Three New Sesquiterpene Glycosides from *Dendrobium nobile* with Immunomodulatory Activity

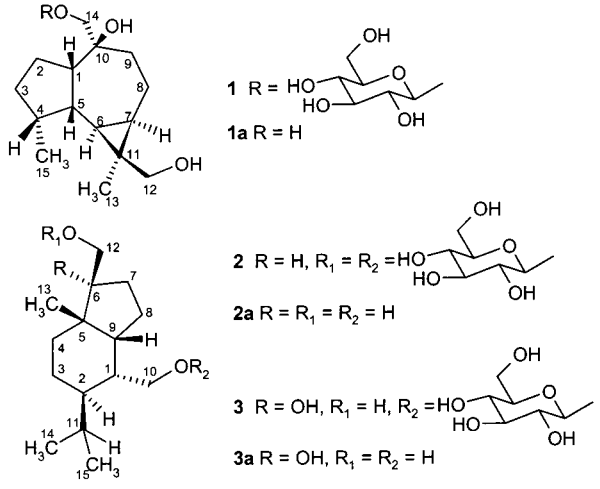
Weimin Zhao,[†,‡] Qinghua Ye,[†] Xiaojian Tan,[§] Hualiang Jiang,[§] Xiaoyu Li,[⊥] Kaixian Chen,[§] and A. Douglas Kinghorn*[,‡]

Shanghai Institute of Materia Medica, Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, People's Republic of China, and Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

Received May 30, 2001

Dendroside A (**1**) and dendronobilosides A and B (**2** and **3**), three new sesquiterpene glycosides, have been isolated from the stems of *Dendrobium nobile*, a plant used in Chinese traditional medicine. Their structures and stereochemistry were determined as 10*β*,12,14-trihydroxyalloaromadendrane 14-*O*-*β*-D-glucopyranoside (**1**), 10,12-dihydroxypicrotoxane 10,12-di-*O*-*β*-D-glucopyranoside (**2**), and 6α,10,12-trihydroxypicrotoxane 10-*O*-*β*-D-glucopyranoside (**3**), respectively, on the basis of spectroscopic and chemical methods. Quantum chemistry calculations were used in support of the structural determination of **1**. Compounds **1** and **2** were found to stimulate the proliferation of murine T and B lymphocytes in vitro, while compound **3** showed inhibitory activity in this same assay.

The stems of *Dendrobium nobile* Lindl. (Chinese name "Jin-Chai-Shi-Hu") (Orchidaceae) are a renowned traditional Chinese medicine used as a tonic, and extracts of the plant have been reported to possess immunostimulant activity.[1] Previously, several sesquiterpene alkaloids[2−4] and phenanthrenoids[5,6] have been identified from this species. Two phenanthrenoids from the aerial parts of the plant have been reported to exhibit antitumor activity.[6] In the present investigation to find additional biologically active compounds from *D. nobile*, three new sesquiterpene glycosides, named dendroside A (**1**) and dendronobilosides A (**2**) and B (**3**), have been isolated and characterized. Herein, we report the isolation, structure determination, and in vitro immunomodulatory activities of compounds **1**−**3**.



Results and Discussion

The fresh stems of *Dendrobium nobile* (2.5 kg) were percolated with 95% EtOH three times at room temperature. After evaporation of ethanol in vacuo, the aqueous residue was extracted with petroleum ether, ethyl acetate, and *n*-butanol, successively. The *n*-butanol extract was subjected to a series of column chromatographic steps, using reversed-phase C_{18} silica gel with a MeOH−water gradient, and finally passage over Sephadex LH-20 with EtOH, to afford pure compounds **1** (40 mg, 0.0016%), **2** (15 mg, 0.0006%), and **3** (70 mg, 0.0028%).

Table 1. ^{13}C NMR (100 MHz) Data of **1**−**3** in C_5D_5N (ppm)

position	**1**	**1** (DMSO-d_6)	**2**	**3**
1	54.4, d	53.4, d	36.6, d	36.7, d
2	24.4, t	32.1, t	39.4, d	39.3, d
3	29.4, t	28.7, t	20.2, t	20.4, t
4	38.4, d	38.9, d	27.3, t	30.3, t
5	40.1, d	39.5, d	42.0, s	46.7, s
6	23.6, d	22.7, d	51.7, d	85.7, s
7	29.6, d	28.9, d	26.5, t	34.6, t
8	18.5, t	17.8, t	22.7, t	21.7, t
9	33.0, t	23.8, t	48.7, d	45.5, d
10	75.1, s	74.1, s	71.5, t	71.9, t
11	25.1, s	24.2, s	27.1, d	27.1, t
12	62.8, t	61.6, t	71.6, t	78.7, d
13	24.5, q	24.1, q	23.9, q	18.1, q
14	79.8, t	78.3, t	22.0, q	21.9, q
15	16.6, q	16.4, q	15.6, q	15.6, q
Glc-1	106.0, d	104.2, d	105.3, d	105.3, d
Glc-2	75.1, d	73.6, d	75.4, d	75.2, d
Glc-3	78.4, d	76.4, d	78.9, d	78.8, d
Glc-4	71.4, d	70.1, d	72.0, d	72.0, d
Glc-5	78.3, d	76.9, d	78.7, d	78.7, d
Glc-6	62.4, t	61.1, t	63.1, t	63.1, t
Glc′-1			104.9, d	
Glc′-2			75.3, d	
Glc′-3			78.8, d	
Glc′-4			72.0, d	
Glc′-5			78.6, d	
Glc′-6			63.2, t	

