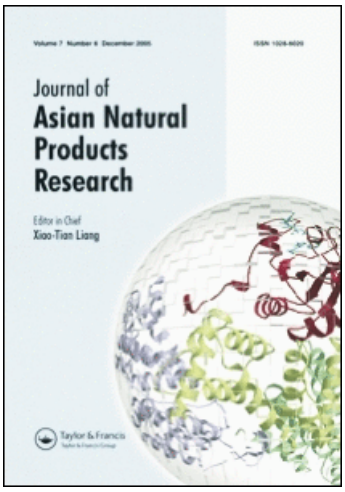

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online Publication Date: 01 July 2008

To cite this Article Hu, Jiang-Miao, Chen, Ji-Jun, Yu, Hong, Zhao, You-Xing and Zhou, Jun(2008)'Two novel bibenzyls from Dendrobium trigonopus',Journal of Asian Natural Products Research,10:7,647 — 651
To link to this Article: DOI: 10.1080/10286020802133605
URL: http://dx.doi.org/10.1080/10286020802133605

Journal of Asian Natural Products Research
Vol. 10, No. 7, July 2008, 647–651

Two novel bibenzyls from *Dendrobium trigonopus*

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(*Received 8 January 2007; final version received 10 May 2007*)

Two novel bibenzyl trigonopols A (**1**) and B (**2**), together with seven known compounds, gigantol (**3**), tristin (**4**), moscatin (**5**), hircinol (**6**), naringenin (**7**), 3-(4-hydroxy-3-methoxyphenyl)-2-propen-1-ol (**8**), and (−)-syringaresinol (**9**), have been isolated from the stems of *Dendrobium trigonopus*, of which compounds **6**, **8**, and **9** were isolated for the first time from this species. The structures of two new compounds were elucidated as *threo*-22-(17-hydroxyl-9-(3-hydroxyl-4-methoxy-phenethyl)-13,16,18-trimethoxy-21*H*-benzo[*c*]chromen-21-yl)ethane-22, 23-diol (**1**) and 9-(4-hydroxyl-3-methoxy-phenethyl)-17-(21-hydroxyl-20-methoxy-phenyl)chroman-11, 16-diol (**2**) on the basis of spectroscopic methods. Trigonopol A was found to exhibit antiplatelet aggregation activity *in vitro* with 67.55% inhibitory ration at 1.4337×10^{-3} M.

Keywords: *Dendrobium trigonopus*; bibenzyl; anti-platelet aggregation activity; trigonopols A and B

1. Introduction

The stems of several *Dendrobium* species (*Orchidaceae*) were used in traditional Chinese medicine as a tonic to nourish the stomach, promote the production of body fluid, and reduce fever [1]. There are about 80 *Dendrobium* species distributed over China [2], but only three species, *Dendrobium candidum* Wall. et Lindl., *D. nobil*e Lindl., and *D. fimbriatum* Hook. var. *oculatum* Hook. have been documented in the Chinese Pharmacopoeia as 'Shi-Hu' [3]. *Dendrobium trigonopus* is abundant in the southwest of China, and a chemical investigation has been reported previously [4]. To find out whether *D. trigonopus* can be a substitute of short resource 'Shi-Hu' or not, further chemical investigation of this plant led to the isolation of nine compounds including four bibenzyls, trigonopol A (**1**), trigonopol B (**2**), gigantol (**3**) [5], and tristin (**4**) [6]; two phenanthrenes, moscatin (**5**) [7] and hircinol (**6**) [7,8]; a flavone naringenin (**7**) [4]; together with two lignins, 3-(4-hydroxy-3-methoxyphenyl)-2-propen-1-ol (**8**) [9,10] and (−)-syringaresinol **9** [11]. This paper describes the isolation and structural elucidation of two novel bibenzyls (**1** and **2**) from *D. trigonopus*.

2. Results and discussion

Repeated column chromatographic purification of the BuOH-soluble portion of the ethanolic extract from the stems of *D. longicornu* on silica gel and Sephadex LH-20 yielded compounds **1** and **2**.

