# Five New Compounds from *Dendrobium longicornu*

**Author** **Jiang-Miao Hu**[1,3], **Ji-Jun Chen**[1], **Hong Yu**[2], **You-Xing Zhao**[1], **Jun Zhou**[1]

**Affiliation**
[1] State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming, P. R. China
[2] Yunnan University, Kunming, P. R. China
[3] Graduate School of the Chinese Academy of Sciences, Beijing, P. R. China

**Key words**
- Orchidaceae
- *Dendrobium longicornu*
- bibenzyl
- phenanthrenes
- lignin glycoside
- anti-platelet aggregation activity

## Abstract

A novel bibenzyl, a new bibenzyl, two new phenanthrenes, and a new lignin glycoside, namely longicornuol A (**1**), 4-[2-(3-hdroxyphenol)-1-methoxyethyl]-2,6-dimethoxyphenol (**2**), 5-hydroxy-7-methoxy-9,10-dihydrophenanthrene-1,4-dione (**3**), 7-methoxy-9,10-dihydrophenanthrene-2,4,5-triol (**4**) and *erythro*-1-(4-*O*-*β*-D-glucopyranosyl-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2,6-dimethoxyphenoxy]-1,3-propanediol (**5**), together with 14 known compounds, were isolated from the stems of *Dendrobium longicornu*. All structures were elucidated by spectroscopic methods (NMR, MS, UV and IR). Anti-platelet aggregation activities of compounds **1 – 5** were also tested.

**Supporting information** available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

**received** April 3, 2007
**revised** January 29, 2008
**accepted** February 25, 2008

**Bibliography**
**DOI** 10.1055/s-2008-1074492
Planta Med 2008; 74: 1–5 © Georg Thieme Verlag KG Stuttgart · New York
Published online ■
ISSN 0032-0943

**Correspondence**
***Prof. Jun ZhouYou-Xing Zhao***
State Key Laboratory of Phytochemistry and Plant Resource in West China
Kunming Institute of Botany
The Chinese Academy of Sciences
Kunming 650204
People's Republic of China
Tel.: +86-871-522-3264
Fax: +86-871-522-3261
jzhou@mail.kib.ac.cn
yxzhao@mail.kib.ac.cn

## Introduction

The stems of several *Dendrobium* species (Orchidaceae) are used as "Shi-Hu" in traditional Chinese medicine to nourish the stomach, promote the production of body fluid and bring down fever [1], [2]. There are about 80 *Dendrobium* species distributed in China. However, only three species (very rare now) are collected in the Pharmacopoeia of China, namely, *D. candidum*, *D. nobile* and *D. fimbriatum* var. *oculatum* [2]. *D. longicornu*, which is not collected in the Pharmacopoeia of China, is a more abundant species in southwestern China, and its chemical constituents have not been reported yet. Our chemical investigation of *D. longicornu* led to the isolation of five new compounds (**1 – 5**; **Fig. 1**), along with fourteen known compounds. The known compounds were identified by comparison of their spectroscopic data with literature. Preliminary anti-platelet aggregation activities of compounds **1 – 5** were also tested. This paper describes the isolation, structural elucidation and anti-platelet aggregation tests of these five new compounds (**1 – 5**).

## Materials and Methods

### General

Melting points were measured on an XRC-1 micro-melting point apparatus and were uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured on a Hitachi UV-3210 spectrophotometer. IR spectra were measured with a Bio-Rad FTS-135 IR spectrometer with KBr pellets. Mass spectra were obtained on a VG Auto Spec-3000 mass spectrometer, 70 eV for EI. 1 D and 2 D NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 spectrometers, with chemical shifts ($\delta$) in ppm relative to trimethylsilane (TMS) as internal standard and coupling constants in Hertz (Hz). Column chromatography was carried out on silica gel (200 – 300 mesh) and TLC was carried out on plates precoated with silica gel (10 – 40 $\mu$m; Qingdao Marine Chemical Ltd.). An RP-18 column (LiChroprep, 40 – 63 $\mu$m; Merck) was applied for reverse-phase chromatography. Sephadex LH-20 was purchased from Amersham Biosciences.

### Plant material

The stems of *Dendrobium longicornu* were collected in January 2004 from Xiaoshao of Kunming in the Yunnan Province, P. R. China, and identified

