Studies on the Constituents of *Gardenia* Species. III.[1] New Iridoid Glycosides from the Leaves of *Gardenia jasminoides* cv. *fortuneana* HARA

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Two new iridoid glycosides, 7β,8β-epoxy-8α-dihydrogeniposide (1) and 8-epiapodantheroside (2), were isolated, together with six known (3—8) and three artifact (9—11) iridoids, from the leaves of *Gardenia jasminoides* cv. *fortuneana* HARA. Their structures were established based on chemical and spectral data.

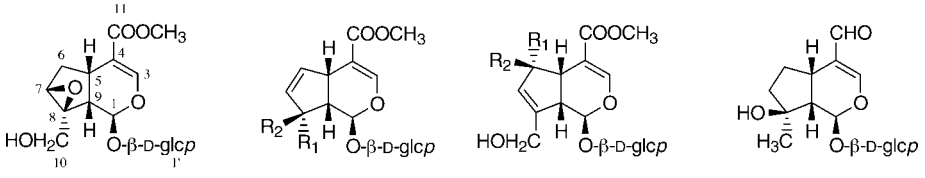
Key words *Gardenia jasminoides*; Rubiaceae; iridoid

We have reported the isolation of 13 new terpenoids from Gardeniae Fructus [the fruit of *Gardenia jasminoides* ELLIS (Rubiaceae)], known as the Shan-zhi-zi (in Chinese) herbal drug, and it has been used for its antiphlogistic, diuretic, and cholagogue effects.[1,2] In the course of further studies on the constituents of *Gardenia* species, we have now examined the iridoid constituents from the leaves of *G. jasminoides* cv. *fortuneana* HARA. This plant does not bear medicinal fruit, and there is no report, so far as we know, on the constituents of this plant. This paper describes the structural elucidation and identification of two new iridoid glycosides (**1**, **2**), isolated along with six known (**3**—**8**) and three artifact (**9**—**11**) iridoids from this plant. The known iridoid glycosides were identified as monotropein methyl ester (=galioside, **3**),[3—5] gardenoside (**4**),[4—6] deacetylasperulosidic acid methyl ester (**5**),[7—9] scandoside methyl ester (**6**),[9—11] geniposide (**7**)[6,12] and ixoroside (**8**),[13] respectively, by direct comparison with authentic samples and/or by comparison of various spectral and chemical data with those reported in the literature. Inouye *et al.* reported the following biosynthetic sequences: **7**→**5**→**3** and **7**→**6**→**4**, that is, **7** was a biosynthetic precursor of both **3** and **4**.[14] To our knowledge, this is the first example of co-occurrence of **3** and **4** in *Gardenia* species.

Compound **1** was obtained as an amorphous powder, $[\alpha]_D$ −43.4° (MeOH). The molecular formula of **1**, $C_{17}H_{24}O_{11}$, was confirmed by high-resolution (HR)-FAB-MS. In the ^{1}H- and ^{13}C-NMR spectra of **1**, signal patterns were similar to those of 6-deoxycatalpol, which also has a 7,8-epoxide,[15] except for the presence of a carbomethoxyl group [δ_H 3.70 (3H, s), δ_C 169.0, 51.9]. The location of the carbomethoxyl group on C-4 was deduced from the ^{1}H-detected heteronuclear multiple-bond connectivity (HMBC) correlation between H-3 [δ_H 7.48 (1H, d, J=0.3 Hz)] and δ_C 169.0. Furthermore, nuclear Overhauser enhancement spectroscopy (NOESY) correlations were observed between H-1 [δ_H 5.26 (1H, d, J=9.5 Hz)]/H-6α [δ_H 1.46 (1H, ddd, J=13.9, 10.2, 1.0 Hz) and H-10$_B$ [δ_H 4.22 (1H, d, J=12.9 Hz)], and H-7 [δ_H 3.48 (1H, br s)]/H-6α and H-10$_A$ [δ_H 3.79 (1H, d, J=12.9 Hz)]. From the above data, the structure of **1** was elucidated as shown in the chart and termed 7β,8β-epoxy-8α-dihydrogeniposide. Compound **1** was isolated from a natural source for the first time, although a partially acetylated derivative of **1** has been synthesized from **7**.[11] To our knowledge, this is the first report of an iridoid glycoside containing a 7,8-epoxide function from *Gardenia* species.

