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Septemloside III: A Nonasaccharide Saponin from the Bark of *Kalopanax septemlobus* (Thunb.) Koidz.

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Abstract: A nonasaccharide saponin**,** new hederagenin saponin, had been isolated from the bark of *Kalopanax septemlobus* (Thunb.) Koidz., and its structure was elucidated by HRESI-MS, NMR experiments and chemical analyses as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-xylopyranosyl-(1→3)-β-D–xylopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside.

Keywords: *Kalopanax septemlobus* (Thunb.) Koidz., hederagenin saponin, septemloside.

Kalopanax septemlobus (Thunb.) Koidz. has been traditionally used for the treatment of rheumatoidal arthritis, neurotic pain and diabetes mellitus in China[1]. Since the isolation and structural determination of hederagenin saponins named kalopanax-saponins A and B had been reporated[2], the constituents such as hederagenin glycosides, syringin, liriodendrin and coniferyl-aldehyde glucosides have been isolated from this plant[3,4]. The phytochemical and pharmacological studies on *Kalopanax* species have intensified in recent years following the discovery of the antidiabetic activity of the constituents in the plant[5], a methanol mixture [6~8] obtained from *K. pictus* was reported to possess antiinflammatory activity, antifungal [9,10] and antirheumatoid arthritis activity[11]. In the course of our antiinflammatory activity investigation on the plant, we found that the ethanol extract of the stem bark of *K. septemlobus* exhibited significant inhibitory activity by our preliminary screening test evaluated against acetic acid-induced writhing, this prompted us to investigate the constituents in the ethanol extract of the plant. We report herein the isolation and structural elucidation of a new nonasaccharide saponin, named as septemloside III (**1**) (**Figure 1**).

The ethanol extract of dried bark of *K. septemlobus* (Thunb.) Koidz. was subjected to repeated column chromatography of normal and reverse silica gel to afford compound **1**. Compound **1** was obtained as a white amorphous solid. mp 241~243℃, $[\alpha]_D^{20}$ -29.6 (C 0.29,

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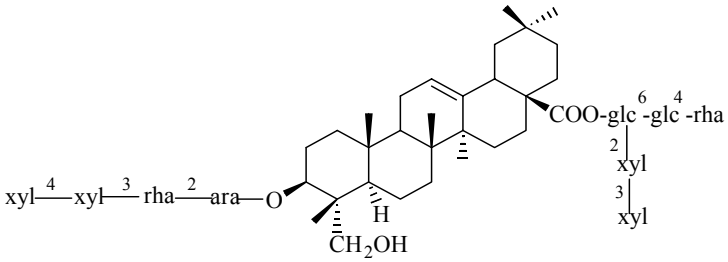
MeOH), its molecular formula was unequivocally established to be C_{79} H_{128} O_{42} by HRESI-MS which showed a quasi-molecular ion peak at *m/z* 1749.8650 $[M+H]^+$. The aglycone of compound **1** could be proved to be hederagenin by direct comparison of ^{13}C NMR spectrum (**Table 1**) with that of the literature[12]. The ^1H NMR spectrum showed signals for six tertiary methyl groups at δ 1.11 (s), 0.93 (s), 0.91(s), 0.82(s), 0.82 (s), and 0.79 (s), and two secondary methyl groups at δ 1.58 (d, *J*=6.0Hz), 1.50 (d, *J*=6.0Hz), which correlated in the HMQC spectrum with eight sp^3 C-atoms at δ(C) 26.30, 14.17, 17.57, 16.27, 23.84, 33.29, 18.69 and 18.59, respectively. It also showed nine anomeric proton signals at δ 4.71 (d, *J*=7.8Hz), 4.87 (d, *J*=7.8Hz), 4.91 (d, *J*=7.8Hz), 5.02 (d, *J*=6.0Hz), 5.13 (d, *J*=7.8Hz), 5.26 (d, *J*=7.2Hz), 5.66 (br, s), 5.96 (d, *J*=8.4Hz), and 6.08 (br, s), which correlated in the HMQC spectrum with ^{13}C NMR signals at δ 103.91, 105.03, 103.83, 104.66, 107.17, 105.26, 102.88, 93.50, and 101.63, respectively. Evaluation of spin-spin couplings and chemical shifts allowed the identification of one α-L-arabinopyranosyl unit, two α-L-rhamno- pyranosyl units, two β-D-glucopyranosyl units, and four β-D-xylopyranosyl units. The ring protons of the monosaccharide residues were assigned starting from the anomeric protons by means of TOCSY experiment. The H_2O layer of mild alkaline hydrolysis of compound **1** yield an oligosaccharide, which afforded monosaccharide on acidic hydrolysis, the monosaccharide was subjected to HPLC analysis [column, Zorbax Carbohydrate Analysis column (250mm×4.6mm, 5μm); solvent, CH_3CN:H_2O=63:37; detector, Alltech ELSD 2000 detector; drift tube temperature, 86.7˚C] to revealed the presence of D-xylose (t_R 6.35 min), D-glucose (t_R 7.67 min), and L-rhamnose (t_R 5.96 min). Acid-hydrolysis of compound **1** yield an aglycone and a sugar residue consisting of D-xylose, D-glucose, L-rhamnose(*vide sppra*), and L-arabinose (t_R 6.93 min) detected by the HPLC method. On the basis of extensive 1D and 2D NMR experiments, along with the ESI-MS^2 fragmentation pathway which displayed two main fragment ions at *m/z* 757 attributable to a pentsaccharide [glc+glc+rha+xyl+xyl+ Na]$^+$, which was further suggested by two fragments of 625 [glc+glc+rha+xyl+Na]$^+$ and 611 [glc+glc+xyl+xyl+Na]$^+$ in the ESI-MS^3 that it contained a rhamnose and a xylose as terminal sugars, and 1037 attributable to [aglycone+ara+rha+xyl+ xyl+Na]$^+$, thus it can be concluded that **1** was a bisdesmosidic saponin with four mono- saccharides linked at C-3(δ 81.28) of the aglycone and the other five monosaccharides linked at C-28(δ 176.68) through an ester bond. This was further confirmed by the HMBC experiment. The manner of the attachment of the units could be unambiguously derived from the HMBC between the proton signal at δ 5.02 with the carbon resonance at δ 81.28, and the proton signal at δ 5.96 with the carbon resonance at δ 176.68, which allowed us to deduce the connective situation between the aglycone with saccharides portions. Thus, the structure of **1** was determined as 3-*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-xylopyrano-syl-(1→3)-β-D-xylopyranosyl-(1→2)-[α-L- rhamnopyrano-syl-(1→4)-β-D-glucopyranosyl-(1→6)]- β-D -glucopyranoside.

