3-EPIOCOTILLOL FROM THE BARK OF *AGLAIA SMITHII* (MELIACEAE) AND TOXIC ACTIVITY AGAINST *ARTEMIA SALINA* AND CYTOTOXIC ACTIVITY AGAINST MURINE LEUKEMIA CELLS *P-388*

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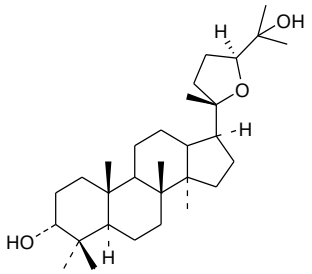
Abstract. *Aglaia smithii* is a higher plant belonging to Meliaceae family and widely distributed in South East Asia. Plants of Meliaceae family were known as a source of cytotoxic (anticancer) substances. In the course of our continuing research for biologically active compounds from Indonesian tropical plants, the methanol extract from bark of *Aglaia smithii* showed significant activity against brine shrimp (*Artemia salina*) and murine leukemia cells P-388. The methanolic extract from the dried bark of *A. smithii* was concentrated and extracted successively with n-hexane, methylene chloride and ethyl acetate. By using Brine Shrimp Lethality Test (BSLT) to follow the separation (bioassay guided isolation), the compounds of n-hexane extract was separated by combination of column and thin layer chromatography to yield a toxic compounds. The chemical structure of active compounds were determined by spectroscopic data and compared with those spectra data reported previously were identified as 3-epiocotillol **(1.)** The toxic activity of compounds (**1)** against brine shrimp (A. salina) were evaluated by Meyer method and showed activity with LC_{50} 13,4 µg/mL, respectively. The cytotoxic activity of compounds (**1)** against murine leukemia cells P-388 were evaluated by Alley Method and showed weak activity with IC_{50} 11 µg/mL.

Keywords: *Aglaia smithii; Meliaceae;* brine shrimp lethality test; murine leukemia cell P-388; 3-epiocotillol

INTRODUCTION

Plants belonging to the genus *Aglaia* are a rich source of tetracyclic triterpenoids of the cycloartane, dammarane and tirucallane series [1] In continuation of our work on plants belonging to this genus collected in Indonesia, we have isolated from the bark of *Aglaia smithii* the known dammaran triterpenoids *3-epiocotillol* (**1).**



(1)

Aglaia smithii is a higher plant belonging to Meliaceae family and widely distributed in South East Asia. Plants of Meliaceae family were known as a source of cytotoxic (anticancer) substances [2-4] . The methanol extract from bark of *A. smithii* showed significant activity against brine shrimp (*Artemia salina*) and murine leukemia cells P-388. This plant has not yet been subjected to any phytochemical or biological investigation. The methanolic extract from the dried bark of *A. smithii* was concentrated and extracted successively with n-hexane, methylene chloride and ethyl acetate. By using Brine Shrimp Lethality Test (BSLT) to follow the separation (bioassay guided isolation), the compounds of n-hexane extract was separated by combination of column and thin layer chromatography to yield two toxic compounds. The toxic activity of compounds (**1)** against brine shrimp *(A. salina)* were evaluated by Meyer method and showed activity with LC_{50} 13,4 µg/mL, The cytotoxic activity of compounds (**1)** against murine leukemia cells P-388 were evaluated by Alley Method and showed weak activity with IC_{50} 11 µg/mL.

EXPERIMENTAL SECTION

Materials

Bark material of *Aglaia smithii* Corr. was collected in Bogor Botanical Garden West Java Indonesia, during October 2006. The specimen was identified and deposited at the Herbarium Bogoriense West Java Indonesia.

Instrumentation

Rotavapor R-200 Buchi with vacum Vac V-500 Buchi. Fischer-Johns Melting Point Apparatus. Spektrofotometer FTIR Shimadzu 8400 and FTIR Spectrum One Perkin Elmer. Spektrofotometer NMR (Nuclear Magnetic Resonance) JEOL JNM ECA-500. Mariner Biospectrometry, Hitachi L 6200, sistem ESI (Electrospray Ionisation), *positive ion mode* and Shimadzu LCMS solution, *negative ion mode*. Bruker SMART APEX Diffractometer.

Procedure

Extraction and isolation

Dried bark of *A. smithii* (3 kg) were extracted exhaustively with methanol at room temperature. The extract (286 g) was diluted with methanol : water (8:2 v/v) and partitioned with *n*-hexane. The extract (13 g) was repeatedly chromatographed on

silica gel yielding compound **(1)** (51 mg) (1) n-hexane/ethyl acetate, 7 : 3, (2) *n*-heksana/aseton, step wise.

Bioassay

The toxic activity of compounds (**1**) against brine shrimp (*A. salina*) were evaluated by Meyer method and showed activity with LC_{50} 13,4 µg/mL. The cytotoxic activity of compounds (**1**) against murine leukemia cells P-388 were evaluated by Alley Method and showed weak activity with IC_{50} 11 µg/mL.