Compound **2** was obtained as an amorphous powder, $[\alpha]_D$ −128.6° (MeOH). The molecular formula of **2**, $C_{17}H_{24}O_{10}$, was confirmed by HR-FAB-MS. Its ^{1}H- and ^{13}C-NMR spectra were similar to those of **4**. The ^{13}C-NMR spectrum of **2**, however, lacked a signal from a C-8 oxygenated quaternary carbon [**4**: δ_C 86.2 (s)], and instead showed a signal of a methine carbon [δ_C 51.0 (d)] in **2**. Furthermore, the ^{1}H-NMR signal of the methine proton [δ_H 3.07 (1H, m)] in **2** was evidently coupled with H-9 (δ_H 2.72), which appeared as a double double doublet. The NOE difference experiment showed that irradiation at H-9 resulted in NOE enhancements at H-5 and H-8 (δ_H 3.07). There was also NOE enhancement between H-1 and H-10$_B$. Consequently, compound **2** was revealed to be the epimer at C-8 of apodantheroside,[16] and the structure of **2** was determined to be 8-epiapodantheroside.

Compound **9** was obtained as an amorphous powder, $[\alpha]_D$ −114.1° (MeOH). The molecular formula of **9**, $C_{18}H_{26}O_{11}$, was confirmed by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **9**, exhibited signal patterns very similar to those of **3**, except for the presence of signals due to a methoxyl group [δ_H 3.27 (3H, s), δ_C 51.8]. The ^{13}C-NMR signal at C-8 of **9** was shifted by +6.3 ppm (δ_C 92.4) in comparison with that of **3**, suggesting that the methoxyl group is located at the 8-



1

2: R_1=CH_2OH, R_2=H
3: R_1=OH, R_2=CH_2OH
4: R_1=CH_2OH, R_2=OH
9: R_1=OCH_3, R_2=CH_2OH

5: R_1=OH, R_2=H
6: R_1=H, R_2=OH
7: R_1=R_2=H
10: R_1=OCH_3, R_2=H
11: R_1=H, R_2=OCH_3

8

Chart 1

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OH group. This finding was supported by the HMBC correlation from δ_H 3.27 to C-8. Consequently, the structure of **9** was determined to be 8-*O*-methylmonotropein methyl ester.

Compounds **10** and **11** were obtained as an amorphous powder, $[\alpha]_D$ +49.0°, −83.0° (MeOH), respectively. Both of the molecular formulas of **10** and **11** were determined to be $C_{18}H_{26}O_{11}$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **10** and **11** exhibited signal patterns very similar to those of **5** and **6**, including the sign of their optical rotations (**5**: $[\alpha]_D$ +17.6°, **6**: $[\alpha]_D$ −47.6°), except for the presence of signals due to a methoxyl group [**10**: δ_H 3.23 (3H, s), δ_C 57.4. **11**: δ_H 3.44 (3H, s), δ_C 57.1], respectively. Comparison of the ^{13}C-NMR spectra of **10** and **11** and **5** and **6**, showed the expected downfield shift of C-6 (+9.6 and +7.8 ppm, respectively), and HMBC correlations between the methoxyl proton and C-6 of **10** and **11** were observed. Consequently, the structures of **10** and **11** were determined to be 6-*O*-methyldeacetylasperulosidic acid methyl ester and 6-*O*-methylscandoside methyl ester, respectively.

Compounds **9**, **10**, and **11** might be artifacts formed from **3**, **5**, and **6** during the extraction and isolation process, respectively. Treatment of compounds **3**, **5**, and **6** with MeOH containing a small amount of HCl (0.03%) at room temperature for 48 h gave compounds **9**, **10**, and **11**, respectively.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO DIP-360 digital polarimeter. UV spectra were recorded with a Beckman DU-64 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-GSX 400 (400 MHz, 100 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. MS were recorded on a JEOL JMS-DX 303 mass spectrometer. GLC was carried out on a Shimadzu GC-7A equipped with a flame ionization detector (FID).

Plant Material *G. jasminoides* cv. *fortuneana* Hara were collected near Sendai, Miyagi prefecture, Japan, in August 2001 and identified by one of the authors (M. Kikuchi). A voucher specimen (No. 2001-8-1) is held in the laboratory of M. Kikuchi.

