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Guaiane-type sesquiterpenoid glucosides from *Gardenia jasminoides* Ellis

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Two new guaiane-type sesquiterpenoid glucosides (1 and 2) were isolated from the fruit of *Gardenia jasminoides* Ellis. Their structures were elucidated to be (1*R*,7*R*,10*S*)-11-*O*-β-D-glucopyranosyl-4-guaien-3-one (1) and (1*R*,7*R*,10*S*)-7-hydroxy-11-*O*-β-D-glucopyranosyl-4-guaien-3-one (2) by one- and two-dimensional NMR techniques (^{1}H NMR, ^{13}C NMR, HSQC, HMBC and NOESY), MS, CD spectrometry and chemical methods.

Keywords: ^{1}H NMR; ^{13}C NMR; 2D NMR; CD; Rubiaceae; *Gardenia jasminoides*; Guaiane-type sesquiterpenoid

Introduction

The genus *Gardenia* (Rubiaceae) contains more than 250 species spread among the warm and tropical regions of the world. Five species are listed in Flora of China and are prescribed in traditional Chinese medicine as sedative, antipyretic, diuretic, cholagogic and anti-inflammatory drugs.[1] *G. jasminoides*, local Chinese name 'Zhi-zi', is one of the most popular *Gardenia* plants and also has been widely used as a yellow dye for staining foods and fabrics.[2,3] A number of iridoid glucosides, crocin, flavones and quinic acid derivatives have been isolated from the fruit of *G. jasminoides* and reported as active components.[4–7] Our precious investigation searching active constituents for anti-Alzheimer's disease led to the isolation of a series of iridoids glucosides and monoterpenoid glucosides.[8,9] The continuous study of this plant revealed two new guaiane-type sesquiterpenoid glucosides, which were rare among the genus *Gardenia*. The extensive application of one-dimensional (1D) NMR (^{1}H and ^{13}C NMR) and two-dimensional (2D) NMR (COSY, HSQC, HMBC and NOESY) techniques resulted in the structure elucidation and the ^{1}H and ^{13}C resonance assignments of the two glucosides. Furthermore, the detailed conformation analysis for the seven-membered ring was discussed based on the NOESY experiments and the coupling constants. The absolute configurations of **1** and **2** were assigned by comparing the results of chemical CD calculations and experimental CD spectra.

Results and Discussion

Compound **1** was obtained as a yellow gum, positive to the Molisch reaction. The HR-ESI-Q-TOF-MS gave $[M + Na]^+$ ion at *m/z* 421.2220, corresponding to the molecular formula $C_{21}H_{34}O_7$, with five degrees of unsaturation. Acid hydrolysis of **1** with 12% hydrochloric acid (HCl) furnished D-glucose, which was identified by gas chromatography analysis of the trimethylsilyl imidazole derivative.[10–12] The ^{1}H and ^{13}C NMR spectroscopic signals of the glucosyl unit were assigned based on the ^{1}H–^{1}H COSY and HSQC experiments. The remaining 15 carbon signals, belonging to four methyls, four methylenes, three methines and four quaternary carbons, suggested a skeleton of sesquiterpenoids. A proton spin system, H-6/H-7/H-8/H-9/H-10(H-14)/H-1, which was deduced from the ^{1}H–^{1}H COSY correlations, permitted us to establish the partial structure of cycloheptane. The unit of α,β-unsaturated cyclopentanone was elucidated by COSY correlations from H-1 to H_2-2 and HMBC cross-peaks at CH_3-15/C-3, C-4, C-5 and H-2/C-1, C-3, C-5. Briefly, a carbon skeleton of guaiane-type sesquiterpenoids was determined by key HMBC correlations at H-6/C-1, 4, 5 and CH_3-12, 13/C-7, 11 (Fig. 1). The location of the glucose residue was established to be at C-11 according to the HMBC cross-peak between the anomeric proton H-1′ (δ 4.51) and C-11 (δ 81.0). From all these observations, the gross structure of **1** was attributed to as shown in Fig. 2. With the constitution defined, a brief discussion of the ^{13}C NMR data is warranted. The low-field chemical shift of the olefinic carbon (C-5 at δ 182.5) was distinctive. It is known that sp^2 carbon atoms of alkenes substituted only by alkyl group absorb in the range of about 100–155 ppm. For **1**, a π electron-accepting substituent (carbonyl group) attached to the double bond induces a α-position deshielding (C-5), which was also attributed to the position of an exocylic bond of the seven-membered ring. Thus, the distinct chemical shift of C-5 was reasonable and characteristic in guaiane-type sesquiterpenoids.