Compound **1** was obtained as a yellow amorphous powder and the molecular formula was assigned as $C_{27}H_{30}O_9$ from positive HR-ESI-MS (*m/z* 521.1782 $[M + Na]^+$), indicating 13 degrees of unsaturation. The ^{1}H NMR

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802133605
http://www.informaworld.com

spectrum of **1** exhibited signals for four methoxy groups at δ_H 3.88 (6H, s, H-24, 25) and 3.93 (6H, s, H-26, 27), and six aromatic protons at δ_H 6.35 (1H, d, $J = 1.4$ Hz, H-14), 6.53 (1H, d, $J = 1.4$ Hz, H-10), 6.69 (1H, s, H-19), 6.71 (1H, d, $J = 1.4$ Hz, H-2), 6.72 (1H, dd, $J = 7.8$, 1.4 Hz, H-6), and 6.86 (1H, d, $J = 7.8$ Hz, H-5) (Table 1). The ^{13}C NMR (DEPT) spectrum showed four CH_3, three CH_2, eight CH, and twelve quaternary carbons. When compared with compound **3**, two methylenes together with twelve aromatic atoms (C-1–14) (Table 1) indicated a bibenzyl skeleton [4], which was confirmed by correlations of H-7 and H-8 (δ_H 2.84, m, 4H) with C-1 (δ_C 133.6), C-2 (δ_C 111.1), C-6 (δ_C 121.0), and C-9 (δ_C 134.4), C-10 (δ_C 109.6), and C-14 (δ_C 104.8) in the HMBC spectrum. The cross-peaks of H-22 with H-23α, H-23β, and H-21 in the COSY spectrum indicated a partial structure $HOCH_2CHOHCHO-$, which was linked to two aromatic groups from key correlations of H-21 with C-11 and C-20 in the HMBC spectrum. Five carbon atoms (C-11, C-12, C-15, C-20, and C-21) together with an oxygen atom formed an oxygen heterocycle ring as shown in Figure 1, accounting for the remaining degree of unsaturation except for three aromatic rings. The coupling constant of H-21/H-22 in the *threo*-isomer (6–8 Hz) is larger than that in the *erythro*-isomer (2–4 Hz) [12], thus, the *threo*-isomer could be indicated from the coupling constant of H-21/H-22 (8.0 Hz) in **1**. Therefore, compound **1** was elucidated to be *threo*-22-(17-hydroxyl-9-(3-hydroxyl-4-methoxy-phenethyl)-13,16,18-

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

	1		**2**	
	δ_C	δ_H	δ_C	δ_H
1	133.6 (C)		134.2 (C)	
2	111.1 (CH)	6.71 (d, $J = 1.4$)	113.0 (CH)	6.81 (d, $J = 1.8$)
3	144.1 (C)		148.2 (C)	
4	146.3 (C)		145.7 (C)	
5	114.2 (CH)	6.86 (d, $J = 7.8$)	115.7 (CH)	6.73 (d, $J = 6.4$)
6	121.0 (CH)	6.72 (dd, $J = 7.8$, 1.4)	121.7 (CH)	6.68 (d, $J = 6.4$, 1.8)
7	38.9 (CH_2)	2.84 (m)	36.9 (CH_2)	2.76 (m)
8	38.0 (CH_2)	2.84 (m)	35.9 (CH_2)	2.76 (m)
9	134.4 (C)		142.9 (C)	
10	109.6 (CH)	6.53 (d, $J = 1.4$)	110.0 (CH)	6.34 (d, $J = 2.0$)
11	143.7 (C)		157.3 (C)	
12	131.0 (C)		101.8 (CH)	6.19 (d, $J = 2.0$)
13	148.4 (C)		156.4 (C)	
14	104.8 (CH)	6.35 (d, $J = 1.4$)	111.3 (C)	
15	135.3 (C)		31.8 (CH_2)	2.97 (dd, $J = 12.8$, 4.4, 1H) 2.62 (dd, $J = 12.8$, 7.2, 1H)
16	147.3 (C)		68.7 (CH)	4.03 (m)
17	134.4 (C)		82.9 (CH)	4.56 (d, $J = 6.4$)
18	147.3 (C)		131.9 (C)	
19	104.1 (CH)	6.69 (s)	111.9 (CH)	7.02 (d, $J = 1.8$)
20	127.3 (C)		148.2 (C)	
21	76.5 (CH)	4.96 (d, $J = 8.0$)	147.4 (C)	
22	78.3 (CH)	4.00 (m)	115.5 (CH)	6.80 (d, $J = 6.4$)
23	61.5 (CH_2)	3.59 (dd, $J = 12.4$, 3.6, 1H) 3.90 (m, 1H)	121.4 (CH)	6.86 (d, $J = 6.4$, 1.8)
24	56.1 (CH_3)	3.88 (s)	56.3 (CH_3)	3.80 (s)
25	56.9 (CH_3)	3.88 (s)	56.3 (CH_3)	3.84 (s)
26	56.4 (CH_3)	3.93 (s)		
27	56.4 (CH_3)	3.93 (s)		

