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Sesquiterpene amino ether and cytotoxic phenols from *Dendrobium wardianum* Warner

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ABSTRACT

A new bibenzyl derivative, dendrocandin V (**1**) and a new sesquiterpene amino ether, wardianumine A (**2**), together with eleven known compounds, including phenanthrenes (denbinobin (**3**), 9,10-dihydro-denbinobin (**4**), mostatin (**5**), loddigesiinols A (**6**)), bibenzyls (moscatilin (**7**), 5-hydroxy-3,4′-dimethoxybibenzyl (**8**), 3,4-dihydroxy-5,4′-dimethoxy bibenzyl (**9**), dendrocandin A (**10**), gigantol (**11**), dendrocandin U (**12**)) and an alkaloids (dihydroshihunine, **13**) were isolated from the EtOH extraction of stems of *Dendrobium wardianum* Warner. Isolation of the new compound **2** indicated that *N*,*N*-dimethylethanolamine as the key adduction in the synthesis of dendroxine and its analogs in *Dendrobium* species. The hypothetical biosynthetic pathway of **2** was then postulated. Inspired by literature and traditional usage of the herbal medicine, some compounds were sent for cytotoxic activity and the results indicated that compounds **1**, **3**, **4**, **5** showed cytotoxic activities against five human cancer cell lines (HL-60, A-549, SMMC-7721, MCF-7, and SW-480) with IC50 from 2.33–38.48 μM. Among those compounds, **3** and **4** showed cell line selectivity with strong activity comparable to DDP.

1. Introduction

The stems of several *Dendrobium* species (Orchidaceae) are used as "Shihu" in traditional Chinese medicine (TCM), which is known to provide medical benefits of promoting secretion of saliva, reducing fever, and enhancing immunity [1]. Many bioactive components of *Dendrobium* are reported, including bibenzyls, sesquiterpenes, phenanthrenes, alkaloids and so on [2].

Dendrobium wardianum Warner are mainly distributed in southern Yunnan, China and some Southeast Asian countries, such as Myanmar, Vietnam, Thailand and northeast India. Because the main producing areas in Tengchong and the shape of the raw medicine, it is also called "Tengchong Shihu" and "Bian huang cao" [3,4]. To date, only alkaloids, sesquiterpenes and flavones has been reported from the species [5]. In continuation of the chemical and pharmacological investigation on *D. wardianum* Warner, a new bibenzyl derivative, dendrocandin V (**1**) and a new sesquiterpene amino ether, wardianumine A (**2**) (Fig. 1), together with eleven known compounds, including phenanthrenes (**3**–**6**), bibenzyls (**7**–**12**), and an alkaloid (**13**) were isolated from the 90% EtOH extract of the stems of this plant. In view of the individual characteristics of alkaloid constituents in *Dendrobium*, an amine compound and a pyrrole alkaloid were obtained for the first time from the species in this study. The results provide a basis for further understanding of the secondary metabolite composition and synthesis pathway of alkaloids in *Dendrobium*.

Alkaloid such as dendrobine was isolated from *Dendrobium nobile* in 1932 [2]. There are 19 alkaloids which derived from picrotoxine sesquiterpene have been isolated from *Dendrobium* species from then on [2]. Reductive amination of sesquiterpene aldehyde precursor with methylamine was assumed as the key step for the formation of this kind of alkaloid [6]. Differed from dendroxine and dendrobine, the last step for the formation of compound **2** may be acetal reaction of (1*R*,2*S*,5*S*,6*R*,7*R*,8*R*,11*S*)-7-Hydroxy-5-(hydroxymethyl)-11-isopropyl-6-methyl-9-oxatricyclo[6.2.1.02,6]undecan-10-one with *N*,*N*-dimethylethanolamine (Scheme 1). Thus, *N*,*N*-dimethylethanolamine observed herein indicated that the moiety as the key adduction in the synthesis of dendroxine and its analogs in *Dendrobium* species. The isolation and elucidation of dendrowadine from the species should confirm it further

