ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 8:4 (2014) 342-347

records of natural products

The New Alkaloids from *Antidesma cuspidatum* M.A.

Berna Elya[1], Roshamur C. Forestrania[1], Mat Ropi[2], Soleh Kosela[3], Khalijah Awang[2], Hanita Omar[2] and A. Hamid A. Hadi[2*]

[1]*Faculty of Pharmacy, University of Indonesia, Kampus UI Depok 16424, Indonesia*

[2]*Department of Chemistry, University Malaya, Kuala Lumpur, Malaysia*

[3]*Department of Chemistry, University of Indonesia, Kampus UI Depok 16424, Indonesia*

(Received October 18, 2013; Revised December 24, 2013; Accepted January 18, 2014)

Abstract: Two new alkaloids, cuspidatin (**1**) and cuspidatinol (**2**), were isolated from *Antidesma cuspidatum* M.A. (Euphorbiaceae). The structures were determined by means of spectroscopic analysis (IR, MS, and NMR spectroscopy). The results of cytotoxicity evaluation showed that compounds **1** and **2** were inhibitory to L1210 cells, with IC_{50} values 8.41 and 6.36 µg/mL respectively.

Keywords: *Antidesma cuspidatum* M.A.; Alkaloids; Euphorbiaceae; Cytotoxic activity.

1. Introduction

Antidesma is a tropical plants genus classified in family Phyllanthaceae (Malpighiales; Euphorbiaceae sensu lato). Most of the genus in South-East Asia is commonly found in the understorey of tropical forests as well as in open vegetation [1]. They have approximately 170 Antidesma species in the world including South-East Asia [2].

Antidesma cuspidatum M.A. is one of the species of Antidesma, which is largely distributed in Malaysia, Borneo, and Indonesia, especially in Sulawesi. The leaves of "Kenida punai", an Indonesia local name of *A. cuspidatum*, have been applied as traditional carminative medicine. The chemical research of genus Antidesma led to the isolation of new alkaloids. Recently, antidesmone, a bicyclic alkaloid, was isolated from Euphorbiaceae species of the East African plants, *A. membranaceum* Müll. Arg. and *A. venosum* E. Mey. Furthermore, belonging to this research, its structure has been revised into (S)-4,8-dioxo-3-methoxy-2-methyl-5-n-octyl-1,4,5,6,7,8-hexahydroquinoline [3]. The other research yield also report a discovery of other compounds indentified as alkaloids from Antidesma. [4,5,6]. These researches indirectly indicate a potential of *A. cuspidatum* as a species which is probably has alkaloid content as well as the other species of genus Antidesma reported.

Medicinal plants have been a useful source for the research of new biological active compounds. Apart from the medicinal effects of traditional herbs, exploratory researches have been made and a wide variety of new biological activities from traditional medicinal plants have recently been reported, including anticancer activity [7]. Based on scientific evidence of natural products in cancer therapy, now days, it had been known that natural resources are playing role of about 60% anticancer drugs [8]. For instance, cytotoxic activities were performed by the compounds from one of the species Antidesma. Four characterized alkaloid compounds of *A. theaitesiamnum* Müll. Arg.

*Corresponding author: E-Mail: berna.elya@farmasi.ui.ac.id; Phone: +628 138 3078647.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 07/05/2014 EISSN:1307-6167

exhibit cytotoxic effects in microgram per milliliter concentration range, with IC_{50} value 0.85, 0.78, and 0.95 µg/mL respectively. All of these compounds used can decrease in intracellular reactive oxygen species resulting in an increase percentage of early apoptotic cells [9]. The facts provide a probability of another species of genus Antidesma to extend cytotoxic activity related antioxidative properties of their alkaloid compound, including alkaloid compound of *A. cuspidatum*. Here we report a structure of alkaloid compound within bark of *A. cuspidatum* as a pilot study of this species. Isolated compound then applied to perform cytotoxicity assay using L1210 murine leukemia cell line. The activity was represented as IC_{50} (the concentration at which growth or activity is inhibited by 50% in certain period).

