



Resorcinols from *Myristica philippensis* Lam.

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ABSTRACT

The dichloromethane extract of the leaves of *Myristica philippensis* Lam. afforded two resorcinols, malabaricone C (1) and malabaricone B (2), and β -sitosterol. The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry. The structure of 2 was deduced by comparison of its ¹H NMR data with those of 1 and confirmed by comparison of its ¹³C NMR data with malabaricone B.

Keywords: *Myristica philippensis*, Myristicaceae, malabaricone B, malabaricone C

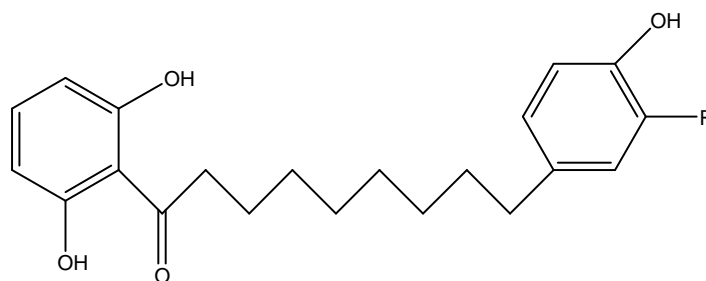
INTRODUCTION

Myristica philippensis is native to the Philippines, but is now cultivated in India, Madagascar, Mauritius, and Martinique. There are no reported chemical studies and medicinal properties of the tree which grows to 300 m altitude in mixed rainforests and in Mt. Makiling, Los Baños, Laguna, Philippines at low to medium altitudes, sometimes along streams [1]. The *M. philippensis* wood is used for light construction. The tree is locally known as duguan which means bloody because of the exudation of red sap when the bark is injured.

There is no reported chemical and biological study on *Myristica philippensis*. However, congeners of the tree have been investigated for their chemical constituents and biological activities. Three new phenolics: ((7S)-8'-(benzo[3',4']dioxol-1'-yl)-7-hydroxy propyl) benzene-2,4-diol, ((7S)-8'-(4'-hydroxy-3'-methoxy phenyl)-7-hydroxypropyl) benzene-2,4-diol and (8R,8'S)-7-(4-hydroxy-3-methoxyphenyl)-8'-methylbutan-8-yl)-3'-methoxy benzene-4',5'-diol), along with four known compounds were isolated from the seeds of *Myristica fragrans*[2]. The major compounds in the nutmeg (*M. fragrans*) oil were sabinene (21.38%), 4-terpineol (13.92%), and myristicin (13.57%) [3]. The leaf oil of *Myristica beddomeii* contained α -pinene (19.59%), *t*-caryophyllene (14.63%) and β -pinene (12.46%); *M. fragrans* afforded sabinene (19.07%), α -pinene (18.04%), 4-terpineol (11.83%), limonene (8.32%) and β -pinene (7.92%); and *Myristica malabarica* afforded *t*-caryophyllene (20.15%), α -humulene (10.17%), nerolidol (9.25%) and δ -cadinene (6.72%) [4]. High antioxidant activity was found in monoterpenoids from *M. fragrans* seed, terpinene-4-ol (3), alpha-terpineol and 4-allyl-2,6-dimethoxyphenol [5]. Malabaricone A-D were first reported as constituents of *M. malabarica* [6]. Another study reported the healing power of malabaricone C isolated from *M. malabarica* against indomethacin-induced gastric ulceration in mice. Malabaricone C was effective in controlling mucin secretion, PGE(2) synthesis and expression of EGF receptor and COX isoforms [7]. The relative healing capacities of malabaricone B and malabaricone C of indomethacin-induced gastric ulceration correlated well with their respective abilities to modulate the angiogenic factors [8]. Malabaricone C from *M. malabarica* showed maximum DPPH scavenging activity and it could prevent both Fe(II)- and 2,2'-azobis(2-amidinopropane) dihydrochloride-induced lipid peroxidation (LPO) of rat liver mitochondria [9]. Another study reported that malabaricone C exhibited stronger antioxidant activity than the commonly used synthetic antioxidant

