Iridoid Glucosides from the Fruit of *Genipa americana*

Masateru ONO,*[,a] Masami UENO,[a] Chikako MASUOKA,[a] Tsuyoshi IKEDA,[b] and Toshihiro NOHARA[b]

[a] *School of Agriculture, Kyushu Tokai University; 5435 Minamiaso, Aso, Kumamoto 869–1404, Japan: and* [b] *Faculty of Medical and Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan.*
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Four new iridoid glucosides, called genamesides A—D, have been isolated from the fruit of *Genipa americana* L. (Rubiaceae), along with four known iridoid glucosides, geniposidic acid, geniposide, gardenoside and genipin-gentiobioside. Their chemical structures were determined on the basis of spectroscopic data.

Key words *Genipa americana*; iridoid glucoside; genameside; Rubiaceae

Genipa americana L. (Rubiaceae) is widely distributed in tropical Central and South America. The fruit of this plant is edible and popular as a source of beverages. The white flesh turns yellow to bluish-purple and finally to jet-black on exposure to air.[1)] The presence of three iridoids, genipin,[2)] geniposidic acid[1)] and geniposide[1)] in this fruit, has been reported. In the course of our studies on the constituents of Peruvian plants,[3)] we have investigated the constituents of the fruit of *G. americana*. The present paper describes the isolation and structural elucidation of four new iridoid glucosides along with four known iridoid glucosides.

The MeOH extract of the fruit of *G. americana* was successively subjected to Diaion HP20 and silica gel column chromatographies, as well as HPLC on ODS, to afford eight iridoid glucosides (**1**—**8**).

Compounds **5**—**8** were identified as geniposidic acid (**5**),[4)] geniposide (**6**),[5)] gardenoside (**7**)[4)] and genipin-gentiobioside (**8**),[6)] respectively, based on their physical and spectral data, although detailed NMR spectral data of **6** and **8** have not been reported in the literature.

Compound **1**, called genameside A, was obtained as a colorless syrup. In the positive FAB-MS, **1** indicated an $[M+H]^+$ ion peak at *m/z* 423. The molecular formula of **1** was determined to be $C_{17}H_{26}O_{12}$ by high-resolution (HR) FAB-MS. The ^{1}H-NMR spectrum of **1** showed signals due to one olefinic proton [δ 7.45 (d, J=1.0 Hz)], one hemiacetal proton [δ 5.68 (d, J=8.0 Hz)], one oxygenated methine proton [δ 4.03 (dd, J=4.0, 7.0 Hz)], two oxygenated methylene protons [δ 3.98 (d, J=12.0 Hz), 3.83 (d, J=12.0 Hz)], one methoxyl group (δ 3.71), two methine protons [δ 3.29 (m), 2.19 (dd, J=8.0, 8.0 Hz)], two methylene protons [δ 2.80 (ddd, J=7.5, 10.0, 14.5 Hz), 1.37 (ddd, J=4.5, 8.0, 14.5 Hz)], and one monosaccharide group. The ^{13}C-NMR spectrum of **1** showed 17 carbon signals, which were composed of signals due to one carboxyl carbon (δ 169.4), two olefinic carbons (δ 152.5, 112.8), one hemiacetal carbon (δ 95.4), one oxygenated methylene carbon (δ 64.4), one oxygenated methine carbon (δ 79.0), one oxygenated quaternary carbon (δ 84.7), one methoxyl carbon (δ 51.7), two methine carbons (δ 49.4, 31.9), one methylene carbon (δ 41.5) and one glucopyranosyl group (δ 100.2, 74.8, 77.9, 71.7, 78.3, 62.9). These ^{1}H- and ^{13}C-NMR signals (Tables 1, 2, respectively) were assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra, and the planar structure of **1**, which was an iridoid glucoside possessing three hydroxyl groups at C-7, C-8 and C-10 of aglycone (Ag), could be determined by these observations. The relative stereochemistry in **1** was examined from its difference NOE spectra, and NOEs were observed between H-5 of Ag and Hβ-6 of Ag, between Hβ-6 of Ag and H-7 of Ag, and between Ha-10 of Ag and H-1 of Ag, as shown in Fig. 1. Acidic hydrolysis of **1** afforded D-glucose which was confirmed by optical rotation using chiral detection in HPLC analysis, and the coupling constant of an anomeric proton signal [δ 4.73 (d, J=8.0 Hz)] indicated the mode of glycosidic linkage of the glucosyl group to be β. Consequently, the structure of **1** was concluded to be 7α-hydroxy-6,7-dihydrogardenoside.