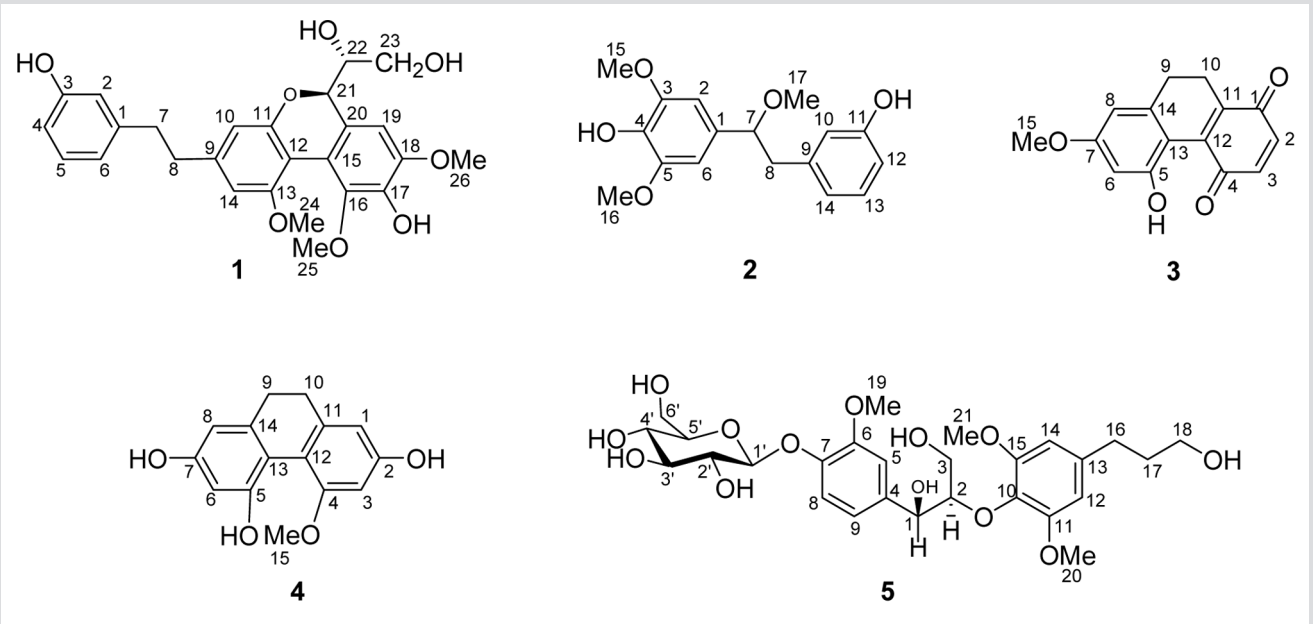


**Fig. 1** Structures of compounds **1 – 5**.

by Professor Hong Yu of the Yunnan University. A voucher specimen (No. Zsh-1) is preserved at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, the Chinese Academy of Science, P. R. China.

## Extraction and isolation

The air-dried stems of the plant (4.5 kg) were powdered and extracted with 95 % aqueous EtOH (18 L × 3) under reflux. The EtOH extract (54 L) was evaporated under reduced pressure and fractionated successively into $CHCl_3$ soluble (76 g) and *n*-BuOH soluble (73 g) fractions. A portion of the $CHCl_3$ extract (70 g) was subjected to silica gel column chromatography (Ø 7 × 90 cm) and eluted with petroleum ether-$Me_2CO$ (4 : 1, 30 L) to give five fractions (A – E). Fraction B (5.2 g) was applied repeatedly to column chromatography over silica gel (Ø 3 × 50 cm, petroleum ether-EtOAc, 3 : 1, 6 L) and then Sephadex LH-20 (Ø 1.7 × 120 cm, $CHCl_3$-MeOH, 1 : 1, 2 L) to afford compounds **6** (30 mg), **7** (11 mg), **8** (20 mg) and **14** (20 mg). Fraction C (3.2 g) was subjected to silica gel column chromatography (Ø 2 × 50 cm, petroleum ether-$Me_2CO$, 7 : 3, 6 L), then to Sephadex LH-20 (Ø 1.7 × 120 cm, $CHCl_3$-MeOH, 1 : 1, 2 L) to obtain compounds **2** (4 mg), **3** (3 mg), **9** (70 mg) and **17** (5 mg). Fraction D was treated the same as Fraction C to afford compounds **13** (8 mg) and **15** (50 mg).

A portion of the *n*-BuOH extract (60 g) was subjected to silica gel column chromatography (Ø 7 × 80 cm) and eluted with $CHCl_3$-MeOH (10 : 1, 25 L) to afford six fractions (I – IV). Fraction II (2.9 g) was subjected to silica gel column chromatography (Ø 2 × 50 cm, $CHCl_3$-MeOH, 30 : 1, 4 L), and then purified further on Sephadex LH-20 (Ø 2 × 120 cm, MeOH, 2 L) to afford compounds **4** (20 mg), **10** (12 mg), **11** (50 mg) and **12** (20 mg). Fraction III (2.1 g) was subjected to column chromatography over silica gel (Ø 2 × 50 cm, $CHCl_3$-MeOH, 20 : 1, 4 L), Sephadex LH-20 (Ø 1.7 × 120 cm, MeOH, 1.5 L) and RP-18 gel (Ø 3 × 30 cm, MeOH-$H_2O$, 7 : 3, 2 L) to yield compound **1** (20 mg). Fraction IV (12.0 g) was treated the same as Fraction III to afford compounds **5** (7 mg), **16** (70 mg), **18** (45 mg) and **19** (6 mg).

