Copacamphane, Picrotoxane and Cyclocopacamphane Sesquiterpenes from *Dendrobium nobile*

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Received December 23, 2007; accepted February 12, 2008; published online March 10, 2008

Dendronobilins K—N, four new sesquiterpenes with copacamphane-type (1), picrotoxane-type (2, 3) and cyclocopacamphane-type (4) skeletons, were isolated from the *n*-BuOH soluble fraction of the 60% ethanol extract of the stems of *Dendrobium nobile*. Their structures were established as 2β,11,12-trihydroxycopacamphan-15-one (1), (2β,3β,4β,5β)-2,4,11,12-tetrahydroxypicrotoxan-3(15)-olactone (2), (2β,3β,5β)-2,11,12,13-tetrahydroxypicrotoxan-3(15)-olactone (3), and (5β,8β)-cyclocopacamphane-5,8,12,15-tetrol (4) on the basis of spectroscopic analysis. Compounds 3 and 4 were inactive in both immunomodulatory and antioxidant bioassay *in vitro*.

Key words *Dendrobium nobile*; Orchidaceae; sesquiterpene; copacamphane-type; picrotoxane-type; cyclopacamphane-type

The dried or fresh stems of several *Dendrobium* species (Orchidaceae) are widely used as a very famous traditional Chinese and folk medicine to nourish the stomach and promote the production of body fluid.[1] *Dendrobium nobile* LINDL. is one of the most popular *Dendrobium* plants and has been recorded in the Chinese Pharmacopoeia (2005 Edition) as one of the original materials of "Shi Hu". Our earlier work on the EtOAc soluble fraction of the 60% EtOH extract of the stems of *D. nobile* led to the isolation of bibenzyls, phenanthrenes, fluorenones and sesquiterpenes, some of which showed antioxidant activity.[2—4] In our further investigation on the *n*-BuOH soluble fraction of the 60% EtOH extract of this plant, four new sesquiterpenes possessing copacamphane-type (**1**), picrotoxane-type (**2**, **3**) and cyclocopacamphane-type (**4**) skeletons were isolated. Herein, we report the isolation and structure elucidation of these four compounds.

Results and Discussion

The 60% EtOH extract of the stems of *D. nobile* was suspended in H_2O, and then partitioned with EtOAc and *n*-BuOH successively. The *n*-BuOH soluble fraction was subjected to repeated column chromatography on silica gel, Sephadex LH-20 and ODS, and further purified by reversed-phase HPLC to afford 4 compounds.

Compound **1** was obtained as colourless oil, $[\alpha]_D^{26}$ +24.2° (c=0.5, MeOH). Its molecular formula was established as $C_{15}H_{24}O_4$ by HR-TOF-MS giving a quasimolecular ion peak $[M+Na]^+$ at m/z 291.1565 and NMR analysis. In the ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1**, 15 carbon signals belonging to three methyls, four methylenes (one oxygenated), four methines (one oxygenated), three quaternary carbons (one oxygenated) and one ketonic carbonyl carbon were observed. Analysis of the ^{1}H–^{1}H COSY and ^{1}H-detected heteronuclear single-quantum correlation (HSQC) spectra of **1** led to the deduction of the fragments C-2–C-3–C-4–C-5 and C-6–C-7–C-8. ^{13}C–^{1}H long-range correlation signals were observed for H-13,14/C-4, C-12; H-10/C-1, C-2, C-6, C-9; H-3/C-1; H-4/C-6, C-15; H-11/C-1, C-9, C-15; H-8/C-15 in the HMBC spectrum of **1**, which suggested the existence of a copacamphane sesquiterpene skeleton.[5] The relative configuration of **1** was determined on the basis of its NOESY spectrum, in which NOE correlations were observed between H-10 and H-2, H-6, H-7α, H-11; H-6 and H-5, H-13(14); H-7α and H-8α; H-4 and H-3β (Fig. 1). Therefore, the structure of compound **1** was established as 2β,11,12-trihydroxycopacamphan-15-one, a new sesquiterpene designated as dendronobilin K.