Compound **1** was obtained as white amorphous powder, with an elemental formula of $C_{21}H_{36}O_8$ determined by HRFABMS (*m*/*z* 439.2321, $[M + Na]^+$). There was evidence of hydroxyl absorption in the IR spectrum at 3396 cm^{-1}. In the ^{13}C NMR spectrum (Table 1), 21 carbon signals were observed, constituted by two methyls, seven methylenes, ten methines, and two quaternary carbons. An anomeric

* To whom correspondence should be addressed. Tel: (312) 996-0914. Fax: (312) 996-4107. E-mail: kinghorn@uic.edu.
† Department of Phytochemistry, Shanghai Institute of Materia Medica.
‡ University of Illinois at Chicago.
§ Department of Medicinal Chemistry, Shanghai Institute of Materia Medica.
⊥ Department of Pharmacology, Shanghai Institute of Materia Medica.

10.1021/np0102612 CCC: $20.00
Published on Web 09/06/2001

Table 2. ^{1}H NMR (400 MHz) Data of **1**−**3** in C_5D_5N (ppm, *J* in Hz)

position	**1**	**1** (DMSO-d_6)	**2**	**3**
1	2.38, m	1.81, m	2.10, m	2.12, m
2	1.65 (α), m, 1.81 (*β*), m	1.40 (α), m, 1.68 (*β*), m	1.28, m	1.32, m
3	1.37 (α), m, 1.82 (*β*), m	1.19 (α), m, 1.70 (*β*), m	1.09, m, 1.35, m	1.18, m, 1.40, m
4	2.00, m	1.88, m	1.10, m, 1.15, m	1.16, m, 1.36, m
5	2.40, m	1.85, m		
6	0.40, dd (9.2, 9.2)	0.18, dd (8.9, 9.0)	2.05, m	
7	0.93, m	0.65, m	1.53, m, 1.92, m	2.02, m
8	1.95 (α), m, 2.25 (*β*), m	0.94 (α), m, 1.55 (*β*), m	1.57, m, 1.86, m	1.65, m, 2.08, m
9	1.70 (α), m, 2.08 (*β*), m	1.40 (α), m, 1.52 (*β*), m	2.15, m	3.00, m
10			3.90, m, 3.90, m	3.88, m, 4.02, m
11			1.90, m	1.88, m
12	4.07, d (11.2)	3.44, m,	3.62, dd (8.9, 8.6)	3.88, m, 4.02, m
	4.16, d (11.2)	3.48, d (4.3)	4.32, m	
13	1.45, s	1.02, s	1.07, s	1.30, s
14	3.78, d (10.4)	3.14, d (10.0)	0.92, d (6.8)	0.88, d (6.7)
	4.06, d (10.2)	3.51, d (10.4)		
15	1.13, d (6.7)	0.92, m	0.82, d (6.8)	0.78, d (6.7)
Glc-1	5.00, d (7.8)	4.10, d (7.8)	4.90, d (7.7)	4.88, d (7.6)
Glc-2	4.12, m	2.95, m	4.09, m	4.00, m
Glc-3	4.30, m	3.14, m	4.31, m	4.25, m
Glc-4	4.29, m	3.04, m	4.27, m	4.25, m
Glc-5	4.05, m	3.06, m	4.04, m	4.00, m
Glc-6	4.45, dd (11.7, 5.4)	3.64, m, 3.42, m	4.46, m, 4.64, m	4.40, dd (5.3, 5.3)
	4.63, dd (11.7, 1.9)			4.58, dd (2.5, 2.3)
Glc′-1			4.90, d (7.7)	
Glc′-2			4.09, m	
Glc′-3			4.31, m	
Glc′-4			4.27, m	
Glc′-5			4.04, m	
Glc′-6			4.46, m, 4.64, m	
OH-10		3.97, s		
OH-12		4.23, m		
Glc-2OH		5.06, d (3.9)		
Glc-3OH		4.92, m		
Glc-4OH		4.88, m		
Glc-6OH		4.50, t (11.5)		