## Isolates

*Longicornuol* A (**1**): yellow amorphous powder ($Me_2CO$); m. p. 127 – 129 °C; $[\alpha]_D^{24}$: + 8.6 (*c* 0.33, MeOH); UV ($CHCl_3$): $\lambda_{max}$ (log $\varepsilon$) = 273 (3.8), 241 (4.3) nm; IR (KBr): $\nu_{max}$ = 3441, 3007, 2937, 2844, 1601, 1511, 1455, 1430, 1341, 1273, 1219, 1116, 1036, 833, 750 $cm^{-1}$; $^1$H-NMR [$(CD_3)_2CO$, 400 MHz]: $\delta$ = 7.07 (1H, dd, *J* = 7.8, 7.7 Hz, H-5), 6.79 (1H, s, H-19), 6.72 (1H, dd, *J* = 2.1, 1.4 Hz, H-2), 6.69 (1H, dd, *J* = 7.7, 1.4 Hz, H-6), 6.65 (1H, dd, *J* = 7.8, 2.1 Hz, H-4), 6.46 (1H, d, *J* = 1.8 Hz, H-14), 6.42 (1H, d, *J* = 1.8 Hz, H-10), 4.91 (1H, b, *J* = 8.0 Hz, H-21), 4.00 (1H, m, H-22), 3.84 (6H, s, H-25, 26), 3.78 (3H, s, H-24), 3.73 (1H, m, *J* = 12.0, 4.8 Hz, H-23$\beta$), 3.50 (1H, m, *J* = 12.0, 6.8 Hz, H-23$\alpha$), 2.80 (4H, m, H-7, 8); $^{13}$C-NMR [$(CD_3)_2CO$, 100 MHz]: see **Table 1**; EI-MS: *m/z* = 468 $[M]^+$, 260, 210, 182, 167, 153, 107, 77; HR-ESI-MS: *m/z* = 491.1678 $[M + Na]^+$ (calcd. for $C_{26}H_{28}O_8Na$: 491.1681).

*4-[2-(3-Hydroxyphenol)-1-methoxyethyl]-2,6-dimethoxyphenol* (**2**): yellow amorphous powder ($CHCl_3$); m. p. 40 – 42 °C; $[\alpha]_D^{24}$: + 7.4 (*c* 0.57, MeOH); UV ($CHCl_3$): $\lambda_{max}$ (log $\varepsilon$) = 329 (3.8), 281.0 (3.7), 274 (3.7), 241 (4.0) nm; IR (KBr): $\nu_{max}$ = 3425, 3000, 2936, 2841, 1612, 1517, 1459, 1427, 1329, 1215, 1153, 1113, 967, 783, 694 $cm^{-1}$; $^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ = 7.12 (1H, dd, *J* = 7.8, 7.8 Hz, H-13), 6.68 (2H, dd, *J* = 7.8, 1.5 Hz, H-12, 14), 6.60 (1H, bs, H-10), 6.45 (2H, bs, H-2, 6), 4.25 (1H, dd, *J* = 7.2, 5.9 Hz, H-7), 3.86 (6H, s, H-15, 16), 3.23 (3H, s, H-17), 3.07 (1H, dd, *J* = 13.6, 7.2 Hz, H-8$\alpha$), 2.83 (1H, dd, *J* = 13.6, 5.9 Hz, H-8$\beta$); $^{13}$C-NMR ($CDCl_3$, 100 MHz): see **Table 1**; EI-MS: *m/z* = 304 $[M]^+$, 208, 197, 182, 167, 107, 77; HR-ESI-MS: *m/z* = 327.1208 $[M + Na]^+$ (calcd. for $C_{17}H_{20}O_5Na$: 327.1208).

*5-Hydroxy-7-methoxy-9,10-dihydrophenanthrene-1,4-dione* (**3**): dark red amorphous powder ($CHCl_3$); m. p. 45 – 47 °C; $[\alpha]_D^{22}$: + 57.6 (*c* 0.02, MeOH); UV ($CHCl_3$): $\lambda_{max}$ (log $\varepsilon$) = 476.5 (3.2), 239.5 (4.0) nm; IR (KBr): $\nu_{max}$ = 3418, 2956, 2924, 2854, 1726, 1663, 1646, 1599, 1561, 1514, 1464, 1379, 1351, 1284, 1194, 1103, 831 $cm^{-1}$; $^1$H-NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.81 (1H, d, *J* = 10.0 Hz, H-2), 6.71 (1H, d, *J* = 10.0 Hz, H-3), 6.36 (1H, d, *J* = 2.0 Hz, H-8), 6.34 (1H, d, *J* = 2.0 Hz, H-6), 3.74 (3H, s, H-11), 2.63 (2H, t, *J* = 6.4, H-10), 2.57 (2H, t, *J* = 6.4, H-9); $^{13}$C-NMR ($CDCl_3$, 100 MHz): see

