SHORT REPORT

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A New Lignan from the Leaves and Stems of *Melaleuca bracteata* F. Muell.

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Abstract: Lignans, such as podophyllotoxin, schisandrin, and silymarin, are known for their notable biological activities. In our study, a new lignan, named melaleucin D (**1**), was isolated from the leaves and stems of the plant *Melaleuca bracteata* F. Muell. The planar structure was determined by combining ^{1}H NMR/^{13}C NMR/ COSY/HSQC/HMBC spectra and HRESIMS data. The absolute configuration was established by comparing the experimental circular dichroism (CD) data with the calculated theoretical ECD data. Compound **1** contained an unusual dioxane moiety. The antibacterial assay revealed that compound **1** did not exhibit a zone of inhibition against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 25922 at a concentration of 256 μg/mL.

Keywords: *Melaleuca bracteata* F. Muell.; lignan; separation; structure elucidation.

1. Plant Source

Leaves and stems of *Melaleuca bracteata* F. Muell. were collected on September 2022 in Hangzhou Botanical Garden (*N* 30°15', *E* 120 07') in Hangzhou City, China. The type specimen (CSH0080226) could be found at Herbarium of Shanghai Chenshan Botanical Garden (CSH). A voucher specimen (MB202209) was stored at Hangzhou Vocational & Technical College.

2. Previous Studies

Lignans, a class of natural products biosynthesized through the dimerization of phenylpropanoid units [1-7]. Some lignans, such as podophyllotoxin, schisandrin, silymarin, and salvianolic acid B, exhibit significant biological activity.

Melaleuca bracteata F. Muell., an evergreen tree belonging to the genus *Melaleuca* in the Myrtaceae family, is extensively distributed across South China. It has been extensively cultivated as an ornamental species for urban landscaping applications, particularly in garden design, roadside vegetation, and residential green spaces. Beyond its horticultural value, the fresh branches and leaves can be processed to yield essential oils possessing notable anti-inflammatory, antimicrobial, and antioxidant activities [8].

Previous studies have predominantly focused on the essential oil of *M. bracteata*, systematic exploration of non-volatile constituents remains limited [8]. A SciFinder database search revealed only three new lignans (melaleucins A-C) and seven known compounds previously isolated from specimens collected at the South China Botanical Garden (Guangzhou, Guangdong Province) [9]. In our

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continuing phytochemical investigation of *M. bracteata* specimens sourced from Hangzhou, Zhejiang Province, we report the isolation and characterization of a new lignan, named melaleucin D (**1**), along with two known phenylpropanoid derivatives (**2** and **3**).

3. Present Study

The dried plant material (branches and leaves, 500 g) was mechanically pulverized and subjected to maceration with 95% ethanol, yielding 38 g of crude extract after solvent removal. The resultant extract was subsequently suspended in distilled water and systematically partitioned using immiscible organic solvents. Sequential liquid-liquid extraction with petroleum ether followed by ethyl acetate afforded corresponding fractions, with the ethyl acetate-soluble portion yielding 12 g of extract.

The ethyl acetate extract was initially subjected to silica gel column chromatography (CC) using a gradient elution of petroleum ether/acetone (10:1→1:1, v/v), yielding eight fractions (EA-1–EA-8). Among these, fraction EA-3 (1.1 g) was further fractionated on a reversed-phase ODS C-18 open column with methanol/water (30%→80%) to afford six subfractions (EA-3A–EA-3F). Subsequent purification of subfraction EA-3C (71 mg) by Sephadex LH-20 gel CC (methanol/chloroform 1:1) afforded compound **1** (3.2 mg). EA-3B (570 mg) was subjected to silica gel column chromatography using a gradient elution of petroleum ether/ethyl acetate (10:1 → 1:1) to afford compound **2** (312 mg). Similarly, EA-2 (78 mg) was purified via silica gel column chromatography with a petroleum ether/ethyl acetate (30:1 → 5:1) as the eluent to give compound **3** (7.2 mg).

Melaleucin D (***1***): Colorless oil; $[\alpha]^{25}_{D}$ −17 (*c* 0.3, MeOH); ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 337.1045 $[M + Na]^{+}$ (calcd. for $C_{18}H_{18}O_5Na^{+}$, 337.1046).

