Taylor & Francis
Taylor & Francis Group

Natural Product Research

Formerly Natural Product Letters

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

Two new pyranoxanthones from the stem bark of *Calophyllum pseudomolle* P.F. Stevens

Mulyadi Tanjung , Tjitjik Srie Tjahjandarie , Ratih Dewi Saputri , Muhammad Fajar Aldin & Hery Purnobasuki

To cite this article: Mulyadi Tanjung , Tjitjik Srie Tjahjandarie , Ratih Dewi Saputri , Muhammad Fajar Aldin & Hery Purnobasuki (2020): Two new pyranoxanthones from the stem bark of *Calophyllum pseudomolle* P.F. Stevens, Natural Product Research

To link to this article: https://doi.org/10.1080/14786419.2020.1808638

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1808638

Taylor & Francis
Taylor & Francis Group

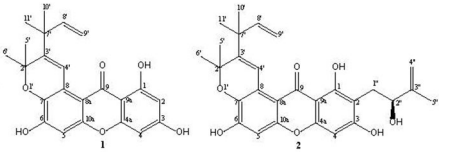
Two new pyranoxanthones from the stem bark of *Calophyllum pseudomolle* P.F. Stevens

Mulyadi Tanjung[a], Tjitjik Srie Tjahjandarie[a], Ratih Dewi Saputri[a], Muhammad Fajar Aldin[a] and Hery Purnobasuki[b]

[a]Natural Products Chemistry Research Group, Organic Chemistry Division, Faculty of Science and Technology, Department of Chemistry, Universitas Airlangga, Surabaya, Indonesia; [b]Faculty of Science and Technology, Department of Biology, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Two new pyranoxanthones, calotetrapterins D (**1**) and E (**2**), were isolated from the stem bark of *Calophyllum pseudomolle* P.F. Stevens along with α-mangostin (**3**). The structures of compounds **1**–**2** were determined based on 1D NMR (^{1}H, ^{13}C) and 2D NMR (HMQC, HMBC), as well as HRESIMS spectroscopic analysis. Compounds **1**–**2** showed moderate activity against HeLa and murine leukaemia P-388 cells.



ARTICLE HISTORY
Received 10 April 2020
Accepted 1 August 2020

KEYWORDS
Calotetrapterins D and E; pyranoxanthones; *Calophyllum pseudomolle*; cytotoxicity

1. Introduction

Calophyllum is one of the largest genus belonging to the Calophyllaceae family (Stevens 1980; Daud et al. 2014; Aminudin et al. 2016) and found mainly in the forest regions of Southeast Asia. The genus produces xanthones (Ito et al. 2002; Tjahjandarie et al. 2019), benzofurans (Tanjung et al. 2018) and chromanone acids (Lim et al. 2015). The phenolic compounds generally have isoprenyl side chains in the aromatic core. The pyranoxanthones were reported to possess anti-corona virus (Shen et al. 2005), anti-proliferative (Ito et al. 2002; Mah et al. 2015) and antimalarial (Hay et al. 2004) properties. *Calophyllum pseudomolle* P.F. Stevens, locally known as Bintangor Kuning, is one endemic plant species in Kalimantan, Indonesia. The morphology of *C. pseudomolle* is similar to that of *C. molle*. The differences lie in the feather on the underside of the leaves and the smaller fruit size, as described by the botanist P.F. Steven (Stevens 1982).

CONTACT Mulyadi Tanjung mulyadi-t@fst.unair.ac.id

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1808638.

The specimen of *C. pseudomolle* was deposited at Herbarium Bogoriense, Bogor, Indonesia. Two new pyranoxanthones, calotetrapterins D (**1**) and E (**2**), were isolated from the stem bark of *C. pseudomolle*. Compounds **1–2** to possess the same pyranoxantone skeleton with those of calotetrapterins A–C (Tanjung et al. 2019). Cytotoxic activity against two cancer cell lines (HeLa and P-388) of **1–3** was also described.

