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A New Lignan Derivative, Lasiocarpone, from The Stembark of *Chisocheton iasiocarpus* (Meliaceae)

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ABSTRACT

A new lignan derivative, named lasiocarpone **(1)**, was isolated from the stembark of *Chisocheton lasiocarpus*. The chemical structure of **1** was determined by extensive NMR and MS spectra analyses as well as by comparing with analogue compound from previous studies. Lasiocarpone showed moderate cytotoxic activity against MCF7 breast cancer cells with an IC_{50} value of 42.5 μM.

Keywords: *Chisocheton lasiocarpus*, Lignan, Lasiocarpone, Meliaceae, MCF7 cancer cell.

INTRODUCTION

The *Chisocheton* plant genera as a second largest plant of Meliaceae family, consisting of 50 plants and widely distributed in the tropics. Previous phytochemical studies of this genera have produced some compounds with interesting biological activity, such as anti-plasmodial[1] limonoid[2], cytotoxic limonoid[3], and anti-inflammatory limonoids[4], NO production inhibitory activity limonoids[5], cell growth inhibitory activity limonoids[6], anti-inflammatory protolimonoids[7], cytotoxic triterpenoid[8] and cytotoxic tetranortriterpenoid[9].

Our previous phytochemical studies on *Chisocheton* plants, we had found new limonoids, pentandrice and dysobinol from *C. pentandrus*[10] and *C. mocrophyllus*[11] as well as a triterpenoid-type lanostane from *C. cumingianus*[12]. In the further study, we focus the stembark of *C. lasiocarpus* that showed significant cytotoxic activity on MCF-7 breast cancer cells *in vitro*.

Chisocheton lasiocarpus is up to 20 m high plant and widely distributed in the tropical regions[13]. Traditionally, the stembark of *C. lasiocarpus* are used for treatment of fever and skin diseases[13-15]. There is

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no phytochemical study on *C. lasiocarpus* previously. In this paper, isolation, structure determination and cytotoxic activity of a new lignan derivative are described.

MATERIAL AND METHOD

Instruments

Optical rotation values were measured with a Perkin-Elmer 341 polarimeter. UV spectrum was measured with Shimadzu-1800 spectrophotometer. IR spectrum was measured on Perkin-Elmer 1760X spectrophotometer. Mass spectra was measured with a Qtof HR-MS XEVotm mass spectrometer instruments. NMR data were recorded on a Bruker Topspin spectrometer and used TMS as an internal standard. Column chromatography on SiO_2 (Merck & Co.). TLC analysis on SiO_2 GF_{254}, stain was observed on UV light and heated on the hotplate after spraying with 10% H_2SO_4 in ethanol.

Plant Material

C. lasiocarpus stembarks were obtained from Bogor Botanical Garden, Indonesia in July 2015. Plant identifications were made from Bogoriense Herbarium, Indonesia. Specimens (No. Bo-1295453) are saved in the Bogoriense Herbarium.

Cytotoxic Assay

Determination of cytotoxic activity is performed according to the procedure described in the previous paper[10]. Harvest suspension of breast cancer cells (MCF-7) by centrifugation. Determine the amount and viability cells (with trypan blue exclusion), and resuspend cells with final 4×10^5 cells / mL supplemented with 10% phosphoric buffer solution (FBS) and 1% Penicillin-Streptomycin. Dispense 50 µL of cell suspension (20,000 cells) into all wells on microplate, then incubated for 24 hours. On different microplate samples were prepared. Samples or standards to be measured, diluted in an EMEM medium containing 10% FBS and 1% Penicillin-Streptomycin, then dilutions. The diluted 50 µL sample was transferred into well on the microplate containing the incubated cell. Then re-incubated for 48 hours. After that, a salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy -methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) reagent was introduced to each microplate of 20 µL and incubated for 2-4 hours until the orange formazan was seen. Furthermore, the colored formazan that has been produced measured its absorbance at a wavelength of 570 nm using a multimode reader. The IC_{50} value was obtained from percentage live cells compared to control (%), versus the tested concentration of compounds (µM).

