

Short Note

9,19-Cyclolanost-24-en-3-one,21,23-epoxy-21,22-dihydroxy (21R, 22S, 23S) from the Leaves of *Lansium domesticum* Corr cv Kokossan

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Abstract: A new cycloartan-type triterpenoid, 9,19-cyclolanost-24-en-3-one,21,23-epoxy-21,22-dihydroxy (21R, 22S, 23S), was isolated from the leaves of *Lansium domesticum* Corr cv kokossan. The chemical structure of **1** was elucidated on the basis of spectroscopic data, X-ray diffraction and comparison with those related compounds previously reported.

Keywords: cycloartan-type triterpenoid; *Lansium domesticum*; Meliaceae

1. Introduction

Lansium domesticum Corr cv kokossan is a species of small tree from the Meliaceae family and widely distributed in Thailand and surrounding countries in Southern Asia [1,2]. Previous phytochemical studies on *L. domesticum* have resulted in the isolation of several types of triterpenoids [3–8], which possess interesting biological activities, such as anticancer [9], antibacterial [10], insecticides [11], antimalarial [12], cosmetic [13] and antifeedant activities [14].

As a part of our studies on triterpenoids compounds from Indonesian Meliaceae plants, we isolated and described two tetranortriterpenoids, kokosanolide A and C and three onoceranoid-type triterpenoids including, kokosanolide B, 8,14-secogammacera-7,14-diene-3,21-dione and 8,14-secogammacera-7,14(27)-diene-3,21-dione from the seeds and the bark of the species kokossan [12]. In further screening for triterpenoid compounds, we found that the ethyl acetate extract from the leaves of *L. domesticum* cv kokossan exhibited the presence of triterpenoid compounds, based on a phytochemical test using the Liebermann-Burchard reagent. We report herein the isolation and structure elucidation of the new cycloartan-type triterpenoid, 9,19-cyclolanost-24-en-3-one,21,23-epoxy-21,22-dihydroxy (21*R*, 22*S*, 23*S*) (**1**) from the leaves of the *L. domesticum* cv kokossan. The chemical structure of compound **1** was established by NMR data and X-ray diffraction, as well as by comparison with those related compounds previously reported.

2. Result and Discussion

The ethyl acetate extract was separated and purified over a chromatography column packed with silica gel 60 by gradient elution. The chromatography column fractions were repeatedly subjected to normal-phase column chromatography and recrystallized in acetone to afford compound **1** (Figure 1). 9,19-cyclolanost-24-en-3-one,21,23-epoxy-21,22-dihydroxy (21*R*, 22*S*, 23*S*) was obtained as a white needle-like crystals from acetone; m.p. 145–147 °C, $[\alpha]_D^{20} -5.2^\circ$ (*c*, 0.5 CHCl₃). The molecular formula of **1** was established to be C₃₀H₄₆O₄ by HR-ESI-TOFMS spectrum $[M + H]^+$ *m/z* 471.3381 (calcd. 470.3396), together with NMR data (Table 1), thus requiring eight degrees of unsaturation. Its UV spectrum exhibited no conjugated group based on maximum absorption at 200 nm. The IR spectrum of **1** exhibited the presences of O-H stretch (3401 cm⁻¹), CH *sp*³ stretch (2928 cm⁻¹), C=O ketone stretch (1699 cm⁻¹), C=C (1453 cm⁻¹), and ether group (1078 cm⁻¹). The ¹H-NMR spectrum exhibited the presences of six methyl groups were resonated at δ_H 0.97, 1.73, 1.66, 1.09, 1.16, and 0.96 for Me-18, Me-26, Me-27, Me-28, Me-29, and Me-30, respectively. The characteristic of C-9 and C-10 cyclopropyl methylene group from a cycloartanone-type triterpenoid resonating at δ_H 0.66 and δ_H 0.70 (*J* = 3.9 Hz), respectively. An olefinic proton (H-24) was observed at δ_H 5.48 (1H, *d*, *J* = 8.4 Hz) together with two geminal vinyl methyls proton were observed at δ_H 1.73 (3H, *s*) and δ_H 1.66 (3H, *s*) which assigned for H-26 and H-27 respectively, thus indicating a $\Delta^{24,25}$ double bond in the side chain. Additional functionalities included the three protons bonded to carbon bearing oxygen were resonated at δ_H 5.17 (1H, *d*, *J* = 5.8 Hz, H-21) as a doublet as well as δ_H 3.96 (1H, *dd*, *J* = 4.5, 6.5 Hz, H-22) and δ_H 4.68 (1H, *dd*, *J* = 6.5, 8.4 Hz, H-23) as double doublet. A total of thirty carbon signals were observed in the ¹³C-NMR spectrum. These were assigned by DEPT and HMQC experiments to two *sp*² carbon signals at δ_C 123.8 and 135.8, one singlet carbon for ketone group at δ_C 215.1, six methyls *sp*³, eight methylenes, three *sp*³ oxygenated carbons at δ_C 101.6, 76.3 and 97.2, and five *sp*³ quaternary carbons. A downfield signal of methine was observed at δ_C 101.6 (δ_H 5.17, 1H, *d*, *J* = 1.2 Hz) corresponding to a hemiketalic carbon. These functionalities accounted for two out of the total eight degrees of unsaturation. The remaining six degrees of unsaturation were consistent to a cycloartan-type structure with one additional ring [15].

