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Subject Area and Category [Agricultural and Biological Sciences](#)
[Plant Science](#)

[Biochemistry, Genetics and Molecular Biology](#)
[Biochemistry](#)

[Chemistry](#)
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Publisher [Taylor & Francis](#)

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ISSN 14786419

Coverage 2003-ongoing

Scope The aim of Natural Product Research is to publish important contributions in the field of natural product chemistry. The journal covers all aspects of research in the chemistry and biochemistry of naturally occurring compounds. The communications include coverage of work on natural substances of land and sea and of plants, microbes and animals. Discussions of structure elucidation, synthesis and experimental biosynthesis of natural products as well as developments of methods in these areas are welcomed in the journal. Finally, research papers in fields on the chemistry-biology boundary, eg. fermentation chemistry, plant tissue culture investigations etc., are accepted into the journal.



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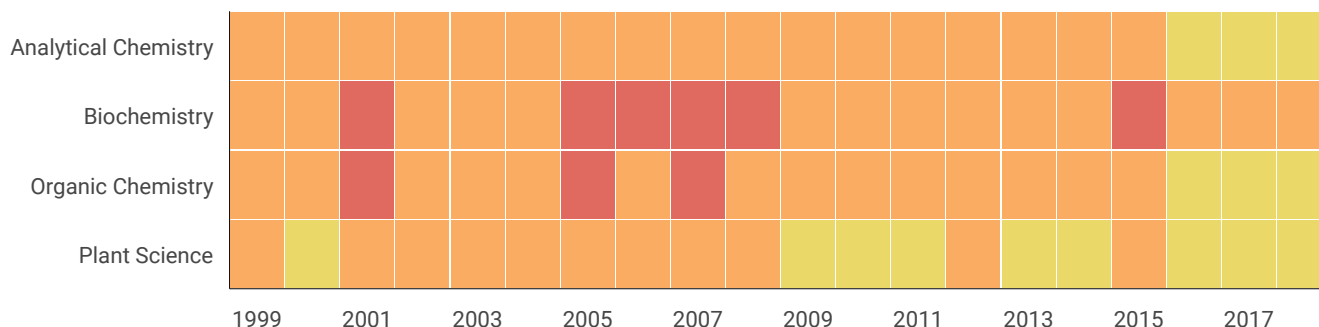


