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Studies of the Constituents of *Gardenia* Species. II.[1] Terpenoids from Gardeniae Fructus

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Four new terpenoids, gardenate A (1), 2-hydroxyethyl gardenamide A (2), (1*R*,7*R*,8*S*,10*R*)-7,8,11-trihydroxyguai-4-en-3-one 8-*O*-*β*-D-glucopyranoside (3) and Jasminoside F (4), were isolated from Gardeniae Fructus. Their structures were established on the basis of spectral analysis.

Key words Gardeniae Fructus; Rubiaceae; iridoid; guaiane-type sesquiterpene; safranal-type monoterpene

In a previous paper,[1] we reported the isolation and structural elucidation of nine new monoterpenoids, gardenamide A, 6*α*-butoxygeniposide, 6*β*-butoxygeniposide, 6″-*O*-*p*-*cis*-coumaroylgenipin gentiobioside, and jasminosides A—E, from Gardeniae Fructus. In the course of further studies on the constituents of this plant, four new terpenoids have been isolated. This paper deals with the structural elucidation and identification of these compounds. The isolation procedure is described in detail in the experimental section.

Compound **1**, named gardenate A, was obtained as an amorphous powder, $[\alpha]_D$ −206.8° (MeOH). Its molecular formula $C_{12}H_{18}O_6$ (obs. *m*/*z* 258.1076) was determined from the high resolution electron impact mass spectrum (HR-EI-MS). The ^{1}H- and ^{13}C-NMR spectra of **1** revealed the presence of a tri-substituted double bond [δ_H: 5.83 (1H, br dd, J=2.7, 1.5 Hz); δ_C: 144.5, 129.8], two oxymethylene [δ_H: 4.03 (2H, br s), 3.82 (1H, dd, J=11.0, 3.9 Hz), 3.69 (1H, dd, J=11.0, 6.8 Hz); δ_C: 63.7, 60.9], two carbomethoxy [δ_H: 3.69, 3.63 (each 3H, s); δ_C: 176.1, 175.1, 52.1, 52.0], three methine [δ_H: 3.49 (1H, ddd, J=8.1, 2.2, 1.0 Hz), 2.83 (1H, ddt, J=11.7, 10.2, 8.1 Hz), 2.71 (1H, ddd, J=11.7, 7.6, 1.0 Hz); δ_C: 53.9, 51.2, 43.1] and methylene [δ_H: 2.46 (1H, dddt, J=15.6, 8.1, 2.7, 1.0 Hz), 2.31 (1H, dddt, J=15.6, 10.2, 4.2, 2.2 Hz); δ_C: 36.4] groups. These data suggested that **1** was an iridoid derivative. Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of **1** were undertaken with the aid of ^{1}H–^{1}H shift correlation spectroscopy (COSY) and ^{1}H-detected multiple-bond correlation (HMBC) experiments and the planar structure of **1** was established to be as shown in Fig. 1. The stereochemistry of **1** was clarified by nuclear Overhauser effect spectroscopy (NOESY) and circular dichroism (CD) spectra. Nuclear Overhauser effect (NOE) was observed between 5-H and 9-H, suggesting these protons are on the same face. The absolute configurations at C-5 and C-9 of **1** were determined by application of the *β*,*γ*-unsaturated ketone Octant rule.[2] The CD spectrum of **1** showed a negative Cotton effect at 222.0 nm ($\Delta\varepsilon$ −12.3), suggesting that both C-5 and C-9 have *S*-configuration. From the above data, the structure of **1** was established to be as shown in Chart 1.[3]

Compound **2** was obtained as an amorphous powder, $[\alpha]_D$ +325.0° (MeOH). Its molecular formula $C_{13}H_{17}NO_5$ (obs. *m*/*z* 267.1109) was determined from the HR-EI-MS. Its ^{1}H- and ^{13}C-NMR spectral patterns were similar to those of gardenamide A,[1] except for the presence of an ethylene moiety (δ_C 61.0, 51.3) in **2**. Furthermore, the ^{13}C-NMR chemical shift at C-3 (δ 140.5) in **2** was shifted downfield by +8.4 ppm compared with that of gardenamide A, suggesting that the 2-hydroxyethyl group is located at the 2-nitrogen. This finding was supported by the HMBC correlations from 1′-H_2 to C-1 and 3-H to C-1′. Furthermore, an NOE interaction was observed between 5-H and 9-H. From the above data, the structure of **2** was deduced to be 2-hydroxyethyl gardenamide A.

