Immunosuppressive Iridoids from the Fruits of *Gardenia jasminoides*

Wen-Liang Chang,*,† Hua-Ying Wang,† Li-Shian Shi,†,‡ Jenn-Haung Lai,§ and Hang-Ching Lin†

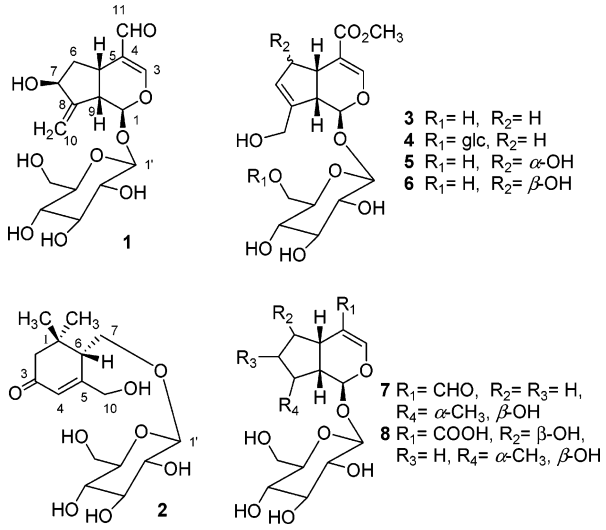
School of Pharmacy, National Defense Medical Center, National Defense University, Taipei, Taiwan 114, Republic of China, and Rheumatology/Immunology and Allergy, Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan 114, Republic of China

Received July 8, 2005

A new iridoid, gardaloside (**1**), and a new safranal-type monoterpene, jasminoside G (**2**), together with 10 known compounds including nine iridoids and a second safranal-type monoterpene, were isolated from the fruits of *Gardenia jasminoides*. The structures of **1** and **2** were established on the basis of spectroscopic evidence. Of these compounds, geniposide (**3**), 6α-hydroxygeniposide (**5**), ixoroside (**7**), and shanzhiside (**8**) showed significant inhibition of IL-2 secretion by phorbol myristate acetate and anti-CD28 monoclonal antibody co-stimulated activation of human peripheral blood T cells.

The dried ripe fruits of *Gardenia jasminoides* Ellis (Rubiaceae) are a traditional Chinese drug used as an antiphlogistic, choleretic, diuretic, hemostatic, and laxative in the treatment of traumatosis by external application.[1] A number of iridoid glycosides and crocetin have been isolated from *G. jasminoides* and reported as active components for increased bile secretion and hepatoprotection.[1−3] In a search for immunosuppressive agents from Chinese herbs, it was found that the MeOH crude extract of *G. jasminoides* specifically inhibited the CD28-co-stimulated activation of human peripheral blood T cells. These extracts showed inhibitory activity at doses of 50, 100, and 200 μg/mL of 52%, 34%, and 37% inhibition of IL-2 secretion, respectively, toward phorbol myristate acetate (PMA) and anti-CD28 monoclonal antibody (anti-CD28 mAb) co-stimulated activation of human peripheral blood T cells. The medium-polar fraction of this plant was investigated and led to the isolation and characterization of a new iridoid, gardaloside (**1**), and a new safranal-type monoterpene, jasminoside G (**2**), together with 10 known compounds including nine iridoids and another monoterpene.

The structures of the known compounds were identified as geniposide (**3**),[4] genipin gentiobioside (**4**),[4] 6α-hydroxygeniposide (**5**),[5] 6β-hydroxygeniposide (**6**),[5] ixoroside (**7**),[6] shanzhiside (**8**),[7] gardenoside (**9**),[8] 7β-hydroxysplendoside (**10**),[9] mussaenosidic acid (**11**),[10] and jasminoside B (**12**),[11] by comparing their spectroscopic data with those reported in the literature. Compounds **10**−**12** were isolated for the first time from this plant. In the present paper, we report the isolation and structural identification of **1** and **2** from *G. jasminoides* and their immunosuppressive effect toward human peripheral blood T cells.



