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

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Received 24 September 2004; Accepted 22 October 2004

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→3)-β-D-xylopyranosyl(1→3)- β - D-glucuronopyranosyloxy]- 22α -O-(2-methylbutiroyloxyolean-12-en-15α,16α,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, fisicidal, 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 saponis 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. ^{1}H 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 CDO_3D and chemical shifs were expressed in δ (ppm), refering to TMS for proton and CDO_3D at δ 49.0 ppm for carbons 13. Preparative HPLC system was performed using a waters 510EF pump, Rheodyne 7125 injector, Waters 481 UV (λ = 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) flowe 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 petroleumether, chloroform, methanol gradually, at room temperature under vacum 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 isocraticaly with solvent of the MeOH/H2O/AcOH (75 : 25 : 0.5), flowe 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; **^{13}C NMR** (**CD_3OD, 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; **^{1}H NMR (CD_3OD, 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 (AB*q*, *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 (AB*q*, *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 moleculer formula $C_{52}H_{84}O_{21}$ which was determined from its positive ion ESIMS (m/z 1067$[M + Na]^+$) and confirmed by ^{13}C NMR and DEPT analysis. The accurate mass of cidenansida I was calculated at m/z 1044 corresponding to $C_{52}H_{84}O_{21.}$ 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 ^{13}C NMR spectrum of cidenasida I showed 48 signals, meaning there are four carbon atoms that overlap each other as according to their moleculer formula. Thus, from the ^{13}C 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 shif 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 quartener 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 assigment 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 oleane-12-en skeleton with an olefinic carbons at δ 126,2 (C-12) and 144,3 (C-13).

Basides 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 (CH_3); 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 (CH_3), H-28 and H-26 (CH_3) 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 3β, 15α, 16α, 22α, 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-methylbutiroiloxy 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-methylbutiroyloxy (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 sapogenins from *Pittosporum undulatum* [5].

Moreover, the presence of three sugar moieties was evidenced by the ^{1}H 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 ot C-3 (δ 92.4) indicated that the threesaccharide 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 glicosidic bonds were $\beta 1 \rightarrow 3$. Based upon the above evidence, Cidenansida I is established as 3-*O*-[β-D-glucopyranosyl ($1 \rightarrow 3$)- β-D-xylopyranosyl($1 \rightarrow 3$)-β-D-glucuronopyranosyloxy]-22α-*O*-(2-methylbutiroyloxy-olean-12-en-15α,16α,28-trihydroxy.

Table 1 NMR data in CD_3OD for aglycon part

Position	δ ^{13}C (ppm)	δ ^{1}H (ppm)	HMBC Correlations
1	40.1	1.74 (*m*), 1.03 (*m*)	H-2, H-3, H-5, H-9, H-25
2	27.1	1.89 (*m*), 1.75 (*m*)	H-1, H-3
3	92.4	3.21 (*dd, 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 (br *d, 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 (*m*), 1.43 (*m*)	H-5, H-7
7	37.1	1.72 (*m*)	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 (*m*)	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 (*m*)	H-9, H-12
12	126.2	5.42 (br *t, 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 (*d, J* = 7 Hz)	H-16, H-27
16	75.0	3.90 (*d, 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 (br *dd, J* = 14; 4 Hz)	H-12, H-16, H-19, H-22, H_{AB}-28
19	47.2	2.40 (*t, J* = 12 Hz), 1.05 (br *d, 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 (*t, J* = 12 Hz), 1.49 (*ddd, J* = 12; 5.8; 1 Hz	H-19, H-22, H-29, H-30
22	72.9	5.39 (*dd, J* = 12; 5.8 Hz)	H-16, H-18, H-21, H_{AB}-28
23	28.3	1.08 (*s*)	H-3, H-5, H-24
24	16.8	0.88 (*s*)	H-3, H-5, H-23
25	16.3	0.99 (*s*)	H-1, H-5, H-9
26	17.9	1.017 (*s*)	H-7, H-9
27	21.0	1.39 (*s*)	H-15
28	63.7	3.28; 3.10 (ABq, *J* = 11 Hz)	H-16, H-18, H-22
29	33.5	0.90 (*s*)	H-19, H-21, H-30
30	25.1	1.020 (*s*)	H-19, H-21, H-29