carbon signal at δ_C 106.0 (d) indicated the existence of one sugar moiety in the structure of **1**. Enzymatic hydrolysis of **1** gave the aglycon **1a**, and glucose was determined as the sugar moiety by co-TLC with an authentic sample. In the ^{1}H NMR spectrum of **1** (Table 2), an anomeric proton signal was found at δ_H 5.00 (1H, d, J = 7.8 Hz), which indicated the glucose unit to be in the *β*-glycosidic form. The absolute configuration of glucose in **1** was found to belong to the D series by GC analysis of a peracetylated thiazolidine derivative.[7] Since there were 15 carbon signals belonging to the aglycon, this was consistent with **1** being a sesquiterpene glycoside. Preliminary inspection of the ^{1}H−^{1}H COSY, TOCSY, and HMQC NMR spectra of **1** enabled the deduction of the following structural fragment in its aglycon: −C-9, C-8, C-7, C-6, C-5, C-4(C-15), C-3, C-2, C-1, C-5−. Further analysis of the ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectra of **1** led to the assignment of an alloaromadendrane skeleton for the aglycon **1a**[8,9] and assignment of all proton and carbon signals in the parent molecule in C_5D_5N (Tables 1 and 2). In the NOESY spectrum of **1**, correlation signals were found between H-6 and H-7; H-6 and H-15; H-1 and H-5; H-4 and H-5; H-14 and H_{glc-1}; H-13 and H-6; and H-13 and H-7. Therefore, the relative configuration of all chiral carbons in the molecule could be determined except for that of the quaternary chiral center at C-10.

To propose the C-10 stereochemistry of **1**, NMR spectra were run with DMSO-d_6 as solvent. On the basis of the analysis of various 2D NMR spectra, all proton and carbon signals of **1** could be assigned (Tables 1 and 2). Due to the formation of a hydrogen bond between the hydroxyl group and DMSO-d_6, the OH-10 proton signal was observed at δ_H 3.97 (1H, s) in ^{1}H NMR spectrum of **1**. Correlation signals between OH-10 and H-1 and between OH-10 and H-5 in the NOESY spectrum suggested that OH-10 is in the *β* configuration.

To confirm the C-10 stereochemistry of **1**, quantum chemistry calculations were carried out on the two possible isomers, with the C-10 hydroxyl in either an α or a *β* configuration. The density functional theory method (B3LYP[10]/ 6-31G*) was employed to optimize the structures using the Gaussian98 program,[11] then NMR calculations were performed on the optimized geomeries at the same level using the gauge-independent atomic orbital (GIAO)[12] method. Meanwhile, the same procedure was applied on TMS to calculate the relative chemical shifts of these two isomers. The plots were made using experimental data versus theoretical values, and it was found that the correlation coefficients (R^2) for the ^{13}C and ^{1}H NMR spectra with the OH-10*β* configuration were 0.9908 and 0.9622, respectively. These values were higher than those for a OH-10α configuration (0.9900 and 0.9477). Some important computational results are summarized in Table 3. It can be seen from Table 3 that for the OH-10*β* configuration the chemical shifts deduced by the B3LYP/6-31G* program are closer to the experimental data actually obtained for **1**, while the corresponding values for the OH-10α configuration were more distant from the experimental values. In the OH-10*β* configuration, since the hydrogens having the same orientation as the hydroxyl are proximal to the oxygen atom, this would result in more downfield chemical shifts due to the deshielding effect of the oxygen atom. Comparing the experimental and theoretical data, it was apparent that the sesquiterpene glycoside **1** has a greater probability of adopting the OH-10*β* configuration. A sesquiterpene with a structure identical to that of **1a** has been

Table 3. Experimentally Observed NMR Data for **1** and Values Obtained by Quantum Chemistry Calculations (ppm)

	^{1}H			^{13}C		
	exptl	calcd values		exptl	calcd values	
no.	value	OH-10α	OH-10β	value	OH-10α	OH-10β
1	2.38	2.66	2.34	54.4	46.4	59.4
2	1.65	2.29	2.14	24.4	26.8	27.3
	1.81	1.65	2.21			
3	1.37	1.59	1.49	29.4	32.4	31.6
	1.82	1.55	1.77			
4	2.00	2.04	2.07	38.4	41.0	39.6
5	2.40	1.96	1.83	40.1	43.2	43.1
6	0.40	1.16	0.79	23.6	25.2	23.3
7	0.93	1.00	0.65	29.6	27.1	28.0
8	1.95	1.77	1.86	18.5	22.9	19.9
	2.25	1.61	2.21			
9	1.70	1.09	1.51	33.0	40.7	37.5
	2.08	2.21	1.88			
10				75.1	74.3	73.6
11				25.1	28.6	25.4

reported recently in two independent studies on the microbial biotransformation of alloaromadendrane-type sesquiterpene substrates.[8,9] Comparison of the ^{1}H and ^{13}C NMR data of **1a** (in CD_3OD) with these reported data further confirmed the structure of the aglycon of **1**. A similar alloaromadendrane sesquiterpene with a hydroxyl group substituted at C-13 instead of C-12 has been identified from *Wyethia arizonica* by Bohlmann et al.[13] Accordingly, compound **1** was determined structurally as 10β,12,14-trihydroxyalloaromadendrane 14-*O*-β-D-glucopyranoside. Since this is a new sesquiterpene glycoside, it has been assigned the trival name dendroside A.

