Three New Monoterpenoids from the Fruit of *Genipa americana*

Masateru ONO,*[,a] Naoki ISHIMATSU,[a] Chikako MASUOKA,[a] Hitoshi YOSHIMITSU,[b] Ryota TSUCHIHASHI,[c] Masafumi OKAWA,[c] Junei KINJO,[c] Tsuyoshi IKEDA,[d] and Toshihiro NOHARA[d]

[a] *Kyushu Tokai University School of Agriculture; 5435 Minamiaso, Aso, Kumamoto 869–1404, Japan:* [b] *Faculty of Pharmaceutical Sciences, Sojo University; 4–22–2 Ikeda, Kumamoto 860–0082, Japan:* [c] *Faculty of Pharmaceutical Sciences, Fukuoka University; 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan: and* [d] *Faculty of Medical and Pharmaceutical Sciences, Kumamoto University; 5–1 Oe-honmachi, Kumamoto 862–0973, Japan.*
Received August 2, 2006; accepted January 17, 2007

Three new monoterpenoids, called genipacetal, genipamide, and genipaol, were isolated from the fruit of *Genipa americana* L. (Rubiaceae), along with the four known iridoids genipin, gardendiol, deacetyl asperulosidic acid methyl ester, and shanzhiside. Their chemical structures were determined on the basis of spectroscopic data.

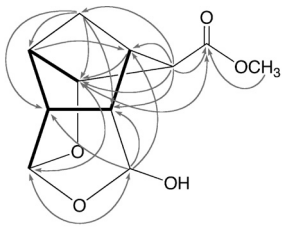
Key words *Genipa americana*; monoterpenoid; iridoid; alkaloid; Rubiaceae

Genipa americana L. (Rubiaceae) is widely distributed in tropical Central and South America. The fruit of this plant is edible and popular as source of beverages. The white flesh turns yellow to bluish-purple and finally to jet-black on exposure to the air.[1)]

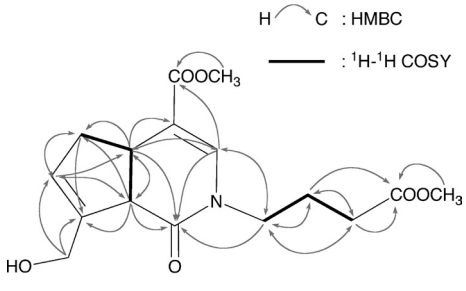
We earlier reported the isolation and structural elucidation of eight iridoid glucosides from the fruit of *G. americana*.[2)] As part of the continuing study of the constituents of this fruit, we now describe the isolation and structural elucidation of three new monoterpenoids (**1**—**3**), along with four known iridoids (**4**—**7**) from this fruit.

Compounds **4**—**7** were identified as genipin (**4**),[3)] gardendiol (**5**),[4)] deacetyl asperulosidic acid methyl ester (**6**),[5)] and shanzhiside (**7**)[6)] based on their physical and spectral data, although detailed ^{1}H- and ^{13}C-NMR spectral data of **6** and **7** have not been reported in the literature.

