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Short Note

3-Hydroxy-8,14-secogammacera-7,14-dien-21-one: A New Onoceranoid Triterpenes from *Lansium domesticum* Corr. cv *kokossan*

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Abstract: A new onoceranoid triterpenes, namely 3-hydroxy-8,14-secogammacera-7,14-dien-21-one (**1**), has been isolated from the fruit peels of *Lansium domesticum* Corr. cv *kokossan*. The structure of **1** was determined on the basis of spectroscopic data including infrared, 1D and 2D-NMR, as well as high resolution mass spectroscopy analysis. Compound **1** showed a weak activity against MCF-7 breast cancer cell lines.

Keywords: *Lansium domesticum*; Meliaceae; MCF-7; triterpene onoceranoid

1. Introduction

Lansium domesticum Corr. (Meliaceae) is a popular plant that widely grows in southeastern Asia [1,2]. *L. domesticum* Corr. cv *kokossan* (Meliaceae) is a higher tree growing up to 30 m in height, commonly called "kokosan" in Indonesia [3]. Several bioactive triterpenoids have been isolated from *L. domesticum* [4–9], of which some have shown potential anticancer [10], antibacterial [11], insecticides [12], antimalarial [13], antimutagenic [14,15], and antifeedant activities [16]. Previously, we isolated six triterpenes from the seeds and bark of *L. domesticum* Corr. cv *kokossan* [2,16]. In this paper, we report the isolation and structural elucidation of the new onoceranoid triterpenes **1** (Figure 1), along with its cytotoxic activity against MCF-7 breast cancer lines.

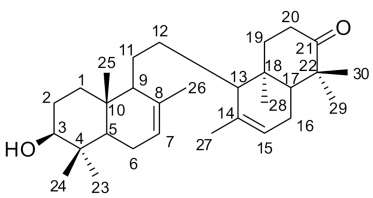

Figure 1. Chemical Structure of compound **1**.

2. Results

Extraction and Isolation

The dried fruit peel (1.7 kg) was extracted with *n*-hexane, ethyl acetate, and methanol three times, respectively, for 24 h, at room temperature. After solvent removal under reduced pressure, we obtained the *n*-hexane (86 g), ethyl acetate (110 g), and methanol (75 g) crude extracts. A portion of *n*-hexane (60 g) was subjected to vacuum liquid chromatography over silica gel using a 10% gradient of *n*-hexane/EtOAc (10:0–0:10) to afford eight fractions (A–H). Fraction D (6.7 g) was subjected to vacuum liquid chromatography, eluted by a 1% gradient of *n*-hexane/EtOAc (100:0–85:15) to afford five combined fractions (D1–D5). Fraction D4 was then subjected to silica gel column chromatography, eluted with *n*-hexane:acetone:methanol (7:2.5:0.5), resulting in four fractions (D4A–D4D). Compound **1** was obtained from fraction D4B as a white crystal (0.27 g).

3-Hydroxy-8,14-secogammacera-7,14-dien-21-one (**1**), white crystal, m.p. 153–155 °C, $[\alpha]^{20}_{D}$ − 3.0° (c 0.1, MeOH), HR-TOF-MS *m*/*z* 463.3569 $[M + Na]^+$ (calcd. for $C_{30}H_{48}O_2Na$, *m*/*z* 463.3552); IR (KBr) ν_{max} (cm^{-1}): 3533, 2932, 1700, 1456; ^{1}H-NMR and ^{13}C-NMR showed in the Table 1. Compound **1** was evaluated for its cytotoxic acitivity against MCF-7 breast cancer cell line, and compared to doxorubicin (35.7 μM) as a positive control. Compound **1** exhibited weak activity against MCF-7 with an IC_{50} value of 717.5 μM.

Table 1. ^{13}C and ^{1}H NMR Spectral Data of Compounds **1** in $CDCl_3$.

Position	δ_C ppm	δ_H ppm (Int, mult., *J* = Hz)
1	37.5	1.14; 1.86 (2H, *m*)
2	27.4	1.65 (2H, *m*)
3	79.1	3.29 (1H, *dd*, 11.3; 4.3)
4	38.7	-
5	51.5	1.62 (1H, *m*)
6	29.8	1.35; 1.50 (2H, *m*)
7	121.7	5.40 (1H, *brs*)
8	135.4	-
9	55.3	1.66 (1H, *m*)
10	36.5	-
11	24.1	1.99; 2.10 (2H, *m*)
12	23.5	1.98; 2.10 (2H, *m*)
13	56.0	1.59 (1H, *m*)
14	134.9	-
15	122.3	5.40 (1H, *brs*)
16	29.9	1.35; 1.50 (2H, *m*)
17	49.6	1.20 (1H, *m*)
18	36.5	-
19	38.4	1.51; 2.15 (2H, *m*)
20	34.7	2.29; 2.77 (2H, *m*)
21	217.0	-
22	47.5	-
23	27.9	1.00 (3H, *s*)
24	15.1	0.87 (3H, *s*)
25	13.3	0.99 (3H, *s*)
26	22.3	1.75 (3H, *s*)
27	22.4	1.72 (3H, *s*)
28	13.6	0.76 (3H, *s*)
29	22.1	1.12 (3H, *s*)
30	25.0	1.08 (3H, *s*)