Extraction and Isolation The fresh leaves of *G. jasminoides* cv. *fortuneana* Hara (1.5 kg) were extracted with MeOH at room temperature for 5 months. Evaporation of the solvent under reduced pressure provided the MeOH extract (55.0 g), and this extract was partitioned between $CHCl_3$ and H_2O. The H_2O-soluble fraction was concentrated under reduced pressure to produce a residue (44.0 g). The residue was passed through a Mitsubishi Diaion HP-20 column, and adsorbed material was eluted with H_2O and MeOH. The MeOH eluate fraction from the HP-20 column was concentrated, the residue (18.0 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (50 : 10 : 1, 30 : 10 : 1), and the eluate was separated into six fractions (frs. 1—6). Fraction 2 was re-chromatographed on Sephadex LH-20 (50% MeOH) and the eluate was separated into nine fractions (frs. 2-1—2-9). Fraction 2-2 was subjected to preparatory HPLC [column, Cosmosil 5C18-AR (10 mm i.d.×25 cm, Nacalai Tesque); mobile phase, MeOH–H_2O (1 : 3); RI detector; flow rate, 1.5 ml/min; column temperature, 35 °C] to give crude compounds **1**—**11**, which were purified by preparatory HPLC [column, Cosmosil 5SL (10 mm i.d.×25 cm, Nacalai Tesque); mobile phase, CH_2Cl_2–MeOH–H_2O (40 : 10 : 1); UV detector, 225 nm; flow rate, 1.5 ml/min; column temperature, 26 °C] to give **1** (18.5 mg), **2** (3.5 mg), **3** (2.8 mg), **4** (148.5 mg), **5** (28.0 mg), **6** (165.0 mg), **7** (27.5 mg), **8** (19.0 mg), **9** (12.5 mg), **10** (35.0 mg), and **11** (14.0 mg), respectively. The known iridoid glycosides were identified as monotropein methyl ester (**3**; $[\alpha]_D^{25}$ −53.1°),[3—5] gardenoside (**4**; $[\alpha]_D^{25}$ −127.1°),[4—6] deacetylasperulosidic acid methyl ester (**5**; $[\alpha]_D^{25}$ +17.6°),[7—9] scandoside methyl ester (**6**; $[\alpha]_D^{25}$ −47.6°),[9—11] geniposide (**7**; $[\alpha]_D^{25}$ +10.3°),[6,12] and ixoroside (**8**; $[\alpha]_D^{25}$ −131.7°),[13] respectively, by direct comparison with authentic samples and/or by comparison of various spectral and chemical data with those reported in the literature.

7β,8β-Epoxy-8α-dihydroxygeniposide (**1**): Amorphous powder. $[\alpha]_D^{25}$ −43.4° (c=0.554, MeOH). UV λ_{max} (MeOH) nm (log ε): 235 (4.02). FAB-MS m/z: 427 $(M+Na)^+$. HR-FAB-MS m/z: 427.1246 (Calcd for $C_{17}H_{24}O_{11}Na$: 427.1217). ^{1}H-NMR (CD_3OD) δ: 7.48 (1H, d, J=0.7 Hz, H-3), 5.26 (1H, d, J=9.5 Hz, H-1), 4.80 (1H, d, J=7.8 Hz, H-1′), 4.22 (1H, d, J=12.9 Hz, H-10$_B$), 3.91 (1H, dd, J=12.0, 2.0 Hz, H-6′$_B$), 3.79 (1H, d, J=12.9 Hz, H-10$_A$), 3.70 (3H, s, 11-COOCH_3), 3.63 (1H, dd, J=12.0, 6.3 Hz, H-6′$_A$), 3.48 (1H, br s, H-7), 2.74 (1H, ddd, J=10.2, 7.8, 7.3 Hz, H-5), 2.55 (1H, dd, J=13.9, 7.8 Hz, H-6β), 2.43 (1H, dd, J=9.5, 7.3 Hz, H-9), 1.46 (1H, ddd, J=13.9, 10.2, 1.0 Hz, H-6α). ^{13}C-NMR (CD_3OD) δ: 169.0 (C-11), 153.1 (C-3), 110.5 (C-4), 100.1 (C-1′), 95.9 (C-1), 78.8 (C-5′), 77.7 (C-3′), 74.9 (C-2′), 71.8 (C-4′), 68.5 (C-8), 63.0 (C-6′), 61.7 (C-10), 60.7 (C-7), 51.9 (11-COOCH_3), 42.3 (C-9), 35.1 (C-6), 31.7 (C-5).

