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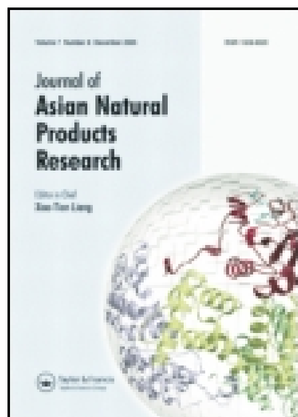

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A new furanoxanthone from the stem bark of *Calophyllum inophyllum*

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The stem bark extracts of *Calophyllum inophyllum* furnished one new furanoxanthone, inophinnin (**1**), in addition to inophyllin A (**2**), macluraxanthone (**3**), pyranojacareubin (**4**), 4-hydroxyxanthone, friedelin, stigmasterol, and betulinic acid. The structures of these compounds were determined by spectroscopic analysis of 1D and 2D NMR spectral data (^{1}H, ^{13}C, DEPT, COSY, HMQC, and HMBC) while EI-MS gave the molecular mass. The new xanthone, inophinnin (**1**), exhibited some anti-inflammatory activity in nitric oxide assay.

Keywords: inophinnin; furanoxanthone; *Calophyllum inophyllum*; Guttiferae

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1. Introduction

Calophyllum has been studied widely for its valuable secondary metabolites. The tree is native to Tropical Asia, East Africa, India, and Australia. *Calophyllum inophyllum* is well recognized as a rich source of biologically active secondary metabolites. Xanthones [1], coumarins [2], triterpenoids [3], and flavonoids [4,5] are biologically active compounds that occur in this species. Previous reports show medicinal uses such as antiseptics, astringents, diuretics, and purgatives [6]. Besides this, it also shows anti-HIV [7,8], antifungal [9], and antimicrobial [10] effects. Some of these compounds have contributed to the drug industry as cancer chemopreventive agents [11]. We report here the isolation and characterization of the new xanthone, inophinnin (Figure 1).

2. Results and discussion

Inophinnin (**1**) is a yellowish crystal with a molecular ion peak at *m/z* 410 in the EI-MS. HR-ESI-MS gave the molecular ion peak at *m/z* 409.1713 $[M - H]^{-}$, corresponding to the molecular formula $C_{24}H_{26}O_{6}$. The melting point of **1** was observed at 166–167°C. The UV spectrum showed absorption maxima at 323, 248, and 210 nm, whereas the FTIR spectrum displayed absorption bands at 3372 cm^{-1} (hydroxyl), 1648 cm^{-1} (conjugated carbon), and 1605 cm^{-1} (aromatic). Both UV and IR spectra suggested that **1** is a xanthone derivative [12].

^{13}C NMR and DEPT spectra indicated the existence of a carbonyl carbon at δ 182.1. Twelve quaternary carbon signals, six of which were oxygenated, were observed. The oxygenated carbon signals appeared at δ 132.2, 142.0, 151.7, 153.4,

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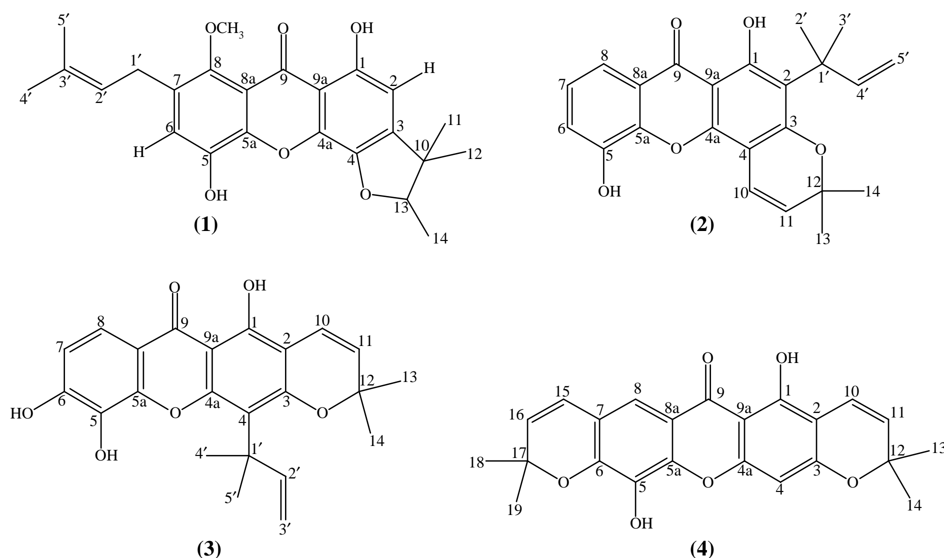