Compound **1**, called genipacetal, was obtained as a colorless syrup and exhibited an $[M+Na]^+$ ion peak at *m*/*z* 249 in the positive FAB-MS; the high-resolution (HR) FAB-MS indicated the molecular formula of **1** to be $C_{11}H_{14}O_5$. The ^{1}H-NMR spectrum of **1** revealed the presence of three oxygenated methine protons [δ 5.74 (d, J=5.0 Hz), 5.30 (s), 4.64 (d, J=5.0 Hz)], one methoxyl group (δ 3.68), five methine protons [δ 3.20 (ddd, J=5.0, 5.0, 9.5 Hz), 2.83 (dd, J=5.0, 5.0 Hz), 2.69 (d, J=1.5 Hz), 2.65 (d, J=4.5 Hz), 2.54 (dd, J=4.5, 9.5 Hz)], and one methylene group [δ 1.74 (d, J=11.0 Hz), 1.67 (dd, J=1.5, 11.0 Hz)]. The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **1** indicated the presence of the partial structure as shown in Fig. 1. The ^{13}C-NMR spectrum of **1** gave signals due to one carboxyl carbon (δ 174.9), two acetal carbons (δ 110.8, 100.6), one oxygenated methine carbon (δ 84.4), one methoxyl carbon (δ 52.5), five methine carbons (δ 54.7, 50.3, 50.2, 49.7, 42.1), and one methylene carbon (δ 38.3). The ^{1}H- and ^{13}C-NMR signals were assigned with the aid of heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) spectra (Tables 1, 2). From the cross-peaks observed in the HMBC spectrum (H-1/C-5, C-8, C-9, and C-10; H-3/C-10 and C-11; H-4/C-3, C-5, C-6, C-9, and C-11; H-5/C-3; H-6/C-3, C-7, C-8, and C-9; H-7/C-5; H-10/C-1 and C-3; and H_3CO/C-11) the planar structure of **1**, which is a monoterpenoid possessing a new skeleton with one methoxycarbonyl group, one five-membered cyclic acetal group, and one five-membered cyclic hemiacetal group, could be suggested (Fig. 1). Ordinary acetylation of **1** with acetic anhydride and pyridine afforded the corresponding monoacetate (**1a**) rather than triacetate. Comparing the chemical shifts of the signals between **1** and **1a**, the signal due to H-1 in **1** was shifted downfield by 0.85 ppm. Thus the presence of a hemiacetal group at C-1 and an epoxy bridge between C-3 and C-10 was established. The relative configurations at the C-5, C-7, C-8, C-9, and C-10 chiral centers were automatically established by forming the epoxy bridge. In the nuclear Overhauser and exchange spectroscopy (NOESY) spectrum of **1**, a key NOE was observed between H-1 and H-4; this indicated that the relative configurations at C-1 and C-4 were as shown in Fig. 2. Furthermore, the coupling constant values for the signals in the ^{1}H-NMR spectrum were analogous to those calculated for the dihedral angles, which was simulated using CAChe CONFLEX,[6)] and the Karplus equation[7)] (Table 3). Consequently, the structure of **1** was concluded to be as shown in Fig. 3.



1



2

Fig. 1. Partial Structure Elucidated by the ^{1}H–^{1}H COSY Spectrum (Bold Lines) and ^{1}H–^{13}C Long-Range Correlations (Arrow) Observed for **1** and **3** in the HMBC Spectra (in CD_3OD, 500 MHz)

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

Table 1. ^{1}H-NMR Data for **1**—**3**, **6**, **7** and **1a** (in CD_3OD, 500 MHz)

Position	1	1a	2	3	6	7
1	5.30 s	6.15 s		4.75 s	5.06 d (8.5)	5.53 d (2.0)
3	4.64 d (5.0)	4.69 d (5.0)	7.34 s	7.52 s	7.65 d (1.5)	7.39 s
4	2.69 d (1.5)	*ca.* 2.72				
5	2.65 d (4.5)	*ca.* 2.72	3.51 ddd (8.5, 8.5, 10.5)	*ca.* 2.89	3.02 ddd (1.5, 6.0, 8.0)	2.97 br d (10.0)
6a	1.74 d (11.0)	1.77 d (11.0)	2.85 ddd (2.5, 8.5, 16.0)	*ca.* 2.91	4.79 m	4.03 br s
6b	1.67 dd (1.5, 11.0)	1.71 d (11.0)	2.19 ddd (2.0, 8.5, 16.0)	1.89 m		
7a	2.83 dd (5.0, 5.0)	2.88 dd (5.0, 5.0)	5.83 d-like (1.0)	5.91 br s	6.02 d-like (1.5)	2.03 dd (6.5, 13.5)
7b						1.84 dd (6.5, 13.5)
8	3.20 ddd (5.0, 5.0, 9.5)	3.23 ddd (5.0, 5.0, 9.5)				
9	2.54 dd (4.5, 9.5)	*ca.* 2.71	3.63 d (10.5)		2.57 dd (8.0, 8.5)	2.59 dd (2.0, 10.0)
10a	5.74 d (5.0)	5.80 d (5.0)	4.38 dd (1.0, 11.5)	4.35 dd (1.5, 15.0)	4.45 dd (1.5, 15.5)	1.27 s
10b			4.33 dd (1.0, 11.5)	4.27 dd (1.5, 15.0)	4.21 br d (15.5)	
11-OCH_3	3.68 s	3.69 s	3.75 s	3.71 s	3.74 s	
4′-OCH_3			3.65 s			
$COCH_3$		2.01 s				
1′a			3.68 ddd (7.0, 7.0, 13.5)		4.72 d (8.0)	4.65 d (8.0)
1′b			3.56 ddd (7.0, 7.0, 13.5)			
2′			1.87 ddt (7.0, 7.0, 7.0)		3.24 dd (8.0, 9.0)	3.18 dd (8.0, 9.0)
3′			2.33 t (7.0)		3.39 dd (9.0, 9.0)	3.37 dd (9.0, 9.0)
4′					*ca.* 3.27	3.26 dd (9.0, 9.0)
5′					*ca.* 3.27	*ca.* 3.31
6′a					3.85 dd (2.0, 12.0)	3.90 dd (1.5, 12.0)
6′b					3.62 dd (6.0, 12.0)	3.65 dd (6.0, 12.0)

δ in ppm from tetramethylsilane (TMS) (coupling constants (J) in Hz are given in parentheses).

