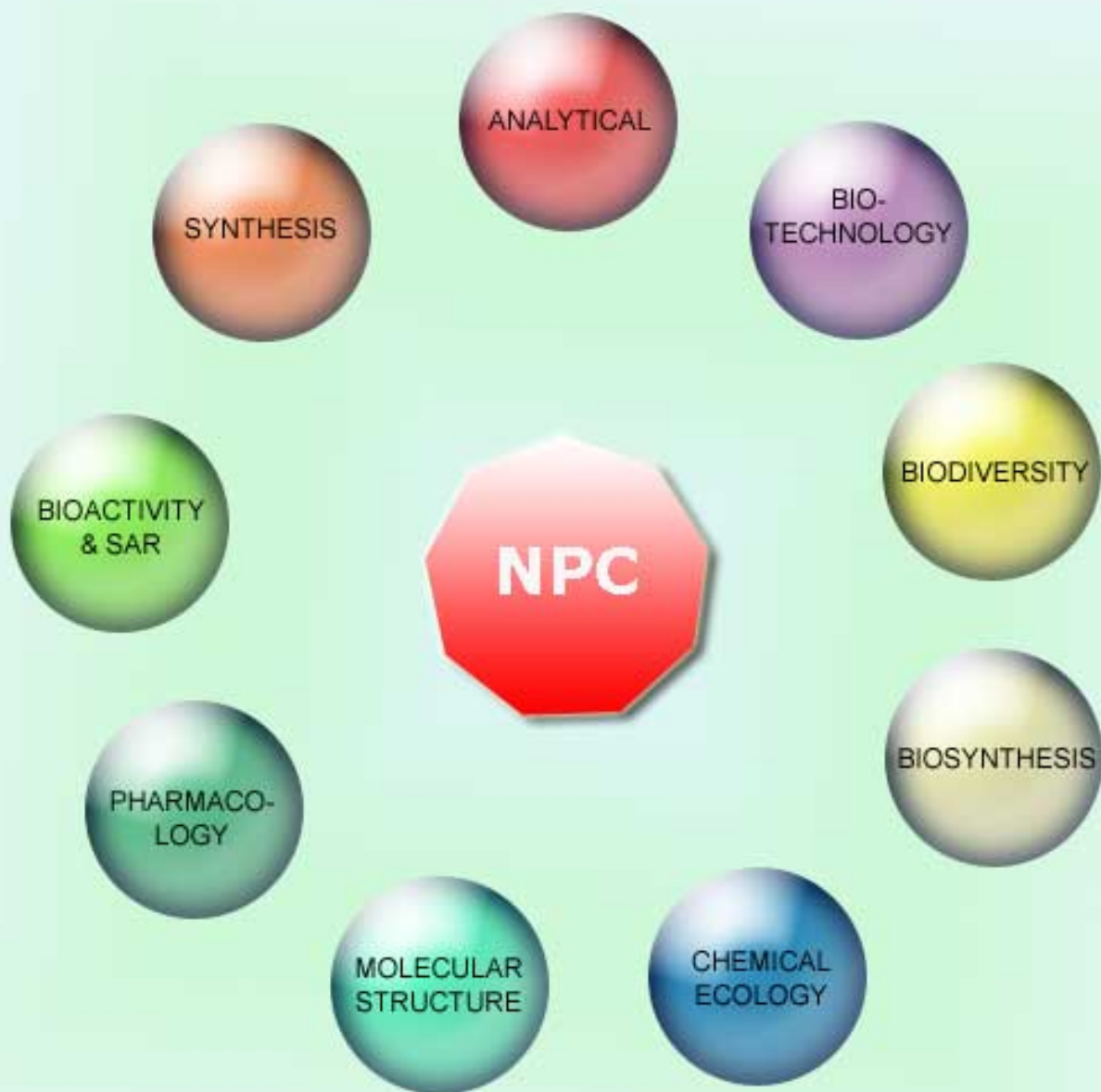


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A New Lignan Dimer from *Mallotus philippensis*

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A new lignan dimer, bilariciresinol (**1**), was isolated from the leaves of *Mallotus philippensis*, along with platanoside (**2**), isovitexin (**3**), dihydromyricetin (**4**), bergenin (**5**), 4-*O*-galloylbergenin (**6**), and pachysandiol A (**7**). Their structures were elucidated by spectroscopic experiments including 1D and 2D NMR and FTICR-MS.