RESULTS

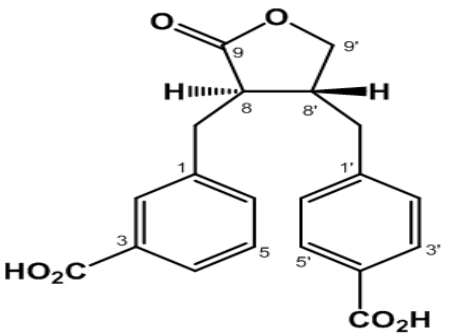
Extraction and Purification

Dried ground stembarks of *C. lasiocarpus* (2.1 kg) were soaked with methanol for three days and filtered. Evaporate of the methanol on vacuum produced a concentrated methanol extract (209.4 g) and subsequently partitioned to *n*-hexane, EtOAc and *n*-BuOH. A portion of EtOAc (28.3 g) was vacuum liquid chromatographed on SiO_2 with *n*-hexane-EtOAc-MeOH as a gradient solvent to yield seven fractions (I-VII). Fraction IV (3.20 g) was column chromatographed on SiO_2 with *n*-hexane-EtOAc (10:0-1:1) as a developing solvent to give seven subfractions (IVa-IVg). Subfraction IVf (420 mg) was further separated by column chromatography on SiO_2 with choroform: methanol (9:1) as a solvent to give five subfractions (IVf.1-5). Subfraction IVf.3 (62.5 mg) was further purified by preparative TLC on SiO_2 GF_{254} with *n*-hexane: ethyl acetate (8:2) as a solvent to give **1** (5 mg) as a minor compound.

Lasiocarpone **(1)**. Yellowish gum; $[\alpha]^D_{20}$ + 25.1° (c 0.1, CH_3OH); UV λ_{max} 280 (ε 2.80); IR (KBr) ν_{max} (cm^{-1}): 3394, 3060, 1731, 1720, 1560, 1464, 1024; ^{1}H- (500 MHz) and ^{13}C NMR (125 MHz) in DMSO-d_6, Table 1. HR-TOFMS *m/z* 355.1015 $[M+H]^+$(Calcd for $C_{20}H_{18}O_6$, *m/z* 354.1025).

DISCUSSION

An aqueous methanol extract was concentrated and partitioned to *n*-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate fraction was column chromatographed on SiO_2 and separated on preparative TLC on SiO_2 GF_{254} produce a new lignan derivative, lasiocarpone **(1)**.



1

Lasiocarpone (1), $[\alpha]^{25}_{D}$ + 25.1° (c 0.1, CH_3OH), was isolated as a yellowish gum with molecular formula, $C_{20}H_{18}O_6$ by high resolution time of flight mass spectra (HR-TOFMS), requiring twelve double bond equivalents. UV absorption of **1** in MeOH exhibited at 280 nm, indicating the presence on benzene ring. IR spectrum showed peaks at 3394, 1731,1720, 1560 and 1024 cm^{-1}, due to the presence of hydroxyl, carboxyl ester lactone, benzene and ether groups. ^{1}H-NMR spectrum of **1** revealed *p*-disubstituted benzene ring at δ_H 7.52 (2H, d, *J*=6.8 Hz, H-2, H-6) and 7.43 (2H, d, *J*=6.4 Hz, H-3, H-5). In ^{1}H-NMR spectrum showed also *m*-disubstituted benzene ring at δH 7.26 [(1H, d, *J*=7.1 Hz, H-4), 7.70 (1H, dd, *J*=7.1, 8.2 Hz, H-5), 7.77 (1H, d, *J*=8.2 Hz, H-6) and 7.18 (1H, s, H-2)]. In addition, deshielded methylene protons at δ_H [4.45 (1H, dd, *J*=7.1, 8.5 Hz), 4.54 (1H, dd, *J*=8.5, 9.4 Hz)], methine signals at δ_H [4.67 (1H, dd, *J*=4.7, 7.1 Hz), 4.19 (1H, m)] and four methylene signals at δ_H 2.87 (1H, dd, *J*=5.5, 13.8 Hz), 3.24 (1H, dd, *J*=7.1, 13.8 Hz), 3.05 (1H, dd, *J*=10.4, 13.5 Hz) and 3.20 (1H, dd, *J*=4.7, 13.5 Hz) also were observed and supporting of a dibenzyl butyrolactone-type lignin[16]. The ^{13}C-NMR spectrum together with DEPT spectrum of **1** displayed twenty carbon signals, including a lactone (δ_C 171.9), two carboxyl carbons (δ_C 167.2, 166.7), four sp^2 quartenary carbons, eight sp^2 methines, an oxygenated sp^3 methylene (δ_C 65.9), two sp^3 methylenes and two sp^3 methines, featuring a dibenzyl butyrolactone-type lignan[16,17]. A NMR data of **1** are similar those of (2*R*,3*R*)-2-(4-hydroxy-3-methoxybenzyl)-3-(3,4,5-trimethoxy)benzylbuyrolactone[17], except the disappear of methoxyl and hydroxyl groups and the appearance of carboxyl group. In order to clarify the position of functional groups, 2D NMR experiments were conducted (Fig. 1). Correlations in H2′-H3′, H5′-H6′, H4-H5-H6, H7-H8 and H7′-H8′-H9′, supporting the presence of a 1, 4-disubtituted and a 1,3-disubtituted benzene ring from a dibenzyl butyrolactone-type lignan.