Table 2. ^{13}C-NMR Data for **1**—**3**, **6** and **7** (in CD_3OD, 125 MHz)

	1	2	3	6	7
1	100.6	171.4	97.3	101.6	94.9
3	84.4	139.2	153.9	155.4	152.3
4	50.3	112.0	110.4	108.3	112.1
5	42.1	38.9	46.5	42.7	41.6
6	38.3	40.9	38.3	75.4	77.8
7	50.2	128.1	128.6	129.9	49.1
8	49.7	144.0	147.6	151.5	79.0
9	54.7	50.2	81.0	45.9	51.8
10	110.8	61.7	59.8	61.7	24.7
11	174.9	168.7	169.8	169.5	171.6
11-OCH_3	52.5	52.0	51.7	51.8	
4′-OCH_3		52.1			
1′		47.9		100.5	99.8
2′		24.9		75.0	74.7
3′		31.7		77.9	78.0
4′		175.0		71.7	71.7
5′				78.5	78.3
6′				62.9	62.9

δ in ppm from TMS.

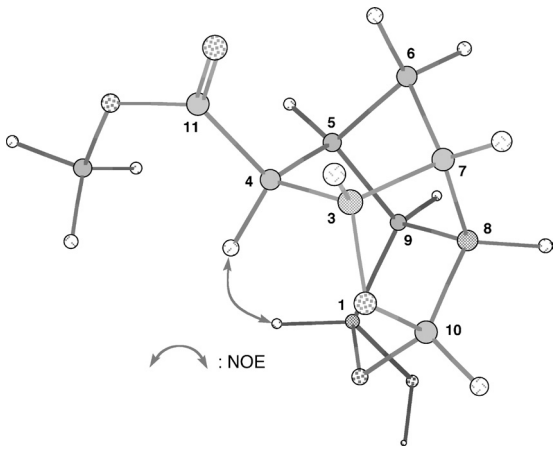


Fig. 2. CAChe Drawings and Key NOE Correlation (Arrow) Observed for **1** in the NOESY Spectrum (in CD_3OD, 500 MHz)

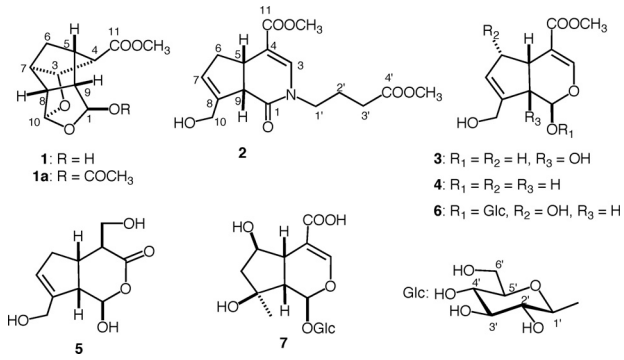


Fig. 3. Structures of **1**—**7** and **1a**

Compound **2**, called genipamide, was obtained as a colorless syrup, and its positive FAB-MS showed an $[M+H]^+$ ion peak at *m/z* 324. The molecular formula of **2** was determined to be $C_{16}H_{21}NO_6$ using HR positive FAB-MS. The ^{1}H-NMR spectrum of **2** indicated signals due to two olefinic protons [δ 7.34 (s), 5.83 (d-like, J=1.0 Hz)], one oxygenated methylene group [δ 4.38 (dd, J=1.0, 11.5 Hz), 4.33 (dd, J=1.0, 11.5 Hz)], and two methoxyl groups (δ 3.75, 3.65). The ^{13}C-NMR spectrum of **2** showed 16 carbon signals, which were composed of signals due to three carboxyl carbons (δ 175.0, 171.4, 168.7), four olefinic carbons (δ 144.0, 139.2, 128.1, 112.0), one oxygenated methylene carbon (δ 61.7), two methoxyl carbons (δ 52.1, 52.0), two methine carbons (δ 50.2, 38.9), and four methylene carbons (δ 47.9, 40.9, 31.7, 24.9). 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**, an iridoidal alkaloid derivative possessing a δ lactam ring and methyl 4-amino butylate group, was characterized as illustrated in Fig. 1. Since the chemical shifts of the signals

