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Natural Product Compounds from *Calophyllum depressinervosum*

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ABSTRACT

Our detailed study on the phytochemistry of the stem bark of *Calophyllum depressinervosum* resulted in the isolation of four xanthones and one coumarin. The xanthones are trapezifolixanthone (1), macluraxanthone (2), ananixanthone (3), caloxanthone C (4) and the coumarin calonolide E2 (5). The structures of these compounds were elucidated using spectroscopic analysis such as 1D and 2D-NMR, GCMS, IR and UV .

Keywords: Natural product compounds, *Calophyllum depressinervosum*

INTRODUCTION

The genus *Calophyllum* comprises 180-200 tree species, which are distributed in the tropical rain forest with some occurring in Malaysia (Cechinel et al, 2009). *Calophyllum depressinervosum* species is one species from this genus which grows abundantly in Malaysia. This species is also known as *Bintagor lekok* by local Malaysians (Whitmore et al., 1973). Previous phytochemical studies have shown that the *Calophyllum* genus is a valuable source of secondary metabolites such as xanthones, coumarins chromenes and flavonoids (Ee et al., 2006). These secondary metabolites have also been shown to give good bioactivities against the HIV virus and they possess good anti-proliferative activities against cancer cell lines (Mah et al., 2015). Many *Calophyllum* species are economically important for the timber industry especially for housing, shipbuilding, furniture, etc. (Sarangwood et al., 2009). This paper reports detailed structural elucidation of trapezifolixanthone (1) and spectroscopic data for macluraxanthone (2), anixanthone (3), caloxanthone C (4) and calanolide E2 (5).

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EXPERIMENTAL

Plant Material

The stem bark of *Calophyllum depressinervosum* was collected from the Sri Aman district in Sarawak, Malaysia, and identified by Associate Professor Dr. Rusea Go from the Biology Department, Universiti Putra Malaysia. A voucher specimen was deposited in the herbarium of Biology Department, Faculty of Science, Universiti Putra Malaysia.

General

Infrared spectra were measured using universal attenuated total reflection (UATR) technique on Perkin-Elmer 100 Series FT-IR spectrometer. EIMS were recorded on a Shimadzu GCMS-QP 5050A spectrometer (column, SGE BPX5 30meter x 0.25 mm I.D x 0.25µm film thickness, temperature, 200ºC). The NMR spectra were obtained using a JEOL 500MHz FTNMR spectrometer using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as internal standard. The UV spectra were recorded in EtOH on a shimadzu UV-160A, UV-Visible Recording Spectrophotometer. The melting points were obtained on a Leica Galen III instrument.

Extraction and Isolation

The dry and powdered stem bark of *Calophyllum depressinervosum* (2.1kg) was extracted three times by soaking in hexane at room temperature for 72 hours. The same procedure was repeated for another solvent, i.e. dichloromethane. Each extract was dried under reduced pressure using a rotary evaporator to obtain the hexane (27.7g) and dichloromethane (26.8g) extracts. These extracts were chromatographed in a silica gel glass column under vacuum using a stepwise gradient system (hexane/dichloromethane, dichloromethane/ethyl acetate and ethyl acetate/methanol). Further purifications of the hexane extract using another silica gel column (gravity) gave trapezifolixanthone (1) (98.9mg), macluraxanthone (2) (157.6mg), ananixanthone (3) (90.7mg) and calanolide E2 (5) (19.4mg). Meanwhile, further purifications on the dichloromethane extract using gravity silica gel column chromatography gave caloxanthone C (4) (16mg).

Trapezifolixanthone (1). Yellow needle crystal; m.p 161-163ºC (literature 140-142ºC, Daud et al., 2014). UV λmax : 3196, 1629, 1575, 1127 (EtOH) nm : 287, 260, 241, 210 EI-MS m/z: 378, 363, 154. ^{1}H NMR (500MHz, $CDCl_3$ and ^{13}C NMR (125MHz, $CDCl_3$) (Table 1).

