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Modification of Allylic Moiety of Eusiderin A to Enhance the Antifeedant Potency

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Abstract
Eusiderin A(**1**) is a neolignan derivative which become major component in *Eusideroxylon zwagery*. This compound possesses potent activity as organic insecticide because the antifungal and antifeedant showed potent activities towards some pests of horticulture pests. This research aimed to modify allilic moiety *via* hydroboration-oxidation, Dess Martin and osmium tetraoxide oxidation. Modification at allilic moiety led to proceed compound (**2-4)**. Based on antifeeding assay against *Epilachna sparsa* disclosed that compound **4** showed eight times higher the activity rather than **1**. Meanwhile compound **2** and **3** demonstrated three time higher the activity than **1**.
Keywords: eusiderin A, oxidation, allilic moiety, antifeedant.

1. Introduction

Eusiderin A**(1)** a neolignan type which isolated as major component from *Eusideroxylon zwagery*. It found out in leaf, bark and root of this plant. Comp.**1** was reported at a concentration of 100 ppm to show significant inhibition, 66% on the mycelial growth of *Tyromeces polutris*(ouzuratake) and 52% against *Coriolus versicolor* (kawaratake) (Wasrin *et al*. 1987). In addition, **1** showed potent antifeedant activity at a concentration of 0.01% against *Epilachna sparsa* and without displayed toxic on the Brine Shrimp Lethality Test (LC50> 500 ppm). It also could prevent *Etiella zinckenella* from destroying soybean, *Glicine max* at a conc. of 0.5%. On the other side of experiment,**1** gave the most effective inhibition ratio against *Fusarium oxysporum f.sp. lycopersici* (49.8%), *Sclerotium rolfsii* (49.6%), and *Rhizoctonia solani*(22.0%) at 5 ppm concentration (Muhaimin *et al.* 2007). This finding leads to the reason why this plant has durable wood. It suggested that eusiderin A play a role in the protection of the plant against insects and fungi.

In continuing our study about structure activity relationship of eusiderin A(**1**) derivatives as antiffedant against *Epilachna spcrsa*. We have evaluated antifeedant potency of comp. **1** along with the congeners to disclose that demethylated methoxyl group at C-5 and C-6 could afford bring about significant increasing the antifeedant activity**.** These results indicated that vicinal diol at C-5 and C-6 play an important role to enhance the antifeedant activity. Meanwhile dioxane ring is necessary to maintain the conformation and stability. However aliphatic elongation on allylic moiety tends to reduce the antifeedant activity (Syamsurizal, 2012). Based on these results, modification of allylic moiety (**1**) is critical point in order to enhance the antifeedant activity. In this study modification of allylic moiety was directed to increase the hydrophilicity so that the antifeedant activity become enhance.

2. Eksperimental

2.1. *General.*
The ^{1}H-NMR spectra was measured with a JEOL GX-500 (1H: 500 MHz, using Me4Si (0 ppm) as internal reference.Chemical Shifts were reported in parts per million (ppm). FAB mass spectra was recorded on a JEOL SX-102. All reactions were carried out under an argon atmosphere unless otherwise indicated. Column chromatography was conducted using silica gel (Fuji Sylisia BW-200). Thin-layer chromatography (TLC) analysis was performed on precoated Kieselgel 60F254 plates (0.25 mm, Merck). The spots were monitored under UV light (254 or 365 nm) and visualized by spraying agents phosphomolibdic acid in ethanolic solution.
2.2. *Isolation and Purification of* **1**.
Sample of the heartwood of *E. zwagery* was collected from Senami Forest, Batanghari District, Jambi Province, Indonesia. The dried heartwood (20 Kg) was ground and extracted three times with MeOH at rt for 6 h and subsequently three times under reflux for 4 h. The MeOH extract (120 g) was fractionated by vacuum liquid chromatography on silica gel using combination of *n*-hexane and ethyl acetate with increasing polarity as eluent to give 6 fractions. Eusiderin A(**1**) was identified on the second and third fraction then crystallized with benzene to afford pure compound of **1** (3.9 gram).
2.2. *Synthesis of **2 -4***
A solution of $NaBH_4$(80 mg) in dehydrated THF(1.6 mL) was treated with $BF_3.Et_2O$ then stirred at rt for 30 min. To the mixture was added dropwise solution of **1** (54 mg) in dehydrated THF(1.6 mL) and stirred at rt for 30 min.

