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Calotetrapterins A-C, three new pyranoxanthones and their cytotoxicity from the stem bark of *Calophyllum tetrapterum* Miq

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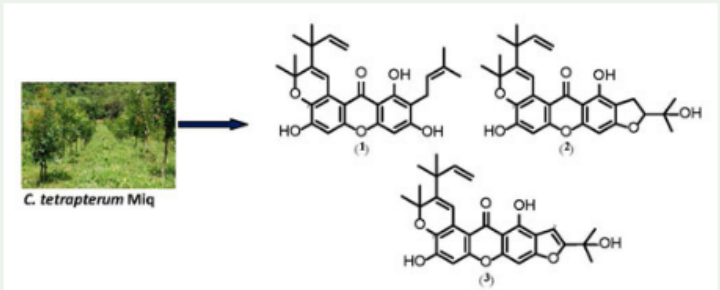
ABSTRACT

Three new pyranoxanthones, calotetrapterins A-C (**1**-**3**) were isolated from the stem bark of *Calophyllum tetrapterum* Miq along with three known xanthones, α-mangostin (**4**), garciniafuran (**5**), and pyranojacareubin (**6**). All structures were elucidated based on their IR, UV, HRESIMS, 1D (^{1}H, ^{13}C) and 2D (HMBC, HMQC) NMR spectral data. Compounds **1**-**6** were tested to P-388 cells for cytotoxic activity, compound 2 exhibited high activity with IC_{50} value 1.0 μM.



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Calotetrapterins A-C; pyranoxanthone; Calophyllum tetrapterum; P-388 cell

1. Introduction

The genus *Calophyllum* (Calophyllaceae) comprises about 198 species found mainly in the restrictive area of Southeast Asia. *Calophyllum* plants are source of phenolic compounds especially xanthones (Ferchichi et al. 2012; Daud et al. 2016), benzofurans (Tanjung et al. 2018) and 4-phenylcoumarins (Zhong et al. 2010) containing isoprenyl as side chain. Isoprenylation of phenolic compounds displays as a major chromophore to increase their cytotoxicity activities against various human cancer cells (Mah et al.

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2015). *Calophyllum tetrapterum* Miq. is one species plant found originated in East Kalimantan, Indonesia. Based on our knowledge, no pyranoxanthones from *C.tetrapterum* has been reported. As part of the phytochemical investigation on *Calophyllum* in Indonesia, six pyranoxanthones including three new pyranoxanthones (**1–3**) were isolated from the stem bark of *C. tetrapterum*. The cytotoxic activity of pyranoxanthones against murine leukemia P-388 is also reported.

