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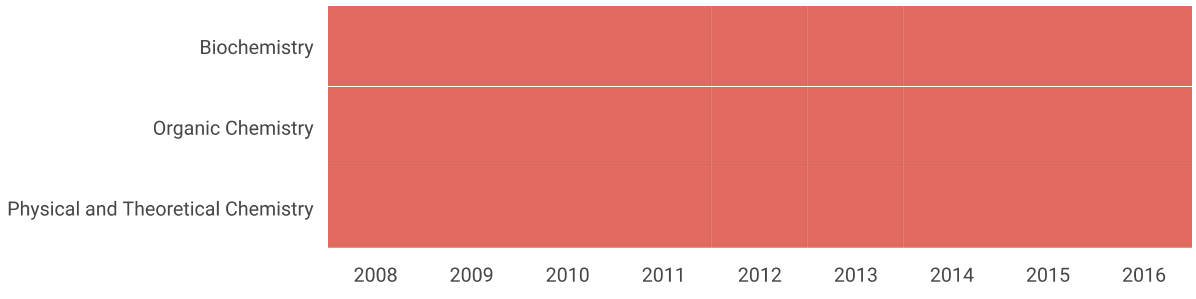

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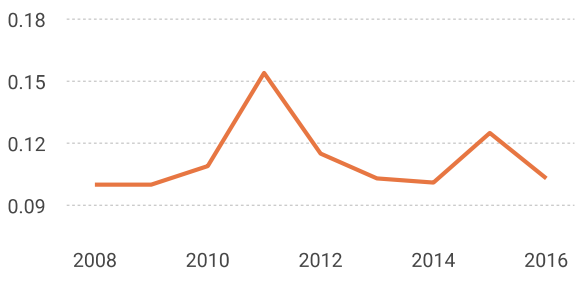

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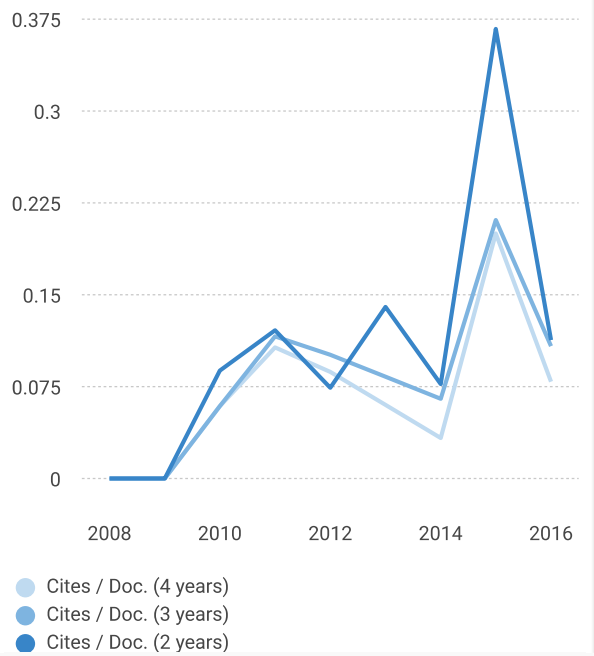

