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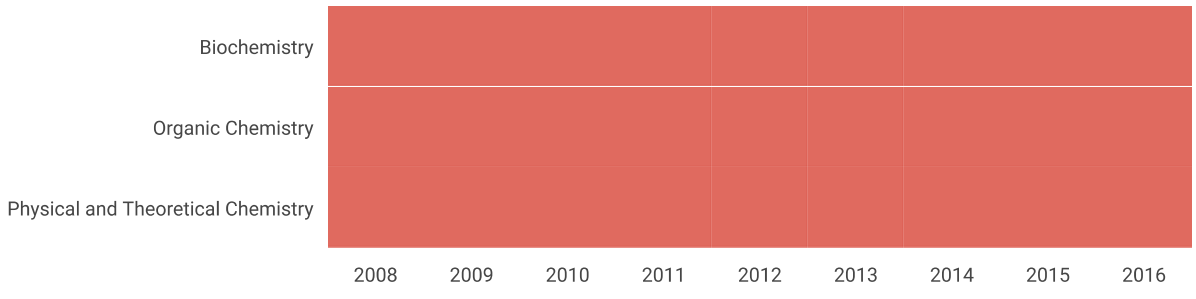

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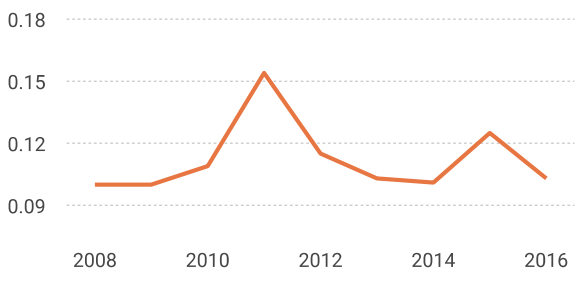

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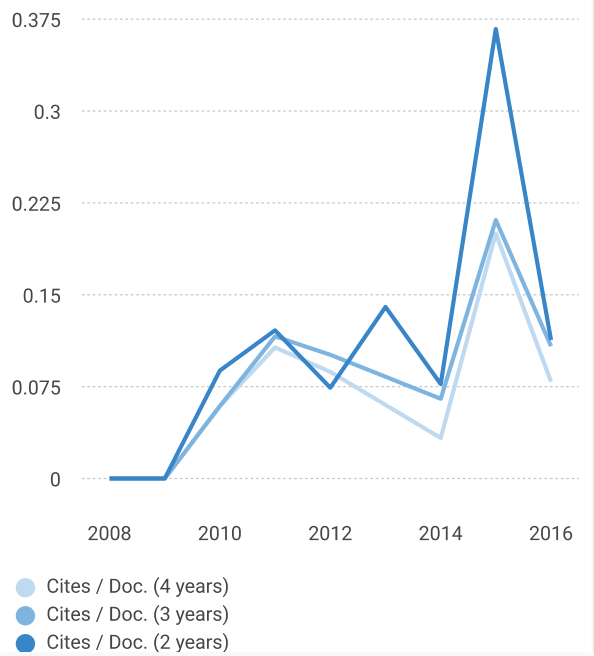