Compound **2** was obtained as colourless oil, $[\alpha]_D^{29}$ +11.5° (c=0.3, MeOH). The HR-TOF-MS (m/z 323.1483, $[M+Na]^+$) and NMR analysis revealed the molecular formula as $C_{15}H_{24}O_6$. The ^{13}C-NMR and DEPT spectra of **2** exhibited 15 carbon signals arising from three methyls, three methylenes (one oxygenated), five methines (two oxygenated), three quaternary carbons (two oxygenated) and one lactonic carbonyl carbon. Analysis of the ^{1}H–^{1}H COSY and HSQC spectra of **2** enabled establishment of the fragments C-2–C-3 and C-5–C-6–C-7–C-8–C-9–C-11. In the ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) spectrum of **2**, ^{13}C–^{1}H long-range correlation signals were observed for H-13,14/C-4, C-12; H-10/C-1, C-2, C-6, C-9; H-3/C-4, C-5, C-15 and H-6/C-15. Thus, compound **2** was

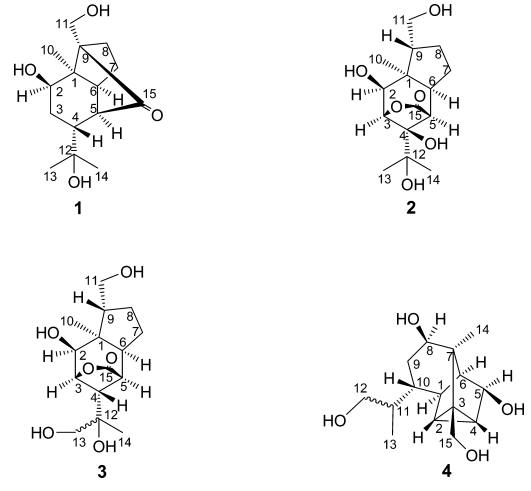

Chart 1. Structures of Compounds **1**—**4**

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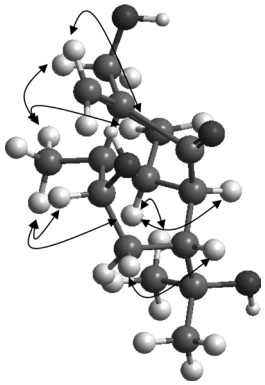

Fig. 1. 3D Model with NOE Correlations of Compound **1**

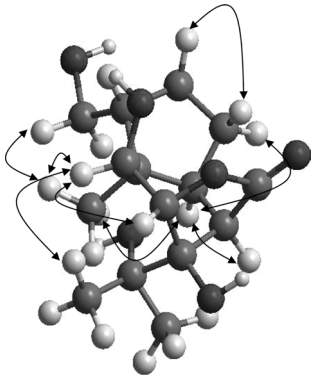

Fig. 2. 3D Model with NOE Correlations of Compound **2**

deduced to be a sesquiterpene possessing a picrotoxane-type skeleton.[6,7] NOE correlations were found between H-2 and H-3, H-10, H-13(14); H-6 and H-5, H-7α, H-10; H-7β and H-8β; H-11 and H-10 in the NOESY spectrum of **2**, which established the relative configuration of **2** (Fig. 2). Based on the above evidence, compound **2** was established as (2β,3β,4β,5β)-2,4,11,12-tetrahydroxypicrotoxan-3(15)-olactone, which is also a new sesquiterpene termed dendronobilin L.

