Asian Journal of Chemistry; Vol. 23, No. 1 (2011), 74-76

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Chemical Constituents of *Artocarpus kemando* (Moraceae)

S.H. TEO[1], G.C.L. EE[1,*], C.K. LIM[2], M. RAHMANI[1] and C.F.J. BONG[3]

[1]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Faculty of Engineering & Science, Universiti Tunku Abdul Rahman, Jalan Genting Klang, 53300 Kuala Lumpur, Malaysia
[3]Department of Crop Science, Faculty of Agriculture & Food Sciences, Universiti Putra Malaysia, Bintulu Campus, 97008 Bintulu, Sarawak, Malaysia

*Corresponding author: Fax: +60 389435380; Tel: +60 389466785, E-mail: gwen@science.upm.edu.my

(*Received*: 9 November 2009; *Accepted*: 21 August 2010) AJC-8990

The stem bark of *Artocarpus kemando* furnished 24-methylenecycloartenyl acetate (fatty acyl ester) (**1**) together with cycloartobiloxanthone (**2**). Compound **1** was obtained for the first time from this species. Structures of both compounds were determined using NMR and MS analysis. A cytotoxic study showed that the crude extract of the stem bark of *Artocarpus kemando* was significantly active against the HL-60 (human promyelocytic leukemia cell) and IMR-32 (human neuroblastoma cell) cell lines. This is the first report on the cyctotoxic activity of *Artocarpus kemando*.

Key Words: *Artocapus kemando*, Moraceae, Triterpenoid, Xanthone, Cytotoxicity.

INTRODUCTION

The genus *Artocarpus* from the Moraceae family which consists of approximately 50 species is widely distributed in the tropical and subtropical regions, including Indonesia[1,2]. Various triterpenes have been previously reported from the genus *Artocarpus*[3]. A majority of the phenolic compounds from this species are primarily flavonoids, apart from the stilbenoids and 2-arylbenzofuran[4]. Flavonoids from this plant and other species of *Artocarpus* have been shown to exhibit antiinflammatory[5], antioxidative[6], antiplatelet aggregation[7] 5α-reductase activities[8], inhibition of cathepsin K[9] and cytotoxicity[10]. These activities are mainly contributed by the phenolic constituents from the plant. The wood is locally used as light-weight hardwood, especially for household utensils and the fruits are edible. This paper reports the isolation and the identification of 24-methylenecycloartenyl acetate (**1**) and cycloartobiloxanthone (**2**) from the stem bark of *Artocarpus kemando* as well as the cytotoxic activities of the extracts.

EXPERIMENTAL

Melting points were determined on a melting point apparatus and are uncorrected. IR spectra were recorded in KBr pellet on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. Mass spectral measurement was obtained on a Shimadzu GCMS-QP5050A spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using TMS as internal standard on a Joel JNM-LA 400 spectrometer at 400 and 100 MHz, respectively. VLC was carried out using Merck Kieselgel 60 PF_{254} (containing gypsum), gravity liquid chromatography with Merck Kieselgel 60 Mesh 230-400 (40-63 μ) and TLC analysis on precoated Si-gel plates (Merck Kieselgel 60 F_{254}, 0.25 mm).

RPMI-1640, fetal bovine serum (FBS), penicillin and streptomycin. Typsin blue, 3-(4,5-dimethulthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were bought from Roche Diagnostics, USA as well as dimethyl sulphoxide (DMSO). Cisplatin, doxorubicin and colchicines were obtained from a chemical supplier.

Samples of the stem bark of *Artocarpus kemando* was collected in May 2007, from Sri Aman, Sarawak in Malaysia.

Extraction and isolation: The milled, air-dried stem bark (4.7 kg) was extracted exhaustively with *n*-hexane and then with ethanol. The *n*-hexane and ethanol extracts, on removal of solvent under reduced pressure, gave light brown residues, weighing 23 and 50 g, respectively. The ethanol extract of *Artocarpus kemando* was chromatographed on a silica gel vacuum column chromatography using a stepwise gradient system (hexane/$CHCl_3$, $CHCl_3$/EtOAc, EtOAc/Me_2O and MeOH) to give 20 fractions **4-6** which appeared as a yellow sticky liquid were combined. It was boiled with methanol and cooled. This afforded 24-methylenecycloartenyl acetate (50 mg) (**1**) after repeated washing with methanol. Recrystallization from methanol afforded a white solid. Combination and purification of fractions **13-16** by column chromatography (SiO_2;

n-hexane/$CHCl_3$, $CHCl_3$/Me_2O and Me_2O gradient) gave subfractions **8** and **10** which showed promising spots on the TLC plate. Elution with $CHCl_3$ and $CHCl_3$/Me_2O (19:1) on both fractions afforded cycloartobiloxanthone (**2**). Structure of compounds **1** and **2** were elucidated using IR, NMR and MS data.