Table 2 NMR data in CD_3OD for sugar part and acyl group

Position	δ ^{13}C (ppm)	δ ^{1}H (ppm)	HMBC Correlations
Glucuronic acid			
1'	105.6	4.56 (*d, J* = 7.6 Hz)	H-3, H-5', H-2'
2'	78.4	3.81 (*dd, J* = 8; 7.6 Hz)	H-3'
3'	86.7	3.76 (*dd, J* = 7; 8 Hz)	H-1', H-2', H-5', H-1''
4'	71.5	3.61 (*m*)	H-3', H-5'
5'	78.4	3.80 (*m*)	
6'	175.2	-	
Xylose			
1''	104.9	4.61 (*d, J* = 7.6 Hz)	H-3', H_{AB}-5''
2''	75.2	3.27 (*t, J* = 8 Hz)	H-3''
3''	78.2	3.32 (*t, J* = 8 Hz)	H-1'', H-2'', H-4'', H-5''
4''	71.0	3.53 (br *ddd, J* = 12, 8, 5.2 Hz)	H-4'', H_{AB}-5''
5''	67.1	3.92 (*dd, J* = 12, 5.2 Hz), 3.26 (*t, J* = 12 Hz)	H-1'', H-3'', H-4''
Glucose			
1'''	103.2	4.96 (*d, J* = 8 Hz)	H-3'', H-2'''
2'''	76.1	3.15 (*dd, J* = 9, 8 Hz)	H-1''', H-3''', H-4'''
3'''	78.2	3.34 (*t, J* = 9 Hz)	H-1''', H-2''', H-4''', H-5'''
4'''	72.5	3.08 (*dd, J* = 9, 8 Hz)	H-3''', HAB-6'''
5'''	75.2	3.29 (m)	H-3'''
6'''	63.6	3.83 (*dd, J* = 12, 2 Hz) 3.55 (*dd, J* = 12, 7 Hz)	H-4'''
Ester			
1''''	178.4	-	H-22, H-2'''', H_{AB}-3''''
2''''	42.9	2.40 (*sext., J* = 6.6 Hz)	H_{AB}-3'''', H-4'''', H-5''''
3''''	27.9	1.70, 1.49 (2 x *ddd, J* = 14, 7, 7.2 Hz)	H-2'''', H-4'''', H-5''''
4''''	12.0	0.92 (*t, J* = 7.2 Hz)	H-2'''', H_{AB}-3''''
5''''	17.0	1.14 (*d, J* = 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, Hα-6 (1.55 ppm), Hα-7, H-9, H-23
H-12	H-18, Hβ-19 (2.41 ppm), $(H\text{-}11)_2$
H-18	H-12, Hβ-19, H-22, HAB-28, H-30
Hα-19	H-27
H-22	H-16, H-18, Hβ-21 (1.48 ppm), HAB-28, H-30
H-23	H-1', H-3, H-5, Hα-6
Hβ-19	H-18
H-24	H-25
H-25	H-24, Hβ-1 (1.65 ppm), Hβ-2 (1.75 ppm), H-11
H-26	Hα-11, H-15, H-24
H-27	Hα-19, H-9
Hβ-6	H-24, H-25, H-26
H-29	Hα-19, Hα-21 (2.09 ppm),
H-30	H-18, Hβ-21, H-22
Sugar Part	
H-1'	H-3, H-3', H-5, H-23
H-1''	H-3', H-3'', Hα-5'' (3.26 ppm)
H-1'''	H-3'', H-3''', H-5'''

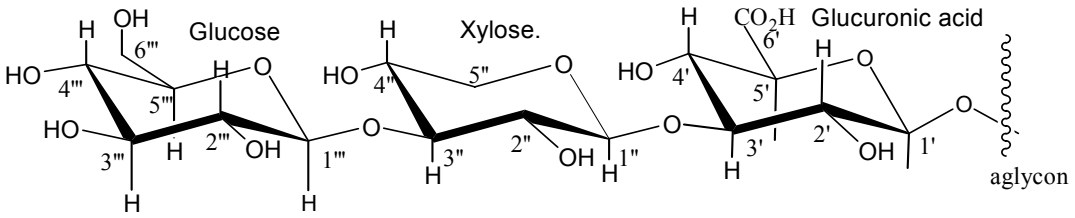
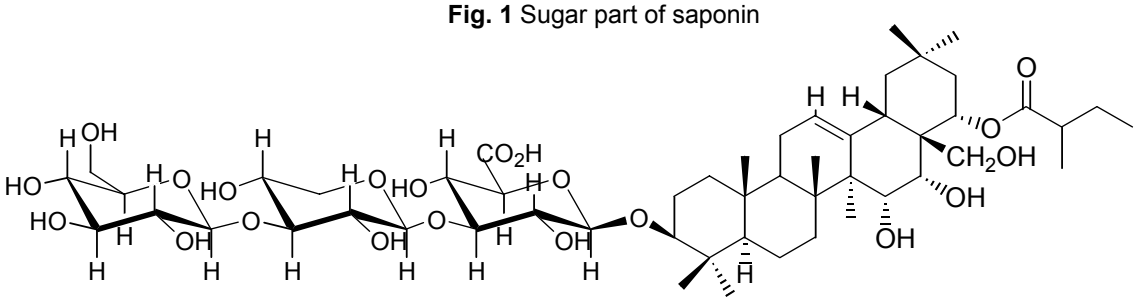


Fig. 1 Sugar part of saponin



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

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

ACKNOWLEDGEMENTS

Thanks are expressed to Professor Damon Ridley and their staff, as Chairman of Organic Chemistry, Sydney University, Australia. School of Chemistry was place execution of this research.
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