A New Isoflavan From the Heartwood of *Dalbergia cochinchinensis*

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**Abstract**

A new isoflavan, named dalbergiacochan A (1), was isolated from the heartwood of *Dalbergia cochinchinensis*, along with two known isoflavans, namely mucronulatol (2) and 2'-O-methylsepiol (3). Their structures were elucidated based on extensive spectroscopic analyses, including one-dimensional (1D) and two-dimensional nuclear magnetic resonance (2D NMR), and mass spectroscopy (MS) data, and the absolute stereochemistry of compound 1 was determined as R-3',6-dihydroxy-2',4',8-trimethoxyisoflavan from its circular dichroism spectrum. Compound 1 was inactive against *Escherichia coli*, *Bacillus thuringiensis*, and *Shigella dysenteriae*.

**Keywords**

*Dalbergia cochinchinensis*, flavonoids, dalbergiacochan A, antibacterial activity, isoflavan

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*Dalbergia cochinchinensis* Pierre ex Laness, family Fabaceae, is well-known as Thailand Rosewood, Siamese Rosewood, and Tracwood. It is a threatened tree yielding valuable hardwood found in Thailand, Vietnam, Laos, and Cambodia. It is currently listed on the International Union for Conservation of Nature (IUCN) Red List as endangered.

In past years, many studies have been conducted on the chemical constituents of *D. cochinchinensis*. Neoflavones, flavonones, isoflavones, chalconones, flavones, flavanes, isoflavane, benzo-furanes, glycosides, phenols, isoflavonequinone, and pterocarpane were isolated from *D. cochinchinensis*. As part of our ongoing study of the chemical constituents of the heartwood of *D. cochinchinensis*, a 95% EtOH crude extract was conducted to isolate more compounds. As a result, a new isoflavon (1) and two known isoflavans (2 and 3) were isolated from the EtOAc-soluble fraction of the 95% EtOH extract of the heartwood (Figure 1). This paper deals with the isolation and structural elucidation of compound 1. In addition, the antimicrobial potential of compound 1 was tested against *Escherichia coli*, *Bacillus thuringiensis*, and *Shigella dysenteriae*.

**Results and discussion**

Compound 1 was obtained as a crystalline solid. Its molecular formula, C18H20O6, was deduced from the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) for the peak at m/z 333.1330 [M+H]+ (calcd for C18H21O6, 333.1338), and the ESI-MS ion peak at m/z 355.0 [M+Na]+, as well as the nuclear magnetic resonance (NMR) spectral data. According to the heteronuclear multiple bond correlation spectroscopy (HMBC) and heteronuclear single quantum coherence (HSQC) correlations, all the 1H and 13C NMR data (Table 1) were assigned (Online_supp.1-3).

The 1H NMR spectrum of 1 showed signals at δ 2.75 (1H, dd, J = 15.8, 5.3 Hz), 2.88 (1H, dd, J = 15.8, 11.1 Hz), 3.45 (1H, m), 3.89 (1H, t, J = 10.3 Hz), and 4.14 (1H, ddd, J = 10.3, 3.2, 2.0 Hz) assigned to the −CH2CH(Ar)CH2O− moiety of an isoflavan skeleton. This skeleton was further supported by 13C NMR data which showed peaks for C-2, C-3, and C-4 at δ 71.2, 33.1, and 32.4, respectively. From the HMBC spectrum of 1, the long-range correlation observed between the

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carbon resonance at δ 147.0 was assigned to C-2′ bearing a methoxyl group. The long-range correlation observed between C-3 at δ 33.1 and the hydrogen resonance at δ 6.64 (1H, d, J = 8.5 Hz), assigned to H-6′, was not substituted. The signals at δ 6.71 (1H, d, J = 8.5 Hz, H-5′) and δ 6.64 (1H, d, J = 8.5 Hz, H-6′) suggested a 2′,3′,4′-trisubstituted B-ring of the isoflavon. The HMBC spectrum of 1 revealed cross-peaks between H-5′ and C-1′ (δ 128.7) and C-3′ (δ 140.7), and between H-6′ and C-2′ (δ 147.0) and C-4′ (δ 147.8), confirming the 2′, 4′-methoxy-3′-hydroxy substitution pattern on the B-ring, similar to that of mucronulatol (Online_supp.4-5).13

As for the A-ring, HMBC correlation (Figure 2) was observed between the proton at H-2 (δ 3.89, 4.14) and the quaternary carbon at δ 148.4, assigned to C-9. The signal at δ 6.55 (1H, s) was assigned to H-5 since it presented HMBC correlations with C-4 (δ 32.4) and C-9 (δ 148.4). In addition, the HMBC correlations from H-5 (δ 6.55) and H-7 (δ 6.39) to C-6 (δ 141.5), and from H-7 (δ 141.5) to C-8 (δ 148.7), along with the singlet aromatic protons at δ 6.55 (1H, s) and δ 6.39 (1H, s) and the analysis of its molecular formula, indicated that C-6 and C-8 of 1 were substituted by a hydroxyl group and a methoxyl group, respectively. Compound 1 exhibited a negative Cotton effect in the region 216-279 nm and a positive Cotton effect in the region 280-305 nm, indicating the (3R)-form (Online_supp.6).14,15 Thus, the structure of compound 1 was concluded to be R-3′,6-dihydroxy-2′,4′,8-trimethoxyisoflavan, which is a new structure, named dalbergiacchan A.