The reaction mixture was cooled to 0°C followed by adding 2M aq.NaOH(0.5 ml) and 30% H_2O_2(2.4 ml) then stirred at rt for 10 min. The reaction mixture was poured into water(100 mL) and extracted with EtOAc. The organic layer was washed with saturated aqueous $Na_2S_2O_3$ and brine, followed by drying over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by chromatography on a silica gel column (1:1 n-hexane-EtOAc) to yield the desired product comp. **2** (46.8 mg).

To a solution of **2** (22.1 mg) in dehydrated CH_2Cl_2(1.1 mL) was added Dess Martin reagent (50.9 mg) and stirred at rt for 30 min. After completion the reaction, the mixture was poured into saturated aqueous $NaHCO_3$ and neutralized with saturated aqueous $Na_2S_2O_3$ and extracted with EtOAc. After being washed with saturated aqueous $NaHCO_3$ and brine. The EtOAc layer was dried over anhydrous $NaSO_4$ and concentrated to dryness. The crude product was purified by chromatography on a silica gel column (2:1 and 1:1, *n*-hexane-EtOAc) to give the desired product **3** (10.3 mg).

To a solution of **1** (100 mg) in dehydrated toluene (2 mL) was added *N*-methylmorpholine (0.13 g), then osmium tetraoxide 0.03 mL(0.98 M in toluene) and stirred at rt for 3 h. The reaction mixture was saturated with solution of Na_2SO_3(aq) and extracted with EtOAc. The solvent was removed under reduced pressure to give crude 122.3 mg. The crude product was purified by chromatography on a silica gel column(3:1 and 1:1, *n*-heksane: EtOAc) to produce the desired product comp.**4** (84.5 mg).

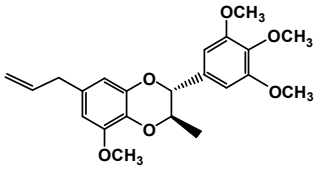
2.3. *Antifeeding Assay*

The test insects, *Epilachna sparsa* collected from vegetables field in Mendalo Darat Village, Jambi Province, Indonesia, where pesticides had not been applied. These insects were maintained in our laboratory under room temperature and fed daily with the leaf of Solanum nigrum until they lay eggs. When the eggs hatched to form the first-instar and reared until the third instar were used for testing. The antifeedant effect of the synthesized compounds against third-instar larvae of *E. sparsa* was determined using a conventional leaf disc method which modified from the assay described by Abdelgaleil and Aswad (Abdelgaleil *et al*. 2005) The leaf disc (60 mm diam) of *Solanum nigrum* was divided into two sectors; the left side immersed in methanol for two seconds as control area and the right side by the same manner with methanol solution of tested compounds as treatment area. The discs were left to dry. Then two of the tested larvae, which starved for 12 h were placed in the center of each Petri dish. The bioassay terminated after 24 h. This choice test was conducted at a range concentrations of 100 to 1000 ppm. Three replicates of each concentration were carried out. The antifeedant percentage was calculated from the following equation: % antifeedant = 100 (C – T/C), where C is the wide area of leaf discs consumed in the control and T is the wide area of leaf discs consumed in the treatment.