2. Materials and Methods

2.1. General

The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated chloroform on a JEOL 400 MHz instrument. Silica gel 60, 70–230 mesh ASTM (Merck 7734) was used for column chromatography. Mayer's reagent was used for alkaloid screening. TLC aluminium sheets (20 × 20 cm Silica gel 60 F_{254}) were used in the TLC analysis. The TLC spots were visualized under UV light (254 and 366 nm) followed by spraying with Dragendorff's reagent for an alkaloid detection.

2.2 Plant Material

The bark of *A. cuspidatum* (Euphorbiaceae), collected from Simpan Sungai Badak Forest, Sintok, Kedah, Malaysia in April 26th 2006, was identified by Mr Teo Leong Eng and Din. A voucher specimen (KL 5230) is deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia and at the Herbarium of the Forest Research Institute, Kepong, Malaysia.

2.3 Extraction and Isolation of the Alkaloid

The dried bark (2.5 kg) of *A. cuspidatum* (Euphorbiaceae) were extracted with hexane (25.0 *l*) for 18 hours. The residual plant material was dried and left for 2 h after moistening with 25% NH_4OH. Then, It macerated with CH_2Cl_2 (25.0 *l*) twice for 3-days. The extract CH_2Cl_2 was concentrated to give residue (7,0 g) that was subjected to column chromatography on silica gel (column dimension = 3 cm, length = 1 m, silica gel 60, 70–230 mesh ASTM; Merck 7734) using CH_2Cl_2 gradually enriched with methanol to yield 58 fractions. Fractions were then recombined on the basis of their TLC behavior to obtain 7 fractions. Fractions 4 (98 mg), afforded an alkaloid identified as cuspidatin (**1**), using PTLC (Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 9:1). Cuspidatinol (**2**) was obtained from fraction 5 (198 mg) by using PTLC (Merck KGaA silica gel 60 F_{254}; CH_2Cl_2-MeOH; 8.5:1.5).

2.4 In Vitro Cytotoxicity Assay

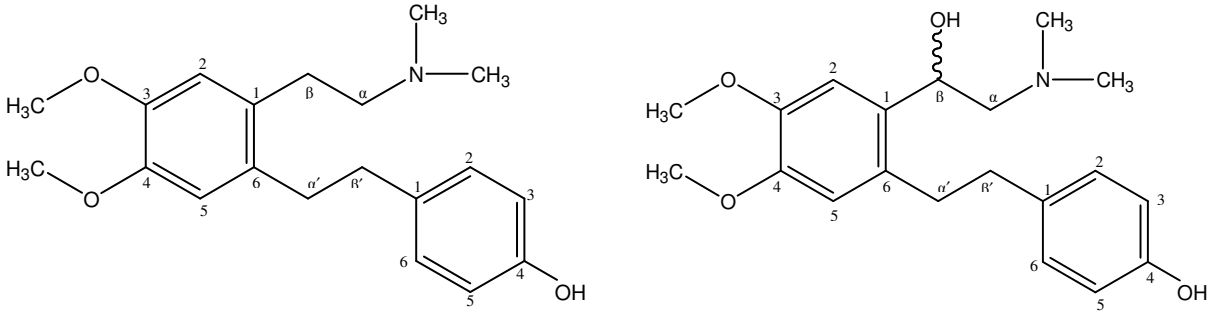
Isolate **1** and **2** were tested its cytotoxic activity against L1210 mouse leukemia cells by *in vitro* technique. L1210 cells were provided by Natural Product PATIR BATAN Laboratory acquired from The Institute of Physical and Chemical Research (RIKEN) Japan. RPMI-1640 was used as media containing glutamine and sodium bicarbonate. A total of 15 mL calf bovine serum was added into 85 mL of medium. All activities were done in a steril room. A total of media containing 2 x 10^5 cells/mL were incorporated into every hole in tissue culture plate. The sample were serially diluted in dimethyl sulfoxide to produce 5 concentrations and each concentration was added to plates in 3 replicates to obtain final isolates concentration of 0 (control), 1, 2, 4, and 8 µg/mL. The plates were incubated for 48 h in 5% CO_2 incubator at 37°C, then the number of cells were counted using Neubauer haemocytometer improved under a microscope. The percentages of inhibition were calculated by comparison of viable cells in the samples with viable cells in the control. The tryptan blue exclusion method was used to determine cell viability. The inhibitory percentage of the test was calculated to

obtain IC_{50}. The IC_{50} value (*inhibitory concentration fifty*) is the concentration of sample that inhibits the proliferation of leukemia L1210 cells in 50% can be obtained from linear regression of logarithmic of test concentration and probit of inhibition percentage. The smaller the IC_{50} value of a test, the higher its cytotoxic activity.