Figure 1. Structures of compounds **1**–**4**.

164.5, and 165.6, whereas the non-oxygenated quaternary carbons resonated at δ 43.7, 103.9, 112.1, 112.7, 132.2, and 133.4. Four methine carbon signals were observed at δ 90.6, 94.0, 113.3, and 122.2, whereas one methylene carbon signal appeared at δ 33.6. Five methyl carbon signals at δ 14.2, 18.0, 21.6, 25.6, and 25.9 were also seen in the DEPT spectrum.

The characterization of chemical shifts for protons at δ 4.00 (d, 2H, H-1′), 5.36 (t, 1H, H-2′), 1.73 (s, 3H, H-5′), and 1.76 (s, 3H, H-4′), and carbons at δ 33.6 (C-1′), 122.2 (C-2′), 133.4 (C-3′), 18.0 (C-5′), and 25.9 (C-4′) suggested the presence of a prenyl moiety in the molecule. This hypothesis was further confirmed by the correlations of the two vinylic methyl proton singlets at δ 1.73 (H-5′) and 1.76 (H-4′) with their neighboring carbons at δ 133.4 (C-3′) and 122.2 (C-2′) in the HMBC spectrum (Figure 2).

The ^{1}H NMR spectrum of **1** which showed a sharp singlet at δ 13.73 (OH-1) supported the existence of a chelated phenolic hydroxyl group, whereas the

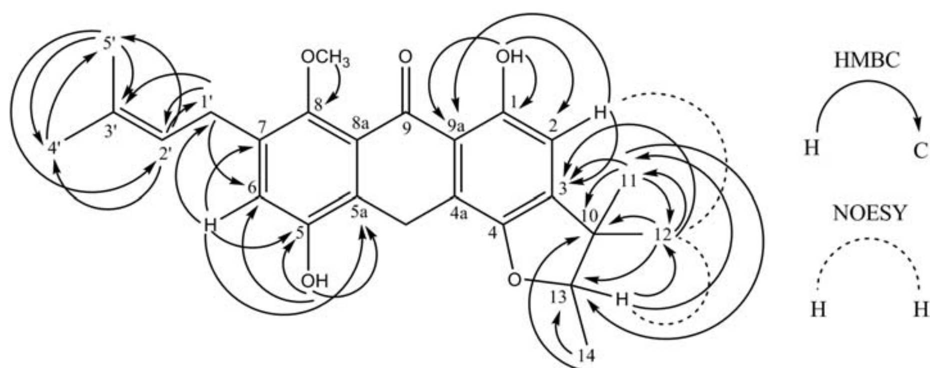

Figure 2. Key HMBC (^{2}J and ^{3}J) and NOESY correlations for compound **1**.

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Table 1. Effect of compound **1** in LPS-induced nitric oxide production on raw cell.