**Table 1** $^{13}$C-NMR spectral data of compounds **1 – 5**

| C | 1 | 2 | 3 | 4 | 5 | C | 1 | | 5 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 144.3 (s) | 132.6 (s) | 185.3 (s) | 109.9 (d) | 73.2 (d) | 18 | 148.7 (s) | 18 | 61.8 (t) |
| 2 | 116.2 (d) | 103.3 (d) | 135.1 (d) | 157.6 (s) | 87.6 (d) | 19 | 106.0 (d) | 19 | 56.5 (q) |
| 3 | 158.2 (s) | 146.9 (s) | 137.3 (d) | 100.0 (d) | 60.9 (t) | 20 | 128.3 (s) | 20 | 56.5 (q) |
| 4 | 113.6 (d) | 133.9 (s) | 185.7 (s) | 155.5 (s) | 137.5 (s) | 21 | 77.2 (d) | 21 | 56.5 (q) |
| 5 | 130.0 (d) | 146.9 (s) | 158.8 (s) | 155.9 (s) | 112.3 (d) | 22 | 79.2 (d) | 1′ | 103.0 (d) |
| 6 | 120.4 (d) | 103.3 (d) | 98.6 (d) | 104.6 (d) | 150.6 (s) | 23 | 61.8 (t) | 2′ | 74.8 (d) |
| 7 | 38.5 (t) | 85.1 (d) | 158.9 (s) | 157.9 (s) | 146.9 (s) | 24 | 56.2 (q) | 3′ | 77.8 (d) |
| 8 | 38.2 (t) | 44.7 (t) | 107.5 (d) | 108.1 (d) | 120.0 (d) | 25 | 56.7 (q) | 4′ | 71.4 (d) |
| 9 | 134.7 (s) | 140.2 (s) | 28.5 (t) | 31.9 (t) | 117.9 (d) | 26 | 56.7 (q) | 5′ | 77.9 (d) |
| 10 | 110.1 (d) | 116.4 (d) | 20.1 (t) | 32.0 (t) | 134.4 (s) | | | 6′ | 62.8 (t) |
| 11 | 145.1 (s) | 155.4 (s) | 139.8 (s) | 143.3 (s) | 154.0 (s) | | | | |
| 12 | 132.5 (s) | 113.1 (d) | 140.9 (s) | 115.1 (s) | 106.7 (d) | | | | |
| 13 | 149.6 (s) | 129.2 (d) | 143.1 (s) | 113.6 (s) | 139.6 (s) | | | | |
| 14 | 106.0 (d) | 121.9 (d) | 112.3 (s) | 142.4 (s) | 106.7 (d) | | | | |
| 15 | 134.7 (s) | 56.3 (q) | 55.8 (q) | 57.3 (q) | 154.0 (s) | | | | |
| 16 | 148.7 (s) | 56.3 (q) | | | 33.2 (t) | | | | |
| 17 | 137.1 (s) | 56.7 (q) | | | 35.4 (t) | | | | |

**Table 1**; EI-MS: $m/z$ = 256 $[M]^+$, 241, 225, 213, 201, 174, 115; HR-ESI-MS: $m/z$ = 279.0625 $[M + Na]^+$ (calcd. for $C_{15}H_{12}O_4Na$: 279.0633).

*2,5,7-Trihydroxy-4-methoxy-9,10-dihydrophenanthrene* (**4**): red amorphous powder ($Me_2CO$); m. p. 92 – 94 °C; $[\alpha]_D^{22}$: + 16.9 (*c* 0.47, MeOH); UV ($CHCl_3$): $\lambda_{max}$ (log $\varepsilon$) = 276.20 (4.1), 239.00 (3.8), 197.80 (3.9) nm; IR (KBr): $\nu_{max}$ = 3424, 2925, 2852, 1587, 1513, 1454, 1435, 1352, 1300, 1264, 1222, 1192, 1155, 1085, 1021, 996, 955, 845 $cm^{-1}$; $^1$H-NMR [$(CD_3)_2CO$, 500 MHz ]: $\delta$ = 6.57 (1H, d, $J$ = 2.3 Hz, H-8), 6.52 (1H, d, $J$ = 2.3 Hz, H-6), 6.35 (1H, d, $J$ = 2.5 Hz, H-1), 6.31 (2H, d, $J$ = 2.5 Hz, H-3), 3.94 (3H, s, H-11), 2.56 (4H, m, H-9, 10); $^{13}$C-NMR [$(CD_3)_2CO$, 100 MHz]: see **Table 1**; EI-MS: $m/z$ = 258 $[M]^+$, 243, 215, 197, 187, 169, 129, 115; HR-ESI-MS: $m/z$ = 281.0790 $[M + Na]^+$ (calcd. for $C_{15}H_{14}O_4Na$: 281.0789).