2. Result and discussion

Calotetrapterin D (**1**) was obtained as a yellow solid, $[\alpha]_D^{20}$ + 0.9 (*c* 0.10 MeOH), m.p. 115–116 °C and HRESIMS measurement represented a pseudo-molecular ion peak [M – H]$^-$ at *m/z* 393.1352, corresponding with the molecular formula was $C_{23}H_{22}O_6$. The UV spectra exhibited four maximum absorptions at λ_{max} 249, 260, 335 and 362 nm, corresponding to a xanthone skeleton (Iinuma et al. 1995; Tanjung et al. 2019). The IR spectrum showed absorption bands for a hydroxy group at 3426 cm^{-1}, a conjugated carbonyl at 1625 cm^{-1} and aromatic ring at 1577 cm^{-1}. The ^{1}H NMR of **1** demonstrated the presence of resonances for two *meta*-couple protons (J = 2.2 Hz) at δ_H 6.21 (H-2), and 6.34 (H-4), a singlet of an isolated aromatic proton at δ_H 6.81 (H-5), and a chelated hydroxy proton at δ_H 13.49 (1-OH). Additionally, the ^{1}H NMR also showed signals due to a side chain of a 2,2-dimethyl-3-(1,1-dimethyl-2-propenyl)pyran ring [δ_H 8.18 (H-4′), 6.03 (dd, J = 10.6; 17.6 Hz; H-8′), δ_H 5.14 (dd, J = 1.1; 17.6 Hz; H-9a′), 5.09 (dd, J = 1.1; 10.6 Hz; H-9b′), 1.51 (H_3-5′/H_3-6′) and 1.42 (H_3-10′/H_3-11′)]. The ^{13}C NMR spectrum (APT experiment, Supporting Information Table S1) of **1** demonstrated the presence of 21 carbon signals accounting for 23 carbon atoms. Furthermore, the HMBC spectrum of **1** showed correlations from 1-OH (δ_H 13.49) to C-1 (δ_C 164.8), C-2 (δ_C 98.8) and C-9a (δ_C 104.1). On the other hand, the *meta*-couple proton at δ_H 6.21 (H-2) correlated to C-1, C-4 (δ_C 94.0) and C-9a while it is the *meta*-couple proton at δ_H 6.34 (H-4) correlated to C-2, C-3 (δ_C 165.5), C-4a (δ_C 158.2) and C-9a, indicating that C-3 carried a hydroxy group. Besides, the isolated aromatic proton at δ_H 6.81 (H-5) showed cross-peaks with C-6 (δ_C 153.7), C-7 (δ_C 137.9), C-8a (δ_C 108.3) and C-10a (δ_C 155.7), which disclosed that C-6 was hydroxylated and the 2,2-dimethyl-3-(1,1-dimethyl-2-propenyl)pyran ring fused to the xanthone B ring at C-7 and C-8 with C-7 being oxygenated. This was confirmed by the increased downfield shift of the olefinic proton at δ_H 8.18 (H-4′), indicating that C-4′ was bonded to C-8. HMBC correlations of H-4′ to C-7 and C-8a supported the sub-structure. Based on the spectroscopic analyses, the structure of calotetrapterin D was established as **1**.

Calotetrapterin E (**2**) was obtained as a yellow solid, $[\alpha]_D^{20}$ − 2.3 (*c* 0.10 MeOH), m.p. 191–193 °C and the HRESIMS spectrum exhibited a pseudo-molecular ion peak [M – H]$^-$ at *m/z* 477.1911, corresponding with the molecular formula of $C_{28}H_{30}O_7$. The UV spectrum (λ_{max} 248, 265, 333 and 363 nm) and IR spectrum (3423, 1645, 1577 cm^{-1}) very similar to those of **1**. The chemical shift of **2** in the 1D NMR spectra (^{1}H, ^{13}C) had similarities with **1**, especially in the aromatic region, and the 2,2-dimethyl-3-(1,1-dimethyl-2-propenyl)pyran ring. The major difference in the ^{1}H NMR spectrum was that an aromatic signal in **1** was replaced by a 2-hydroxy-3-methyl-3-butenyl side chain. Consequently, the ^{1}H NMR spectrum of compound **2** showed signals due to a singlet at δ_H 6.39 (H-4) in the aromatic region and a 2-hydroxy-3-methyl-3-butenyl