Compound **3** was obtained as an amorphous powder, $[\alpha]_D$ −18.5° (MeOH). The FAB-MS exhibited an ion at *m*/*z* 457 $(M+Na)^+$. The ^{1}H-NMR spectral pattern closely resembled that of 8-epi-torilolone 8-*O*-*β*-D-glucopyranoside obtained by alkaline hydrolysis of 11-*O*-acetyl-8-epi-torilolone 8-*O*-*β*-D-glucopyranoside, which was isolated from *Torillis japonica* Fruit.[4] The ^{13}C-NMR spectrum of **3**, however, lacked a signal for the quaternary carbon at C-7 in 8-epi-torilolone 8-*O*-*β*-D-glucopyranoside and instead showed a signal characteristic of an oxygenated quaternary carbon at δ 80.3 in **3**. Furthermore, the location of an additional hydroxyl group at C-7 in **3** was suggested by downfield shifts of the signals due to C-6 [δ 33.0 (+3.7 ppm)], C-8 [δ 89.1 (+7.7 ppm)] and C-11 [δ 78.1 (+5.5 ppm)] on comparison of the ^{13}C-NMR spectrum of **3** and 8-epi-torilolone 8-*O*-*β*-D-glucopyranoside. Detailed analyses of the ^{1}H- and ^{13}C-NMR spectra of **3** were undertaken with the aid of ^{1}H–^{1}H COSY and HMBC experiments (Fig. 1). From these data, **3** was suggested to be a guaiane-type sesquiterpenoid, having a hydroxyl group at C-7. The stereochemistry of **3** was clarified from the NOESY experiment. NOEs were observed between 10-H/1-H and 8-H, suggesting that they were all on the same face (*β*) of the molecule. The isopropanol group at C-7 was determined to be on the same side as 8-H from the observed NOE interactions between 8-H/12-CH_3 (δ 1.39), one of the methylene protons at C-6 (δ 2.26)/8-H and 12-CH_3, and one of the methylene protons at C-6 (δ 2.99)/13-CH_3 (δ 1.36) and 15-CH_3 (δ 1.72). The absolute configuration at C-1 of **3** was determined as *R* from the CD curve and the sign of the optical rotation. The CD spectrum of **3** showed a positive Cotton effect at 312.0 nm ($\Delta\varepsilon$ +0.32) and a negative Cotton effect at 241.0 nm ($\Delta\varepsilon$ −3.2), which is a very similar CD curve to that of (1*R*)-4-guaien-3-one type sesquiterpenoids.[5] Furthermore, the optical rotation ($[\alpha]_D$ −18.5°) of **3** exhibited the same sign when compared with (1*R*)-4-guaien-3-one type sesquiterpenoids.[4,6] Therefore, **3** was determined to be (1*R*,7*R*,8*S*,10*R*)-7,8,11-trihydroxyguai-4-en-3-one 8-*O*-*β*-D-glucopyranoside.