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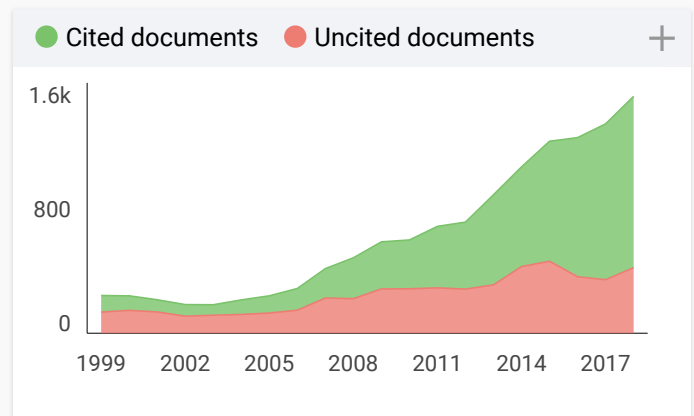
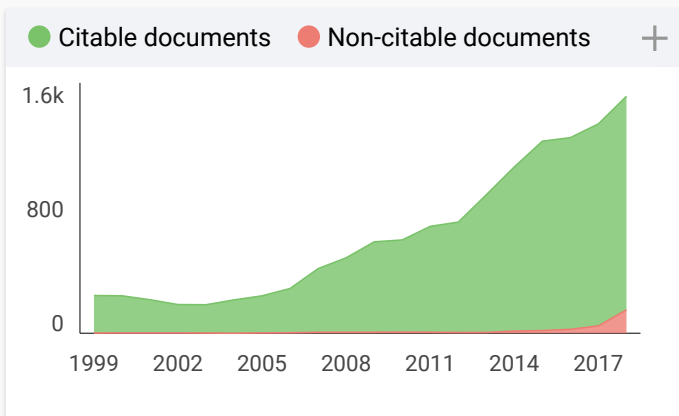
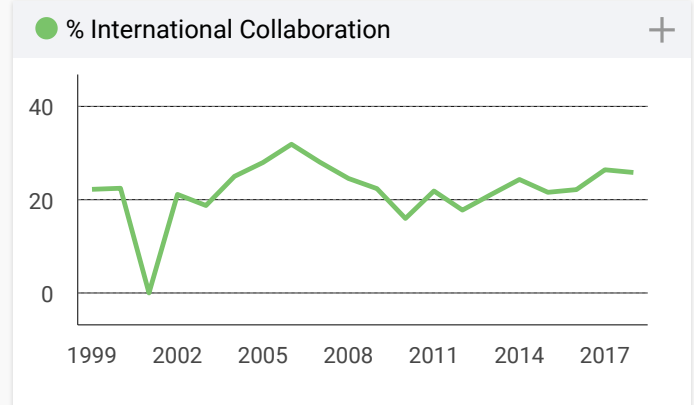
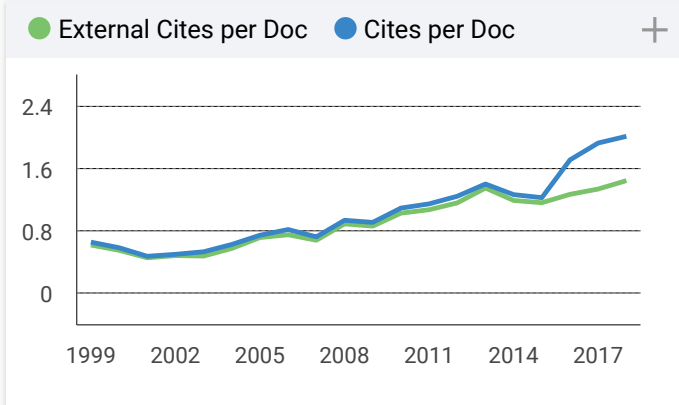
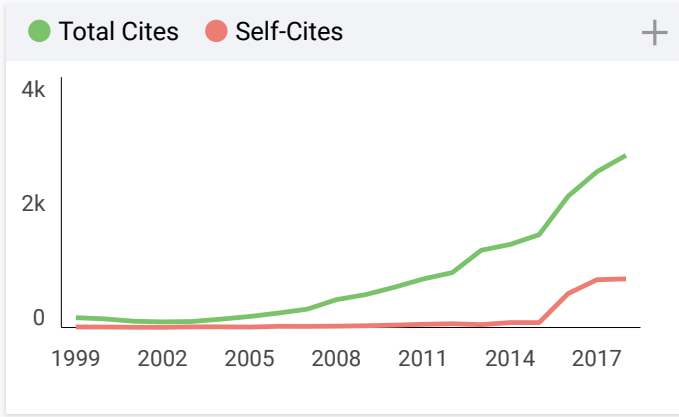
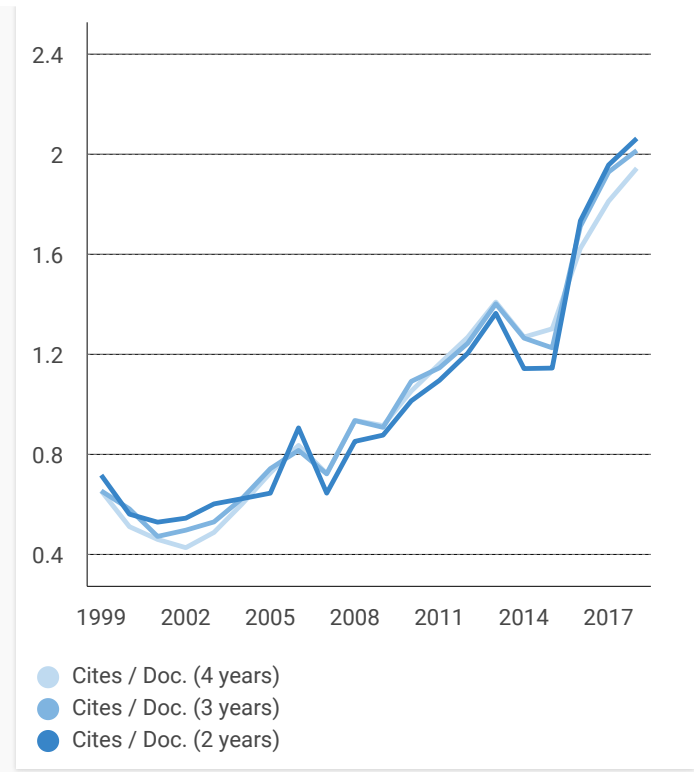
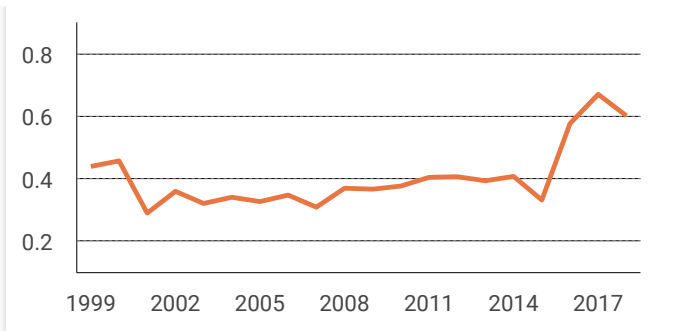