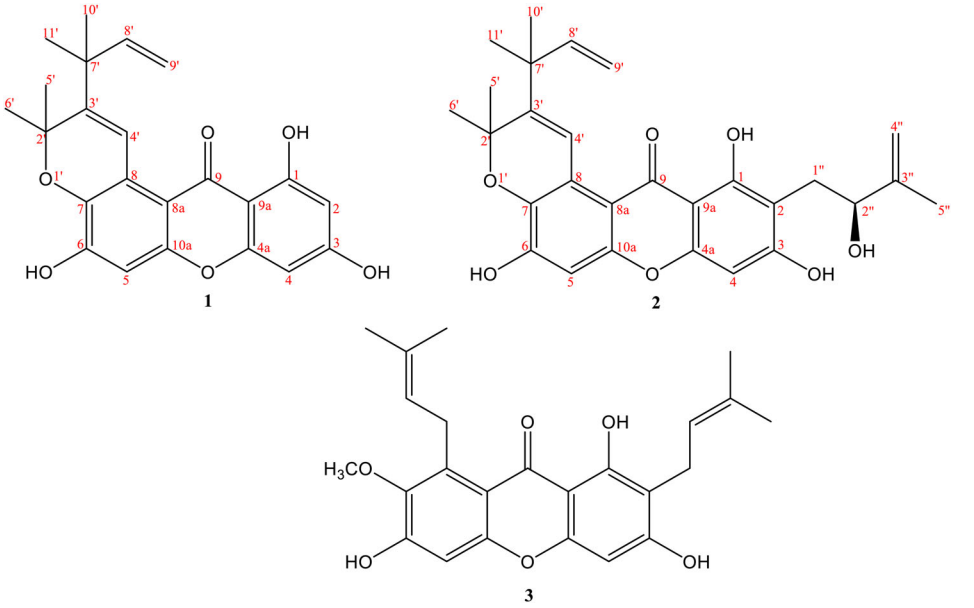


Figure 1. Chemical structures of **1–3**.

group consisting of a methylene benzylic group [δ_H 3.16 (dd, $J = 2.0$; 15.2 Hz; H-1a″), 2.93 (dd, $J = 7.8$; 15.2 Hz; H-1b″)], an oxy-methine group [δ_H 4.43 (d, $J = 7.3$ Hz; H-2″)], an exomethylene group [δ_H 4.97 (H-4a″) and 4.86 (H-4b″)] and an allylic methyl group [δ_H 1.87 (H-5″]. The assignment of the C_5 unit at C-2 was confirmed by HMQC and HMBC spectra. The HMBC spectrum of **2** showed correlations of 1-OH (δ_H 13.82) to C-1 (δ_C 161.2), C-2 (δ_C 107.5) and C-9a (δ_C 108.6) while the benzylic methylene protons (H-1″) of the C_5 side chain at δ_H 3.16, and 2.93 correlated to C-1, C-2, C-3 (δ_C 163.3), C-2″ (δ_C 77.7) and C-3″ (δ_C 146.8), indicating the C_5 group was attached to C-2. From the spectroscopic analyses, the structure of calotetrapterin E was established as **2**. The absolute configuration at C-2″ was determined as *S* using the Mosher's esterification method based on the values of $\Delta\delta$ ($\delta_S - \delta_R$) from the chemical shift of ^{1}H NMR (Lee et al. 2007).

The NMR spectra of α-mangostin (**3**) showed chemical shifts that were almost identical to the previously reported values (Tanjung et al. 2019). The structures of compounds **1–3** are shown in Figure 1.

All of the compounds (**1–3**) were *in vitro* assessed for their cytotoxic activity toward two cancer cell lines (HeLa and P-388) employing the MTT method (Saputri et al. 2018, 2019). Compounds **1**–**2** showed moderate activity against HeLa and P-388 cells, and compound **3** was inactive (Supporting Information Table S1). Compound **2** was slightly more cytotoxic than compound **1** against HeLa and P-388 cells. The presence of a 2-hydroxy-3-methyl-3-butenyl side chain at C-2 of **2** increases anticancer activity.

3. Experimental

3.1. Plant material

The stem bark of *C. pseudomolle* P.F. Stevens was collected in Nov 2017 in Sungai Mendawak, Anak Sungai Kapuas, District Kubu Raya, West Kalimantan, Indonesia

and identified by Mr. I. Rachman, a senior botanist from Herbarium Bogoriense The voucher specimen (CP 20171104) was deposited at Herbarium Bogoriense, Bogor, Indonesia.

3.2. Extraction and isolation

The air-dried stem bark of *C. pseudomolle* (1.5 kg) was extracted with MeOH using maceration for two days at room temperature. Evaporation of the solvent with an evaporator gave a MeOH extract (200 g). The MeOH extract was suspended in H_2O (9:1 v/v) and then partitioned with *n*-hexane and then EtOAc (three times) to give *n*-hexane extract (15 g), and EtOAc extract (3.1 g). The EtOAc extract was subjected to VLC using a gradient elution of *n*-hexane-EtOAc (from 9:1 to 3:7) to afford three fractions (A–C). Fraction B purified using centrifugal planar chromatography using *n*-hexane-$CHCl_3$ (from 7:3 to 3:7), $CHCl_3$, $CHCl_3$-EtOAc (9:1) to give calotetrapterin D (**1**, 9 mg). Fraction C was purified by the same methods [eluents *n*-hexane-acetone (from 19:1 to 4:1)] to afford compound calotetrapterin E (**2**, 7 mg) and α-mangostin (**3**, 20 mg).

