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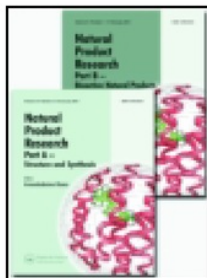
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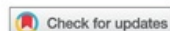
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Airlanggins A-B, two new isoprenylated benzofuran-3-ones from the stem bark of *Calophyllum soulattri*

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ABSTRACT

Two new isoprenylated benzofuran 3-ones, airlanggin A (**1**) and B (**2**) along with two known xanthenes, ananixanthone (**3**) and trapezifolixanthone (**4**) were isolated from the stem bark of *Calophyllum soulattri*. Structures of all the compounds were elucidated using extensive spectroscopic methods, including UV, IR, HRESIMS, 1D and 2D NMR. Compounds **1–4** were evaluated for their cytotoxicity against P-388 cells, showing that compound **3** was the most active with IC₅₀ 0.68 µg/mL and compound **1** showed moderate activity with IC₅₀ 5.80 µg/mL.

24

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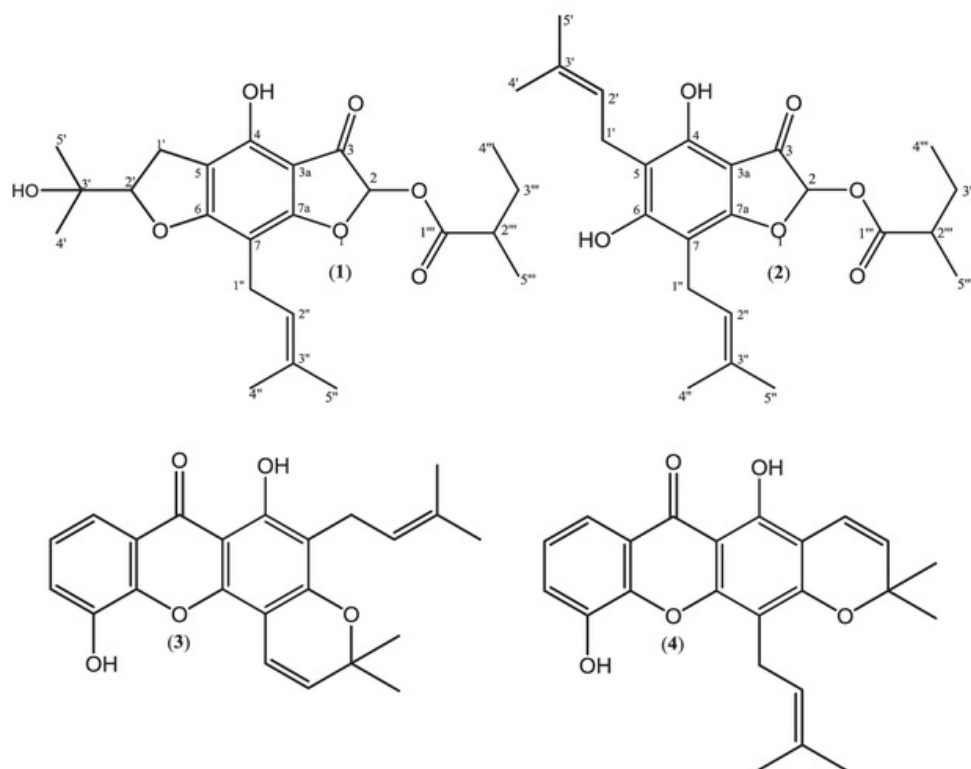
1. Introduction

Calophyllum soulattri locally known 'bintangor' belongs to the Clusiaceae family. *Calophyllum* is widely distributed in Asia, Australia, Africa and Polynesia. In Indonesia, the aqueous decoction of stem bark or leaves of this plant has been used to treat inflammation and rheumatism (Heyne 1987) (Figure 1). The *Calophyllum* genus has been known to produce a variety of

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51

Figure 1. Compounds 1–4 isolated from the stem bark of *Calophyllum soulattri*.

42

xanthenes (Mah et al. 2015; Daud et al. 2016), coumarins (Zhong et al. 2010; Daud et al. 2014) and chromanone acids (Lim et al. 2015). In continuation of a phytochemical investigation of *Calophyllum* plants in Indonesia, we wish to report the isolation and structural elucidation of two isoprenylated benzofuran 3-ones, airlanggin A (**1**) and B (**2**) from the stem bark of *C. soulattri*. Compounds **1** and **2** are the first example of natural products from *C. soulattri* apart from the previously reported xanthenes (Mah et al. 2015). The cytotoxic properties against murine leukaemia P-388 of isolated compounds from this plant are also reported.