3. Discussion

The molecular composition of **1** was proposed as $C_{30}H_{48}O_2$ with seven degrees of unsaturation, based on HR-TOF-MS and NMR spectral data. Mass spectra showed molecular ion peak at *m*/*z* 463.3569 $[M + Na]^+$, with calculated *m*/*z* 463.3552 for $C_{30}H_{48}O_2Na$. The IR spectrum exhibited bands at ν_{max} (cm^{-1}) 3533 (hydroxy), 2932 (C-H stretching of aliphatic), 1700 (ketone), and 1456 (*gem* dimethyl).

The ^{13}C NMR data (Table 1) with DEPT and HSQC experiments (Figures S2–S4) revealed the presence of total of 30 carbon signals, which were classified as eight methyls, eight methylenes, seven methines (two olefinic and one oxygenated), and seven quaternary carbons (two olefinic and one carbonyl). The two trisubstituted double bonds and the carbonyl in a system with seven degrees of unsaturation suggested that **1** possess a tetracyclic structure. Previous studies supported that evidence, indicating that compound **1** has an onoceranoid-type triterpenoid [3,14].

The ^1H NMR spectrum of **1** (Figure S1) displayed the presence of eight methyl groups at δ_H (ppm) 1.00 (3H, Me-23), 0.87 (3H, Me-24), 0.99 (3H, Me-25), 1.75 (3H, Me-26), 1.72 (3H, Me-27), 0.76 (3H, Me-28), 1.12 (3H, Me-29), and 1.08 (3H, Me-30). Two olefinic protons (H-7 and H-15) were observed at δ_H (ppm) 5.45 and 5.42 (each 1H, brs), together with two vinyl methyls proton resonating at δ_H (ppm) 1.75 (3H, s, H-26) and δ_H 1.72 (3H, s, H-27), indicating two trisubstituted double bonds of **1**. One oxygenated methine signal was also observed at δ_H 3.29 ppm (1H, dd, H-3).

The structure of **1** was further defined by ^1H-^1H COSY (correlated spectroscopy) and HMBC (heteronuclear multiple bond connectivity) spectra (Figure 2, Figures S5 and S6). The ^1H-^1H COSY spectra showed couplings between H-1 (δ_H 1.14)/H-2 (δ_H 1.65), H-2 (δ_H 1.65)/H-3 (δ_H 3.29), H-9 (δ_H 1.66)/H-11 (δ_H 2.10), H-11 (δ_H 2.10)/H-12 (δ_H 1.98), H-12 (δ_H 1.98)/H-13 (δ_H 1.59), and H-19 (δ_H 1.51)/H-20 (δ_H 2.77), supporting the presence of a onoceranoid-type triterpenoid structure. The oxygenated methine bearing a hydroxy group was located at C-3 by the HMBC correlations of H_2-1 (δ_H 1.86), H_3-23 (δ_H 1.00) and H_3-24 (δ_H 0.87) to C-3 (δ_C 79.1). The $\Delta^{7,8}$ and $\Delta^{14,15}$ double bonds were assigned by the HMBC correlations from H_3-26 (δ_H 1.75) to C-7 (δ_C 121.7), C-8 (δ_C 135.4), C-9 (δ_C 55.3); and H_3-27(δ_H 1.72) to C-13 (δ_C 56.0), C-14 (δ_C 134.9), C-15 (δ_C 122.3). The ketone group was attached to C-21 by the HMBC correlations of H_2-20 (δ_H 2.77) and H_3-29 (δ_H 1.12) to C-21 (δ_C 217.0). This analysis indicated that compound **1** was similar to 3*β*-hydroxyonocera-8(26),14-dien-21-one [5], uniquely differing on the double bond position in the B ring. Based on the coupling constants of H-3 (dd, *J* = 11.4; 4.3 Hz), the configuration of the hydroxyl group at C-3 was indicated in the *β*-equatorial position [5,17]. From the analyses, compound **1** was determined as 3-hydroxy-8,14-secogammacera-7,14-dien-21-one.