Compound **3** was obtained as colourless oil, $[\alpha]_D^{29}$ +4.8° (c=1.0, MeOH). The molecular formula of $C_{15}H_{24}O_6$ was determined by HR-TOF-MS (*m/z* 323.1478, $[M+Na]^+$) and NMR analysis. Fifteen carbon signals due to two methyls, four methylenes (two oxygenated), six methines (two oxygenated), two quaternary carbons (one oxygenated) and one lactonic carbonyl carbon were observed in the ^{13}C-NMR and DEPT spectra of **3**. The ^{13}C-NMR data of **3** were similar to those of **2** except for the loss of a methyl as well as a hydroxyl-substituted quaternary carbon and the appearance of a methine and a hydroxymethyl group. Elucidation of ^{1}H–^{1}H COSY, HSQC and HMBC spectra of **3** also deduced a picrotoxane-type sesquiterpene skeleton similar to that of **2**. The difference between these two compounds lays in the substitution of a hydroxyl group at C-13 in **3** instead of at C-4 in **2**. In the NOESY spectrum of **3**, NOE correlations were found between H-2 and H-3, H-10, H-14; H-5 and H-6, H-13; H-10 and H-6, H-11; H-6 and H-7α; H-7β and H-8β, which determined the relative configuration of all chiral carbons in the molecule except for C-12 (Fig. 3). However, we could not determine the configuration at C-12, for it lies outside of a ring with a freely rotating C-4–C-12 single bond and the NMR

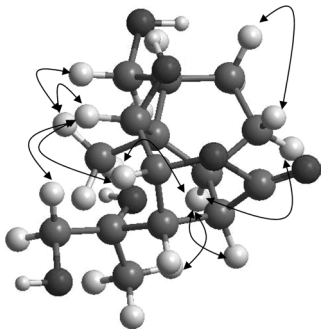

Fig. 3. 3D Model with NOE Correlations of Compound **3**

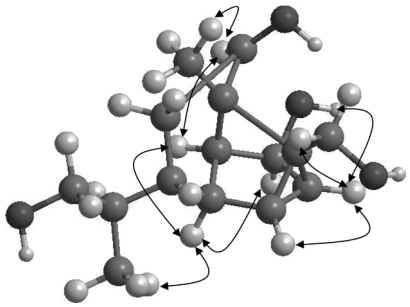

Fig. 4. 3D Model with NOE Correlations of Compound **4**

data are insufficient to establish its configuration. Thus, compound **3** was established as (2β,3β,5β)-2,11,12,13-tetrahydroxypicrotoxan-3(15)-olactone. To the best of our knowledge, compound **3** is a new sesquiterpene called dendronobilin M.

Compound **4** was obtained as colourless oil, $[\alpha]_D^{26}$ +12.6° (c=1.0, MeOH). The HR-TOF-MS (*m/z* 291.1589, $[M+Na]^+$) and NMR analysis indicated the molecular formula as $C_{15}H_{24}O_4$. The ^{13}C-NMR and DEPT spectra of **4** showed the presence of two methyls, three methylenes (two oxygenated), eight methines (two oxygenated) and two quaternary carbons. Analysis of the ^{1}H–^{1}H COSY and HSQC spectra of **4** enabled the deduction of the fragments C-2–C-4–C-5–C-6–C-1, C-8–C-9–C-10 and C-12–C-11–C-13. ^{13}C–^{1}H long-range correlation signals were found at H-13/C-10, C-11, C-12; H-14/C-3, C-7, C-8; H-15/C-2, C-3; H-6/C-2; H-5/C-3; H-1/C-2, C-3, C-9 in the HMBC spectrum of **4**, which exhibited a cyclocopacamphane type sesquiterpene skeleton.[4,8] In order to elucidate the relative configuration of **4**, the NMR spectra were also measured in DMSO-d_6. Interpretation of ^{1}H–^{1}H COSY, HSQC and HMBC spectra of **4** resulted in the assignments of all proton and carbon signals. NOE correlations were observed between H-1 and H-5, H-6, H-13; H-8 and H-6, H-14; H-4 and H-2, H-15, OH-5 in the NOESY spectrum (Fig. 4). Then the relative configuration of all chiral carbons in the molecule except for C-11 was determined. Based on the above evidence, compound **4** was established as (5β,8β)-cyclocopacamphane-5,8,12,15-tetrol. It is a new sesquiterpene, and was assigned the name dendronobilin N.

