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Larvicidal Anthraquinones and Triterpenes from *Ploiarium alternifolium* (Theaceae)

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Chemical studies on the stem bark of *Ploiarium alternifolium* (Theaceae) have yielded the anthraquinone emodin, and its derivative a methoxy bearing anthraquinone. These 2 compounds have not been reported to be present in this plant. Also isolated from the same plant was ploariquinone, a geranyl anthraquinone. Separation of the triterpenoid-containing fraction gave the phenolic oleanane benzoate. Structural elucidations of these compounds were achieved using ^{1}H NMR, ^{13}C NMR, DEPT, COSY and HETCOR experiments while MS gave the molecular masses. The crude extracts were found to be moderately bioactive against the larvae of *Aedes aegypti*. This paper reports the isolation and identification of these compounds as well as bioassay data for the crude extracts.

Key Words: Larvicidal, Anthraquinones, Triterpenes, *Ploiarium alternifolium*.

INTRODUCTION

The genus *Ploiarium* belongs to the Theaceae family. *Ploiarium alternifolium* (Vahl.) Melanch is a cicada tree and it is locally known as "Jinggau" in Sarawak. "Jinggau" is one of the most common trees in secondary forests and on sandy and acid soils in Southern Sarawak, Malaysia. The leaves are eaten raw as salad and have a pleasant sharp taste. *P. alternifolium* is recognized as a hard, heavy red wood with indistinct soft tissue and rays. The wood is commonly used as fence and pepper posts. It is also popularly used as firewood[1].

P. alternifolium has been found through phytochemical studies to contain secondary metabolites that can be grouped as geranyl anthraquinones, anthraquinonyl xanthones, triterpenoid benzoates and bixanthones[2]. Anthraquinone is the main group of the quinones. It is widely found in liken, fungi and higher stage plants. Rubiaceae, Polygonacecae, Leguminosae and Liliaceae families are rich in anthraquinones. Besides the natural quinones, a dianthraquinone has also been isolated. This dianthraquinone was also synthesized from the oxidative coupling phenol reaction.

EXPERIMENTAL

Plant material

The stem bark of *P. alternifolium* was collected from the Sri Aman division in Sarawak, East Malaysia. The plants were identified at the Herbarium, Forest Department Headquarters, Kuching, Sarawak.

UV spectra were measured in ETOH and $CDCl_3$ and the IR spectra were obtained in KBr discs. ^1H NMR spectra were measured at 400 MHz. Separation by column chromatography was carried out using Silica gel Merck 9385.

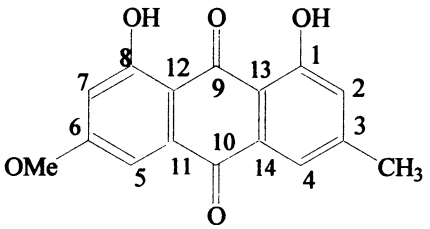
Extraction of *Ploiarium alternifolium*: The finely ground air-dried stem bark (1.7 kg) was extracted succesively with *n*-hexane, ethyl acetate and ethanol for 48 h. The extracts were filtered and concentrated down under reduced pressure in a rotary evaporator. About *ca.* 4.5 g of hexane extract was obtained. The hexane extract was purified by column chromatography using hexane, hexane-ethyl acetate and methanol. This yielded 3β-benzoyloxyolean-11-en-13β, 28-olide (0.3 g) and ploiariquinone A (0.2 g). The crude ethyl acetate extract (18.0 g) which was purified using column chromatography and PLC gave emodin (1.3 g), euxanmodin C (1.1 g) and 1,8-dihydroxy-3-methoxy-6-methyl-anthraquinone (0.1 g). The ethanol extract (121.0 g) which was purified using column chromatography gave emodin (0.2 g) and euxanmodin C (0.4 g).

Larvicidal assay: Investigations on the larvicidal activity of samples on *Aedes aegypti* were carried out using the method recommended by WHO (1980)[3]. A standard stock solution of 5000 ppm was prepared by dissolving 25 mg extract in 5 mL of absolute ethanol. A test solution was made by pipetting a sample of the stock solution into 25 mL of chlorine-free tap water in glass containers. The test solutions were made at concentrations (50, 100 and 150 ppm) as required. A control was prepared by using 1.5 mL of absolute alcohol in chlorine-free water. The test sample was made up to 50 mL with chlorine-free water. Ten late third instar mosquito larvae were introduced into each glass by a dropper. A little larvae food (roasted cow's liver) was added. Mortality of the mosquito larvae was evaluated after 24 h. A series of a least 5 concentration in duplicates were needed to obtain LC_{50} and LC_{90}. Results were analyzed using the Probit Analysis Programme.

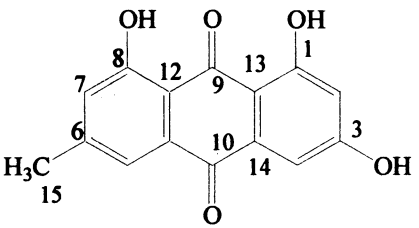
Antimicrobial Activity: Four microorganisms were used, *i.e.*, *Bacillus subtilis* mutant (B28), *Bacillus subtilis* wild type (B29), *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (ATCC 60690). All the microorganisms were cultured in the appropriate broth (nutrient broth for bacterial and potato dextrose broth for fungi) at 30°C overnight and calibrated the concentrations of the cultures using turbidometrically at a wavelength of 600 nm to obtain 10^5–10^6 colony forming units (CFU) per mL. Antimicrobial activity was qualitatively determined by a modified disc diffusion method as described previously by Mackeen *et al.*[4]

