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Original Research Article

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 ^{1}H and ^{13}C 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), ephemeranthoquinone (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-, ^{13}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.

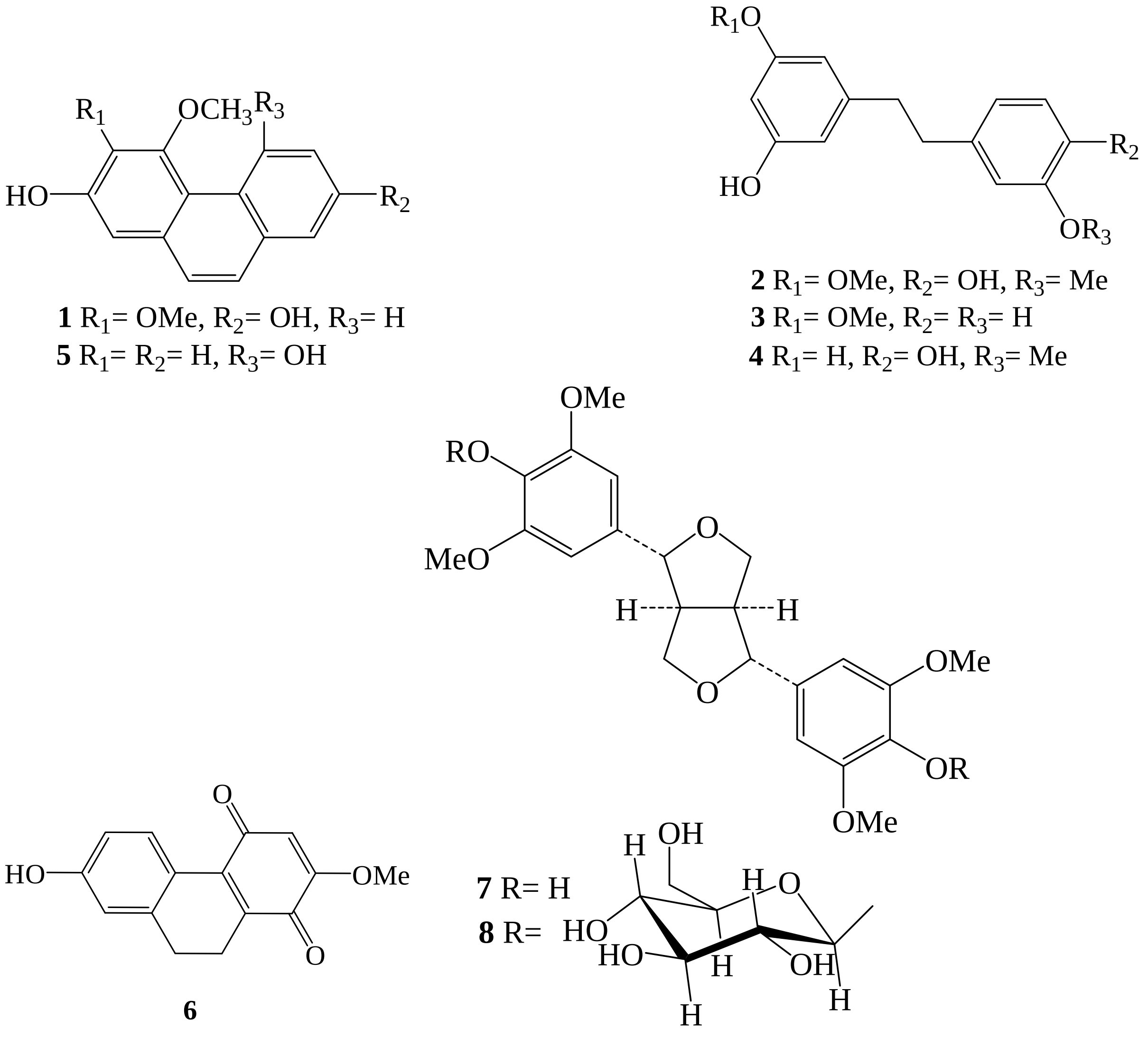

Fig 1: Structures of compounds isolated from *Dendrobium hongdie*

The $CHCl_3$ 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/acetone 20:1) was subjected to column chromatography on silica gel (petroleum ether/acetone 10:1, v/v portion) to yield 9 (150 mg). Fraction 3 (5 g, eluted with petroleum ether/acetone 10:1) was subjected to column chromatography using MeOH/$CHCl_3$ (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/acetone 8:1) was chromatographied using MeOH/$CHCl_3$ (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/acetone 8:2) was purified over Sephadex LH-20 using MeOH/$CHCl_3$ (2:3) to yield 4 (30 mg). The EtOAc soluble fraction (30 g) was separated on silica gel column, eluting with $CHCl_3$/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 50 % MeOH fraction (28 g) was applied on a column chromatography (silica gel, gradient $CHCl_3$/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 Qstar Pulsa LC/TOF mass spectrometer,) and ^{1}H-, ^{13}C-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 phenanthraquinone, two lignans and a sterol) as follows:

Compound 1: White amorphous powder. EIMS *m/z* 270 ([M]$^+$, 100). ^{1}H NMR (CD_3COCD_3) ・: 7.53, 7.50 (each 1H, J= 8.9), 9.33 (1H, d, J= 9.2), 7.19 (1H, dd, J= 9.2, 2.8), 7.24 (1H, d, J= 2.8), 7.15 (1H, s), 3.98, 4.01 (each 3H, s). ^{13}C NMR (CD_3COCD_3) ・: 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. ^{1}H NMR ($CDCl_3$) ・: 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, OCH_3-3''), 3.82 (3H, s, OCH_3-5'), 2.82 (4H, m, CH_2-1, 2). ^{13}C NMR ($CDCl_3$) ・: 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 (OCH_3-5'), 56.2 (OCH_3-3''), 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. ^{1}H NMR (CD_3COCD_3) ・: 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, 5'-OCH_3), 2.82 (4H, m, 1,2-CH_2). ^{13}C NMR (CD_3COCD_3) ・: 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 (OCH_3-5'), 38.5 (C-1), 38.2 (C-2). Comparison with the data shown in literature [9], the compound was identified as be batatasin III.

Compound 4: Reddish gum.. EIMS: *m/z* (%) 260 (M$^+$, 22), 137 (100), 123 (5), 107 (2), 94 (50), 77 (3); ^{1}H NMR [$(CD_3)_2CO$] ・: 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, d, *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-CH_2), 2.81 (2H, m, 2-CH_2); ^{13}C NMR [$(CD_3)_2CO$] ・: 159.6 (s, C-3'), 159.6 (s, C-5'), 148.5 (s, C-3''), 145.7 (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-CH_2), 38.3 (t, 1-CH_2). 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); ^{1}H NMR [$(CD_3)_2CO$] ・: 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-OMe); ^{13}C NMR [$(CD_3)_2CO$] ・: 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-OMe). Comparison with the data shown in literature [11], the compound was identified as be moscatin.

Compound 6: Reddish powder. EIMS: *m*/*z* 256 [M^+, 100], 241, 213, 171, 115, 69. ^{1}H NMR spectrum (C_5D_5N) ・: 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-CH_2) 2.62 (2H, m, 10-CH_2). ^{13}C NMR (C_5D_5N) ・: 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-OCH_3), 28.5 (t, C-9), 21.3 (t, C-10). Comparison with the data shown in literature [11], the compound was identified as be ephemeranthoquinone.

Compound 7: White crystals. ^{1}H NMR ($CDCl_3$) ・: 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, 9'b), 3.90 (2H, dd, *J* = 8.8, 2.8 Hz, H-9a, 9'a), 3.88 (12H, s, 3, 3', 5, 5'-OCH_3), 3.08 (2H, m, H-8, 8'); ^{13}C NMR ($CDCl_3$) ・: 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'-OCH_3), 54.3 (d, C-8, 8'). Comparison with the data shown in literature [12], the compound was identified as be (-)-syringaresinol.

Compound 8: White amporous powder. FAB-MS *m*/*z* 765 ([M+Na]$^+$). ^{1}H NMR (C_5D_5N) ・: 6.93 (4H, s), 3.79 (12H, s). ^{13}C NMR (C_5D_5N) ・: 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 liriodendrin.

Compound 9: White amporous powder. EIMS *m*/*z:* 412 [M]$^+$ (100), 369, 351, 300, 271, 255, 213, 145, 91. ^{1}H NMR ($CDCl_3$) ・: 5.37 (1H, m, H-6), 3.55 (1H, m, H-3), 0.69 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) ・: 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 *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 ephemeranthoquinone (6), lignans (-)-syringaresinol (7) and liriodendrin (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 ephemeranthoquinone on the spontaneous contraction of the guinea-pig ileum, spasmolytic, 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), ephemeranthoquinone (6), (-)-syringaresinol (7), liriodendrin (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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