RESULTS AND DISCUSSION

The dammarane, 3-epiocotillol **(1)** were isolated from the n-hexane extract of *A. smithtii* bark by chromatography on silica gel. They have been found previously in *A. lawii* leaves [5]. However, they have been described in other genera of the family Meliaceae and were identified on the basis of comparison of their spectral data with literature values [1,5]. They all possess the same 20S,24-epoxy-25-hydroxy chain at C-17.

3-Epiocotillol

3-epiocotillol showed an $[M-H]^+$ peak at *m/z* 461 in the LC-MS, coresponding to molecular formula $C_{30}H_{52}O_3$.

Recent detailed NMR studies have unambiguously demonstrated that the 24R and 24S isomer can be easily distinguished by the resonances of C-24 (δC 83.2 for the R-isomer and δC 86.5 for the S-isomer) [5]. The chemical shifts and coupling patterns of the H-24 differ also from each other (δH3.7, J =7 and 7 Hz and δH 3.6, J = 10 and 5.5 Hz, respectively) (Table 1). Position hydroxyl group at C-3 and gemdimethyl at C-4 with HMBC correlation H-28/C-3, C-5, C-29; H-29/C-28,C-5,C-3. The IR showed the absorption of a hydroxyl group at 3300 cm^{-1}.

In the ^{13}C NMR appeared signal of the eight carbon methyl, ten carbon methylen, two carbon methyn, two carbon methyn oxygenated, two carbon quartener oxygenated and six carbon quartener, corresponding with pentacyclic structure for dammaran scheleton with tetrahydrofuran ring on the side chain. The1H NMR exhibited the signal an oxymethineat δ 3.63 typical of the H-24 in 20S,24S-epoxy-25-hydroxydammaranes. The 1D and 2D (COSY, HMQC, HMBC) NMR data were in agreement with the structure depicted in Figure 1, especially the diagnostic HMBC correlations Me-21/C-17, C-20,C-22 and Me-26, Me-27/C-24. C-20 was assigned the S configuration similar to most of the dammarane triterpenes particularly those isolated from the *Aglaia* genus

Table 1 ^{13}C (125 MHz) and ^{1}H NMR (500 MHz) data for 3-epiocotillol ($CDCl_3$)

Posisi C	□□C-NMR. □c (mult.)	^{1}H-NMR □H, (ΣH, mult., *J* (Hz))	HMBC (H→C)
1	33,8 (t)	1,4;1,44 (2H,m)	C-3,C-5
2	25,6 (t)	1,51(1H,m) ;1,57 (1H,m)	
3	76,5 (d)	3,39 (1H,t,J = 2,6)	C-1
4	37,5 (s)	-	
5	49,7 (d)	1,26 (1H,m)	C-4,C-10,C-29
6	18,4 (t)	1,41(2H,m)	C-4,C-8
7	34,9 (t)	1,29 (2H,m)	
8	40,8 (s)	-	
9	50,8 (d)	1,45 (1H,m)	C-8
10	37,8 (s)	-	
11	21,8 (t)	1,54 (2H,m)	C-13
12	27,2 (t)	1,15 (2H,m)	
13	43,0 (d)	1,61 (1H,m)	C-12,C-18
14	50,3 (s)	-	
15	31,6 (t)	1,45 (1H,m) ;1,06 (1H,m)	C-30
16	26,0 (t)	1,88 (2H,m)	C-20
17	50,0 (d)	1,47 (1H,m)	
18	16,3 (q)	0,96 (3H,s)	C-8,C-7,C-13,C-14
19	16,7 (q)	0,86 (3H,s)	C-1, C-5,C-10
20	86,8 (s)	-	
21	24,3 (q)	1,14 (3H,s)	C-17,C-20,C-22
22	35,4 (t)	1,23 (2H,m)	
23	26,5 (t)	1,76 (2H,m)	
24	86,4 (d)	3,63 (1H,dd,*J*=5,5 dan 10,4)	
25	70,4 (s)	-	
26	28,0 (q)	1,18 (3H,s)	C-24,C-25, C-27
27	24,2 (q)	1,11 (3H,s)	C-24,C-25,C-26
28	28,5 (q)	0,93 (3H,s)	C-3,C-4,C-5,C-29
29	22,3 (q)	0,83 (3H,s)	C-3,C-4,C-28
30	15,7 (q)	0,88 (3H,s)	C-7,C-8, C-14,C-15

CONCLUSION

1. The toxic and cytotoxic 3-epiocotillol was isolated from the stem bark of *Aglaia smithii* with bioactivity (Brine Shrimp Lethality Test) guided chromatografic fractionation.
2. The toxic activity of compounds (**1)** against brine shrimp (*A. salina*) showed activity with LC_{50} 13.,4 µg/mL, The cytotoxic activity of compounds (**1)** against murine leukemia cells P-388 showed weak activity with IC_{50} 11 µg/mL

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