Compound **2**, called genameside B, was obtained as a colorless syrup, and its positive FAB-MS showed the same $[M+H]^+$ ion peak as that of **1** at *m/z* 423. The ^{1}H- and ^{13}C-NMR signals of **2** were very similar to those of **1**. In the same manner as for **1**, these ^{1}H- and ^{13}C-NMR signals were examined in detail, and the planar structure of **2** was elucidated to be the same as that of **1**. On acidic hydrolysis, **2** afforded D-glucose, and the β-glycosidic linkage was determined based on the coupling constant of the anomeric proton. In comparing the chemical shifts of ^{1}H-NMR signals in CD_3OD between **1** and **2**, the signals due to Hα-6 of Ag, Hβ-6 of Ag and H-9 of Ag in **2** were shifted by +0.45, −0.62 and +0.25 ppm, respectively. Therefore, **2** was recognized as an isomer at C-7 and/or C-8 of Ag in **1**. In the difference NOE spectra of **2**, NOEs were observed between H-5 of Ag and Hβ-6 of Ag, between Hα-6 of Ag and H-7 of Ag, and between H-5 of Ag and H-9 of Ag, while no correlation between H-1 of Ag and H-10 of Ag was detected (Fig. 1). Jensen and Nielsen reported that the chemical shift value (in CD_3OD) of the C-9 of Ag in **7**, and its epimer at C-8 of Ag, galioside, was δ 52.4 and 45.4, respectively, and that the chemical shift of C-9 of Ag may be used to determine the configuration at C-8 of Ag in 8,10-dihydroxy type iridoid glucosides: fourteen 8α,10-dihydroxy iridoid glucosides indicated the chemical shift of C-9 of Ag at a mean of δ 44.8±1.7 [in D_2O (glucosides) or $CDCl_3$ (acetates)]; in contrast, four 8β,10-dihydroxy iridoid glucosides showed that at δ 50.7±0.7.[7)] The chemical shift of C-9 of Ag in **1** and **2** was δ 49.4 and 45.4, respectively. From the above evidence, **2** was concluded to be a stereoisomer of **1** at C-7 and C-8 of Ag.

Compound **3**, called genameside C, was obtained as an amorphous powder, and showed the same $[M+H]^+$ ion peak

* To whom correspondence should be addressed. e-mail: mono@ktmail.ktokai-u.ac.jp

Table 1. ^{1}H-NMR Data for **1**—**4**, **6** and **8** (in CD_3OD, 500 MHz)

