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A New Pyranoxanthone Inophyllin B from *Calophyllum inophyllum*

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Abstract – A new prenylated pyranoxanthone, inophyllin B (**1**), was isolated from the roots of *Calophyllum inophyllum* (Guttiferae). Together with this compound was also isolated the known pyranoxanthone brasilixanthone B (**2**) and two common triterpenes friedelin (**3**) and sitosterol (**4**). Structural elucidations of these compounds were achieved through ^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC experiments. The molecular mass was determined using MS techniques. The crude extract indicated weak toxicity to the larvae of *Aedes aegypti*. We report here the isolation, structural elucidation and bioassay data for Inophyllin B and brasilixanthone B.
Keywords – *Calophyllum inophylum*, pyranoxanthone, inophyllin B, brasilixanthone B, toxicity, *Aedes aegypti*

Introduction

Calophyllum inophyllum which belongs to the Guttiferae family is known as 'bintangor' by the locals in Malaysia. It is a tall and leafy tree with big branches. Several species of this genus are known to be used in folk medicine[1]. This genus has been found to be rich in xanthones[2,3], coumarins[4,5], chromenes[6] and flavonoids[7]. Recently several coumarins isolated from two *Calophyllum* species were found to inhibit HIV-1 replication and cytopathicity[8]. Detail chemical studies on the root bark of *Calophyllum inophyllum* were investigated and this gave a new prenylated pyranoxanthone inophyllin B, brasilixanthone B and two known triterpenes, friedelin and sitosterol.

Experimental

Plant material – The roots of *Calophyllum inophyllum* were collected from University Putra Malaysia campus grounds. The plant was identified by Dr Rusea Go of Biology Department, University Putra Malaysia. The voucher specimen is kept in the Herbarium of the Biology Department, University Putra Malaysia.

General – ^{1}H and ^{13}C NMR spectra were recorded on a JEOL FTNMR 400 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as the internal standard. EIMS and CIMS was recorded on a Shimadzu GCMS QP5000 instrument equipped with a direct injection probe. UV spectra were recorded in $CHCl_3$ on a Shimadzu UV-2100 spectrophotometer while IR spectra were recorded on a Perkin-Elmer FTIR model 1725x spectrophotometer.

Extraction and isolation – The air-dried root bark (1.6 kg) was ground and the powder extracted twice with hexane for at least 48 hours. The same plant material was extracted twice with chloroform followed by methanol. The extracts were filtered and evaporated to dryness under vacuum to give 24.8 g of crude hexane extract, 46.7 g of crude chloroform extract and 64.3 g of crude methanol extract. Direct recrystallization of a crystalline compound from the crude hexane extract led to the isolation of a new xanthone, inophyllin B (**1**)(10 mg). The balance of the crude hexane extract was subjected to a column chromatography (silica gel 9385) using various solvents mainly hexane, hexane-chloroform, chloroform, chloroform-ethyl acetate, ethyl acetate and ethyl acetate-methanol with a 10% stepwise gradient. This gave a total of 30 fractions. Fraction 10, VJB with hexane-chloroform (1:9) eluent afforded sitosterol. Fraction 6 was further rechromatographed with solvent system of hexane-chloroform (3:2) to give 5 fractions. Fraction 3, VJA yielded friedelin. The same procedure was applied to the crude chloroform extract and further work-up of fraction VJ10 by repeated column chromatography using chloroform-ethyl acetate (2:3) led to the isolation of Brasilixanthone B (5 mg).

Inophyllin B (1) – 1,5,6-trihydroxy-2-(1,1-dimethyl-2-propenyl)-6',6'-dimethylpyrano[2',3':3,4]-xanthone. Yellow needles, m.p.176°C. UV ($CHCl_3$) λ_{max} nm (log ε) 376, 294, 250; IR ν_{max} cm^{-1} (KBr): 3432, 2970, 1654, 1610, 1592. EI-MS *m/z* (rel. int.) 394 (25), 364 (24), 363 (100), 154 (21), 43 (15), and 41 (21); HREIMS : 394.1414 (calc. for $C_{23}H_{22}O_6$: 394.4218; ^{1}H NMR (400 MHz,

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Table 1. Proton and carbon connectivities obtained from ^{1}H-^{13}C HSQC and HMBC experiments for inophyllin B

