A New Bibenzyl from *Dendrobium secundum*

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From the aerial parts of *Dendrobium secundum*, a new bibenzyl, namely 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl, was isolated, along with four known compounds which included brittonin A, moscatilin, syringaresinol, and ferulic acid. All of these isolates showed appreciable DPPH free radical scavenging potential, except for brittonin A, which was devoid of activity.

Key words: Dendrobium secundum, Bibenzyl, Free Radical Scavenging

Introduction

The genus *Dendrobium* (Orchidaceae) is represented by more than 1100 species widely distributed throughout Asia, Europe, and Australia, and there are about 150 species of *Dendrobium* in Thailand (Seidenfaden, 1985). *Dendrobium* plants have been reported to elaborate several classes of secondary metabolites including bibenzyls, alkaloids, phenanthrenes, fluorenones, sesquiterpenes, coumarins, steroids, and polysaccharides (Zhang *et al.*, 2007; Chen Y. *et al.*, 2008; Ito *et al.*, 2010).

As part of our continuing studies on bioactive phenolics from Thai medicinal plants (Sritularak and Likhitwitayawuid, 2009; Sritularak *et al.*, 2010), we examined the constituents of *Dendrobium secundum* (Blume) Lindl. (Orchidaceae), a native plant of Thailand with no previous reports on its secondary metabolites. In this study, an MeOH extract prepared from the aerial parts of this plant was found to possess antioxidative potential, showing 75% DPPH (2,2-diphenyl-1-picrylhydrazyl) reduction at the concentration of 100 μg/ml. A subsequent chemical investigation resulted in the isolation of a new bibenzyl, namely 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl (**1**), as well as four known compounds. Each of these isolates was evaluated for its DPPH free radical scavenging activity.

Results and Discussion

Compound **1** was isolated as a yellow amorphous solid. The positive HR-ESI mass spectrum exhibited an $[M + Na]^+$ ion at *m/z* 313.1049 (calcd. for $C_{16}H_{18}NaO_5$ 313.1052), suggesting the molecular formula $C_{16}H_{18}O_5$. The IR spectrum showed absorption bands for hydroxy (3419 cm^{-1}) and aromatic (1609, 1463 cm^{-1}) groups, respectively. The UV absorptions at 219 and 281 nm were indicative of a bibenzyl structure (Zhang *et al.*, 2007). This was supported by the presence of two pairs of methylene protons at δ_H 2.78 ppm (H_2-α') and δ_H 2.75 ppm (H_2-α), which correlated to the carbon atoms at δ_C 37.7 ppm (C-α') and δ_C 38.2 ppm (C-α), respectively, in the HSQC spectrum. The ^1H-^1H COSY spectrum of **1** (Table I) showed an ABM coupling system representing three aromatic protons for ring B [δ_H 6.60 ppm (1H, d, *J* = 2.0 Hz, H-2′), δ_H 6.65 ppm (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), and δ_H 6.80 ppm (1H, d, *J* = 8.0 Hz, H-5′)], similar to that of moscatilin (**2**), a bibenzyl first isolated from *Dendrobium moscatum* (Majumder and Sen, 1987). This was confirmed by the NOESY correlation between H-2′ and the methoxy protons at δ_H 3.83 ppm (3H, s). The fact that the molecular mass of **1** is 14 amu lower than that of **2** suggests that **1** has only one methoxy group at ring A. This methoxy group should be located at C-3 of ring A, as supported by the NOESY interaction between H-2 [δ_H 6.21 ppm (1H, d *J* = 2.0 Hz)] and MeO-3 protons [δ_H 3.80 ppm (3H, s)]. This was corroborated by the HMBC correlations from C-α to H-2 and H-6, and from C-α' to H-2′ and H-6′. Thus, **1** was identified as 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl (Fig. 1).

The identification of moscatilin (**2**) (Majumder and Sen, 1987), brittonin A (**3**) (Asakawa *et al.*,

Table I. ^{1}H NMR and ^{13}C NMR data of **1** (in $CDCl_3$).