Macluraxanthone (2). Yellow needle crystal; m.p 170-174ºC (literature 170-172ºC, Iinuma et al., 1994). UV λ_{max} : 3417, 2971, 1582, 1193 (EtOH) nm : 346, 295, 251, 204 EI-MS m/z: 394, 379, 339, 162. ^{1}H NMR (500MHz, $CDCl_3$): δ13.89 (s, 1-OH), δ7.57 (d , 1H, *J*=8.05Hz, H-8), δ6.97 (d, 1H, *J*=8.05Hz, H-7), δ6.67 (d, 1H, *J*=9.10Hz, H-10), δ6.50 (dd, 1H, *J*=10.8&17.2Hz, H-2'), δ5.69 (d, 1H, *J*=9.10Hz, H-11), δ5.02 (d, 1H, *J*=17.2Hz, H-3b'), δ4.86 (d, 1H, *J*=10.8Hz, H-3a'), δ1.71 (s, 6H, H-4'&H-5'), δ1.46 (s, 6H, H-13&H-14). ^{13}C NMR (125 MHz, $CDCl_3$) δ180.9 (C-9), δ158.6 (C-1), δ156.1 (C-4a), δ155.2 (C-3), δ151.7 (C-2'), δ151.1 (C-5), δ145.9 (C-5a), δ132.9 (C-6), δ127.5 (C-11), δ116.2 (C-8), δ115.5 (C-10), δ113.5 (C-8a), δ113.4 (C-4), δ112.9 (C-7), δ106.6 (C-3'), δ104.8 (C-2), δ102.8 (C-9a), δ78.2 (C -12), δ41.0 (C-1'), δ29.2 (C-4'&C-5'), δ27.2 (C-13&14).

Anixanthone (3). Yellow needle crystal; m.p 168-170ºC (literature 170-171ºC, Joaquim et al., 1998). UV $\lambda_{max:}$ 3217, 2918, 1573 (EtOH) nm : 302, 283, 261 EI-MS m/z: 378, 363, 335, 154. ^{1}H NMR (500MHz, $CDCl_3$): δ13.22 (s, 1-OH), δ7.78 (d, 1H, *J*=8.02 Hz, H-8),7.30(d,1H, *J*=8.02 Hz, H-6), δ7.23 (t, 1H, *J*=8.02, H-7), δ6.79 (d, 1H, *J*=10.3Hz, H-10), δ5.65 (d,1H, *J*=10.3Hz, H-11), δ5.25 (t, 1H, *J*=6.87Hz, H-2'), δ3.36 (d, 2H, *J*=6.87Hz, H-1'), δ1.81(s, 3H, H-4'), δ1.68 (s, 3H, H-5'), δ1.49 (s, 6H, H-13&H-14). ^{13}C NMR (125 MHz, $CDCl_3$) δ180.8 (C-9), δ160.6 (C-1), δ158.6 (C-3), δ149.3 (C-4a), δ144.3 (C-8a), δ144.1 (C-10a), δ131.7 (C-3'), δ127.5 (C-11), δ124.0 (C-7), δ 121.9 (C-2'), δ121.2 (C-5), δ120.1 (C-6), δ117.2 (C-8), δ115.0 (C-10), δ112.3 (C-2), δ103.2 (C-9a), δ100.7 (C-4), δ78.1 (C-12), δ28.2 (C-14&C-13), δ25.8 (C-5'), δ21.3 (C-1'), δ18.0 (C-4').

Caloxanthone C (4). Yellow needle crystal; m.p 211-213ºC (literature 217ºC, Iinuma et al.,1994). UV $\lambda_{max:}$ 3436 ,2935, 1600, 1594, (EtOH) nm : 387, 294, 284, 233 EI-MS m/z: 378, 363, 335, 154. ^{1}H NMR (500MHz, $CDCl_3$): δ13.42 (s, 1-OH), δ7.72(d, 1H, *J*=9.15 Hz, H-8), δ7.23 (t, 1H, *J*=9.15Hz, H-7), δ7.20 (d, 1H, *J*=9.15Hz, H-6), δ6.78 (d, 1H, *J*=10.3Hz, H-10), δ6.77 (dd, 1H, *J*=10.3&17.2Hz, H-2'), δ5.63 (d, 1H, *J*=10.3Hz, H-11), δ5.24 (d, 1H, *J*=17.2Hz, H-3b'), δ5.07 (d, 1H, *J*=10.3Hz, H-3a'), δ1.64 (s, 6H, H-4'&H-5'), δ1.51 (s, 6H, H-13&H-14). ^{13}C NMR (125 MHz, $CDCl_3$) δ181.4 (C-9), δ159.4 (C-3), δ156.7 (C-1), δ155.8 (C-2'), δ154.0 (C-4a), δ145.4(C-5), δ144.2 (C-5a), δ127.4 (C-11), δ124.2 (C-7), δ120.5 (C-8a), δ119.7 (C-6), δ116.1 (C-8), δ116.0 (C-10), δ113.2 (C-4), δ105.6 (C-2), δ104.1 (C-3'), δ103.6 (C-9a), δ78.4 (C-12), δ41.4 (C-1'), δ28.3 (C-4'&C-5'), δ28.0 (C-13&C-14).