Proton resonance	^{1}J connectivity	^{2}J onnectivity	^{3}J connectivity
13.83(1-OH)		157.01(C-1)	103.36 (C-9a)
7.54(H-7)	113.38(C-7)	151.31(C-6)	146.31(C-5)
6.93(H-8)	116.82(C-8)	113.83(C-8a)	133.20(C-10a)
6.65(H-11)	116.21 (C-11)		78.60(C-13), 159.2(C-3)
5.62(H-12)	127.75(C-12)	78.60(C-13)	105.41(C-4)
1.45(H-14)	27.86(C-14)		127.75(C-12), 27.86(C-15)
1.45(H-15)	27.86(C-15)		127.75(C-12), 27.86(C-14)
1.69(H-17)	29.80(C-17)		113.99(C-2), 29.80(C-18), 152.85(C-19)
1.69(H-18)	29.80(C-18)		113.99(C-2), 29.80(C-17), 152.85(C-19)
6.48(H-19)	152.85(C-19)	106.73(C-20)	29.80(C-17), 29.80(C-18)
5.01(H-20)	106.73(C-20)	152.85(C-19)	41.52(C-16),

$CDCl_3$): δ 13.83 (OH-1, s), δ 7.54 (1H, d, *J*=8.2 Hz, H-7), δ 6.93 (1H, d, *J*=8.2 Hz, H-8), δ 6.65 (1H, d, *J*=10.1 Hz, H-11), δ 5.62 (1H, d, *J*=10.1 Hz, H-12), δ 1.45 (6H, s,14-CH_3, 15-CH_3); δ 1.69 (6H, s, 17-CH3, 18-CH_3), δ 6.48 (1H, dd, *J*=17.4 Hz, 11.9 Hz, H-19), δ 5.01 (1H, brd, *J*=17.4 Hz, H-20), δ 4.85 (1H, brd, *J*=11.9 Hz, H-20), δ 6.41 (OH-5, s), ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.01 (C-1), δ 113.99 (C-2), δ 159.21 (C-3), δ 105.41 (C-4), δ 155.5 (C-4a), δ 146.31 (C-5), δ 151.31 (C-6), δ 113.38 (C-7), δ 116.82 (C-8), δ 113.83 (C-8a), δ 181.45 (C-9), δ 103.36 (C-9a), δ 133.2 (C-10a), δ116.21(C-11), δ 127.30(C-12), δ 78.90 (C-13), δ 27.86(C-14,C-15), δ 41.52 (C-16), δ 29.80 (C-17,C-18), δ 152.85 (C-19), δ 106.73 (C-20).

Brasilixanthone B (2) – Yellow needles, m.p. 181-182°C. EIMS *m/z* 392 for molecular formula $C_{23}H_{20}O_6$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.62 (s, 1-OH), δ 6.26 (1H, s, H-4), δ 6.27 (1H, s, H-5), δ 6.83 (1H, s, 6-OH), δ 5.57 (1H, d, *J*=10 Hz, H-11), δ 6.72 (1H, d, *J*=10 Hz, H-12), δ 1.47 (6H, s, 14-CH_3, 15-CH_3), δ 1.50 (6H, s, 19-CH_3, 20-CH_3), δ 8.02 (1H, d, *J*=10 Hz, H-16), δ 5.82 (1H, d, *J*=10 Hz, H-17). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.49 (C-1), δ 103.85 (C-2), δ 153.03 (C-3), δ 94.25 (C-4), δ 157.75 (C-4a), δ 102.40 (C-5), δ 150.91 (C-6), δ 136.83 (C-7), δ 119.67 (C-8), δ 108.52 (C-8a), δ 182.45 (C-9), δ 104.36 (C-9a), δ 159.92 (C-10a), δ 108.52 (C-11), δ 132.32 (C-12), δ 77.32 (C-13), δ 28.32 (C-14), δ 28.32 (C-15), δ 120.92 (C-16), δ 127.21 (C-17), δ 77.32 (C-18), δ 27.31 (C-19), δ 27.31 (C-20).

Toxicity assay – Investigations on the toxicity of samples on *Aedes aegypti* were carried out using the method recommended by WHO (1980) [9] with slight modifications. A standard stock solution of 5000 μg ml^{-1} was prepared. Test solutions were made by pipetting samples of the stock solution into 250 ml glass containers and made up to a total volume of 50 ml. A control was prepared by using 1.5 ml of absolute alcohol in chlorine-free water. Ten late third instar mosquito larvae were introduced into the glasses and a little larvae food added. A series of at least 5 concentrations in duplicates were needed to obtain LC_{50} and LC_{90} values. Results were analysed using the Probit Analysis Programme

Results and Discussion

Inophyllin B (**1**) was isolated as yellow crystals with a melting point of 176°C. EIMS gave a molecular ion peak at *m/z* 394 indicating a molecular formula of $C_{23}H_{22}O_6$. HRMS: 394.1414. This compound gave typical IR bands for xanthones at 3432, 2970, 1654, 1610, 1592 cm^{-1}. The UV spectrum gave a maximum absorption at 294 nm. The ^{1}H NMR spectrum showed the presence of one chelated hydroxyl group at δ 13.44. Two one proton doublets with a coupling constant value of 10.1 Hz each was observed at δ 5.62 and δ 6.65. These two proton signals were observed to be coupled to each other in the COSY spectrum. This indicated a pair of ortho-coupled protons in either ring A or ring B. Thus these two signals were assigned to the protons attached to C-12 and C-11. Another pair of one-proton each signals which are also ortho-coupled as observed in the COSY spectrum was seen at δ 7.54 and δ 6.93. Hence another AB system was obvious to be present in the other xanthone ring. A hydroxyl signal which appeared as a sharp singlet at δ 6.41 was assigned to C-5. Another hydroxyl singlet was observed at δ 6.41. This was assigned to position 6 in the xanthone ring.