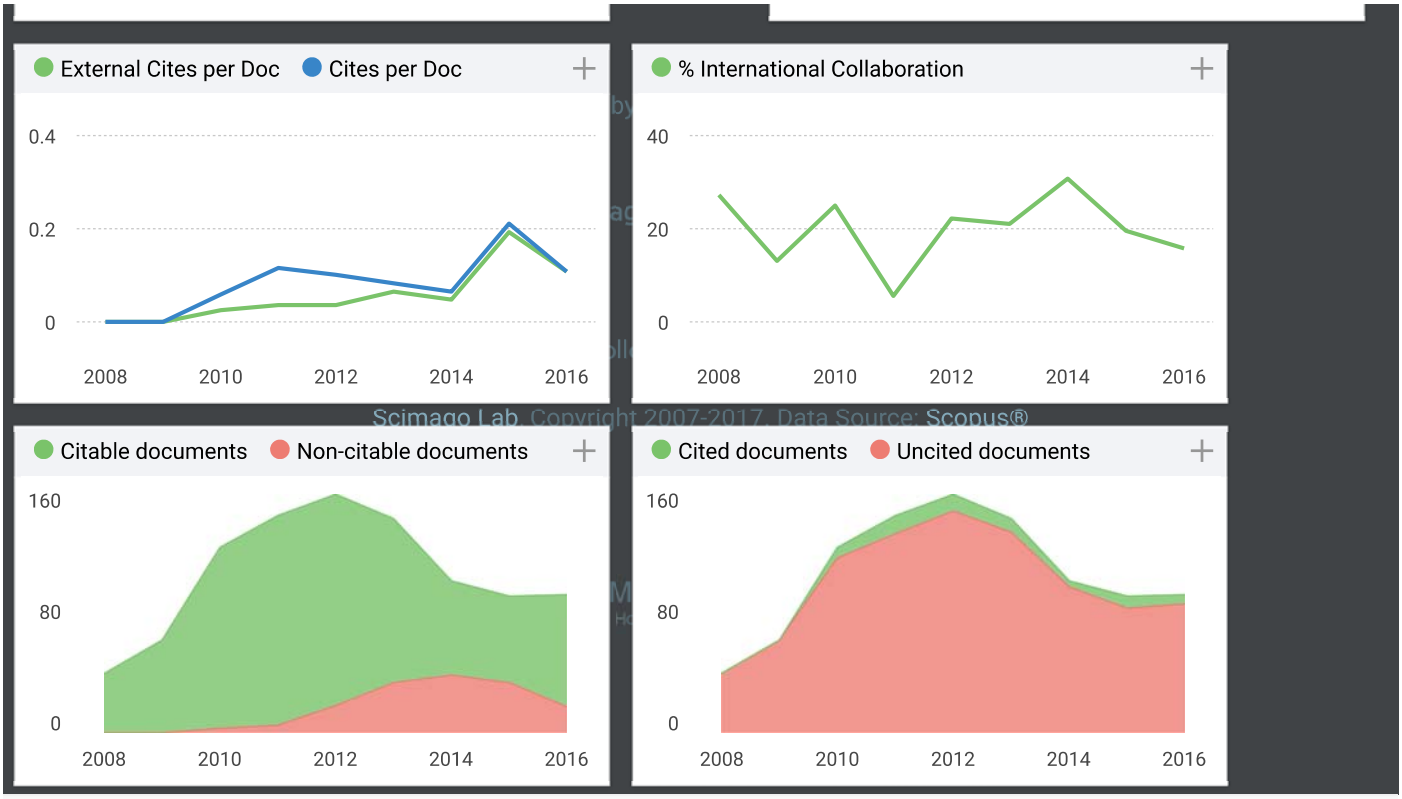

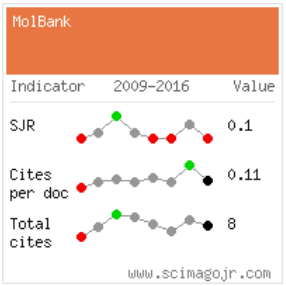

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5,9,11-Trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)pyrano[2,3-a]xanthen-12(2*H*)-one from the Stem Bark of *Calophyllum pseudomole*

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5,9,11-Trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)-pyrano[2,3-a]xanthen-12(2*H*)-one (**1**) was isolated from the stem bark of *Calophyllum pseudomole*. The structure of **1** was established by spectroscopic analysis which included UV, IR, HRESIMS and NMR experiments. View Full-Text (/1422-8599/2016/3/M906/htm)

Keywords: 5,9,11-trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″butenyl)- pyrano[2,3-a]xanthen-12(2*H*)-one (/search?q=5%2C9%2C11-trihydroxy-2%2C2-dimethyl-10-%283%E2%80%B2-methyl-2%E2%80%B2-butenyl%29-3-%282%E2%80%B3-methyl-3%E2%80%B3butenyl%29-%20pyrano%5B2%2C3-a%5Dxanthen-12%282H%29-one); xanthone (/search?q=xanthone); *Calophyllum pseudomole* (/search?q=Calophyllum%20pseudomole)

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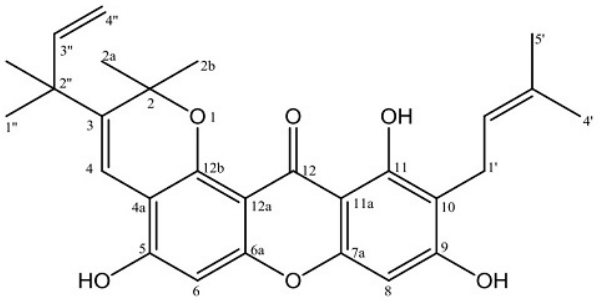