3. Results and Discussion

3.1. Structure elucidation

Cuspidatin or 4-[2-(2-(dimethylamino) ethyl)-4,5-dimethoxyphenethyl]phenol (Figure 1), noticed as compound **1**, was obtained as a brownish amorphous solid. The LC-MS spectrum the molecular ion peak at *m/z* 330.2077 $[M + H]^+$, thus suggesting formula of $C_{20}H_{27}NO_3$. IR spectrum emerged strong absorption at 3383 cm^{-1} indicating of hydroxyl group. The ^{1}H-NMR spectrum (Table 1) showed one proton singlet at δ 6.60 in aromatic region which is characteristic of 1,3,4,6-tetrasubstituted phenyl ring (ring A), with two methoxyl group (6H, s, δ 3.81). Another set of 2 H doublets at δ_H 6.83 and 6,72 is due to the 1,4-disubstituted benzene ring system ($\alpha_2\beta_2$, $J_{\alpha\beta}$ = 8.3 Hz, H-2′, H-6′ and H-3′, H-5′ respectively). The remaining resonances were for the aliphatic protons H-α and H-β observed as multiplets (due to possible conformational preferences) at δ_H 3.15 and 2.89 respectively; H-α′ and H-β′ (overlapped as a broad singlet) at 2.79, two NMe groups (6H, s, δ 2.47) , and one hydroxyl group at δ_H 4.76. The ^{13}C-NMR spectrum of compound **1** (Table 1) shows signals of 12 aromatic carbons constituting the two phenil ring, two methoxyl group (δ_C 55.94 and 56.01 ppm) four methylene of which one carbon (C-α 59.60 ppm) is N-linked and two other NMe groups. The characterization above is confirmed by the HMBC spectrum (Figure 2).



1 **2**

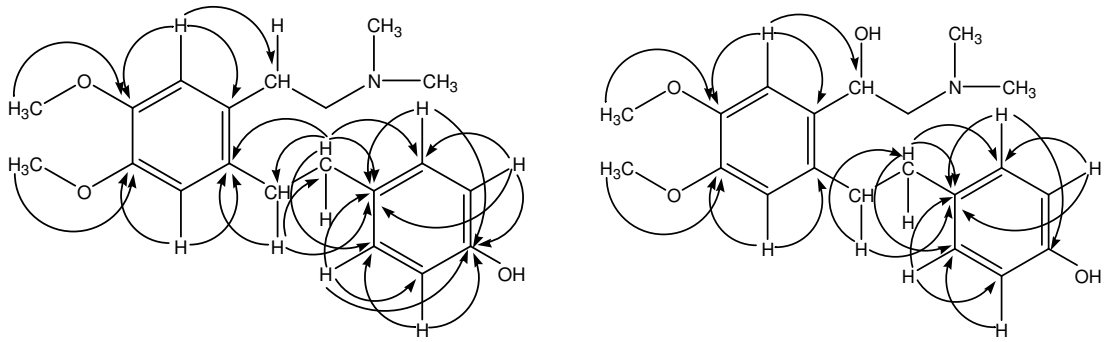
Figure 1. Structure of compounds **1** and **2** from *A. cuspidatum*.

Cuspidatinol or compound **2** (Figure 1) was isolated as a brownish amorphous solid with the molecular ion peak at *m/z* 346.2107 $[M + H]^+$ indicating formula of $C_{20}H_{27}NO_4$. IR spectroscopy data revealed the presence of hydroxyl at 3385 cm^{-1}. The comparison of ^{13}C-NMR and ^{1}H-NMR showed that the difference between compound **1** (Table 1) and **2** (Table 2) only at C-β of **1** which is replaced by hydroxyl group in compound **2**. Proton H-β revealed a singlet peak (torsional angle 90^o to H-α). The correlation of the protons and carbons which sustained the characterization of compound **2** was mentioned in HMBC spectral data (Figure 2).