RESULTS AND DISCUSSION

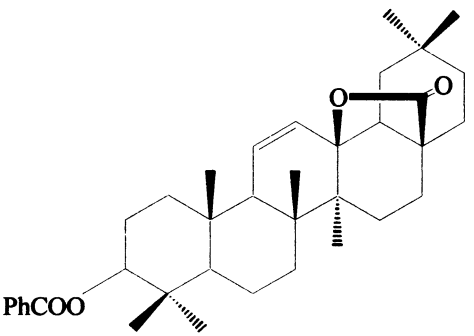
Yellow needles of 1,8-dihydroxy-3-methoxy-6-methylanthraquinone which melted at 209–210°C was isolated from the ethyl acetate extract. Emodin was isolated as orange coloured crystals with an m.p. of 256–257°C while 3β-benzoyloxyolean-11-en-13β, 28-olide was isolated as white crystals with a melting point of 178–180°C. The spectral data for emodin and 3β-benzoyloxyolean-11-en-13β, 28-olide are in agreement with literature[2, 5].



1,8-dihydroxy-3-methyl-6-methoxy-anthraquinone



Emodin



3-β-Benzoyloxyolean-11-en-13β,28-olide

1,8-Dihydroxy-3-methoxy-6-methylanthraquinone was isolated as yellow needle crystals with a melting point 209–210°C (Lit. m.p. 208–209°C)[6]. Strong IR absorptions were observed at 1676 cm^{-1} which was due to the carbonyl group at C-10 and 1630 cm^{-1} due to the chelated carbonyl group at C-9. The presence of hydroxyl groups with a strong and broad absorption at 3436 cm^{-1} was also observed. Mass spectral data gave a molecular ion peak at m/z 284 which corresponds to the molecular formula $C_{16}H_{12}O_5$. The ^1H NMR spectrum shows the presence of two lowfield chelated hydroxyls at δ 12.33 and δ 12.13. These signals were assigned to the hydroxyl groups attached to C-1 and C-8.

Two singlets at 7.09 and 7.63 were assigned to H-5 and H-7. The two meta-coupled doublets at 6.69 (J = 2.6 Hz) and 7.37 (J = 2.6 Hz) were assigned to H-2 and H-4 respectively. A three hydrogen singlet at 3.94 indicates the existence of a methoxy group, while another three hydrogen singlet at 2.64 shows the presence of a methyl group. The presence of these two groups was confirmed by DEPT experiment. This compound differs from emodin where C-3 carries a methoxy group. The ^{13}C NMR spectrum gives a total of 16 carbons. The DEPT experiment confirmed the presence of 4 —CH groups, 1 —CH_3 group and a methoxy group. Again the anthraquinone skeleton was confirmed by the presence of the two carbonyls at 191.1 and 182.2. The HETCOR spectrum correlates protons at H-2, H-4, H-7 and H-5 to the carbon signals at 106.8, 108.2, 121.3 and 124.5 respectively. Hence these carbons were carefully assigned. Other carbons were assigned by comparison with chemical shift values in emodin[5].

TABLE-1
^{13}C NMR (100 MHz, $CDCl_3$) AND ^{1}H NMR (400 MHz, $CDCl_3$) ASSIGNMENTS FOR 1,8-DIHYDROXY-3-METHOXY-6-METHYLANTHRAQUINONE

Carbon	δ	Proton	δ	J (Hz)
C-1	165.2	OH-1	12.33, s	–
C-2	106.8	H-2	6.69, d	2.6
C-3	166.6			
C-4	108.2	H-4	7.37, d	2.6
C-5	124.5	H-5	7.06, brs	
C-6	148.4			
C-7	121.3	H-7	7.63, brs	
C-8	162.5	OH-8	12.13, s	
C-9	191.1			
C-10	182.2			
C-11	110.3			
C-12	135.3			
C-13	133.0			
C-14	113.5			
OCH_3	56.1	OCH_3	3.94, s	
CH_3	22.2	CH_3	2.46, s	

TABLE-2
LC_{50} AND LC_{90} VALUES OF CRUDE EXTRACTS ON *AEDES AEGYPTI* ACTIVITY

Plant	LC_{50} (μg/mL)	LC_{50} (μg/mL)
P. alternifolium (hexane extract)	95.0	139.9
P. alternifolium (ethyl acetate)	129.4	242.0
P. alternifolium (ethanol extract)	131.6	200.9

TABLE-3
DIAMETER INHIBITION ZONE (mm) OF THE CRUDE EXTRACTS

Sample	Bacteria (nm)			
Crude extracts	MRSA	B29	B28	60690
P. alternifolium (Hexane)	6.5	7.0	7.0	6.5
P. alternifolium (EtOAc)	9.0	10.0	9.0	10.0
P. alternifolium (EtOH)	8.0	10.0	9.0	9.5

Standard antibiotic gentamycin 10 (μg/disc) was used against *P. aeruginosa* (24 mm diameter inhibition zone) bacteria

MRSA : Methicillin resistant *Staphylococcus aureus*
B_{29} : *Bacillus subtilis* (wild type)
B_{28} : *Bacillus subtilis* mutant
ATCC 60690 : *Pseudomonas aeruginosa*

Tables 2 and 3 give the larvicidal and anti-bacterial screening results for the three crude extracts of *Ploiarium alternifolium*. The larvae of *Aedes aegypti* were moderately susceptible to the three crude extracts with the hexane extract being the most larvicidal among the three. It gave an LC_{50} value of 95.0 μg/mL. All three crude extracts indicated weak activity against the bacteria B28, B29, ATCC, 60690 and MRSA with less than 10 mm inhibition zone.

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