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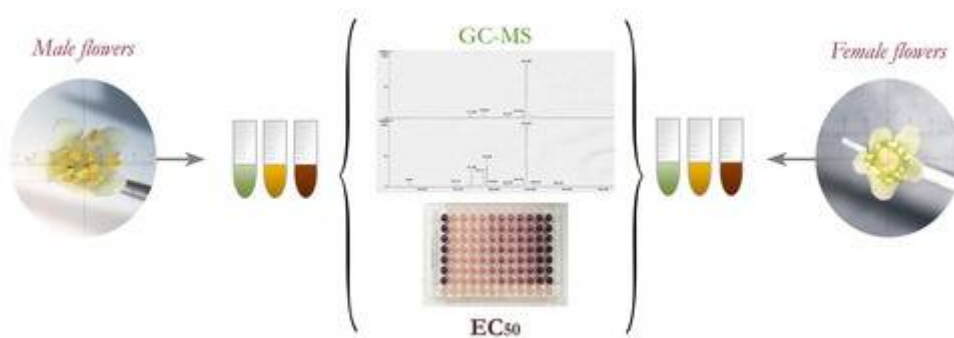
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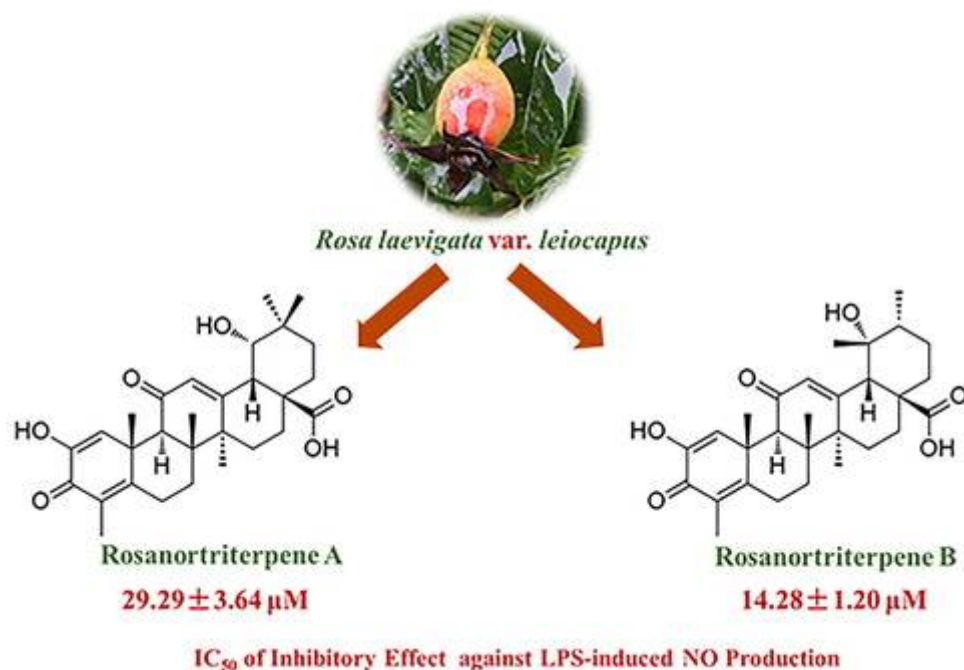


