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Identification of Cytotoxic Compound from *Artocarpus communis* Leaves Against P-388 Cells

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Abstract: In the course of continuing research for finding bioactive compounds from Indonesian plants, the leaves of *Artocarpus communis* was extracted by ethanol. This extract partitioned with *n*-hexane-water (1:4) and then water extract was partitioned with dichloromethane. Dichloromethane extract was purified by column chromatography techniques on silica gel to afford yellow crystal (F-1). Based on LC-MS, ^{1}H-NMR and ^{13}C-NMR (1D and 2D) spectra and compared with previous spectral data, it was identified as prenylated flavonoid, 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl] 1-propanone. This compound showed significant cytotoxicity against murine P-388 leukemia cells.

Key words: *Artocarpus communis*, prenylated flavonoid, cytotoxicity

INTRODUCTION

The genus *Artocarpus* (Moraceae), an exceptionally rich source of prenylated flavonoids, consists of approximately 50 species that are indigenous to the region of Southeast Asia, including Indonesia. Different compounds isolated from some species of *Artocarpus* have been shown to exhibit interesting biological properties (Lemmens *et al*., 1995; Nomura *et al*., 1998). Some of these compounds show interesting biological activities, such as cytotoxic (Nomura and Hano, 1998), antimalarial activity (Boonlaksiri *et al*., 2000), inhibition of tyrosinase and melanin biosynthesis (Likhitwitayawuid and Stritularak, 2001; Shimizu *et al*., 1998) and of 5α-reductase (Shimizu *et al*., 2000). Thus, in a continuation of our studies on the chemistry of Indonesian plants, the chemical constituents of *A. communis* have been investigated. In this paper, we report the isolation, structure elucidation and biological evaluation of prenylated flavonoid from dichloromethane extract of the leaves of this species. The structure of this compound was elucidated on the basis of spectroscopic data including 2-D NMR. The isolated compound exhibited cytotoxicity against P-388 cells.

MATERIALS AND METHODS

This research was conducted at Natural Product and Pharmaceutical Laboratory, Research Centre for Chemistry, Indonesian Institute of Sciences (LIPI) on 2006-2007.

General experimental procedures: ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM ECA-500 spectrometer, operating at 500 MHz (^{1}H-) and 125.76 MHz (^{13}C-), using TMS (Tetra Methyl Silane) as an internal standard. MS were obtained with Mariner Biospectrometry Spectrometer using ESI System (Electro Spray Ionization) and positive ion mode. Column chromatography was carried out using Merck Silica gel 60 (70-230 mesh ASTM) and TLC (Thin Layer Chromatography) analysis on precoated Silica gel plates (Merck Kieselgel 60 F 254, 0.25 mm).

Plant material: Sample of the leaves of *Artocarpus communis* was collected in March 2006, from plantation trees growing in Parung, Bogor, Indonesia. The plant was identified by staff at Biology Laboratory, Institute of Technology Bandung, West Java, Indonesia and a voucher specimen has been deposited at Biology Laboratory.

Extraction, isolation and identification: The dried leaves (4.95 kg) of *A. communis* were extracted exhaustively using macerator with ethanol 70%. The ethanol extracts (250 g) were concentrated using vacuum rotary evaporator and then partitioned with hexane-water (1:4). Water extracts added with dichloromethane and then dichloromethane extract was fractionated by column chromatography on silica gel using gradient elution (hexane-ethyl acetate), were resulted 80 fractions. Fractions 6-17 (F-1) were re-crystallized to give yellow crystal.

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F-1 was identified using LC-MS and NMR (^{1}H-NMR, ^{13}C-NMR, DEPT 135, HMQC and HMBC) Spectrometer, to give prenylated flavonoid, named 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone.

Cytotoxicity assay (Alley *et al.*, 1988): P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3×10^4 cells cm^{-3}. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable concentrations of samples were prepared using PBS (phosphoric buffer solution, pH 7.30-7.65). Control wells received only DMSO. The assay was terminated after an 48 h incubation period by adding MTT reagent [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h of incubation was conducted. Optical density was read by using a microplate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (FM). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

RESULTS AND DISCUSSION

The dried leaves of *A. communis* were macerated with ethanol and the ethanol extract was fractionated by using hexane-water (1:4) and then dichloromethane. The product of dichloromethane extract was fractionated by column chromatography to give a number of fractions that contained a major compound. The LC-MS spectrum of compound F-1 gave an $[M]^+$ ion at 409.546 with a molecular formula of $C_{25}H_{28}O_5$ (Fig. 1). The analysis of its NMR data and comparison with reference showed in Table 1. From the ^{13}C-NMR spectrum, F-1 indicated 25 carbons, including 8 sp^2 methine carbons, 4 methylene carbons, 3 methyl groups and 10 quartenery carbons.

