite MB-3 column and the eluate was concentrated under reduced pressure. The syrup thus obtained was subjected to Avicel cellulose TLC [n-butanol–pyridine–H_2O (6:4:3), aniline hydrogen phthalate reagent], and only a spot corresponding to glucose (Rf 0.37) was detected on the chromatogram.

Treatment of 1 with HCl——A solution of 1 (5 mg) in 2 N HCl (1 ml) was heated on a water bath for 3 h. After cooling, the solution was extracted with EtOAc (1 ml), and the EtOAc-soluble portion was examined by silica gel TLC [benzene–ethyl formate–formic acid (5:4:1)]. A spot of a phenolic compound was detected, and its Rf value (0.59) coincided with that of protocatechuic acid. The aqueous layer was treated with Amberlite MB-3 and the neutral solution was concentrated under reduced pressure to a syrup, which was trimethylsilylated with N-trimethylsilylimidazole and analyzed by gas chromatography [t_R 10.86 and 17.24 (glucose); column, 1.5% SE-30; carrier gas, nitrogen; column temperature, 155 °C; flow rate, 60 ml/min].

K₃[Fe(CN)₆] Oxidation of 1——$K_3[Fe(CN)_6]$ (160 mg) was added to a solution of 1 (200 mg) and Na_2CO_3 (90 mg) in H_2O (50 ml) at room temperature. After being stirred for 1.5 h, the mixture was neutralized with 1 N HCl and applied to an MCI gel column. Elution with increasing concentrations of MeOH in H_2O (0—45%) afforded mangiferin (10) (7 mg) and isomangiferin (11) (12 mg), which were identified by comparison of the ^{1}H-NMR and IR spectra with those of authentic specimens, and mmp determination.

Compound 2 (2)——A yellow amorphous powder, $[\alpha]_D^{31}$ −50.0° (c=0.8, MeOH). *Anal.* Calcd for $C_{26}H_{23}O_{13} \cdot 1/2H_2O$: C, 56.42; H, 4.55. Found: C, 56.48; H, 4.65. ^{1}H-NMR (acetone-d_6) δ: 4.50 (1H, dd, J=13, 2 Hz, H-6''), 4.70 (1H, dd, J=13, 4 Hz, H-6''), 5.00 (1H, d, J=9 Hz, H-1''), 5.98 (1H, s, H-5), 6.83 (1H, d, J=8 Hz, H-5'), 6.89 (2H, d, J=9 Hz, p-hydroxybenzoyl-H-3,5), 7.18 (1H, dd, J=8, 2 Hz, H-6'), 7.26 (1H, d, J=2 Hz, H-2'), 7.86 (1H, d, J=9 Hz, p-hydroxybenzoyl-H-2,6). ^{13}C-NMR: Table I.

Enzymatic Hydrolysis of 2 with Crude Hesperidinase——A solution of 2 (50 mg) in H_2O (10 ml) was incubated with crude hesperidinase at 35 °C overnight. The reaction mixture was evaporated to dryness, and the residue was treated with EtOH. The EtOH-soluble portion was subjected to MCI gel column chromatography with 30% MeOH to furnish a yellow amorphous powder (20 mg), which was identified as compound 1 by comparison of the ^{1}H-NMR and IR spectra with those of an authentic sample. Further elution with 40% MeOH gave another product as colorless needles (5 mg), mp 216—217 °C, IR ν_{max}^{KBr} cm^{-1}: 1665, 1590. This substance was shown to be identical with p-hydroxybenzoic acid by mmp and IR comparison.

Compound 3 (3)——A yellow amorphous powder, $[\alpha]_D^{24}$ −181.3° (c=0.4, MeOH). *Anal.* Calcd for $C_{33}H_{28}O_{17} \cdot 2H_2O$: C, 54.10; H, 4.40. Found: C, 54.39; H, 4.36. UV λ_{max}^{MeOH} nm (log ε): 258 (4.38), 270 (4.38), 295 (4.21), 325 (4.08). ^{1}H-NMR (acetone-d_6) δ: 4.48—4.76 (2H, m, H-6''), 5.27 (1H, d, J=10 Hz, H-1''), 5.54 (1H, t, J=10 Hz, H-2''), 5.88 (1H, s, H-5), 6.80 (1H, d, J=8 Hz, H-5'), 6.88 (2H, d, J=9 Hz, p-hydroxybenzoyl-H-3,5), 7.07 (2H, s, galloyl-H), 7.07—7.25 (2H, m, H-2',6'), 7.88 (2H, d, J=9 Hz, p-hydroxybenzoyl-H-2,6). ^{13}C-NMR: Table I.