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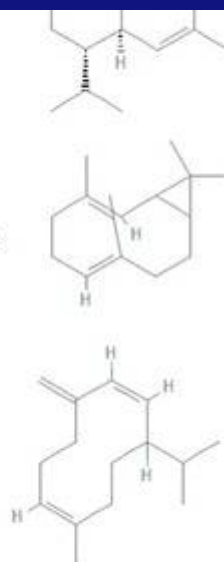
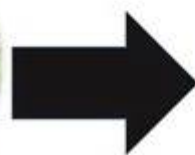
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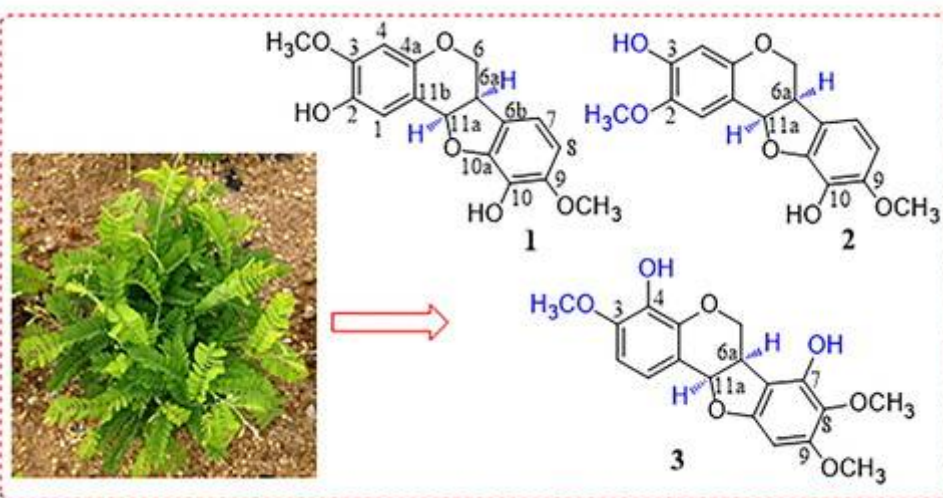
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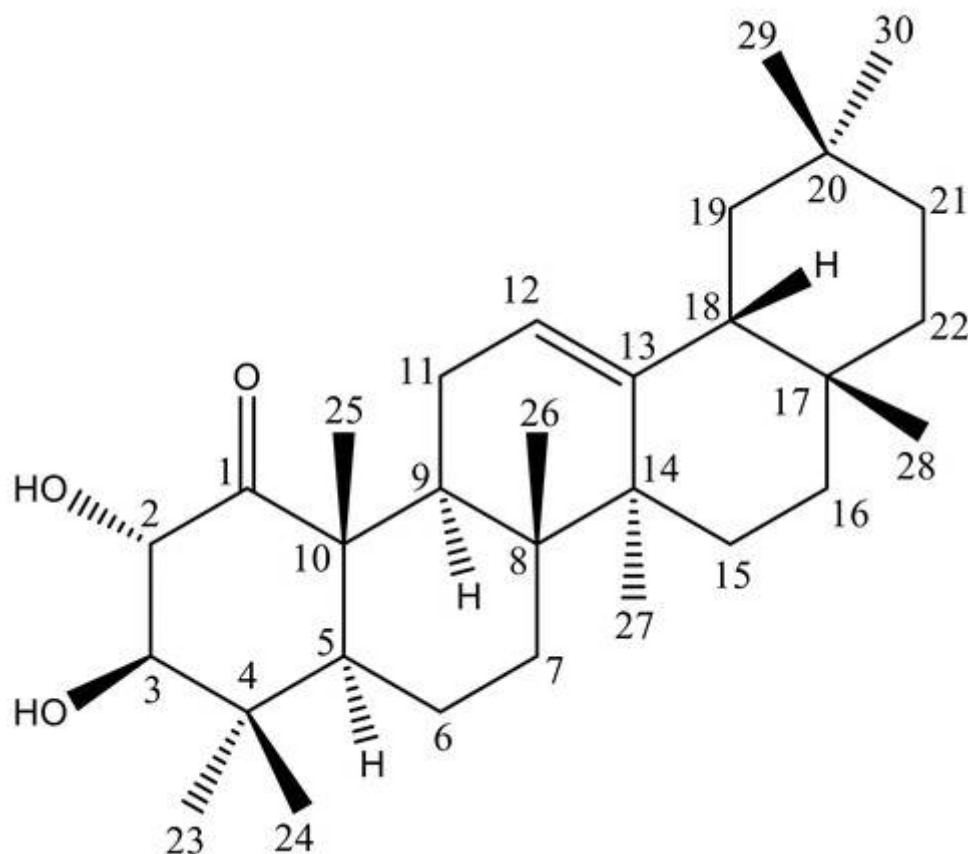
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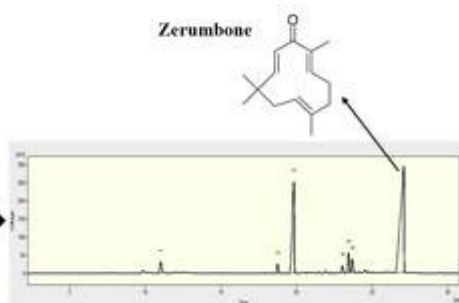
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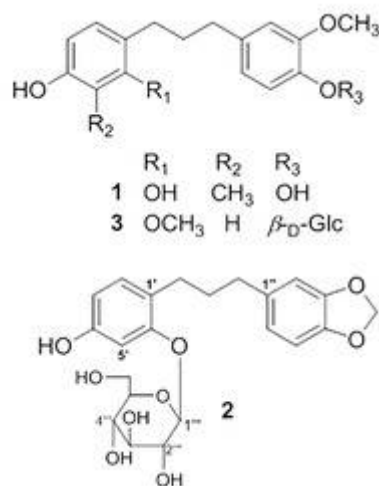
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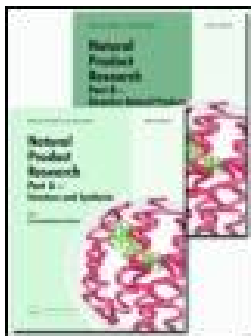
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Calodioscurins A and B, two new isoprenylated xanthenes from the stem bark of *Calophyllum dioscurii* P.F. Stevens

Tjitjik Srie Tjahjandarie, Mulyadi Tanjung, Dhaniar Farah Rahmania, Churin In Rhidoma & Ratih Dewi Saputri

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Calodioscurins A and B, two new isoprenylated xanthenes from the stem bark of *Calophyllum dioscurii*

P.F. Stevens

Tjitjik Srie Tjahjandarie, Mulyadi Tanjung, Dhaniar Farah Rahmania, Churin In Rhidoma and Ratih Dewi Saputri

Natural Products Chemistry Research Group, Organic Chemistry Division, Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Two new isoprenylated xanthenes, calodioscurin A (**1**) and B (**2**) were isolated from the stem bark of *Calophyllum dioscurii* P.F. Stevens along with two known isoprenylated 4-phenylcoumarins, apetalolide (**3**) and methyl inophyllum P (**4**). The structures of two new compounds were determined based on their HRESIMS, IR, UV, 1D and 2D NMR spectral data. Compounds **1–4** were assayed on P-388 cells, compound **2** showed IC₅₀ value 11.5 μM and categorised moderate activity.

ARTICLE HISTORY

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Calodioscurins A and B; isoprenylated xanthone; *Calophyllum dioscurii*; P-388 cell



1. Introduction

Calophyllum dioscurii P.F. Stevens (Calophyllaceae) is one species of endemic plant from Indonesia. The decoction of leaves and stem bark of this plant were used to treat fever and skin disease (Heyne 1987). *Calophyllum* plants were known to yield phenolic

CONTACT Tjitjik Srie Tjahjandarie  tjitjiktjahjandarie@fst.unair.ac.id

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compounds especially isoprenylated benzofurans, isoprenylated xanthenes (Tanjung et al. 2018a, 2018b) and isoprenylated 4-phenylcoumarins (Zou et al. 2010). Isoprenylation of xanthenes as a major compound on *Calophyllum* genus shows to increase cytotoxic activities toward variety of human cancer cells (Mah et al. 2015). Based on literature study, no isoprenylated xanthone from *C. dioscurii* was reported yet. In continuation of our research on *Calophyllum* plants from Indonesia, two new isoprenylated xanthenes (calodioscurins A and B) were isolated from the stem bark of *C. dioscurii*. All of isolated compounds also reported the cytotoxic assayed toward murine leukemia cells P-388.