Table 3. Calculated and Observed ^1H–^1H Coupling Constants of **1**

	Dihedral angle (°)	Calculated (Hz)	Observed (Hz)
H-1/H-9	−88.5	−0.3	0.0
H-3/H-4	−106.6	0.6	0.0
H-3/H-7	32.4	5.8	5.0
H-4/H-5	77.9	0.1	0.0
H-5/Ha-6	63.9	1.4	0.0
H-5/Hb-6	−62.6	1.5	0.0
H-5/H-9	44.8	4.0	4.5
Ha-6/H-7	−61.6	1.6	0.0
Hb-6/H-7	63.6	1.4	0.0
H-7/H-8	−33.0	5.7	5.0
H-8/H-9	−16.5	7.5	9.5
H-8/H-10	16.9	7.5	5.0

due to H-5 and H-9 were similar to each other, the configurations at C-5 and C-9 could not be determined using NOESY spectral data. However, the coupling constant value between H-5 and H-9 and chemical shift values of the signals due to C-5 and C-9 were imposable on those of gardemide A (**9**)[8] and 2-hydroxyethyl gardenamide A (**10**),[9] and therefore the relative configurations at C-5 and C-9 were concluded to be identical with those of **9** and **10**. The structure of **2** was therefore defined as 2-(3-methoxycarbonyl-propyl) gardenamide A.

Compound **3**, called genipaol, was obtained as a colorless syrup and exhibited an $[M-H]^-$ ion peak at *m/z* 241 in the negative FAB-MS. The molecular formula of **3** was determined to be $C_{11}H_{14}O_6$ using HR negative FAB-MS. The ^1H- and ^{13}C-NMR spectra of **3** were similar to those of **4**, apart from the appearance of the signal due to one oxygenated quaternary carbon (δ 81.0) and lack of the signals due to one methine group. From these data, **3** was considered to be 5- or 9-hydroxy genipin. In the HMBC spectrum, a key correlation was observed between the signal due to H-3 and the signal due to methine carbon (δ 46.5, C-5). The NOESY spectrum gave correlations between H-1 and H_2-10. Although the configuration of **3** has not been confirmed, taking account of a biogenetic point of view, it is probably the same as that of **4**.[10] Thus **3** was considered to be 9-hydroxygenipin.

To the best of our knowledge, **1**—**3** are new monoterpenoids, and **5**—**7** are the first examples of isolation from the fruit of *G. americana*.

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

Extraction and Isolation The dried and powdered fruit of *G. americana* (284 g) was extracted with MeOH (450 ml) five times 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 on a Diaion HP20 column, eluted with H_2O, 60% MeOH, 80% MeOH, MeOH, and acetone, to give fractions (frs.) 1—7. Fraction 4 (23.6 g) was subjected to silica gel column chromatography (CC) eluted with a gradient of mixtures of $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.2, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0) to give frs. 4.1—4.10. Fractions 4.2 (498 mg) and 4.3 (581 mg) were each subjected to Chromatorex ODS CC eluted with a gradient of mixtures of MeOH–H_2O (starting from 30% MeOH to 100% MeOH) to furnish **4** (16 mg) from fr. 4.2, and **3** (9 mg) and frs. 4.3.1—4.3.4 from fr. 4.3. HPLC (COSMOSIL 5C18 AR-II, Nacalai Tesque Inc., 20 mm i.d.×250 mm) of fr. 4.3.1 (100 mg) eluted with 30% MeOH gave **5** (10 mg). Fraction 4.8 (2803 mg) was subjected to silica gel CC using a gradient of mixtures of $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.2, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0) as eluents to furnish frs. 4.8.1—4.8.7. A part (320 mg) of fr. 4.8.6 (1823 mg) was subjected to HPLC (COSMOSIL SIL-06, Nacalai Tesque Inc., 20 mm i.d.×250 mm) eluted with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) to afford frs. 4.8.6.1—4.8.6.11. HPLC (COSMOSIL 5C18 AR-II) of 4.8.6.10 (43 mg), using 25% MeOH as eluent, gave **6** (11 mg). Fraction 4.10 (9142 mg) was chromatographed on a silica gel column using a gradient of mixtures of $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.2, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0) as eluents to afford frs. 4.10.1—4.10.8. A part (79 mg) of fr. 4.10.8 (4101 mg) was subjected to HPLC (COSMOSIL SIL-06) using $CHCl_3$–MeOH–H_2O (6 : 4 : 1) as eluent to afford **7** (34 mg). Fraction 5 (5.0 g) was subjected to Chromatorex ODS CC eluted with a gradient of mixtures of MeOH–H_2O (30% MeOH, 40% MeOH, 50% MeOH, MeOH) to afford frs. 5.1—5.5. Fraction 5.1 (4545 mg) was chromatographed on a silica gel column using a gradient of mixtures of $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.2, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1) as eluents to give frs. 5.1.1—5.1.9. Fraction 5.1.3 (151 mg) was subjected to HPLC (COSMOSIL 5C18 AR-II) using 35% MeOH as eluent to furnish **1** (20 mg). Chromatography of fr. 5.1.4 (179 mg) on a Chromatorex ODS column eluted with a gradient of mixtures of MeOH–H_2O (30% MeOH, 40% MeOH, 50% MeOH, MeOH) gave **2** (15 mg).

