Isolation of Stilbenoids and Lignans from *Dendrobium hongdie*

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**Abstract**

**Purpose:** To isolate and characterize chemical compounds of biological importance from the whole plant of *Dendrobium hongdie*.

**Methods:** The whole plants of *Dendrobium hongdie* was extracted with ethanol (EtOH) and separated using silica gel, Sephadex LH-20 and MCI gel to isolate the pure compounds. Characterization of the isolated compounds was achieved using ¹H and ¹³C nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).

**Results:** Nine compounds including two phenanthrenes, three bibenzyls, a phenanthraquinone, two lignans and a sterol were isolated from the extract. The structures of the compounds were elucidated as nudol (1), gigantol (2), batatasin III (3), tristin (4), moscatin (5), ephemeralanthoquinone (6), (-)-syringaresinol (7), liriodendrin (8) and β-sitosterol (9).

**Conclusion:** Nine compounds have been successfully isolated from *D. hongdie* for the first time. This plant is a potential source of some useful phytochemicals.

**Keywords:** *Dendrobium hongdie*, Isolation, Stilbenoids, Lignans, Phytochemicals

**INTRODUCTION**

Stilbenoids such as phenanthrenes, bibenzyls, and phenanthraquinones are regarded as important secondary metabolites occurring exclusively in the plants of Orchidaceae, which was distributed in tropical and subtropical areas and consist of about 700 genus and 20000 species [1]. The genus *Dendrobium* is represented by more than 1100 species widely distributed throughout Asia, Europe, and Australia, and there are about 80 species of *Dendrobium* in China. The stems of a number of *Dendrobium* species was used as precious healthy foods and nutrients in traditional Chinese medicine [2]. Previous phytochemical investigations on the plants of *Dendrobium* genus such as *D. nobile*, *D. crysotoxum* and *D. densiflorum*, have led to the isolation of bibenzyls, phenanthrenes, alkaloids, polysaccharides, fluorenones, sesquiterpenes, coumarins and others, mainly bibenzyls and phenanthrenes, with antitumor, antimutagenic, immunomodulatory, antioxidant and platelet aggregation inhibitory activities [3-7]. *D. hongdie* was a hybrid of *D. nobile* and *D. phalaenopsis* developed by Yunnan Inmol Laboratory of Technology, China. Previously there was no report on its chemical constituents. In the course of our search for bioactive natural products from medicinal plants in Yunnan of China, we investigated the plant. From the whole plants of...
D. hongdie, nine compounds including two phenanthrenes, three bibenzyls, one phenanthraquinone, two lignans and a sterol were isolated and their structures were identified by $^1$H-$^1$C-NMR and MS spectrum.

**EXPERIMENTAL**

**Plant material**

The whole plant of *D. hongdie* was collected from Yunnan Inmol Laboratory of Technology, Kunming, China and identified by one of the authors, Prof. Hong Yu (School of Life Science, Yunnan University, Kunming, China).

**Extraction**

The air-dried powdered whole plants (4 kg) were exhaustively extracted with 95 % EtOH six times at room temperature (each for 24 h). The extract obtained was concentrated to dryness under reduced pressure in a rotary evaporator to yield the EtOH extract (180 g, 4.5 %), which was dissolved in water (1 L) and extracted with CHCl$_3$ and EtOAc successively to yield CHCl$_3$ (65 g, 1.62 %), EtOAc (30 g, 0.75 %) and water soluble fractions (90 g, 2.25 %).

**Isolation of compounds**

Silica gel (200-300 mesh, Qingdao Marine Chemical Co., China), Sephadex LH-20 (25-100 μm, Pharmacia Fine Chemical Co. Ltd.) and MCI gel CHP 20P (75-150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography (CC), and silica gel GF254 was used for TLC (Qingdao Marine Chemical Co., China). Solvents were of industrial purity and re-distilled prior to use.