Compound **2** was obtained as white amorphous powder, with an elemental formula of $C_{27}H_{48}O_{12}$ determined by HRFABMS (*m*/*z* 587.3017, $[M + Na]^+$). An IR hydroxyl peak was observed at 3386 cm^{-1}. In the ^{13}C NMR spectrum of **2**, 27 carbon signals including three methyls, eight methylenes, 15 methines, and one quaternary carbon were observed (Table 1). Enzymatic hydrolysis of **2** yielded glucose and its aglycon **2a**. In the ^{1}H NMR spectrum of **2** (Table 2), two anomeric proton signals were found to overlap at δ_H 4.90, with both appearing as a doublet with an identical coupling constant ($J = 7.7$ Hz). In turn, in the ^{13}C NMR spectrum (Table 1), two anomeric carbon signals were observed at δ_C 105.3 (d) and 104.9 (d), respectively. Therefore, it was concluded that the two glucose units both exhibit a β-glycosidic linkage to the aglycon **2a**. Analysis of ^{1}H−^{1}H COSY, TOCSY, and HMQC spectra of **2** led to the deduction of the following fragments in its aglycon: −C-14, C-11, C-15−; −C-4, C-3, C-2, C-1(C-10), C-8, C-9−, and −C-12, C-6, C-7−. Further analysis of the NMR data of **2** revealed a picrotoxane-type sesquiterpene skeleton.[14,15] The relative configuration of **2** was established according to its NOESY spectrum, in which correlation signals were found between H-10 and H-2; H-1 and H-11, H-13, H-14, and H-15; and H-13 and H-9 and H-12. To the best of our knowledge, the aglycon of **2** obtained by enzymatic hydrolysis is a new compound, and assignments of the ^{1}H and ^{13}C NMR signals of **2a** have been made on the basis of its ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, and HMBC NMR spectra. The positions of attachment of the two glucose units with the aglycon were determined to be at C-10 and C-12 according to correlation signals in the HMBC spectrum of **2**. Thus, the structure of **2** was assigned as 10,12-dihydroxypicrotoxane 10,12-di-*O*-β-D-glucopyranoside. Compound **2** is a new sesquiterpene diglycoside and has been given the trival name dendronobiloside A.

Table 4. Effect of Compounds **1**−**3** on Murine Lymphocyte Proliferation Induced by Concanavalin A (ConA) or Lipopolysaccharide (LPS) (5 μg/mL)[a]

		OD at 570 nm	
group		with Con A	with LPS
	concentration (M)	mean ± SD	mean ± SD
control		0.57 ± 0.03	0.58 ± 0.02
astragaloside I	10^{-7}	0.59 ± 0.04	0.65 ± 0.05
	10^{-6}	0.63 ± 0.02↑	0.65 ± 0.01↑
	10^{-5}	0.63 ± 0.04↑	0.63 ± 0.02↑
control		0.47 ± 0.01	0.45 ± 0.01
1	10^{-7}	0.64 ± 0.03↑↑	0.45 ± 0.02
	10^{-6}	0.62 ± 0.03↑	0.52 ± 0.01↑↑
	10^{-5}	0.50 ± 0.03	0.45 ± 0.04
2	10^{-7}	0.46 ± 0.02	0.49 ± 0.00↑
	10^{-6}	0.53 ± 0.03	0.50 ± 0.03
	10^{-5}	0.60 ± 0.03↑	0.56 ± 0.03↑↑
control		0.80 ± 0.02	0.56 ± 0.03
3	10^{-7}	0.68 ± 0.04↓	0.54 ± 0.04
	10^{-6}	0.68 ± 0.05↓	0.51 ± 0.06
	10^{-5}	0.71 ± 0.02↓	0.57 ± 0.04

[a] For protocols used, see Experimental Section ($n = 3$; ↑, ↓ $p < 0.05$; ↑↑, ↓↓ $p < 0.01$ compared with control group).