Cell cultures: Two human cancer cell lines were used. They were HL-60 and the IRM-32 cell lines. The HL-60 cell line was obtained from National Cancer Institute (NCI), USA and IRM-32 was provided by Health Science Research Resources Bank, Japan. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5 % fetal bovine serum (FBS), 100 μ mL^{-1} penicillin and 100 g/mL streptomycin by using 25 cm^2 flask in a 37 ºC incubator with 5 % CO_2.

Cytotoxicity assay: The exponential growing HL-60 and IRM-32 cells were obtained by plating each well with 100 μL of stock culture (1×10^5 cells/mL) in 96-well μL plate and incubated at 37 ºC for 24 h. The stock solution was prepared at a concentration of 10 mg/mL by dissolving 10 mg of sample (compound) in 10 mL of DMSO. Serial dilution of the stock solution in the growth medium provided seven sample solutions at concentrations of 2.5, 5.0, 7.5, 10.0, 20.0, 30.0 and 40.0 μg/mL. The growth medium was removed from the wells, washed well and the treated with 100 μL of varying concentration of sample solution. Controls were made containing only untreated cell population in 100 μL of growth medium.

MTT Assay: HL-60 and IRM-32 cells growing exponentially in 96-well micro plate were treated with a range of concentrations (2.5-40.0 μg/mL) of each sample for 3 days. Each concentration of sample was performed in triplicate and the culture plate was incubated for 3 days at 37 ºC, 5 % CO_2 and 90 % humidity. After treatment, 10 μL of the MTT labeling reagent (0.5 mg/mL) was added to each well. The plate was then incubated further for 4 h at 37 ºC with 5 % CO_2. After that 100 μL of the solubilized solution was added to each well and the plate was allowed to stand overnight in the incubator at 37 ºC with 5 % CO_2. Absorbance was measuared using ELISA spectrophotometer (EL_x 800) at wavelength 550 nm. The percentage of MTT reduction was calculated comparing the absorbance of treated cells to that of untreated control cells.

24-Methylenecycloartenyl acetate (1): White solid; m.p. 116-119 ºC (lit.[11] 114-117 ºC); IR (KBr, ν_{max}, cm^{-1}): 2942 (OH), 1734 (C=O group of an ester), 1456 (C=C), 1374, 1248, 1094 (C-O); EIMS m/z: 483 $[M]^+$, 422, 408, 175, 125, 109, 95, 81, 69; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.57 (1H, m, H-3), 0.97 (1H, s, H-18), 0.34 (1H, d, *J* = 3.7, H-19a), 0.58 (1H, d, *J* = 3.7, H-19b), 0.90 (1H, d, *J* = 4.6, H-21), 1.04 (1H, d, *J* = 1.8, H-26), 1.03 (1H, d, *J* = 2.8, H-27), 0.90 (1H, d, *J* = 4.6, H-28), 0.85 (1H, d, *J* = 4.6, H-29), 0.85 (1H, s, H-30), 4.66, 4.71 (2H, s, H-31) and ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.57 (C-1), 25.78 (C-2), 80.63 (C-3), 39.41 (C-4), 47.12 (C-5), 20.90 (C-6), 28.12 (C-7), 47.80 (C-8), 20.90 (C-9), 25.92 (C-10), 26.46 (C-11), 35.50 (C-12), 45.25 (C-13), 48.77 (C-14), 32.82 (C-15), 26.77 (C-16), 52.21 (C-17), 17.95 (C-18), 29.75 (C-19), 36.10 (C-20), 18.28 (C-21), 34.95 (C-22), 31.29 (C-23), 156.83 (C-24), 33.76 (C-25), 21.85 (C-26), 21.98 (C-27), 19.27 (C-28), 15.12 (C-29), 25.39 (C-30), 105.92 (C-31'), 21.31 ($\underline{CH_3}$-CO), 170.95 (CH_3-$\underline{CO}$).