Calanolide E2 (5). Yellowish oil. UV λ_{max} : 2952, 1624, 1128 (EtOH) nm : 311, 297, 281, 240 EI-MS m/z: 388, 329, 271, 215, 107. ^{1}H NMR (500MHz, $CDCl_3$): δ12.37 (s, 7-OH), δ6.59 (d, 1H, *J*=10.31Hz, H-9), δ5.45 (d, 1H, *J*=10.31Hz, H-10), δ4.50 (m, 1H, H-3'), δ3.68 (m, 1H, H-4), δ2.84 (dd, 1H, *J*=9.16&14.89Hz, H-3a), δ2.67 (dd, 1H, *J*=9.16&14.89Hz, H-3b), δ2.53 (m, 1H, H-2'), δ1.81 (m, 1H, H-14a), δ1.46 (m, 1H, H-14b), δ1.43 (s, 3H, H-12), δ1.35 (s, 3H, H-13), δ1.34 (d, 3H, *J*=5.73Hz, H-4'), δ1.15 (m, 2H, H-15), δ1.12 (d, 3H, *J*=6.87Hz, H-5'), δ0.85 (t, 3H, *J*=6.87Hz, H-16). ^{13}C NMR (125 MHz, $CDCl_3$) δ201.1 (C-1'), δ179.3 (C-2), δ160.1 (C-8a), δ159.9 (C-5), δ157.3 (C-7), δ125.7 (C-10), δ115 (C-9), δ108 (C-4a), δ102.6 (C-6), δ101.0 (C-8), δ78.2 (C-11), δ76.1 (C-3'), δ44.2 (C-2'), δ38.6 (C-3), δ35.5 (C-14), δ30.5 (C-4), δ28.5 (C-13), δ28.1 (C-12), δ20.8 (C-15), δ16.3 (C-4'), δ14.0 (C-16), δ9.3 (C-5').

RESULTS AND DISCUSSION

Trapezifolixanthone (1) (98.9 mg) was isolated as yellow needle crystals with a melting point of 145-148ºC (literature 140-142ºC, Daud et al., 2014). The EIMS spectrum showed a molecular ion peak at 378, which is consistent with the molecular formula $C_{23}H_{22}O_5$. The ion fragment peak at m/z 363 was due to the loss of a methyl group. The fragment ion peak at m/z 154 was due to the loss of a pyrano ring and the prenyl group that are attached to the xanthone skeleton.

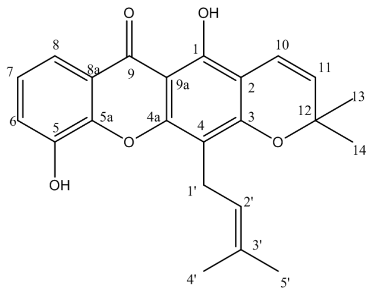
The FTIR spectrum for compound 1 gives typical IR absorptions for xanthones at 3196 cm^{-1}, 1629 cm^{-1}, 1575 cm^{-1}, and 1127 cm^{-1}. The strong absorptions at 3196 cm^{-1} and 1629 cm^{-1} were representative for hydroxyl and conjugated carbonyl stretching. Meanwhile, the absorption at 1575 cm^{-1} was due to the stretching of an aromatic group.

The ^{1}H NMR spectrum for compound 1 exhibited the presence of one chelated hydroxyl group at δ13.05 (OH-1). The presence of a 3-methylbut-2-enyl substituent in compound 1 was indicated by the ^{1}H NMR signals at δ5.23 (t, 1H, *J*=5.73Hz, H-2'), δ3.49 (d, 2H, *J*=5.73Hz, H-1'), δ1.85 (s, 3H, H-5') and δ1.71 (s, 3H, H-4'). In the COSY experiment, the nature of the allylic coupling systems within the prenyl moiety was clearly demonstrated. It showed COSY couplings between the olefinic proton C-2' and the benzylic proton of C-1'. The signal at δ3.49 (H-1') in the proton NMR showed a long range coupling with the carbon signal at δ107.1 (C-4) , δ153.8 (C-4a) and δ158.3(C-3) in the HMBC spectrum hence confirming the prenyl unit to be positioned at C-4.

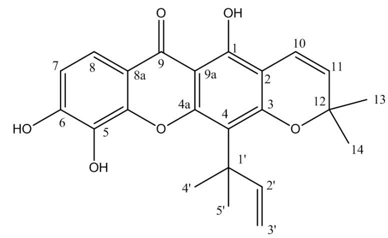
The ^{1}H NMR spectrum for compound 1 also revealed the presence of a pyrano ring attached to the xanthone skeleton. The ring ^{1}H NMR spectrum shows a pair of ortho-coupled proton with a coupling constant value of 10.3Hz at δ6.74 and δ5.61 for H-11and H-10. The HMBC spectrum shows a cross peak for the proton signal at δ1.47 (H-13 and H-14) with the carbon signal at δ78.4 (C-12). This indicates the two methyls to be directly bonded to the carbon at δ78.4 (C-12). The ^{2}J correlation of the proton at δ5.61 (H-10) with the carbon signal at δ104.8 (C-2) suggests the pyrano ring to be attached to the carbons at δ104.8 (C-2) and δ158.3 (C-3).