BHT [10]. Furthermore, malabaricones A and B isolated from *M. malabarica* exhibited antileishmanial activity [11]. An earlier study reported the isolation of two resorcinols, malabaricones B and C from the dried seed covers of *M. fragrans* which exhibited strong antifungal and antibacterial activities [12]. A recent study reported that malabaricone C from *M. fragrans* exhibited an anti-inflammatory effect through the inhibition of NF- κ B activation by inhibiting interconnected ROS/Akt/IKK/NF- κ B signaling pathways [13]. The molecular mechanism of the anti-inflammatory activity of a natural diarylnonanol, malabaricone C has been reported [14]. Furthermore, malabaricone C from nutmeg (*M. fragrans*) inhibits PDGF-induced proliferation and migration of aortic smooth muscle cells through induction of heme oxygenase-1 [15]. *M. fragrans* was also reported as an aphrodisiac agent [16] and an antidepressant [17]. Malabaricone C exhibited cytotoxicity ($IC_{50}=5.26\pm 1.20 \mu\text{M}$) against the MCF-7 human breast cancer cell line. The malabaricone C-induced killing of the MCF-7 cells followed an apoptotic pathway involving oxidative damage to the cellular DNA [18]. Malabaricone C isolated from *Myristica cinnamomea* inhibited violacein production by *Chromobacterium violaceum* CV026 and it also inhibited the quorum sensing-regulated pyocyanin production and biofilm formation in *Pseudomonas aeruginosa* PAO [19]. Malabaricones B and C isolated from *Myristica crassa* were reported to possess significant inhibitory activity on acetylcholinesterase [20]. Malabaricone C exhibited high nematocidal activity against *Toxocara canis* with MLC of 6-10 μM [21]. *Myristica maingayi* afforded malabaricones B and C with IC_{50} values of 3 and 4 mg ml^{-1} , respectively [22]. *Myristica gigantea* [23] and *Myristica dactyloides* [24] also afforded malabaricones B and C.

We report herein the isolation and structure elucidation of malabaricone C (**1**), malabaricone B (**2**) and β -sitosterol from the leaves of *M. philippensis*. This is the first report on the isolation of these compounds from *M. philippensis*.



1 R = OH
2 R = H

EXPERIMENTAL SECTION

General Experimental Procedure

NMR spectra were recorded on a Varian VNMRS spectrometer in CDCl_3 at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. Column chromatography was performed with silica gel 60 (70-230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F₂₅₄ and the plates were visualized by spraying with vanillin/ H_2SO_4 solution followed by warming.

Sample Collection and Isolation

The leaves of *Myristica philippensis* Lam. used in this study were collected from Mt. Makiling Forest Reserve, Los Baños, Laguna, Philippines in May 2013. The sample was authenticated at the University of the Philippines, Los Baños, College, Laguna.

The CH_2Cl_2 extract of the air-dried leaves (332g) of *M. philippensis* was ground in a blender, soaked in CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford the crude extract (8 g) which was chromatographed in increasing proportions of acetone in CH_2Cl_2 at 10% increment. A glass column 18 inches in height and 1.0 inch internal diameter was used for the fractionation of the crude extracts. Five milliliter fractions were collected. Fractions with spots of the same *R_f* values were combined and rechromatographed in appropriate solvent systems until TLC pure isolates were obtained. A glass column 12 inches in height and 0.5 inch internal diameter was used for the rechromatography. Two milliliter fractions were collected. Final purifications were conducted using Pasteur pipettes as columns. One milliliter fractions were collected.

The 50% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (2 \times) in 15% acetone in CH_2Cl_2 to afford β -sitosterol (10 mg) after washing with petroleum ether. The 60% to 80% acetone in CH_2Cl_2 fractions were combined and rechromatographed in $\text{CH}_3\text{CN}:\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (1:1:8 by volume). The less polar fractions were combined and rechromatographed (3 \times) in $\text{CH}_3\text{CN}:\text{Et}_2\text{O}:\text{CH}_2\text{Cl}_2$ (1:1:8 by volume) to afford **2** (8

mg) after washing with petroleum ether, followed by Et₂O. The more polar fractions were combined and rechromatographed (4×) in CH₃CN:Et₂O:CH₂Cl₂ (1.5:1.5:7 by volume) to afford **1** (5 mg) after washing with petroleum ether, followed by Et₂O.

RESULTS AND DISCUSSION

The dichloromethane extract of the air-dried leaves of *Myristica philippensis* afforded two resorcinols, **1** and **2**, and β-sitosterol. The structure of **1** was elucidated by extensive 1D and 2D NMR spectroscopy and confirmed by mass spectrometry and comparison of its ¹³C NMR data with those of malabaricone C [19]. The structure of **2** was deduced by comparison of its ¹H NMR data with **1** and confirmed by comparison of its ¹³C NMR data with those of malabaricone B [20]. β-Sitosterol was identified by comparison of its ¹H NMR data with those reported in the literature [25].

The resorcinols, malabaricone C (**1**) and malabaricone B (**2**) isolated from *M. philippensis* have been previously reported as constituents of *M. fragrans* [12], *M. maingayi* [22], *M. gigantea* [23], *M. dactyloides* [24] and *M. crassa* [20], while **1** was also reported as a constituent of *M. malabarica* [6] and *M. cinnamomea* [19]. Thus, these resorcinols which were reported to exhibit various biological activities [7-22] are found in the genus *Myristica* of the family Myristicaceae.

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