The CD spectrum showed a positive Cotton effect at 313.0 nm and a negative Cotton effect at 232.8 nm (Fig. 3), suggesting that C-

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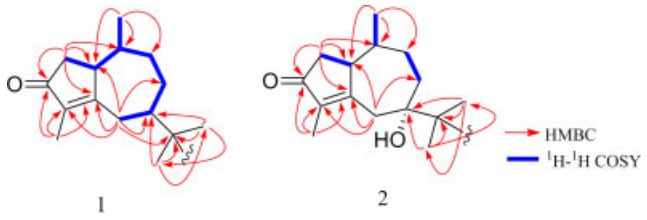

Figure 1. Key HMBC (→) and COSY (———) correlations of 1 and 2.

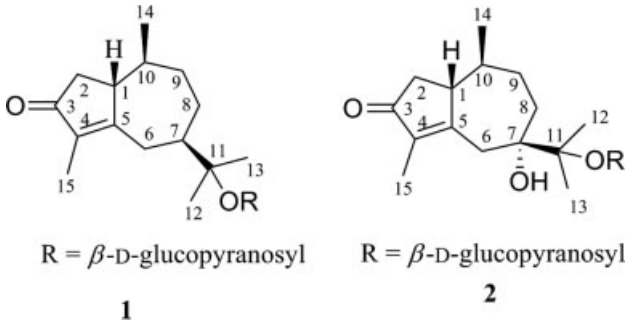

Figure 2. The structures of compounds 1 and 2.

1 has *R* configuration and H-1 was located as β orientation.[13,14] In NOESY spectrum, the significant correlations that were observed at H-1/CH_3-14 indicated β configuration for CH_3-14. Proton signal at δ 2.12 (1H, *t*, J = 11.5 Hz) for H-6b showed triplet, implying that there were a homoallylic coupling for H-6b/H-6a and a vicinal coupling for H-6b/H-7. The same coupling constant (J = 11.5 Hz) for H-6b/H-7 and H-6b/H-6a suggested H-6b were oriented β-quasi-axial, H-7 were oriented α-quasi-axial. From the above analyses, **1** appears more stable in conformation with the subsistent of C-7 equatorially oriented and cycloheptane ring in slightly distorted chair conformation, which was found in most sesquiterpenoids with a known stereochemistry[15,16] and confirmed by our NOESY experiment (Fig. 4).

In this study, the absolute configuration of C-1was assigned as *R* by comparing the CD data with the reported. To further confirm the deduction, CD computation for model compound (R = Me in **1**) was performed at the B3LYP/6-311++G(2d,p) level.[17,18] The shapes of the computed CD and experimental CD are almost the same (Fig. 3). The different $\Delta\varepsilon$ after 300 nm between the observed and the computed may be that the R was methyl used in the model computations instead of the sugar residue. As the size of Me is smaller than sugar residue, this may affect the geometry that had the lowest energy. The difference between the two geometries (R = Me and sugar residue) may lead to the CD differences between the recorded and the experimental

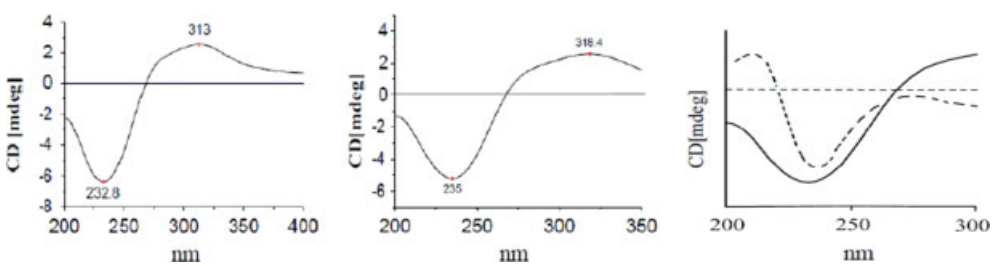

Figure 3. The CD spectrum of compounds 1–2 and computed CD curve.

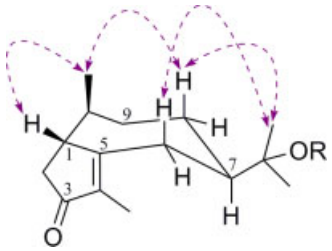

Figure 4. Key NOESY () correlations of 1.

one. However, the major $\Delta\varepsilon$ values near 240 nm are almost the same between the experimental and computational results. This exhibited the absolute configuration for **1** is the same as the predicted as above. Based on the results, **1** was elucidated as (1*R*,7*R*,10*S*)-11-*O*-β-D-glucopyranosyl-4-guaien-3-one.