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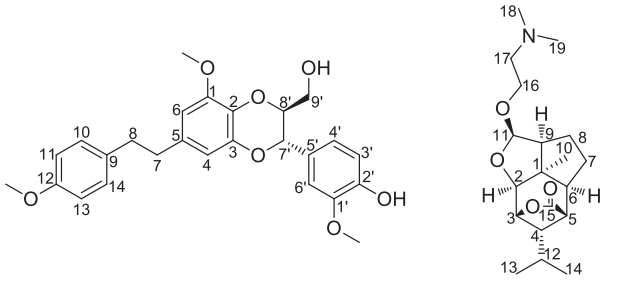
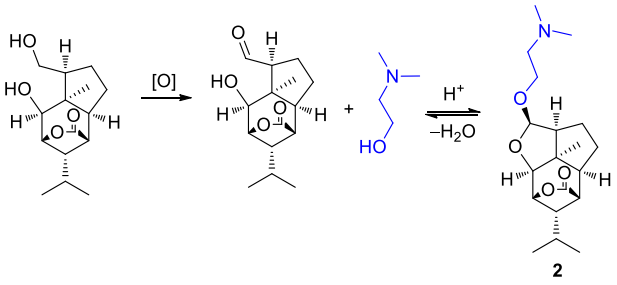


Dendrocandin V (**1**) Wardianumine A (**2**)

Fig. 1. Structures of compounds **1** and **2**.



Scheme 1. Possible biosynthetic pathway of wardianumine A.

[5].

According to the relevant research, bibenzyls and phenanthrenes have a good cytotoxic activity, thus, some of these isolated compounds were sent for cytotoxic activity assay. This manuscript will describe the isolation and elucidation process and the bioassay results of isolated compounds from the stems of *D. wardianum*.

2. Results and discussion

The damp-dry stems of *D. wardianum* Warner (19 kg) were refluxed with 90% EtOH four times and the ethanol solution was concentrated and then extracted successively with EtOAc and BuOH. Further purification of the EtOAc and BuOH fractions were carried out by column chromatography (CC) with silica gel (200–300 mesh), sephadex LH-20, RP-8 and RP-18 CC. This let us to obtain two new compounds, dendrocandin V (**1**) and wardianumine A (**2**), together with eleven known compounds such as denbinobin (**3**) [7], 9,10-dihydro-denbinobin (**4**) [8], mostatin (**5**) [9], loddigesiinols A (**6**) [10], moscatilin (**7**) [11], 5-hydroxy-3,4′-dimethoxybibenzyl (**8**) [12], 3,4-dihydroxy-5,4′-dimethoxy bibenzyl (**9**) [13], dendrocandin A (**10**) [14], gigantol (**11**) [15], dendrocandin U (**12**) [16], dihydroshihunine (**13**) [17]. Structures of these compounds were elucidated by ^{1}H, ^{13}C NMR, and MS data analyses and comparing with those reported in the literature.

Dendrocandin V (**1**): light yellow oil (MeOH), possessed a molecular formula of $C_{26}H_{28}O_7$ as established by the (+)-HR-ESI-MS at *m/z* 475.1730 $[M + Na]^+$ (calcd as 475.1727), indicating thirteen degrees of unsaturation. The IR spectrum showed absorption bands at 3436, 1607, 1512 and 1456 cm^{-1} ascribable to hydroxyl and aromatic functional groups. The UV spectrum exhibited the absorption maxima at 278 and 204 nm. The ^{1}H and ^{13}C NMR spectra indicated the presence of three methoxyl groups [δ_H 3.79 (3H, s), 3.87 (3H, s) and 3.75 (3H, s); δ_C 56.7, 56.6 and 55.8], three methylene groups [δ_H 2.76 (2H, m), 2.81 (2H, m), 3.69 (1H, dd, J = 2.4, 12.6 Hz) and 3.48 (1H, dd, J = 4.8, 12.6 Hz); δ_C 39.3, 38.4 and 62.3], and two oxygenated methine groups [δ_H 4.85 (1H, d, J = 8.4 Hz) and 4.00 (1H, m); δ_C 77.7 and 80.0] (Table 1). Furthermore, the signals of nine aromatic protons were observed in the ^{1}H NMR spectrum, and the signals of 18 aromatic carbons (9 protonated carbons and 9 quaternary carbons) were shown in the ^{13}C NMR spectra. Also, comparison of the ^{1}H and ^{13}C NMR spectra for **1** with those of dendrocandin U [16] indicated that compound **1** also contains three aromatic rings. The cross-peaks of δ_H 2.76 (2H, m, H-7) with δ_C 110.8 (C-4), 135.7 (C-5), 106.4 (C-6), 38.4 (C-8); δ_H 2.81 (2H, m, H-8) with δ_C 39.3 (C-7), 135.2 (C-9), 130.7 (C-10, 14) in the HMBC spectrum (Fig. 2) showed the presence of a bibenzyl unit. In addition, The HMBC correlation peaks between δ_H 6.88 (1H, dd, J = 1.8, 7.8 Hz, H-4′) with δ_C 148.4 (C-2′), 112.1 (C-6′) and 77.7 (C-7′), and between δ_H 4.85 (1H, d, J = 8.4 Hz, H-7′) with δ_C 121.8 (C-4′), 129.7 (C-5′), 112.1 (C-6′), 80.0 (C-8′) and 62.3 (C-9′) deduced the presence of a phenylpropane unit. By comparing the ^{1}H and ^{13}C NMR spectra of **1** with those of dendrocandin U, compound **1** was deduced to lack one hydroxyl. From the HMBC correlations it can be dedicated that the methoxy (δ_H 3.79; δ_C 56.7) was linked to C-1 (δ_C 149.4), another methoxy (δ_H 3.87; δ_C 56.6) was linked to C-1′ (δ_C 149.9) and the last methoxy (δ_H 3.75; δ_C 55.8) was linked to C-12 (δ_C 159.5). The coupling constant ($J_{7',8'}$ = 8.4 Hz) between H-7'and H-8′ indicated the *threo* configuration of the chiral centers of the dioxane ring. The optical rotation of compound **1** (− 14.7222) was similar to that of dendrocandin U (− 4.7) indicating that both compounds may have the same trans-configurations at C-7′ and C-8′. Therefore, compound **1** was elucidated as 9-[7-[7′-(2′-hydroxy-1′-dimethoxyphenyl)-8′-hydroxymethyl-1-methoxy-7′,8-dihydrobenzo[2a,3a]dioxin-5-yl]ethyl]-12-methoxylbenzene,