*Erythro-1-(4-O-β-D-Glucopyranosyl-3-methoxyphenyl)-2-[4-(3-hydroxypropyl)-2,6-dimethoxyphenoxy]-1,3-propanediol* (**5**): yellow amorphous powder ($Me_2CO$); m. p. 205 – 207 °C; $[\alpha]_D^{22}$: + 3.2 (*c* 0.59, MeOH); UV ($CHCl_3$): $\lambda_{max}$ (log $\varepsilon$) = 274 (2.9), 240 (3.2) nm; IR (KBr): $\nu_{max}$ = 3426, 2924, 2854, 1592, 1512, 1463, 1423, 1332, 1269, 1226, 1124, 1074, 1027, 823, 663 $cm^{-1}$; $^1$H-NMR [$(CD_3)_2CO$, 400 MHz]: $\delta$ = 7.11 (1H, dd, $J$ = 8.5, 2.5 Hz, H-9), 7.09 (1H, d, $J$ = 2.5 Hz, H-5), 6.89 (1H, d, $J$ = 8.5 Hz, H-8), 6.58 (2H, bs, H-12, 14), 4.99 (1H, t, $J$ = 4.7 Hz, H-1), 4.86 (1H, d, $J$ = 7.04 Hz, H-1′), 4.21 (1H, m, H-2), 3.82 (9H, s, H-19, 20, 21), 3.85 (1H, m, H-3$\beta$), 3.62 (2H, m, H-6′), 3.55 (2H, t, $J$ = 6.0 Hz, H-18), 3.46 (2H, m, H-2′, 5′), 3.44 (1H, dd, $J$ = 13.2, 6.5 Hz, H-3$\alpha$), 3.42 (2H, m, H-3′, 4′), 2.64 (2H, t, $J$ = 7.5 Hz, H-16), 1.81 (2H, m, H-17); $^{13}$C-NMR [$(CD_3)_2CO$, 125 MHz]: see **Table 1**; FAB-MS (negative ion): $m/z$ = 569 $[M - H]^-$, 473, 407, 339, 283, 255; HR-ESI-MS: $m/z$ = 593.2227 $[M + Na]^+$ (calcd. for $C_{27}H_{38}O_{13}Na$: 593.2210).

### Platelet preparation and aggregation

Blood was collected from adult New Zealand white rabbits through a polyethylene cannula placed in the common carotid artery by a 10 mL syringe. The first few milliliters of blood were discarded, and the rest was diluted 10-fold with 3.8 % trisodium citrate. After equilibrating at room temperature for 30 minutes, the citrated blood was centrifuged for 10 minutes at 1000 rpm at 24 °C to obtain platelet-rich plasma (PRP). 1 mL of diluted blood contained 100 μL Chrono-Lume reagent (Chrono-Log Co.) and 20 μL testing sample were placed in a glass cuvette and subsequently incubated in the aggregometer (SHANDA PA-196) at 37 °C for 5 minutes. ADP (10 μL, 250 μM; Sigma, purity 95 %) 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 release from the blood platelets was calculated based on ATP standards. All tests were completed within 3 hours after the blood collection. Ticlopidine (TCP, purity ≥ 99 %; Sigma), an anti-platelet aggregation agent, was used as a positive control.

### Supporting information

The mass and NMR spectra of compounds **1 – 5** can be seen in the Supporting Information (**Fig. 1S – 15S**).

## Results and Discussion

Compound **1** was obtained as a yellow amorphous powder, exhibiting a molecular ion peak at $m/z$ = 468 in its EI-MS. The molecular formula of $C_{26}H_{28}O_8$ was deduced from the positive HR-ESI-MS ($m/z$ = 491.1678 $[M + Na]^+$) and its NMR data, indicating 13 degrees of unsaturation. The $^{13}$C-NMR spectrum exhibited 26 signals, including three methoxy carbons and 18 aromatic carbons. Two methylene carbons [$\delta_C$ = 38.5 (C-7), 38.2 (C-8)] were attached to the corresponding benzylic protons at $\delta_H$ = 2.80 (4H, m, H-7 and H-8). These two methylenes, together with the existence of two aromatic rings, indicated a bibenzyl nucleus [3], [4], which was confirmed by the correlations [H-7/C-2 and C-6, H-8/C-10 and C-14, H-10/C-12] in the HMBC spectrum. A partial structure -OCHCHOHCH$_2$OH [$\delta_C$ = 77.2 (C-21), 79.2 (C-22), 61.8 (C-23)] was deduced from the cross-peaks [H-22/H-21, H-22/H-23$\alpha$, H-22/H-23$\beta$] in the COSY spectrum. This fragment was linked to C-11 and C-20 inferred from the key HMBC correlations [H-21/C-11, H-21/C-15, H-21/C-19, H-22/C-20, H-23/C-21], which indicated the formation of a heterocyclic ring. The existence of such a heterocyclic ring was confirmed further by the remaining one degree of unsaturation. Because the coupling

constant of H-21 and H-22 in the *threo*-isomer (6 – 8 Hz) is larger than that in the *erythro*-isomer (2 – 4 Hz) [5], compound **1** was proposed as the *threo*-isomer on the basis of the coupling constant ($J$ = 7.96 Hz). Therefore, compound **1** was determined as *threo*-1-{9-hydroxy-3-[2-(3-hydroxyphenyl)-ethyl]-1,8,10-trimethoxy-6*H*-benzo[*c*]chromen-6-yl}-ethane-1,2-diol, named longicornuol A.