Table 1. ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz), and HMBC spectroscopic data of compound **1** ($CDCl_3$, δ in ppm, *J* in Hz).

Position	Chemical shift (δ) (ppm)			HMBC
	^{1}H-NMR	^{13}C-NMR		
1	-	129.65	(C)	-
2	6.60 (*s*)	112.86	(CH)	1, 3, β
3	-	147.72	(C)	-
4	-	147.28	(C)	-
5	6.60 (*s*)	112.73	(CH)	4, 6
6	-	127.88	(C)	-
β′	2.79 *	31.93	(CH_2)	α′, 6, 1′, 6′, 2′
α′	2.79 *	37.30	(CH_2)	β′, 6, 1′
β	2.89 *	34.66	(CH_2)	-
α	3.15 *	59.60	(CH_2)	-
1′	-	132.67	(C)	-
2′	6.83 (*d*, *J* = 8.3 Hz)	131.95	(CH)	1′, 4′
3′	6.72 (*d*, *J* = 8.3 Hz)	115.58	(CH)	1′, 2′, 4′
4′	-	155.15	(C)	-
5′	6.72 (*d*, *J* = 8.3 Hz)	115.58	(CH)	6′, 4′
6′	6.83 (*d*, *J* = 8.3 Hz)	131.95	(CH)	1′, 5′, 4′
NMe $_{(2)}$	2.47 (*sb*)	43.76	(CH_3)	-
3-OMe	3.81 (*s*)	55.94	(CH_3)	3
4-OMe	3.87 (*s*)	56.01	(CH_3)	4
4′-OH	4.76 (*s*)	-		-
β-OH	-	-		-

Asterisks (*) mark overlapping signals



1 **2**

Figure 2. Key HMBC correlations (H → C) of compound **1** and **2**.

Table 2. ^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz), and HMBC spectroscopic data of compound **2** ($CDCl_3$, δ in ppm, *J* in Hz).

Position	Chemical shift (δ) (ppm)		HMBC
	^{1}H-NMR	^{13}C-NMR	
1	-	129.81 (C)	-
2	6.61 (*s*)	112.99 (CH)	1, 3, β
3	-	147.62 (C)	-
4	-	148.31 (C)	-
5	6.61 (*s*)	112.99 (CH)	4, 6
6	-	126.14 (C)	-
β′	2.81 *	26.06 (CH_2)	1′, 6′ 2′
α′	2.81 *	29.79 (CH_2)	β′, 1′
β	3.16 *	37.49 (CH_2)	-
α	2.91 *	34.72 (CH_2)	-
1′	-	129.81 (C)	-
2′	6.82 (*d*, *J* = 8.3 Hz)	132.04 (CH)	1′, 4′
3′	6.71 (*d*, *J* = 8.3 Hz)	115.84 (CH)	1′, 2′
4′	-	156.27 (C)	-
5′	6.71 (*d*, *J* = 8.3 Hz)	115.84 (CH)	6′
6′	6.82 (*d*, *J* = 8.3 Hz)	132.04 (CH)	1′, 5′
NMe $_{(2)}$	2,44 *sb*	56.30 (CH_3)	-
3-OMe	3.82*s*	56.04 (CH_3)	3
4-OMe	3.88*s*	57.73 (CH_3)	4
4′-OH	4.75*s*	-	-
β-OH	4.75*s*	-	-

Asterisks (*) mark overlapping signals

3.2 Cytotoxic activity

The compound **1** and **2** were evaluated their cytotoxic activity against L1210 mouse leukemia cells by in vitro technique. The results of cytotoxicity assessment showed that compound **1** and **2** exhibit cytotoxicity with IC_{50} 8.41 and 6.36 µg/mL respectively.

Acknowledgments

We thank to Center of Natural Product and Department of Chemistry, University Malaya, Kuala Lumpur Malaysia for the research grant.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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