Genipacetal (**1**): Colorless syrup. $[\alpha]_D^{32}$ +100.3° (c=2.0, MeOH). Positive FAB-MS *m/z*: 249 $[M+Na]^+$, negative FAB-MS *m/z*: 225 $[M-H]^-$, HR positive FAB-MS *m/z*: 249.0737 $[M+Na]^+$ (Calcd for $C_{11}H_{14}O_5Na$: 249.0739). ^1H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Genipamide (**2**): Colorless syrup. $[\alpha]_D^{32}$ +207.7° (c=1.4, MeOH). Positive FAB-MS *m/z*: 324 $[M+H]^+$, HR positive FAB-MS *m/z*: 324.1440 $[M+H]^+$ (Calcd for $C_{16}H_{22}NO_6$: 324.1447). ^1H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Genipaol (**3**): Colorless syrup. $[\alpha]_D^{27}$ +101.4° (c=1.1, MeOH). Negative FAB-MS *m/z*: 241 $[M-H]^-$, HR negaitive FAB-MS *m/z*: 241.0724 $[M-H]^-$ (Calcd for $C_{11}H_{13}O_6$: 241.0710). ^1H-NMR spectral data: see Table 1. ^{13}C-NMR spectral data: see Table 2.

Acetylation of 1 Compound **1** (5 mg) in Ac_2O–pyridine (1 : 1, 1 ml) was left to stand at room temperature overnight. After removal of the reagent under a stream of N_2, the residue was partitioned between ether and H_2O. The ether layer was concentrated to give **1a** (5 mg).

1a: Syrup. ^1H-NMR spectral data: see Table 1.

Computional Methods Calculations were performed using CAChe (Version 4.1.1) with extended MM2 parameters[12] (Fujitsu Co., Japan), which was run on a Macintosh Powerbook G3/400. Convergence was obtained when the difference in the energies between two successive interactions was less than 0.00001 kcal/mol. Drawing was performed using the Chem3D program (Cambridge Scientifics Computing Inc., Cambridge, MA, U.S.A.).

Acknowledgments We express our appreciation to Mr. K. Takeda of Kumamoto University for measurement of the NMR spectra. We thank Mr. H. Harazono of Fukuoka University for measurement of the FAB-MS.

References

1) Ueda S., Iwahashi Y., Tokuda Y., *J. Nat. Prod.*, **54**, 1677—1680 (1991).
2) Ono M., Ueno M., Masuoka C., Ikeda T., Nohara T., *Chem. Pharm. Bull.*, **53**, 1342—1344 (2005).
3) Jensen S. R., *Phytochemistry*, **22**, 1761—1765 (1983).
4) Zhao W. M., Xu J. P., Qin G. W., *Phytochemistry*, **37**, 1079—1082 (1994).
5) Inouye H., Takeda Y., Saito S., Nishimura H., Sakuragi R., *Yakugaku Zasshi*, **94**, 577—586 (1974).
6) Goto H., Osawa E., *Tetrahedron Lett.*, **33**, 1343—1346 (1992).
7) Karplus M., *J. Chem. Phys.*, **30**, 11—15 (1959).
8) Machida K., Onodera R., Furuta K., Kikuchi M., *Chem. Pharm. Bull.*, **46**, 1295—1300 (1998).
9) Machida K., Kazumi O., Ishii M., Kakuda R., Yaoita Y., Kikuchi M., *Chem. Pharm. Bull.*, **48**, 746—748 (2000).
10) Horeau A., Nouaille A., *Tetrahedron Lett.*, **22**, 1939—1942 (1971).
11) Ono M., Nishida Y., Masuoka C., Li J.-C., Okawa M., Ikeda T., Nohara T., *J. Nat. Prod.*, **67**, 2073—2075 (2005).
12) Allinger N. L., *J. Am. Chem. Soc.*, **99**, 8127—8134 (1977).