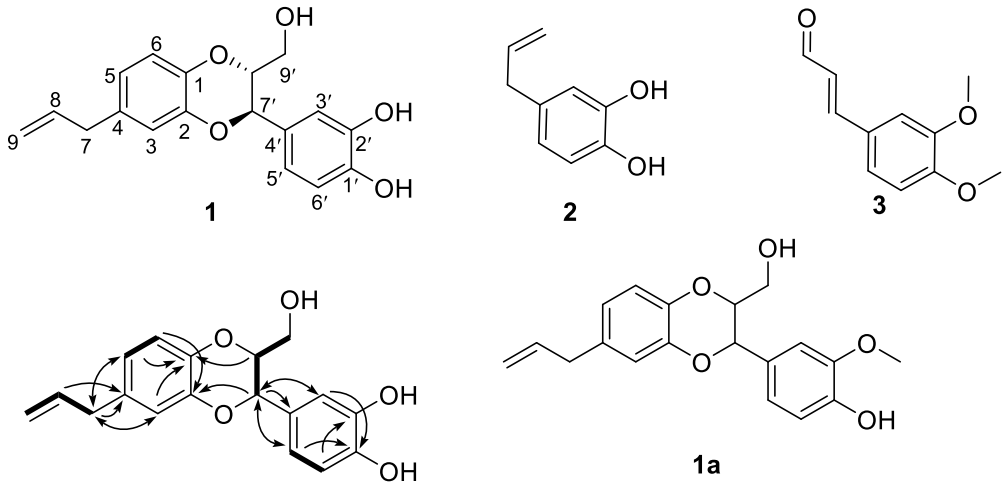

Figure 1. The structure of **1–3**, the analogue **1a**, and ^{1}H-^{1}H COSY of **1**

The HRESIMS of compound **1** in positive ionization mode displayed a sodiated molecular ion $[M + Na]^{+}$ at *m/z* 337.1045, which was consistent with the molecular formula $C_{18}H_{18}O_5$ (calcd 337.1046), corresponding to 10 double-bond equivalences.

Acquisition of the ^{1} H NMR in acetone-d_6 revealed the presence of six aromatic protons for two distinct AMX spin systems indicative of two trisubstituted aromatic rings [δ_H 6.82 (1H, d, *J* = 8.2 Hz, H-6), 6.72 (1H, d, *J* = 1.9 Hz, H-3), 6.68 (1H, dd, *J* = 8.2, 1.9 Hz, H-3) and 6.87 (1H, d, *J* = 8.0 Hz, H-3′), 6.96 (1H, d, *J* = 1.6 Hz, H-6′), 6.83 (1H, d, *J* = 8.0, 1.6 Hz, H-4′)], three olefinic protons displaying characteristic splitting patterns for a terminal double bond [δ_H 5.06 (1H, ddt, *J* = 17.0, 1.9, 1.6 Hz, H-9a), 5.00 (1H, ddt, *J* = 10.1, 1.9, 1.2 Hz, H-9b), and 5.94 (1H, ddt, *J* = 17.0, 10.1, 6.8 Hz, H-

8)], two protons at δ_H 3.28 (2H, ddd, *J* = 6.8, 1.6, 1.2 Hz, H_2-7) for a methylene that coupled to the protons of the adjacent terminal double bond, two mutually coupled protons at δ_H 4.89 (1H, d, *J* = 8.0 Hz) and 3.99 (1H, ddd, *J* = 8.0, 4.2, 4.2 Hz) suggesting vicinal coupling in a constrained environment (e.g., cyclic or rigid structure). Additionally, two mutually coupled doublets of doublets were observed at δ 3.72 (1H, dd, *J* = 12.3, 2.2 Hz) and 3.49 (1H, dd, *J* = 12.3, 4.2 Hz), exhibiting a diagnostic geminal coupling constant (12.3 Hz) that confirms the diastereotopic nature of the methylene protons.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Resonances of **1** and the ^{1}H NMR resonances of analogue **1a** in Acetone-d_6 (δ in ppm)

No.	**1**		**1a**
	δ_H (mult. *J* in Hz)	δ_C	δ_H
1		142.9	
2		144.8	
3	6.72, d (1.9)	117.6	6.6-7.1
4		133.8	
5	6.68, dd (8.2, 1.9)	122.0	6.6-7.1
6	6.82, d (8.2)	117.4	6.6-7.1
7	3.28, ddd (6.8, 1.6, 1.2)	40.0	3.17
8	5.94, ddt (17.0, 10.1, 6.8)	138.9	5.8-6.2
9	5.06, ddt (17.0, 1.9, 1.6) 5.00, ddt (10.1, 1.9, 1.2)	115.6	4.9-5.2
1′		146.5	
2′		146.0	
3′	6.87, d (8.0)	115.5	6.6-7.1
4′	6.83, dd (8.0, 1.6)	129.7	6.6-7.1
5′		120.2	
6′	6.96, d (1.6)	116.0	6.6-7.1
7′	4.89, d (8.0)	77.0	
8′	3.99, ddd (8.0, 4.2, 2.2)	79.5	3.4-4.2
9′	3.72, dd (12.3, 2.2) 3.49, dd (12.3, 4.2)	61.9	3.4-4.2
			3.93, OCH_3

