An Insecticidal Compound from *Barringtonia asiatica*

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Abstract
One oleanane glycoside was successfully isolated from the seeds of *Barringtonia asiatica*. The structure of this compound was determined by one- and two- dimensional ^{1}H- and ^{13}C-NMR and also by direct comparison with standard compound. This compound showed the highest insecticidal activity against *Crocidolomia pavonana*. The result showed that *B. asiatica* seeds have the most active insecticidal compound with LC_{50} value of 290 ppm that is potential for natural insecticide application.

Keywords: *Barringtonia asiatica*, oleanane glycoside, insecticidal activity, *Crocidolomia pavonana*

Abstrak
Satu buah glikosida oleanan berhasil diisolasi dari biji *Barringtonia asiatica*. Struktur senyawa ini ditentukan oleh ^{1}H- dan ^{13}C-NMR satu dan dua dimensi serta oleh perbandingan langsung dengan standar. Senyawa ini menunjukkan aktivitas insektisida yang paling tinggi terhadap *Crocidolomia pavonana*. Dari penelitian ini diketahui bahwa biji *B. asiatica* mengandung senyawa insektisida yang paling aktif dengan nilai LC_{50} sebesar 290 ppm yang potensial untuk aplikasi insektisida.

Kata Kunci: *Barringtonia asiatica*, glikosida oleanan, aktivitas insektisida, *Crocidolomia pavonana*

Introduction

Currently, insecticidal compounds become very important substances in agricultural field, since they are needed for controlling insect pests. The harmful effect of synthetic insecticides on the environment has stimulated researches on finding new natural insecticidal compounds that more environmental friendly.

One of potential source of natural insecticidal compound is *Barringtonia asiatica*. The seeds of this plant have been used to stupefy fish and octopus in many Pacific islands (Etoh, 2001). Recent finding suggest that methanol extract from this plant

also has an insecticidal activity against *Crocidolomia pavonana* (Dono & Sujana, 2007).

The structure of active insecticidal compound from *B. asiatica* has not been investigated yet. Research investigating the structure of the active compound need to be carried out.

Therefore the objectives of this research is to isolate and determine the structure of insecticidal compound from *B. asiatica.*

Methodology

General

^{1}H-NMR (500 MHz), ^{13}C NMR (500 MHz), and 2D-NMR spectra were recorded in CD_3OD on a JEOL JNM A-500 spectrometer using TMS as internal standard Chromatographic separations were carried out on silica gel G60 and Chromatorex ODS adsorbens.

Plant Material

Plant materials used in this research were seeds of *Barringtonia asiatica*. Plant materials were taken from Kecamatan Jatinangor, Kabupaten Sumedang, West Java.

Bioindicator

Bioindicator used in this research was the second instar larvae of *Crocidolomia pavonana* Fabricius (age 2 hour after skin replacement). The larvae were taken from field colony developed in Propagation Room at Department of Plant Protection, Faculty of Agriculture, Padjadjaran University.

Isolation and Characterization

Fresh seeds of *B. Asiatica* (100 g) are washed, cutted into small pieces, and dried for several days. Dry plant materials were used as starting material for extraction. Maceration process was stopped until the extract has no color. Further, the yielded methanol extract was evaporated with rotary evaporator at temperature 55–60°C and at 580-600 mmHg to get crude methanol extract (40 g). Then, the methanol extract was applied to a column of Si-gel G60 by liquid vacuum chromatography method. Gradual elution was carried out with chloroform followed by various mixtures of

chloroform: MeOH (9.5:0.5, 9:1, 8.5:1.5, 8:2, 7.5:2.5, and 7:3), and chloroform-MeOH-H_2O (7:3:0.5). Total 17 fractions were collected and fractions giving similar spots on TLC were combined to give 7 fractions. The active F7 fraction (872 mg) were subjected to be rechromatographed over silica gel that were eluted with $CHCl_3$:MeOH:H_2O (7:3:0.5). The eluates were combined on the basis of TLC analysis to provide 6 fractions. Then, the active F73 fraction (100 mg) was subjected to C-18 gradient chromatography. Gradual elution was carried out with MeOH:H_2O (3:7) followed by the increasing MeOH by 5% until achieving MeOH:H_2O (7:3). The separation process resulted in the most active fraction, F734 (11 mg).