2. Result and discussion

Calotetrapterin A (**1**) showed absorption bands at λ_{max} 247, 264, 322 nm consimilar with a xanthone chromophore (Tanjung et al. 2018). The HRESIMS spectrum displayed negative ion peak $[M\text{-}H]^-$ at *m/z* 461.1971 appropriate with a molecular formula of $C_{28}H_{30}O_6$. The IR spectrum of **1** showed strong absorption for hydroxyl (3423 cm^{-1}), conjugated carbonyl (1622 cm^{-1}), and aromatic (1577 and 1460 cm^{-1}) groups. The ^1H NMR spectrum of **1** demonstrated two aromatic signals at δ_H 6.40 (H-4) and 6.77 (H-5) recommended for a 1,2,3,6,7,8-hexasubstituted xanthone (Azebaze et al. 2004). Additionally, the ^1H NMR spectrum of **1** also showed the signals of hydroxyl group, 3-methyl-2-butenyl (isoprenyl), 3-methyl-1-butenyl, and a monosubstituted 2,2-dimethylpyrano ring confirmed by HMBC spectrum. A signal at δ_H 13.77 is the signal a hydroxyl group at C-1 of xanthone structure. The presence of 3-methyl-2-butenyl side chain signals showed a methylene signal at δ_H 3.34 (2H, d, $J = 7.3$ Hz, H-1′), a vinylic at δ_H 5.27 (1H, tm, $J = 7.3$ Hz, H-2′), and two methyls at δ_H 1.63 (3H, s, H-4′), and 1.77 (3H, s, H-5′). The signal of a monosubstituted 2,2-dimethylpyrano ring demonstrated a vinylic at δ_H 8.19 (1H, s, H-4′), and a gem dimethyl at 1.49 (6H, s, H-5′/H-6′). A downfield signal at δ_H 8.19 indicating for a vinylic from influenced by anisotropic factor from carbonyl group (Azebaze et al. 2004). Furthermore, the signal of 3-methyl-1-butenyl side chain showed a vinylic at δ_H 6.02 (1H, dd, $J = 10.6$; 17.5 Hz, H-8′), a methylene terminal [δ_H 5.16 (1H, dd, $J = 1.1$; 17.5 Hz, H-9′a) and δ_H 5.08 (1H, dd, $J = 1.1$; 10.6 Hz, H-9′b)], and a gem dimethyl at δ_H 1.41 (6H, s, H-10′/H-11′). The ^{13}C NMR spectrum (APT experiment) of **1** demonstrated the existence of six methyl carbons, two methylene carbons, five methine carbons and 15 quaternary carbons (including one carbonyl carbon and six oxyaryl carbons). The location of hydroxyl, 3-methyl-2-butenyl side chain, 3-methyl-1-butenyl side chain, and a monosubstituted 2,2-dimethylpyrano ring was confirmed with HMQC and HMBC spectra. The signal of a chelated hydroxyl at δ_H 13.77 (1-OH) showed correlation to C-1 (δ_C 161.5), C-2 (δ_C 110.9), C-9a (δ_C 103.8) and methylene signal of 3-methyl-2-butenyl side chain at H-1′ (δ_H 3.34) correlated to C-2 (δ_C 110.9), C-3 (δ_C 162.9), C-2′ (δ_C 123.4), C-3′ (δ_C 131.4) showing that isoprenyl side chain located at C-2. The signal aromatic at δ_H 6.40 (H-4) showed correlation with to C-2 (δ_C 110.9), C-3 (δ_C 162.9), C-4a (δ_C 155.9), and C-9a (δ_C 103.8) supported that an isoprenyl at C-2. Furthermore, the signal at δ_H 6.77 (H-5) correlated to three oxyarils [C-6 (δ_C 154.1), C-7 (δ_C 137.6)], C-10a (δ_C 153.3), and a quaternary carbon at C-8a (δ_C 108.4). Consequently, a monosubstituted 2,2-dimethylpyrano ring attached to aromatic at C-7 and C-8. The location of a monosubstituted 2,2-dimethylpyrano ring attached to C-7 and C-8 was supported by the long-range correlations of the proton signal at δ_H 8.19 (H-4′) to C-7 (δ_C 137.6), C-8a (δ_C 108.4), C-2′ (δ_C 80.3), C-7′ (δ_C 42.7) unequivocally located the

3-methyl-1-butenyl side chain at C-3′. The existence of long-range correlations of a gem dimethyl at δ_H 1.41 (H-10′/H-11′) to C-3′ (δ_C 149.7), C-7′ (δ_C 42.7), C-8′ (δ_C 147.9), C-11′/C-10′ (δ_C 42.7) and the signal of a methylene terminal at δ_H 5.16, and δ_H 5.08 (H-9′) correlated to C-7′ (δ_C 42.7), C-8′ (δ_C 147.9) obviously placed the 3-methyl-1-butenyl side chain at C-3′. Based on the above-mentioned spectral orientation, the structure of calotetrapterin A was established as **1**.