Table 1
^{1}H and ^{13}C NMR spectral data of compound **1** (J in Hz and δ in ppm).

No.	δ_H[a]	δ_C[b]
1		149.4
2		132.7
3		145.7
4	6.39 (1H, d, J = 1.8)	110.8
5		135.7
6	6.35 (1H, d, J = 1.2)	106.4
7	2.76 (2H, m)	39.3
8	2.81 (2H, m)	38.4
9		135.2
10	7.07 (1H, d, J = 9.0)	130.7
11	6.80 (1H, m)	114.8
12		159.5
13	6.80 (1H, m)	114.8
14	7.07 (1H, d, J = 9.0)	130.7
1′		149.9
2′		148.4
3′	6.83 (1H, d, J = 7.8)	116.4
4′	6.88 (1H, dd, J = 1.8, 7.8)	121.8
5′		129.7
6′	6.99 (1H, dd, J = 1.8)	112.1
7′	4.85 (1H, d, J = 8.4)	77.7
8′	4.00 (1H, m)	80.0
9′	3.69 (1H, dd, J = 2.4, 12.6) 3.48 (1H, dd, J = 4.8, 12.6)	62.3
1-OCH_3	3.79 (3H, s)	56.7
1′-OCH_3	3.87 (3H, s)	56.6
12-OCH_3	3.75 (3H, s)	55.8

[a] ^{1}H NMR recorded at 600 MHz.
[b] ^{13}C NMR recorded at 150 MHz in CD_3OD.

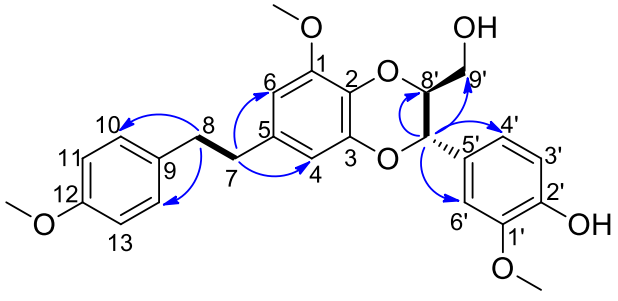


Fig. 2. The main ^{1}H–^{1}H COSY(—) and HMBC( ) of compound **1**.

Table 2
^{1}H and ^{13}C NMR spectral data of compound **2** (J in Hz and δ in ppm).

No.	δ_H[a]	δ_C[b]
1		51.2
2	4.02 (1H, d, J = 4.0)	84.1
3	4.70 (1H, dd, J = 4.5, 5.0)	80.7
4	2.21 (1H, m)	52.7
5	2.53 (1H, m,)	44.7
6	2.15 (1H, m)	44.2
7	1.96 (1H, m)	32.5
	1.50 (1H, s)	
8	1.83 (1H, m)	29.6
	1.46 (1H, m)	
9	2.32 (1H, m)	63.7
10	1.50 (3H, s)	32.2
11	4.96 (1H, d, J = 1.5)	109.9
12	1.88 (1H, m)	25.5
13	1.00 (3H, dd, J = 6.5, 12.0)	20.9
14	1.00 (3H, dd, J = 6.5, 12.0)	21.5
15		180.7
16	3.81 (1H, m)	66.5
	3.51 (1H, m)	
17	2.56 (2H, m)	59.6
18	2.27 (3H, s)	45.9
19	2.27 (3H, s)	45.9

[a] ^{1}H NMR recorded at 500 MHz.
[b] ^{13}C NMR recorded at 125 MHz in DMSO.

named dendrocandin V.