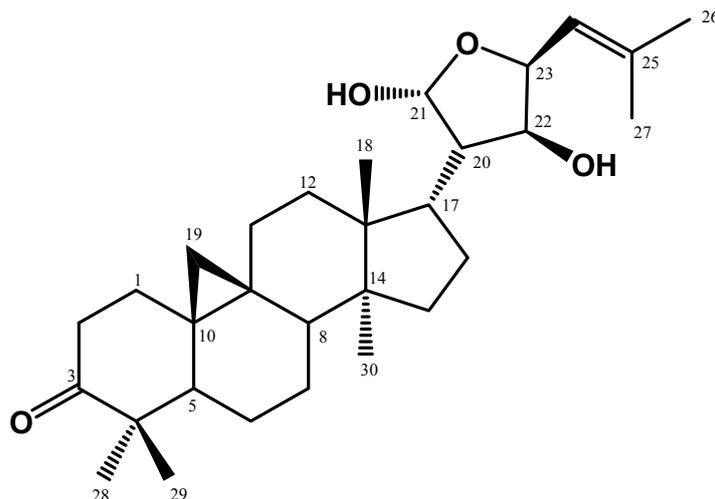


Figure 1. 9,19-Cyclolanost-24-en-3-one,21,23-epoxy-21,22-dihydroxy (21*R*, 22*S*, 23*S*) (**1**).

The gross structure of **1** was deduced from the ^1H - ^1H COSY and HMBC spectra (Figure 2). The ^1H - ^1H COSY spectrum showed coupling between H-17/H-20, H-20/H-21, H-20/H-22 and H-22/H-23, supporting the presence of a cycloartane triterpenoid structure. A long-range correlation was exhibited in the HMBC spectrum, between methine *sp*² proton signal at δ_{H} 5.48 (H-24) and oxygenated carbon signal at δ_{C} 97.2 (C-23), as well as a correlation between δ_{H} 2.19 (H-20) and δ_{H} 4.82 (H-22) to a downfield signal of methine at δ_{C} 101.6 (C-21) which suggests that the furan ring was built at C-21, C-20, C-22 and C-23, respectively. The position of olefinic carbon signals δ_{C} 123.8 (C-24) and δ_{C} 35.8 (C-25) were confirmed by long-range correlation of *gem*-dimethyl vinyl group (Me-26 and Me-27), whereas the position of the C-3 carbonyl was confirmed by showing correlations of H-2, Me-28 and Me-29 to the C-3 carbonyl group (δ_{C} 215.1). The NMR data of **1** was similar to those of argenteanone [16], the main difference was stereochemistry at C-21, C-22 and C-23. The structure of compound **1** was further confirmed by X-ray diffraction analysis (Figure 3). The asymmetric unit of compound **1** contain one unit of 3-(2,4-dihydroxy-5-(2-methylprop-1-en-1-yl)tetrahydrofuran-3-yl)-2a,5a,8,8-tetramethyltetradecahydrocyclo-penta[*a*]-cyclopropana[*e*]-phenanthren-9(1*H*)-one plus half unit of water molecule. It was crystallized in *P2*₁ space group with $a = 13.4825(2)$ Å, $b = 6.2433(1)$ Å, $c = 17.0838(3)$ Å, $\beta = 109.3068(8)^\circ$ and $Z = 4$. The molecular structure contains three six-membered rings of cyclohexane, two five membered rings, a cyclopentane and oxolane, and one cyclopropane. Three six membered rings, cyclopentane and cyclopropane are *trans* fused when the cyclopropane is in axial position relative to the mean plane cyclohexane and cyclopentane, therefore confirming the absolute configuration of **1**. Therefore, compound **1** was determined as a new cycloartan-type triterpenoid and was named 24(*E*)-cyclolanost-24-en-3-one, 21,23-epoxy-21,22-dihydroxy (21*R*, 22*S*, 23*S*) (**1**).