8-Epiapodantheroside (**2**): Amorphous powder. $[\alpha]_D^{25}$ −128.6° (c= 0.0715, MeOH). UV λ_{max} (MeOH) nm (log ε): 235 (3.92). FAB-MS m/z: 389 $(M+H)^+$, 411 $(M+Na)^+$. HR-FAB-MS m/z: 389.1413 (Calcd for $C_{17}H_{25}O_{10}$, 389.1448). ^{1}H-NMR (CD_3OD) δ: 7.40 (1H, d, J=1.5 Hz, H-3), 6.01 (1H, ddd, J=5.8, 2.4, 2.2 Hz, H-6), 5.78 (1H, dt, J=5.8, 2.2 Hz, H-7), 5.66 (1H, d, J=4.1 Hz, H-1), 4.67 (1H, d, J=7.8 Hz, H-1′), 3.88 (1H, dd, J=12.2, 2.0 Hz, H-6′$_B$), 3.71 (3H, s, 11-COOCH_3), 3.71 (1H, m, H-10$_B$), 3.65 (1H, dd, J=12.2, 5.8 Hz, H-6′$_A$), 3.53 (2H, m, H-5, H-10$_A$), 3.07 (1H, m, H-8), 2.72 (1H, ddd, J=8.3, 8.3, 4.1 Hz, H-9). ^{13}C-NMR (CD_3OD) δ: 169.1 (C-11), 152.5 (C-3), 135.1 (C-7), 132.9 (C-6), 112.2 (C-4), 99.9 (C-1′), 95.4 (C-1), 78.5 (C-5′), 78.0 (C-3′), 74.8 (C-2′), 71.7 (C-4′), 63.8 (C-10), 62.8 (C-6′), 51.7 (11-COOCH_3), 51.0 (C-8), 43.3 (C-9), 40.2 (C-5).

8-*O*-Methylmonotropein Methyl Ester (**9**): Amorphous powder. $[\alpha]_D^{25}$ −114.1° (c=0.377, MeOH). UV λ_{max} (MeOH) nm (log ε): 235 (3.98). FAB-MS m/z: 441 $(M+Na)^+$. HR-FAB-MS m/z: 441.1358 (Calcd for $C_{18}H_{26}O_{11}Na$, 441.1372). ^{1}H-NMR (CD_3OD) δ: 7.38 (1H, d, J=1.2 Hz, H-3), 6.28 (1H, dd, J=5.9, 2.7 Hz, H-6), 5.80 (1H, dd, J=5.9, 1.9 Hz, H-7), 5.78 (1H, d, J=2.0 Hz, H-1), 4.62 (1H, d, J=8.1 Hz, H-1′), 3.88 (1H, dd, J=12.0, 1.7 Hz, H-6′$_B$), 3.71 (3H, s, 11-COOCH_3), 3.69 (2H, m, H-5, 6′$_A$), 3.68 (1H, d, J=12.0 Hz, H-10$_B$), 3.55 (1H, d, J=12.0 Hz, H-10$_A$), 3.27 (3H, s, 8-OCH_3), 2.78 (1H, dd, J=9.0, 2.0 Hz, H-9). ^{13}C-NMR (CD_3OD) δ: 168.8 (C-11), 151.9 (C-3), 138.0 (C-6), 133.0 (C-7), 111.7 (C-4), 99.8 (C-1′), 94.2 (C-1), 92.4 (C-8), 78.5 (C-5′), 78.0 (C-3′), 74.7 (C-2′), 71.6 (C-4′), 65.8 (C-10), 62.7 (C-6′), 51.8 (8-OCH_3), 51.2 (11-COOCH_3), 46.5 (C-9), 38.8 (C-5).