Compounds **3** and **4** were measured for their immunomodulatory and antioxidant activities *in vitro*, but the results were both inactive.

Experimental

General Procedures Optical rotations were measured using a Jasco P-

Table 1. ^{1}H-NMR (400 MHz) Data for Compounds **1**—**3** in CD_3OD

Position	**1**	**2**	**3**
2	4.05 (1H, dd, 11.0, 6.7 Hz)	4.55 (1H, d, 1.6 Hz)	4.39 (1H, d, 1.5 Hz)
3	(α) 2.03 (1H, m)	4.47 (1H, t, 1.5 Hz)	4.65 (1H, m)
	(β) 1.47 (1H, m)		
4	1.80 (1H, m)		2.47 (1H, m)
5	2.24 (1H, m)	2.38 (1H, dd, 3.8, 1.4 Hz)	2.49 (1H, m)
6	2.62 (1H, d, 4.5 Hz)	2.94 (1H, m)	2.95 (1H, m)
7	(α) 1.95 (1H, m)	(α) 1.98 (1H, m)	(α) 2.00 (1H, m)
	(β) 1.28 (1H, m)	(β) 1.78 (1H, m)	(β) 1.77 (1H, m)
8	(α) 1.77 (1H, m)	(α) 1.22 (1H, m)	(α) 1.23 (1H, m)
	(β) 1.16 (1H, m)	(β) 1.87 (1H, m)	(β) 1.87 (1H, m)
9		2.70 (1H, m)	2.65 (1H, m)
10	1.17 (3H, s)	1.10 (3H, s)	1.13 (3H, s)
11	3.94 (1H, d, 11.9 Hz)	3.56 (1H, dd, 10.7, 8.9 Hz)	3.54 (1H, dd, 10.6, 9.4 Hz)
	3.45 (1H, d, 11.9 Hz)	3.37 (1H, dd, 10.7, 4.9 Hz)	3.38 (1H, m)
12			
13	1.23 (3H, s)	1.28 (3H, s)	3.44 (1H, d, 10.9 Hz)
			3.37 (1H, m)
14	1.22 (3H, s)	1.29 (3H, s)	1.27 (3H, s)

Table 2. ^{1}H-NMR (400 MHz) Data for Compound **4**

Position	**4** (CD_3OD)	**4** (DMSO-d_6)
1	1.77 (1H, m)	1.62 (1H, m)
2	1.31 (1H, m)	1.11 (1H, m)
4	1.11 (1H, m)	0.97 (1H, m)
5	3.81 (1H, m)	3.65 (1H, m)
6	1.37 (1H, m)	1.18 (1H, m)
8	3.44 (1H, m)	3.27 (1H, m)
9	(α) 1.67 (1H, m)	1.56 (2H, m)
	(β) 1.75 (1H, m)	
10	1.51 (1H, m)	1.36 (1H, m)
11	1.54 (1H, m)	1.38 (1H, m)
12	3.57 (1H, dd, 10.7, 4.0 Hz)	3.38 (1H, m)
	3.40 (1H, m)	3.21 (1H, m)
13	0.95 (3H, d, 6.5 Hz)	0.83 (3H, d, 6.3 Hz)
14	1.39 (3H, s)	1.28 (3H, s)
15	4.11 (1H, d, 12.1 Hz)	3.91 (1H, dd, 11.9, 3.6 Hz)
	3.23 (1H, d, 12.1 Hz)	3.11 (1H, dd, 11.8, 6.8 Hz)
OH-5		4.65 (1H, d, 3.2 Hz)
OH-8		4.89 (1H, d, 7.4 Hz)
OH-12		4.28 (1H, t, 5.2 Hz)
OH-15		4.97 (1H, dd, 7.0, 3.8 Hz)