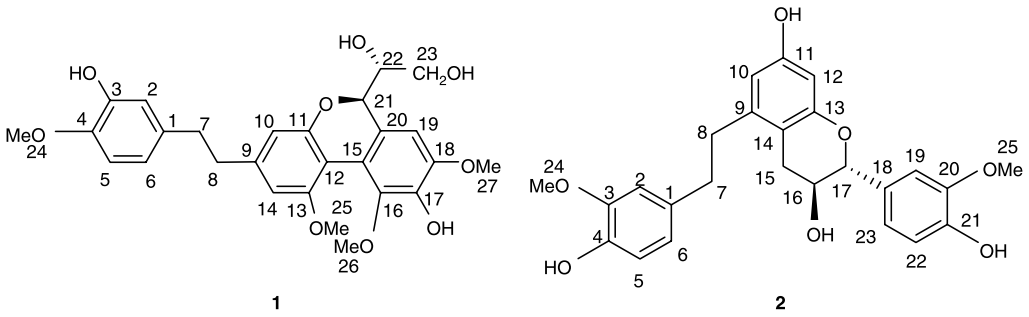

Figure 1. Structures of compounds **1** and **2**.

trimethoxy-21*H*-benzo[*c*]chromen-21-yl) ethane-22,23-diol (**1**).

Compound **2** was obtained as a yellow amorphous powder and its molecular formula $C_{25}H_{26}O_7$ was deduced from the HR-FAB-MS (positive) (*m/z* 439.1778 $[M + H]^+$), indicating 13 degrees of unsaturation. The ^{1}H NMR spectrum of **2** showed two methoxy groups at δ_H 3.84 (3H, s, H-25) and 3.80 (3H, s, H-24), two oxymethine protons at δ_H 4.03 (1H, m, H-16) and 4.56 (1H, d, $J = 6.4$ Hz, H-17), and eight aromatic protons, including two trisubstituted aromatic moieties at δ_H 6.19 (1H, d, $J = 2.0$ Hz, H-12), 6.34 (1H, d, $J = 2.0$ Hz, H-10), 6.68 (1H, dd, $J = 6.4$, 1.8 Hz, H-6), 6.73 (1H, d, $J = 6.4$ Hz, H-5), 6.81 (1H, d, $J = 1.8$ Hz, H-2), 6.80 (1H, d, $J = 6.4$ Hz, H-22), 6.86 (1H, dd, $J = 6.4$, 1.8 Hz, H-23), and 7.02 (1H, d, $J = 1.8$ Hz, H-19) (Table 1). The ^{13}C NMR (DEPT) spectrum revealed twenty-five carbon atoms, including two CH_3, three CH_2, and three aromatic rings. Two methylenes together with twelve aromatic atoms (C-1–14) (Table 1) indicated a bibenzyl skeleton as in **1**, which was further confirmed by long-range correlations between H-7 and H-8 (δ_H 2.76, m, 4H) with C-1 (δ_C 134.2), C-2 (δ_C 113.0), C-6 (δ_C 121.7), and C-9 (δ_C 142.9), C-10 (δ_C 110.0), and C-14 (δ_C 111.3) in the HMBC spectrum. The correlations between H-16/H-15α, H-16/H-15β, and H-16/H-17 in the COSY spectrum indicated that **2** contained the partial structure —CH_2CHOHCHO—. From the HMBC spectrum, key correlations of H-17 with C-13 (δ_C 156.4), C-18 (δ_C 131.9), and C-19 (δ_C 111.9), H-15 with C-14 (δ_C 111.3), C-13 (δ_C 156.4), and C-9 (δ_C 142.9) indicated that the segment —CH_2CHOHCHO— and an oxygen atom together with two quaternary carbons (C-13 and C-14) formed an oxygen heterocycle ring as shown in Figure 1, corresponding to a remaining unsaturation degree except for three aromatic rings. A *trans*-orientation at the C-16/C-17 bond can be proposed from the chemical shift of H-17 at δ_H 4.56 (d, $J = 6.4$ Hz) and its optical rotation (+7.1), which was consistent with (+)-catechin [13]. Thus, compound **2** was determined as 9-(4-hydroxyl-3-methoxy-phenethyl)-17-(21-hydroxyl-20-methoxy-phenyl)-chroman-11,16-diol (**2**) (Figure 2).