In addition, the two known compounds 1 and 2 were identified to be mucronulatol (2)16,17 and 2′-O-methylsepiol (3)18,19 by analysis of NMR data and comparison with those of the related compounds.

Compound 1 was evaluated for its antibacterial activity against both Gram-positive (E coli and B thuringienis) and Gram-negative bacteria (S dysenteriae). Erythromycin was used as a positive control. Compound 1 was found to be inactive against all the tested bacteria (MIC ≥ 500 μg/mL).

### Experimental

#### General

1H NMR and 13C NMR spectra, along with HMBC experiments, were recorded on a Bruker-DRX-400 (400 and 100 Hz) NMR spectrometer. Chemical shifts (δ) are given in ppm relative to tetramethylsilane (TMS) as an internal reference and coupling constants (J) in Hz. ESI-MS was measured on an MDS-SCIEX-API-2000 liquid chromatography with tandem mass spectrometry (LC/MS/MS) instrument. Normal phase silica gel (100–200 mesh and 200–300 mesh, Qingdao Marine Chemical Ltd ) and Sephadex LH–20 (GE Healthcare Bio-Sciences AB) were used for column chromatography. Thin layer chromatography (TLC) was performed on GF254 plates (Qingdao Marine Chemical Ltd) and compounds were...
detected at 254 nm. Solvents were evaporated under reduced pressure using a rotary evaporator (Eyela-N-1001, Tokyo Rikakai Co. Ltd).

Plant material
The heartwood of D. cochinchinensis was imported from Laos by Foshan Jiahe Wood Furniture Co., Ltd in 2011 and identified by Deng Yun-fei, South China Botanical Garden, Chinese Academy of Sciences. A voucher specimen (No. 201000816) was deposited at the herbarium of South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China.

Extraction and isolation
The powdered heartwood of D. cochinchinensis was extracted with 95% EtOH (3×10 L) at room temperature for 3 days each. After evaporation under reduced pressure, the pooled crude EtOH extract (881.4 g) was suspended in H2O (2 L) and then partitioned with EtOAc (3×2.5 L) to afford an EtOAc-soluble (475.8 g) extract. This was fractionated by partitioning with EtOAc-soluble (475.8 g) extract. This was fractionated by

1H NMR (500 MHz, (CD3)2CO): 8.53 (1 H, s, 7-OH), 7.57 (1 H, s, 3′-OH), 6.96 (1 H, d, J = 8.2 Hz, H-5), 6.80 (1 H, d, J = 8.5 Hz, H-6′), 6.75 (1 H, d, J = 8.5 Hz, H-5′), 6.60 (1 H, s, H-4), 6.42 (1 H, dd, J = 8.2, 2.5 Hz, H-6), 6.34 (1 H, d, J = 2.3 Hz, H-8), 4.95 (2 H, d, J = 1.1 Hz, H-2), 3.80 (3 H, s, H-2′-OCH3), and 3.86 (3 H, s, H-4′-OCH3); 13C NMR (125 MHz, (CD3)2CO): 159.6 (C-7), 156.0 (C-9), 149.7 (C-4′), 146.9 (C-2′), 140.8 (C-3′), 128.5 (C-5), 126.6 (C-1′), 129.3 (C-3), 122.5 (C-4), 118.9 (C-6′), 117.1 (C-10), 109.7 (C-6), 108.4 (C-5′), 103.7 (C-8), 69.1 (C-2), 60.7 (C-2′-OCH3), and 56.8 (C-4′-OCH3).

Bacterial assay
Compound 1 was screened for antibacterial activity using E. coli ATCC 8739 was obtained from Guangdong Institute of Microbiology, and B thuringiensis GDMCC 800070 and S dysenteriae GDMCC 804814 from Microbiology Laboratory, College of Food, South China Agricultural University. Minimum inhibition concentrations (MIC) were determined by a two-fold serial dilution method using Mueller Hinton broth according to the Clinical and Laboratory Standards Institute recommendations (CLSI, 2002). Erythromycin was used as a standard antibacterial agent.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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2. Statement of Human and Animal Rights:
   This article does not contain any studies with human or animal subjects.
3. Statement of Informed Consent:
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Supplemental material

Supplemental material for this article is available online.

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