Compound **2** was isolated as a yellow gum, which gave positive results to Molisch reaction. Its positive HR-ESI-Q-TOF-MS showed a pseudomolecular ion $[M + Na]^+$ at *m/z* 437.2123 (calcd 437.2151) compatible with molecular formula $C_{21}H_{34}O_8$ (M_w = 414). The ^{1}H and ^{13}C spectra of **2** were very similar to those of **1**, except that H-7 in **1** was replaced by hydroxy substituent in **2**. This was further confirmed by the high-frequency chemical shift of C-7 (δ 78.2) and the molecular formula evidence. The structure of **2** was assigned by detailed elucidation of ^{1}H–^{1}H COSY, HSQC and HMBC spectra (Fig. 1). The relative and absolutely configuration of **2** was determined as the same as **1** by the analysis of the NOESY and CD spectroscopic data (Fig. 3). Thus, **2** was elucidated as (1*R*,7*R*,10*S*)-7-hydroxy-11-O-β-D-glucopyranosyl-4-guaien-3-one.

Experimental

General procedure

Optical rotations were determined on a JASCO P-1020 digital polarimeter in CH_3OH. IR spectra were measured on a JASCO FT/IR-480 plus spectrometer. UV spectra were recorded in CH_3OH using a JASCO V-550 UV/Vis spectrometer. ESI-MS and HR-ESI-Q-TOF-MS spectra were obtained on a FINIGAN LCQ Advantage MAX mass spectrometer and Micromass Q-TOF mass spectrometer, respectively. The analytical HPLC was performed on a Dionex system equipped with a Dionex PDA-100 diode-array detector using a RP-18 column (5 μm, 4.6 mm × 250 mm; Purospher STAR). The preparative HPLC was carried on a Varian instrument equipped with UV detector (VARIAN Prostar 325, USA) and a RP-18 column (5 μm, 20 mm × 250 mm; Purospher STAR). Column chromatography was carried on macroporous adsorptive resins D101 (250–300 μm; Tianjin Pesticide Factory, China), silica gel (200–300 mesh; Qingdao Haiyang Chemical Group Corporation,

Table 1. NMR spectroscopic data (400 MHz for ^{1}H and 100 MHz for ^{13}C) for **1** and **2**

	1		**2**	
Position	δ_H (ppm), *J* (Hz)	δ_C (ppm)	δ_H (ppm), *J* (Hz)	δ_C (ppm)
1	3.04 br. s	48.4	3.02 br. s	48.5
2	2.35 dd (18.3, 6.0); 2.05 dd (18.1, 3.5)	38.3	2.32 m	38.6
3		212.2		213.4
4		137.4		142.3
5		182.5		175.4
6	3.26 m; 2.12 t (11.5)	30.7	3.08 m; 2.52 d (12.6)	36.5
7	1.47 m	53.0		78.2
8	1.99 br. m; 1.34 m	32.1	2.35 dd (13.2, 5.9); 1.80 br. d (12.1)	30.9
9	1.42 m; 1.09 m	31.0	1.76 m; 1.23 m	27.3
10	2.21 m	35.0	2.15 m	35.8
11		81.0		89.2
12	1.30 s	25.6	1.40 s	25.7
13	1.27 s	22.2	1.26 s	25.6
14	0.98 d (7.1)	20.4	0.96 d (7.0)	19.8
15	1.71 d (2.1)	7.8	1.70 d (2.0)	9.8
Glc-1′	4.51 d (7.7)	98.6	4.58 d (7.6)	98.0
2′	3.18 m	75.5	3.19 m	76.4
3′	3.36 m	78.6	3.09 m	77.7
4′	3.26 m	71.9	3.16 m	71.9
5′	3.24 m	77.6	3.29 m	78.8
6′	3.83 dd (12.1, 2.0); 3.63 dd (11.8, 5.4)	63.0	3.74 dd (11.5, 2.2); 3.46 dd (11.6, 5.7)	63.3

Qingdao, China), Sephadex LH-20 (Amersham Biosciences AB), Toyopearl HW-40 (Toyo Soda MFG) and ODS (60–80 μm; Merck). TLC was performed on silica gel GF_{254} plates (Qingdao Haiyang Chemical Group Corporation).

NMR spectra

NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer equipped with a 5-mm BBO z-gradient probe head. ^{1}H, ^{13}C NMR spectra (400 and 100 MHz, respectively) and 2D NMR experiments (COSY, HSQC, HMBC, NOESY) were recorded at 300 K using standard Bruker pulse programs (XWin-NMR version 3.5).

About 3.0–10.0 mg samples were dissolved in 0.6 ml deuterated solvents (CD_3OD), which was used as the internal lock. The chemical shifts were given in δ (ppm) scale and referenced to the solvent signal (δ (CHD_2OD) = 3.31 ppm for ^{1}H NMR and δ (CD_3OD) = 49.0 ppm for ^{13}C NMR).

