

A TRITERPENOID SAPONIN FROM SEEDS OF KOLOWE (*Chydenanthus excelsus*)**Laode Rijai**

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ABSTRACT

A triterpenoid saponin have been isolated from *n*-butanol fraction of the seeds of kolowe (*Chydenanthus excelsus*). The structure was determined as 3-O-[β -D-glucopyranosyl(1 \rightarrow 3)- β -D-xylopyranosyl(1 \rightarrow 3)- β -D-glucuronopyranosyloxy]-22 α -O-(2-methylbutyroyloxyolean-12-en-15 α ,16 α ,28-trihydroxy). Structure elucidation was accomplished by NMR (HMBC, HMQC/HSQC, ROE, ROESY, TOCSY) methods, ESIMS, and IR spectroscopic.

Keywords: *Chydenanthus excelsus*, Lecythidaceae, Triterpenoid saponin

INTRODUCTION

The seeds of kolowe (*C. excelsus*) species (Lecythidaceae) are important source of poison fish in Kamaru, Buton island, Indonesia, and Andaman island, Birma [1]. These seeds of species have been using as substance catch sea fishes in Kamaru, Buton Island, Indonesia. These extracts of methanol, *n*-butanol, and ethylacetate were as fungisidal, fiscidal, and antifeedant [2]. We now report one triterpenoid saponin from the seeds of plant that have been determined their structure. Identification of other saponins from seeds of plant was conducted by using isolation and NMR experiment to determine of their structures. These saponin will be reported later.

EXPERIMENTAL SECTION**General Experimental Procedures**

IR spectra were determined using a Shimadzu 8400S FTIR spectrometer and by Nujol mull technique. ESIMS were conducted using Finningan MAT LCQ mass spectrometer. ^1H and ^{13}C NMR spectra were recorded using Bruker Avance 400 at 400 MHz for proton and 100 MHz for carbon 13. All the NMR data were measured in CDCl_3 and chemical shifts were expressed in δ (ppm), referring to TMS for proton and CDCl_3 at δ

49.0 ppm for carbons 13. Preparative HPLC system was performed using a waters 510EF pump, Rheodyne 7125 injector, Waters 481 UV ($\lambda = 254$ nm) and Waters R410 RI detector, Column (Alltech Alltima C-18 reverse phase, 30 cm x 2.2 cm id, particle size, 10 μm), mobile phase methanol/water/acetate acid (75 : 25 : 0.5) flow rate 11.25 ml/min.

Plant Material

Kolowe (*C. excelsus*) was collected from Kamaru, Buton island, Indonesia on January 2001, and was identified in Laboratory of Bogoriense herbarium, Indonesian Institute of Sciences, Bogor, Indonesia. The specimen of the plant is kept in the herbarium of this.

Extraction and Isolation

The powdered dried seeds of *C. excelsus* (1.6 kg) were extracted with MeOH five times (2 L, each) at room temperature. The methanol extract was concentrated to dryness under reduced pressure to give a dark-brown residue (950 g). The methanol extract (500 g) defatted with petroleum ether, chloroform, methanol gradually, at room temperature under vacuum by open column chromatography. The methanol extract was concentrated under reduced pressure to give a dark-brown residue (475.5 g). The methanol extract (250 g) was partitioned between *n*-

butanol and water afforded in the organic layer the crude saponins fraction (145.5 g), was subjected to preparative HPLC. The *n*-butanol fraction (550 mg) was separated and purification repeated by preparative HPLC afforded a pure saponin (51 mg). Other saponins are using separation and purification by preparative HPLC. The extract of *n*-butanol (550 mg) in preparative HPLC was eluted isocratically with solvent of the MeOH/H₂O/AcOH (75 : 25 : 0.5), flow rate 11.25 ml/min.