H	1	2	3	4	6	8
Ag-1	5.68 d (8.0)	5.53 d (3.5)	5.13 d (8.0)	5.25 d (6.5)	5.16 d (7.5)	5.14 d (8.0)
Ag-3	7.45 d (1.0)	7.41 s	7.51 d (2.0)	7.50 d (1.0)	7.51 d (1.5)	7.51 d (1.5)
Ag-5	3.29 m	3.15 ddd (8.0, 8.0, 8.0)	3.19 ddd (8.0, 8.0, 8.0)	*ca.* 3.21	3.18 ddd (8.0, 8.0, 8.0)	*ca.* 3.18
Ag-6a	2.80 ddd (7.0, 10.0, 14.5)	2.18 ddd (3.0, 7.5, 13.5)	2.82 dd (8.0, 16.5)	2.83 dd (8.5, 16.5)	2.82 dd (8.0, 16.5)	2.83 dd (8.5, 16.5)
Ag-6b	1.37 ddd (4.0, 8.0, 14.5)	1.82 ddd (5.0, 8.0, 13.5)	2.08 dddd (2.0, 2.0, 8.0, 16.5)	2.11 br dd (5.5, 16.5)	2.09 dddd (1.5, 1.5, 8.0, 16.5)	2.17 dddd (1.5, 1.5, 8.5, 16.5)
Ag-7	4.03 dd (4.0, 7.0)	3.94 dd (3.0, 5.0)	5.80 br s	5.88 br s	5.80 s	5.85 s
Ag-9	2.19 dd (8.0, 8.0)	2.44 dd (3.5, 8.0)	2.72 dd (8.0, 8.0)	2.95 dd (7.5, 7.5)	2.72 dd (7.5, 8.0)	2.71 dd (7.9, 7.9)
Ag-10a	3.98 d (12.0)	3.78 d (11.5)	4.31 br d (14.0)	4.61 d (13.0)	4.31 br d (14.5)	4.32 br d (14.5)
Ag-10b	3.83 d (12.0)	3.72 d (11.5)	4.18 dd (2.0, 14.0)	4.27 d (13.0)	4.18 br dd (1.5, 14.5)	4.19 br dd (1.5, 14.5)
OCH_3	3.71 s	3.70 s	3.71 s	3.71 s	3.71 s	3.71 s
Glc-1	4.73 d (8.0)	4.67 d (8.0)	4.74 d (8.0)	4.72 d (8.0)	4.71 d (8.0)	4.71 d (8.0)
Glc-2	3.23 dd (8.0, 9.0)	3.20 dd (8.0, 9.0)	*ca.* 3.30	*ca.* 3.26	3.22 dd (8.0, 9.0)	3.23 dd (8.0, 9.0)
Glc-3	3.39[a)]	3.37[a)]	3.55 dd (9.0, 9.0)	*ca.* 3.39	3.39[a)]	3.39 dd (9.0, 9.0)
Glc-4	*ca.* 3.30	*ca.* 3.31	3.58 dd (9.0, 9.0)	*ca.* 3.31	*ca.* 3.29	*ca.* 3.26
Glc-5	*ca.* 3.30	*ca.* 3.31	3.43 ddd (2.5, 4.0, 9.0)	*ca.* 3.30	*ca.* 3.29	3.52 ddd (1.5, 7.0, 9.0)
Glc-6a	3.86 d (11.5)	3.86 d (11.5)	3.89 dd (2.5, 11.5)	3.87 dd (1.0, 11.5)	3.86 d (11.5)	4.11 dd (1.5, 12.0)
Glc-6b	3.67[a)]	*ca.* 3.68	3.83 dd (4.0, 11.5)	*ca.* 3.66	3.64 dd (3.5, 11.5)	3.77 dd (7.0, 12.0)
Glc′-1			4.41 d (8.0)	4.35 d (8.0)		4.37 d (8.0)
Glc′-2			3.23 dd (8.0, 9.0)	*ca.* 3.26		*ca.* 3.17
Glc′-3			3.38 dd (9.0, 9.0)	*ca.* 3.39		*ca.* 3.28
Glc′-4			*ca.* 3.30	*ca.* 3.31		*ca.* 3.31
Glc′-5			*ca.* 3.35	*ca.* 3.30		*ca.* 3.28
Glc′-6a			3.88 dd (2.5, 11.5)	3.87 dd (1.0, 11.5)		3.86 br d (11.5)
Glc′-6b			3.66 dd (5.5, 11.5)	*ca.* 3.68		3.65 dd (3.5, 11.5)

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses). *a*) Signals were deformed by virtual coupling.

Table 2. ^{13}C-NMR Data for **1**—**4**, **6** and **8** (in CD_3OD, 125 MHz)

C	1	2	3	4	6	8
Ag-1	95.4	96.3	98.4	97.9	98.3	98.8
Ag-3	152.5	152.6	153.3	153.4	153.3	153.3
Ag-4	112.8	113.7	112.6	112.8	112.6	112.4
Ag-5	31.9	31.4	36.6	36.0	36.6	36.7
Ag-6	41.5	40.5	39.7	39.8	39.7	39.7
Ag-7	79.0	79.3	128.4	130.6	128.4	129.0
Ag-8	84.7	84.5	144.8	141.5	144.8	144.8
Ag-9	49.4	45.4	47.0	47.1	47.1	47.1
Ag-10	64.4	66.4	61.4	69.1	61.4	61.5
Ag-11	169.4	169.4	169.5	169.7	169.5	169.6
OCH_3	51.7	51.7	51.7	51.9	51.7	51.7
Glc-1	100.2	100.6	100.3	100.3	100.4	100.6
Glc-2	74.8	74.7	74.9	74.9	74.9	74.8
Glc-3	77.9	78.0	76.9	77.9	77.9	77.8
Glc-4	71.7	71.4	80.6	71.7	71.6	71.7
Glc-5	78.3	78.4	76.2	78.1	78.3	77.9
Glc-6	62.9	62.6	61.8	62.8	62.7	69.8
Glc′-1			104.6	104.6		104.8
Glc′-2			74.6	75.3		75.1
Glc′-3			77.9	78.0		78.0
Glc′-4			71.4	71.4		71.7
Glc′-5			78.1	78.4		77.8
Glc′-6			62.5	62.9		62.8

δ in ppm from TMS.