2. Result and discussion

Phytochemical study on the ethyl acetate extract from the stem bark of *C. soulattri* yielded two new isoprenylated benzofuran 3-ones, namely airlanggin A (**1**), airlanggin B (**2**) and two xanthenes, namely ananixanthone (**3**) (Daud et al. 2016) and trapezifolixanthone (**4**) (Mah et al. 2015).

41

Airlanggin (**1**) was isolated as a yellow solid, $[\alpha]_D^{20} + 11.6$ (c 0.10 MeOH), showed a quasi-molecular ion $[M + H]^+$ at m/z 419.2070 corresponding to the molecular formula $C_{23}H_{30}O_7$. The UV maximum absorption at λ_{max} 218 (4.43), 261 (4.28) and 301 (4.00) nm possesses dihydrobenzofuran and benzofuran-3-one chromophore (Wang et al. 2017). The IR spectrum of **1** indicated absorptions for hydroxyl (3421 cm^{-1}), conjugated carbonyl (1668 cm^{-1}) and aromatic (1585 and 1419 cm^{-1}) groups, respectively.

The ^1H NMR spectrum of **1** showed a singlet proton signal of acetal group at δ_H 6.02 (H-2) typical of pentasubstituted benzofuran-3-one (Jiang et al. 2008). The ^1H NMR spectrum of **1**

showed a chelated hydroxyl δ_{H} 12.87 (4-OH), and a 2-(1-hydroxy-1-methylethyl) dihydrofuran group at δ_{H} 4.76 (1H, t, $J = 8.8$ Hz, H-2'), 3.20 (1H, dd, $J = 9.6; 15.8$ Hz, H-1a'), 3.10 (1H, dd, $J = 7.8; 15.8$ Hz, H-1b'), 1.33 (3H, s, H-4') and 1.21 (3H, s, H-5'). In addition, compound **1** showed a 3-methyl-2-butenyl proton signals at δ_{H} 5.20 (1H, t, $J = 7.1$ Hz, H-2''), 3.37 (2H, d, $J = 7.2$ Hz, H-1''), 1.68 (3H, s, H-4''), 1.78 (3H, s, H-5''), a 2-methyl-1-butanoyl proton signals at δ_{H} 2.60 (1H, m, H-2'''), 1.63 (2H, m, H-3'''), 1.29 (3H, d, $J = 6.9$ Hz, H-5''') and 0.93 (3H, t, $J = 7.4$ Hz, H-4''').

The ^{13}C NMR spectrum of **1** showed 23 carbon signals and their assignments were determined by HMQC and HMBC spectra. One carbonyl carbon signal at δ_{C} 183.3 and three signals of oxyaryl carbons at δ_{C} 154.7, 163.8, 155.5, are characteristic for C-3, C-4, C-6 and C-7a of a benzofuran-3-one structure. The ^{13}C NMR spectrum of **1** showed signals of a benzofuran-3-one nucleus (δ_{C} 183.3, 163.8, 155.5, 154.7, 108.2, 107.0, 105.9, 102.1), a 3-methyl-2-butenyl chain (δ_{C} 132.1, 121.9, 25.8, 22.2, 18.0), a 2-(1-hydroxy-1-methylethyl) dihydrofuran (δ_{C} 91.3, 72.1, 27.2, 25.7, 23.9) and a 2-methyl-1-butanoyl chain (δ_{C} 173.3, 40.6, 27.7, 18.1, 11.7).