Bioassay

Insecticidal activity bioassay of sample against *C. pavonana* was carried out by residue method of leaves as reported by Prijono (2003). Sample was dissolved in methanol to get concentration of 1% and the mixture was added by alkylarylpolyglycoleter 400 $g.L^{-1}$ as attacher and Tween 80 as emulsifier for about 1 $mL.L^{-1}$. Control was added by mixing of methanol 4%, alkylarylpolyglycoleter 400 $g.L^{-1}$ and Tween 80 for about 1 $mL.L^{-1}$. Every treatment was treated by triplicate experiments. Two pieces of lettuce leaves with 4×4 cm^2 size were placed into a solvent and then being dried. After removing the solvent, two pieces of leaves were added into the Petri dish with diameter of 9 cm that was sealed by filter paper. Then, ten larvae were placed into every Petri dish. Larvae were treated with leaves for 48 hours. Everyday, larvae were treated with leaves with no new treatment until larvae achieving the fourth instars stage. Observation was carried out everyday since 48 hours after the treatment until larvae get into fourth instars. Numbers of *C. povanana* larvae died were counted. The larvae mortality of *C. pavonana* was counted by using equation:

$$P = \frac{a}{b} \times 100\%$$

P = Mortality (%)

a = Number of *C. pavonana* larvae that are

died

b = Number of *C. pavonana* larvae that are tested

If the number of *C. pavonana* larvae in control that are died is less than 20%, the mortality for every treatment is corrected by using Abbot equation (Finney, 1971):

$$Pt = \frac{Po - Pc}{100 - Pc} \times 100\%$$

Pt = Corrected mortality (%).

Po = Mortality for every treatment (%).

Pc = Control mortality (%).

Lethal concentration test (LC)

The test is intended to determine the correlation between concentrations with mortality of sample againts *C. pavonana* larvae. By the correlation of the concentration, we can get sub lethal concentration. The test is carried out by using residue method of leaves that has been explained by Prijono *et al*. (2001). The sample is tested to five concentration level that is expected can kill 10-90% tested insect that is determined in preliminary test. The correlation between sample concentrations with mortality of *C. pavonana* larvae is determined by probit analysis (Finney, 1971). The sample is dissolved in methanol until achieving the expected concentration. The mixture is added by alkylarylpolyglycoleter 400 $g.L^{-1}$ as attacher and Tween 80 as emulsifier for about 1 $mL.L^{-1}$. Control is added by mixing methanol 4%, alkylarylpolyglycoleter 400 $g.L^{-1}$ and Tween 80 for about 1 $mL.L^{-1}$ together. Every treatment is three times. Two pieces of letucce leaves with 4x4 cm size are immersed in mixture of solvent and then dried. After the solvent evaporating, two pieces of leaves are placed in the petri dish with diameter 9 cm that is sealed with filter paper. Then, 10 of second instar larvae of *C. pavonana* are placed into every petri dish. Larvae are given with leaves for 48 hours. Each day, larvae are given with leaves with no new treatment until achieving fourth instar. Observation is done regularly since 48 hours after treatment until larvae get into fourth instar.

Results and Discussions

Isolation and structure determination of insecticidal compound

A ^{13}C-NMR spectrum of F734 revealed the presence of 52 resonances (Table 1). Among these are three resonances observed at δ 105.6, 103.3, and 104.7 ppm, which are consistent with the presence of three sugar anomeric carbons. One additional resonance seen at 178.5 ppm fall in the region normally associated with carbonyl group of ester. Two slightly lower field signals (144.5 and 126.3 ppm) are indicative of one C=C bonded system. Comparison of the ^{13}C resonances of the isolated compound with those of the previously reported saponin containing 2-methylbutirate (Herlt *et al.*, 2002). All signals showed a very close correlation indicating the same oleanane triterpenoid, 2-methylbutyryl, and sugar moieties except the carboxyl carbon of the glucuronic acid that was not observed in the ^{13}C-NMR spectrum. This signal was not observed also in spectrum of Herlt *et al.* (2002) until the compound was esterified to afford a methyl ester. From this ester, the carboxyl carbon was readily observable.

The 500 MHz ^{1}H-NMR spectrum in CD_3OD of F734 showed seven methyl groups on quarternary carbons and an olefinic carbon at 5.41 ppm, while methylene protons at 3.74 and 3.82 ppm indicated hydroxyl methyl substituents. Three anomeric sugar protons were observed at 3.49, 3.67, and 3.97 ppm with their coupling constants indicating that the sugars were β-linked both to the aglycon and to one another. All proton signals in F734 were not quite superimposed with those of previously reported saponin (Herlt *et al.*, 2002) since a different solvent was used in this NMR measurement. But the data was quite similar with those of previously repoted acutanguloside A-F, other saponins isolated from another species of *Barringtonia*, *B. acutangula* (Mills *et al.*, 2005).