Calotetrapterin B (**2**) was obtainted also as a yellow solid, showed UV (λ_{max} 245, 268, 320), and IR (3330, 1616, 1571, 1463 cm^{-1}) absorptions very resemblant with **1**. Its molecular formula was established as $C_{28}H_{30}O_7$ showed $[M+H]^+$ ion at *m/z* 479.2076 by the HRESIMS. The NMR spectrum (^1H and ^{13}C) of **2** had very consimilar with **1**. The major difference, the ^1H and ^{13}C NMR of **2** showed a 2-(1-hydroxy-1-methylethyl)dihydrofuran ring attached to C-2 and C-3. The location of a 2-(1-hydroxy-1-methylethyl)dihydrofuran ring was assigned by HMBC and HMQC spectrum. The long-range correlations a chelated hydroxyl group at δ_H 13.67 (1-OH) to C-1 (δ_C 158.7), C-2 (δ_C 108.8), C-9a (δ_C 103.8), and the signal of a methylene of dihydrofuran ring at δ_H 3.15 (H-3) correlated to C-2 (δ_C 108.8), C-4′ (δ_C 71.4) showed that a 2-(1-hydroxy-1-methylethyl)dihydrofuran ring attached to C-2 and C-3. The signal of oxymethine at δ_H 4.82 (H-2) correlated to C-5′ (δ_C 25.9),C-6′ (δ_C 25.6) supporting that the location of a 2-(1-hydroxy-1-methylethyl)dihydrofuran fused at C-2 and C-3. Other HMBC correlations of **2** consistent with structure of calotetrapterin B.

Calotetrapterin C (**3**) was obtained also as a yellow solid. Its molecular formula was established as $C_{28}H_{28}O_7$ with HRESIMS spectra by means of ion peak $[M+H]^+$ at *m/z* 477.1911. The NMR (^1H and ^{13}C) spectra data of **3** were identically to those **2**. The main difference, in the NMR (^1H, ^{13}C) of **3** displayed a 2-(1-hydroxy-1-methylethyl)furan ring and dertemined based on HMBC and HMQC measurement. The HMBC long-range correlations of a chelated hydroxyl at 1-OH (δ_H 14.25) exhibited that cross peaks with C-1 (δ_C 156.8), C-2 (δ_C 113.7), and C-9a (δ_C 105.6). The signal of a vinylic of furan ring at H-3′ (δ_H 6.86) correlated to C-2 (δ_C 113.7), C-3 (δ_C 160.0), C-2′ (δ_C 165.6) and a gem dimethyl at H-5′/H-6′ (δ_H 1.62) showing correlations with C-2′ (δ_C 165.6), C-4′ (δ_C 67.9). The long-range correlations of δ_H 6.86 and δ_H 1.62 with carbon signals were supported the location of a 2-(1-hydroxy-1-methylethyl)furan ring fused at C-2 and C-3 on xanthone skeleton. Based on the above NMR data, structure **3** was established as calotetrapterin C.

Three known xanthones, α-mangostin (**4**), garciniafuran (**5**), pyranojacareubin (**6**) by 1D (^1H, ^{13}C) and 2D (HMQC, HMBC) NMR, HRESIMS data very resemblant with published data (Chae et al. 2012, Shiozaki et al. 2013).

The cytotoxic activity of compounds (**1**-**6**) were evaluated for their cytotoxicity using cell viability in murine leukemia P-388 with MTT method. These compounds displayed IC_{50} values of 5.4 ± 0.6, 1.0 ± 0.2, 4.1 ± 0.4, 212.0 ± 1.1, 93.5 ± 1.3, and 71.2 ± 1.2 μM, respectively. Those cytotoxic data suggested that all of new compounds (**1**-**3**) showed high activity and known compounds (**4**-**6**) were inactive. Influence of a pyrano ring fused at C-7 and C-8 along with a 3-methyl-1-butenyl side chain attached at C-3′′ suggested as a key factor to enhance cytotoxic effect (Ito et al. 2002). Hence, the lipophilicity of a 3-methyl-1-butenyl side chain on pyrano ring contributes to damage the cell membranes of P-388 cells. The main difference between the three new compounds (**1**-**3**) be located in the substituent at C-2 and C-3. The existence of a

dihydrofuran ring of compound **2** tend to be more active than a furan ring of compound **3** fused at C-2 and C-3. However, influence of a furan ring of compound **3** slighly more active than the existence of the isoprenyl side chain at C-2 and hydroxyl group at C-3 of compound **1**.

3. Experimental

3.1. Plant material

The fresh stem barks of *C. tetrapterum* were collected from Mendawak River, East Kalimantan, Indonesia in Apr 2016. The plant was authenticated by Mr. Ismail Rachman, botanist from the Herbarium Bogoriense, LIPI, Bogor. A specimen (CT 65798) was deposited as a reference.

