SHORT REPORT

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A New Chromanone Derivative from *Calophyllum inophyllum* Resin and Its Antibacterial Activity

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Abstract: A new chromanone derivative, calophylloidic acid B (**2**), was isolated from *Calophyllum inophyllum* resin in Lombok, Indonesia. The structure of the new compound was elucidated as a structural isomer of calophylloidic acid A (**1**), which we had previously isolated from Indonesian *C. inophyllum* resin. It exhibited potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Keywords: Chromanone; *Calophyllum inophyllum;* calophylloidic acid; Indonesia; antibacterial activity.

1. Plant Source

In October 2019, *Calophyllum inophyllum* resin was collected from the arboretum of the Research and Development Institute of Non-Timber Forest Product Technology, Lombok Barat District, West Nusa Tenggara Barat Province, Indonesia, and was identified by taxonomists at the Herbarium Bogoriense, Indonesia. The voucher specimen (accession number: BO-1880537) is preserved in the arboretum of the Research and Development Institute of Non-Timber Forest Product Technology, Lombok Barat District, West Nusa Tenggara Barat Province, Indonesia.

2. Previous Studies

The genus *Calophyllum* is predominantly distributed in the tropical regions of the world, such as India, Malaysia, Indonesia, and Madagascar, where > 130 species have been reported [1-3]. The plant species produce various secondary metabolites to resist tropical environmental factors, such as

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pests, microorganisms, and ultraviolet radiation. Chromanones, coumarins, and xanthones have been previously isolated from *Calophyllum* genus as key secondary metabolites [4-6]. Due to rich secondary metanolite profile of the genus, *Calophyllum* sp. seed oils as well as trunk bark, root, and leaf decoctions have been used as traditional medicines since ancient times owing to their beneficial biological effects, such as antibacterial [4], anti-human immunodeficiency virus [5], and antitumor [6] activities.

In our previous study, we isolated a new compound, calophylloidic acid A (**1**), which is the main component of *Calophyllum inophyllum* resin from Lombok, Indonesia (Figure 1) [7]. We found that *C. inophyllum* resin and **1** exhibited strong antibacterial activity. In that study, LC-MS analysis revealed the molecular ions showing the same *m/z* value as that of **1**, suggesting the presence of other structural isomers of **1** in the resin. To effectively use *C. inophyllum* resin, further component analysis and biological evaluations are required. Therefore, we performed compositional analysis of *C. inophyllum* resin using chiral column chromatography and assessed the antibacterial activity of the isolated compounds.

3. Present Study

The EtOH extracts of *C. inophyllum* resin from our previous study were used [7]. These extracts (15.4 g) were analyzed using silica gel (n-hexane/ethyl acetate 3:1, 0.1% acetic acid) via open column chromatography to obtain 10 fractions (Frs.1–10). Fr. 3 (46.3 mg of 2.2 g) was separated via chiral column chromatography (Daicel CHIRALPAK IG, 5 μm, 10 × 250 mm; H_2O/acetonitrile 33:67, 0.1% formic acid) to obtain four fractions. Further, Fr. 3-1 (24.9 mg) was separated via chiral column chromatography (CHIRALPAK IG, H_2O/acetonitrile 33:67, 0.1% formic acid) to isolate calophylloidic acid B (**2**) (10.8 mg).