Compound **4**, named jasminoside F, was purified as its

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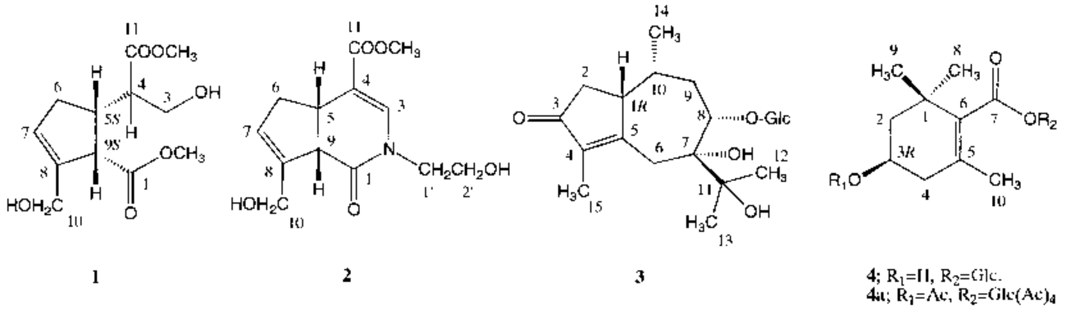
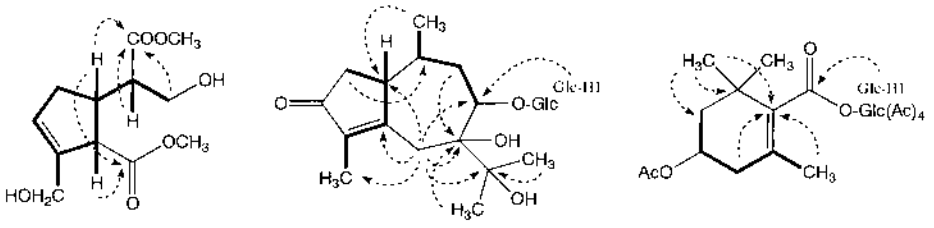


1 **2** **3** **4**; R$_1$=H, R$_2$=Glc. **4a**; R$_1$=Ac, R$_2$=Glc(Ac)$_4$

Chart 1



1 **3** **4a**

Fig. 1. The Main HMBC Correlations

Heavy lines indicate partial structures inferred from ^{1}H–^{1}H COSY.

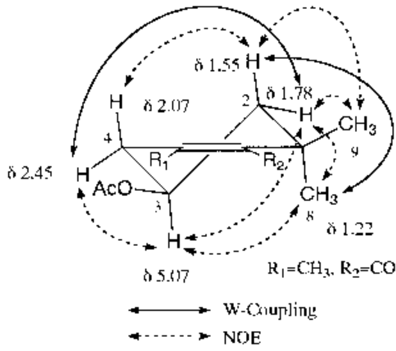


Fig. 2. NOE Correlations and W-Couplings of **4a**

pentaacetate (**4a**), $[\alpha]_D$ $-24.1°$ (MeOH). The ^{1}H- and ^{13}C-NMR spectral data of **4a** indicated structural similarity with picrocinic acid [4-(β-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxylic acid] isolated from the fruit of *Gardenia jasminoides* ELLIS forma *grandiflora* (LOUR.) MAKINO.[7] However, the anomeric carbon signal in **4a** was shifted upfield of *ca.* 10 ppm (δ 91.5) in comparison with that of an etherial bonded one. From this finding, including the number of acetyl groups of **4a**, the glucosyl group is on the ester moiety at C-7, and not on the hydroxyl moiety at C-3. The location of the glucosyl group on the ester moiety at C-7 was further supported from the HMBC spectrum; that is, the anomeric proton (Glc-H$_1$) showed an HMBC correlation with the ester carbonyl carbon (δ 167.8). From the above data, including the other HMBC correlations of **4a** (Fig. 1), the planar structure of **4** was established to be as shown in Fig. 1. The relative configuration of C-3 was determined from the ^{1}H–^{1}H COSY and NOESY experiments. As shown in Fig. 2, the ^{1}H–^{1}H COSY and NOESY spectra indicated 3-H to be quasi-axial with respect to the half-chair form of the cyclohexene. Furthermore, an NOE was observed between one of the quasi-axial methyl protons (δ 1.22) at C-1 and 3-H, showing that they were on the same face of the molecule. The absolute configuration of **4a** was determined from the CD spectrum. The CD spectrum of **4a** showed a negative Cotton effect at 237.4 nm ($\Delta\varepsilon$ -2.1), suggesting that the methylene proton (δ 2.07) at C-4 and the methyl protons (δ 1.22) at C-1 are β and α-quasi-axial, respectively.[8] Therefore, the absolute configuration at C-3 of **4a** was deduced as *R*. This was further confirmed by comparison of the sign of the optical rotation of similar monoterpenoids.[9] From this evidence, the full structure of **4** was established as shown in Chart 1.