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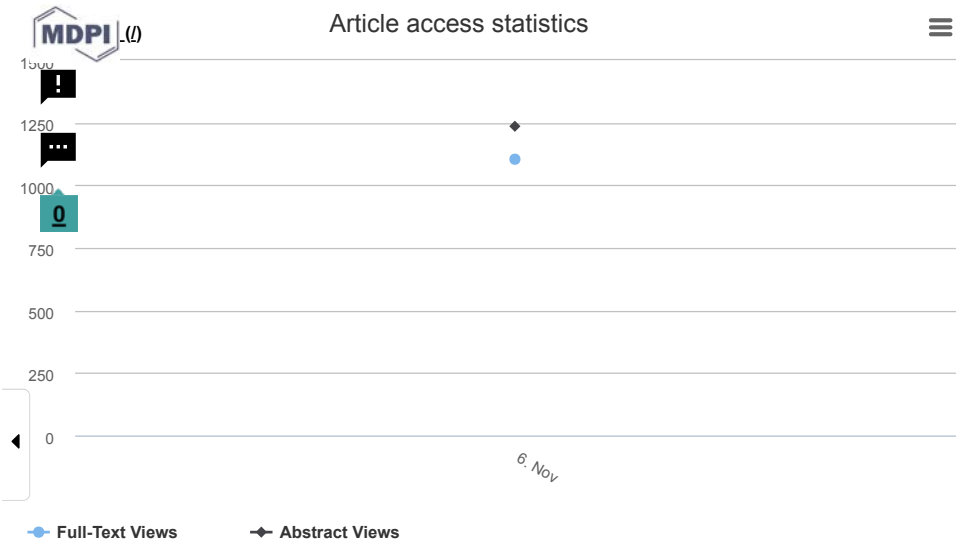

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5,9,11-Trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)pyrano[2,3-a]xanthen-12(2*H*)-one from the Stem Bark of *Calophyllum pseudomole*

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Abstract: 5,9,11-Trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)-pyrano[2,3-a]xanthen-12(2*H*)-one (**1**) was isolated from the stem bark of *Calophyllum pseudomole*. The structure of **1** was established by spectroscopic analysis which included UV, IR, HRESIMS and NMR experiments.

Keywords: 5,9,11-trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″butenyl)-pyrano[2,3-a]xanthen-12(2*H*)-one; xanthone; *Calophyllum pseudomole*

1. Introduction

The *Calophyllum* genus (Clusiaceae) comprises more than 180 species found mainly in Southeast Asia. This genus has been shown to produce a number of secondary metabolites, particularly xanthones [1–3], coumarins [4–6], chromanone acids [7–9], and flavonoids [10]. In Indonesia, the local name of *Calophyllum* is 'bitangor' [11].

Herein, we report the isolation and structural elucidation of a new isoprenylated xanthone, 5,9,11-trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)-pyrano[2,3-a]xanthen-12(2*H*)-one (**1**) (Figure 1) from the stem bark of *Calophyllum pseudomole* as well as its antioxidant activity.

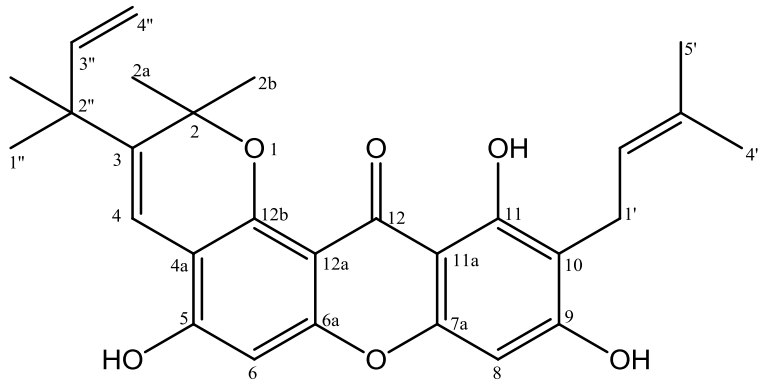

Figure 1. Structures of 5,9,11-trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)pyrano[2,3-a]xanthen-12(2*H*)-one (**1**).