A white amorphous powder (51 mg): **ESIMS** ($M + Na$)⁺ *m/z* (% rel.): 1067 (100); **FTIR**_(Nujol): V_{max} (cm⁻¹): 3385; 3184; 2725; 2671; 2359; 2341; 1733; 1684; 1653; 1558; 1306; 1155; 1076; 1020; 966; 721; ¹³C NMR (CD₃OD, 100 MHz) δ (ppm): 12,0; 16,3; 16,8; 17,0; 17,9; 19,5; 21,0; 24,7; 25,1; 27,1; 27,9; 28,3; 32,3; 33,5; 37,1; 37,9; 40,1; 40,4; 42,0; 42,2; 42,9; 45,6; 47,6; 48,1; 48,7; 56,6; 63,6; 63,7; 67,1; 68,5; 71,0; 71,5; 72,5; 72,9; 75,0; 75,2; 76,1; 78,2; 78,4; 86,7; 92,4; 103,2; 104,9; 105,6; 126,2; 144,3; 178,4; 175,2; ¹H NMR (CD₃OD, 400 MHz) δ (ppm): 0,80 (br *d*, *J* = 12 Hz); 0,88 (*s*); 0,90 (*s*); 0,92 (*t*, *J* = 7,2 Hz); 0,99 (*s*); 1,017 (*s*); 1,02 (*s*); 1,03 (*m*); 1,05 (br *d*, *J* = 12 Hz); 1,08 (*s*); 1,14 (*d*, *J* = 6,8 Hz); 1,39 (*s*); 1,43 (*m*); 1,49 (*ddd*, *J* = 12; 5,8; 1 Hz); 1,56 (*m*); 1,60 (*m*); 1,70 (*ddd*, *J* = 14; 7; 7,2 Hz); 1,72 (*m*); 1,74 (*m*); 1,75 (*m*); 1,89 (*m*); 1,94 (*m*); 2,17 (*t*, *J* = 12 Hz); 2,52 (br *dd*, *J* = 14; 4 Hz); 3,08 (*dd*, *J* = 9; 8 Hz); 3,10 (ABq, *J* = 11 Hz); 3,15 (*dd*, *J* = 9; 8 Hz); 3,21 (*dd*, *J* = 11,6; 4 Hz); 3,26 (*t*, *J* = 12 Hz); 3,27 (*t*, *J* = 8 Hz); 3,28 (ABq, *J* = 11 Hz); 3,29 (*m*); 3,32 (*t*, *J* = 8 Hz); 3,34 (*t*, *J* = 9 Hz); 3,53 (br *ddd*, *J* = 12; 8; 5,2 Hz); 3,55 (*dd*, *J* = 12; 7 Hz); 3,61 (*m*); 3,76 (*dd*, *J* = 7; 8 Hz); 3,77 (*d*, *J* = 7 Hz); 3,80 (*m*); 3,81 (*dd*, *J* = 8; 7,6 Hz); 3,83 (*dd*, *J* = 12; 2 Hz); 3,90 (*d*, *J* = 7 Hz); 3,92 (*dd*, *J* = 12; 5,2 Hz); 4,56 (*d*, *J* = 7,6 Hz); 4,61 (*d*, *J* = 7,6 Hz); 4,96 (*d*, *J* = 8 Hz); 5,39 (*dd*, *J* = 12; 5,8 Hz); 5,42 (br *t*, *J* = 3,6 Hz).

RESULTS AND DISCUSSION

The powdered dried seeds of *C. excelsus* were extracted with methanol. The methanol extract was defatted with petroleum ether and chloroform gradually. The methanol extract was partitioned between *n*-BuOH and water. The *n*-BuOH fraction was subjected to chromatographic purification repeated by HPLC preparative, afforded a barigenol A1 triterpenoid saponin.

