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

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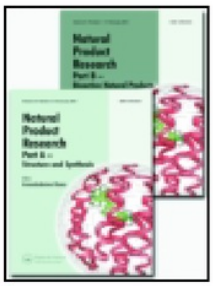
Submission date: 02-Sep-2019 06:39PM (UTC+0800)
Submission ID: 1166208378
File name: ones_from_the_stem_bark_of_Calophyllum_dioscurii_P_F_Stevens.pdf (865.14K)
Word count: 3176
Character count: 14276



Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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To cite this article: Tjitjik Srie Tjahjandarie, Mulyadi Tanjung, Dhaniar Farah Rahmania, Churin In Rhidoma & Ratih Dewi Saputri (2019): Calodioscurins A and B, two new isoprenylated xanthones from the stem bark of *Calophyllum dioscurii* P.F. Stevens, Natural Product Research, DOI: 10.1080/14786419.2019.1643864

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ABSTRACT

Two new isoprenylated xanthones, 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 IC_{50} value 11.5 μM and categorised moderate activity.

ARTICLE HISTORY
Received 11 June 2019
Accepted 4 July 2019

KEYWORDS
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

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1643864.
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compounds especially isoprenylated benzofurans, isoprenylated xanthones (Tanjung et al. 2018a, 2018b) and isoprenylated 4-phenylcoumarins (Zou et al. 2010). Isoprenylation of xanthones 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 xanthones (calodiocurins 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 (Iinuma 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-OCH_3). 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 methylen 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-OCH_3) 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 methylen 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 ^{1}H 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-4′a), 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-1′a) 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 xanthones (**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 B_1-B_4. Compound **1** (8 mg) was isolated from subfraction B_2 by radial planar chromatography using *n*-hexane-$CHCl_3$ (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,

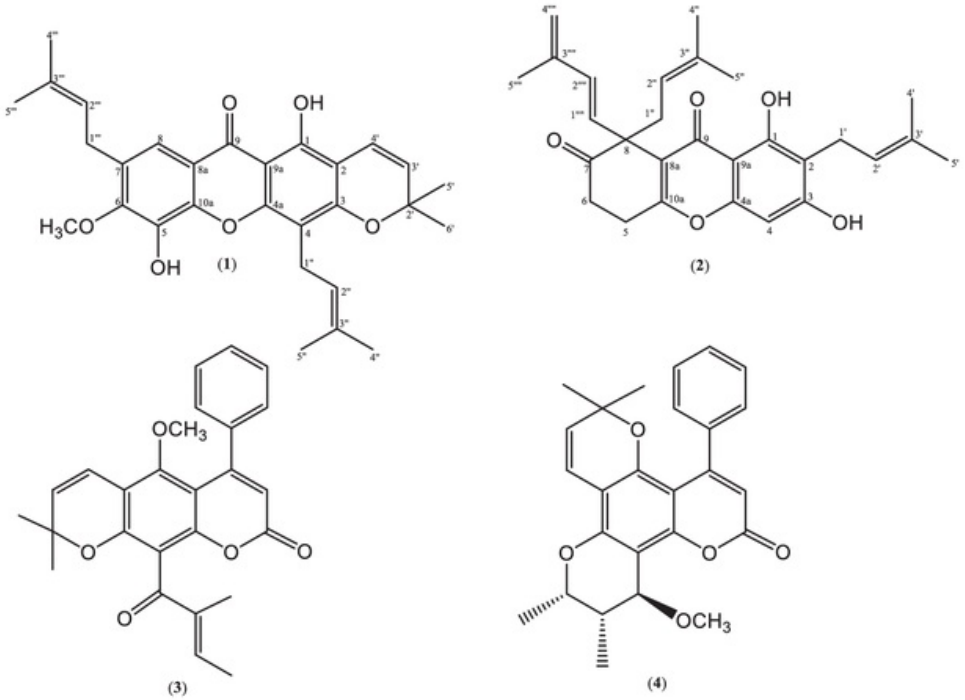

Figure 1. Xanthones and 4-phenylcoumarins from *C. dioscurii*.

1604, 1579 and 1159. ^{1}H-NMR (400 MHz, $CDCl_3$) 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-OCH_3), 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″). ^{13}C-NMR (100 MHz, $CDCl_3$), 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-OCH_3), 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 $C_{29}H_{33}O_6$ 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. ^{1}H-NMR (400 MHz, $CDCl_3$) 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-4′a), 4.88 (1H, *s*, H-4′b), and 1.82 (3H, *s*, H-5′). ^{13}C-NMR (100 MHz, $CDCl_3$), 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 $C_{28}H_{33}O_5$ 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 xanthones, 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.

Funding

This research was supported by Research Grant of Universitas Airlangga, Indonesia (Penelitian Hibah Mandat, Universitas Airlangga, 2019).

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