Compound **2** was obtained as a yellow amorphous powder. Its molecular formula was assigned as $C_{17}H_{20}O_5$ from the positive HR-ESI-MS ($m/z$ = 327.1208 $[M + Na]^+$) and its NMR spectra, indicating 8 degrees of unsaturation. In the $^{13}$C-NMR spectrum, seventeen carbons were observed, including three methoxys, one methylene, seven methines and six quaternary carbons. In the $^1$H-NMR spectrum, four proton signals at $\delta_H$ = 7.12 (1H, dd, $J$ = 7.8, 7.8 Hz, H-13), 6.68 (2H, dd, $J$ = 7.8, 1.5 Hz, H-12, 14) and 6.60 (1H, dd, $J$ = 1.5, 1.5 Hz H-10) indicated a 1, 3-disubstituted aromatic ring; two other aromatic proton signals at $\delta_H$ = 6.45 (2H, bs, H-2, 6) indicated a 1,3,4,5-tetrasubstituted aromatic ring (**Fig. 1**). An ethyl fragment was inferred from the correlation between the methine [$\delta_H$ = 4.25 (H-7)] and methylene [$\delta_H$ = 3.07 and 2.83 (H-8)] in the COSY spectrum. Thus, compound **2** was elucidated as 4-[2-(3-hdroxyphenol)-1-methoxyethyl]-2,6-dimethoxyphenol on the basis of HMBC correlations [H-7/C-2, H-7/C-6, H-7/C-9, H-8/C-10, H-8/C-14, H-8/C-1, $CH_3O$-3/C-3, $CH_3O$-5/C-5 and $CH_3O$-7/C-7] and by comparison of the NMR data with those of aloifol I [3].

Compound **3** was obtained as a dark red amorphous powder. The molecular formula was assigned as $C_{15}H_{12}O_4$ according to the HR-ESI-MS ($m/z$ = 279.0625 $[M + Na]^+$) and NMR spectra, indicating 10 degrees of unsaturation. In the $^{13}$C-NMR spectrum, 15 carbon signals were observed, including one methoxy, two methylenes, four methines, and eight quaternary carbons. Two of the eight quaternary carbons should be carbonyl carbons according to their chemical shifts at $\delta_C$ = 185.3 (C-1) and 185.7 (C-4). The two carboxyls, two methylenes [$\delta_C$ = 28.5 ($CH_2$, C-9), 20.1 ($CH_2$, C-10)] and ten olefinic carbons indicated a skeleton of 9,10-dihydrophenanthrenedione. A methoxy group was discerned from the proton signals at $\delta_H$ = 3.74 (3H, s) in the $^1$H-NMR spectrum and it was linked to C-7 from the HMBC correlation [H-15/C-7]. A broad peak at 3418 cm$^{-1}$ in the IR spectrum presented the existence of a hydroxy, which was linked to C-5 observed from the HMBC correlation [H-6/C-5 ($\delta_C$ = 158.8)]. The whole structure was established by the COSY correlation [H-2/H-3] and HMBC correlations [H-2/C-1, H-3/C-4, H-9/C-8, H-9/C-13, H-10/C-1, H-10/C-12] and by comparison with ochrone A [6]. Thus, compound **3** was elucidated as 5-hydroxy-7-methoxy-9,10-dihydrophenanthrene-1,4-dione.

Compound **4** was obtained as a red amorphous powder. The molecular formula was assigned as $C_{15}H_{14}O_4$ from the HR-ESI-MS ($m/z$ = 281.0790 $[M + Na]^+$) and NMR data, indicating 10 degrees of unsaturation. The $^{13}$C-NMR spectrum revealed 15 carbon atoms, including 12 aromatic carbons, a methoxy, and two secondary carbons ($\delta_C$ = 31.9, 32.0). These two methylene carbons were consistent with four protons [$\delta_H$ = 2.56 (4H, m)] in the $^1$H-NMR spectrum. The existence of two aromatic rings and two methylenes indicated a 9,10-dihydrophenanthrene skeleton. Four signals [$\delta_H$ = 6.57 (1H, d, $J$ = 2.3 Hz, H-8), 6.52 (1H, d, $J$ = 2.3 Hz, H-6), 6.35 (1H, d, $J$ = 2.5 Hz, H-1), 6.31 (2H, d, $J$ = 2.5 Hz, H-3)] in the $^1$H-NMR spectrum indicated that the two aromatic rings were both 1,3,4,5-tetrasubstituted. A methoxy group [$\delta_H$ = 3.74 (3H, s, H-11)] was linked to C-4 observed from the HMBC spectrum [$CH_3O$-4/C-4 ($\delta_C$ = 155.5)] and a hydroxy was attached to C-2 deduced from the HMBC correlations [H-1/C-2 ($\delta_C$ = 157.6), H-3/C-2, H-3/C-4]. Two other hydroxys were linked to C-5 and C-7, which was also derived from the HMBC spectrum [H-6/C-5 ($\delta_C$ = 155.9), H-6/C-7 ($\delta_C$ = 157.9), H-8/C-7]. The whole structure was proved further by the HMBC correlations [H-9/C-8, H-9/C-13, H-10/C-1, H-10/C-12] and the comparison with 4-methoxy-9,10-dihydrophenanthrene-2,3,7-triol [7]. Therefore, **4** was elucidated as 2,5,7-trihydroxy-4-methoxy-9,10-dihydrophenanthrene.