The ^{13}C NMR and DEPT spectra for (1) exhibited 23 carbon signals, which consist of four methyls, one methylene, six methines and eleven quaternary carbons including one carbonyl signal at δ181.1 (C-9). The carbons at δ181.1 (C-9), δ158.3 (C-3), δ156.1 (C-1), δ153.8 (C-4a), δ144.5 (C-5a) and δ144.3 (C-5) were shifted downfield due to the electronegative element oxygen, making them more deshielded. The carbon at δ181.1 (C-9) peak is generally weak due to slow relaxation of the quaternary carbon and is highly deshielded.

The COSY spectrum for (1) shows protons coupled to each other, indicating the positions of adjacent protons. One doublet and triplet signals with coupling constant 8.02Hz were observed at δ7.73 (H-8) and δ7.23 (H-7) indicating the ortho-coupled proton in a benzene ring. The meta-coupled protons in the left benzene ring at δ7.73 (H-8) and δ7.28 (H-6) signal were assigned to



(1)



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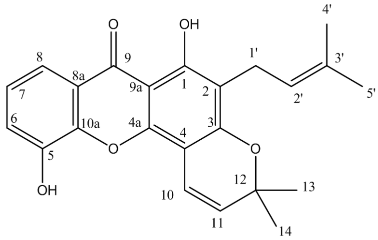

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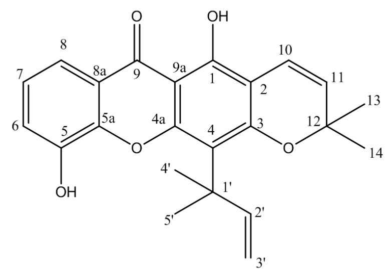

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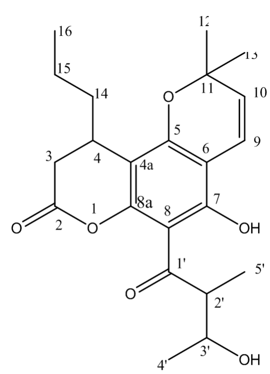

(5)

Figure 1. Structures of xanthones and coumarin

their respective carbons at signals δ116.9 (C-8) and δ119.9 (C-6) via the HMQC spectrum.

Based on the information given by the 1D and 2D NMR data, compound 1 was identified as trapezifolixanthone previously isolated from *Calophyllum hosei* (Daud et al., 2014).

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Table 1
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$)

Position	δ_H	δ_C	HMBC, δ
1	13.05 (s, OH)	156.1	156.1 (C-1), 104.8 (C-2)
2		104.8	
3		158.3	
4		107.1	
4a		153.8	
5	5.74 (s, OH)	144.3	144.3 (C-5), 119.9 (-6)
5a		144.5	
6	7.28 (d, 1H, *J* = 8.02Hz)	119.9	144.3 (C-5), 116.9 (C-8)
7	7.23 (t, 1H, *J* = 8.02Hz)	124.0	144.3 (C-5), 120.9 (C-8a)
8	7.73 (d, 1H, *J* = 8.02Hz)	116.9	144.5 (C-5a), 119.9 (C-6)
8a		120.9	
9		181.1	
9a		103.1	
10	5.61 (d, 1H, *J* = 10.3Hz)	127.6	104.8 (C-2), 78.4 (C-12)
11	6.74 (d, 1H, *J* = 10.3Hz)	115.8	158.3 (C-3), 78.4 (C-12)
12		78.4	
13	1.47 (s, 6H)	28.4	127.6 (C-10), 78.4 (C-12)
14			28.4 (C-13&C-14)
1'	3.49 (d, 2H, *J* = 5.73Hz)	21.8	158.3 (C-3), 153.8 (C-4a), 131.7 (C-3'), 122.7 (C-2'), 107.1 (C-4)
2'	5.23 (t, 1H, *J* = 5.73Hz)	122.7	25.7 (C-4'), 18.0 (C-5')
3'		131.7	
4'	1.71 (s, 3H)	25.7	131.7(C-3'),122.7(C-2'), 18.0(C-5')
5'	1.85 (s, 3H)	18.0	131.7(C-3'),122.7(C-2'), 25.7(C-4')

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