2. Result and discussion

Calodioscurin A (**1**) showed positive ion peak $[M + H]^+$ at m/z 477.2268 corresponding to the molecular formula $C_{29}H_{32}O_6$ based on the information provided at HRESIMS spectrum. Four absorption bands at λ_{max} 217 (3.03), 269 (3.08), 289 (3.20) and 325 (2.81) nm showed that of **1** possesses xanthone chromophore (Ito et al. 2002). The IR spectrum of **1** indicated absorptions for hydroxyl (3411 cm^{-1}), conjugated carbonyl (1649 cm^{-1}), aromatic (1604 and 1579 cm^{-1}) and ether (1159 cm^{-1}) groups. A signal of aromatic in the ^1H NMR spectrum of **1** at δ_{H} 7.58 (H-8) is typical for 1,2,3,4,5,6,7-heptasubstituted xanthone (Linuma et al. 1997). Additionally, compound **1** showed the proton signals of hydroxyl group, methoxyl group, isoprenyl, and 2,2-dimethylpyrano ring that confirmed by 2D-NMR spectrum. Two signals of hydroxyl group demonstrated at δ_{H} 13.19 (1-OH), δ_{H} 5.64 (5-OH), and a methoxyl signal at δ_{H} 3.99 (6-OCH₃). Furthermore, compound **1** also showed a 2,2-dimethylpyrano ring signal at δ_{H} 6.74 (1H, d, $J = 10.0$ Hz, H-4), 5.60 (1H, d, $J = 10.0$ Hz, H-3), 1.48 (6H, s, H-5'/H-6) as well as two isoprenyl signals at δ_{H} 5.29 (1H, t, $J = 7.2$ Hz, H-2), 5.26 (1H, t, $J = 7.6$ Hz, H-2'), 3.51 (2H, t, $J = 7.2$ Hz, H-1'), 3.41 (2H, t, $J = 7.6$ Hz, H-1'), 1.87 (3H, s, H-5), 1.76 (3H, s, H-5'), 1.74 (3H, s, H-4), and 1.71 (3H, s, H-4'). The ^{13}C NMR (APT experiment) spectrum, compound **1** showed the existence of seven methyl carbon signals (including one methoxyl carbon), two methylene carbon signals, five methine carbon signals and 15 quaternary carbon signals (including one carbonyl carbon, one oxycarbon and six oxyaryl carbons). The location of two hydroxyls, a methoxyl, two isoprenyl, and a 2,2-dimethylpyrano ring was established by HMQC and HMBC spectra. A signal of hydroxyl at δ_{H} 13.19 (1-OH) demonstrated correlation to C-1 (δ_{C} 156.1), C-2 (δ_{C} 104.6), C-9a (δ_{C} 103.3), whilst a vinyl signal of a 2,2-dimethylpyrano ring at δ_{H} 6.74 (H-4) correlated to C-3 (δ_{C} 158.1), C-2' (δ_{C} 78.2) and a vinyl signal at δ_{H} 5.60 (H-3) correlated to C-2 (δ_{C} 104.6), C-2' (δ_{C} 78.2) showing that 2,2-dimethylpyrano ring fused at C-2 and C-3. The appearance of long-range correlations of a methylene at δ_{H} 3.51 (H-1) to C-3 (δ_{C} 158.1), C-4 (δ_{C} 107.3), C-4a (154.2), C-2' (δ_{C} 122.6), C-3' (δ_{C} 131.7), and the signal of a gem dimethyl at δ_{H} 1.87 (H-5) and δ_{H} 1.71 (H-4) correlated to C-2' (δ_{C} 122.6), C-3' (δ_{C} 131.7) obviously located the isoprenyl side chain at C-4. A signal of hydroxyl group at δ_{H} 5.64 (5-OH) showed correlation with three oxyaryl carbons [C-5 (δ_{C} 137.0), C-6 (δ_{C} 149.9), C-10a (δ_{C} 143.3)], and a signal of methoxyl at δ_{H} 3.99 (6-OCH₃) correlated to C-6 (δ_{C} 149.9) supported that a hydroxyl attached at C-5 and a methoxyl at C-6. A signal of a methylene at δ_{H} 3.41 (H-1) correlated to C-6 (δ_{C} 149.9), C-7 (δ_{C} 116.5), C-2' (δ_{C}

121.8), C-3' (δ_C 131.7) indicating that isoprenyl located at C-7. A signal of aromatic at δ_H 7.58 (H-8) correlated to C-6 (δ_C 149.9), C-9 (δ_C 180.9), C-10a (δ_C 143.3), and C-1' (δ_C 28.5) was supported the location of isoprenyl at C-7. From HRESIMS, 1D and 2D-NMR spectra, compound **1** was established as calodioscurin A.