To clarify the pharmacological usage of *Dendrobium* species, their antiplatelet aggregation activities have been tested previously [14,15]. In our investigation on components with antiplatelet aggregation activity from *D. trigonopus*, compound **1** was found to exhibit antiplatelet aggregation activity in a preliminary screening test *in vitro* (Table 2) with a moderate inhibitory ratio of 67.55% at 1.434×10^{-3} M.

3. Experimental

3.1 General experimental procedures

Melting points were measured using an XRC-1 micromelting apparatus and are uncorrected. IR spectra were obtained on a Bio-Rad FTS-135 infrared spectrometer using KBr pellets. UV spectra were taken on a Hitachi UV-3210 spectrophotometer. Optical rotations were measured using a Horiba SEAP-300 polari-

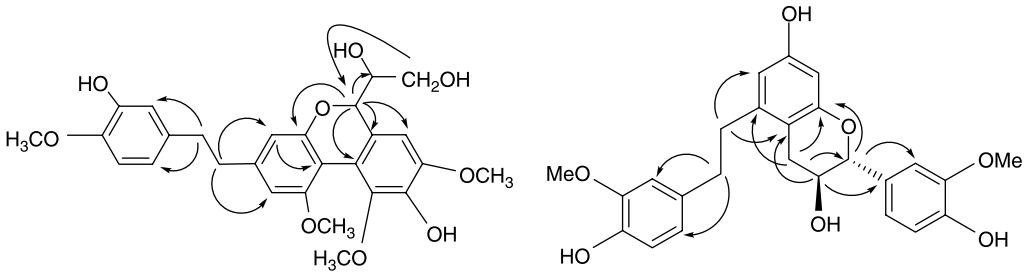

Figure 2. Selected HMBC correlations of **1** and **2**.

meter. MS and HR-MS were recorded on a VG Auto Spec-3000 mass spectrometer. 1D and 2D NMR spectra were measured on a Bruker AM-400 or DRX-500 spectrometer with TMS as the internal standard. Silica gel (200–300 mesh) for column chromatography (CC) and TLC was obtained from Qindao Marine Chemical Factory, Qingdao, China.

3.2 Plant material

The stems of *D. trigonopus* were collected and identified by Professor Hong Yu from Simao (Yunnan province) in January 2006. A voucher specimen (No. Zsh-002) has been preserved at State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, China.

3.3 Extraction and isolation

The air-dried stems of the plant (3.0 kg) were powdered and extracted with 95% aqueous EtOH (15 L × 3) under reflux. The EtOH extract (45 L) was evaporated under reduced pressure and fractionated successively into EtOAc-soluble (93 g) and *n*-BuOH-soluble (80 g) fractions. The EtOAc extract was subjected to CC [silica gel (200–300 mesh, 1200 g), petroleum ether/Me_2CO (7:3–0:10)] to give fractions I–VI, fraction II (3.2 g) was subjected to CC [silica gel, petroleum ether/Me_2CO (4:1)] to afford fractions a–e; fraction c (0.4 g) was purified by CC (Sephadex LH-20, $CHCl_3$/MeOH, 1:1, v/v) to afford compounds **5** (100 mg) and **6** (50 mg). Fraction III was treated as fraction II to provide compounds **3** (2.3 g), **4** (0.4 g), and **8** (20 mg). Fraction IV (5.0 g) was further separated by CC [silica gel, petroleum ether/Me_2CO (7:3)] to yield a yellow compound, and purified by CC [Sephadex LH-20, $CHCl_3$/MeOH (1:1, v/v)] to afford compound **7** (20 mg). Fraction V (9.0 g) was subjected to CC [silica gel, petroleum ether/Me_2CO (7:3, v/v)] to afford compound **9** (1.5 g). The *n*-BuOH extract (80 g) was subjected to CC [silica gel, 200–300 mesh, 1200 g, $CHCl_3$/CH_3OH (10:1)] to give five fractions A–E. Fraction A (16 g) was separated by CC [silica gel, petroleum ether/Me_2CO (8:2–5:5)] and then purified by CC (Sephadex LH-20, MeOH) to produce compounds **1** (6 mg) and **2** (5 mg).