Gardaloside (**1**) was obtained as a pale yellowish oil. The HRFABMS of **1** exhibited a sodiated molecular ion peak at *m/z* 381.1167 $[M + Na]^+$, consistent with the molecular formula $C_{16}H_{22}O_9$. The IR absorption band at 1626 cm^{-1} indicated the presence of an aldehyde group, which was confirmed by a ^1H NMR signal at δ 9.19 (s) and a resonance at δ 193.0 in the ^{13}C NMR spectrum. The NMR data of **1** were similar to ixoroside (**7**),[5] except for the presence of ^1H NMR signals at δ 5.35 (2H, s) and 4.31 (1H, m) in conjunction with ^{13}C NMR resonances at δ 152.4, 112.8, and 73.9, corresponding to an exocyclic methylene group and an oxygenated methine proton in **1**. Two additional functional groups were observed, an oxygenated methine proton at C-7 and an exocyclic methylene group at C-8, based on the key HMBC correlations observed from H-9 (δ 3.05) to C-10 (δ 112.8) and C-6 (δ 39.0) and from H-7 (δ 4.31) to C-10 (δ 112.8). These assignments were further supported by exocyclic methylene protons at δ 5.35 that showed long-range HMBC correlations with C-8 (δ 152.4), C-7 (δ 73.9), and C-9 (δ 44.8) and correlations exhibited between H-7 (δ 4.31) and H-9 (δ 3.05) in its NOESY spectrum. An anomeric proton signal at δ 4.64 (1H, d, J = 7.9 Hz) suggested the presence of a sugar residue with β-configuration and H-1 (δ 5.65) in the α-configuration. The relative configuration of a C-7 hydroxyl group was assigned as β, on the basis of the NOE correlation exhibited between H-7 (δ 4.31) and H-1 (δ 5.65). Thus, the structure of compound **1** was deduced as (1*S*,4a*S*,6*S*,7a*S*)-1,4a,5,6,7,7a-hexahydro-6-hydroxy-7-methylene-1-(*O*-β-D-glucopyranosyl)-cyclopenta[*c*]pyran-4-carbaldehyde.

Jasminoside G (**2**) was obtained as a pale yellowish oil. The HRFABMS of **2** showed a peak at *m/z* 347.1708 $[M + H]^+$ corresponding to the molecular formula $C_{16}H_{26}O_8$. The UV, IR, and ^1H NMR spectral data of **2** were similar to that of jasminoside B (**12**),[10] except the attachment of a β-D-glucopyranosyl moiety is in a different position. The

* To whom correspondence should be addressed.Tel: +886-2-87923100, ext. 18879. Fax: 886-2-87923169. E-mail: wlchang@ndmctsgh.edu.tw.
† National Defense University.
‡ Current address: Department of Biotechnology, National Formosa University, Huwei, Yunlin, Taiwan, Republic of China.
§ Tri-Service General Hospital.

10.1021/np0580816 CCC: $30.25
Published on Web 10/28/2005

attachment of the β-D-glucopyranosyl moiety in **2** was found to be at the C-7 position, on the basis of C-7 (δ 70.0) being shifted downfield by δ 7.89 and C-6 (δ 48.1) being shifted upfield by δ 2.54 compared with ^{13}C NMR data of jasminoside B (**12**). The HMBC spectrum showed a correlation of H-1′ (δ 4.22) to C-7 (δ 70.0) and a correlation between methyl protons (δ 1.14) and H-7 (δ 4.10 and 3.84) in its NOSEY spectrum, supporting the attachment of a β-D-glucopyranosyl moiety at the C-7 position. Thus, the structure of compound **2** was deduced as (*S*)-3-(hydroxymethyl)-5,5-dimethyl-4-[(*O*-β-D-glucopyranosyl)methyl]cyclohex-2-enone.

In the biological assay, six major iridoid compounds (**3**−**8**) showed significant inhibition of IL-2 secretion by human T cell-induced CD28-co-stimulated activation at 100 μg/mL. These compounds possess an iridoid glucoside moiety. The iridoid glycosides may be a kind of pharmacophore of the inhibitor of CD28-co-stimulated pathway speculative statement. Among them, compounds **3**, **5**, **7**, and **8** showed significant inhibition of IL-2 secretion at 50 μg/mL (Table 2). Compounds **7** and **8**, which possess a hydroxyl moiety at C-8, showed more potent activity than those of the other iridoid glucosides. The presence of a hydroxyl functionality at the C-8 position in iridoid glucosides is probably responsible for enhancing their inhibition activities.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 370 digital polarimeter. IR spectra were recorded in KBr disks on a Perkin-Elmer 983 G spectrophotometer. UV spectra were obtained on a Shimadzu UV-160 spectrometer. ^{1}H and ^{13}C NMR spectra were determined on a Bruker AM-500 spectrometer using DMSO-d_6 and MeOH-d_4, with TMS as internal standard, and 2D NMR spectra were recorded by using the Bruker standard pulse programs. FABMS were measured on a JEOL JMX-HX110 mass spectrometer.