Concentration of **1** (μM)	Concentration of NO (μM)	Percentage of inhibition
100.00	6.98 ± 0.13	61.42
50.00	7.64 ± 0.15	57.77
25.00	8.92 ± 0.18	50.69
12.50	14.74 ± 0.09	18.52
6.25	16.32 ± 0.15	9.78

Note: Each value of concentration of NO is mean ± S.E.M.

broad singlet at δ 6.34 (OH-5) was due to a free hydroxyl group. Moreover, the proton at δ 13.73 has long-range correlations with the carbons at δ 94.0 (C-2), 103.9 (C-9a), and 164.5 (C-1) establishing its location to be at C-1. An aromatic proton at δ 6.24 has ^{1}J correlation with C-2 at δ 94.0 and long-range correlations with the quaternary carbons at δ 103.9 (C-9a) and 112.1 (C-3). This information demonstrated that position 2 carries a proton. Meanwhile, a broad singlet at δ 6.34 displayed cross peaks with C-6 at δ 113.3, C-5 at 132.2, and C-5a at 132.2 and 153.4. Therefore, this free hydroxyl was assigned to C-5 (Figure 2).

Another sharp singlet observed at δ 4.00 (s, 3H) suggested the presence of a methoxyl group which is attached to C-8. This signal gave a ^{1}J correlation with 8-OCH_3 at δ 61.9 and a ^{3}J correlation with C-8 at δ 142.0 via HMQC and HMBC spectra, respectively. An aromatic proton was observed at δ 6.82 (H-6). This proton was assigned to the carbon at δ 113.3 according to the HMQC spectrum. It was correlated with C-1′ at δ 33.6, C-7 at 112.7, C-5 at 132.2, and C-5a at 153.4. This aromatic proton was thus confirmed to be located at C-6.

Two methyl singlets (δ 1.31 and 1.60), one methyl doublet (δ 1.42, $J = 6.4$ Hz), and a methine quartet (δ 4.53, $J = 6.4$ Hz) suggested the presence of a trimethyldihydrofuran ring. The fusion of this furan ring at the oxygenated C-4 and C-3 was confirmed by the long-range correlations of H-11 (δ 1.31) and H-12 (δ 1.60) with C-3 (δ 112.1) and between H-14 (δ 1.42) and C-10 (δ 43.7). Other HMBC correlations are shown in Figure 1. Meanwhile, the NOESY experiment showed correlations of H-13/H-12 and H-12/H-2 suggesting that their orientations are identical. The HMBC and NOESY correlations for **1** are shown in Figure 1. Therefore, the structure of compound **1** was characterized as 1,5-dihydroxy-8-methoxy-7-(3′,3′-dimethyl-2′-propenyl)-4″,4″,5″-trimethylfurano-[2″,3″:4,3]-xanthone and named inophinnin.

Anti-inflammatory tests on **1** indicated some anti-inflammatory activity. Table 1 shows the nitric oxide assay results of compound **1**.

3. Experimental

3.1 General experimental procedures

Melting points were measured using Leica Galen III microscope, equipped with Testo 720 temperature recorder and are uncorrected. Ultraviolet spectra were recorded in EtOH on a Shimadzu UV-160A, UV–VIS Recording Spectrophotometer. Infrared spectra were measured using the universal attenuated total reflection technique on a Perkin-Elmer 100 Series FT-IR spectrometer. NMR spectra were obtained using a JEOL FTNMR 400 MHz spectrophotometer using tetramethylsilane as an internal standard. EI-MS were recorded on a Shimadzu GC-MS model QP2010 Plus spectrophotometer.

3.2 Plant material

The stem bark of *C. inophyllum* was collected from Universiti Putra Malaysia

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campus grounds. This plant was identified by Dr Rusea Go from the Department of Biology, Faculty of Science, Universiti Putra Malaysia. A voucher specimen was deposited in the herbarium of Biology Department, Faculty of Science, Universiti Putra Malaysia (voucher specimen RG200).