The ^{13}C NMR spectra of **1** ($CDCl_3$, 150 MHz), supported by DEPT135 and HSQC spectra, indicated the presence of four sp^3 carbons and 14 sp^2 carbons. The four sp^3 carbons were assigned to two oxymethines (δ_C 79.5, 77.0) and two methylene including one oxygenated (δ_C 61.9, 40.0). The sp^2 carbons were attributed to seven methines (δ_C 138.9, 129.7, 122.0, 117.6, 117.4, 115.5, 116.0), one olefinic methylene (δ_C 115.6), six quaternary carbons (δ_C 146.5, 146.0, 144.8, 142.9, 133.8, 129.7). These assignments collectively account for the 12 carbons comprising the two benzene rings and the two carbons forming the terminal double bond mentioned previously. The structure of compound **1** was determined through integrated interpretation of 2D NMR data, including COSY, HSQC, and HMBC spectra. Two three-carbon units including an allyl group and a substituted glycerol moiety were assigned by the COSY correlations of CH_2-7/CH-8/CH-9 and CH_2(OH)-7′/CH(O)-8/CH(O)-9. The former was linked to one benzene ring at C-4, while the later was connected to the other benzene ring at C-4′, indicating the presence of two phenylpropanoid units. Given the presence of one remaining double-bond equivalence, the structure necessitated an additional ring. A careful examination of the HMBC data revealed that the two phenylpropanoid moieties were connected via a dioxane unit, as determined by the HMBC correlations of H-8′ to C-1 and H-7′ to C-2. Thus, the planar structure of **1** was determined to be the demethylated derivative of a synthetic compound **1a** [10].

The *trans*-configuration of H-7′/H-8′ was deduced by the vicinal coupling constant value (*J* = 8.0 Hz) between these two protons [9]. The absolute configuration of the asymmetric centers C-7 and

C-8 was assigned to be 7*R* and 8*R* by comparisons of the experimental CD spectrum with the theoretical CD spectrum (which was obtained by boltzmann-weighted average of calculated theoretical CD data of three lowest conformers) calculated using the TDDFT method based on B3LYP/6-31G* level using a Solvation Model based on density (Figure 2). Compound **1** was designated as melaleucin D in accordance with established natural product nomenclature conventions (melaleucins A–C from *Melaleuca bracteate*) [9].

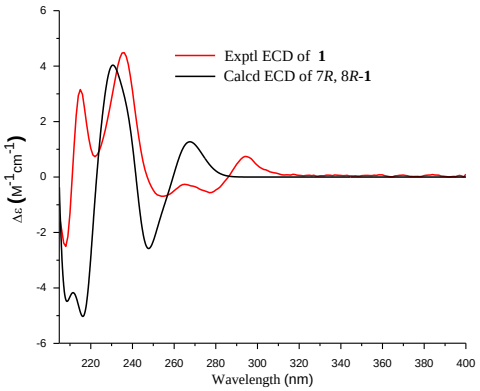

Figure 2. Experimental ECD spectrum of compound 1 and the calculated ECD spectrum of 7R,8R-1

The known compounds **2** and **3** was determined to be hydroxychavicol (**2**) [11] and 3,4-dimethoxycinnamaldehyde (**3**) [12]. Biosynthetically, compound **1** is proposed to originate from hydroxychavicol (**2**) via an intermolecular cyclization process that constructs the dihydrobenzo[b][1,4]dioxane scaffold [9].

Up to now, the phytochemical investigations of *M. bracteata* has led to the characterization of 13 specialized metabolites, comprising five lignans (melaleucins A–D, 3′-methoxymiliumollin), three phenylpropanoids (methyl eugenol, hydroxychavicol, 3,4-dimethoxycinnamaldehyde), aromatic derivatives (3,4,5-trimethoxy-benzoic acid methyl ester), triterpenoid (betulinic acid), cyclohexenoid (vomifoliol), steroids (β-sitostenone, β-sitosterol). Notably, the lignans and phenylpropanoids exhibited structural elaboration from hydroxychavicol, a key phenylpropanoid precursor. This exclusive occurrence of hydroxychavicol-derived architectures within the genus *Melaleuca* establishes them as chemosystematic markers, validating its taxonomic placement in *Myrtaceae*. The absence of these pathway-specific metabolites in congeneric species underscores *M. bracteata's* distinct biochemical evolution.

In light of reported antimicrobial efficacy displayed by structural analogues against *Staphylococcus aureus* ATCC 6538 [9], we evaluated compounds **1**–**3** for antibacterial activity using the Oxford cup method against both Gram-positive (*S. aureus* ATCC 6538) and Gram-negative (*Escherichia coli* ATCC 25922) strains. Notably, no inhibition zones were observed for any tested compounds at a concentration of 256 μg/mL.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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