Plant Material. "Shanzhizi", the fruits of *Gardenia jasminoides* Ellis, was supplied from Chien-Yuan Co., Taipei, Taiwan, during September 2002. The plant was identified by one of the authors (H.C.L.) at National Defense Medical Center, where a voucher specimen (NDMCP No. 910901) has been deposited.

Extraction and Isolation. The dried and powdered fruits (9.87 kg) of *G. jasminoides* were extracted with Me_2CO and MeOH (20 L × 4), successively at room temperature. The MeOH extracts were concentrated under reduced pressure to yield a black syrup (763.6 g), which was dissolved in 95% MeOH/H_2O (1 L), then partitioned (1:1) with *n*-hexane to give the *n*-hexane-soluble fraction (163.6 g) and a 95% MeOH/H_2O-soluble fraction (600.0 g). The 95% MeOH/H_2O-soluble fraction (300.0 g) was subjected to medium-pressure liquid chromatography (MPLC) (C_8 column; 50% MeOH/H_2O) to afford four fractions. The first fraction (194.0 g) was subjected to further MPLC (C_8 column; 30% MeOH/H_2O) to produce three subfractions. The first subfraction was chromatographed on a Lobar (RP-8) column (15% MeOH/H_2O) to give genipin gentibioside (**4**, 5.40 g) and 6α-hydroxygeniposide (**5**, 282.0 mg). The second subfraction was chromatographed by preparative HPLC (C_{18} column; mobile phase: 15% MeOH/H_2O; flow rate: 10 mL/min) to give nine compounds, 6β-hydroxygeniposide (**6**, 113.4 mg, t_R 36.6 min), ixoroside (**7**, 127.7 mg, t_R 28.9 min), shanzhiside (**8**, 120.4 mg, t_R 20.5 min), gardenoside (**9**, 16.2 mg, t_R 28.5), 7β-hydroxysplendoside (**10**, 9.3 mg, t_R 16.6 min), jasminoside B (**12**, 25.0 mg, t_R 31.3 min), mussaenosidic acid (**11**, 3.5 mg, t_R 26.0 min), gardaloside (**1**, 3.7 mg, t_R 23.3 min), and jasminoside G (**2**, 5.0 mg, t_R 30.5 min). The second subfraction was chromatographed on a Lobar (RP-8) column (30% MeOH/H_2O) to give geniposide (**3**, 22.1 g).

Gardaloside (1): pale yellow oil; $[\alpha]^{23}_D$ −69.6° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.9) nm; IR (KBr) ν_{max} 3367 (OH), 2927, 1626 (CHO), 1410, 1242, 1157 cm^{-1}; ^{1}H and ^{13}C NMR (MeOH-d_4, 500 and 125 MHz), see Table 1; FABMS (positive mode) *m/z* 381 $[M + Na]^+$ (12), 358 $[M + H]^+$ (2), 329 (20), 289 (11), 176 (100); HRFABMS *m/z* 381.1167 $[M + Na]^+$ (calcd for $C_{16}H_{22}NaO_9$, 381.1161).

Table 1. NMR Spectral Data for Compounds **1** and **2**[a]

	1		**2**	
position	δ_H[b]	δ_C	δ_H[b]	δ_C
1	5.65 d (3.8)	97.7		36.4
2			2.67 d (17.2)	49.8
			2.04 d (17.2)	
3	7.36 s	164.5		202.6
4		124.1	6.13 s	123.6
5	3.18[c]	29.5		168.7
6	2.16 ddd (12.6, 6.3, 4.4)	39.0	2.29 t (1.8, 8.2)	48.1
	1.86 ddd (12.6, 7.2, 7.2)			
7	4.31[c]	73.9	4.10 dd (11.1, 5.8)	70.0
			3.84 dd (11.1, 5.2)	
8		152.4	1.14 s	27.2
9	3.05[c]	44.8	1.04 s	28.9
10	5.35 s	112.8	4.46 dd (17.6, 1.4)	65.1
			4.18 dd (17.6, 1.3)	
11	9.19 s	193.0		
1′	4.67 d (7.9)	100.1	4.22 d (7.8)	104.5
2′	3.18[c]	74.7	3.12 d (7.8)	75.1
3′	3.25[c]	78.0	3.31[c]	78.0
4′	3.26[c]	71.6	3.24[c]	71.6
5′	3.32[c]	78.5	3.24[c]	78.2
6′	3.90 dd (11.7, 5.3)	62.8	3.86[c]	62.8
	3.63[c]		3.64 dd (11.7, 5.3)	

