Antifeedant Terpenoid from The Bark of *Lansium domesticum* Corr cv. Kokossan (Meliaceae)[1)]

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ABSTRACT

In the course of our continuing search of novel antifeedant compounds from Indonesian plants, the methanolic extract of bark of *Lansium domesticum* showed significant antifeedant activity against the fourth instars of *Epilachna sparsa*. The methanolic extract of the bark of *L. domesticum* was concentrated and extracted with ethyl acetate. The ethyl acetate extract exhibited an antifeedant activity toward *E. sparsa*. By using the antifeedant activity to follow the separations, the ethyl acetate fraction was separated by combination of column chromatography on Kieselgel 60 to afford two antifeedant compounds **1** and **2**.

Based on spectroscopic evidences and comparison with those related data previously reported indicated that isolated compound as triterpenoids with molecular skeleton similar with those onoceranoid. An antifeedant activity of compounds **1** and **2** showed activity as 99% and 85% against the fourth instars of *E. sparsa* at concentration of 1%.

Keywords: *Lansium domesticum,* Meliaceae, triterpenoids, antifeedant activity, *Epilachna sparsa*

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INTRODUCTION

Pest management in agriculture, forestry and managed landscapes has often relied on toxic, broad apectrum insecticides with negative impacts on natural enemies, pollinators and other non-target organism. And continuous use of specific insecticides has frequently resulted in the development of resistance in the very pests targeted for population suppression. The concept of using antifeedant as crop protectants is intuitively attractive. Most plant defensive chemicals *discourage* insect herbivory, either by deterring feeding and oviposition or by impairing larval growth, rather than killing insect outright. Insect antifeedant is a behaviour modifying substance that deters feeding through a direct action on peripheral sensilla (= taste organs) in insect (Isman *et al.*, 2002).

Lansium domesticum Corr (Meliaceae) is a popular fruit in southern Asia. The plant family Meliaceae is noted for the production of useful bitter principles which are insect anifeedant and growth-reducing substances with low mammalian toxicity (Omar *et al.*, 2005). Phytochemical investigation of the fruit peel of *L. domesticum* revealed the presence of lansic acid, 3β-hydroxyonocera-8(26),14-dien-21-one and 21α-hydroxyonocera-8(26),14-dien-3-one (Tanaka *et al*, 2002). The seed contain tetranortriterpenoids named dukunolides A-F (Nishizawa *et al.*, 1985, 1989). The bark contain antifeedant triterpenoid namely isoonoceratriene, 3-keto-22-hydroxyonoceradiene, onoceradienedione, lansiolic acid, lansiolic acid A and 3-keto lansiolic acid. These compounds showed antifeedant activity against *Sitophilus oryzae*.

In our continuing search on antifeedant compounds from Indonesian plants, the methanolic extract of bark of *L. domesticum* cv kokossan showed significant antifeedant activity against the fourth instars of *Epilachna sparsa*. In this research, we report the isolation and characterization of two antifeedant triterpenes, onoceradienedione **(1)** and 14-hydroxy-7-onoceradienedione **(2)**.

EXPERIMENTAL

General Experimental Procedure. UV and IR spectra were measured with System Perkin Elmer Spectrum One. ^{1}H and ^{13}C-NMR spectra were recorded with a JEOL JNM ECA-500, operating at 500 and 400 MHz (^{1}H), 125 and 100 MHz (^{13}C), using residual and deutered solvent peaks as internal standards. Vacuum liquid chromatography was carried out using Kieselgel 60.

Plant Material. Samples of the bark *L. domesticum* cv kokossan were collected in March 2006 from Cililin, Bandung, Indonesia. The plant was identified by the staff at Department of Biology, Padjadjaran University.

Bioassay. The bioassay was performed according to a method developed by Schwinger *et al.* (1983). All test were carried out with *Epilachna sparsa* on *Solanum nigrum*. A methanolic solution of the substance was brushed on one half of the leaf and the other half was treated with pure methanol. A test duration for up 24 h with two larvae in one petri dish (choice test).

Extraction and Isolation. The dried and milled bark of *L. domesticum* cv kokossan (3 kg) was extracted exhaustively by methanol at room temperature. The methanol extract (200 g) was partitioned between *n*-hexane and 10% aqueous methanol, and the aqueous phase was further extracted with ethyl acetate. The ethyl acetate-soluble fraction was subjected to Kieselgel 60 column eluted with 0-100% dichloro metane/*n*-hexane. The fraction eluted with dichloro metane/*n*-hexane (3:7) contained **(1)**. The fraction was further separated by Kieselgel 60 column eluted with ethyl acetate/*n*-hexane (0,5:9,5) to yield **(1)** (52,5 mg). The fraction eluted with dichloro metane/*n*-hexane (6:4) contained **(2).** The fraction was further separated by Kieselgel 60 column eluted with acetone/*n*-hexane (1:9) to yield **(2) (**10,7 mg).