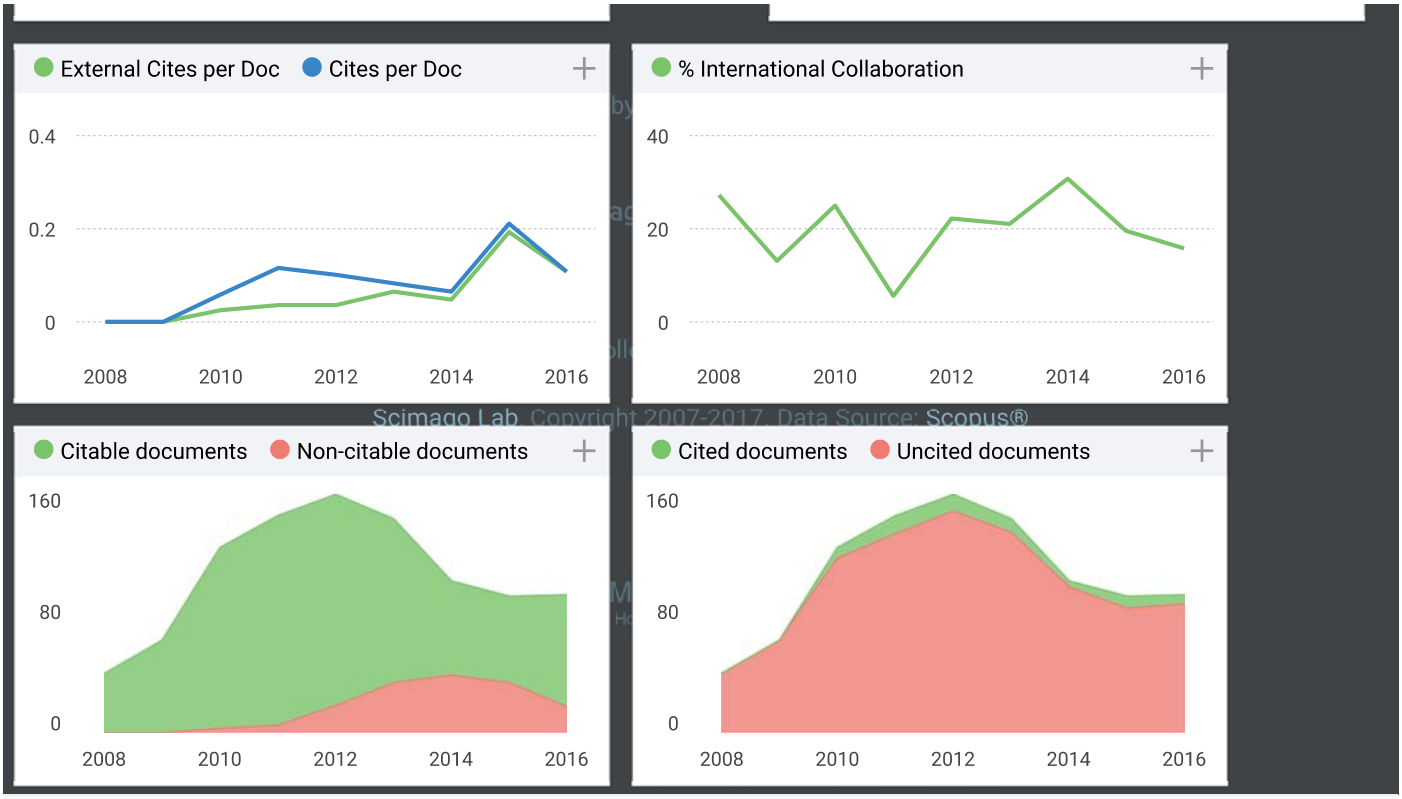

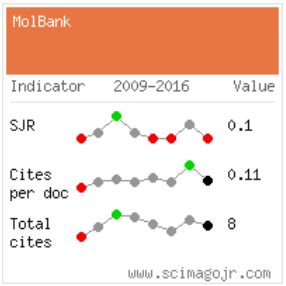

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Abstract

A new pyranocoumarin, namely 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut- 3″en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2*H*,12*H*-pyrano[2,3-a]xanthen-12-one **1**, was isolated from the stem barkof *Calophyllum pesudomole*. The structure of compound **1** was elucidated based on its ultaraviolet (UV); infrared (IR); high resolution electro spray ionization mass spectrometry (HRESIMS); 1D and 2D nuclear magnetic resonance (NMR) spectral data. View Full-Text (/1422-8599/2017/4/M961/htm)

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5,9,11-Trihydroxy-10-(2′′-hydroxy-3′′-methylbut-3′′-en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2*H*,12*H*-pyrano[2,3-a]xanthen-12-one from *Calophyllum pseudomole*

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Abstract: A new pyranocoumarin, namely 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut-3″en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-one **1**, was isolated from the stem barkof *Calophyllum pesudomole*. The structure of compound **1** was elucidated based on its ultaraviolet (UV); infrared (IR); high resolution electro spray ionization mass spectrometry (HRESIMS); 1D and 2D nuclear magnetic resonance (NMR) spectral data.

Keywords: 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut-3″-en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-one; *Calophyllum pseudomole*; pyranoxanthone

1. Introduction

The genus *Calophyllum* belongs to the Clusiaceae family which comprises about 180 species found mainly in Southeast Asia. This plant isendemic to Kalimantan Island, Indonesia. This genus has been shown to possess a number of secondary metabolites such as xanthones [1–3], coumarins [4,5], and chromanone acids [6,7]. Many of these compounds have shown a wide range of biological and pharmacological properties such as anti-HIV [8,9], anticancer [10,11], and antimalarial properties [12].