3.2. Extraction and isolation

The dried stem barks of *C. tetrapterum* (1.8 kg) was extracted successively at room temperature with MeOH over a period of two days, and then evaporation of the solvent under reduced pressure gave a dark brown residue (125 g). The extract was redissolved in MeOH-H_2O (9:1) and partitioned with *n*-hexane (32 g) and EtOAc (26 g) fractions. A part of EtOAc fraction (25 g) was subjected to VLC chromatography over silica gel and eluted with *n*-hexane-EtOAc (from 9:1 to 1:1) to give three fractions A-C. TLC analysis of fraction A (2.5 g) showed no phenolic compounds with UV light, therefore analysis was not continued. Fraction B (3.6 g) was fractionated with CC chromatography, eluted with *n*-hexane-EtOAc (from 19:1 to 7:3) gave two subfractions B_1-B_2. Subfraction B_1 (325 mg) was purified by planar radial chromatography using *n*-hexane-$CHCl_3$ (from 4:1 to 1:1) to yielded compound **5** (10 mg) and compound **6** (15 mg). Subfraction B_2 (410 mg) was purified by planar radial chromatography using *n*-hexane-acetone (from 19:1 to 4:1) to obtain compound **2** (13 mg) and compound **3** (18 mg). Fraction C (4.5 g) was separated by CC chromatography and eluted with *n*-hexane-EtOAc (from 4:1 to 1:1) to produce three subfractions C_1-C_3. Subfraction C_3 was purified by planar radial chromatography using *n*-hexane-EtOAc (from 9:1 to 3:7) to yielded compound **1** (8 mg) and compound **4** (9 mg).

3.3. Spectral data

Calotetrapterin A (**1**): yellow solid, UV/Vis (MeOH) λ_{max} (nm) (log ε): 247 (4.64), 264 (4.60), and 322 (4.38). IR (KBr) ν (cm^{-1}): 3423, 2972, 2923, 2852, 1622, 1577, 1460 and 1188. ^{1}H-NMR (400 MHz, acetone-d_6) δ_H ppm: 13.77 (1H, *s*, 1-OH), 6.40 (1H, *s*, H-4), 6.77 (1H, *s*, H-5), 3.34 (2H, *d*, $J = 7.3$ Hz, H-1), 5.27 (1H, *tm*, $J = 7.3$ Hz, H-2), 1.63 (3H, *s*, H-4), 1.77 (3H, *s*, H-5), 8.19 (1H, *s*, H-4), 1.49 (6H, *s*, H-5′/H-6′), 6.02 (1H, *dd*, $J = 10.6$; 17.5 Hz, H-8′), 5.16 (1H, *dd*, $J = 1.1$; 17.5 Hz, H-9á), 5.08 (1H, *dd*, $J = 1.1$; 10.6 Hz, H-9b), 1.41 (6H, *s*, H-10′/H-11′). ^{13}C-NMR (100 MHz, acetone-d_6), δ_C ppm: 161.5 (C-1), 110.9 (C-2), 162.9 (C-3), 93.2 (C-4), 155.9 (C-4a), 102.9 (C-5), 154.1 (C-6), 137.6 (C-7), 122.8 (C-8), 108.4 (C-8a), 183.1 (C-9), 103.8 (C-9a), 153.3 (C-10a), 21.9 (C-1), 123.4 (C-2), 131.4 (C-3), 25.9 (C-4), 17.8 (C-5), 80.3 (C-2), 149.7 (C-3), 118.8 (C-4), 27.3 (C-5′/C-6′), 42.7 (C-7), 147.9 (C-8),

112.2 (C-9′), 28.6 (C-10′/C-11′). HRESIMS: *m/z* $[M-H]^-$ calcd. for $C_{28}H_{30}O_6$ 461.1964, found 461.1971.