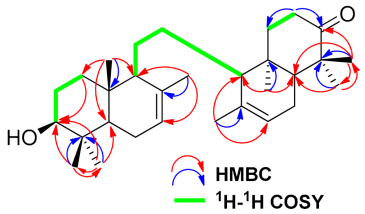

Figure 2. ^1H-^1H COSY and HMBC correlations of compound **1**.

4. Materials and Methods

4.1. General Experimental Procedures

Mass spectrum was recorded on a waters Xevo QTOFMS (Waters, Milford, MA, USA). IR spectrum was measured on a One Perkin Elmer infrared-100 (Waltham, MA, USA) in KBr. NMR data were recorded on a Brucker spectrometer (Billerica, MA, USA) at 400 MHz for ^1H and 120 MHz for ^{13}C using TMS as an internal standard. Chromatographic separations were carried out on silica gel G60 (Merck, Darmstadt, Germany) and RP18 (Merck). TLC plates were precoated with silica gel GF254 (Merck, 0.25 mm) and detection was achieved by spraying with 10% (*v*/*v*) H_2SO_4 in ethanol, followed by heating.

4.2. Plant Material

The fruit peels of *L. domesticum* Corr. cv *kokossan* (Meliaceae) was collected from Cililin, West Java, Indonesia in April 2018. The plant was identified and deposited in The Herbarium of Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia (Identification Number: 195/HB/08/2018).

4.3. Cytotoxic Bioassay

Cytotoxicity of the compound against MCF-7 human breast cancer cells was measured using MTT (*Methyl Thiazoldiphenyl-Tetrazoliumbromide*) assay. Stock culture was grown in flasks, containing Roswell Park Memorial Institute (RPMI) medium, respectively supplemented with 10% (*v*/*v*) feta bovine serum (FBS) and 1% (*v*/*v*) penicillin-streptomicin as an antibiotic. The culture was incubated at 37 °C for 24 h. The medium was changed, and tumor cells were detached and seeded in 96-well microliter plates. After, 24 h, compounds were added to the wells. After 48 h, cell viability was determined by measuring the metabolic conversion of yellow salt or 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color product resulting from reduction in viable cells. Insoluble formazan was diluted with DMSO. The MTT assay results were read using spectrophotometer UV at 450 nm. Compound **1** was evaluated at eight concentrations (15.9; 34.0; 70.3; 140.7; 283.6; 567.3; 1134.6; 2269.1 μM) in 100% DMSO with the final concentration of DMSO of 2.5% (*v*/*v*) in each well. Doxorubicin was used as a positive control. IC_{50} values were calculated by the linear regression method using Microsoft Excel software.

5. Conclusions

A new onoceranoid triterpene, namely 3-hydroxy-8,14-secogammacera-7,14-dien-21-one (**1**), was isolated from fruit peels of *L. domesticum* Corr. cv *kokossan* (Meliaceae). This compound exhibited weak cytotoxic activity against MCF-7 human breast cancer cell lines with IC_{50} value of 717.5 μM.

Supplementary Materials: The following are available online, Figure S1. ^{1}H-NMR spectrum of **1** (500 MHz in $CDCl_3$), Figure S2. ^{13}C-NMR spectrum of **1** (125 MHz in $CDCl_3$), Figure S3. DEPT-135°_spectrum of **1** (125 MHz in $CDCl_3$), Figure S4. HSQC Spectrum of **1**, Figure S5. ^{1}H-^{1}H COSY spectrum of **1**, Figure S6. HMBC spectrum of **1**, Figure S7. Infrared Spectrum of **1** (in KBr), Figure S8. HR-TOF-MS Spectrum of **1**, Figure S9. TLC Profile of **1**.

Author Contributions: Conceptualization, T.M. and U.S.; methodology, N.K.P. and Z.; software validation, S.F.; formal analysis, S.F.; investigation, R.M. and J.A.A.; resources, T.M. and M.Y.; data curation, N.K.P. and Z.; writing—original draft preparation, Z.; writing—review and editing, J.A.A. and M.Y.; visualization, R.M.; supervision, T.M., M.Y., and U.S.; project administration, N.K.P.; funding acquisition T.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Techavuthiporn, C. *Langsat–Lansium Domesticum. Exotic Fruits Reference Guides*; Rodrigues, S., de Brito, E.S., Silva, E.d.O., Eds.; Academic Press: London, UK, 2018; pp. 279–283.
2. Mayanti, T.; Tjokronegoro, R.; Supratman, U.; Mukhtar, M.R.; Awang, K.; Hadi, A.H.A. Antifeedant triterpenoids from the seeds and bark of *Lansium domesticum* cv *kokossan* (Meliaceae). *Molecules* **2011**, *16*, 2785–2795. [CrossRef]
3. Dong, S.H.; Zhang, C.R.; Dong, L.; Wu, Y.; Yue, J.M. Onoceranoid-type triterpenoids from *Lansium domesticum*. *J. Nat. Prod.* **2011**, *74*, 1042–1048. [CrossRef] [PubMed]