Calodioscurin B (**2**) was established the molecular formula $C_{28}H_{33}O_5$ deduced from a positive ion peak $[M + H]^+$ at 449.2327 of HRESIMS spectrum. From IR spectrum revealed the presence of hydroxyl (3448 cm^{-1}), conjugated carbonyl (1651 cm^{-1}), aromatic (1627 and 1579 cm^{-1}) and ether (1186 cm^{-1}). Five absorption bands at λ_{max} 217 (3.33), 234 (3.12), 255 (3.02), 263 (2.98) and 299 (2.69) nm, indicated that compound **2** was a typical for a modified xanthone (Ito et al. 2002). The ^1H NMR spectrum, compound **2** showed a signal of aromatic at δ_H 6.34 (H-4), and a signal of methylene at δ_H 2.91 (H-5) as well as a methylene splitted into two signals at δ_H 2.88 (H-6a), and δ_H 2.53 (H-6b) confirmed a modified xanthone. Additionally, compound **2** showed signals of two hydroxyl groups, two isoprenyl side chains, and a 2-methyl-1,3-butadienyl side chain that were confirmed by 2D-NMR spectrum. Two hydroxyl signals showed at δ_H 13.27 (1-OH), and δ_H 6.24 (3-OH). Two isoprenyl side chain signals showed two methylene signals at δ_H 3.45 (2H, d, $J = 7.0$ Hz, H-1), a methylene splitted into two signals [δ_H 3.24 (1H, dd, $J = 8.0; 14.0$ Hz, H-1a), δ_H 3.04 (1H, dd, $J = 8.0; 14.0$ Hz, H-1b)]; two vinylic signals [δ_H 5.28 (1H, tm, $J = 7.0$ Hz, H-2', δ_H 4.68 (1H, tm, $J = 7.9$ Hz, H-2)], and four methyl signals [δ_H 1.78 (3H, s, H-4'), 1.84 (3H, s, H-5'), δ_H 1.52 (3H, s, H-4), 1.50 (3H, s, H-5)]. The presence of 2-methyl-1,3-butadienyl side chain showed two vinylic signals [δ_H 5.81 (1H, dd, $J = 8.0; 14.0$ Hz, H-1), δ_H 6.06 (1H, dd, $J = 8.0; 14.0$ Hz, H-2)], a methylene terminal splitted into two signals [δ_H 4.94 (1H, s, H-4a), δ_H 4.88 (1H, s, H-1b)] and a methyl signal at δ_H 1.82 (3H, s, H-5). The ^{13}C NMR spectrum, compound **2** showed 28 carbon signals completely separated. Compound **2** showed 13 carbon signals of a modified xanthone nucleus [δ_C 206.7, δ_C 180.8, δ_C 164.2, δ_C 161.4, δ_C 159.5, δ_C 155.4, δ_C 117.7, δ_C 109.7, δ_C 105.0, δ_C 93.6, δ_C 56.1, δ_C 35.1, δ_C 27.5], 10 carbon signals of two isoprenyl side chains [δ_C 136.5, δ_C 135.2, δ_C 121.1, δ_C 119.5), δ_C 33.1, δ_C 26.1, δ_C 26.0, δ_C 21.6, δ_C 18.0, δ_C 17.9] and five carbon signals of a 2-methyl-1,3-butadienyl side chain (δ_C 141.4, δ_C 133.6, δ_C 131.3, δ_C 117.5, δ_C 18.7). The placement of two hydroxyl groups, a carbonyl of cyclohexanone, a 2-methyl-1,3-butadienyl side chain, and two isoprenyl side chains on a modified xanthone was confirmed by HMQC and HMBC spectra. A hydroxyl group signal at δ_H 13.27 (1-OH) correlated to C-1 (δ_C 159.5), C-2 (δ_C 109.7), C-9a (δ_C 105.0), and a methylene signal of isoprenyl side chain at δ_H 3.45 (H-1) showed correlation to C-1 (δ_C 159.5), C-2 (δ_C 109.7), C-3 (δ_C 161.4), C-2' (δ_C 121.1), C-3' (δ_C 136.5), consequently an isoprenyl side chain located at C-2. A hydroxyl group signal at δ_H 6.24 (3-OH) correlated to C-3 (δ_C 161.4), and C-4 (δ_C 93.6) indicated that a hydroxyl group placed at C-3. Long-range correlations of a signal of aromatic at δ_H 6.34 (H-4) to C-2 (δ_C 109.7), C-3 (δ_C 161.4), C-4a (δ_C 155.4), C-9a (δ_C 105.0) were confirmed a isoprenyl at C-2 and a hydroxyl at C-3. A methylene of cyclohexanone signal on a modified xanthone at δ_H 2.91 (H-5) showed long-range correlations to C-6 (δ_C 35.1), C-7 (δ_C 206.7), C-8a (δ_C 117.7), and C-10a (δ_C 164.2) indicated that a carbonyl placed at C-7. The methylene signal of another isoprenyl at δ_H 3.24 (H-1a) and δ_H 3.04 (H-1b) correlated to carbonyl at C-7 (δ_C 206.7), a quaternary carbon at C-8 (δ_C 56.1), and C-2' (δ_C 119.5), C-3' (δ_C 135.2) was assigned the isoprenyl side chain

at C-8. A vinyl signal of 2-methyl-1,3-butadienyl at δ_{H} 5.81 (H-1) correlated to quaternary carbon at C-8 (δ_{C} 56.1), C-3' (δ_{C} 141.4), C-4' (δ_{C} 117.5), and C-5' (δ_{C} 18.7) confirmed a 2-methyl-1,3-butadienyl side chain at C-8. Therefore, the structure of compound **2** was assigned as calodioscurin B.