All of aromatic protons were correlated to carboxyl and used for assigment of the carboxyl groups were attached in *meta* and *para* orientation, respectively. Furthermore, a correlation between an oxygenated methylene at δ_H 4.45 to C-9 (δ_C 171.9) and C-8′(δ_C 50.3) and a methine proton at δ_H 4.67 to C-9 (δ_C 171.9) were used to assign a lactone ring at C-8, C-9, C-9′ and C-8′, which characteristic for butyrolactone-type of lignan. Correlation from methylene protons at δ_H 3.07 and 2.87 to aromatic carbons at δ_C 137.8 (C-1), 138.2 (C-1′) and to methine carbons at δ_C 54.8 (C-8) and 50.3 (C-8′), supporting the dibenzyl butyrolactone-type of lignan. It was clearly confirmed that compound **1** contains a 3-carboxybenzyl at C-9 and 4′-carboxybenzyl unit C-9′ on a butyrolactone skeleton and the relative configuration of the dibenzyl units is *trans*. A relative stereochemistry of C-8 and C-8′ was supported also by NOESY spectra. There are no crosspeak between H-8 and H-8′ In the NOE spectra, consequently both protons are *trans*-orientation.

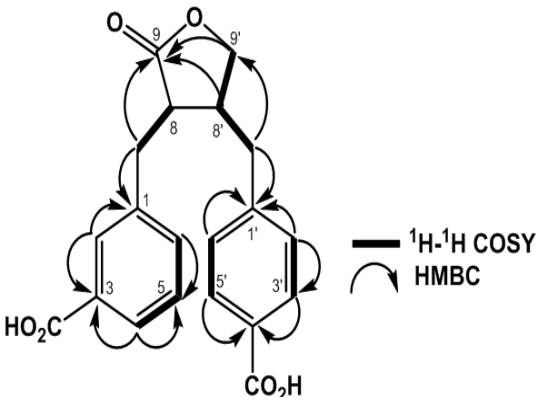


Fig. 1. Selected correlations for 1

This results was supported also by comparing to those of *trans*-dibenzylbutyrolactone[18] and the specific optical rotation of 1 ($[\alpha]^{20}_{D}$ -24.1°, c 0.1, CH_3OH) is same negative sign to that of the previously reported, (2*R*,3*R*)-2-(4-hydroxy-3-methoxybenzyl)-3-(3,4,5-trimethoxy) benzylbuyrolactone ($[\alpha]^{18}_{D}$ -25.1° (c 0.55, EtOH)][17]. Therefore, compound **1** was elucidated to be a new dibenzyl butyrolactone-type lignan, (2*R*,3*R*)-5-carboxybenzyl-4′-carboxybenzylbutyrolactone and was named lasiocarpone.

Lasiocarpone **(1)** was checked its cytotoxic activity on MCF-7 breast cancer cells according to a method described[10,19] and cisplatin (IC_{50} 27.0 μM) was used as a positive control[20]. Lasiocarpone

Table 1: NMR data for Lasiocarpone

Position of C	δ_C (mult.)	δ_H (ΣH., Integral., *J=Hz*)
1	137.8 (s)	-
2	129.6 (d)	7.18 (1H, s)
3	134.0 (s)	-
4	126.9 (d)	7.26 (1H, *d*, 7.1)
5	127.6 (d)	7.70 (1H, *dd*, 7.1, 8.2)
6	128.5 (d)	7.77 (1H, *d*, 8.2)
7	36.4 (t)	3.05 (1H, *dd*, 10.4, 13.5) 3.20 (1H, *dd*, 4.7, 13.5)
8	54.5 (d)	4.67 (1H, *dd*, 4.7, 7.1)
9	171.8 (s)	-
1′	138.0 (s)	-
2′	131.5 (d)	7.52 (1H, *d*, 6.8)
3′	128.6 (d)	7.43 (1H, *d*, 6.4)
4′	134.8 (s)	-
5′	128.6 (d)	7.40 (1H, *d*, 6.4)
6′	131.5 (d)	7.60 (1H, *d*, 6.8)
7′	36.7 (t)	2.87 (1H, *dd*, 5.5,13.8) 3.24 (1H, *dd*, 7.1, 13.8)
8′	50.3 (d)	4.19 (1H, m)
9′	65.9 (t)	4.45 (1H, *dd*, 7.1, 8.5) 4.54 (1H, *dd*, 8.5, 9.4)
4′-CO_2H	166.7 (s)	-
5-CO_2H	167.2 (s)	-

displayed an IC_{50} value of 42.5 µM against MCF-7 breast cancer cell, indicating showed moderate cytotoxic activity.

CONCLUSION

A new dibenzyl butyrolactone-type lignan derivative, lasiocarpone **(1)**, was isolated from the stembark of *Chisocheton lasiocarpus* as a minor compound. This investigation confirm that *Chisocheton* genus is capable to produce a lignan derivative.

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