The presence of a 1,1-dimethylallyl substituent in inophyllin B (**1**) was obvious from the set of signals consisting of one-proton doublet of doublet at δ 6.48 (1H, dd, *J*=17.4 and 11.9 Hz), two one-proton doublet of doublets at δ 5.01 (1H, d, *J*=17.4 Hz), δ 4.85 (1H, d, *J*=11.9 Hz) and one six protons singlet at δ 1.69. A pair of broad doublet at δ 4.85 and δ 5.01 gave correlations with a hydrogen doublet of doublet signal at δ 6.48. The ^{1}H NMR spectrum also showed the presence of 4 methyl groups and this was confirmed by the DEPT spectrum which also confirmed the presence of one CH_2 group. The HMBC also gave ^{3}J linkages of the CH group at δ 6.48 to two methyl groups at δ 1.69 hence confirming the prenyl moiety. Thus, the doublet of doublet at δ 6.48 was assigned to H-19 while the the 2 broad doublets at δ 5.01 and δ 4.85 were assigned to H-20 and the two

methyls at δ 1.69 were assigned to 17-CH_3 and 18-CH_3. The position of the prenyl moiety at C-2 was further confirmed from HMBC which gave ^3J correlations between carbon signal at δ 113.99 (C-2) and H-17 and H-18 (methyls 17 and 18).

However, the total number of carbon atoms as obtained from the ^{13}C NMR spectrum was 23 hence there should be another ring in the xanthone skeleton. The pyrano ring was obvious from the HMBC spectrum which indicated the protons at C-11 and C-12 to be correlated to C-4 and C-3 via a ^2J and ^3J coupling. HMBC also gave ^2J correlation between the peak at δ 5.62 (H-12) and δ 78.90 (C-13). Correlations were also observed between C-13 (δ 78.90) and H-11 (δ 6.65) through a ^3J coupling. The detail correlations as observed in the COSY, HSQC and HMBC spectra are summmarised in Table 1.

Innophyllin B (**1**) was thus assigned to a pyranoxanthone with the IUPAC name 1,5,6-trihydroxy-2-[1,1-dimethyl-2-propenyl]-6'dimethylpyrano[2',3':3,4]-xanthone.

Brasilixanthone B (**2**) was isolated as yellow crystals from the hexane extract of *Calophyllum inophyllum*. It gave a melting point of 181-182°C. The EIMS spectrum gave a molecular ion peak at *m/z* 392 implying the molecular formula $C_{23}H_{20}O_6$. The ^1H NMR spectrum gave 2 pairs of 1 proton signals at δ 5.57, δ 6.72, δ 5.82 and δ 8.02. All these four doublets had a *J* value of 10 Hz each. The COSY spectrum indicated coupling between the signals at δ 5.57 and δ 6.72 and also between the signals at δ 5.82 and δ 8.02.

Hence the molecule has 2 pairs of ortho-coupled protons. Two one proton singlet at δ 6.26 and δ 6.27 implies two non-coupled protons bonded to the xanthone ring. The ^1H NMR spectrum also showed the presence of 4 methyl groups at δ 1.47 and δ 1.50. The ^{13}C NMR spectrum gave a total carbon count of 23 implying the xanthone to possess 2 pyrano rings. This was further confirmed by the total number of quarternary carbon count of 10. The HSQC and HMBC spectra gave further evidence to the identity of the compound (**2**) to be brasilixanthone B. The rest of the ^{13}C NMR data are in agreement with published data[10].

Table 2. LC_{50} values for the crude extracts of *Calophyllum inophyllum* and inophyllin B against the larvae of *Aedes aegypti*.

Extract	LC_{50} (μg ml^{-1})
Hexane	>150
Ethanol	>150
Inophyllin B	>100
Azadirachta indica [11]	1.2-18.0

Table 2 gives the toxicity test result for the crude extracts and pure inophyllin B. The high LC_{50} value of > 150 μg ml^{-1} for the crude extracts as compared to that of the standard, *Azadirachta indica* indicates it to be not toxic to the larvae of *Aedes aegypti* Similarly the high LC_{50} value of > 100 μg ml^{-1} for inophyllin B also shows that the compound is not toxic to the larvae of *Aedes aegypti*. The toxicity of brasilixanthone B was not evaluated due to the shortage of the pure compound.

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