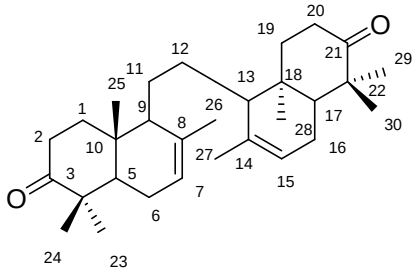
Onoceradienedione (1): colorless crystal, m.p 143-144^{o}C, IR (KBr) ν_{max} 3040, 2962, 2854, 1708, 1450-1384, 1662-1608, 1296-1107, 887 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H 5.42(1H, s, H-7 and H-15), 2.72 and 2.25 (2H, dt, H-1 and H-19), 2.09 (2H, m, H-6 and H-16), 1.93 (2H, m, H-11 dan H-12), 1.72 (3H, s, H-26 and 27), 1.65 (1H, m, H-9 and H-13), 1.58(1H, dd, H-5 and H-17), 1.32 and 1.35 (2H, m, H-2 and H-20), 1.08 (3H, s, H-24 and H-30), 1.04 (3H, s, H-23 and H-29),0.97 (3H, s, H-25 and H-28); ^{13}C-NMR ($CDCl_3$) δ_C see Table 1.

14-hydroxy-7-onoceradienedione (2): colorless amorphous solid, IR (KBr) ν_{max} 3444, 2962-2931, 1705, 1698, 1458, 1338, 1315, 1261-1076, 802 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H 7.3 (1H, s), 5.4 (1H, s), 2.75, 2.73, 2.70 (2H, dt), 2.6 (2H, m), 2.1 (3H, m), 1,9 (2H, m), 1.8 (2H, m), 1.7 (2H, m), 1.6-1.4 (6H, m), 1.3 (3H, s), 1.09 (3H, s), 1.08 (3H, s), 1.05 (3H, s), 1.01 (3H,s), 0.96 (3H, s), 0.92 (3H,s); ^{13}C-NMR ($CDCl_3$) δ_C see Table 1.

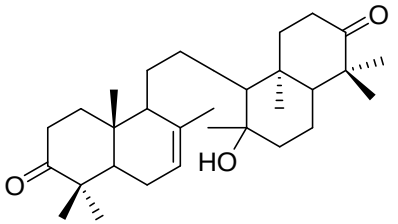
DISCUSSION

Compound **1** was obtained as colorless crystal, m.p 143-144^{o}C. The IR spectrum of **1** showed a strong absorption band at 1708 cm^{-1} and weak absorption band at 1662-1608 cm^{-1} , indicating the presence of ketone and alkene groups. The ^{1}H-NMR spectrum of **1** showed signals due to four tertiary methyls, and its ^{13}C-NMR spectrum aided by HMQC experiments revealed the presence of trisubstituted olefin (δ_C 135.3 and 122.1), a ketone (δ_C 217). The four methylenes (δ_C 38.5, 34.8, 30.1, 24.2), two methines (δ_C 55.6, 51.6) and two tertiary carbon (δ_C 47.6, 36.7) were confirmed by the ^{13}C-NMR and DEPT spectra. The number of odd hydrogen indicating the C-NMR signals due to symmetrical compound. These observation together with a detailed comparison of the spectral data with those previously reported led us to identify **1** as onoceradienedione, a symmetrical triterpene (see Table 1).

Compound **2** was obtained as colorless amorphous solid. The IR spectrum of **1** showed a sharp absorption band at 3444 cm^{-1} and strong absorption band at 1705 cm^{-1} indicating the presence of hydroxyl, alkene and ketone groups. The ^{1}H and ^{13}C-NMR spectrum of **2** showed were similar to those of **1** except for the presence of hydroxyl group on C-14 (δ_C 74,0). Therefore, it may be concluded that **2** is 14-hydroxy-7-onoceradienedione.



(1)



(2)

Table 1. ^{13}C-NMR data of onoceradienedione (**1**), 14-hydroxy-7-onoceradienedione (**2**)

Position	δ_C (**1**)	δ_C (**2**)
1	38.5	38.4
2	34.8	34.1
3	217.0	217.1
4	47.6	47.6
5	51.6	51.6
6	30.1	31.3
7	122.1	121.7
8	135.3	135.7
9	55.6	55.2
10	36.7	36.6
11	24.2	21.5
12	24.2	21.5
13	55.6	61.8
14	135.3	74.0
15	121.1	44.2
16	38.5	28.9
17	51.6	55.5
18	36.7	36.6
19	30.1	38.5
20	34.8	34.7
21	217.0	216.9
22	47.6	47.6
23	25.1	25.1
24	22.3	22.2
25	13.5	13.4
26	22.5	22.3
27	22.5	24.1
28	13.5	15.1
29	22.3	21.3
30	25.1	26.4

CONCLUSION

Two triterpenoids, namely onoceradienedione (**1**) and 14-hydroxy-7-onoceradienedione (**2)** had been isolated for the first time from the bark of of *L. domesticum* cv kokossan. These compounds showed antifeedant activity as 99% and 85% against the fourth instars of *E. sparsa* at concentration of 1%.

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