Table 3. ^{13}C-NMR (100 MHz) Data for Compounds **1**—**4** in CD_3OD

Position	**1**	**2**	**3**	**4**	**4** (DMSO-d_6)
1	55.4 (s)	50.2 (s)	51.4 (s)	42.5 (d)	42.5 (d)
2	73.2 (d)	74.5 (d)	73.4 (d)	20.8 (d)	20.8 (d)
3	30.6 (t)	90.4 (d)	86.1 (d)	31.2 (s)	31.6 (s)
4	46.8 (d)	82.8 (s)	50.4 (d)	24.5 (d)	25.1 (d)
5	56.0 (d)	55.5 (d)	47.3 (d)	78.4 (d)	78.0 (d)
6	44.7 (d)	47.7 (d)	46.6 (d)	51.1 (d)	51.4 (d)
7	24.9 (t)	26.3 (t)	26.4 (t)	51.1 (s)	51.5 (s)
8	29.1 (t)	28.3 (t)	28.2 (t)	75.4 (d)	75.1 (d)
9	64.1 (s)	47.0 (d)	46.7 (d)	33.2 (t)	34.0 (t)
10	15.2 (q)	21.7 (q)	22.3 (q)	38.0 (d)	38.1 (d)
11	58.2 (t)	63.6 (t)	63.5 (t)	40.0 (d)	40.4 (d)
12	73.3 (s)	73.6 (s)	72.6 (s)	66.3 (t)	66.2 (t)
13	29.9 (q)	27.2 (q)	70.4 (t)	15.3 (q)	16.8 (q)
14	28.5 (q)	26.5 (q)	24.8 (q)	15.9 (q)	17.6 (q)
15	222.7 (s)	181.4 (s)	182.0 (s)	61.0 (t)	60.8 (t)

1020 polarimeter. NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). ESI-MS spectra were obtained on a Bruker Esquire 2000 mass spectrometer. HR-TOF-MS spectra were performed on a Micromass mass spectrometer. The analytical and preparative HPLC were performed on a Shimadzu Pak with RI detector using a Shim-pack VP-ODS column (4.6×250 mm) and a Shim-pack PREP-ODS column (20×250 mm), respectively. Column chromatography was carried out on silica gel (200—300 mesh, Qingdao Haiyang Chemical Group Corp., Qingdao, China), Sephadex LH-20 (Amersham Biosciences AB) and ODS (60—80 μm, YMC) as packing materials. Silica gel G was used for analytical TLC.

Plant Material The fresh stems of *D. nobile* were collected from Yunnan province in 2004 and identified by Ms. Li-Ping Xiao of Hongkong Kadoorie Farm and Botanic Garden. A voucher specimen (YZXDN-2004) is deposited at the Key Laboratory for Research & Development of New Drugs from Traditional Chinese Medicine & Natural Products in Shenzhen, China.

Extraction and Isolation The powdered air-dried stems of *D. nobile* (5 kg) were refluxed with 60% EtOH three times. After evaporation of solvent *in vacuo*, the residue (210 g) was suspended in H_2O, and partitioned with EtOAc and *n*-BuOH successively. The *n*-BuOH extract (45 g) was first subjected to column chromatography on silica gel eluting with $CHCl_3$/MeOH (100 : 0→0 : 100) to afford 9 fractions. Fraction 4 (4.8 g) was passed over a Sephadex LH-20 column ($CHCl_3$–MeOH, 1 : 1) and then chromatographed on silica gel MPLC by gradient elution with cyclohexane/acetone (9 : 1→0 : 1) to give 6 subfractions. Subfraction 3 (755 mg) was applied to an ODS column eluting with MeOH/H_2O (3 : 7→7 : 3). The fraction eluted with 40% MeOH was further purified by preparative HPLC (30% MeOH) to yield compound **1** (4.5 mg). Fraction 5 (3.9 g) was passed over Sephadex LH-20 with $CHCl_3$/MeOH (1 : 1) as eluent and then chromatographed on ODS eluting with MeOH/H_2O (3 : 7→6 : 4) to give 7 subfractions. Compounds **2** and **3** (3.0, 11.6 mg) were finally obtained from the eluent of 30% MeOH by purification with preparative HPLC (20% MeOH). Fraction 6 (6.8 g) was applied to an ODS column and eluted with MeOH/H_2O (2 : 7→7 : 3). The fraction obtained with 20% MeOH was purified by preparative HPLC (15% MeOH) to yield compound **4** (11.9 mg).