Experimental

Optical rotations were determined with a JASCO DIP-360 digital polarimeter. UV Spectra were recorded with a Beckman DU-64 spectrometer. The CD spectra were obtained with a JASCO J-700 spectropolarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM-EX 270 (270 and 67.8 MHz, respectively) and JEOL JNM-GSX 400 (400 and 100 MHz, respectively) spectrometers. Chemical shifts are given in the δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; dd, double doublet; q, quartet; ddd, double double doublet; dddd, double double double doublet; m, multiplet; br, broad). MS were recorded on a JEOL JMS-DX 303 mass spectrometer (FAB-MS were obtained with glycerol as the matrix). Column chromatography was carried out on Kieselgel 60 (Merck; 70—230 mesh), Sephadex LH-20 (Pharmacia Fine Chemicals) and Cosmosil 75C$_{18}$-OPN (Nacalai Tesque). A preparative HPLC system (pump, CCPD; detecter, UV-8010) using Cosmosil 5C18-AR (Nacalai Tesque, 10 mm i.d.×25 cm) and TSK-gel Silica-60 (Tosoh, 7.8 mm i.d.×30 cm) columns was used. TLC was carried out with precoated Kieselgel 60 plates (Merck) and detection was achieved by spraying with 5% H_2SO_4 followed by heating.

Isolation Procedure Dried fruit of *Gardenia jasminoides* ELLIS forma *grandiflora* (LOUR.) MAKINO (1.0 kg) collected in Guangxi prefecture(広西), China were extracted with MeOH under reflux for 5.0 h. The MeOH extract was concentrated under reduced pressure and the residue was suspended in water (400 ml). The suspension was successively extracted with CHCl$_3$, Et$_2$O, EtOAc and *n*-BuOH. The CHCl$_3$-soluble fraction was concentrated under reduced pressure to produce a residue (29.8 g). The residue was chromatographed on a silica-gel column using *n*-hexane–EtOAc (4 : 1—1 : 1) and eluate was separated into twelve fractions (frs. 1—12). Fraction 11 was rechromatographed on a C18 open column using MeOH–H$_2$O (2 : 1) and the eluate was separated into twelve fractions (frs. 11-1—11-12). Fractions 11-2 and 11-3 were subjected to prep. HPLC [Cosmosil 5C18-AR column, MeOH–H_2O (1 : 1), flow rate: 1.5 ml/min, 220 nm] to give four peaks (peaks 1—4), respectively. Peak 1 was purified by prep. HPLC [Cosmosil 5C18-AR column, MeOH–H_2O (1 : 2), flow rate: 1.4 ml/min; 220 nm. TSK-gel Silica-60 column, *n*-hexane–acetone (3 : 2), flow rate: 1.5 ml/min; 205 nm] to give **1** (6.0 mg). Peak 2 was purified by prep. HPLC [Cosmosil 5C18-AR column, MeOH–H_2O (1 : 2), flow rate: 1.5 ml/min; 220 nm] to give **2** (3.0 mg). The *n*-BuOH-soluble fraction was concentrated under reduced pressure to produce a residue (51.0 g). The residue was chromatographed on a silica-gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and the eluate was separated into thirteen fractions (frs. 1—13). Fraction 3 was rechromatographed on a Sephadex LH-20 column using 50% MeOH and the eluate was separated into seventeen fractions (frs. 3-1—3-17). Fraction 3-2 was subjected to prep. HPLC [Cosmosil 5C18-AR column, MeOH–H_2O (1 : 1), flow rate: 1.2 ml/min, 242 nm] to give **3** (10.0 mg). Fraction 6 was rechromatographed on a Sephadex LH-20 column using 50% MeOH and the eluate was separated into seven fractions (frs. 6-1—6-7). Fraction 6-3 was subjected to prep. HPLC [Cosmosil 5C18-AR column, MeOH–H_2O (1 : 2), flow rate: 1.2 ml/min; 234 nm] to give crude **4**, which was acetylated with Ac_2O in pyridine. After the usual work-up, the crude product was purified by prep. HPLC [Cosmosil 5C18-AR column, MeOH–H_2O (3 : 1), flow rate: 1.5 ml/min; 230 nm] to give **4a** (4.0 mg).