The placement of 2-(1-hydroxy-1-methylethyl) dihydrofuran, 3-methyl-2-butenyl, 2-methyl-1-butanoyl and hydroxyl groups in benzofuran-3-one skeleton was established by HMQC and HMBC spectra. Long-range correlation was observed in dihydrofuran HMBC spectrum of **1** between the proton signal of acetal group at δ_{H} 6.02 (H-2) with two carbonyl [δ_{C} 183.3 (C-3), 173.3 (C-1''')], and a quaternary carbons [δ_{C} 105.9 (C-3a)] showed ester group attached at C-2 from benzofuran-3-one structure. A chelated hydroxyl proton at δ_{H} 12.87 correlated to C-3a (δ_{C} 105.9), C-4 (δ_{C} 154.7) and C-5 (δ_{C} 108.2) showing that a hydroxyl group was placed at C-4. Furthermore, two proton signals of methylene (δ_{H} 3.20 and 3.10) has correlation with a quaternary carbon at δ_{C} 108.2 (C-5), a methine carbon at δ_{C} 91.3 (C-2') and two oxyaryl carbons [δ_{C} 154.7 (C-4), 163.8 (C-6)] which showed that 2-(1-hydroxy-1-methylethyl) dihydrofuran group fused ring at C-5 and C-6. The proton signal of methylene at δ_{H} 3.37 (H-1'') from isoprenyl (3-methyl-2-butenyl) group showed long-range correlations with two oxyaryl carbons [δ_{C} 163.8 (C-6), 155.5 (C-7a)], two quaternary carbons [δ_{C} 102.1 (C-7), 132.1 (C-3'')] and a methine carbon at δ_{C} 121.9 (C-2'') revealed that (3-methyl-2-butenyl) group attached at C-7. The presence of long-range correlations between the proton signal of a methine (δ_{H} 2.60) was correlated to a methine carbon at δ_{C} 107.0 (C-2), a methylene at δ_{C} 27.7 (C-3'''), a carbonyl (ester) at δ_{C} 173.3 (C-1''') and two methyl carbons [δ_{C} 18.1 (C-5'''), 11.7 (C-4''')] reinforces the position of 2-methyl-1-butanoyl at C-2. Therefore, compound **1** was identified as hydroxy-6-(2-hydroxypropan-2-yl)-8-(3-methylbut-2-en-1-yl)-3-oxo-2,3,5,6-tetrahydrobenzo[1,2-b:5,4-b']difuran-2-yl 2-methyl-butanoate and given the trivial name airlanggin A. The stereochemistry of the proton at C-2 in **1** was not determined.

Airlanggin B (**2**) was isolated as a white solid, $[\alpha]_{\text{D}}^{20} +5.6$ (c 0.31 MeOH), has molecular formula of $\text{C}_{23}\text{H}_{30}\text{O}_6$ deduced from the $[\text{M} + \text{H}]^+$ ion at m/z 403.2121. The UV spectrum (λ_{max} 218, 262 and 302 nm) and IR spectrum (3201, 1651, 1581 and 1502 cm^{-1}) absorptions were very similar to those of **1**.

The ^1H and ^{13}C NMR of **2** had very similar to those of **1**. However, the major difference in the ^1H and ^{13}C NMR signals of **2** showed the isoprenyl (3-methyl-2-butenyl) group was attached at C-5 and hydroxyl at C-6. The ^1H NMR spectrum of **2** showed a 3-methyl-2-butenyl proton signals at δ_{H} 5.24 (1H, t, $J = 7.2$ Hz, H-2'), 3.43 (2H, d, $J = 6.5$ Hz, H-1'), 1.83 (3H, s, H-4'), 1.76 (3H, s, H-5') at C-5 and a hydroxyl group 6-OH at δ_{H} 6.30. The placement of isoprenyl (3-methyl-2-butenyl) group was confirmed by HMBC spectrum. The presence of proton signal

of methylene from 3-methyl-2-butenyl group at δ_{H} 3.43 (H-1') has correlation with two oxyaryl carbons [δ_{C} 159.3 (C-6), 153.6 (C-7a)], two quaternary carbons [δ_{C} 109.8 (C-5), 135.6 (C-3')] and a methine carbon at δ_{C} 121.5 (C-2') showed that isoprenyl (3-methyl-2-butenyl) group attached at C-5. Furthermore, the placement of isoprenyl (3-methyl-2-butenyl) at C-5 was reinforced by the correlation between proton signal of hydroxyl group at δ_{H} 6.30 (6-OH) with a hydroxyl carbon at δ_{C} 159.3 (C-6) and two quaternary carbons [δ_{C} 109.8 (C-5), 105.5 (C-7)]. From the above spectral evidence, the structure of airlanggin B was assigned as **2**. The stereochemistry of the proton at C-2 in **2** was not determined.

Airlanggins A (**1**) and B (**2**) are the first reported naturally occurring isoprenylated benzofuran 3-one. Plausible biosynthetic pathways of **1** and **2** are proposed from acylphloroglucinol as precursor. The oxidation reaction of acylphloroglucinol to produce 2-hydroxy-benzofuran-3-one. According to this pathway, 2-hydroxy-benzofuran-3-one by selective reaction of isoprenylation, cyclization and esterification to produce **1** and **2**.