Compound **5** was obtained as a yellow amorphous powder. Its molecular formula was assigned as $C_{27}H_{38}O_{13}$ from its HR-ESI-MS ($m/z$ = 593.2227 $[M + Na]^+$) and NMR data. In the $^{13}$C-NMR spectrum, 27 carbon signals were observed, indicating the existence of three methoxys, four methylenes, two methines, two aromatic rings and a glucopyranoside fragment. Four methylenes, two methines and two aromatic rings were linked as the aglycone of nymphaeoside A [8] inferred from the key HMBC correlations [H-1/C-4, H-2/C-4, H-2/C-10, H-16/C-13, H-16/C-18]. A $\beta$-glucopyranoside moiety was attached to C-7, which can be deduced from the coupling constant of its anomeric proton [1H, d, $J$ = 7.04 Hz, H-1′] and the HMBC correlation [H-1′/C-7]. Because the coupling constant of H-1 and H-2 in the *threo*-isomer (6 – 8 Hz) is larger than in the *erythro*-isomer (2 – 4 Hz) [5], **5** was an *erythro*-isomer due to the coupling constant of the benzylic proton ($J$ = 4.7 Hz) [10]. Therefore, compound **5** was elucidated as *erythro*-1-(4-*O*-$\beta$-D-glucopyranosyl-3-methoxyphen-

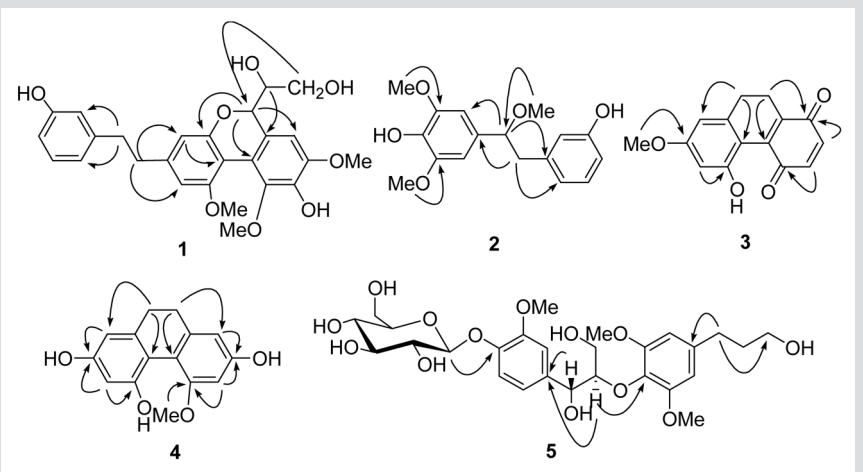


**Fig. 2** Key HMBC (H → C) correlations of compounds **1 – 5**.

**Table 2** Anti-platelet aggregation activity of compounds **1 – 5** (TCP: ticlopidine)

| Sample | Concentration ($\mu$M) | ADP ($\mu$M) | A (1′) (%) | A (max) (%) |
|---|---|---|---|---|
| Control | N/A | 8.93 | 0.00 | 0.00 |
| **1** | 1525.6 | 8.93 | 69.42 | 73.59 |
| **2** | 2348.7 | 8.93 | 85.66 | 83.48 |
| **3** | 2789.1 | 8.93 | 96.13 | 83.30 |
| **4** | 2767.4 | 8.93 | 57.34 | 63.16 |
| **5** | 1252.6 | 8.93 | 30.34 | 33.16 |
| TCP | 1088.4 | 8.93 | 100 | 100 |

yl)-2-[4-(3-hydroxypropyl)-2,6-dimethoxyphenoxy]-1,3-propanediol.

The fourteen known compounds were identified as batatasin [11], gigantol [12], moscatilin [13], aloifol I [3], tristin [14], 3,3′,4-trihydroxybibenzyl [15], (3*S*,4*S*,5*R*)-3,4,5-trihydroxy-1-cyclohexenecarboxylic acid [16], naringenin [17], methyl $\beta$-orsellinate [18], episyringaresinol [19], episyringaresinol 4″-*O*-$\beta$-D-glucopyranoside [5], 3-(3-methoxy,4-hydroxyphenyl)-1-propanol [20], 9-$\beta$-D-ribofuranosyl-9*H*-purin-6-amine [21] and eugenyl *O*-$\beta$-D-glucopyranoside [22] by comparison of their spectroscopic data with literature values.

Anti-platelet aggregation activity is an important indication for "Shi-Hu" to treat blood diseases. Since several compounds isolated from *Dendrobium* species have already been reported to exhibit such activity [23], [24], compounds **1 – 5** were tested for anti-platelet aggregation activity on New Zealand white rabbit platelets *in vitro* (● **Table 2**). Ticlopidine (TCP) was used as a positive control and its 100% inhibition concentration was 1088.44 $\mu$M/mL. Compounds **1**, **2**, **3**, **4** and **5** exhibited weak anti-platelet aggregation activities.

## Acknowledgements

The authors thank Dr Tina Tingya Dong, Professor Karl W. Tsin, Dr Zhi-Yong Jiang, Department of Biology, Hongkong University of Science and Technology for the anti-plate aggregation activity tests. The authors are also 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 of the spectral measurements.