Compound **3** was obtained as white amorphous powder, with an elemental formula of $C_{21}H_{38}O_8$ determined by HRFABMS (*m*/*z* 441.2451, $[M + Na]^+$). In the ^{13}C NMR spectrum, 21 carbon signals including three methyls, seven methylenes, nine methines, and two quaternary carbons were observed (Table 1). On enzymatic hydrolysis of **3**, glucose was obtained as the only sugar component. The ^{1}H NMR spectrum of **3** revealed only one anomeric proton signal at δ_H 4.88 (1H, d, $J = 7.6$ Hz). Therefore, the glucose unit was assigned in the β configuration. The ^{13}C NMR data of **3** were similar to those of **2**, except for the loss of a methine group and a set of signals belonging to a glucose moiety, along with the appearance of a hydroxyl-substituted quaternary carbon signal at δ_C 85.7 (s). Analysis of the ^{1}H−^{1}H COSY and HMQC spectra of **3** led to the deduction of the fragments −C-14, C-11, C-15− and −C-4, C-3, C-2, C-1(C-10), C-9, C-8, C-7−. Further analysis of the HMBC spectrum of **3** permitted the aglycon to also be assigned with a picrotoxane-type skeleton.[14,15] The difference between the aglycons of **2** and **3** lay in the hydroxyl substitution at C-6 in the structure of **3**. The relative configuration of the aglycon of **3** was determined according to the NOESY spectrum, in which correlation signals were found between H-10 and H-2; H-9 and H-1; H-13 and H-1, H-9, and H-12; and H-1 and H-14 and H-15. The aglycon of **3** obtained by enzymatic hydrolysis also is a new compound, and assignments of ^{1}H and ^{13}C NMR signals of **3a** have been made on the basis of its ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, and HMBC NMR spectra. The ^{13}C−^{1}H long-range correlation signal between C-10 and H_{glc-1} in the HMBC spectrum and the correlation signal between H_{10} and H_{glc-1} in the NOESY spectrum of **3** indicated the linkage of the glucose unit to C-10 of the aglycon. Accordingly, the structure of **3** was established as 6α,10,12-trihydroxypicrotoxane 10-*O*-β-D-glucopyranoside. Compound **3** is a new sesquiterpene glycoside and has been given the trival name dendronobiloside B.

In a preliminary in vitro pharmacological evaluation (Table 4), it was found that dendroside A (**1**) stimulated significantly ($p < 0.01$) the proliferation of T lymphocytes of mice at concentrations of 1×10^{-7} M. Dendroside A (**1**) and dendronobiloside A (**2**) stimulated significantly ($p < 0.01$) the proliferation of B lymphocytes of mice at concentrations of 1×10^{-5} and 1×10^{-6} M, respectively. In contrast, dendronobiloside B (**3**) was found to inhibit the

proliferation of T lymphocytes of mice at concentrations of 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} M ($p < 0.05$).[16] Astragaloside I was used as a positive control and exhibited stimulant activity toward the proliferation of T and B lymphocytes at concentrations of 1×10^{-5} and 1×10^{-6} M ($p < 0.05$).[17] Previously, polysaccharides from *Dendrobium* species have been reported to stimulate the proliferation of T and B lymphocytes of mice.[18] This is the first report of small molecules with immunomodulatory activities from a *Dendrobium* species.

Experimental Section

General Experimental Procedures. The melting point (uncorrected) was determined on a Kofler apparatus. The optical rotations were measured with a Horiba Sepa-300 polarimeter. The IR spectra were recorded using a Perkin-Elmer 577 spectrometer. NMR spectra were run in C_5D_5N, DMSO-d_6, or $CDCl_3$ on a Bruker AM-400 spectrometer with TMS as internal standard. LRFABMS measurements were made with a Varian MAT 212 instrument, and the HRFABMS data were obtained on a VG 7070-HF spectrometer. ESIMS (negative-ion mode) and APCIMS (positive-ion mode) were measured using a Finnigan LCQ-DECA instrument. GC analysis was performed with a Shimadzu model GC-9A instrument equipped with an OV-17 column (3.1 mm i.d. × 2 m) and a He flame ionization detector and temperature programming from 170 to 210 °C at a rate of 10 °C/min; injection temperature, 270 °C; carrier gas, He (50 mL/min). Column chromatographic separations were carried out using silica gel H60 (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China) and RP-18 (100−200 mesh, Tianjin No. 2 Chemical Reagent Factory, Tianjin, People's Republic of China) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) were used for analytical TLC.