Cycloartobiloxanthone (2): Dark yellow solid; m.p. 271-273 ºC (lit.[12] 270 ºC) IR (KBr, ν_{max}, cm^{-1}): 3434 (brs), 2976, 1650, 1476, 1358, 1272; EIMS m/z (rel. int.): 434 $[M]^+$, 421, 419, 391, 377, 361, 347, 332, 293, 203, 189, 177, 152, 135, 105, 91, 77, 69, 54. ^{1}H and ^{13}C NMR data (Table-1).

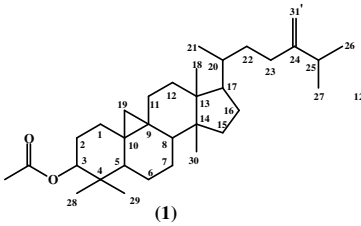
TABLE-1
^{1}H AND ^{13}C NMR DATA FOR 2 (400 AND 100 MHz, ACETONE-d_6)

Position	^{1}H (δ)	^{13}C (δ)	HMBC correlations
2	–	161.4	
3	–	112.7	
4	–	181.4	
4a	–	105.3	
5	–	162.6	
6	6.11 (1H, s)	99.9	162.6(C-5), 159.5(C-7), 101.8(C-8), 105.3(C-4a)
7	–	159.5	
8	–	101.8	
8a	–	152.0	
9	6.90 (1H, d, *J* = 10.0 Hz)	115.9	159.5(C-7), 152.0(C-8a), 78.7(C-11)
10	5.65 (1H, d, *J* = 10.0 Hz)	127.9	101.8(C-8), 78.7(C-11)
11	–	78.7	
12	1.43 (3H, s)	28.4	127.9(C-10), 78.7(C-11), 28.2(C-13)
13	1.43 (3H, s)	28.2	127.9(C-10), 78.7(C-11), 28.4(C-12)
14	2.34 ($1H_b$, t, *J* = 15.6 Hz) 3.19 ($1H_a$, dd, *J* = 15.6, 7.3 Hz)	20.3	161.4(C-2), 112.7(C-3), 181.4(C-4), 47.5(C-15), 133.7(C-2') 161.4(C-2), 112.7(C-3), 47.5(C-15), 93.7(C-16)
15	3.40 (1H, dd, *J* = 15.6, 7.3 Hz)	47.5	20.3(C-14), 93.7(C-16), 22.8(C-17), 28.2(C-13),133.7(C-2')
16	–	93.7	
17	1.31 (3H, s)	22.8	47.5(C-15), 93.7(C-16), 28.2(C-13)
18	1.63 (3H, s)	28.2	47.5(C-15), 93.7(C-16), 22.8(C-17)
1'	–	104.9	
2'	–	133.7	
3'	–	137.9	
4'	–	146.9	
5'	6.39 (1H, s)	105.3	104.9(C-1'), 137.9(C-3'), 146.9(C-4'), 151.5(C-6')
6'	–	151.5	
1-OH	13.36 (1-OH, s)	–	

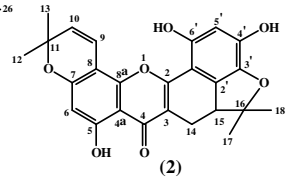
RESULTS AND DISCUSSION

Compound (**1**) was obtained from the ethanol extract as a white solid with m.p. 116-119 ºC (lit.[11] 114-117 ºC). The compound showed signals corresponding to a cyclopropane ring, characteristic of a cycloartane skeleton in the NMR spectrum. It gave a molecular mass of 482 which corresponded to the molecular formula $C_{33}H_{54}O_2$.

The ^{13}C NMR assignment of (**1**) was made by performing HSQC and DEPT NMR experiments and by comparison with the corresponding data of (**1**) from *Euphorbia broteri*[11]. The ^{13}C NMR spectrum also showed a total of 33 resonances, comprising 8 methyls, 12 methylenes, 6 methines and 7 quaternary carbon atoms which are in agreement with the molecular formula.



(1)



(2)

Based on literature search, this is the first isolation of 24-methylenecycloartenyl acetate (**1**) from this *Artocarpus* species. This compound was further confirmed by comparison of spectroscopic data with literature values[11].