Gardenate A (1) An amorphous powder, $[\alpha]_D^{26}$ −206.8° (c=0.46, MeOH). EI-MS *m/z*: 258 M^+. HR-MS *m/z*: 258.1076 (M^+) (Calcd for $C_{12}H_{18}O_6$: 258.1104). ^{1}H-NMR (400 MHz, CD_3OD) δ: 5.83 (1H, br dd, J=2.7, 1.5 Hz, 7-H), 4.03 (2H, br s, 10-H_2), 3.82 (1H, dd, J=11.0, 3.9 Hz, 3-H_B), 3.69 (1H, dd, J=11.0, 6.8 Hz, 3-H_A), 3.69 (3H, s, 11-$COOCH_3$), 3.63 (3H, s, 1-$COOCH_3$), 3.49 (1H, ddd, J=8.1, 2.2, 1.0 Hz, 9-H), 2.83 (1H, ddt, J=11.7, 10.2, 8.1 Hz, 5-H), 2.71 (1H, ddd, J=11.7, 6.8, 3.9 Hz, 4-H), 2.46 (1H, dddt, J=15.6, 8.1, 2.7, 1.0 Hz, 6-H_β), 2.31 (1H, dddt, J=15.6, 10.2, 4.2, 2.2 Hz, 6-H_α). ^{13}C-NMR (100 MHz, CD_3OD) δ: 175.1 (C-1), 63.7 (C-3), 51.2 (C-4), 43.1 (C-5), 36.4 (C-6), 129.8 (C-7), 144.5 (C-8), 53.9 (C-9), 60.9 (C-10), 176.1 (C-11), 52.1, 52.0 ($COO\underline{C}H_3$). CD (c=3.59×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): −12.3 (222.0).

2-Hydroxyethyl Gardenamide A (2) An amorphous powder, $[\alpha]_D^{26}$ +325.0° (c=0.16, MeOH). EI-MS *m/z*: 267 M^+. HR-MS *m/z*: 267.1109 (M^+) (Calcd for $C_{13}H_{17}NO_5$: 267.1107). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.39 (1H, s, 3-H), 5.83 (1H, br dd, J=2.9, 1.5 Hz, 7-H), 4.37 (2H, br s, 10-H_2), 3.74 (3H, s, 11-$COOCH_3$), 3.67 (5H, m, 9-H, 1′,2′-H_2), 3.52 (1H, ddd, J=8.5, 8.5, 8.3 Hz, 5-H), 2.84 (1H, dddd, J=14.6, 8.3, 2.9, 1.5 Hz, 6-H_B), 2.22 (1H, ddt, J=14.6, 8.5, 2.2 Hz, 6-H_A). ^{13}C-NMR (100 MHz, CD_3OD) δ: 171.6 (C-1), 140.5 (C-3), 111.0 (C-4), 38.9 (C-5), 40.8 (C-6), 128.2 (C-7), 143.9 (C-8), 50.2 (C-9), 61.8 (C-10), 168.9 (C-11), 52.0 ($COO\underline{C}H_3$), 51.3 (C-1′), 61.0 (C-2′).