A DEPT experiment showed 24 methine, 11 methylene, and 9 methyl carbon atoms, giving a total of 44 protonated atoms and 8

quarternary carbons. The methine proton signal of H-5 resonating at 0.79 ppm was at an unusually high field position, indicative of an oleanane triterpenoid. This resonance was similar with that of the previously reported ranuncoside VIII (Burton *et al*, 2003). HMBC data enabled virtually all of the heteronuclear connectivities within the aglycone structure to be established, including the respective attachment site of the 2-methylbutyryl groups. Indication that each linkage was a β-linkage was confirmed by diaxial coupling constants of the three anomeric proton (7.95, 7.95, and 6.70 Hz). HMBC data were also crucial in establishing the connectivity of the sugar moieties. The HMBC cross peaks clearly showed the connectivity of the three sugar residues in the glycone moiety to be 3-*O*-β-galactopyranosyl (1→3)-[β-glucopyranosil (1→2)]-β-glucuronopranosyl. All of the sugars in F734 showed the same relative stereochemistry which was assumed to be the common D forms. These results correspond to the findings recently reported by Herlt *et al*. (2002) for two other saponins found in *Barringtonia asiatica*. Since one- and two dimensional ^{1}H-NMR and ^{13}C-NMR spectral data of F734 was identical to those of the previously reported saponin (Herlt *et al.*, 2002) (Mills *et al.*, 2005), F734 was identified as 3-*O*-{β-galactopyranosyl (1→3)-[β-glucopyranosil (1→2)]-β-glucuronopiranosyloxy}-22-O-(2-methyl-1-oxobutoxy)-15,16,28-trihydroxy-(3β,15α,16α,22α)-olean-12-ene.

Table 1 ^{13}C-NMR data (in CD_3OD) for *B.asiatica* saponin

Carbon	δC F734 (ppm)	δC (ppm) [a]	Carbon	δC F734 (ppm)	δC (ppm) [a]
aglycon			glucoronic acid		
1	40.2	38.9	1’	105.6	105.3
2	27.1	27.2	2’	78.2	78.8
3	92.1	89.4	3’	86.8	87.5
4	39.1	39.6	4’	70.5	71.7
5	56.8	55.4	5’	77.4	77.2
6	19.6	18.8	6’	-	170.6
7	37.2	36.7	Glucose		
8	40.5	41.5	1”	103.3	103.8
9	48.2	47.1	2”	76.3	76.3
10	37.9	36.7	3”	78.3	78.4
11	24.8	23.9	4”	72.3	72.4
12	126.3	124.8	5”	77.4	77.7
13	144.5	144.5	6”	63.7	63.1
14	42.4	47.1	Galactose		
15	68.6	67.5	1’”	104.7	105.2
16	75.2	74.7	2’”	72.9	72.9
17	45.8	45.2	3’”	75.2	75.3
18	42.3	41.6	4’”	70.5	70.0
19	47.6	47	5’”	78.7	77.2
20	32.5	31.9	6’”	63.6	61.9
21	41.9	41.5	Ester		
22	72.6	72.0	1’”’	178.5	176.2
23	28.4	27.9	2’”’	43.0	41.8
24	17.0	16.7	3’”’	28.1	26.7
25	16.4	15.8	4’”’	12.2	11.8
26	17.9	17.5	5’”’	17.1	16.7
27	21.1	21.3			
28	62.6	62.8			
29	33.7	33.5			
30	25.2	25.2			

[a] Data from ref. (Herlt *et al.*, 2002)

Compound F734 was evaluated for its insecticidal activity against the larvae of *Crocidolomia pavonana*. The LC_{50} values of this compound was determined by Probit analysis (Finney, 1971) and the data show that the LC_{50} value of F734 is 290 ppm. By comparing this value with the commercial insecticides, betacylflutrin (11.3 ppm) and

spinosad (0.322 ppm), F734 is less active than both of them. In order to enhance its activity, derivating synthesis of this compound should be carried out in the next research.

