SUPPLEMENTARY MATERIAL

Sesquiterpenes Produced by *Pestalotiopsis microspora* HF 12440 Isolated from *Artocarpus heterophyllus*

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**Abstract:** A drimane-type sesquiterpene, (+)-dendocarbin L (1) together with two bisabolane-type sesquiterpenes, (+)-sydonic acid (2) and (+)-sydowic acid (3) were isolated from the mycelium of *Pestalotiopsis microspora* HF 12440, an endophytic fungus from the stem of *Artocarpus heterophyllus*. The structures of all compounds were elucidated using spectroscopic methods and by comparison with the literature. Compound 1 was isolated from the fungi for the first time, compounds 2 and 3 were firstly obtained from this endophytic fungus. Compound 3 showed cytotoxicity (IC₅₀ 2.56 μg/mL) against murine leukemia P-388 cells.

**Keywords:** *Artocarpus heterophyllus*, cytotoxicity, *Pestalotiopsis microspora*, sesquiterpene

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List of Supplementary Material

Experimental

Figure S1. The $^1$H-NMR spectrum of 1
Figure S2. The $^{13}$C-NMR spectrum of 1
Figure S3. The HSQC spectrum of 1
Figure S4. The HMBC spectrum of 1
Figure S5. The $^1$H-NMR spectrum of 2
Figure S6. The $^{13}$C-NMR spectrum of 2
Figure S7. The HSQC spectrum of 2
Figure S8. The HMBC spectrum of 2
Figure S9. The $^1$H-NMR spectrum of 3
Figure S10. The $^{13}$C-NMR spectrum of 3
Figure S11. The HSQC spectrum of 3
Figure S12. The HMBC spectrum of 3
Experimental

General Experimental Procedures

Vacuum liquid chromatography, column chromatography and thin layer chromatography used silica gel 60 G, silica gel Kieselgel 60 (0.063-0.200 mm) and silica gel 60 F$_{254}$ (Merck, Darmstadt, Germany), respectively. Spots were detected under UV and sprayed with 5% vanillin solution. Autopol IV polarimeter (Rudolph Research Analytical, New Jersey, United States) was used to determine the values of optical rotation. NMR spectroscopic data were recorded at 500 MHz for $^1$H and 125 MHz for $^{13}$C on Agilent Varian with CDCl$_3$ and (CD$_3$)$_2$CO as the solvents and tetramethylsilane (TMS) as the internal standard (Agilent Technologies, Santa Clara, United States). The HR/ESI/TOF-MS was recorded on LCT Premier XE (Waters Corporation, Milford, United States).

Fungal Material

The fungal *P. microspora* was isolated from the stem of *A. heterophyllus*. The fresh stem of *A. heterophyllus* was collected from the garden of Labtek VII (School of Pharmacy), Bandung Institute of Technology, Indonesia. Identification of fungal strain was conducted at Indonesian Culture Collection (InaCC), Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI) based on molecular protocol including isolation of genomic DNA, amplification and sequencing. A fungal strain was deposited at Natural Product Research Laboratory, Bandung Institute of Technology with code BTG-1.

Fermentation, Extraction and Isolation

*P. microspora* was cultivated in the 250 mL Erlenmeyer flask (200 flasks) containing 50 mL potato dextrose broth media and incubated at 28 $^0$C. After two weeks, the cells were separated from media using Buchner funnel. The cells were extracted with methanol (three times) to obtain 4.8 g crude extract. The crude extract was subjected to vacuum liquid chromatography over silica gel eluted with dichloromethane and acetone (100:0 to 0:100) gave six fractions, A-F. Fraction B (256 mg) was separated using silica gel column chromatography to obtain compound 1 (6 mg). 122 mg fraction D was separated with column chromatography over silica gel eluted with dichloromethane and ethyl acetate (8.5 to 4.5-4:6) to give compound 3 (7 mg). Fraction E (130 mg) was fractionated with silica gel column chromatography eluted with chloroform and methanol (9.5:0.5 to 8.5:1.5) to give 24 sub-fractions, E.1-E.24. Sub-
fraction E.18-E.20 (10 mg) were combined and purified using column chromatography over silica gel to yield compound 2 (3.5 mg).

(+)-Dendocarbin L (1)
Colorless solid. \([\alpha]_D^{25} +12.5^0\) (c.0.0008, CHCl3). \(^1\)H and \(^{13}\)C NMR matched with the literature (Sultana et al. 2011). HR/ESI/TOF-MS \(m/z 267.1446 \text{[M + H]}^+\) (calc. \([\text{M + H]}^+\) for \(C_{15}H_{22}O_4\), 267.1440).

(+)-Sydonic acid (2)
Yellow solid. \([\alpha]_D^{25} +4.0^0\) (c.0.0008, CHCl3). \(^1\)H and \(^{13}\)C NMR matched with the literature (Kudo et al. 2009). HR/ESI/TOF-MS \(m/z 265.1446 \text{[M - H]}^+\) (calc. \([\text{M - H]}^+\) for \(C_{15}H_{22}O_4\), 265.1440).

(+)-Sydowic acid (3)
Yellow solid. \([\alpha]_D^{25} +22.8^0\) (c.0.0011, CHCl3). \(^1\)H and \(^{13}\)C NMR matched with the literature (Serra, 2000). HR/ESI/TOF-MS \(m/z 265.1441 \text{[M + H]}^+\) (calc. \([\text{M + H]}^+\) for \(C_{15}H_{20}O_4\), 265.1440).

**Murine Leukemia P388 Assay**
All pure compounds (1-3) were tested their cytotoxicity against murine leukemia P388 cells using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide) assay as previously reported (Sahidin et al., 2005; Yang et al., 2008). The cells were seeded in 96-well plates (cell density 3 x 10⁴ cells/cm³). All compounds (1-3) were added in various concentrations and incubated for 48 h where the crude extract and compounds were dissolved in DMSO (Dimethyl sulfoxide). After 48 h incubation, 10 µL MTT reagent was added into each sample and then incubated for 4 h. The MTT-stop solution containing SDS (Sodium Dodecyl Sulfate) was added and the incubation was continued for 24 h. Optical density was read with a microplate reader at 550 nm. IC₅₀ values were taken from the plotted graph of percentage live cells compared to control. The control was made from MTT solution and DMSO (without cells and medium).
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The $^1$H-NMR data of 1–3 were measured at 500 MHz. The $^{13}$C-NMR data of 1–3 were measured at 125 MHz.

**Figure S1.** The $^1$H-NMR spectrum of 1

**Figure S2.** The $^{13}$C-NMR spectrum of 1
Figure S3. The HSQC spectrum of 1

Figure S4. The HMBC spectrum of 1
Figure S5. The $^1$H-NMR spectrum of 2

Figure S6. The $^{13}$C-NMR spectrum of 2
Figure S7. The HSQC spectrum of 2

Figure S8. The HMBC spectrum of 2
Figure S9. The $^1$H-NMR spectrum of 3

Figure S10. The $^{13}$C-NMR spectrum of 3
Figure S11. The HSQC spectrum of 3

Figure S12. The HMBC spectrum of 3