Calotetrapterin B (**2**): yellow solid, UV/Vis (MeOH) λ_{max} (nm) (log ε): 245 (4.62), 268 (4.59), and 320 (4.36). IR (KBr) ν (cm^{-1}): 3330, 2958, 2952, 2856, 1616, 1571, 1463 and 1172. ^{1}H-NMR (400 MHz, acetone-d_6) δ_H ppm: 13.67 (1H, *s*, 1-OH), 6.29 (1H, *s*, H-4), 6.80 (1H, *s*, H-5), 4.82 (1H, *d*, *J* = 7.9; 9.4 Hz, H-2), 3.15 (2H, *t*, *J* = 8.2 Hz, H-3), 1.24 (3H, *s*, H-5), 1.29 (3H, *s*, H-6), 8.18 (1H, *s*, H-4), 1.50 (6H, *s*, H-5′/H-6′), 6.03 (1H, *dd*, *J* = 10.6; 17.5 Hz, H-8′), 5.16 (1H, *dd*, *J* = 1.1; 17.5 Hz, H-9á), 5.08 (1H, *dd*, *J* = 1.1; 10.6 Hz, H-9b′), 1.41 (6H, *s*, H-10′/H-11′). ^{13}C-NMR (100 MHz, acetone-d_6), δ_C ppm: 158.7 (C-1), 108.8 (C-2), 167.8 (C-3), 88.7 (C-4), 158.1 (C-4a), 102.9 (C-5), 153.4 (C-6), 137.8 (C-7), 122.7 (C-8), 108.3 (C-8a), 183.3 (C-9), 103.8 (C-9a), 154.6 (C-10a), 92.8 (C-2), 27.0 (C-3′), 71.4 (C-4′), 25.9 (C-5′), 25.6 (C-6′), 80.4 (C-2′), 149.8 (C-3′), 118.7 (C-4′), 27.3 (C-5′/C-6′), 42.7 (C-7′), 147.8 (C-8′), 112.3 (C-9′), 28.7 (C-10′/C-11′). HRESIMS: *m/z* $[M+H]^+$ calcd. for $C_{28}H_{30}O_7$ 479.2070, found 479.2076.

Calotetrapterin C (**3**): yellow solid, IR (KBr) ν (cm^{-1}): 3450, 2958, 2927, 2858, 1620, 1573, 1461 and 1172. ^{1}H-NMR (400 MHz, acetone-d_6) δ_H ppm: 14.25 (1H, *s*, 1-OH), 7.05 (1H, *s*, H-4), 6.78 (1H, *s*, H-5), 6.86 (1H, *s*, H-3′), 1.62 (6H, *s*, H-5′/H-6′), 8.19 (1H, *s*, H-4′), 1.52 (6H, *s*, H-5′/H-6′), 6.04 (1H, *dd*, *J* = 10.6; 17.6 Hz, H-8′), 5.18 (1H, *dd*, *J* = 1.1; 17.6 Hz, H-9á), 5.10 (1H, *dd*, *J* = 1.1; 10.6 Hz, H-9b′), 1.43 (6H, *s*, H-10′/H-11′). ^{13}C-NMR (100 MHz, acetone-d_6), δ_C ppm: 156.8 (C-1), 113.7 (C-2), 160.0 (C-3), 90.2 (C-4), 154.1 (C-4a), 102.9 (C-5), 152.8 (C-6), 137.8 (C-7), 122.0 (C-8), 108.1 (C-8a), 182.2 (C-9), 105.6 (C-9a), 154.9 (C-10a), 165.6 (C-2), 98.1 (C-3′), 67.9 (C-4′), 29.2 (C-5′/C-6′), 80.4 (C-2′), 149.8 (C-3′), 118.7 (C-4′), 27.3 (C-5′/C-6′), 42.5 (C-7′), 147.9 (C-8′), 112.3 (C-9′), 28.7 (C-10′/C-11′). HRESIMS: *m/z* $[M+H]^+$ calcd. for $C_{28}H_{28}O_7$ 477.1913, found 477.1911.

3.4. Cytotoxic assay

All of compounds (**1**-**6**) were assayed cytotoxic activity against murine leukemia P-388 cell in accordance with the MTT colorimetric method as erenow described (Tanjung et al. 2018; Saputri et al. 2018).

4. Conclusions

In summary, three new pyranoxanthones, calotetrapterins A-C (**1**-**3**) were isolated from the stem bark of *C. tetrapterum* Miq together with three known xanthones, α-mangostin (**4**), garciniafuran (**5**) and pyranojacareubin (**6**). Compound **2** showed high activity against murine leukemia P-388.

Disclosure statement

The authors proclaim no potential conflict of interest.

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