The ^{1}H-NMR spectra suggested the existence of two aromatic rings, ring A and B. At ring A have three proton aromatics, revealed a clear AMX system as indicated by the proton resonances at δ_H 6.38 (H-2, *d*, *J* 2.45 Hz) and 6.37 (H-4, *dd*, *J* 2.45 and 8.5 Hz) and at 7.54 (H-5, *d*, *J* 8.5 Hz) and 6.37 (1 H, *dd*, *J* 2.45 and 8.5 Hz). The down field chemical shift at 12.85 (*s*) indicated the presence of hydroxyl group formed a hydrogen bond with a carbonyl group (C-7) at 204.14 ppm. The multiplicity of carbons were assigned by the DEPT-135 experiment and correlation of the chemical H and C shift for all protonated carbons was determined based on the HMQC spectrum. The presences of the functional groups above were suggested by the long range coupling HMBC experiment as summarized in the Fig. 2. Partial structure A was confirmed by the presence of the long range coupling (HMBC correlation) of the aromatic proton H-2 at δ_H 6.38 to C-1, C-2, C-3, C-4 and C-6. The other aromatics proton H-4 at δ_H 6.37 and H-5 at δ_H 7.54 also indicated the presence of long range coupling between C-2, C-6 and C-1, C-7.

At ring B, there are *ortho*-coupled aromatic protons at δ_H 6.60 (H-11, *d*, *J* 8.5 Hz) and 6.72 (H-12, *d*, *J* 8.5 Hz). The presence of a methyl group in a singlet at δ_H 1.38

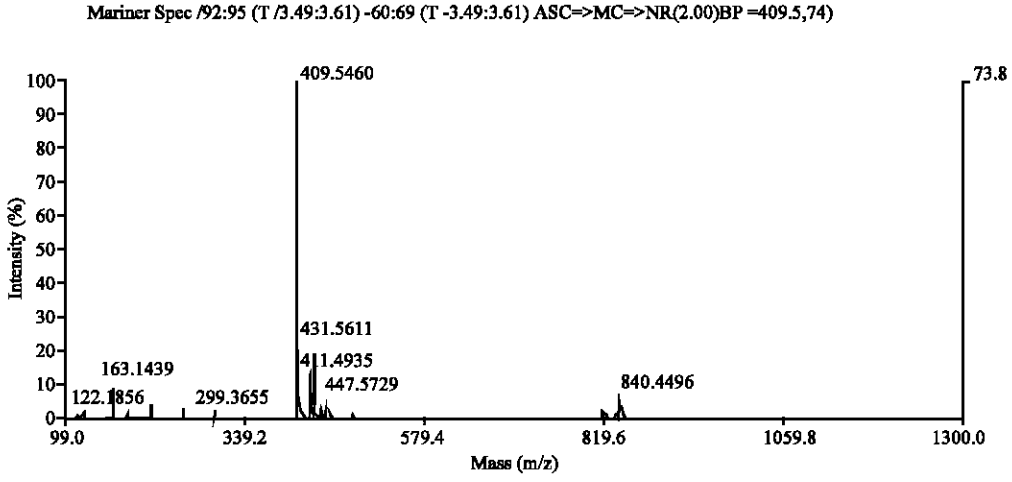

Fig. 1: LC-MS spectrum of compound F-1 from dichloromethane fraction of *A. communis* leaves

Table 1: Comparison of ^{13}C and ^{1}H NMR assignment between compound F-1 and reference

	^{13}C and ^{1}H NMR assignment from compound F-1		^{13}C and ^{1}H NMR assignment from reference (McLean *et al*., 1996)	
Carbon	δ_C (ppm)	δ_H (ppm) (ΣH, multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)
1	165.2		165.15	
2	103.62	6.38 (1H, *d*, 2.45)	103.51	6.37 (2.0)
3	163.48		163.14	
4	108.27	6.37 (1H, *dd*, 2.45; 8.5)	107.97	6.34 (8.8; 2.0)
5	132.45	7.54 (1H, *d*, 8.5)	132.27	7.55 (8.8)
6	113.66		113.59	
7	204.14		203.89	
8	39.77	3.1 (2H, *q*)	39.65	3.08 (*m*)
9	26.66	2.9 (2H, *m*)	26.49	2.97 (*m*)
10	128.08		127.91	
11	121.29	6.60 (1H, *d*, 8.5)	121.14	6.61 (8.2)
12	114.71	6.72 (1H, *d*, 8.5)	114.53	6.73 (8.2)
13	143.11		143.02	
14	139.72		139.56	
15	119.22		119.03	
16	119.49	6.54 (1H, *d*, 9.8)	119.36	6.54 (10.2)
17	130.3	5.63 (1H, *d*, 9.8)	130.12	5.63 (10.2)
18	78.9		78.76	
19	40.89	1.7 (2H, *m*)	40.77	1.70 (*m*)
20	22.94	2.09 (2H, *m*)	22.8	2.08 (*m*)
21	123.99	5.08 (1H, *t*)	123.84	5.08 (7.0)
22	132.1		131.95	
23	25.8	1.66 (3H, *s*)	25.66	1.65 (*bs*)
24	17.78	1.57 (3H, *s*)	17.64	1.56 (*bs*)
25	26.20	1.38 (3H, *s*)	26.07	1.38 (s)
1-OH		12.85 (1H, *s*)		12.81 (*s*)
3-OH				7.20 (*bs*)
13-OH				5.58 (*bs*)