The cytotoxic activity of compounds **1–4** was evaluated for their cytotoxicity by MTT assay against murine leukaemia P-388. These compounds exhibited IC_{50} values of 5.80 ± 0.12 , 37.08 ± 0.90 , 0.68 ± 0.01 and 5.14 ± 0.23 $\mu\text{g/mL}$, respectively. The structure–activity anticancer relationship against murine leukaemia P388 between the isoprenylated benzofuran-3-one and isoprenylated xanthone compounds is discussed according to the class of compounds.

Those cytotoxic data for isoprenylated benzofuran-3-one suggested that compound **1** have moderate activity and compound **2** was inactive. The cyclization between hydroxyl (6-OH) with isoprenyl (3-methyl-2-butenyl) to be 2-(1-hydroxy-1-methylethyl) dihydrofuran group of **1** enhances activity than isoprenyl (3-methyl-2-butenyl) at C-5 and hydroxyl at C-6 of **2**. For isoprenylated xanthone, compound **3** has high activity and compound **4** showed moderate activity. Compound **3** and **4** are isomers which difference the placement of isoprenyl and pirano groups in C-2 and C-3. The placement of isoprenyl at C-2 and pirano at C-3 of **3** enhances activity.

9

3. Experimental

3.1. General

NMR spectra were measured on a JEOL JNM-ECA 400 MHz FTNMR spectrophotometer (Tokyo, Japan) in CDCl_3 with TMS as the internal standard. Mass spectra were measured on an ESI-TOF Waters LCT Premier XE producing pseudo-molecular ions, $[\text{M} + \text{H}]^+$ positive ion mode (Santa Clara, CA, USA). UV spectra were recorded in MeOH on a Shimadzu series 1800 UV-vis spectrophotometer (Kyoto, Japan). IR spectra were recorded in KBr on a One Perkin Elmer instrument (Waltham, MA, USA). Column chromatography and radial chromatography were carried out using silica gel 60 and silica gel 60 PF₂₅₄ (Merck, Darmstadt, Germany). Optical rotations were determined with a Perkin Elmer Polarimeter Model 341.

3

3.2. Plant material

The stem bark of *C. soulattri* was collected from the conserved forest of Batam Island, Riau Island, Indonesia on Dec 2015, and was identified by Mr. Ismail Rachman from the Herbarium Bogoriense, Bogor. A voucher specimen (PL 69321) was deposited in Herbarium Bogoriense,

Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia.

3.3. Extraction and isolation

The air-dried stem bark of *C. soulattri* (2.6 kg) was successively twice (each for 48 h) by maceration in methanol, and then evaporated under reduced pressure to give a dark brown residue (560 g). The extract was redissolved in MeOH-water (15:1) and partitioned with *n*-hexane (201 g) and ethyl acetate (73 g) fractions. A part of ethyl acetate fraction (70 g) was subjected to vacuum liquid chromatography over silica gel and eluted with *n*-hexane-ethyl acetate (from 9:1 to 3:7) to give fractions A-C. Fraction B (5.14 g) was then subjected to column chromatography and eluted with *n*-hexane-ethyl acetate (from 9:1 to 7:3) to produce subfractions B₁-B₂. Subfraction B₂ was purified by planar radial chromatography using *n*-hexane-chloroform (from 9:1 to 7:3) to yielded compound **2** (12 mg) and **4** (20 mg). Fraction C (6.99 g) was refractionated using column chromatography and eluted *n*-hexane-ethyl acetate (from 9:1 to 3:7) to produce subfractions C₁-C₃. Subfraction C₃ was purified by planar radial chromatography using *n*-hexane-chloroform (from 9:1 to 1:1) to yielded compound **1** (4 mg) and **3** (9 mg).