Compound (**2**) was purified as a least polar yellow pigment with R_f value 0.39 in $CHCl_3/Me_2O$ (4:1), m.p. 271-273 ºC (lit.[12] 270 ºC). Mass spectrometry revealed a $[M]^+$ m/z 434 and analyzed for the molecular formula $C_{25}H_{22}O_7$. The IR spectrum showed hydroxyl and xanthone carbonyl absorptions at 3434 and 1650 cm^{-1}, respectively.

The ^1H NMR spectrum of (**2**) exhibited a low field singlet signal for a chelated hydroxyl group at δ 13.36 (1-OH). The superimposed singlet at δ 1.43 (6H, s) indicated 2 chromene methyl groups, together with 2 doublet signals at δ 6.90 (1H, d, J = 10.0 Hz) and δ 5.65 (1H, d, J = 10.0 Hz), each due to olefinic protons indicating the presence of a 2,2-dimethyl chromene. Other than that, 2 singlet signals at δ: 6.11 and 6.39 are assigned to H-6 and H-5', respectively. Furthermore, the upfield singlet signals at δ 1.31 (3H, s, H-17) and δ 1.63 (3H, s, H-18) were, respectively attributed to two tetrahydrofuran methyl groups.

The ^{13}C NMR spectrum showed one signal of a conjugated carbonyl group at δ 181.4, signals of 12 substituted aromatic carbons at δ: 161.4 (C-2), 112.7 (C-3), 105.3 (C-4a), 162.6 (C-5), 159.5 (C-7), 101.8 (C-8), 152.0 (C-8a), 104.9 (C-1'), 133.7 (C-2'), 137.9 (C-3'), 146.9 (C-4') and 151.5 (C-6') which were assigned accordingly based on information from the HMQC experiment. Meanwhile, 2 signals at δ: 99.9 and 105.3 were assigned to 2 protonated aromatic carbons which are C-6 and C-5', respectively.

The DEPT spectrum showed the presence of 25 carbons which consisted of four methyl carbons, one methylene carbon, four methine carbon and 16 quartenary carbons. The signal for the carbonyl group at δ 181.4 was assigned to C-4.

In the HMBC spectrum, the singlet at δ 6.11 gave cross peaks with 4 carbon signals at δ: 105.3, 162.6, 159.5 and 101.8, which further confirmed the assignment of δ: 6.11 to H-6. Besides that, the location of the two tetrahydrofuran methyl groups (17-CH_3 and 18-CH_3) were further elucidated by the long-range ^3J correlations between δ 1.63 (methyl proton at H-18) with δ 47.5 (C-15) and δ 93.7 (C-16) and δ 22.8 (C-17) *via* ^2J and ^3J correlations, respectively. The presence of the pyrano ring attached to C-7 and C-8 was deduced from the long-range connectivity between the doublet at δ 6.90 (H-9) and δ 78.7 *via* a ^3J correlation and between δ 5.65 (H-10) and δ 101.8 also *via* a ^3J correlation in the HMBC spectrum. Hence, the signals at δ: 78.7 and 101.8 were assigned to C-11 and C-8, respectively and δ: 5.65 and 6.90 to H-10 and H-9, respectively. This assignment was further confirmed by the coupling between δ: 6.90 and 5.65 in the ^1H-^1H COSY experiment. Compound **2** was therefore assigned cycloartobilixanthone. The spectral data are summarized in Table-1.

Cytotoxic activities towards the HL-60 and IMR-32 cell lines were conducted. All the crude extracts indicated cytotoxicities towards both cell lines. The acetone extract gave LC_{50} values of 15 and 13 μg mL^{-1} towards the HL-60 and IMR-32 cell lines, respectively. The ethanol extract on the other hand gave a much lower LC_{50} value of 12 μg mL^{-1} towards the IMR-32 cell line. However, the methanol extract showed a slightly higher LC_{50} value of 17.5 μg mL^{-1} towards the HL-60 cell line. The cytotoxic results of the crude extracts are summarized in Table-2.

TABLE-2
CYTOTOXIC ACTIVITIES OF ETHANOL, ACETONE AND METHANOL EXTRACT AGAINST HL-60 AND IMR-32 CELL LINES

	IC_{50} Value (μg mL^{-1})	
Compounds	HL-60	IMR-32
Ethanol extract	31.5	12.0
Acetone extract	15.0	13.0
Methanol extract	17.5	N/A

N/A: Not active.

ACKNOWLEDGEMENTS

The authors wish to thank Associate Professor Dr. Jegak Uli for collection of plant samples and the Ministry of Higher Education for financial support under the FRGS research fund.

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