![Figure 1: Structures of compounds isolated from *Dendrobium hongdie*](image-url)
The CHCl₃ soluble fraction (65 g) was separated on a silica gel column, eluting with petroleum ether containing increasing amounts of EtOAc (1:0, 20:1, 10:1, 8:1, 8:2, 7:3, 1:1) to obtain 7 fractions. Fraction 2 (6 g, eluted with petroleum ether/acetonitrile 20:1) was subjected to column chromatography on silica gel (petroleum ether/acetonitrile 10:1, v/v portion) to yield 9 (150 mg). Fraction 3 (5 g, eluted with petroleum ether/acetonitrile 10:1) was subjected to column chromatography using MeOH/CHCl₃ (2:3) on Sephadex LH-20 to yield 2 (2 g), 3 (16 mg) and 6 (8 mg). Fraction 4 (3.5 g, eluted with petroleum ether/acetonitrile 8:1) was chromatographed using MeOH/CHCl₃ (2:3) and Sephadex LH-20 to obtain 1 (30 mg) and 5 (25 mg). Similarly, fraction 5 (1.3 g, eluted with petroleum ether/acetonitrile 8:2) was purified over Sephadex LH-20 using MeOH/CHCl₃ (2:3) to yield 4 (30 mg). The EtOAc soluble fraction (30 g) was separated on silica gel column, eluting with CHCl₃/acetone 8:2 to produce 7 (100 mg). The water soluble phase after extraction with EtOAc was subjected on a MCI chromatography, eluting with water with increasing amounts of MeOH (0 %, 50 %, 90 %, 100 %) to furnish 3 fractions. The 70 % MeOH fraction (28 g) was applied on a column chromatography (silica gel, gradient CHCl₃/MeOH) (1.0:20:1, 10:1, 8:1, 8:2, 7:3, 0:1) to yield 8 (300 mg).

**Identification of compounds**

Identification of compounds was carried out using mass spectroscopy (MS, API Ostar Pulsa LC/TOF mass spectrometer,) and ¹H, ¹³C-NMR spectra on Bruker DRX-500 spectrometer.

**RESULTS**

The EtOH extract from the whole plants of *D. hongdie* yielded nine compounds (two phenanthrenes, three bibenzyls, one phenanthroquinone, two lignans and a sterol) as follows:

**Compound 1:** White amorphous powder. EIMS m/z 270 [M⁺]. ¹H NMR (CD₂COCD₂) : 7.53, 7.50 (each 1H, J = 8.9), 9.33 (1H, d, J = 9.2), 7.19 (1H, d, J = 9.2, 2.8), 7.24 (1H, d, J = 2.8), 7.15 (1H, s), 3.98, 4.01 (each 3H, s). ¹³C NMR (CD₂COCD₂) : 155.0, 151.3, 149.0, 142.0, 133.7, 129.4, 128.0, 126.8, 126.2, 123.5, 118.3, 116.7, 111.6, 108.9, 60.4, 59.2. Comparison with the data shown in literature [8], the compound was identified as be nudol (Fig 1).

**Compound 2:** Yellow gum. EIMS m/z 274 [M⁺]. 137 (100), 122, 107, 94, 77. ¹H NMR (CD₂COCD₂) : 6.35 (1H, s, H-4'), 6.31 (2H, s, H-2', 6'), 6.88 (1H, d, J = 8.0, H-5'), 6.72 (1H, d, J = 8.0, H-6'), 6.67 (1H, s, H-2'), 3.88 (3H, s, OCH₃-3'), 3.82 (3H, s, OCH₃-5'), 2.82 (4H, m, CH₂-1, 2). ¹³C NMR (CD₂COCD₂) : 161.7 (C-5'), 157.8 (C-3'), 147.2 (C-3'), 145.4 (C-1'), 144.5 (C-4'), 134.7 (C-1'), 121.9 (C-6'), 115.2 (C-2'), 112.2 (C-5'), 109.5 (C-2'), 107.6 (C-6'), 100.0 (C-4'), 56.8 (OCH₃-5'), 56.2 (OCH₃-3'), 39.1 (C-2'), 38.1 (C-1'). Comparison with the data shown in literature [6, 8], the compound was identified as be gigantol.

**Compound 3:** Yellow gum. EIMS m/z 244 [M⁺]. 137 (100), 107, 77. ¹H NMR (CD₂COCD₂) : 7.12 (1H, dd, J = 7.7, 7.7, H-5'), 6.73 (1H, d, J = 7.7, H-6'), 6.72 (1H, dd, J = 7.7, 2.4, H-4'), 6.71 (1H, m, H-2'), 6.39 (1H, s, H-4), 6.30 (2H, s, H-2', 6'), 3.72 (3H, s, OCH₃), 2.82 (4H, m, 1, 2-CH₂). ¹³C NMR (CD₂COCD₂) : 161.9 (C-5'), 159.3 (C-3'), 158.2 (C-3'), 145.2 (C-1'), 144.4 (C-1'), 130.2 (C-5'), 120.5 (C-6), 116.3 (C-2'), 113.7 (C-4'), 108.9 (C-2'), 106.3 (C-6'), 99.9 (C-4'), 55.4 (OCH₃-5'), 38.5 (C-1'), 38.2 (C-2). Comparison with the data shown in literature [9], the compound was identified as be batacasin III.