For ^{1}H NMR, 8–16 transients were acquired with a 2.0-s relaxation delay, 32K data points, 3205 and 4006 Hz spectral width for **1** and **2** and 90° pulses (14.00 μs at 0 dB). FIDs were Fourier transformed with LB = 0.1 Hz and the spectra were zerofilled to 32K points. For ^{13}C NMR, 3000–10 000 transients requiring 2–8 h acquisition time were acquired with 2.0-s relaxation delay and 24 149 Hz spectral width and the 90° pulse (7.5 μs at −4 dB). Fourier transformed with LB = 1.0 Hz and the spectra were zerofilled to 32K points. The resulting spectra were manually phased, baseline corrected, calibrated and integrated using Xwin-NMR software.

^{1}H–^{1}H magnitude-mode ge-2D COSY spectrum was recorded over 1K data points in F2 and 256 data points in F1, using a 2.0-s relaxation delay and 4000 Hz spectral width in both dimensions. Window function for COSY spectra was Qsine (SSB = 0). The phase-sensitive ge-2D HSQC spectrum was recorded over 1K data points in F2 and 256 data points in F1, using a 2.5-s relaxation delay, 2394 Hz spectral width in F2 and 14 087 Hz in F1. Magnitude-mode ge-2D HMBC spectrum was recorded over 2K data points in F2 and 256 data points in F1, using a 2.0-s relaxation delay and 4000 Hz spectral width in F2 and 24 149 Hz in F1. The optimized coupling constants for HSQC and HMBC spectra were $^1J_{(C, H)}$ = 140 Hz and $^nJ_{(C, H)}$ = 10 Hz, respectively. Qsine (SSB = 2) was used for the window function of HSQC and HMBC processing. The phase-sensitive NOESY experiment was obtained using a mixing time of 300 ms, a 2.0-s relaxation delay and the 4000 Hz spectral width for both dimensions. The spectra were zerofilled to 1K data points, and Qsine (SSB = 3) was used for the window function.

Plant material

The fruit of *G. jasminoides* was collected from Guangzhou Qingping Medical Material Market, China, in April 2007 and authenticated by Professor Danyan Zhang, Guangzhou Chinese Medicine University. A voucher specimen (20 070 417) is deposited in the Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou, China.

Extraction and isolation

Dried fruit of *G. jasmonoides* (8.0 kg) was refluxed with 60% (v/v) EtOH for three times. After evaporation of EtOH *in vacuo*, the aqueous residue was subjected to column chromatography over D101 eluted with EtOH/H_2O to yield five fractions. Fraction 3 (EtOH/H_2O, 50 : 50 v/v) was separated by silica gel eluted with $CHCl_3$/MeOH gradiently to yield subfractions 3–7 ($CHCl_3$/MeOH, 9 : 1 v/v), which was submitted to repeated ODS, Toyopearl HW-40 columns eluted with MeOH/H_2O, followed by preparative HPLC (MeOH/H_2O, 4 : 6 v/v) to yield compounds **1** (3.0 mg) and **2** (15.7 mg).

(1R,7R,10S)-11-O-β-D-glucopyranosyl-4-guaien-3-one (**1**)

Yellow gum; $[\alpha]_D^{25.6} - 21.2$ (c 0.5, MeOH); UVλ_{max} (MeOH) nm (log ε): 244 (4.17); IR (KBr) ν_{max} (cm^{-1}) 3415, 2925, 1679, 1632, 1456, 1382, 1078; CD ($c = 0.025$, MeOH): 232.8 nm ($\Delta\varepsilon - 3.40$), 313 nm ($\Delta\varepsilon + 1.32$); ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) (Table 1); HR-ESI-Q-TOF-MS *m/z* 421.2220 $[M + Na]^+$ (calculated for $C_{21}H_{34}O_7Na$, 421.2202).

(1R,7R,10S)-7-hydroxy-11-O-β-D-glucopyranosyl-4-guaien-3-one (**2**)

Yellow gum; $[\alpha]_D^{23.2} - 15.8$ (c 0.5, MeOH); UVλ_{max} (MeOH) nm (log ε): 244 (4.24); IR (KBr) ν_{max} (cm^{-1}): 3441, 2926, 1644, 1454, 1393, 1038; CD ($c = 0.025$, MeOH): 235 nm ($\Delta\varepsilon - 3.00$), 318.4 nm ($\Delta\varepsilon + 1.36$); ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) (Table 1); HR-ESI-Q-TOF-MS *m/z* 437.2123 $[M + Na]^+$ (calculated for $C_{21}H_{34}O_8Na$, 437.2151).

Computational

The effect of the sugar moiety on ECD should be weak due to its flexibility and the lack of double bond near the stereogenic centers. ECD was computed using reasonable simplified model which is to use OMe to replace the sugar residue. Conformational search was performed using Amber force field and the conformations with low energy from 0 to 3 kcal/mol were optimized at the B3LYP/3-21G(d) level. Optimization for the selected lowest geometry was then performed at the B3LYP/6-311+G(d,p) level. This geometry was then used in ECD computations at the B3LYP/6-311++G(2d,p) level.

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