Plant Material. The plant material was collected in the suburbs of Chongqing in September 1998 and identified by Prof. Ming Zhang of Chongqing Institute of Traditional Chinese Medicine, Chongqing, Sichuan Province, People's Republic of China. A voucher specimen (No. SIMMW9809) is deposited at the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. The fresh stems of *Dendrobium nobile* (2.5 kg) were percolated with 95% EtOH (4000 mL × 3) at room temperature. The filtrate was concentrated in vacuo. The residue was partitioned with water and petroleum ether, EtOAc, and *n*-BuOH (1000 mL × 3), successively. The *n*-BuOH extract (15.0 g) was subjected to column chromatography over RP-18 eluted with water, and 20%, 40%, 60%, and 100% MeOH−water in sequence to afford five fractions (F1−F5). F4 was chromatographed further over an RP-18 column with 40%−60% MeOH in water as eluent and finally purified through a Sephadex LH-20 column eluted with EtOH to afford dendroside A (**1**, 40 mg, 0.0016% w/w) and dendronobilosides A (**2**, 15 mg, 0.0006%) and B (**3**, 70 mg, 0.0028%).

Dendroside A (1): white amorphous powder; mp 145−147 °C; $[\alpha]_D^{14}$ −48.6° (*c* 0.1, MeOH); IR ν_{max} 3396 (br, OH), 2949, 1641, 1454, 1383, 1265, 1163, 1078, 1028, 631 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; LRFABMS (positive ion mode) *m/z* 417 $[M + H]^+$ and 439 $[M + Na]^+$; HRFABMS (positive-ion mode) *m/z* 439.2321 $[M + Na]^+$ (calcd for $C_{21}H_{36}O_8Na$, 439.2307).

Enzymatic Hydrolysis of Dendroside A (1). Compound **1** (10 mg) and β-cellulase (10 mg; Lizhu Dongfeng Bio-Tech Co. Ltd., Shanghai, People's Republic of China) were dissolved in 5 mL of H_2O and kept at 37 °C for 3 days. The aqueous solution was then concentrated to dryness and chromatographed over silica gel, with $CHCl_3$−acetone (1:1) as eluent, to give **1a** (5 mg), and then with $CHCl_3$−MeOH−water (7:3:0.5) as eluent, to give glucose (2 mg), which was compared with an authentic standard by co-TLC (EtOAc−MeOH−H_2O−HOAc, 13:3:3:4, R_f 0.46).

Compound 1a: colorless gum; $[\alpha]_D^{24}$ −15.5° (*c* 0.2, MeOH); IR ν_{max} 3417 (br, OH), 2953, 2868, 1647, 1454, 1383, 1111, 1080, 1028 cm^{-1}; ^1H and ^{13}C NMR data of **1a** in CD_3OD were consistent with those of 10β,12,14-trihydroxyalloaromadendrane reported in the literature:[8] ^1H NMR (C_5D_5N, 400 MHz) δ 4.15 (1H, d, *J* = 11.1 Hz, H-12a), 4.05 (1H, d, *J* = 11.1 Hz, H-12b), 3.78 (1H, d, *J* = 10.3 Hz, H-14a), 3.68 (1H, d, *J* = 10.3 Hz, H-14b), 2.41 (1H, m, H-5), 2.40 (1H, m, H-1), 2.25 (1H, m, H-8a), 2.08 (1H, m, H-9a), 2.00 (1H, m, H-4), 1.95 (1H, m, H-8b), 1.82 (1H, m, H-2a), 1.81 (1H, m, H-3a), 1.79 (1H, m, H-9a), 1.65 (1H, m, H-2b), 1.40 (3H, s, H-13), 1.37 (1H, m, H-3b), 1.07 (3H, d, *J* = 6.8 Hz, H-15), 0.90 (1H, m, H-7), 0.38 (1H, dd, *J* = 9.1, 9.1 Hz, H-6); ^{13}C NMR (C_5D_5N, 100 MHz) δ 76.1 (s, C-10), 71.3 (t, C-14), 63.2 (t, C-12), 54.5 (d, C-1), 40.4 (d, C-5), 38.9 (d, C-4), 33.1 (t, C-9), 29.9 (d, C-7), 29.8 (t, C-3), 25.4 (s, C-11), 24.9 (t, C-2), 24.8 (q, C-13), 24.0 (d, C-6), 19.1 (t, C-8), 17.0 (q, C-15); ESIMS (negative-ion mode) *m/z* 253 $[M - H]^-$; APCIMS (positive-ion mode) *m/z* 255 $[M + H]^+$.

Dendronobiloside A (2): white amorphous powder; $[\alpha]_D^{20}$ −62.9° (*c* 0.6, MeOH); IR ν_{max} 3386 (br, OH), 2956, 2873, 1645, 1466, 1385, 1078, 1031 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 587 $[M + Na]^+$; HRFABMS *m/z* 587.3017 $[M + Na]^+$ (calcd for $C_{27}H_{48}O_{12}Na$, 587.3042).

Dendronobiloside B (3): white amorphous powder; $[\alpha]_D^{20}$ −63.6° (*c* 0.5, MeOH); IR ν_{max} 3396 (br, OH), 2929, 2874, 1645, 1456, 1383, 1163, 1078, 1028 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; FABMS *m/z* 419 $[M + H]^+$ and *m/z* 441 $[M + Na]^+$; HRFABMS *m/z* 441.2451 $[M + Na]^+$ (calcd for $C_{21}H_{38}O_8Na$, 441.2463).