Two known 4-phenylcoumarins, apetalolide (**3**) and methyl inophyllum P (**4**) from HRESIMS, 1D and 2D-NMR spectra identically with available spectra data (Zou et al. 2010).

Compounds (**1–4**) (Figure 1), the cytotoxic effect against P-388 cells were assessed by MTT method, showing IC_{50} values of 11.5 ± 0.78 , 26.38 ± 0.92 , 53.9 ± 0.36 , and 83.9 ± 1.12 μM , respectively (Tanjung et al. 2018a, 2018b). The structure-activity relationship against P-388 cells of compounds (**1–4**) were discussed based on the type of compounds. Those cytotoxic effects for isoprenylated xanthenes (**1–2**) more than active compared to isoprenylated 4-phenylcoumarins (**3–4**). The cytotoxic effect suggested that compound **2** showed moderate activity, compound **1** displayed weak activity, and compounds (**3–4**) were inactive. The structure of **2** is a modified xanthone undergoes a keto-enol tautomerism reaction which is suggested to increase the cytotoxic effect of P-388 cells (Ito et al. 2002).

3. Experimental

3.1. Plant material

Collecting fresh sample of *C. dioscurii* was obtained from the Batam conserved forest, Riau Island, Indonesia on Dec 2015 by Mr. Ismail Rachman. The specimen (CD 00025) was identified in Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

3.2. Extraction and isolation

The dried powdered stem bark of *C. dioscurii* (2.1 kg) was extracted with MeOH two times (each 5 L, 3 days) at room temperature. Evaporation of the solvent with rotavapor gave the MeOH extract (430 g). The MeOH extract was suspended in water (9:1 v/v), and then partitioned with *n*-hexane (18 g) and EtOAc (13.5 g) respectively. A part of EtOAc extract (13 g) was chromatographed on polyamide and then eluted with *n*-hexane- EtOAc (from 9:1 to 3:7) to give three fractions. Fraction B (435 g) was chromatographed with the same method and eluted with *n*-hexane- EtOAc (from 9:1 to 4:1) gave four subfractions B₁-B₄. Compound **1** (8 mg) was isolated from subfraction B₂ by radial planar chromatography using *n*-hexane-CHCl₃ (from 9:1 to 7:3). Subfraction C (610 mg) was purified by planar radial chromatography using *n*-hexane-acetone (from 19:1 to 4:1) to yield compound **2** (9 mg), compound **3** (11 mg), and compound **4** (7 mg).

3.3. Spectral data

Calodioscurin A (**1**): yellow solid, UV/Vis (MeOH) λ_{max} (nm) (log ϵ): 217 (3.03), 269 (3.08), 289 (3.20) and 325 (2.81) nm. IR (KBr) ν (cm^{-1}): 3411, 2972, 2925, 2852, 1649,

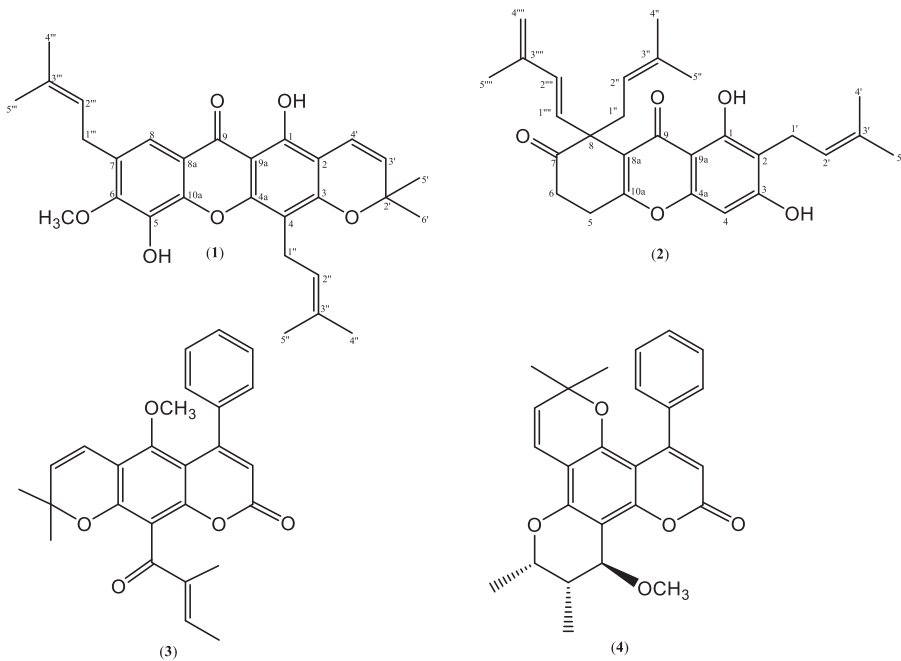


Figure 1. Xanthones and 4-phenylcoumarins from *C. dioscurii*.