3.3. Spectral data

Calotetrapterin D (**1**): yellow solid, m.p. 115–116 °C, UV/Vis (MeOH) λ_{max} (log ε) (nm): 249 (4.40), 260 (4.48), 335 (4.38) and 362 (3.76). IR (KBr) ν (cm^{-1}): 3426, 2970, 1625, 1577, 1560, 1460, 1164. ^{1}H-NMR (400 MHz, acetone-d_6) δ_H ppm: 13.49 (1H, *s,* 1-OH), 6.21 (1H, *d*, $J = 2.2$ Hz, H-2), 6.34 (1H, *d*, $J = 2.2$ Hz, H-4), 6.81 (1H, *s,* H-5), 8.18 (1H, *s,* H-4′), 1.51 (6H, *s,* H-5′/H-6′), 6.03 (1H, *dd*, $J = 10.6$; 17.6 Hz, H-8′), 5.14 (1H, *dd*, $J = 1.1$; 17.6 Hz, H-9a′), 5.09 (1H, *dd*, $J = 1.1$; 10.6 Hz, H-9b′) and 1.42 (6H, *s,* H-10′.H-11′). ^{13}C-NMR (100 MHz, acetone-d_6), δ_C ppm: 164.8 (C-1), 98.8 (C-2), 165.5 (C-3), 94.0 (C-4), 158.2 (C-4a), 103.1 (C-5), 153.7 (C-6), 137.9 (C-7), 122.9 (C-8), 108.3 (C-8a), 183.2 (C-9), 104.0 (C-9a), 155.7 (C-10a), 80.5 (C-2′), 150.0 (C-3′), 118.8 (C-4′), 27.4 (C-5′/C-6′), 42.8 (C-7′), 148.0 (C-8′), 112.4 (C-9′) and 28.7 (C-10′/C-11′). HRESIMS: *m/z* $[M + H]^+$ calcd for $C_{23}H_{21}O_6$ 393.1338, found 393.1352.

Calotetrapterin E (**2**): yellow solid, m.p. 191–193 °C, UV/Vis (MeOH) λ_{max} (log ε) (nm): 248 (4.41), 265 (4.49), 333 (4.39) and 363 (3.79). IR (KBr) ν (cm^{-1}): 3423, 2970, 1645, 1577, 1460, 1188. ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H ppm: 13.82 (1H, *s,* 1-OH), 9.15 (1H, *s,* 4-OH), 6.14 (1H, *s,* 6-OH), 6.39 (1H, *s*, H-4), 6.81 (1H, *s,* H-5), 8.13 (1H, *s,* H-4′), 1.54 (3H, *s,* H-5′), 1.52 (3H, *s,* H-6′), 5.98 (1H, *dd*, $J = 10.5$; 17.6 Hz, H-8′), 5.13 (1H, *dd*, $J = 1.1$; 17.6 Hz, H-9a′), 5.08 (1H, *dd*, $J = 1.1$; 10.5 Hz, H-9b′), 1.43 (6H, *s,* H-10′.H-11′), 3.16 (1H, *dd*, $J = 2.0$; 15.2 Hz, H-1a″), 2.93 (1H, *dd*, $J = 7.8$; 15.2 Hz, H-1b″), 4.43 (1H, *d*, $J = 7.3$ Hz, H-2″), 4.97 (1H, *s*, H-4a″), 4.86 (1H, *s*, H-4b″) and 1.87 (3H, *s*, H-5″). ^{13}C-NMR (100 MHz, $CDCl_3$), δ_C ppm: 161.2 (C-1), 107.5 (C-2), 163.3 (C-3), 94.6 (C-4), 155.9 (C-4a), 101.9 (C-5), 150.7 (C-6), 135.8 (C-7), 121.8 (C-8), 108.6 (C-8a), 182.6 (C-9), 103.5 (C-9a), 153.4 (C-10a), 80.7 (C-2′), 148.9 (C-3′), 118.1 (C-4′), 27.5 (C-5′/C-6′), 42.2 (C-7′), 146.9 (C-8′), 112.1 (C-9′), 28.4 (C-10′), 28.3 (C-11′), 28.1 (C-1″), 77.7 (C-2″), 146.8 (C-3″), 110.3 (C-4″) and 18.8 (C-5″). HRESIMS: *m/z* $[M - H]^-$ calcd for $C_{28}H_{29}O_7$ 477.1913, found 477.1911.