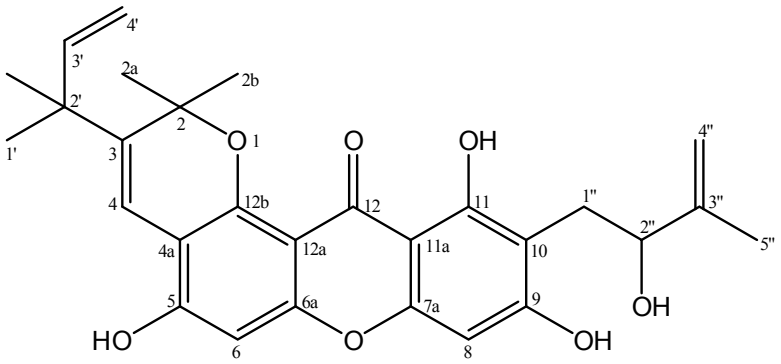

Figure 1. Structures of 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut-3″-en-1-yl)-2,2- dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-one.

In continuation of our phytochemical investigation on bioactive xanthone, we wish to report the isolation and structural elucidation of a new pyranoxanthone, 5,9,11-trihydroxy-10-(2″-hydroxy-

3″-methylbut-3″-en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-one (Figure 1) from the stem bark of *Calophyllum pseudomole*. The cytotoxicity against murine leukemia P-388 and antiplasmodial activity against *Plasmodium falciparum* of **1** are also briefly described.

2. Result and Discussion

5,9,11-Trihydroxy-10-(2″-hydroxy-3″-methylbut-3″-en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-onewas isolated as a yellow solid, and its UV absorption maxima at λ_{max} 245, 268, and 320 nm which is characteristic of xanthone chromophore [2]. Based on HRESIMS (see data supplementary Figure S1), compound **1** showed deprotonated molecule ion $[M-H]^-$ at *m*/*z* 477.1917corresponding to the molecular formula $C_{28}H_{30}O_7$. The IR spectrum (data supplementary Figure S2) showed absorption for hydroxyl (3423 cm^{-1}), conjugated carbonyl (1645 cm^{-1}), and aromatic (1577 and 1460 cm^{-1}) groups [13]. The ^{1}H-NMR spectrum (Table 1, data supplementary Figure S3) of **1** showed the presence of a singlet of chelated hydroxyl at δ_H13.82 (11-OH), a singlet of hydroxyl at δ_H 6.14 (5-OH), and two singlets of the isolated aromatic proton at δ_H 6.81 (H-6) and δ_H 6.39 (H-8) which is typical for a xanthone with six substituents [1]. The ^{1}H-NMR spectrum also revealed the presence of a substituent of a monosubstitued 2,2-dimethylpyrano, 2-methylbut-3-en-2-yl, and 2-hydroxy-3-methylbut-3-en-1-yl group. The presence of a monosubstitued 2,2-dimethylpyrano was determined from resonances of vinyl at δ_H 8.13 (s, 1H), and two signals of methyl at δ_H 1.54 (s, 3H) and 1.52 (s, 3H). The ^{1}H-NMR spectrum of 2-methylbut-3-en-2-yl showed the presence of a methyl at δ_H 1.43 (s, 6H), a vinyl at δ_H 5.98 (dd, 1H), and a methylene at δ_H 5.13 (d, 1H) and δ_H 5.08 (d, 1H). The ^{1}H-NMR spectrum of 2-methylbut-3-en-2-yl also showed the presence of a methyl at δ_H 1.87 (s, 3H), two proton signals of methylene at δ_H 3.16 (dd, 1H) and 2.93 (dd, 1H), a methyne at δ_H 4.43 (d, 1H), and two proton signals of vinilyc methylene at δ_H 4.97 (s, 1H) and 4.86 (s, 1H).