The saponin was a white amorphous powder, had a molecular formula C₅₂H₈₄O₂₁ which was determined from its positive ion ESIMS (*m/z* 1067[M + Na]⁺) and confirmed by ¹³C NMR and DEPT analysis. The accurate mass of cidenansida I was calculated at *m/z* 1044 corresponding to C₅₂H₈₄O₂₁. The IR spectrum of saponin shows

absorptions at 3385 cm⁻¹ (OH), 966 cm⁻¹ and 721 cm⁻¹ (pyran), 1155 cm⁻¹ (ether pyran). The ¹³C NMR spectrum of cidenansida I showed 48 signals, meaning there are four carbon atoms that overlap each other as according to their molecular formula. Thus, from the ¹³C NMR spectrum of saponin showed the presence 52 carbon atoms in the molecule (Table 1). Of the 52 carbon atoms, 30 were assigned to the aglycon part, 17 to the oligosaccharide moiety, and the remaining five to an acyl group (Table 2).

Among the 30 carbons of the aglycon, seven were assigned to the methyl carbons at δ 28.3, 16.8, 16.3, 17.9, 21.0, 33.5, and 25.1 ppm, and the corresponding methyl proton singlets were identified by a HMQC and HSQC experiment. The chemical shift methyl proton singlets are at δ 0.88, 0.90, 0.99, 1.017, 1.08, 1.39, and 1.020, along with one olefinic proton at δ 5.42 ppm. Six saturated quaternary carbons were found at δ 40.4, 42.2, 37.9, 48.7, 45.6, and 32.3 ppm, while five carbons bearing oxygen were found at δ 92.4 (assigned to C-3), 68.5, 75.0, 72.9, and 63.7 ppm. Structure assignment was initiated from the long-range coupling networks observed between the methyl proton and the adjacent carbons from a HMBC experiment. Extensive NMR analyses showed the aglycon was of an olean-12-en skeleton with an olefinic carbons at δ 126,2 (C-12) and 144,3 (C-13).

Besides the two hydroxyls at C-3 and C-28, the other three groups were located at C-15, C-16, and C-22. Their configuration were determined using the ROE and ROESY. The spatial proximity observed between H-3 and H-23 (CH₃); H-3 and H-5; H-15, H-16 and H-26, H-27; H-15, H-16 and H-28 indicated the β -orientation of the hydroxyl at C-3 and α -orientation of the hydroxyl at C-15 and C-16, respectively. The ROE and ROESY observed between H-22 and H-18, H-22 and H-30 (CH₃), H-28 and H-26 (CH₃) indicated the α -orientation of the hydroxyl at C-22, and D/E ring was *cis*-configuration. From the above evidences, the aglycon was identified as 3 β , 15 α , 16 α , 22 α , and 28 (methylenhydroxy) pentanol, an compound named cidenansida I. An acyl group was also mapped out from HH-COSY, HMQC/HSQC, and TOCSY correlation and identified as 3-methylbutyrioloxyl esterified to C-22 hydroxyl as established from the long-range HMBC coupling between H-22 (δ 5.39 ppm, *dd*, *J* = 12; 5.8 Hz) and C-1 (178,4 ppm) of the acyl (Figure 2), and confirmed by the lowfield signal of H-22, indicative for acylation. In the TOCSY experiment to signal at δ 0.92 ppm (H-5 of the acyl group) given correlation related to proton signals at δ 1.49, 1.70, 2.40, and 1.14 ppm, thus greatly simplifying the mapping of the spin system. These

correlation of signals by a HH-COSY, HMQC/HSQC, and HMBC experiment, the acyl group are 3-methylbutyryloxy (Fig 1). The NMR spectra of cidenansida I aglycon were confirmed by a saponins from *Maesa laxiflora* [3] and *Barringtonia asiatica* [4], while the acyl group by saponin from *Pittosporum undulatum* [5].