Keywords: *Mallotus philippensis*, Euphorbiaceae, lignan, bilariciresinol.

The *Mallotus* species are a rich source of biologically active compounds such as phloroglucinols, tannins, terpenoids, coumarins, benzopyrans, and chalcones [1]. In the course of our systematic phytochemical investigations of *Mallotus* species, we reported several flavonoids, triterpenes, benzopyrans, flavonolignans, and megastigmane derivatives possessing significant NF- κ B inhibition, cytotoxic effects against several human cancer cell lines, and antiradical activity [2].

In line with this, we studied the chemical constituents of *Mallotus philippensis* (Lamk.) Muell.-Arg. (Euphorbiaceae, common name: kamala tree, Vietnamese name: Canh kien), which is abundant throughout Vietnam. The leaves and stem bark of this plant are traditionally used to treat acne and other cutaneous diseases. The fruit glands are used as medicine against syphilis, dropsy, and gastric diseases. Decoctions of the roots are employed to treat acute dysentery, swollen fauces and throat, epilepsy, and diarrhea. The seeds are also used in Thai folk medicine against dizziness and nausea [3]. In the present paper, we report the isolation and structural elucidation of a new lignan dimer, bilariciresinol (**1**), along with six

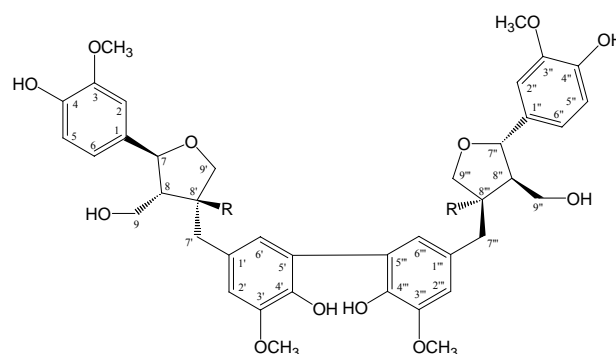


Figure 1: Structures of **1** (R = H) and **1a** (R = OH).

Known compounds (**2-7**) from the leaves of *M. philippensis*. Compound **1** was obtained as a white amorphous powder. The ¹H NMR spectrum showed signals of three ABX-type aromatic protons [δ_{H} 6.92 (1H, d, $J = 2.0$ Hz), 6.78 (1H, d, $J = 8.0$ Hz), and 6.79 (1H, dd, $J = 8.0, 2.0$ Hz)] and two *m*-coupled [δ 6.83 and 6.75 (each 1H, d, $J = 2.0$ Hz)], indicating one 1,3,4-trisubstituted and one 1,3,4,5-tetrasubstituted aromatic ring. Two methoxyl groups were identified by proton signals at δ 3.83 and 3.88 (each 3H, s). In addition, the presence of an oxymethine (δ 4.76, 1H, d, $J = 6.5$ Hz),

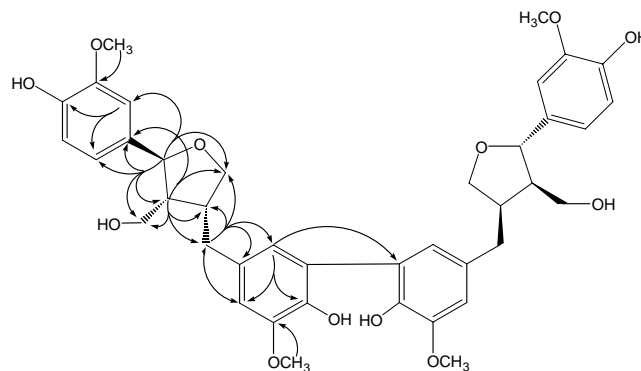
Table 1: The NMR spectral data of **1**[#].