Wardianumine A (**2**): light yellow oil (DMSO), possessed a molecular formula of $C_{19}H_{31}NO_4$ as established by the (+)-HR-ESI-MS at m/z 338.2330 $[M + H]^+$ (calcd as 338.2326), indicating five degrees of unsaturation. The characteristic IR absorption at 1780 cm^{-1} was ascribed to the lactonic carbonyl group. In the ^{13}C NMR spectrum (Table 2) of **2**, five methyls, four methylenes, eight methines, and two quaternary carbons were identified. Analysis of the $^{1}H-^{1}H$-COSY and HSQC date enabled the establishment of the fragments C(2)-C(3), C(5)-C(6)-C(7)-C(8)-C(9)-C(11), and C(13)-C(12)-C(14). The constitution of **2** was deduced on the basis of the HMBC plot (Fig. 3), in which the $^{1}H,^{13}C$ long-range correlations Me(10)/C(1), C(2), C(6), and C(9), H-C(3)/C(1), C(5), and C(15), and H-C(6)/C(1), C(9), C(10), and H-C(11)/C(1), C(2), C(16) were observed. Thus compound **2** possess a picrotoxane sesquiterpene skeleton [5]. In addition, in the HMBC plot, the $^{1}H,^{13}C$ long-range correlations H-C(11)/C(9), C(16), H-C(16)/C(11), C(17), H-C(17)/C(16), C(18),C-(19), H-C(18)/C(17), C(19), H-C(19)/C(17), C(18) were observed, deduced the presence of O-*N*,*N*-dimethylethlethanamine at C-11. In the ROESY plot (Fig. 3), the ROE correlations H-C(2)/H-C(3), H-C(4), CH_3(10), and H-C(12), H-C(6)/H-C(5), H_α-C(7), H-C(9), and H-C(12), H-C(9)/H-C(11) allowed to determine the relative configuration of all chiral C-atoms of **2**. Therefore,

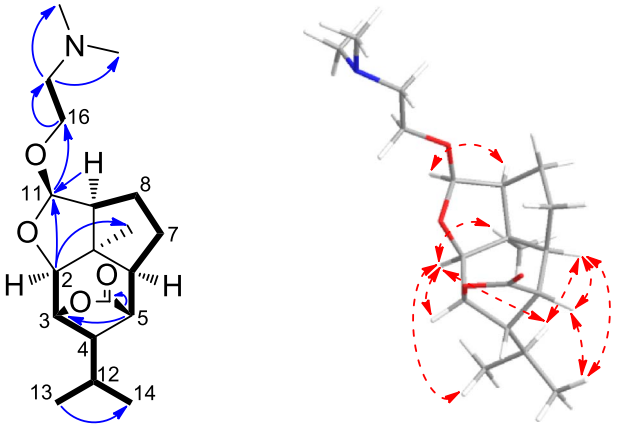


Fig. 3. Key $^{1}H-^{1}H$ COSY(−), HMBC(↷) and ROESY(↶) of compound **2**.

compound **10** was elucidated as (2β, 3β, 5β, 11β)-2,11-epoxy-11-O-*N*,*N*-dimethylethanamine-picrotoxano-3(15)-lactone, named wardianumine A. The hypothetical biosynthetic pathway of **2** was postulated as shown in Scheme 1.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a JASCO P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401PC spectrometer. IR spectra were recorded on a Bruker FT-IR Tensor 27 spectrometer using KBr pellets. ESI-MS (including HR-ESI-MS) were recorded on a Waters Auto Premier P776 spectrometer. 1D and 2D NMR spectra were recorded on Bruker Avance III 600 MHz, Bruker DRX-500 MHz spectrometers with TMS as the internal standard. Column chromatography (CC) was carried out using silica gel (200–300 mesh) and TLC was carried out on plates precoated with silica gel (10–40 μm, Qingdao Marine Chemical Ltd., Qingdao, China), and spots were visualized with UV light at 254, 365 nm, RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), and Sephadex LH-20 was purchased from Amersham Biosciences.

3.2. Plant material

The *D. wardianum* Warner plants were purchased in November 2015 from Puer, Yunnan Province, and identified by Dr. Jiang-Miao Hu at the Institute of Botany, Chinese Academy of Sciences (CAS). A voucher specimen (No. Zsh-7) was preserved at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, CAS, PR China.