*Calophylloidic acid B **(2)**:* Yellow gum; $[\alpha]_D^{28}$ = – 23.6° (*c* = 0.23, $CHCl_3$); UV (acetonitrile): λ_{max} (log ε): 263 (3.66), 309 (3.98); IR ν_{max} (KBr): = 3333, 3110, 2961, 2360, 1704, 1647, 1542, 1488, 1286, 1240 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.88 (3H, t, *J* = 7.3 Hz, H-18), 1.16 (3H, d, *J* = 7.2 Hz, H-12), 1.24 (2H, m, H-17), 1.34 (3H, d, *J* = 6.5 Hz, H-11), 1.46 (3H, s, H-5′), 1.46 (1H, m, H-16a), 1.50 (3H, s, H-5″), 1.52 (3H, s, H-4′), 1.54 (3H, s, H-10″), 1.65 (3H, s, H-9″), 1.78 (1H, m, H-16b), 1.88 (2H, m, H-6″), 1.98 (1H, m, H-2″), 2.10 (2H, m, H-1″), 2.50 (2H, m, H-1′), 2.60 (1H, dq, *J* = 3.6, 7.2 Hz, H-3), 2.72 (1H, dd, *J* = 6.6, 15.8 Hz, H-14a), 2.85 (1H, dd, *J* = 8.2, 15.8 Hz, H-14b), 3.51 (1H, m, H-13), 4.38 (1H, dq, *J* = 3.6, 6.5 Hz, H-2), 4.41 (1H, m, H-4a″), 4.48 (1H, m, H-4b″), 4.78 (1H, t, *J* = 7.9 Hz, H-2′), 4.89 (1H, t, J =6.7 Hz, H-7″), 16.27 (1H, s, 4-OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 9.9 (CH_3, C-12), 14.1 (CH_3, C-18), 16.3 (CH_3, C-11), 17.2 (CH_3, C-5″), 17.7 (CH_3, C-5′), 17.9 (CH_3, C-10″), 20.9 (CH_2, C-17), 25.7 (CH_3, C-4′), 25.7 (CH_3, C-9″), 30.7 (CH, C-13), 33.1 (CH_2, C-6″), 34.9 (CH_2, C-16), 37.9 (CH_2, C-14), 39.9 (CH, C-3), 40.5 (CH_2, C-1″), 42.7 (CH_2, C-1′), 45.1 (CH, C-2″), 59.8 (C, C-6), 74.3 (CH, C-2), 103.2 (C, C-10), 113.1 (CH_2, C-4″), 114.1 (C, C-8), 117.1 (CH, C-2′), 122.3 (CH, C-7″), 132.1 (C, C-8″), 135.3 (C, C-3′), 147.3 (C, C-3″), 164.4 (C, C-9), 177.1 (C, C-15), 188.7 (C, C-4), 196.6 (C, C-7), 200.4 (C, C-5); HRESIMS: *m/z* 527.3369 $[M + H]^+$ (calcd. 527.3367 for $C_{32}H_{47}O_6$).

Antibacterial activities of **2** against *Staphylococcus aureus* and *Escherichia coli* were evaluated as previously described [7]. Minimal inhibitory concentration (MIC) was also determined. Briefly, **2** at a concentration of 5–2560 μg/mL prepared in DMSO was diluted with each bacterial solution and incubated at 37 °C for 24 h. Subsequently, bacterial growth was assessed based on turbidity. MIC was defined as the lowest concentration at which no turbidity was observed. Compound **2** exhibited potent antibacterial activity against *S. aureus* and *E. coli* with MIC values of 16 and 8 μg/mL, respectively, which were the same activity as **1**. Recently, we have reported the improvement of water solubility and antibacterial activity of **1** via inclusion complexation with cyclodextrins [11]. Compound **2** is also expected to improve the water solubility and antibacterial activity through the formation of the inclusion complex with cyclodextrins.

Compound **2** was isolated via preparative HPLC using chiral stationary phases column (Figure 1). The ^{13}C NMR spectroscopic data revealed that **2** possessed 32 carbon atoms, and its HSQC correlations indicated the presence of eight methyl, seven methylene, six methine, and 11 quaternary carbons.

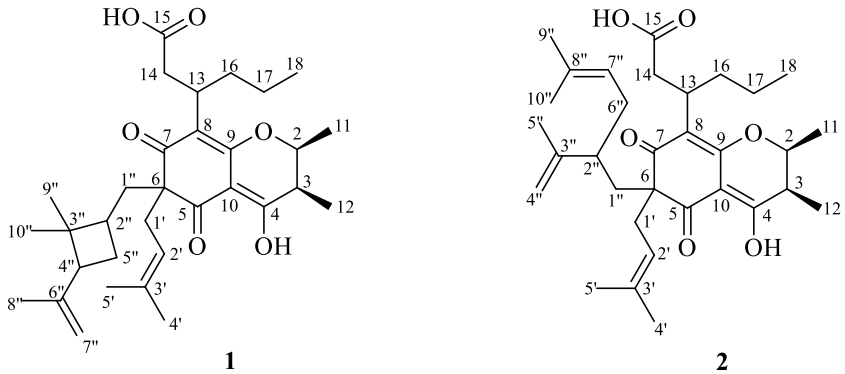

Figure 1. Structures of calophylloidic acid A (**1**) and B (**2**)