Enzymatic Hydrolysis of Dendronobilosides A (2) and B (3). Compounds **2** (10 mg) and **3** (15 mg) were dissolved in 5 mL of H_2O, and β-cellulase (10 mg) was added to each solution and kept at 37 °C for 7 days. The aqueous solutions were then extracted with EtOAc, and each EtOAc extract was chromatographed over silica gel, with $CHCl_3$−acetone (5:1) used to afford **2a** (3 mg) and $CHCl_3$−MeOH (9:1) used to afford **3a** (8 mg). Each residual aqueous solution was concentrated to dryness and chromatographed over Si gel, with $CHCl_3$−MeOH−H_2O (7:3:0.5) as eluent, to give glucose (3 mg) from both **2** and **3**, which were identified by co-TLC with an authentic standard.

Compound 2a: colorless gum; $[\alpha]_D^{20}$ −22.0° (*c* 0.25, MeOH); IR ν_{max} 3331 (br, OH), 2955, 2872, 1674, 1464, 1385, 1082, 1032 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 3.82 (1H, m, H-12), 3.77 (1H, m, H-10), 3.51 (1H, dd, *J* = 8.8, 8.8 Hz, H-10), 3.40 (1H, dd, *J* = 9.5, 9.6 Hz, H-12), 1.91 (1H, m, H-8), 1.88 (1H, m, H-9), 1.84 (1H, m, H-7), 1.82 (1H, m, H-6), 1.80 (1H, m, H-11), 1.78 (1H, m, H-1), 1.64 (1H, m, H-7), 1.45 (1H, m, H-3), 1.42 (1H, m, H-8), 1.28 (1H, m, H-2), 1.20 (1H, m, H-4), 1.12 (1H, m, H-3), 1.06 (3H, s, H-13), 0.90 (3H, d, *J* = 7.0 Hz, H-14), 0.80 (3H, d, *J* = 6.6 Hz, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 64.6 (t, C-10), 64.5 (t, C-12), 53.9 (d, C-6), 48.3 (d, C-9), 41.9 (s, C-5), 38.9 (d, C-2), 38.4 (d, C-1), 27.3 (t, C-4), 26.9 (d, C-11), 25.6 (t, C-8), 23.9 (q, C-13), 22.4 (t, C-7), 21.7 (q, C-14), 19.8 (t, C-3), 15.4 (q, C-15); EIMS *m/z* 240 $[M]^+$ and *m/z* 222 $[M - H_2O]^+$.

Compound 3a: colorless gum; $[\alpha]^{20}{}_D$ −68.5° (*c* 0.21, MeOH); IR ν_{max} 3415 (br, OH), 2955, 1655, 1456, 1381, 1159, 1068, 1047 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) δ 4.09 (1H, dd, *J* = 4.7, 4.8 Hz, H-10), 4.02 (1H, d, *J* = 10.9 Hz, H-12), 3.90 (1H, d, *J* = 10.7 Hz, H-12), 3.65 (1H, t, *J* = 9.2, 9.9 Hz, H-10), 3.11 (1H, m, H-9), 2.10 (1H, m, H-8), 2.08 (2H, m, H-7), 2.04 (1H, m, H-11), 2.00 (1H, m, H-1), 1.76 (1H, m, H-8), 1.44 (1H, m, H-3), 1.42 (1H, m, H-4), 1.40 (1H, m, H-2), 1.38 (3H, s, H-13), 1.22 (1H, m, H-3), 1.20 (1H, m, H-4), 0.91 (3H, d, *J* = 6.9 Hz, H-14), 0.82 (3H, d, *J* = 7.0 Hz, H-15); ^{13}C NMR (C_5D_5N, 100 MHz) δ 85.3 (s, C-6), 65.9 (t, C-12), 63.2 (t, C-10), 46.4 (s, C-5), 45.2 (d, C-9), 39.2 (d, C-2), 38.8 (d, C-1), 34.4 (t, C-7), 30.0 (t, C-4), 26.8 (d, C-11), 21.5 (q, C-14), 21.3 (t, C-8), 20.2 (t, C-3), 17.8 (q, C-13), 15.3 (q, C-15); ESIMS *m/z* 279 $[M + Na]^+$.