2. Result and Discussion

5,9,11-Trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)pyrano[2,3-a]xanthen-12(2*H*)-one (**1**) was isolated as a yellow solid, m.p. 160–162 °C. The molecular formula of compound is $C_{28}H_{30}O_6$, whereas that of the deprotonated molecule $[M - H]^-$ is $C_{28}H_{29}O_6$ at *m*/*z* 461.1971 (calcd. 461.1964) by the HRESIMS. The UV spectrum exhibited four absorption bands characteristic of a xanthone chromophore at λ_{maks} 247, 264, 322 and 396 nm [1]. The IR spectrum showed absorption bands at ν_{max} 3423, 1622, and 1460 cm^{-1} indicating the presence of a hydroxyl, conjugated carbonyl and aromatic groups, respectively. The ^{1}H-NMR (Table 1) spectrum showed the presence of a chelated hydroxyl group (δ_H 13.77, 11-OH) and two isolated aromatic proton signals at δ_H 6.77 (1H, s, H-6) and 6.40 (1H, s, H-8) suggest that compound **1** is similar to a xanthone with six substituents [1]. The ^{1}H-NMR also revealed signals due to 3′-methyl-2′-butenyl group [δ_H 1.63 (3H, s, H-4′), 1.77 (3H, s, H-5′), 3.34 (2H, d, *J* = 7.3 Hz, H-1′), 5.27 (1H, t, *J* = 7.3 Hz, H-2′)], ring of 2,2-dimethylpyrano monosubstituent group at δ_H 1.49 (6H, s, H2a/H-2b), 8.19 (1H, s, H-4) and 1,1-dimethylalyl group [δ_H 1.41 (6H, s, H-1″, 2″-CH_3), 5.08 (1H, dd, *J* = 1.1; 10.6 Hz, H-4″a), 5.16 (1H, dd, *J* = 1.1; 17.5 Hz, H-4″b), 6.02 (1H, dd, *J* = 10.6; 17.5 Hz, H-3″)]. The ^{13}C-NMR spectrum (Table 1) of **1**, 26 carbon signals representing 28 carbon atoms were observed. The HMBC spectrum, the chelated hydroxyl group (δ_H 13.77, 11-OH) correlated with three quaternary carbons [δ_C 161.5 (C-11), 110.9 (C-10), 103.8 (C-11a)], and two carbons being further correlated to the isolated aromatic (δ_H 6.40), indicating that the *para*-position of the hydroxyl group was unsubstituted. The presence of long-range correlations in the HMBC spectrum between methylen group at δ_H 3.34 on the isoprenyl group with three aromatic carbon signals at δ_C 162.9 (C-9), 161.5 (C-11), 110.9 (C-10) and two vinyl carbon signals at δ_C 131.4 (C-3′), 118.8 (C-2′), indicated that an isoprenyl is attached at C-10 proton. Furthermore, a proton signal of an aromatic (δ_H 6.77, H-6) correlated with three quaternary carbons [δ_C 154.1 (C-5), 153.3 (C-6a), 108.4 (C-4a)] showed 2,2-dimethylpyrano group were fused at C-4a and C-12b. The presence of long-range correlations between vinyl group at δ_H 8.19 the that 2,2-dimethylpyrano group with four quaternary carbons [δ_C 137.6 (C-3), 108.4 (C-4a), 80.3 (C-2), 42.7 (C-2″)] showed that 1,1-dimethylalyl group attached at C-3. Therefore, compound **1**, was elucidated as 5,9,11-trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)pyrano[2,3-a]xanthen-12(2*H*)-one. Other HMBC correlations consistent with the structure **1** are shown in Table 1 and Figure 2. To our knowledge, compound **1** has not been reported previously as a novel natural product.

On antioxidant evaluation against DPPH radical scavenging, compound **1** exhibited IC_{50} values 76 μg/mL more active than apigenin as control positive (IC_{50} 130 μg/mL). Those antioxidant data suggested that compound **1** has high activity.

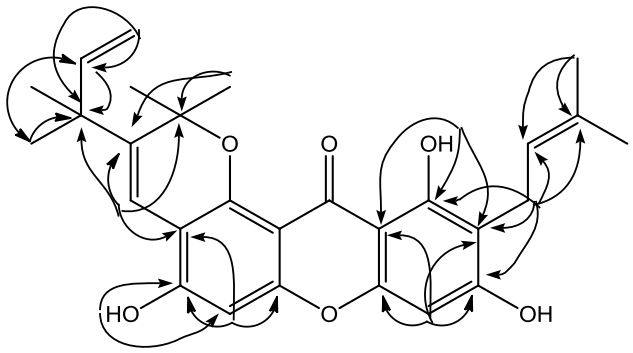

Figure 2. Selected HMBC correlations for **1**.