6-*O*-Methyldeacetylasperulosidic Acid Methyl Ester (**10**): Amorphous powder. $[\alpha]_D^{25}$ +49.0° (c=1.09, MeOH). UV λ_{max} (MeOH) nm (log ε): 234 (3.85). FAB-MS m/z: 441 $(M+Na)^+$. HR-FAB-MS m/z: 441.1358 (Calcd for $C_{18}H_{26}O_{11}Na$, 441.1372). ^{1}H-NMR (CD_3OD) δ: 7.61 (1H, d, J=1.2 Hz, H-3), 6.17 (1H, br d, J=2.0 Hz, H-7), 4.96 (1H, d, J=8.8 Hz, H-1), 4.70 (1H, d, J=7.8 Hz, H-1′), 4.47 (1H, dd, J=15.9, 1.5 Hz, H-10$_B$), 4.37 (1H, br ddd, J=6.1, 2.0, 1.0 Hz, H-6), 4.20 (1H, d, J=15.9 Hz, H-10$_A$), 3.82 (1H, dd, J=12.0, 2.0 Hz, H-6′$_B$), 3.74 (3H, s, 11-COOCH_3), 3.67 (1H, dd, J=12.0, 5.3 Hz, H-6′$_A$), 3.23 (3H, s, 6-OCH_3), 3.08 (1H, ddd, J=7.4, 6.1, 1.2 Hz, H-5), 2.53 (1H, dd, J=8.8, 7.4 Hz, H-9). ^{13}C-NMR (CD_3OD) δ: 169.5 (C-11), 155.1 (C-3), 152.9 (C-8), 127.6 (C-7), 108.2 (C-4), 101.8 (C-1), 100.8 (C-1′), 85.0 (C-6), 78.3 (C-5′), 77.9 (C-3′), 75.0 (C-2′), 71.4 (C-4′), 62.6 (C-6′), 61.8 (C-10), 57.4 (6-OCH_3), 51.8 (11-COOCH_3), 46.0 (C-9), 42.1 (C-5).

6-*O*-Methylscandoside Methyl Ester (**11**): Amorphous powder. $[\alpha]_D^{25}$ −83.0° (c=0.532, MeOH). UV λ_{max} (MeOH) nm (log ε): 235 (3.80). FAB-MS m/z: 441 $(M+Na)^+$. HR-FAB-MS m/z: 441.1358 (Calcd for $C_{18}H_{26}O_{11}Na$, 441.1372). ^{1}H-NMR (CD_3OD) δ: 7.40 (1H, d, J=0.7 Hz, H-3), 5.83 (1H, br t, J=2.0 Hz, H-7), 5.63 (1H, d, J=2.9 Hz, H-1), 4.59 (1H, d, J=8.1 Hz, H-1′), 4.28 (1H, dd, J=15.4, 0.8 Hz, H-10$_B$), 4.18 (1H, br d, J=15.4 Hz, H-10$_A$), 4.17 (1H, br s, H-6), 3.89 (1H, dd, J=12.0, 2.0 Hz, H-6′$_B$), 3.72 (3H, s, 11-COOCH_3), 3.65 (1H, dd, J=12.0, 5.9 Hz, H-6′$_A$), 3.44 (3H, s, 6-OCH_3), 3.30 (2H, m, H-5, 9). ^{13}C-NMR (CD_3OD) δ: 169.1 (C-11), 153.6 (C-3), 149.7 (C-8), 127.4 (C-7), 110.5 (C-4), 100.0 (C-1′), 95.2 (C-1), 90.1 (C-6), 78.5 (C-5′), 78.0 (C-3′), 74.7 (C-2′), 71.6 (C-4′), 62.8 (C-6′), 60.5 (C-10), 57.1 (6-OCH_3), 51.7 (11-COOCH_3), 47.6 (C-9), 39.2 (C-5).

Determination of Absolute Structures of Glucosyl Moieties in 1—11 Each of compounds **1**—**11** (*ca.* 1 mg) was refluxed with 4% HCl for 4 h. The reaction mixture was neutralized with Ag_2O, filtered, and excess Ag^+ in the filtrate was removed with H_2S. The solution was concentrated *in vacuo* and dried to give a glucose residue that was subjected to preparation of the corresponding thiazolidine derivative, followed by trimethylsilylation and GLC analysis, according to the reported procedure.[17] GLC conditions: column, G-column (Kagakuhin Kensa Kyokai, 1.2 mm i.d.×40 m); column temperature, 240 °C; carrier gas, N_2 (30 ml/min). D-glucose, t_R 39.4 min (ref.: L-glucose, t_R 41.2 min).

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References and Notes

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