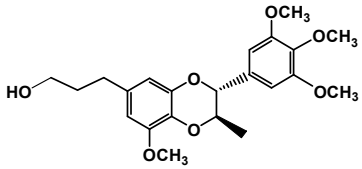
3. Results and Discussion

Modification of allylic moiety(**1**) was conducted by taking into account of the previous results that as increased hydrophobicity of allylic chain led to reduce antifeedant activity of **1**. Therefore need to be calculated the clogP values in order to reduce the hydrophobicity on the contrary to enhance the hydrophilicity. ClogP is a parameter correlated to the permeability of drugs and is thus believed to be an important index in predicting the biological activity (Murakami *et al*. 2003). When clogP value is small bring about the hydrophilicity increse and otherwise. In this study, allylic moiety(**1**) was oxidated in order to minimize the clogP value thus reduced the hydrophobicity or to improve the hydrophilicity. It is expected that access of oxidated analogs (**1**) into the target cell compartment increases due to more hydrophilic than (**1**).

Oxidation of allylic moiety (**1**) required two consecutive reaction steps that is hydroboration by $NaBH_4$ and oxidation with hydrogen peroxide 30% so that a primary alcohol by anti-markovnikov mechanism was yield 91% comp.**2** (98.3 mg). Molecular mass of **2** was determined by FAB-MS mass spectrum (m/z), 405 $(M + H)^+$ where the molecular mass is greater than (**1**) 18 unit indicated oxygenation of the allylic chain occured. These results were confirmed using ^{1}H-NMR data in which the loss of olefin chain (**1**) signals at δ 5.94 ppm (1H, ddt like, J = 17, 7.3, 6.7 Hz H-10) and 5.07 ppm (2H, like dd, J = 18, 10 Hz, H-11), then appeared the aliphatic signal at 3.88 ppm δ (2H, t, J = 7.8 Hz, H-11) and 1.87 ppm (2H, m, H-10). Besides that the proton signal at H-9 also changed significantly at chemical shift (0.68 ppm, upfield). Whereas proton signals relative to the other positions unchanged the chemical shift. The structure of comp. **2** as seen in **Figure 1**.



eusiderin A(1)



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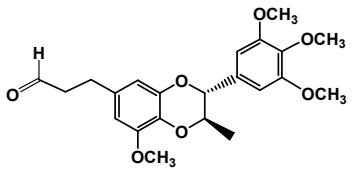

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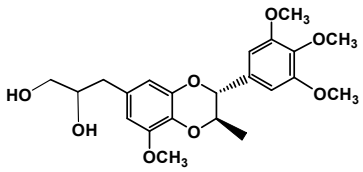

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Figure **1** . Structure of **1** and synthesized analogs

Dess Martin oxidation is specifically converting primary alcohols to aldehydes. By using Dess Martin reagent is expected to avoid further oxidation of aldehydes to carboxylic acids, because it is acidic reagents in which the functional groups plays a role in preventing further oxidation of the aldehyde (**3**). Comp.**3** was successfully synthesized with a yield (84%). The structure is determined from the mass spectral data FAB-MS (m/z), 403 $(M + H)^+$ where 16 unit is greater than comp. **1**. ^{1}H-NMR data was significantly changed down filed at H-11, δ 9.82 (s) known as typical aldehyde position. Significant changed were also observed up field at H-10, δ 2.76 (t, J = 6.3 Hz like) and H-9 at δ 2.88 (t, J = 6.1 Hz) where position of other signals not change significantly. The overall signal of the compound (**3**) as shown in **Table 1**.

Tabel.1 ^{1}H-NMR Data of **(1)** and the derivatives **(2-4)** in CDCl3 (500 MHz)