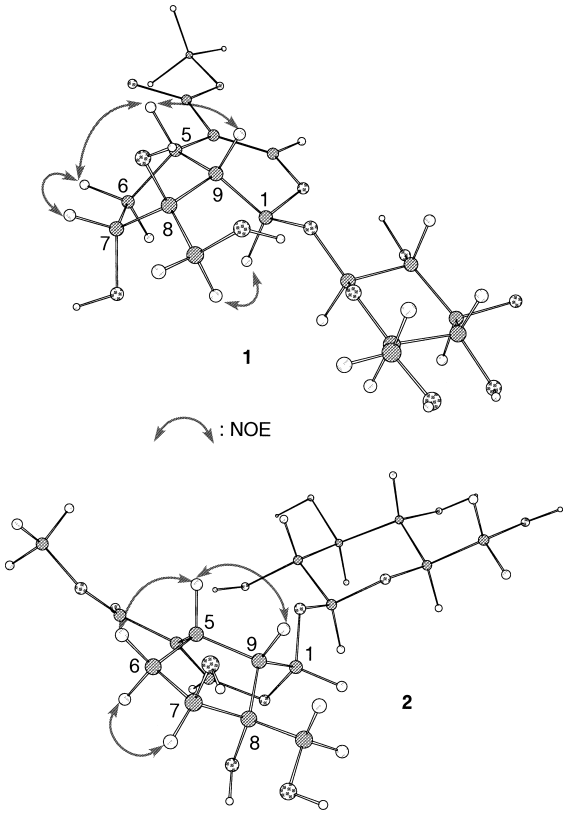
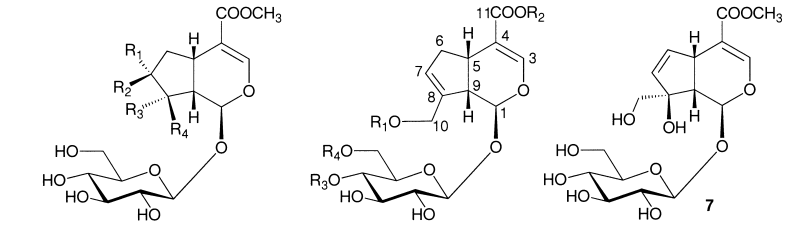


Fig. 1. Selected NOE Correlations Observed in Difference NOE Spectra of **1** and **2**

as that of **8** at *m*/*z* 551 in the positive FAB-MS. The ^{1}H- and ^{13}C-NMR spectra, which were assigned by techniques similar to those of **1**, were imposable on those of **8**; in particular, the signals due to the Ag moiety were almost superimposable, and **3** gave D-glucose on acidic hydrolysis. Therefore, **3** was believed to be a positional isomer of **8** in the glycosidic linkage of terminal glucosyl group (Glc′). In the ^{13}C-NMR spectrum of **3**, compared with that of **6**, signals due to the C-4 of the inner glucosyl group (Glc) indicated a glycosilation shift[8,9] by +9.0 ppm. These data suggested that **3** is a positional isomer of **8**, with Glc′ situated at C-4 of Glc. Moreover, HMBC correlation was observed between H-1 of Glc′ and C-4 of Glc, and the coupling constant of the anomeric



1: $R_1 = R_4 = OH$, $R_2 = H$, $R_3 = CH_2OH$
2: $R_1 = H$, $R_2 = R_3 = OH$, $R_4 = CH_2OH$
3: $R_1 = R_4 = H$, $R_2 = CH_3$, $R_3 = Glc$
4: $R_1 = Glc$, $R_2 = CH_3$, $R_3 = R_4 = H$
5: $R_1 = R_2 = R_3 = R_4 = H$
6: $R_1 = R_3 = R_4 = H$, $R_2 = CH_3$
8: $R_1 = R_3 = H$, $R_2 = CH_3$, $R_4 = Glc$

Glc: β-D-glucopyranosyl

Fig. 2. Structures of **1**—**8**

proton signal indicated the mode of glycosodic linkage of Glc′ to be *β*. The structure of **3** was therefore defined as genipin *β*-cellobioside.

Compound **4**, called genameside D, was obtained as a colorless syrup, and its positive FAB-MS indicated the same $[M+H]^+$ ion peak as those of **3** and **8** at *m/z* 551. The ^{1}H- and ^{13}C-NMR spectra of **4** were similar to those of **3** and **8**, and these signals were assigned as shown in Tables 1 and 2 by techniques similar to those of **1**, suggesting **4** to be a diglucoside of genipin. Further, acidic hydrolysis of **4** gave D-glucose, and the coupling constant of anomeric protons indicated the mode of glycosidic linkages of both glucose units to be *β*. In comparing the chemical shifts of the signals in the ^{13}C-NMR spectra of **4** and **6**, the signals due to C-10 of Ag showed the glycosylation shift[8,9] by 7.7 ppm. Moreover, the correlations between H-1 of the glucosyl group (Glc) and C-1 of Ag, and between H-1 of another glucosyl group (Glc′) and C-10 of Ag were observed in the HMBC spectrum of **4**. Thus, **4** was concluded to be 10-*O*-*β*-D-glucopyranosyl geniposide.