Table 1. NMR data (500 MHz for ^1H and 125 MHz for ^{13}C , in acetone- d_6) for **1**.

Position	^{13}C -NMR	^1H -NMR
	δ_{C} ppm (mult., ppm)	δ_{H} ppm (Int, mult., $J = \text{Hz}$)
1	33.9 (t)	1.86 (1H, <i>m</i>) 1.60 (1H, <i>m</i>)
2	37.8 (t)	2.21 (1H, <i>ddd</i> , 3.9, 6.5, 10.4) 2.73 (1H, <i>ddd</i> , 6.0, 6.0, 10.4)
3	215.1 (s)	-
4	50.7 (s)	-
5	46.7 (d)	1.93 (1H, <i>m</i>)
6	22.1 (t)	1.59 (1H, <i>m</i>) 1.50 (1H, <i>m</i>)
7	28.1 (t)	1.20 (1H, <i>m</i>) 1.86 (1H, <i>m</i>)
8	48.8 (d)	1.64 (1H, <i>m</i>)
9	21.7 (s)	-
10	26.9 (s)	-
11	26.7 (t)	1.93 (1H, <i>m</i>) 1.07 (1H, <i>m</i>)
12	32.8 (t)	1.57 (1H, <i>m</i>) 1.64 (1H, <i>m</i>)
13	48.7 (s)	-
14	46.5 (s)	-
15	36.3 (t)	1.05 (1H, <i>m</i>) 1.34 (1H, <i>m</i>)
16	26.9 (t)	1.86 (1H, <i>m</i>) 2.00 (1H, <i>m</i>)
17	49.2 (d)	1.57 (1H, <i>m</i>)
18	19.4 (q)	0.97 (1H, <i>s</i>)
19	27.1 (t)	0.70 (1H, <i>d</i> , 3,9) 0.66 (1H, <i>d</i> , 3,9)
20	59.4 (d)	2.19 (1H, <i>m</i>)
21	101.6 (d)	5.17 (1H, <i>d</i> , 5,8)
22	78.2 (d)	3.96 (1H, <i>dd</i> , 4.5, 6.5)
23	79.0 (d)	4.68 (1H, <i>dd</i> , 6.5, 8.4)
24	123.8 (d)	5.48 (1H, <i>d</i> , 8,4)
25	135.8 (s)	-
26	26.1 (q)	1.73 (3H, <i>s</i>)
27	18.4 (q)	1.66 (3H, <i>s</i>)
28	21.1 (q)	1.09 (3H, <i>s</i>)
29	19.7 (q)	1.16 (3H, <i>s</i>)
30	19.4 (q)	0.96 (3H, <i>s</i>)

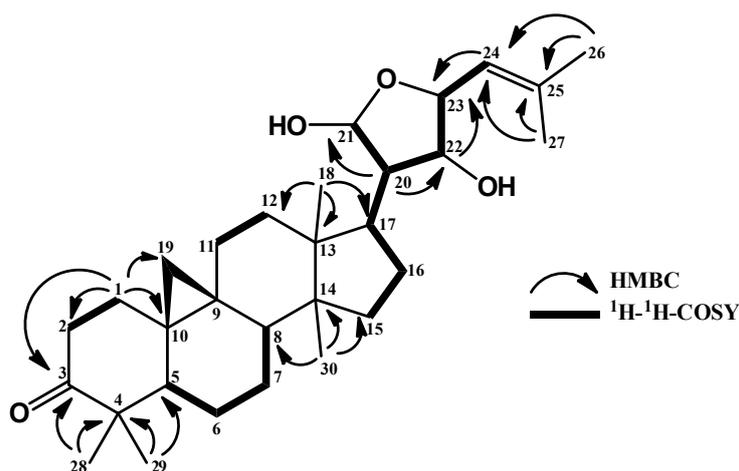


Figure 2. Selected HMBC and ^1H - ^1H -COSY correlations for **1**.