**Compound 4:** Reddish gum.. EIMS: m/z (%) 260 (M⁺, 22), 137 (100), 123 (5), 107 (2), 94 (50), 77 (3); ¹H NMR [(CD₂CO)CO] : 6.80 (1H, d, J = 1.9, H-2'), 6.76 (1H, d, J = 8.0, H-5') 6.67 (1H, dd, J = 8.0, 1.9, H-6'), 6.28 (2H, m, J = 2.1, H-2', 6'), 6.26 (1H, t, J = 2.1, H-4'), 3.79 (3H, s, 3''-OMe), 2.88 (2H, m, 1-CH₂), 2.81 (2H, m, 2-CH₂); ¹³C NMR [(CD₂CO)CO] : 159.6 (s, C-3'), 159.6 (s, C-5'), 148.5 (s, C-3'), 147.5 (s, C-1'), 145.8 (s, C-4'), 134.8 (s, C-1'), 116.1 (d, C-5'), 122.1 (d, C-6'), 113.4 (d, C-2'), 108.5 (d, C-6'), 108.5 (d, C-2'), 101.7 (d, C-3'), 56.7 (q, 3''-OMe), 39.3 (t, 2-CH₂), 38.3 (t, 1-CH₂). Comparison with the data shown in literature [10], the compound was identified as be tristin.

**Compound 5:** Colorless powder. EIMS: m/z (%) 240 (M⁺, 100), 225 (49), 197 (45), 139 (18); ¹H NMR [(CD₂CO)CO] : 9.57 (1H, s, 5-OH), 9.09 (1H, s, 2-OH), 7.62 (1H, d, J = 8.8, H-9), 7.50 (1H, d, J = 8.8, H-10), 7.43 (1H, dd, J = 7.6, 7.6, H-7), 7.40 (1H, dd, J = 7.6, 1.8, H-6), 7.13 (1H, dd, J = 7.6, 1.8, H-8), 7.09 (1H, d, J = 2.5, H-1), 7.01 (1H, d, J = 2.5, H-3), 4.15 (3H, s, 4-O-Me); ¹³C NMR [(CD₂CO)CO] : 157.4 (C-4), 156.4 (C-2), 155.2 (C-5), 137.2 (C-10a), 135.0 (C-8a), 129.8 (C-7), 127.5 (C-9), 127.0 (C-10), 121.1 (C-8), 119.9 (C-4b), 117.1 (C-6), 114.0 (C-4a), 107.9 (C-1), 102.7 (C-3), 58.7 (4-O-Me). Comparison with the data shown in literature [11], the compound was identified as be moscatin.

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**Compound 6**: Reddish powder. EIMS: m/z 256 [M^+, 100], 241, 213, 171, 115, 69. 1^H NMR spectrum (CD2D2N) · : 8.42 (1H, d, J= 8.6, H-5), 7.16 (1H, d, J= 8.6, H-6), 7.04 (1H, s, H-8), 6.05 (1H, s, H-3), 3.66 (3H, s, MeO-2), 2.66 (2H, m, 9-CH2) 2.62 (2H, m, 10-CH2). 13C NMR (CD2D2N) · : 188.5 (s, C-4), 182.0 (s, C-1), 161.7 (s, C-7), 159.5 (s, C-2), 142.7 (s, C-8a), 137.5 (s, C-10a), 136.9 (s, C-4a), 123.2 (s, C-4b), 133.6 (d, C-5), 116.7 (d, C-8), 115.3 (d, C-5), 108.7 (d, C-2), 56.8 (q, 3-OCH3), 28.5 (t, C-9), 21.3 (t, C-10). Comparison with the data shown in literature [11], the compound was identified as be ephemeralanthoquinone.