As far as we know, **1**—**4** are new iridoid glucosides, and **7** and **8** are the first examples of the isolation from the fruit of *Genipa americana*, although **7** was isolated form the callus and cell suspension cultures of *G. americana*,[1] and the hexaacetate of **2** was previously synthesized from pentaacetate of **6**.[7] Although the absolute configurations of Ag moieties of **1**—**4** have not been confirmed because of the lability of Ag of **1**—**4** on the acidic hydrolysis, they are probably the same as that[10] of **6** from a biogenetic point of view.

Experimental

All the instruments and materials used were the same as those cited in a previous report,[11] unless otherwise specified.

Plant Material The fruit of *Genipa americana* L. was purchased in October 1993 from Fundation pala la Investigation Technologica del Recurso Agrobiologico Andio, a research institute of Andes agricultural bioresources in Peru, and was identified by Sokurates Shiota, Executive Director, Fundation pala la Investigation Technologica del Recurso Agrobiologico Andio.

Extraction and Isolation The dried and powdered fruit of *Genepia americana* (284 g) was extracted with MeOH (450 ml×5) under reflux for 1 h, and the solvent was removed under reduced pressure to give a syrup (97.5 g). The MeOH extract was chromatographed over Diaion HP20 (H_2O, 60% MeOH, 80% MeOH, MeOH, acetone) to give fractions (frs.) 1—7. Chromatography of fr. 4 (23.6 g) over silica gel [Art. 7734, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] furnished **6** (4253 mg) and frs. 8—16. Fraction 15 (2468 mg) was subjected to silica gel column chromatography [Art. 9385, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to afford frs. 17—21. HPLC (COSMOSIL 5C18 AR-II, Nacalai Tesque, Inc., 20 mm i.d.×250 mm, 30% MeOH) of fr. 19 (868 mg) furnished **2** (16 mg) and frs. 22—26. Fraction 25 (37 mg) was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1)] to give **7** (25 mg). Fraction 20 (494 mg) and fr. 21 (782 mg) were each subjected to HPLC under the same conditions as for fr. 19 to give **1** (23 mg), **3** (28 mg) and frs. 27—28 from fr. 20, and **5** (29 mg), **8** (260 mg), **4** (55 mg) and frs. 42—43 from fr. 21.

Genameside A (**1**): Colorless syrup. $[\alpha]_D^{26}$ −91.9° (*c*=2.4, MeOH). Positive FAB-MS *m/z*: 423 $[M+H]^+$. HR positive FAB-MS *m/z*: 445.1428 $[M+Na]^+$ (Calcd for $C_{17}H_{26}O_{12}Na$: 445.1322). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Genameside B (**2**): Colorless syrup. $[\alpha]_D^{26}$ −63.8° (*c*=1.9, MeOH). Positive FAB-MS *m/z*: 423 $[M+H]^+$. HR positive FAB-MS *m/z*: 445.1348 $[M+Na]^+$ (Calcd for $C_{17}H_{26}O_{12}Na$: 445.1322). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Genameside C (**3**): White powder. $[\alpha]_D^{26}$ −0.4° (*c*=3.3, MeOH). Positive FAB-MS *m/z*: 551 $[M+H]^+$. HR positive FAB-MS *m/z*: 573.1905 $[M+Na]^+$ (Calcd for $C_{23}H_{34}O_{15}Na$: 573.1795). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Genameside D (**4**): Colorless syrup. $[\alpha]_D^{26}$ +3.3° (*c*=6.0, MeOH). Positive FAB-MS *m/z*: 551 $[M+H]^+$. HR positive FAB-MS *m/z*: 573.1821 $[M+Na]^+$ (Calcd for $C_{23}H_{34}O_{15}Na$: 573.1795). ^{1}H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Acidic Hydrolysis of 1—4 Compounds **1** (1 mg), **2** (1 mg), **3** (1 mg) and **4** (3 mg) in 2 N HCl were each heated at 95 °C for 1 h. The reaction mixture was neutralized with 4 N NaOH and then evaporated under reduced pressure to give a residue. The residue was extracted with MeOH and the MeOH extract was analyzed by HPLC under the following condition: column, YMC pack Polyamine II (YMC Co., Ltd., 4.6 mm i.d.×250 mm); solvent, 80% CH_3CN; flow rate, 0.8 ml/min; column temperature, 35 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; column oven, JASCO CO-2060. The retention time and optical activity of each of the samples were identical with those [t_R (min): 16.6; optical activity: positive] of D-glucose. However, the Ag of **1**—**4** couldn't be detected by TLC.

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