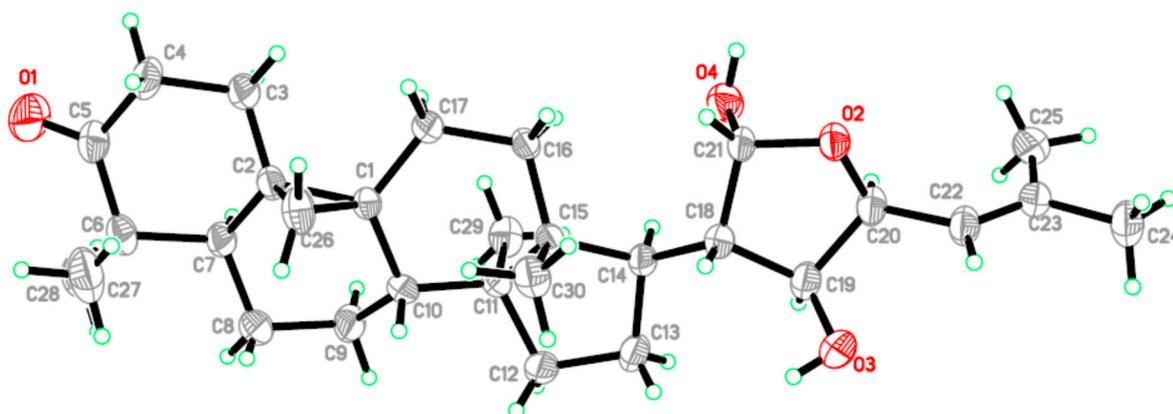


Figure 3. ORTEP drawing for **1**.

3. Experimental Section

3.1. General

Melting points were measured on an electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on an ATAGO AP-300 automatic polarimeter. The UV spectrum spectra were recorded on Shimadzu series 1800 spectrophotometer (Kyoto, Japan). The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR (Waltham, MA, USA) in KBr. HR-ESI-TOFMS spectra were obtained with a Waters LCT Premier XE mass spectrometer instruments (Santa Clara, CA, USA). ^1H and ^{13}C -NMR spectra were obtained with a JEOL JNM A-500 spectrometer using TMS as internal standard (Tokyo, Japan). Chromatographic separations were carried out on silica gel 60 (Merck, Darmstadt, Germany). TLC plates were precoated with silica gel GF₂₅₄ (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in ethanol, followed by heating. Crystallographic data were collected on a Bruker SMART APEXII CCD area-detector diffractometer with a graphite monochromated Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$) at 100.0 K with the Oxford Cryosystem Cobra low-temperature attachment (Billerica, MA, USA).

3.2. Plant Material

The leaves of *L. domesticum* cv kokossan were collected in Cililin District, Bandung, West Java Province, Indonesia in July 2013. The plant was identified by the staff of the Laboratory of Plant Taxonomy, Department of Biology, Padjadjaran University, Indonesia. A voucher specimen (No. 10188) was deposited at the herbarium of the Padjadjaran University.

3.3. Extraction and Isolation

The dried leaves (2.0 kg) were extracted with methanol exhaustively (5 L) at room temperature for 3 days. After removal of the solvent under reduced pressure, the viscous concentrate of MeOH extract (49.7 g) was first suspended in H₂O and then partitioned successively with *n*-hexane and EtOAc. A portion of ethyl acetate (16.7 g) was subjected to column chromatography over silica gel using a gradient of *n*-hexane/EtOAc (10:0–0:10) to afford 22 fractions (A01–A22). Fraction A06 (1.9 g) eluted by *n*-hexane/EtOAc = 6:4 was subjected to silica gel column chromatography, eluted with the mixtures of *n*-hexane/CHCl₃ (10:0–8:2) as eluting solvents to afford 17 fractions (B01–B17). The Fraction of B06 to B07 were combined and to give the white crystal (30.0 mg) after crystallized using acetone solvent.

NMR, IR and HRMS spectra for the title compound are available in the Supplementary Information.

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Author Contributions

Unang Supratman designed the whole experiment and contributed to the manuscript. Tri Mayanti and Julinton Sianturi researched data and wrote the manuscript, Desi Harneti and Darwati analyzed the NMR and LCMS/MS spectra, Mohamad Mustaqim Rosli and Hoong-Kun Fun analyzed the X-ray diffraction. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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