3.3. Extraction and isolation

The damp-dry stems of *D. wardianum* Warner (19 kg) were refluxed with 90% EtOH four times and the ethanol solution was concentrated and then extracted successively with AcOEt and BuOH. The AcOEt extract (250 g) was first subjected to CC (silica gel, $CHCl_3$/MeOH 50:1 → 0:1, v/v): Fractions 1–6. Fr. 3 (11.422 g) was purified by CC (petroleum ether/acetone 2:1, v/v), Sephadex LH-20 chromatography ($CHCl_3$/MeOH 1:1) to afford compounds **3** (1 mg), **4** (2 mg), **7** (12 mg), **8** (2 mg), **9** (47 mg). Fr. 4 (4.9 g) was subjected to CC (silica gel, petroleum ether/acetone 3:1 → 2:1, 5:2, 3:2, $CHCl_3$/MeOH 90:1, v/v), Sephadex LH-20 chromatography (MeOH/H_2O 9:1, v/v) and then to HPLC (60% MeOH, 65% MeOH, 70% MeOH) to afford compounds **5** (4 mg), **6** (1 mg), **10** (5 mg), **11** (21 mg), **1** (4 mg), **12** (6 mg). Among them, **1** is a new compound. The BuOH extract (400 g) was first subjected to CC (silica gel, $CHCl_3$/MeOH 8:1 → 0:1, v/v): Fractions 1–7. Fr. 4 (80 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 8:1 → 0:1, $CHCl_3$/MeOH 8:1 and 3% diethylamine, Sephadex LH-20 chromatography (MeOH/H_2O 9:1, v/v), petroleum ether/acetone 6:1 and 3% diethylamine) to afford compounds **2** (87 mg), **13** (6 mg), and **2** is a new compound.

3.31 Dendrocandin V (**1**): Light yellow oil, [α]20 D-14.7222 (*c* 0.120, MeOH); UV (MeOH) λ_{max} (log ε_{max}): 204 (4.6), 223 (4.3), 278 (3.5) nm; IR (KBr) ν_{max} (cm^{-1}): 3436, 2932, 1607, 1512, 1456, 1242, 1118, 1033, 825, 588 cm^{-1}; ESI-MS m/z: 475 $[M + Na]^+$; HR-ESI-MS m/z: 475.1730 $[M + Na]^+$ (calcd for $C_{26}H_{28}O_7Na$, 475.1727).

3.32 Wardianumine A (**2**): Light yellow oil, [α]20 D + 7.1014 (*c* 0.230, MeOH); IR (KBr): 2963, 2768, 1779, 1461, 1357, 1190, 1116, 970 cm^{-1}; ESI-MS m/z: 338 $[M + H]^+$; HR-ESI-MS m/z: 338.2330 $[M + H]^+$ (calcd for $C_{19}H_{31}NO_4H$, 338.2326).

3.4. Cytotoxicity assay

The following human tumor cell lines were used: HL-60, A-549,

Table 3
The cytotoxicity assay results of compounds **1**, **3**, **4**, **5**.

Compound	HL-60	A-549	SMMC-7721	MCF-7	SW480
	IC50 ± SD (μM)				
1	–	–	–	38.48 ± 1.16	–
3	3.08 ± 0.12	19.68 ± 1.12	–	13.13 ± 0.47	16.81 ± 0.13
4	2.33 ± 0.12	14.79 ± 0.64	14.84 ± 0.41	3.63 ± 0.03	6.66 ± 0.71
5	–	16.29 ± 0.25	–	23.75 ± 0.82	18.97 ± 1.04
DDP	3.88 ± 0.08	34.16 ± 0.28	13.79 ± 0.30	35.23 ± 0.92	33.99 ± 1.45
Taxol	< 0.008	< 0.008	< 0.008	< 0.008	< 0.008

SMMC-7721, MCF-7, and SW-480, which were obtained from ATCC (Manassas, VA, USA). All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-5 (3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H–tetrazolium (MTS) (Sigma, St. Louis, MO, USA).24Briefly, 100 μL of adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1×10^5 cells/mL in 100 μL medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTS (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS-50% DMF after removal of 100 μL medium. The optical density of the lysate was measured at 490 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC50 value of each compound was calculated by the Reed and Muench's method [18]. The date of cytotoxicity assay were shown in Table 3.

Acknowledgments

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Appendix A. Supplementary data

1D and 2D NMR, HREIMS, HRESIMS, IR, UV and [α]20 D spectra of compounds **1-2** are available as Supporting Information. Supplementary data associated with this article can be found in the online version, at doi:http://dx.doi.org/10.1016/j.fitote.2017.08.015.

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