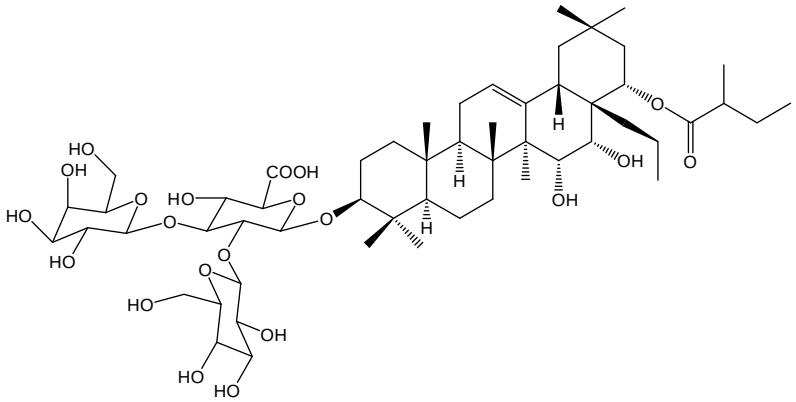

Figure 1 Structure of insecticidal compound from *B. asiatica* (F734)

Saponin (F734): Amorphous white powder (11 mg); ^{1}H-NMR: δ 0.93 (1H, d, *J*=7.35, H-1), 1.63 (1H, d, *J*=12.25, H-1), 1.72 (1H, m, H-2), 1.95 (1H, m, H-2), 3.23 (1H, dd, *J*=4.3; 11.6, H-3), 0.79 (1H, bd, *J*=11.6, H-5), 1.44 (1H, m, H-6), 1.54 (1H, m, H-6), 1.73 (2H, m, H-7), 1.59 (1H, m, H-9), 1.84 (1H, s, H-11), 1.95 (1H, m, H-11), 5.41 (1H, bt, H-12), 3.77 (1H, d, *J*=4.9, H-15), 3.89 (1H, d, *J*=4.9, H-16), 2.52 (1H, dd, *J*=14.05;4.05, H-18), 1.16 (1H, m, H-19), 2.19 (1H, m, H-19), 1.30 (1H, d, *J*=19.55, H-21), 1.92 (1H, s, H-21), 5.38 (1H, t, *J*=6.1, H-22), 1.08 (3H, s, H-23), 0.88 (3H, s, H-24), 0.99 (3H, s, H-25), 1.02 (3H, s, H-26), 1.39 (3H, s, H-27), 3.74 (1H, m, H-28), 3.82 (1H, bm, H-28), 0.92 (3H, s, *J*=3.05, H-29), 1.02 (3H, s, H-30), 4.49 (1H, bd, *J*=6.7, H-1'), 3.35 (1H, d, *J*=9.15, H-2'), 3.82 (1H, bm, H-3'), 3.82 (1H, bs, H-4'), 3.69 (1H, d, *J*=7.3 H-5'), 4.67 (1H, d, *J*=7.95, H-1"), 3.63 (1H, t, *J*=7.95, H-2"), 3.49 (1H, dd, *J*=3.05, 9.8, H-3"), 3.82 (1H, bm, H-4"), 3.44 (1H, m, H-5"), 3.30 (1H, m, H-6"), 3.56 (1H, q, *J*=7.35, H-6"), 4.97 (1H,

d, *J*=7.95, H-1‴), 3.15 (1H, t, *J*=6.8, H-2‴), 3.82 (1H, bm, H-3‴), 3.09 (1H, t, *J*=7,95, H-4‴), 3.61 (1H, m, H-5‴), 3.09 (1H, t, *J*=7.95, H-6‴), 3.88 (1H, d, *J*=4.3, H-6‴), 2.41 (1H, sept., *J*=7.35, H-2⁗), 1.49 (2H, m, H-3⁗), 0.96 (3H, m, H-4⁗), and 1.12 (3H, d, *J*=6.75, H-5⁗); ^{13}C-NMR (see Table 1).

Conclusions

The most active insecticidal compound has been successfully isolated from methanolic extract of *B. asiatica* seeds with LC_{50} value is 290 ppm. The structure of this compound has been successfully determined which resulting an oleanane saponin, (3-*O*-{β-galactopyranosyl (1→3)-[β-glucopyranosil (1→2)]-β-glucuronopiranosyloxy}-22-O-(2-methyl-1-oxobutoxy)-15,16,28-trihydroxy-(3β,15α,16α,22α)-olean-12-ene).

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