(1*R*,7*R*,8*S*,10*R*)-7,8,11-Trihydroxyguai-4-en-3-one 8-*O*-β-D-Glucopyranoside (3) An amorphous powder, $[\alpha]_D^{26}$ −18.5° (c=0.27, MeOH). FAB-MS *m/z*: 457 $[M+Na]^+$. UV λ_{max}^{MeOH} nm (log ε): 242.0 (4.07). ^{1}H-NMR (270 MHz, CD_3OD) δ: 4.52 (1H, d, J=7.9 Hz, Glc-H_1), 4.05 (1H, dd, J=10.7, 2.8 Hz, 8-H), 3.84 (1H, dd, J=11.7, 2.2 Hz, Glc-H_{6B}), 3.64 (1H, dd, J=11.7, 5.3 Hz, Glc-H_{6A}), 3.13 (1H, dd, J=9.0, 7.9 Hz, Glc-H_2), 2.99 (1H, d, J=13.0 Hz, 6-H_α), 2.90 (1H, m, 1-H), 2.34 (1H, dd, J=17.4, 6.0 Hz, 2-H_B), 2.26 (1H, d, J=13.0 Hz, 6-H_β), 2.16 (1H, q, J=7.6 Hz, 10-H), 2.07 (1H, dd, J=17.4, 3.9 Hz, 2-H_A), 1.88 (1H, dd, J=13.9, 2.8 Hz, 9-H_B), 1.72 (3H, d, J=2.2 Hz, 15-CH_3), 1.64 (1H, dd, J=13.9, 10.7 Hz, 9-H_A), 1.39 (3H, s, 12-CH_3), 1.36 (3H, s, 13-CH_3), 1.03 (3H, d, J=7.6 Hz, 14-CH_3). ^{13}C-NMR (67.8 MHz, CD_3OD) δ: 48.0 (C-1), 38.5 (C-2), 211.9 (C-3), 141.6 (C-4), 175.7 (C-5), 33.0 (C-6), 80.3 (C-7), 89.1 (C-8), 34.9 (C-9), 31.1 (C-10), 78.1 (C-11), 26.7 (C-12), 26.2 (C-13), 20.7 (C-14), 9.0 (C-15), 105.9 (Glc-C_1), 75.2 (Glc-C_2), 78.2 (Glc-C_3), 71.6 (Glc-C_4), 78.3 (Glc-C_5), 62.8 (Glc-C_6). CD (c=1.3×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): +0.32 (312.0), −3.2 (241.0).

Jasminoside F Pentaacetate (4a) An amorphous powder, $[\alpha]_D^{26}$ −24.1° (c=0.29, MeOH). FAB-MS *m/z*: 579 $[M+Na]^+$. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.84 (1H, d, J=8.3 Hz, Glc-H_1), 5.18 (1H, dd, J=10.0, 9.3 Hz, Glc-H_4), 5.14 (1H, dd, J=9.3, 8.3 Hz, Glc-H_2), 5.26 (1H, t, J=9.3 Hz, Glc-H_3), 5.07 (1H, m, 3-H), 4.29 (1H, dd, J=12.2, 4.9 Hz, Glc-H_{6B}), 4.13 (1H, dd, J=12.2, 2.4 Hz, Glc-H_{6A}), 3.86 (1H, ddd, J=10.0, 4.9, 2.4 Hz, Glc-H_5), 2.45 (1H, dd, J=17.4, 6.0 Hz, 4-H_α), 2.07 (1H, m, 4-H_β), 1.99, 2.01, 2.04, 2.05, 2.06 (each 3H, s, $COCH_3$), 1.78 (1H, dd, J=12.3, 2.3 Hz, 2-H_α), 1.68 (3H, s, 10-CH_3), 1.55 (1H, m, 2-H_β), 1.22 (3H, s, 8-CH_3), 1.04 (3H, s, 9-CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 35.4 (C-1), 42.8 (C-2), 67.5 (C-3), 37.0 (C-4), 133.26 (C-5), 133.35 (C-6), 167.8 (C-7), 28.4 (C-8), 28.6 (C-9), 21.3 (C-10), 91.5 (Glc-C_1), 70.1 (Glc-C_2), 72.7 (Glc-C_3), 68.1 (Glc-C_4), 73.0 (Glc-C_5), 61.6 (Glc-C_6), 170.6, 170.5, 170.1, 169.4, 169.0 ($\underline{C}OCH_3$), 20.75, 20.65, 20.58, 20.5 ($CO\underline{C}H_3$). CD (c=3.17×10^{-4} M, MeOH) $\Delta\varepsilon$ (nm): −2.1 (237.4).

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