The molecular formula of **2** was determined to be $C_{32}H_{46}O_6$ using HRESIMS ($[M + H]^+$ at *m/z* 527.3369 and $[M – H]^–$ at *m/z* 525.3223) (Figure S10). Further, 1D and 2D NMR spectroscopic data of **2** were found to be similar to compound **1** and its structural analogs [4,7]. Upon comparing the ^{13}C NMR data of **1** and **2**, we revealed that C-9″ (δ_C 24.7) and C-10″ (δ_C 24.0) of **1**, which are the characteristic features of the four-membered ring structure, were not observed in **2**. Meanwhile, the NMR signals of H-4″ (δ_H 4.41, 4.48) were assigned to two terminal methylene protons from the double bond. The presence of an isopropenyl group from H-5″ (δ_H 1.50) to C-2″ (δ_C 45.1), C-3″ (δ_C 147.3), and C-4″ (δ_C 113.1) was confirmed based on HMBC correlation. The signals of two methyl groups H-9″ (δ_H 1.65) and H-10″ (δ_H 1.54) and the vinyl proton H-7″ (δ_H 4.89) suggested the presence of a prenyl group. The presence of a prenyl group was also confirmed based on HMBC correlation from H-9″ and H-10″ to C-7″ (δ_C 122.3). The HMBC correlations from H-1″ (δ_H 2.10) to C-5 (δ_C 200.4), C-6 (δ_C 59.8), C-7 (δ_C 196.6), C-2″ (δ_C 45.1), C-3″ (δ_C 147.3), and C-6″ (δ_C 33.1) indicated that the lavandulyl group is bound at C-6. The ^1H-^1H COSY, HSQC, and HMBC experiments of **2** enabled the allocation of all signals and determination of the planar structure (Figure 2).

The relative structures of dimethyl chromanone were identified as *cis*-isomers by comparing the chemical shifts of **2** ($\delta_{C\text{-}2}$ 74.3, $\delta_{C\text{-}3}$ 39.9, $\delta_{C\text{-}11}$ 16.3, and $\delta_{C\text{-}12}$ 9.9) and **1** ($\delta_{C\text{-}2}$ 74.4, $\delta_{C\text{-}3}$ 40.6, $\delta_{C\text{-}11}$ 16.3, and $\delta_{C\text{-}12}$ 9.8) [7], J value (3.6 Hz) between H-2 and H-3 of **2**, and NOESY correlations of H-2/H-3 and H-11/H-12 (Figure 2). In our previous study, we determined the absolute configuration of C-2 and C-3 of **1** as (2*S*, 3*R*) using time-dependent density functional theory and electronic circular dichroism (ECD) calculations [7]. In the current study, the absolute configuration of C-2 and C-3 of **2** was determined as (2*S*, 3*R*) in a similar manner by comparing the ECD data of **1** and **2** (Figure S11). However, the absolute configurations of C-6, C-13, and C-2″ are unknown. Therefore, **2** was identified as a new chromanone derivative and named as "calophylloidic acid B". Several chromanone derivatives have been isolated from *Calophyllum* species [3-5, 8-10]. They contain an isoprene unit and a monoterpene group at C-6 position of the unique coumarin ring system. The synthetic pathway for these compounds, including calophyllodic acids (**1** and **2**), is unknown yet.