N ^o	δ _c ^{a, b}	DEPT	δ _H ^{a, c} mult. (<i>J</i> in Hz)
1 (1'')	135.77	C	-
2 (2'')	110.75	CH	6.92 d (2.0)
3 (3'')	148.96	C	-
4 (4'')	147.00	C	-
5 (5'')	116.00	CH	6.78 d (8.0)
6 (6'')	119.86	CH	6.79 dd (8.0, 2.0)
7 (7'')	84.02	CH	4.76 d (6.5)
8 (8'')	53.99	CH	2.40 m
9 (9'')	60.50	CH ₂	3.82 dd (11.0, 7.0) 3.65 dd (11.0, 7.0)
1' (1''')	133.27	C	-
2' (2''')	112.28	CH	6.83 d (2.0)
3' (3''')	149.47	C	-
4' (4''')	142.79	C	-
5' (5''')	127.06	C	-
6' (6''')	124.68	CH	6.75 d (2.0)
7' (7''')	33.78	CH ₂	2.53 dd (13.0, 12.0) 2.97 dd (13.0, 5.0)
8' (8''')	43.80	CH	2.77 m
9' (9''')	73.57	CH ₂	4.03 dd (8.0, 7.0) 3.78 dd (8.0, 7.0)
3 (3'')-OCH ₃	56.42	CH ₃	3.83 s
3' (3''')-OCH ₃	56.66	CH ₃	3.88 s

^arecorded in CD₃OD, ^bat 125 MHz, ^cat 500 Hz, [#]all the data were assigned by HSQC and HMBC experiments.

Two oxymethylene (δ 3.82/3.65, each 1H, dd, *J* = 11.0, 7.0 Hz and 4.03/3.78, each 1H, dd, *J* = 8.0, 7.0 Hz), and a methylene (δ 2.53, 1H, dd, *J* = 13.0, 12.0 Hz and 2.97, 1H, dd, *J* = 13.0, 5.0 Hz) suggested a 4,4',9'-trihydroxy-3,3'-dimethoxy-7,9'-epoxy lignan [4].

The ¹³C NMR spectrum of **1** exhibited 20 carbon signals, in which the two methoxyl groups were indicated by signals at δ 56.42 and 56.66. Twelve carbon signals in range of δ 110.75 - 149.47 confirmed the two aromatic rings. In addition, one oxymethine and two oxymethylene groups were determined by signals at δ 84.02 (CH), 73.57 (CH₂), and 60.50 (CH₂), respectively. All carbons were assigned to relevant protons by an HSQC experiment and the results are summarized in Table 1. The NMR data of **1** were similar to those of (+)-lariciresinol [4]. The differences of the spectral data between the two compounds were only observed in ring B. The easily visible changes were the presence of a quaternary carbon C-5 (δ 127.06) in **1** instead of a methine (δ 116.5) in (+)-lariciresinol [4]. A strongly downfield-shifted (+10.56 ppm) C-5 (and C-5'') suggested that two lariciresinol units were linked in a magnetically symmetric mode between C-5 and C-5'' [5], which was confirmed by FTICR-MS peak at *m/z* 741.28712 [*M* + Na]⁺ (calcd. for C₄₀H₄₆O₁₂Na, 741.28870) corresponding to a molecular formula of C₄₀H₄₆O₁₂ (*M* = 718).

**Figure 2:** Key HMBC correlations of **1**.

The NMR data of **1** were first assigned by comparison with those of (+)-lariciresinol [4] and **1a** [5] and further confirmed by an HMBC experiment (Figure 2). The relative configuration of **1** was determined by the good agreement of its ¹³C NMR data, as well as its ¹H NMR multiplicities and coupling constants with those of (+)-lariciresinol [4]. Thus, **1** was elucidated to be a new compound, bilariciresinol (Figure 1).