Moreover, the presence of three sugar moieties was evidenced by the ^1H and ^{13}C NMR that displayed three sugar anomeric proton at δ 4.56 (*d*, $J = 7.6$ Hz), 4.61 (*d*, $J = 7.6$ Hz), 4.96 (*d*, $J = 8.0$ Hz) and carbons at δ 105.6, 104.9, 103.2 ppm (Table 3), respectively. The lowfield chemical shifts of C-3 (δ 92.4) indicated that the trisaccharide chain was connected to this position, whose sequence was determined by a combination of HH-COSY, DEPT, HMBC, HSQC/HMQC, TOCSY 1D, ROE and ROESY. Starting from the anomeric protons of each sugar unit, all the hydrogens within each spin system were assigned by TOCSY, HH-COSY, HMQC/HSQC, and HMBC. Furthermore, ROE and

ROESY experiment across the glycosidic bonds, also revealed the 1,3 and 1,5 diaxial relationship for glucuronic acid, glucose, and xylose. Thus, greatly simplifying the mapping of the spin system. On the basis of the assigned protons, the ^{13}C resonances of each sugar unit identified by HSQC/HMQC, HH-COSY, and HMBC. These data led to the identification of the three monosaccharide units as β -xylose, β -glucose, and β -glucuronic acid. The inter-sugars linkages were established from the following HMBC correlations: H-1 of the glucose with C-3 of xylose; H-1 of the xylose with C-3 of glucuronic acid, and H-1 of glucuronic acid with C-3 of aglycon. On the basis coupling constant from H-3, H-1', H-1'', and H-1''' along with ROE and ROESY experiment result. all configuration of glycosidic bonds were $\beta 1 \rightarrow 3$. Based upon the above evidence, Cidenansida I is established as 3-O- $[\beta$ -D-glucopyranosyl (1 \rightarrow 3)- β -D-xylopyranosyl(1 \rightarrow 3)- β -D-glucuronopyranosyloxy]-22 α -O-(2-methylbutyryloxy)-olean-12-en-15 α ,16 α ,28-trihydroxy.

Table 1 NMR data in CD_3OD for aglycon part

Position	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm)	HMBC Correlations
1	40.1	1.74 (<i>m</i>), 1.03 (<i>m</i>)	H-2, H-3, H-5, H-9, H-25
2	27.1	1.89 (<i>m</i>), 1.75 (<i>m</i>)	H-1, H-3
3	92.4	3.21 (<i>dd</i> , $J = 11.6$; 4 Hz)	H-1', H-5, H-23, H-24
4	40.4	-	H-2, H-3, H-5, H-6, H-23, H-24
5	56.6	0.80 (<i>br d</i> , $J = 12$ Hz)	H-1, H-3, H-6, H-7, H-9, H-23, H-23, H-24, H-25
6	19.5	1.56 (<i>m</i>), 1.43 (<i>m</i>)	H-5, H-7
7	37.1	1.72 (<i>m</i>)	H-5, H-6, H-9, H-26
8	42.2	-	H-6, H-7, H-9, H-11, H-15, H-26, H-27
9	48.1	1.60 (<i>m</i>)	H-1, H-5, H-7, H-11, H-12, H-25, H-26
10	37.9	-	H-1, H-2, H-5, H-6, H-9, H-11, H-25
11	24.7	1.94 (<i>m</i>)	H-9, H-12
12	126.2	5.42 (<i>br t</i> , $J = 3.6$ Hz)	H-9, H-11, H-18
13	144.3	-	H-11, H-12, H-15, H-18, H-19, H-27
14	48.7	-	H-7, H-7, H-9, H-15, H-16, H-18, H-26, H-27
15	68.5	3.77 (<i>d</i> , $J = 7$ Hz)	H-16, H-27
16	75.0	3.90 (<i>d</i> , $J = 7$ Hz)	H-15, H-18, H-22, H _{AB} -28,
17	45.6	-	H-15, H-16, H-18, H-19, H-21, H-22, H _{AB} -28
18	42.2	2.52 (<i>br dd</i> , $J = 14$; 4 Hz)	H-12, H-16, H-19, H-22, H _{AB} -28
19	47.2	2.40 (<i>t</i> , $J = 12$ Hz), 1.05 (<i>br d</i> , $J = 12$ Hz)	H-18, H-21, H-29, H-30
20	32.3	-	H-18, H-19, H-21, H-22, H-29, H-30
21	42.0	2.17 (<i>t</i> , $J = 12$ Hz), 1.49 (<i>ddd</i> , $J = 12$; 5.8; 1 Hz)	H-19, H-22, H-29, H-30
22	72.9	5.39 (<i>dd</i> , $J = 12$; 5.8 Hz)	H-16, H-18, H-21, H _{AB} -28
23	28.3	1.08 (<i>s</i>)	H-3, H-5, H-24
24	16.8	0.88 (<i>s</i>)	H-3, H-5, H-23
25	16.3	0.99 (<i>s</i>)	H-1, H-5, H-9
26	17.9	1.017 (<i>s</i>)	H-7, H-9
27	21.0	1.39 (<i>s</i>)	H-15
28	63.7	3.28; 3.10 (<i>ABq</i> , $J = 11$ Hz)	H-16, H-18, H-22
29	33.5	0.90 (<i>s</i>)	H-19, H-21, H-30
30	25.1	1.020 (<i>s</i>)	H-19, H-21, H-29