3.3 *Extraction and isolation*

Approximately 3 kg of air-dried stem bark of *C. inophyllum* was ground into fine powder and extracted successively with *n*-hexane, dichloromethane, ethyl acetate, and methanol for 72 h. The extracts were evaporated to dryness under vacuum to give 80.7 g of hexane extract, 21.7 g of dichloromethane extract, and 40.1 g of ethyl acetate extract. Part of each extract was subjected to column chromatography over silica gel and eluted with stepwise gradient system using *n*-hexane, chloroform, ethyl acetate, and methanol. Further purifications of the hexane extract resulted in friedelin (450 mg) and betulinic acid (14 mg). Meanwhile, purification of the dichloromethane extract afforded a new furanoxanthone, inophinnin (**1**) (9 mg), inophyllin A (**2**) (5 mg), caloxanthone C (**3**) (14 mg), pyranojacareubin (**4**) (6 mg) and stigmasterol (22 mg). Compound **1** was obtained from a mixture of *n*-hexane–chloroform (1:4) eluate, followed by several further purifications using chromatotron and eluting with a chloroform–methanol (4.9:0.1) mixture. Lastly, 4-hydroxyxanthone (8 mg) was obtained from the purification of the ethyl acetate extract.

3.3.1 *Inophinnin (1)*

A yellow crystal; m.p. 166–167°C; UV (EtOH) λ_{max} nm (log ε): 323 (5.12), 248 (5.01), 210 (4.94); IR ν_{max} cm^{-1}: 3372, 2926, 1648, 1605; ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.73 (OH-1, s), 6.82 (1H, s, H-6), 6.34 (OH-5, s), 6.24 (1H, s, H-2), 5.36 (1H, t, $J = 6.9$ Hz, H-2′), 4.53 (1H, q, $J = 6.4$ Hz, H-13), 4.00 (2H, d, $J = 6.9$ Hz, H-1′), 4.00 (3H, s, 8-OCH_3), 1.76 (3H, s, H-4′), 1.73 (3H, s, H-5′), 1.60 (3H, s, H-12), 1.42 (3H, d, $J = 6.4$ Hz, H-14), 1.31 (3H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$): δ 182.1 (C-9), 165.6 (C-4), 164.5 (C-1), 153.4 (C-5a), 151.7 (C-4a), 142.0 (C-8), 133.4 (C-3′), 132.2 (C-5 and C-8a), 122.2 (C-2′), 113.3 (C-6), 112.7 (C-7), 112.1 (C-3), 103.9 (C-9a), 94.0 (C-2), 90.6 (C-13), 61.9 (8-OCH_3), 43.7 (C-10), 33.6 (C-1′), 25.9 (C-4′), 25.6 (C-12), 21.6 (C-11), 18.0 (C-5′), 14.2 (C-14); EI-MS *m/z* (rel. int.): 410 [M^+] (61), 395 (45), 381 (7), 367 (100), 352 (10), 337 (14), 325 (9), 176 (10). HR-ESI-MS: *m/z* 409.1713 $[M - H]^-$ (calcd for $C_{24}H_{26}O_6$, 410.1730).

3.4 *Anti-inflammatory assay*

Raw cells (2×10^6 cells/ml) were seeded in a 96-well plate and incubated for 2 h. They were then treated and induced with 10 μg/ml lipopolysaccharide (LPS) in the presence of compound **1** and made up to a final volume of 100 μl and further incubated for 24 h. Griess reagent (50 μl) was added to react with 50 μl of cell-free culture supernatant and incubation was carried out for 10 min at room temperature. Readings at OD = 550 nm were taken. A fresh culture medium was used as blank. The results were expressed as mean ± S.E.M.

4. Conclusion

The stem bark of *C. inophyllum* furnished one new furanoxanthone, inophinnin (**1**) along with four other xanthones inophyllin A (**2**), macluraxanthone (**3**), pyranojacareubin (**4**), 4-hydroxyxanthone and three common triterpenes, friedelin, stigmasterol, and betulinic acid. Inophinnin (**1**) showed potent anti-inflammatory activity.

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