Partial Hydrolysis of 3 with Tannase——A solution of 3 (7 mg) in H_2O (0.3 ml) was incubated with tannase at 37 °C for 30 min. The reaction mixture was subjected to MCI gel column chromatography with 50% MeOH to furnish gallic acid and a hydrolysate (3 mg), the ^{1}H-NMR spectrum of the latter being identical with that of compound 2.

Compound 4 (4)——Pale yellow needles (H_2O), mp 193—194 °C, $[\alpha]_D^{24}$ −143.8° (c=0.4, MeOH). *Anal.* Calcd for $C_{33}H_{28}O_{17} \cdot 2H_2O$: C, 54.10; H, 4.40. Found: C, 54.26; H, 4.26. UV λ_{max}^{MeOH} nm (log ε): 258 (4.47), 270 (4.48), 295 (4.30), 325 (4.17). ^{1}H-NMR (acetone-d_6) δ: 4.64 (2H, br s, H-6''), 5.28 (1H, d, J=10 Hz, H-1''), 5.54 (1H, t, J=10 Hz, H-2''), 5.88 (1H, s, H-5), 6.76 (1H, d, J=8 Hz, H-5'), 6.78 (2H, d, J=9 Hz, p-hydroxybenzoyl-H-3,5), 6.96—7.20 (2H, m, H-2',6'), 7.15 (2H, s, galloyl-H), 7.82 (2H, d, J=9 Hz, p-hydroxybenzoyl-H-2,6). ^{13}C-NMR: Table I.

Enzymatic Hydrolysis of 4 with Crude Hesperidinase——A solution of 4 (150 mg) in H_2O (5 ml) was incubated with crude hesperidinase at 37 °C overnight, and the reaction mixture was treated as described for compound 2 to afford gallic acid (13 mg), p-hydroxybenzoic acid (10 mg) and compound 1 (40 mg).

Partial Hydrolysis of 4 with Tannase——A solution of 4 (100 mg) in H_2O (5 ml) was treated as described for compound 3 to give gallic acid and a hydrolysate (4a) (60 mg), a yellow amorphous powder, $[\alpha]_D^{24}$ −140.6° (c=0.6, MeOH). *Anal.* Calcd for $C_{26}H_{24}O_{13} \cdot H_2O$: C, 55.52; H, 4.66. Found: C, 55.69; H, 4.99. ^{1}H-NMR (acetone-d_6) δ: 5.19 (1H, d, J=10 Hz, H-1''), 5.52 (1H, t, J=10 Hz, H-2''), 5.92 (1H, s, H-5), 6.82 (3H, d, J=9 Hz, H-5', p-hydroxyben-

zoyl-H-3,5), 7.09 (1H, br d, $J = 8$ Hz, H-6'), 7.20 (1H, br s, H-2'), 7.82 (2H, d, $J = 9$ Hz, p-hydroxybenzoyl-H-2,6).

Compound 5 (5)——A yellow amorphous powder, $[\alpha]_D^{30}$ $-48.8°$ ($c = 0.4$, MeOH). *Anal.* Calcd for $C_{40}H_{32}O_{23} \cdot H_2O$: C, 53.46; H, 3.81. Found: C, 53.81; H, 4.05. ^{1}H-NMR (acetone-d_6) δ: 4.68 (2H, br s, H-6''), 5.41 (1H, d, $J = 10$ Hz, H-1''), 5.63 (1H, t, $J = 10$ Hz, H-2''), 5.83 (1H, t, $J = 9$ Hz, H-3''), 5.92 (1H, s, H-5), 6.79 (1H, d, $J = 8$ Hz, H-5'), 6.96, 7.05, 7.17 (each 2H, s, galloyl-H). ^{13}C-NMR: Table I. Tannase hydrolysis of **5** in the same way as described for **3** gave gallic acid and compound **1**.

Compound 6 (6)——A yellow amorphous powder, $[\alpha]_D^{24}$ $-135.0°$ ($c = 1.0$, MeOH). *Anal.* Calcd for $C_{33}H_{28}O_{18} \cdot 2H_2O$: C, 52.95; H, 4.31. Found: C, 52.91; H, 4.32. UV λ_{max}^{MeOH} nm (log ε): 278 (4.53), 324 (4.17). ^{1}H-NMR (acetone-d_6) δ: 4.64 (2H, br s, H-6''), 5.25 (1H, d, $J = 10$ Hz, H-1''), 5.53 (1H, t, $J = 10$ Hz, H-2''), 5.88 (1H, s, H-5), 6.78 (2H, d, $J = 9$ Hz, H-3',5'), 7.05, 7.13 (each 2H, s, galloyl-H), 7.50 (2H, d, $J = 9$ Hz, H-2',6'). ^{13}C-NMR: Table I.