Configuration of D-Glucose Obtained on the Hydrolysis of 1−3. A solution in pyridine (100 μL) of each hydrolyzed sugar from **1**−**3** (0.04 mol/L) and L-cysteine methyl ester hydrochloride (0.06 mol/L) was mixed and warmed at 60 °C for 1 h. Acetic anhydride (150 μL) was then added, and each

mixture warmed at 90 °C for another 1 h. After evaporation of pyridine and acetic anhydride in vacuo, each residue was dissolved in acetone (350 μL) and the solution (1 μL) was subjected to GLC.[7] A peak for a peracetylated thiazolidine derivative with retention time at 9.39 min was observed for all three samples, which was identical to the derivative of authentic D-glucose prepared in the same manner.

Lymphocyte Proliferation Test. The prepared spleen cells of mice (4×10^5) were seeded into each well of a 96-well microplate, and 5 μg/mL of concanavalin A (Con A, from *Canavalia ensiformis* Type III, Sigma) or lipopolysaccharide (LPS, from *Escherichia coli*, Sigma) was added alone or in combination with various concentrations of compounds **1**−**3**. The plates were cultured at 37 °C with 5% CO_2 in a humidified atmosphere for 48 h. Cell proliferation was assayed by the MTT method.[16] Briefly, 4 h before the termination of each culture, 20 μL of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was added to each well (the final MTT concentration was 0.5 μg/mL). At the end of the culture stage, 100 μL of dissolving solution (10% SDS−50% *N*,*N*-dimethylformamide) was added, then the plates were incubated for a further 6−7 h in the incubator to let the purple formazan dye dissolve. Absorbance at a wavelength of 570 nm in each well was determined in a multiwell spectrophotometer. Results were the means of triplicate samples ± SD. Independent two-tailed Student *t*-tests were performed. Astragaloside I was used as a positive control.[17]

Acknowledgment. W.-M.Z. thanks the Chinese Academy of Sciences for financial support. We gratefully acknowledge additional financial support from the National Natural Science Foundation of the People's Republic of China (grant 29725203) and the "863" High Technology Program of the People's Republic of China (grant 863-103-04-01). We thank Prof. Ming Zhang of Chongqing Insitute of Traditional Chinese Medicine, Sichuan Province, People's Republic of China, for the collection and identification of the plant material. We acknowledge Dr. K. Fagerquist, Mass Spectrometry Facility, Department of Chemistry, University of Minnesota, Minneapolis, MN, for the high-resolution mass spectral data. The authors are grateful to the Network Information Center, Chinese Academy of Sciences, for providing computational facilities.

References and Notes

(1) Jiangsu New Medical College. *Dictionary of Chinese Medicines*; Shanghai Scientific and Technologic Press: Shanghai, 1986; pp 586−590.
(2) Chen, K. K.; Chen, A. L. *J. Biol. Chem.* **1935**, *111*, 653−665.
(3) Suzuki, I.; Nakashima, T.; Nagasawa, N. *Chem. Pharm. Bull.* **1965**, *13*, 713−715.
(4) Okamota, T.; Natsume, M.; Onaka, T.; Uchimaru, F.; Shimizu, M. *Chem. Pharm. Bull.* **1972**, *20*, 418−421.
(5) Talapatra, B.; Mukhopadhyay, P.; Chaudhury, P.; Talapatra, S. K. *Indian J. Chem. Soc.* **1982**, *21B*, 386-387.
(6) Lee, Y. H.; Park, J. D.; Baek, N. I.; Kim, S. I.; Ahn, B. Z. *Planta Med.* **1995**, *61*, 178−180.
(7) Hara, S.; Okabe, H.; Mihashi, K. *Chem. Pharm. Bull.* **1987**, *35*, 501−506.
(8) Miyazawa, M.; Uemura, T.; Kameoka, H. *Phytochemistry* **1995**, *40*, 793−796.
(9) Lima, D. P.; Carnell, A. J.; Roberts, S. M. *J. Chem. Res., Synop.* **1999**, 396−397.
(10) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.
(11) *Gaussian 98*, Revision A.7; Guassian, Inc.: Pittsburgh, PA, 1998.
(12) Wolinski, K.; Hilton, J. F.; Pulay, P. *J. Am. Chem. Soc.* **1990**, *112*, 8251−8260.
(13) Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Planta Med.* **1984**, *50*, 195−196.
(14) Fischer, N. H.; Olivier, E. J.; Fischer, H. D. *Prog. Chem. Org. Nat. Prod.* **1979**, *38*, 268−273.
(15) Inubushi, Y.; Tsuda, Y.; Katarao, E. *Chem. Pharm. Bull.* **1966**, *14*, 668−671.
(16) Niks, M.; Otto, M. *J. Immunol. Methods* **1990**, *130*, 149−151.
(17) Bedir, E.; Pugh, N.; Calis, I.; Pasco, D. S.; Khan, I. A. *Biol. Pharm. Bull.* **2000**, *23*, 834−837.
(18) Zhao, Y. L.; Wang, S. L.; Li, X. Y. *Acta Bot. Yunnan.* **1994**, *16*, 392−396.

NP0102612