**Compound 7**: White crystals. 1H NMR (CDCl3) · : 6.57 (4H, s, H-2, 2’, 6, 6’), 5.56 (2H, br s, 4, 4’-OH), 4.72 (2H, d, J = 4.5 Hz, H-7, 7’), 4.28 (2H, dd, J = 9.0, 7.0 Hz, H-9b, 9b), 3.90 (2H, dd, J = 8.8, 2.8 Hz, H-9a, 9a), 3.88 (12H, s, 3, 3’, 5, 5’, OCH3), 3.08 (2H, m, H-8, 8’), 13C NMR (CDCl3) · : 147.2 (s, C-3, 3’, 5, 5’), 134.3 (s, C-4, 4’), 132.1 (s, C-1, 1’), 102.8 (d, C-2, 2’), 102.7 (d, C-6, 6’), 86.1 (d, C-7, 7’), 71.8 (t, C-9, 9’), 56.4 (q, 3, 3’, 5, 5’-OCH3), 54.3 (d, C-8, 8’). Comparison with the data shown in literature [12], the compound was identified as be (-)-syringaresinol.

**Compound 8**: White amorphous powder. FAB-MS m/z 765 ([M+Na]+). 1H NMR (CDCl3) · : 6.93 (4H, s), 3.79 (12H, s). 13C NMR (CDCl3) · : 153.9, 138.2, 135.2, 105.0, 104.9, 86.1, 78.6, 78.3, 76.0, 72.2, 71.6, 62.6, 56.7, 54.7. Comparison with the data shown in literature [13], the compound was identified as be liroindendrin.

**Compound 9**: White amorphous powder. EIMS m/z: 412 [M]+, 399, 384, 379, 365, 347, 213, 145, 91. 1H NMR (CDCl3) · : 5.37 (1H, m, H-6), 3.55 (1H, m, H-3), 0.69 (3H, s, H-18); 13C NMR (CDCl3) · : 140.8 (s, C-5), 121.7 (d, C-6), 71.8 (d, C-3), 56.8 (d, C-14), 56.1 (d, C-17), 50.2 (d, C-9), 45.8 (C-24), 42.3 (t, C-4, s, C-13), 39.8 (t, C-12), 37.3 (t, C-1), 36.5 (s, C-10), 36.2 (d, C-20), 34.0 (t, C-22), 31.9 (t, C-7, d, C-8), 31.6 (t, C-2), 29.2 (d, C-25), 28.3 (t, C-16), 26.1 (t, C-23), 24.3 (t, C-15), 23.1 (t, C-28), 21.2 (t, C-11), 19.84 (q, C-26), 19.4 (q, C-19), 19.1 (q, C-27), 18.8 (q, C-21), 12.0 (q, C-18), 11.9 (q, C-29). Based on the data and a direct comparison with an authentic sample, 9 was identified as be β-sitosterol.

**DISCUSSION**

Some compounds have been isolated from the plants of Orchidaceae which usually contain stilbenoids such as phenanthrenes and bibenzyls as characteristic compounds. Some of the isolated compounds have been found to show significant anti-tumor, anti-inflammatory and platelet anti-aggregation activities. In the present study, nine compounds have been isolated from the EtOH extract of the whole plants of D. hongdie. Detailed analysis of the 1H and 13C NMR showed that their structures were phenanthrenes nudol (1) and moscatin (5), bibenzyls gigantol (2), batatasin III (3) and tristin (4), phenanthraquinone ephemeralanthoquinone (6), lignans (-)-syringaresinol (7) and liroindendrin (8) and sterol β-sitosterol (9) respectively. All the compounds were isolated from D. hongdie for the first time. Some of the isolated compounds have been evaluated for biological properties and significant results were reported. Examples are the inhibition of ephemeralanthoquinone on the spontaneous contraction of the guinea-pig ileum, spasmylytic, allelopathic and inhibitory activities of batatasin III on germination and radicle growth, and cytotoxicity of tristin against human stomach cancer SGC-7901 [6,14-19].

**CONCLUSION**

Nine compounds namely, two phenanthrenes, three bibenzyls, one phenanthraquinone, two lignans and a sterol have been isolated from the whole plants of D. hongdie for the first time, and their structures were elucidated as nudol (1), gigantol (2), batatasin III (3), tristin (4), moscatin (5), ephemeralanthoquinone (6), (-)-syringaresinol (7), liroindendrin (8) and β-sitosterol (9). The results provided some understanding on the chemical constituents of the plant and also showed that the plant could be a potential source of some useful phytochemicals.

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