Proton	**1**	**2**	**3**	**4**
H-2	4,55(d, J = 7,9 Hz)	4,55(d, J =7,3 Hz)	4,56(d, J =7,2 Hz)	4,56(d, J =7,9 Hz)
H-3	4,10(dq, J =7,9, 6,1Hz)	4,09(m)	4,11(m)	4,06-4,15(m)
H-4	6,57 (s)	6,58 (s)	6,57 (s)	6,57 (brs)
H-8	6,57 (s)	6,58 (s)	6,57 (s)	6,57 (brs)
H-9	3,30(d, J =6,7 Hz)	2,62(t, J =7,3 Hz)	2,62 (t, J = 6,1 Hz).	2,70(dt like, J = 8,2, 2,9 Hz)
H-10	5,94 (ddt, J =17, 7,3, 6,7Hz)	1,87 (m)	2,76 (t like J = 6,3 Hz)	4,06-4,15(m)
H-11	5,07(dd, J =18, 10Hz)	3,88 ppm (t, J = 7,8 Hz)	9,82 (s)	3,72(dd, J = 10,8, 2,9 Hz, H-11a) 3,54(dd, J = 11,0, 7,0 Hz, H-11b)
H-3’	6,38 (brs)	6,39(brs)	6,38(d, J =2,0 Hz)	6,41 (d, J =1,8Hz)
H-5’	6,49 (brs)	6,48(brs)	6,47(d, J = 2,0 Hz)	6,50 (d, J = 1,8Hz)
H-3”	1,26 (d, 6,1Hz)	1,25 (d, J = 6,4Hz)	1,26 (d, J =7,0Hz)	1,27 (d, J = 5,9 Hz)
5-OCH_3	3,88(s)	3,88(s)	3,88(s)	3,88(s)
6-OCH_3	3,89(s)	3,89(s)	3,89(s)	3,88(s)
7- CH_3	3,88(s)	3,88(s)	3,88(s)	3,88(s)
6’-OCH_3	3,75(s)	3,85(s)	3,86(s)	3,88(s)

Pinacol formation at the allylic chain was come about *via* oxidation with osmium tetraoxsideby utilizing *N*-methylmorpholine as catalyst. This reaction occured very quickly which successfully synthesized compound **4** with a yield of 81%. The structure was determined from the mass spectral data FAB-MS (m / z), 421 $(M + H)^+$, 34 unit greater than comp. (**1**). ^{1}H-NMR data showed allylic chain converted to vicinal diol at position C-11 and C-10. Pinacol group indicated by the appearance of signals at δ 3.72 (dd, J = 10.8, 2.9 Hz, H-11a) and 3.54 (dd, J = 11.0, 7 , 0 Hz, H-11b). Then signal upfiled at δ 4.06 to 4.15 (m, H-10). Position H-9 signal observed up filed at δ 2.70 (dt like, J= 8.2, 2.9 Hz). These specific signals ensured the structure of comp **4**.

Modification of allylic moiety (**1**) was intended to reduce the clogP value in order to increase the hydrophilicity. By promoting the hydrophilicity is expected to enhance accumulation of comp.(**2-4**) in the site target of *E.sparsa* such that appetite to eat the leaves of *S. nigrum* reduced. These eusiderin A congener(**1-4**) was tested the antifeedant activity against *E. sparsa* showed comp. **4** was ten times higher than comp.(**1**), however comp. **2** and **3** demonstrated enhancement three times than **1** (The antifeedant activity results shown in

Table 2). Thus as the higher hydrophilicity of **1** brought about enhanced the antifeedant potency.

Table 2. Antifeedant Activities of (**1-4**)

Compounds	clogP value	Antifeedant AC_{90},(%)[a]	Enhancement
eusiderin A (**1**)	4.12	0,88	-
2	3.19	0.30	3 X
3	2,72	0.33	3 X
4	2.23	0.11	8 X
azadirachtin	-1.38	0,02	

[a)] AC_{90}: concentration where 90% of tested leaf unconsumed
[b)] positif control

4. Conclusion

Modifications at allylic moiety of **1** was crucial point to enhance the antifeedant potency. This study was successfull to synthesize compounds (**2-4**). Based on the evaluation of antifeedant effect against *E. sparsa* found that the comp.(**4**) was eight times stronger than (**1**). Similarly, the analogs **2** and **3** which showed increased activity three times from (**1**). Thus as the higher hydrophilicity of **1** brought about enhanced the antifeedant potency.

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