Position	δ_H	δ_C	HMBC (correlation with ^{1}H)
1	-	130.4	2*, 6*, α'
2	6.21 d (2.0)	103.5	6, α
3	-	146.6	2*, 3-OMe
4	-	133.7	2
5	-	143.7	6*
6	6.42 d (2.0)	108.6	2, α
1′	-	133.8	2′*, 5′, α, α'*
2′	6.60 d (2.0)	111.2	6′, α'
3′	-	146.2	2′*, 5′, 3′-OMe
4′	-	143.7	2′, 5′*, 6′
5′	6.80 d (8.0)	114.1	6′*
6′	6.65 dd (8.0, 2.0)	121.0	2′, 5′*, α'
α	2.75 m	38.2	2, 6, α'*
α'	2.78 m	37.7	2′, 6′, α*
3-OMe	3.80 s	56.1	-
3′-OMe	3.83 s	55.9	-

* Two-bond coupling.

1976), syringaresinol (**4**) (Vermes *et al.*, 1991), and ferulic acid (**5**) (Dobhal *et al.*, 1999) was done through comparison of their spectroscopic data with reported values.

With regard to their DPPH free radical scavenging activity, compounds **1**, **2**, **4**, and **5** exhibited appreciable activity [IC_{50} (15.87 ± 1.48), (5.14 ± 0.18), (11.38 ± 0.24), (37.52 ± 0.47) μM, respectively], as compared with the positive controls quercetin and Trolox [IC_{50} (2.47 ± 0.08), (11.68 ± 0.44) μM, respectively]. Compound **3** was inactive (showing 12.53% inhibition at 100 μg/ml), as expected, due to the lack of free OH groups. It should be noted that moscatilin (**2**) had higher activity than Trolox. This compound has been extensively studied for its anticancer potential (Ho and Chen, 2003; Chen T. H. *et al.*, 2008; Tsai *et al.*, 2010).

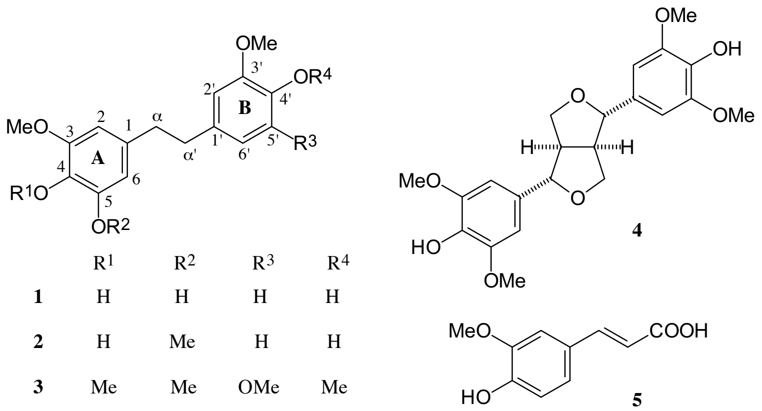
Material and Methods

General procedures

UV spectra were obtained on a Milton Roy Spectronic 3000 Array spectrophotometer (Rochester, Monroe, NY, USA) and IR spectra on a Perkin-Elmer FT-IR 1760X spectrophotometer (Norwalk, Fairfield, CT, USA). Mass spectra were recorded on a Micromass LCT mass spectrometer (ESI-TOF-MS) (Manchester, UK). NMR spectra were recorded on a Bruker Avance DPX-300 FT-NMR spectrometer (Rheinstetten, Germany) or a Varian Unity INOVA-500 NMR spectrometer (Palo Alto, Santa Clara, CA, USA). Microtiter plate reading was performed on a Perkin-Elmer Victor3 1420 multilabel counter.

Plant material

The stems of *D. secundum* were purchased from Jatujak market, Bangkok, Thailand, in September 2009, and identified by Prof. Thatree Phadungcharoen (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand). A voucher specimen (DS/BS-092552) is deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.