3.4. Cytotoxic assay

Cytotoxicity of compounds (**1**–**3**) against P-388 (murine leukaemia cells), and HeLa (human cervical cancer cells), were appraised using the MTT methods (Ito et al. 2002; Saputri et al. 2018). Artonin E was used as the positive control.

3.5. Mosher's esterification methods

R and *S*-MTPA ester of compound **2** were prepared using the Mosher's esterification methods previously reported (Lee et al. 2007).

4. Conclusions

Calotetrapterins D (**1**) and E (**2**), two new pyranoxanthone derivatives, were isolated from the stem bark of *C. pseudomolle* and showed moderate cytotoxicity against HeLa and P-388 cells.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The research is supported by the Research Grant of Penelitian Hibah Mandat, Universitas Airlangga, Indonesia.

References

Aminudin NI, Ahmad F, Taher M, Zulkifli RM. 2016. Incrassamarins A-D: four new 4-substituted from *Calophyllum incrassatum* and their biological activities. Phytochem Lett. 16:287–293.

Daud SB, Ee GCL, Malek EA, Teh SS, See I. 2014. A new coumarin from *Calophyllum hosei*. Nat Prod Res. 28(19):1534–1538.

Hay A-E, Helesbeux J-J, Duval O, Labaied M, Grellier P, Richomme P. 2004. Antimalarial xanthones from *Calophyllum caledonicum* and *Garcinia vieillardii*. Life Sci. 75(25):3077–3085.

Iinuma M, Tosa H, Tanaka T, Yonemori S. 1995. Two xanthones from roots of *Calophyllum inophyllum*. Phytochemistry 38(3):725–728.

Ito C, Itoigawa M, Mishina Y, Filho VC, Mukainaka T, Tokuda H, Nishino H, Furukawa H. 2002. Chemical constituents of *Calophyllum brasiliensis*: structure elucidation of seven new xanthones and their cancer chemopreventive activity. J Nat Prod. 65(3):267–272.

Lee J, Lee D, Jang DS, Nam J-W, Kim J-P, Park KH, Yang MS, Seo E-K. 2007. Two new stereoisomers of tetrahydrofuranoid lignans from the flower buds of *Magnolia fargesii*. Chem Pharm Bull. 55(1):137–139.

Lim CK, Subramaniam H, Say YH, Jong VYM, Khaledi H, Chee CF. 2015. A new chromanone acid from the stem bark of *Calophyllum teysmannii*. Nat Prod Res. 29(21):1970–1977.

Mah SH, Ee GCL, Teh SS, Sukari MA. 2015. *Calophyllum inophyllum* and *Calophyllum soulattri* source of anti-proliferative xanthones and their structure-activity relationships. Nat Prod Res. 29(1):98–101.

Saputri RD, Tjahjandarie TS, Tanjung M. 2018. Meliglabrin, a new flavonol derivative from the leaves of *Melicope glabra* (Blume) T.G. Hartley. Nat Prod Sci. 24(3):155–158.

Saputri RD, Tjahjandarie TS, Tanjung M. 2019. Two novel coumarins bearing an acetophenone derivative from the leaves of *Melicope quercifolia*. Nat Prod Res. 1–6. https://doi.org./10.1080/14786419.2019.1644634

Shen Y-C, Wang L-T, Khalil AT, Chiang LT, Cheng PW. 2005. Bioactive pyranoxanthones from the roots of *Calophyllum blancoi*. Chem Pharm Bull. 53:224–247.

Stevens PF. 1980. A revision of the old world species of *Calophyllum* (Guttiferae). J Arnold Arbor. 61:117–699.

Stevens PF. 1982. Species of Calophyllum in Indonesia. Document Herbarium Bogoriensis. Bogor, Indonesia: Center of Biological Research and Development.

Tanjung M, Rachmadiarti F, Prameswari A, Agyani VUW, Saputri RD, Tjahjandarie TS, Syah YM. 2018. Airlanggins A-B, two new isoprenylated benzofuran-3-ones from the stem bark of *Calophyllum soulattri*. Nat Prod Res. 32(13):1493–1498.

Tanjung M, Tjahjandarie TS, Saputri RD, Kurnia BD, Rachman MF, Syah YM. 2019. Calotetrapterins A-C, three new pyranoxanthones and their cytotoxicity from the stem bark of *Calophyllum tetrapterum* Miq. Nat Prod Res. 1–6.

Tjahjandarie TS, Tanjung M, Rahmania DF, Rhidoma CI, Saputri RD. 2019. Calodioscurins A and B, two new isoprenylated xanthones from the stem bark of *Calophyllum dioscurii* P.F. Stevens. Nat Prod Res. 1–6.