Table 2 NMR data in CD₃OD for sugar part and acyl group

Position	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm)	HMBC Correlations
Glucuronic acid			
1'	105.6	4.56 (<i>d</i> , <i>J</i> = 7.6 Hz)	H-3, H-5', H-2'
2'	78.4	3.81 (<i>dd</i> , <i>J</i> = 8; 7.6 Hz)	H-3'
3'	86.7	3.76 (<i>dd</i> , <i>J</i> = 7; 8 Hz)	H-1', H-2', H-5', H-1''
4'	71.5	3.61 (<i>m</i>)	H-3', H-5'
5'	78.4	3.80 (<i>m</i>)	
6'	175.2	-	
Xylose			
1''	104.9	4.61 (<i>d</i> , <i>J</i> = 7.6 Hz)	H-3'', H _{AB} -5''
2''	75.2	3.27 (<i>t</i> , <i>J</i> = 8 Hz)	H-3''
3''	78.2	3.32 (<i>t</i> , <i>J</i> = 8 Hz)	H-1'', H-2'', H-4'', H-5''
4''	71.0	3.53 (<i>br ddd</i> , <i>J</i> = 12, 8, 5.2 Hz)	H-4'', H _{AB} -5''
5''	67.1	3.92 (<i>dd</i> , <i>J</i> = 12, 5.2 Hz), 3.26 (<i>t</i> , <i>J</i> = 12 Hz)	H-1'', H-3'', H-4''
Glucose			
1'''	103.2	4.96 (<i>d</i> , <i>J</i> = 8 Hz)	H-3''', H-2'''
2'''	76.1	3.15 (<i>dd</i> , <i>J</i> = 9, 8 Hz)	H-1''', H-3''', H-4'''
3'''	78.2	3.34 (<i>t</i> , <i>J</i> = 9 Hz)	H-1''', H-2''', H-4''', H-5'''
4'''	72.5	3.08 (<i>dd</i> , <i>J</i> = 9, 8 Hz)	H-3''', H _{AB} -6'''
5'''	75.2	3.29 (<i>m</i>)	H-3'''
6'''	63.6	3.83 (<i>dd</i> , <i>J</i> = 12, 2 Hz) 3.55 (<i>dd</i> , <i>J</i> = 12, 7 Hz)	H-4'''
Ester			
1''''	178.4	-	H-22, H-2''''', H _{AB} -3''''
2''''	42.9	2.40 (<i>sext.</i> , <i>J</i> = 6.6 Hz)	H _{AB} -3''''', H-4''''', H-5''''
3''''	27.9	1.70, 1.49 (2 x <i>ddd</i> , <i>J</i> = 14, 7, 7.2 Hz)	H-2''''', H-4''''', H-5''''
4''''	12.0	0.92 (<i>t</i> , <i>J</i> = 7.2 Hz)	H-2''''', H _{AB} -3''''
5''''	17.0	1.14 (<i>d</i> , <i>J</i> = 6.8 Hz)	H-2''''', H _{AB} -3''''