	R1	R2	R3	R4
1	H	H	H	H
2	H	Me	H	H
3	Me	Me	OMe	Me

Fig. 1. Chemical structures of 4,5,4′-trihydroxy-3,3′-dimethoxybibenzyl (**1**), moscatilin (**2**), brittonin A (**3**), syringaresinol (**4**), and ferulic acid (**5**).

Extraction and isolation

Dried powdered stems of *D. secundum* (1.6 kg) were extracted with MeOH (3 × 10 l) at room temperature to give a viscous mass of dried extract (206 g) after evaporation of the solvent. This material was subjected to vacuum-liquid chromatography (VLC) on silica gel (*n*-hexane/EtOAc and CH_2Cl_2/MeOH gradient) to give 8 fractions. Fraction 6 (2.2 g) was separated by column chromatography (CC) (silica gel; *n*-hexane/EtOAc, 8:2) and then purified on a Sephadex LH-20 column (CH_2Cl_2/MeOH, 1:1) to give 5 fractions. Separation of fraction 2 (183 mg) was performed on silica gel (CH_2Cl_2/MeOH, 99.5:0.5) to afford brittonin A (**3**) (110 mg). Fraction 7 (16.8 g) was separated by VLC over silica gel (CH_2Cl_2/MeOH gradient) to give 6 fractions (A – F). Fraction C (2.7 g) was separated by CC (silica gel; *n*-hexane/EtOAc, 7:3) and then further purified on a Sephadex LH-20 column (MeOH) to give 7 fractions. Fraction 3 (87 mg) was further subjected to repeated CC over silica gel (CH_2Cl_2) to yield moscatilin (**2**) (37 mg). Fraction D (1.3 g) was separated by CC (silica gel; CH_2Cl_2/acetone, 97:3) to give 11 fractions (I – XI). Separation of fraction V (57 mg) by CC (silica gel; *n*-hexane/EtOAc, 4:1) gave **1** (5 mg). Fraction VII (200 mg) was separated on Sephadex LH-20 (MeOH) and then on silica gel (*n*-hexane/EtOAc, 7:3) to give 5 fractions. Separation of fraction 4 (47 mg) by CC (silica gel; CH_2Cl_2/MeOH, 99.6:0.4) gave syringaresinol (**4**) (22 mg). Fraction IX (89 mg) was chromatographed on a Sephadex LH-20 column (MeOH) and then on a silica gel column (CH_2Cl_2/MeOH, 8.5:1.5) to give ferulic acid (**5**) (7 mg).

4,5,4'-Trihydroxy-3,3'-dimethoxybibenzyl (**1**): Yellow amorphous solid. – UV (MeOH): λ = 281 (3.4), 219 (4.1) nm. – IR (film): υ = 3419, 2934, 1721, 1609, 1515, 1463, 1455, 1202 cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): see Table I. – ^{13}C NMR (75 MHz, $CDCl_3$): see Table I. – HR-ESI-MS: m/z = 313.1049 $[M + Na]^+$ (calcd. for $C_{16}H_{18}NaO_5$ 313.1052).

DPPH radical scavenging method

The free radical scavenging effect of the samples was assessed by measuring their ability to decolour a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (Sigma, St. Louis, MO, USA) as previously described (Likhitwitayawuid *et al.*, 2006; Sritularak *et al.*, 2010). Briefly, test samples were initially prepared as a solution in EtOH (1000 μg/ml). Each compound was first tested at the concentration of 100 μg/ml. An IC_{50} value was determined for the compound that showed more than 50% inhibition. For IC_{50} analysis, two-fold serial dilutions were performed to give seven concentrations. The test was done by addition of the sample solution (20 μl) to the solution of 50 μM DPPH in EtOH (180 μl) in a 96-well microtiter plate. The reaction mixture was incubated at room temperature for 30 min, and then its absorbance at 510 nm was measured with a microplate reader. Quercetin (Sigma) and Trolox® were used as positive controls.

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