The ^{13}C-NMR spectrum (Table 1, Figure S4 data supplementary) of **1** showed 28 carbon signals and their assignments were determined by heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) spectra. The placement of hydroxyl, a monosubstitued 2,2-dimethylpyrano, 2-methylbut-3-en-2-yl, and 2-hydroxy-3-methylbut-3-en-1-yl group was confirmed by HMQC and HMBC spectra(Table 1). Based on the HMBC spectrum, a chelated hydroxyl signal at δ_H 13.82 (11-OH) was correlated with two quaternary carbons [δ_C 107.4 (C-10); 103.4 (C-11a)], and an oxyaryl carbon δ_C 161.1 (C-11) indicated that C-10 has a substitutent. Two proton signals of methylene (H-1″) of the 2-hydroxy-3-methylbut-3-en-1-yl group at δ_H3.18 and δ_H 2.95 showed long-range correlations with two oxyaryl carbons [δ_C 161.1; 163.2 (C-9)], two quaternary carbons [δ_C 107.4; 146.7 (C-3″)], and a methine carbon of alcohol [δ_C 77.5 (C-2″)]. The correlations showed that the 2-hydroxy -3-methylbut-3-en-1-ylgroup attached at C-10. The position of 2-hydroxy-3-methylbut-3-en-1-yl group at C-10 is reinforced by the correlation of the isolated aromatic proton signals at δ_H6.39 (H-8) with two oxyaryl carbons [δ_C 155.8 (C-7a); 163.2 (C-9)], and two quaternary carbons [δ_C107.4 (C-10); 103.4(C-11a)]. Furthermore, the proton signal of isolated aromatic at δ_H 6.81 (H-6) correlates with two oxyaril carbons [δ_C 153.4 (C-5), 150.6 (C-6a)] and a quaternary carbon signal (δ_C 108.5, C-4a), which showed that 2,2-dimethylpyrano was fused at C-4a and C-12b and thehydroxyl group attached at C-5 from the xanthone skeleton. The position of the hydroxyl group at C-5 is reinforced by the correlation of the hydroxyl proton signal at δ_H 6.15 (5-OH) with an oxyaryl carbon [δ_C 153.4 (C-5)], and a methine carbon [δ_C 101.8 (C-6)]. The methine signal of the vinyl group at δ_H 8.13 (H-4) of a monosubstitued 2,2-dimethylpyrano group showed long-range correlations with four quarternary carbons [δ_C 80.6 (C-2), 135.7 (C-3), 108.5 (C-4a), 42.1 (C-2′)] which showed thata 2-methyl but-3-en-2-yl group attached at C-3. The HMBC correlation of compound 1 can seen on Figure 2. Therefore, compound **1** was identified as 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut-3″-en-1-yl) -2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-one and a novel compound.

Table 1. NMR spectroscopic data of 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut-3″-en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-one in $CDCl_3$.

No.C	δ_H (Mult, *J* in Hz)	δ_C	HMBC
2	-	80.6	-
2a	1.54 (s, 3H)	27.4	C-2; C-2b; C-12b
2b	1.53 (s, 3H)	27.4	C-2; C-2a; C-12b
3	-	135.7	-
4	8.13 (s, 1H)	118.0	C-2; C-3; C-4a;C-2′
4a	-	108.5	-
5	-	150.6	-
6	6.81 (s, 1H)	101.8	C-3; C-4a; C-5; C-6a
6a	-	153.4	-
7a	-	155.8	-
8	6.39 (s, 1H)	94.5	C-7a; C-9; C-10; C-11a
9	-	163.2	-
10	-	107.4	-
11	-	161.1	-
11a	-	103.4	-
12	-	182.5	-
12a	-	121.7	-
12b	-	148.8	-
1′	1.43 (s, 3H)	28.3	C-2′; C-3′;2′-CH_3
2′	-	42.7	-
2′-CH_3	1.43 (s, 3H)	28.2	C-1′; C-2′; C-3′
3′	5.98 (dd, 10.6; 17.6, 1H)	146.8	C-1′; C-2′; 2′-CH_3
4′	5.08 (d, 10.6, 1H) 5.13 (d, 17.6, 1H)	112.0	C-2′; C-3′
1″	3.16 (d, 15.1, 1H) 2.93 (dd, 7.6; 15.1, 1H)	28.1	C-9; C-10;C-11; C-2″; C-3″
2″	4.42 (d, 7.3, 1H)	77.5	C-10; C-1″; C-3″; C-4″; C-5″
3″	-	146.7	-
4″	4.97 (s, 1H) 4.86 (s, 1H)	110.2	C-2″; C-5″
5″	1.87 (s, 3H)	18.7	C-2″; C-3″; C-4″
5-OH	6.15 (s, 1H)	-	C-4a; C-5; C-6
9-OH	9.15 (s, 1H)	-	-
11-OH	13.48 (s, 1H)	-	C-10; C-11; C-11a