4. Nishizawa, M.; Nishide, H.; Hayashi, Y.; Kosela, S. The structure of lansioside A: A novel triterpene glycoside with amino-sugar from *Lansium domesticum*. *Tetrahedron Lett.* **1982**, *23*, 1349–1350. [CrossRef]
5. Nishizawa, M.; Nishide, H.; Kosela, S.; Hayashi, Y. Structure of lansiosides: Biologically active new triterpene glycosides from *Lansium domesticum*. *J. Org. Chem.* **1983**, *48*, 4462–4466. [CrossRef]
6. Tanaka, T.; Ishibashi, M.; Fujimoto, H.; Okuyama, E.; Koyano, T.; Kowiyhayakorn, T.; Hayashi, M.; Komiyama, K. New onoceranoid constituents from *Lansium domesticum*. *J. Nat. Prod.* **2002**, *65*, 1709–1711. [CrossRef] [PubMed]
7. Habaguchi, K.; Watanabe, M.; Nakadaira, Y.; Nakanishi, K.; Kaing, A.K.; Lim, F.L. The full structures of lansic acid and its minor congener, an unsymmetric onoceradienedione. *Tetrahedron Lett.* **1986**, *34*, 3731–3734. [CrossRef]
8. Mayanti, T.; Supratman, U.; Mukhtar, M.R.; Awang, K.; Ng, S.W. Kokosanolide from the seed of *Lansium domesticum* Corr. *Acta Crystallogr.* **2009**, *E65*, o750.
9. Supratman, U.; Mayanti, T.; Awang, K.; Mukhtar, M.R.; Ng, S.W. 14-Hydroxy-8,14-secogammacera-7-ene-3,21-dione from the bark of *Lansium domesticum* Corr. *Acta Crystallogr.* **2010**, *E66*, o1621.
10. Manosroi, A.; Jantrawut, P.; Sainakham, M.; Manosroi, W.; Manosroi, J. Anticaner activities of the extract from longkong (*Lansium domesticum*) young fruits. *Pharm. Biol.* **2012**, *50*, 1397–1407. [CrossRef] [PubMed]
11. Ragasa, C.Y.; Labrador, P.; Rideout, J.A. Antimicrobial terpenoid from *Lansium domesticum*. *Philipp. Agric. Sci.* **2006**, *89*, 101–105.
12. Leatemia, J.A.; Isman, M.B. Insecticidal activity of crude seed extracts of *Annona.* spp., *Lansium domesticum* and *Sandoricum koetjape* against lepidopteran larvae. *Phytopatasitica* **2004**, *32*, 30–37. [CrossRef]
13. Saewan, N.; Sutherland, J.D.; Chantrapromma, K. Antimalarial tetranortriterpenoids from the seed of *Lansium domesticum* Corr. *Phytrochemistry* **2006**, *67*, 2288–2293. [CrossRef] [PubMed]
14. Matsumoto, T.; Kitagawa, T.; Teo, S.; Anai, Y.; Ikeda, R.; Imahori, D.; Ahmad, H.S.; Watanabe, T. Structures and antimutagenic effects of onoceranoid-type triterpenoids from the leaves of *Lansium domesticum*. *J. Nat. Prod.* **2018**, *81*, 2187–2194. [CrossRef] [PubMed]
15. Matsumoto, T.; Kitagawa, T.; Ohta, T.; Yoshida, T.; Imahori, D.; Teo, S.; Ahmad, H.S.; Watanabe, T. Structures of triterpenoids from the leaves of *Lansium domesticum*. *J. Nat. Med.* **2019**, *73*, 727–734. [CrossRef] [PubMed]
16. Mayanti, T.; Sianturi, J.; Harneti, D.; Darwati; Supratman, U.; Rosli, M.M.; Fun, H.K. 9,19-Cyclolanost-24-en-3-one,21,23-epoxy-21,22-dihydroxy (21*R*, 22*S*, 23*S*) from the leaves of *Lansium domesticum* Corr cv *kokossan*. *Molbank* **2015**, *2015*, M880. [CrossRef]
17. Hou, Y.; Cao, S.; Brodie, P.J.; Miller, J.S.; Birkinshaw, C.; Andrianjafy, M.N.; Andriantsiferana, R.; Rasamison, V.E.; Tendyke, K.; Shen, Y.; et al. Euphane triterpenoids of *Cassipourea lanceolata* from the Madagascar rainforest. *Phytochemistry* **2010**, *71*, 669–674. [CrossRef] [PubMed]

Sample Availability: Samples of the compounds **1** are available from the authors.