Hydrolysis of 6 with Tannase——A solution of **6** (200 mg) in H_2O (10 ml) was treated with tannase at 37 °C for 1 h. Work-up as described in the case of **3** gave gallic acid and a hydrolysate (**6a**) as a yellow amorphous powder, $[\alpha]_D^{24}$ $+45.8°$ ($c = 0.9$, MeOH). *Anal.* Calcd for $C_{19}H_{20}O_{10} \cdot 1/4H_2O$: C, 55.27; H, 5.00. Found: C, 55.37; H, 5.48. FAB-MS m/z 409 ([M + H]$^+$). UV λ_{max}^{MeOH} nm (log ε): 296 (4.06), 314 (4.10). IR ν_{max}^{KBr} cm^{-1}: 3400, 1630, 1605. ^{1}H-NMR (acetone-d_6) δ: 4.94 (1H, d, $J = 10$ Hz, H-1''), 6.02 (1H, s, H-5), 6.84 (2H, d, $J = 9$ Hz, H-3',5'), 7.64 (2H, d, $J = 9$ Hz, H-2',6'). ^{13}C-NMR: Table I.

Compound 7 (7)——A yellow amorphous powder, $[\alpha]_D^{30}$ $-47.4°$ ($c = 0.7$, MeOH). *Anal.* Calcd for $C_{40}H_{32}O_{22} \cdot 1/2H_2O$: C, 54.99; H, 3.81. Found: C, 54.84; H, 4.00. ^{1}H-NMR (acetone-d_6) δ: 4.68 (2H, br s, H-6''), 5.41 (1H, d, $J = 10$ Hz, H-1''), 5.62 (1H, t, $J = 10$ Hz, H-2''), 5.81 (1H, t, $J = 9$ Hz, H-3''), 5.91 (1H, s, H-5), 6.80 (2H, d, $J = 9$ Hz, H-3',5'), 6.96, 7.05, 7.16 (each 2H, s, galloyl-H), 7.51 (2H, d, $J = 9$ Hz, H-2',6'). ^{13}C-NMR: Table I. Hydrolysis of **7** with tannase in a manner similar to that described for **3** gave gallic acid and a hydrolysate (**6a**).

Compound 8 (8)——A yellow granular solid (H_2O), $[\alpha]_D^{30}$ $-35.2°$ ($c = 0.2$, MeOH). *Anal.* Calcd for $C_{26}H_{22}O_{15} \cdot 1/2H_2O$: C, 53.52; H, 3.97. Found: C, 53.32; H, 4.21. UV λ_{max}^{MeOH} nm (log ε): 218 (4.33), 240 (4.19), 257 (4.27), 271 (4.06), 315 (3.91). ^{1}H-NMR (acetone-d_6 + D_2O) δ: 4.56 (2H, br s, H-6'), 5.01 (1H, d, $J = 10$ Hz, H-1'), 6.41 (1H, s, H-4), 6.91 (1H, s, H-5), 7.14 (2H, s, galloyl-H), 7.51 (1H, s, H-8). Hydrolysis of compound **8** with tannase as described for **3** furnished gallic acid and mangiferin (**10**).

Enzymatic Transformation of 1 to 10 and 11 with a Crude Enzyme Preparation——A mixture of fresh leaves of *M. indica* (26 g) and polyamide (24 g) in buffer solution (100 ml) (pH 7) consisting of KH_2PO_4 (10 mM) and KOH was macerated for 5 min in a Waring blender at 0 °C. The macerate was squeezed through three layers of muslin and the filtrate was centrifuged at 7000 rpm. The precipitate was removed by decantation, and the supernatant was treated at -20 °C with acetone (400 ml). After 30 min, the resulting precipitates (67 mg) were collected by filtration, and again dissolved in 5 mM phosphate buffer (pH 7) (10 ml). A part (1 ml) of this solution was added to a solution of compound **1** (5 mg) in H_2O (0.5 ml) and the mixture was incubated at 37 °C for 24 h. The reaction products were analyzed by cellulose TLC (2% HOAc) and HPLC [TSK gel -ODS 120T (reverse-phase); solvent, 15% acetonitrile–water; flow rate, 0.75 ml/min] to identify compound **1** (Rf 0.67, t_R 3.7 min), mangiferin (Rf 0.16, t_R 4.6 min) and isomangiferin (Rf 0.10, t_R 5.2 min). On the other hand, similar treatment of compound **1** with heat-treated (100 °C, 1 h) enzyme solution gave only the starting material (Fig. 2).

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