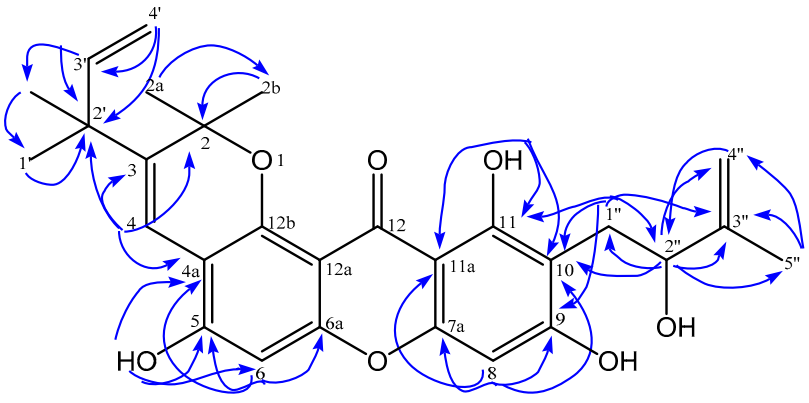

Figure 2. Selected HMBC correlation for 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut-3″-en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xathen-12-one.

The cytotoxic activityof compound **1** against murine leukemia P-388 cells, as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay, showed IC_{50} values of 4.10 μg/mL andwas categorized as moderate activity.The antiplasmodial activity of **1**against *P. falciparum* showed IC_{50} values of 2.88 μg/mL and was categorized as moderate activity.

3. Experiment Section

3.1. General

NMR spectra were recorded on a JEOL 400 ECA spectrophotometer (Tokyo, Japan) in $CDCl_3$ at 400 (^1H) and 100 (^{13}C) MHz using tetramethyl silane (TMS) as the internal standard. The mass spectra were recorded using a Waters LCT Premier XE (Santa Clara, CA, USA). The UV spectra were measured with a Shimadzu series 1800 spectrophotometer (Kyoto, Japan). The IR spectra were recorded with Perkin–Elmer spectrum-100 FT-IR (Waltham, MA, USA). Column chromatography and planar radial chromatography were carried out using silica gel 60 G 1.07734.1000 and Si gel 60 PF_{254} 1. 07749.1000 (Merck, Darmstadt, Germany).

3.2. Plant Material

The stem bark of *C. Pseudomole*was collected in Sungai Mendawak, anak Sungai Kapuas, District Kubu Raya, Kalimantan, Indonesia on April 2015. The specimen was identified at the Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

3.3. Extraction and Isolation

The dried stem bark of *C. pseudomole* (3.0 kg) was macerated in MeOH twice for 2 days each time. After evaporation of the solvent in a rotary evaporator, 260 g of pale brown semi-solid was obtained. Further, the MeOH extract was partitioned first with *n*-hexane (1:1 *v/v)*; water was added (10% *v/v*) in the second step to increase the polarity; then the MeOH extract was partitioned with EtOAc (1:1 *v/v*). The EtOAc extract (35 g)was subjected to column chromatography over silica gel and eluted with *n*-hexane-ethyl acetate (from 9:1 to 1:1) to give fractions A–C. Fraction C was then subjected further to column chromatography and eluted with *n*-hexane-EtOAc (from 9:1 to 1:1) to produce three subfractions C_1–C_3. Subfraction C_2 was purified by planar radial chromatography using*n*-hexane-$CHCl_3$ (from 3:7 to 7:3), $CHCl_3$ and $CHCl_3$-EtOAc 9:1 to yield compound 5,9,11-trihydroxy-10-(2″-hydroxy-3″-methylbut-3″-en-1-yl)-2,2-dimethyl-3-(2′-methylbut-3′-en-2′-yl)-2H,12H-pyrano[2,3-a]xanthen-12-one (18 mg).

3.4. Cytotoxic Assay

The cytotoxic properties of **1** against murine leukemia P-388 cells were evaluated according to the MTT method as previosly described [14–16]. Artonin E was used as the positive control.

3.5. Antiplasmodial Assay

The antiplasmodial properties of **1** against *P. falciparum* were evaluated according to the Trager–Jensen method as previosly described [17,18]. Chloroquine was used as the positive control.

Supplementary Materials: The following are available online at 1422-8599/2017/4/M961, IR, HRESIMS, 1D and 2D NMR spectra are reported in the supplementary materials as Figures S1–S6.

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Author Contributions: Mulyadi Tanjung designed the whole experiment of bioactivity and wrote the manuscript. Tjitjik Srie Tjahjandarie researched data, analyzed the NMR and HRESIMS spectra and contributed to the manuscript, Ratih Dewi Saputri designed the whole experiment. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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