3.4. Spectral data

Airlanggin A (**1**): yellow solid, UV/Vis (MeOH) λ_{\max} (nm) (log ϵ): 218 (4.43), 261 (4.28) and 301 (4.00). IR (KBr) ν_{\max} (cm⁻¹): 3421, 2970, 2933, 2875, 1668, 1631, 1585, 1419 and 1195. ¹H NMR (400 MHz, CDCl₃) δ_{H} ppm: 6.02 (1H, s, H-2), 12.87 (1H, s, -OH), 3.20 (1H, dd, *J* = 9.6, 15.8 Hz, H-1'a), 3.10 (1H, d, *J* = 7.8, 15.8 Hz, H-1'b), 4.76 (1H, t, 8.8 Hz, H-2'), 1.33 (3H, s, H-12), 1.21 (3H, s, H-5'), 3.37 (2H, d, 7.2 Hz, H-1''), 5.20 (1H, t, 7.1 Hz, H-2''), 1.68 (3H, s, H-4''), 1.73 (3H, s, H-5''), 2.60 (1H, m, H-2'''), 1.63 (2H, m, H-3'''), 0.93 (3H, t, 7.4 Hz, H-4'''), 1.29 (3H, d, 6.9 Hz, H-5'''). ¹³C NMR (100 MHz, CDCl₃) δ_{C} ppm: 107.0 (C-20), 183.3 (C-3), 105.9 (C-3a), 154.7 (C-4), 108.2 (C-5), 163.8 (C-6), 102.1 (C-7), 155.5 (C-7a), 27.2 (C-1'), 91.3 (C-2'), 72.1 (C-3'), 25.7 (C-4'), 23.9 (C-5'), 22.2 (C-1''), 121.9 (C-2''), 132.1 (C-3''), 25.8 (C-4''), 18.0 (C-5''), 173.3 (C-1'''), 40.6 (C-2'''), 27.7 (C-3'''), 11.7 (C-4'''), 18.1 (C-5'''). HRESIMS: *m/z* [M + H]⁺ calcd. for C₂₃H₃₁O₇ 419.2070, found 419.2070.

Airlanggin B (**2**): white solid, UV/Vis (MeOH) λ_{\max} (nm) (log ϵ): 218 (4.47), 262 (4.33) and 302 (3.98). IR (KBr) ν_{\max} (cm⁻¹): 3201, 2977, 2922, 2850, 1651, 1610, 1581, 1502 and 1290. ¹H NMR (400 MHz, CDCl₃) δ_{H} ppm: 6.02 (1H, s, H-2), 13.04 (1H, s, 4-OH), 6.30 (1H, s, 6-OH), 3.43 (2H, d, 6.5 Hz, H-1'), 5.24 (1H, t, 7.2 Hz, H-2'), 1.76 (3H, s, H-4'), 1.83 (3H, s, H-5'), 1.45 (2H, d, 6.2 Hz, H-1''), 5.21 (1H, t, 6.8 Hz, H-2''), 1.72 (3H, s, H-4''), 1.82 (3H, s, H-5''), 2.60 (1H, m, H-2'''), 1.62 (2H, m, H-3'''), 0.93 (3H, t, 7.4 Hz, H-4'''), 1.28 (3H, d, 7.0 Hz, H-5'''). ¹³C NMR (100 MHz, CDCl₃) δ_{C} ppm: 106.9 (C-2), 183.3 (C-3), 105.0 (C-3a), 157.1 (C-4), 109.8 (C-5), 159.3 (C-6), 105.5 (C-7), 153.6 (C-7a), 21.9 (C-1'), 121.5 (C-2'), 135.6 (C-3'), 26.0 (C-4'), 18.0 (C-5'/5''/5'''), 21.7 (C-1''), 17.9 (C-2''), 133.7 (C-3''), 25.9 (C-4''), 173.4 (C-1'''), 40.6 (C-2'''), 27.7 (C-3'''), 11.7 (C-4'''). HRESIMS: *m/z* [M + H]⁺ calcd. for C₂₃H₃₁O₆ 403.2125, found 403.2121.

Ananixanthone (**3**): yellow solid. The ¹H and ¹³C NMR spectral data are consistent with publish data (Daud et al. 2016).

Trapezifolixanthone (**4**): yellow solid. The ¹H and ¹³C NMR spectral data are consistent with publish data (Mah et al. 2015).

3.5. Cytotoxic assay

Cytotoxic properties of the isolated compounds **1–4** against murine leukaemia P-388 cells were evaluated according to the MTT method as previously described (Tanjung et al. 2010, 2012, 2017). Artonin E was used as the positive control.

4. Conclusions

The phytochemical investigation of the stem bark of *C. soulattri* gave two new isoprenylated benzofuran 3-ones, airlangin A (**1**) and B (**2**) were first time found in natural compounds.

Supplementary material

¹H NMR, ¹³C NMR, COSY, HMQC, HMBC, HRESIMS and IR spectra are reported in the supplementary materials as Figures S1–S15 and related to the following articles are available online.

5 Disclosure statement

No potential conflict of interest was reported by the authors.

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