*3.3.1 Trigonopol A (**1**)*

Yellow amorphous powder (acetone), mp 53–55°C; $[\alpha]_D^{24} + 5.2$ ($c = 0.55$, MeOH); UV ($CHCl_3$) λ_{max} (log ε): 241.6 (4.2) and

Table 2. Anti-platelet aggregation activity of **1**.

Sample	Concentration (M)	ADP (M)	A (1′) (%)	A (max) (%)
Control	N/A	8.93×10^{-3}	0.00	0.00
1	1.434×10^{-3}	8.93×10^{-3}	62.42	67.55
TCP	1.088×10^{-3}	8.93×10^{-3}	100	100

TCP, ticlopidine; A (1′), the maximum inhibition (%) of platelet aggregation in 1 min; A (max), the maximum inhibition (%) of platelet aggregation in 5 min.

280.2 (3.8) nm; IR (KBr) ν_{max}: 3441, 2935, 1614, 1602, 1513, 1462, 1340, 1219, 1116, 830, 735 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz] and ^{13}C NMR spectral data [$(CD_3)_2CO$, 100 MHz], see Table 1; EI-MS (70 eV) *m/z*: 498 (26, M^+), 361 (8), 331 (13), 210 (73), 182 (31), 167 (62), 153 (26), 137 (100), 122 (16), 107 (11), 91 (9), and 77 (10); HR-ESI-MS *m/z* 521.1782 $[M + Na]^+$ (calcd for $C_{27}H_{30}O_9Na$, 521.1787).

3.3.2 *Trigonopol B (2)*

Yellow amorphous powder (acetone), mp 55–57°C; $[\alpha]_D^{26} + 7.1$ ($c = 0.12$, MeOH); UV (MeOH) λ_{max} (log ε): 205.4 (4.9), 282.4 (3.9), 330.2 (3.1) nm; IR (KBr) υ_{max}: 3425, 2929, 2853, 1704, 1615, 1515, 1454, 1431, 1367, 1272, 1234, 1138, 1032, and 820 cm^{-1}; ^1H NMR [$(CD_3)_2CO$, 400 MHz] and ^{13}C NMR [$(CD_3)_2CO$, 100 MHz] spectral data, see Table 1; EI-MS (70 eV) *m/z*: (438 12, M^+), 273 (22), 166 (38), and 137 (100); HR-FAB-MS (positive) *m/z*: 439.1778 $[M + H]^+$ (calcd for $C_{25}H_{26}O_7$, 439.1757).

3.4 *Antiplatelet aggregation activity*

Blood was collected from adult New Zealand white rabbits and the blood was diluted to 10-fold with 3.8% trisodium citrate. After equilibrating at room temperature for 30 min, the citrated blood was centrifuged for 10 min at 1000 rpm (24°C) to obtain platelet-rich plasma. Subsequently, 1 ml of diluted blood contained 100 μl Chrono-Lume reagent (Chrono-Log Co., Havertown, PA, USA) and 20 μl testing sample were placed in a glass cuvette and subsequently incubated in the aggregometer (SHANDA PA-196 from Shanghai) at 37°C for 5 min. ADP (10 μl, 250 μM) was added to induce platelet aggregation. Each aggregation was recorded until the maximal extent of aggregation was reached. The extent of aggregation was determined from the maximum height of response in Ohms (Ω), and the rate of aggregation determined from the slope of the steepest part of the curve. The measurement of ATP released from the blood platelets was calculated based on ATP standards. All tests were completed within 3 h after the blood collection. Ticlopidine (TCP), an anti-platelet aggregation compound, was used as a positive control. The result was showed in Table 2.

Acknowledgements

The authors wish to express their thanks to Dr Tina Tingya Dong, Professor Karl W. Tsin, Dr Jiang-Zhi-Yong, Department of Biology, Hong Kong University of Science and Technology for their help extended in carrying out antiplatelet aggregation activity. The authors are grateful to the members of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, for all the spectral measurements.

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