The known compounds **2-7** were characterized as platanoside [6], isovitexin [7], dihydromyricetin [8], bergenin [9], 4-*O*-galloylbergenin [9], and pachysandiol A [10], respectively, by detailed analyses of their NMR and ESI-MS data and comparison of them with reported values. Platanoside was isolated for the first time from a *Mallotus* species. This is the first report of these compounds from *M. philippensis*.

Experimental

General: Optical rotation was determined on a JASCO DIP-1000 KUY polarimeter. All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ¹H and 125 MHz for ¹³C), and chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. The ESI-MS was obtained on an AGILENT 1200 SERIES LC-MSD Trap spectrometer. The high resolution mass spectra were obtained using a Variant 910 FT-ICR mass spectrometer. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0.040 - 0.063 mm, Merck) or YMC RP-18 resins (30 - 50 μm, Fujisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F₂₅₄ (Merck 1.05715) or RP₁₈ F_{254s} (Merck) plates. Compounds were visualized by spraying with 10% H₂SO₄ and heating for 5 minutes.

Plant materials: The leaves of *M. philippensis* were collected in Trang Dinh, Lang Son Province, Vietnam during February, 2009 and identified by Dr. Ninh Khac Ban, Institute of Ecology and Biological Resources,

Vietnam Academy of Science and Technology. A voucher specimen (No TD34) was deposited at the Herbarium of the Institute of Natural Products Chemistry.

Extraction and isolation: The air dried leaves of *M. philippensis* (5 kg) were exhaustively extracted (three times, each 60 min) with hot MeOH (40-50 °C) under ultrasonic conditions to obtain 180 g of MeOH residue. This was suspended in water and partitioned in turn with *n*-hexane, CHCl₃ and ethyl acetate giving 45, 35, and 25 g of the corresponding extracts. The CHCl₃ extract (35 g) was submitted to a silica gel CC using step wise elution of CHCl₃-MeOH (from 50/1 to 1/1, v/v) to give seven fractions, C1-C7. Fraction C3 (5 g) was further separated on a silica gel CC using CHCl₃-acetone 20/1 (v/v) to obtain compound **7** (23.5 mg). The new compound **1** (14 mg) was purified from fraction C5 (3.7 g) by using a silica gel CC with CHCl₃-MeOH-H₂O 8/1/0.1 (v/v/v) as eluent. The ethyl acetate extract (25 g) was separated into nine fractions, E1-E9, by a silica gel CC using step wise elution of CHCl₃-MeOH (from 10/1 to 1/1, v/v). Compound **4** (20 mg) was isolated from fraction E3 (2.1 g) after subjecting it to a silica gel CC eluting with CHCl₃-MeOH 8/1. Further separation of

fraction E5 (5 g) by a silica gel CC using CHCl₃-acetone-H₂O 1/1/0.05 (v/v/v) as eluent, followed by a silica gel CC with CHCl₃-MeOH-H₂O 5/1/0.1 (v/v/v) to obtain compounds **5** (6.0 mg) and **6** (19.0 mg). Fraction E6 (3.7 g) afforded compounds **2** (12 mg) and **3** (9 mg) after using a silica gel CC eluting with CHCl₃-acetone-H₂O 1/2/0.1 (v/v/v), followed by an YMC RP-18 CC eluting with MeOH-H₂O 1.5/1 (v/v).

Bilariciresinol (**1**)

[α]_D: +26 (*c* 0.50, MeOH).

R_f: 0.45 (CHCl₃-MeOH-H₂O, 3.5:1:0.1).

¹H (500 MHz, CD₃OD): Table 1.

¹³C NMR (125 MHz, CD₃OD): Table 1.

ESIMS: *m/z* 741 [M + Na]⁺ (positive).

FTICR-MS: *m/z* 741.28712 [M + Na]⁺; calcd for C₄₀H₄₆O₁₂Na: 741.28870.

14 mg (2.8 × 10⁻⁴ % of dried weight).

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