Table 3 Result of 1 D ROE experiment for Cidenansida I

Proton Irradiated	Protons with enhanced signal
Aglycon	
H-5	H-5, H α -6 (1.55 ppm), H α -7, H-9, H-23
H-12	H-18, H β -19 (2.41 ppm), (H-11) ₂
H-18	H-12, H β -19, H-22, H _{AB} -28, H-30
H α -19	H-27
H-22	H-16, H-18, H β -21 (1.48 ppm), H _{AB} -28, H-30
H-23	H-1', H-3, H-5, H α -6
H β -19	H-18
H-24	H-25
H-25	H-24, H β -1 (1.65 ppm), H β -2 (1.75 ppm), H-11
H-26	H α -11, H-15, H-24
H-27	H α -19, H-9
H β -6	H-24, H-25, H-26
H-29	H α -19, H α -21 (2.09 ppm),
H-30	H-18, H β -21, H-22
Sugar Part	
H-1'	H-3, H-3', H-5, H-23
H-1''	H-3', H-3'', H α -5'' (3.26 ppm)
H-1'''	H-3'', H-3''', H-5'''

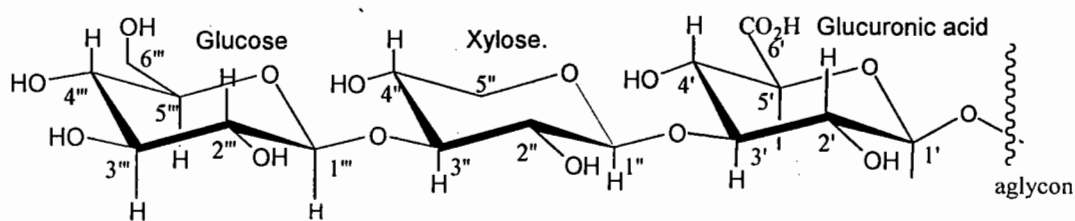
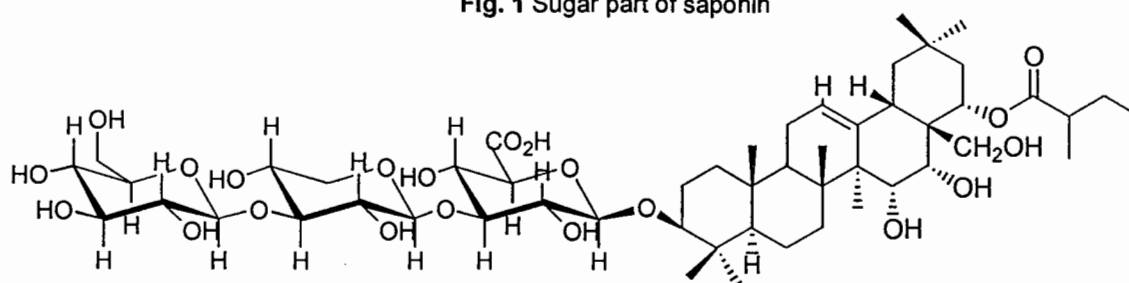


Fig. 1 Sugar part of saponin



3-O-[β-D-glucopyranosyl(1→3)-β-D-xylopyranosyl(1→3)-β-D-glucuronopyranosyloxy]-22α-O-(2-methylbutyroyloxyolean-12-en-15α,16α, 28-trihydroxy.

Fig. 2 A triterpenoid saponin from seeds of Kolowe (*C. excelsus*)

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