## References

1 Dictionary of Chinese Medicines Shanghai: Shanghai Science and Technology Press; 1986: 586
2 Pharmacopoeia of China, Part I Beijing: Chemistry and Industry Press; 2005: 62
3 *Juneja RK, Sharma SC, Tandon JS.* Two substituted bibenzyls and a dihydrophenanthrene from *Cymbidium aloifolium*. Phytochemistry 1987; 26: 1123 – 5
4 *Letcher RM, Nhamo LRM.* Chemical constituents of the Combretaceae part III. Substituted phenanthrenes, 9, 10-dihydrophenanthrenes and bibenzyls from the heartwood of *Combretum psidioides*. J Chem Soc [Perkin I] 1972; 23: 2941 – 6
5 *Li XC, Barnes DL, Khan IA.* A new lignan glycoside from *Eleutherococcus senticosus*. Planta Med 2001; 67: 776 – 8
6 *Bhaskar MU, Rao LJM, Rao NSP, Rao PRM.* Ochrone A, a novel 9,10-dihydro-1,4-phenanthraquinone from *Coelogyne ochracea*. J Nat Prod 1991; 54: 386 – 9
7 *Leong YW, Kang CC, Harrison LJ, Powell AD.* Phenanthrenes, dihydrophenanthrenes and bibenzyls from the orchid *Bulbophyllum vaginatum*. Phytochemistry 1997; 44: 157 – 65
8 *Zhang ZZ, ElSohly HN, Li XC, Khan SI, Broedel Jr SE, Raulli RE et al.* Phenolic compounds from *Nymphaea odorata*. J Nat Prod 2003; 66: 548 – 50
9 *Yoshikawa K, Sugawara S, Arihara S.* Phenylpropanoids and other secondary metabolites from fresh fruits of *Picrasma quassioides*. Phytochemistry 1995; 40: 253 – 6
10 *Miyase T, Ueno A, Takizawa N, Kobayashi H, Oguchi H.* Studies on the glycosides of *Epimedium grandiflorum* Morr var. *thunbergianum* (Miq.) Nakai . Chem Pharm Bull 1987; 35: 3713 – 9
11 *Bai L, Masukawa N, Yamaki M, Takagi S.* Two bibenzyl glucosides from *Pleione bulbocodioides*. Phytochemistry 1997; 44: 1565 – 7
12 *Leslie C, Jamieson SV.* Dihydrostilbenes of cannabis, synthesis of cannipren. J Chem Soc [Perkin I] 1982; 7: 1467 – 75
13 *Majumder PL, Sen RC.* Moscatilin, a bibenzyl derivative from the orchid *Dendrobium moscatum*. Phytochemistry 1987; 26: 2121 – 4
14 *Majumder PL, Pal S.* Cumulantin and tristin, two bibenzyl derivatives from the orchids *Dendrobium cumulatum* and *Bulbophyllum triste*. Phytochemistry 1993; 32: 1561 – 5
15 *Anton H, Schoeneborn R, Mues R.* Bibenzyls and bisbibenzyls from a neotropical *Plagiochila* species. Phytochemistry 1999; 52: 1639 – 45
16 *Geiger H, El-Dessouki S, Seeger T.* (3*S*,4*S*,5*R*)-3,4,5-Trihydroxy-1-cyclohexene-carboxylic acid from *Sequoiadendron giganteum*. Phytochemistry 1995; 40: 1705 – 7
17 *Cioffi G, Escobar LM, Braca A, De Tommasi N.* Antioxidant chalcone glycosides and flavanones from *Maclura* (Chlorophora) *tinctoria*. J Nat Prod 2003; 66: 1061 – 4
18 *Hylands PJ, Ingolfsdottir K.* The isolation of methyl $\beta$-orsellinate from *Stereocaulon alpinum* and comments on the isolation of 4, 6-dihydroxy-2-methoxy-3-methylacetophenone from *Stereocaulon* species. Phytochemistry 1985; 24: 127 – 9
19 *Chang FR, Chao YC, Teng CM, Wu TC.* Chemical constituents from *Cassytha filiformis* II. J Nat Prod 1998; 61: 863 – 6
20 *Cardona ML, Garcia B, Pedro JR, Sinisterra JF.* Flavonoids, flavonolignans and a phenylpropanoid from *Onopordon corymbosum*. Phytochemistry 1990; 29: 629 – 31
21 *Ranganathan R.* A novel purine nucleoside synthesis: 9-$\beta$-D-arabinofuranosyl-ademine. Tetrahedron Lett 1975; 13: 1185 – 8
22 *Takeda Y, Ooiso Y, Masuda T, Honda G, Otsuka H, Sezik E et al.* Iridoid and eugenol glycosides from *Nepeta cadmea*. Phytochemistry 1998; 49: 787 – 91
23 *Chen CC, Wu LG, Ko FN, Teng CM.* Antiplatelet aggregation principles of *Dendrobium loddigesii*. J Nat Med 1994; 57: 1271 – 4
24 *Fan CQ, Wang W, Wang YP, Qin GW, Zhao WM.* Chemical constituents from *Dendrobium densiflorum*. Phytochemistry 2001; 57: 1255 – 8