[a] Measured in MeOH-d_4. [b] Figures in parentheses denote *J* values (Hz). [c] Overlapped signals.

Table 2. Effect of Compounds from *G. jasminoides* on PMA and Anti-CD28 mAb-Induced IL-2 Secretion

	IL-2 secretion (100% cell survival)[a]	
compound	50 μg/mL	100 μg/mL
control	539 ± 170	539 ± 170
3	249 ± 97	216 ± 97
4	510 ± 161	370 ± 51
5	253 ± 11	171 ± 40
6	322 ± 105	186 ± 74
7	231 ± 45	120 ± 26
8	5 ± 5	5 ± 25

[a] 100% survival rate of T cells compared with the control in the MTT assay is shown.

Jasminoside G (2): pale yellow oil; $[\alpha]^{23}_D$ −75.6° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 237 (3.70) nm; IR (KBr) ν_{max} 3413 (OH), 1648 (C=O), 1384, 1078, 1038 cm^{-1}; ^{1}H and ^{13}C NMR (MeOH-d_4, 500 and 125 MHz), see Table 2; FABMS (positive mode) *m/z* 369 $[M + Na]^+$ (28), 347 $[M + H]^+$ (47), 329 (100), 319 (10); HRFABMS *m/z* 347.1708 $[M + H]^+$ (calcd for $C_{16}H_{27}O_8$, 347.1706).

Immunosuppression Assay. T lymphocytes were purified from whole blood by negative selection. The PMA and anti-CD28 monoclonal antibodies stimulated mimicked CD28 co-stimulation. The determination of IL-2 concentration of CD28-co-stimulated T cells was performed by ELISA assay.[12] The cytotoxicity was determined by MTT colorimetric assay.

Acknowledgment. We are grateful to the National Science Council, the Republic of China, for support of this research.

References and Notes

(1) Tang, W.; Eisenbrand, G. *Chinese Drugs of Plant Origin−Gardenia jasminoides Eillis*; Springer-Verlag: Berlin, 1992; pp 539−543.
(2) El-Naggar, L. J.; Beal, J. L. *J. Nat. Prod.* **1980**, *43*, 649−707.
(3) Parmar, V. S.; Sharma, S. K.; Poonam. *J. Sci. Ind. Res.* **2000**, *59* (11), 893−903.
(4) Endo, T.; Taguehi, H. *Chem. Pharm. Bull.* **1973**, *21*, 2684−2688.

(5) Miyagoshi, M.; Amagaya, S.; Ogihara, Y. *Planta Med.* **1987**, *53*, 462−464.
(6) Takeda, Y.; Nishimura, H.; Inouye, H. *Phytochemistry* **1975**, *14*, 2647−2650.
(7) Inouye, H.; Saito, S.; Shingu, T. *Tetrahedron Lett.* **1970** (41), 3581−3584.
(8) Bailleul, F.; Delaveau, P.; Rabron, A.; Plat, M.; Koch, M. *Phytochemistry* **1977**, *16*, 723−726.
(9) Jensen, S. R.; Nielsen, B. J. *Phytochemistry* **1982**, *21*, 1623−1629.
(10) Damtoft, S.; Hansen, S. B.; Jacobsen, B.; Jensen, S. R.; Nielsen, B. J. *Phytochemistry* **1984**, *23*, 2387−2389.
(11) Machida, K.; Onodera, R.; Furuta, K.; Kikuchi, M. *Chem. Pharm. Bull.* **1998**, *46*, 1295−1300.
(12) Lai, J. H.; Ho, L. J.; Kwan, C. Y.; Chang D. M.; Lee, T. C. *Transplantation* **1999**, *68*, 1383−1392.

NP0580816