Table 1. NMR spectroscopic data of 5,9,11-trihydroxy-2,2-dimethyl-10-(3′-methyl-2′-butenyl)-3-(2″-methyl-3″-butenyl)pyrano[2,3-a]xanthen-12(2*H*)-one in acetone-d_6.

No. C	δ_H (Mult, *J* Hz)	δ_C	HMBC
2	-	80.3	-
2a	1.49 (s, 3H)	27.3	C-2; C-2b
2b	1.49 (s, 3H)	27.3	C-2; C-2a
3	-	137.6	-
4	8.19 (s, 1H)	118.8	C-2; C-3; C-4a; C-2″
4a	-	108.4	-
5	-	154.1	-
6	6.77 (s, 1H)	102.9	C-4a; C-5; C-6a
6a	-	153.3	-
7a	-	155.9	-
8	6.40 (s, 1H)	93.2	C-7a; C-9; C-10; C-11a
9	-	162.9	-
10	-	110.9	-
11	-	161.5	-
11a	-	103.8	-
12	-	183.1	-
12a	-	122.8	-
12b	-	149.7	-
1′	3.34 (d, 7.3, 2H)	21.9	C-9; C-10; C-11; C-2′; C-3′
2′	5.27 (t, 7.3, 1H)	123.4	C-1′; C-4′, C-5′
3′	-	131.4	-
4′	1.63 (s, 3H)	25.9	C-2′; C-3′; C-5′
5′	1.77 (s, 3H)	17.8	C-2′; C-3′; C-4′
1″	1.41 (s)	28.6	C-2″; C-3″, 2″-CH_3
2″	-	42.7	-
2″-CH_3	1.41 (s)	28.6	C-1″; C-2″, C-3″
3″	6.02 (dd, 10.6; 17.5, 1H)	147.9	C-1″; C-2″
4″	5.16 (dd, 1.1; 17.5, 1H) 5.08 (dd, 1.1; 10.6, 1H)	112.2	C-2″, C-3″
11-OH	13.77 (s, 1H)	-	C-10; C-11; C-11a

3. Experimental Section

3.1. General

The UV spectrum was measured with Shimadzu series 1800 spectrophotometer (Kyoto, Japan). The IR spectrum was recorded with Perkin-Elmer spectrum-100 FT-IR (Waltham, MA, USA). NMR spectra were recorded on a JEOL 400 ECA spectrophotometer (Tokyo, Japan) in acetone-d_6 at 400 (^{1}H) and 100 (^{13}C) MHz using TMS as the internal standard. The mass spectra were recorded using a Waters LCT Premier XE (Santa Clara, CA, USA). Column chromatography and radial chromatography were carried out using silica gel 60 and silica gel 60 PF_{254} (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 sample was identified and deposited in 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 methanol twice for 4 days, and then evaporated under reduced pressure to give a dark brown residue (120 g). Further, the methanol extract was partitioned first with *n*-hexane. The methanol extract was mixed with water (10% *v*/*v*) to increase the polarity and then partitioned with ethyl acetate. The ethyl acetate extract (24 g) was subjected to column chromatography over silica gel and eluted with *n*-hexane-ethyl acetate (from 9:1 to 3:7) to give fractions A–D. Fraction B showed the most potent antioxidant activity. Fraction B was then subjected to column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 7:3) to produce subfractions B_1–B_3. Subfraction B2 was purified by planar radial chromatography using *n*-hexane-acetone (from 9:2 to 4:1) to yield compound **1** (16 mg).

3.4. DPPH Radical Scavenging

The antioxidant assay of compound **1** against DPPH (2,2-diphenyl-1-picrihidrazil) radical was measured by UV spectrometer at λ 517 nm as described previously [12–14]. The inhibition percentage (%) of radical scavenging activity was calculated using the following equation:

$$\text{Inhibition}\,(\%) \;=\; (A_o - A_s/A_o) \times 100 \qquad (1)$$

where A_o is the absorbance of the control reaction (containing all reagents except the active compound), and A_s is the absorbance of the active compound.

Supplementary Materials: HRESIMS, ^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, IR and UV spectra are reported in the supplementary materials at www.mdpi.com/1422-8599/2016/3/M906.

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