Spectra compound F-1 recorded at 500 MHz for ^{1}H spectrum and 125 MHz for ^{13}C spectrum in $CDCl_3$ and reference's spectra recorded at 400 MHz. The values are in ppm and *J* values (Hz) in parentheses. Abbreviations for NMR signal are as follows: *s* = singlet, *d* = doublet, *t* = triplet. Correlation of chemical shift H and C were assigned, based on the HMQC spectra

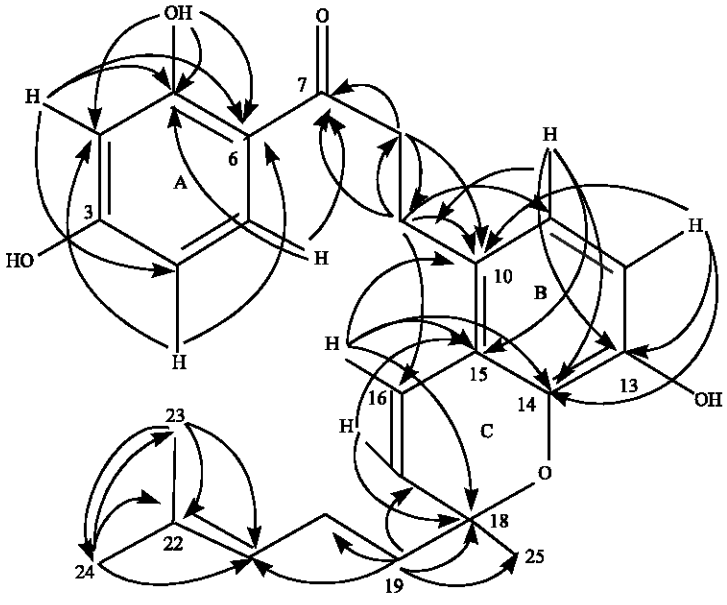

Fig. 2: HMBC experiment of compound F-1

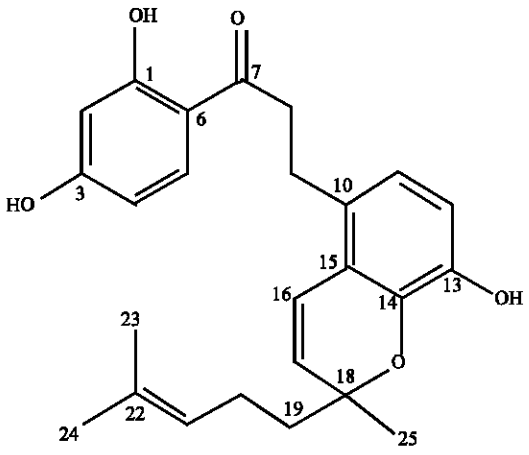

Fig. 3: Molecular structure compound F-1 [1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1- benzopyran-5-yl]-1-propanone]

(CH_3) and two cis-olefinic protons in doublets at δ_H 5.63 and 6.54 (each, *J* 9.8 Hz) and a quaternary carbon at δ_C 78.7 (*s*) implied the presence of the dimethylchromene group (ring C). The presence of two methylene group in the spectrum at δ_C 3.1 and 2.9 and the presence of long range coupling to carbonyl group C-7 at 204.14, indicated the presence of propanone group, which connected to ring A and B.

The ^{1}H-NMR spectrum data also indicated the presence of isoprenyl group at δ_H 5.08 (1H, *t*, H-21), 2.09 (2H, *m*, H-20), 1.7 (2H, *m*, H-19), 1.66 (3H, *s*, H-23), 1.57 (3H, *s*, H-24), 1.38 (3H, *s*, H-25). This isoprenyl was located in ring C based on the presence of long range coupling between H-19 at δ_H 1.7 to C-18 at δ_C 78.9 (*s*) and C-17 at δ_C 130.30 (*d*).

From 1D- and 2D- NMR spectrum, MS data and comparison with previous spectral data (Koshihara *et al*., 1988; McLean *et al*., 1996), compound F-1 was identified as prenylated flavonoid, named 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (Fig. 3).

The cytotoxicity of this compound was evaluated according to the method previously described (Alley *et al*., 1988). This compound exhibited significant active in the murine P388 leukemia cells bioassay with IC_{50} 6.7 μg mL^{-1}.

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