A new chromanone derivative

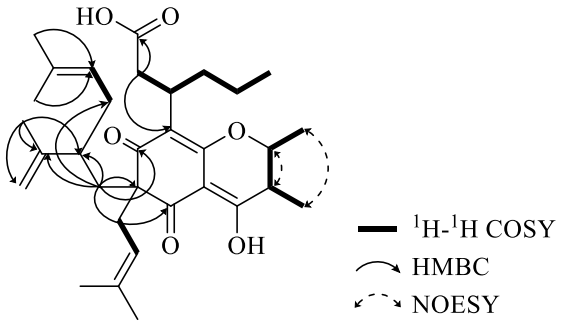

Figure 2. Key ^{1}H-^{1}H-COSY, HMBC, and NOESY correlations for calophylloidic acid B (**2**)

In conclusion, a new chromanone derivative, calophylloidic acid B (**2**), was isolated and its chemical structure elucidated by 1D and 2D NMR techniques and Mass spectral data for the first time. The compound **2** and its derivatives might be considered as potential antibacterial agents against *S. aureus* and *E. coli*.

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Supporting Information

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

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References

[1] M. Govindasamy, S. Ramalingam, R. Dhairiyasamy and S. Rajendran (2022). Investigation on thermal and storage stability of the *Calophyllum inophyllum* ester with natural leaf extract as antioxidant additive, *Energy* **253**, 124117.

[2] V. Vigneshwar, S.Y. Krishnan, R.S. Kishna, R. Srinath, B. Ashok and K. Nanthagopal (2019). Comprehensive review of *Calophyllum inophyllum* as a feasible alternate energy for CI engine applications, *Renew. Sustain. Energ. Rev.* **115**, 109397.

[3] H. Wang, Q.Y. Sun, F.M. Yang, C.L. Long, Y.H. Wang, G.H. Tang, F.W. Zhao, H.M. Niu, Q.Q. Huang, J.J. Xu, Y. Wataya and L.J. Ma (2010). Chromanone derivatives from the pericarps of *Calophyllum polyanthum*, *Helv. Chim. Acta* **93**, 2183-2188.

[4] F. Cottiglia, B. Dhanapal, O. Sticher and J. Heilmann (2004). New chromanone acids with antibacterial activity from *Calophyllum brasiliense*, *J. Nat. Prod.* **67**, 537-541.

[5] Y. Kashman, K. R. Gustafson, R.W. Fuller, J.H. Cardellina 2nd, J.B. McMahon, M.J. Currens, R.W. Buckheit Jr, S.H. Hughes, G.M. Cragg and M.R. Boyd (1992). The calanolides, a novel HIV-inhibitory class of coumarin derivatives from the tropical rainforest tree, *Calophyllum lanigerum*, *J. Med. Chem.* **35**, 2735-2743.

[6] M.C. Yimdjo, A.G. Azebaze, A.E. Nkengfack, A.M. Meyer, B. Bodo and Z.T. Fomum (2004). Antimicrobial and cytotoxic agents from *Calophyllum inophyllum*, *Phytochemistry* **65**, 2789-2795.

[7] S. Mizuno, R. Miyata, K. Mukaide, S. Honda, A. Sukito, M. Sahlan, T. Taniguchi and S. Kumazawa (2022). New compound from the plant origin of propolis from Lombok, Indonesia and its antibacterial activity, *Results Chem.* **4**, 100276.

[8] Y.-C. Shen, M.-C. Hung, L.-T. Wang and C.-Y. Chen (2003). Inocalophyllins A, B and their methyl esters from the seeds of *Calophyllum inophyllum*, *Chem. Pharm. Bull.* **51**, 802-806.

[9] L.M. Lemos, R.B. Oliveira, B.L. Sampaio, G.V. Ccana-Ccapatinta, F.B. Da Costa and D.T.O. Martins (2016). Brasiliensic and isobrasiliensic acids: isolation from *Calophyllum Brasiliense* Cambess. And anti-*Helicobacter pylori* activity, *Nat. Prod. Res.* **30**, 2720-2725.

[10] S. Tip-pyang and J. Sichaem (2021). Two new chromanone acid derivatives from *Calophyllum inophyllum*, *Chem. Nat. Compd*. **57**, 265-268.