Dendronobilin K (**1**): Colourless oil; $[\alpha]_D^{26}$ +24.2° (c=0.5, MeOH); ESI-MS *m/z* 291 $[M+Na]^+$, 559 $[2M+Na]^+$; HR-TOF-MS *m/z* 291.1565 (Calcd for $C_{15}H_{24}O_4Na$, 291.1572); ^{1}H- and ^{13}C-NMR, see Tables 1 and 3, respectively.

Dendronobilin L (**2**): Colourless oil; $[\alpha]_D^{29}$ +11.5° (c=0.3, MeOH); ESI-MS *m/z* 323 $[M+Na]^+$, 623 $[2M+Na]^+$, 299 $[M-H]^-$, 335 $[M+Cl]^-$, 599 $[2M-H]^-$; HR-TOF-MS *m/z* 323.1483 (Calcd for $C_{15}H_{24}O_6Na$, 323.1471); ^{1}H- and ^{13}C-NMR, see Tables 1 and 3, respectively.

Dendronobilin M (**3**): Colourless oil; $[\alpha]_D^{29}$ +4.8° (c=1.0, MeOH); ESI-MS *m/z* 323 $[M+Na]^+$, 335 $[M+Cl]^-$; HR-TOF-MS *m/z* 323.1478 (Calcd for $C_{15}H_{24}O_6Na$, 323.1471); ^{1}H- and ^{13}C-NMR, see Tables 1 and 3, respectively.

Dendronobilin N (**4**): Colourless oil; $[\alpha]_D^{26}$ +12.6° (c=1.0, MeOH); ESI-MS *m/z* 291 $[M+Na]^+$, 303 $[M+Cl]^-$; HR-TOF-MS *m/z* 291.1589 (Calcd for $C_{15}H_{24}O_4Na$, 291.1572); ^{1}H- and ^{13}C-NMR, see Tables 2 and 3, respec-

tively.

Acknowledgements The authors thank Ms. Li-Ping Xiao, Hongkong Kadoorie Farm and Botanic Garden, for identification of the plant materials. We are grateful to the Shanghai Institute of Materia Medica of the Chinese Academy of Science for measuring the HR-TOF-MS. Thanks are also given to Ling Li of Key Laboratory for Research & Development of New Drugs from Traditional Chinese Medicine & Natural Products in Shenzhen for ESI-MS spectra.

References

1) Jiangsu New Medical College, "Dictionary of Chinese Herb Medicines," Shanghai Scientific and Technologic Press, Shanghai, 1986, p. 586.
2) Zhang X., Gao H., Wang N. L., Yao X. S., *J. Asian Nat. Prod. Res.*, **8**, 113—118 (2006).
3) Zhang X., Xu J. K., Wang J., Wang N. L., Kurihara H., Kitanaka S., Yao X. S., *J. Nat. Prod.*, **70**, 24—28 (2007).
4) Zhang X., Liu H. W., Gao H., Han H. Y., Wang N. L., Wu H. M., Yao X. S., Wang Z., *Helv. Chim. Acta*, **90**, 2386—2394 (2007).
5) Zhao C. S., Liu Q. F., Halaweish F., Shao B. P., Ye Y. Q., Zhao W. M., *J. Nat. Prod.*, **66**, 1140—1143 (2003).
6) Dahmen J., Leander K., *Phytochemistry*, **17**, 1949—1952 (1978).
7) Zhao W. M., Ye Q. H., Dai J. Q., Martin M. T., Zhu J. P., *Planta Med.*, **69**, 1136—1140 (2003).
8) Ye Q. H., Zhao W. M., *Planta Med.*, **68**, 723—729 (2002).