1604, 1579 and 1159. ¹H-NMR (400 MHz, CDCl₃) δ_H ppm: 7.58 (1H, *s*, H-8), 13.19 (1H, *s*, 1-OH), 5.64 (1H, *s*, 5-OH), 3.99 (3H, *s*, 6-OCH₃), 5.60 (1H, *d*, *J* = 10.0 Hz, H-3), 6.74 (1H, *d*, *J* = 10.0 Hz, H-4), 1.48 (6H, *s*, H-5'/H-6'), 3.51 (2H, *d*, *J* = 7.2 Hz, H-1'), 5.29 (1H, *d*, *J* = 7.2 Hz, H-2'), 1.71 (3H, *s*, H-4'), 1.87 (3H, *s*, H-5'), 3.41 (2H, *d*, *J* = 7.2 Hz, H-1'), 5.26 (1H, *d*, *J* = 7.6 Hz, H-2'), 1.74 (3H, *s*, H-4'), and 1.76 (3H, *s*, H-5'). ¹³C-NMR (100 MHz, CDCl₃) δ_C ppm: 156.1 (C-1), 104.6 (C-2), 158.1 (C-3), 107.3 (C-4), 154.2 (C-4a), 137.0 (C-5), 149.9 (C-6), 61.1 (6-OCH₃), 116.5 (C-7), 116.2 (C-8), 120.8 (C-8a), 180.9 (C-9), 103.3 (C-9a), 143.3 (C-10a), 78.2 (C-2'), 127.4 (C-3'), 115.8 (C-4'), 28.4 (C-5'/C-6'), 21.7 (C-1'), 122.6 (C-2'), 131.5 (C-3'), 25.8 (C-4'), 18.0 (C-5'), 28.5 (C-1'), 121.8 (C-2'), 131.7 (C-3'), 25.9 (C-4') and 17.9 (C-5'). HRESIMS: *m/z* [M + H]⁺ calcd. for C₂₉H₃₃O₆ 477.2277, found 477.2268.

Calodioscurin B (**2**): yellow solid, UV/Vis (MeOH) λ_{max} (nm) (log ε): 217 (3.33), 234 (3.12), 255 (3.02), 263 (2.98) and 299 (2.69) nm. IR (KBr) ν (cm⁻¹): 3448, 2966, 2923, 2854, 1651, 1627, 1460, and 1186. ¹H-NMR (400 MHz, CDCl₃) δ_H ppm: 6.34 (1H, *s*, H-4), 2.91 (2H, *m*, H-5), 2.88 (1H, *m*, H-6a), 2.53 (1H, *m*, H-6b), 13.27 (1H, *s*, 1-OH), 6.24 (1H, *s*, 3-OH), 3.45 (2H, *d*, *J* = 7.0 Hz, H-1), 5.28 (1H, *d*, *J* = 7.0 Hz, H-2), 1.78 (3H, *s*, H-4), 1.84 (3H, *s*, H-5), 3.24 (1H, *dd*, *J* = 8.0; 14.0 Hz, H-1'a), 3.04 (1H, *dd*, *J* = 8.0; 14.0 Hz, H-1'b), 4.68 (1H, *d*, *J* = 7.9 Hz, H-2'), 1.52 (3H, *s*, H-4'), 1.50 (3H, *s*, H-5'), 5.81 (1H, *d*, *J* = 16.0 Hz, H-1), 6.06 (1H, *d*, *J* = 16.0 Hz, H-2), 4.94 (1H, *s*, H-4a), 4.88 (1H, *s*, H-4b), and 1.82 (3H, *s*, H-5'). ¹³C-NMR (100 MHz, CDCl₃) δ_C ppm: 159.5 (C-1), 109.7 (C-2), 161.4 (C-3), 93.6 (C-4), 155.4 (C-4a), 27.5 (C-5), 35.1 (C-6), 206.7 (C-7), 56.1 (C-8), 117.7 (C-8a), 180.8 (C-9), 105.0 (C-9a), 164.2 (C-10a), 21.6 (C-1'), 121.1 (C-2'), 136.5 (C-3'), 26.1 (C-4'), 18.0 (C-5'), 33.1 (C-1'), 119.5 (C-2'), 135.2 (C-3'), 26.0 (C-4'), 17.9 (C-5'), 131.3 (C-1'), 133.6 (C-2'), 141.4 (C-3'), 117.5 (C-4'), and 18.7 (C-5'). HRESIMS: *m/z* [M + H]⁺ calcd. for C₂₈H₃₃O₅ 449.2328, found 449.2327.

3.4. Cytotoxic assay

Effect of compounds (**1–4**) against P-388 cells (human murine leukaemia) were evaluated in pursuance of the MTT colorimetric method as anteriorly described (Saputri et al. 2018).

4. Conclusions

In this research, two new isoprenylated xanthenes, calodioscurin A (**1**) and B (**2**) were found the first time on natural compounds from the stem bark of *C. dioscurii*.

Disclosure statement

No conflict of interest on author's team.

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