Table 1 NMR data of compound **1** (δ ppm)

No.	Hedera-genin	**1**		No.	**1**	
		^{13}C	^{1}H		^{13}C	^{1}H
1	38.9	39.28	1.42(m, 1H) 0.93(m, 1H)	5	69.69	4.58(m, 1H)
2	27.6	28.97	2.05(m,1H) 1.11(m, 1H)	6	18.59	1.50(d, 3H, *J*=6.0Hz)
3	73.7	81.28	4.17(m, 1H)	xyl-1	107.17	5.13(d, 1H, *J*=7.8Hz)
4	42.9	43.70		2	75.51	3.99(dd, 1H, *J*=8, 8.4Hz)
5	48.8	48.36	1.65(m, 1H)	3	75.56	4.05#
6	18.7	18.18	1.58(m, 1H) 1.44(m, 1H)	4	76.60	4.22#
7	33.0	32.58	1.76(m, 1H) 1.68(m, 1H)	5	64.88	4.22#, 3.56(d, 1H, *J*=9.6Hz)
8	39.8	40.08		xyl-1	103.91	4.71(d, 1H, *J*=7.8Hz)
9	48.2	47.92	1.62(m, 1H)	2	74.14	3.88(d, 1H, *J*=5.4Hz)
10	37.3	36.98		3	78.00	4.06#
11	23.8	23.99	1.81(m, 1H) 1.76(m, 1H)	4	71.07	4.08#
12	122.7	122.82	5.26(br s, 1H)	5	67.54	4.21(d,1H,*J*=9.6Hz),3.57(d,1 H,*J*=5.4Hz)
13	145.0	144.56				
14	42.2	42.17		C-28		
15	28.4	28.97	2.05(m, 1H) 1.11(m, 1H)	glc-1	93.50	5.96(d, 1H, *J*=8.4Hz)
16	23.8	23.18	1.95(m, 1H) 1.89(m, 1H)	2	79.29	4.11(dd, 1H, *J*=9.6, 6.6Hz)
17	46.7	47.21		3	78.37	4.17#
18	42.0	41.63	3.03(dd,1H,*J*=13.8,4.0Hz)	4	70.46	4.18#
19	46.5	46.43	1.59(m, 1H) 1.45(m, 1H)	5	75.70	3.92(m, 1H)
20	31.0	30.87		6	68.98	4.50(br d, 1H), 4.22#
21	34.3	34.16	1.18(m, 1H) 0.93(m, 1H)	xyl-1	105.26	5.26(d, 1H, *J*=7.2Hz)
22	33.3	33.29	1.55(m, 1H) 1.07(m, 1H)	2	75.70	3.92(dd, 1H, *J*=7.8, 7.8Hz)
23	68.2	64.03	4.04(m, 1H) 3.80(brd, 1H)	3	75.13	4.05#
24	13.1	14.17	0.93(s, 3H)	4	76.04	4.28#
25	16.0	16.27	0.82(s, 3H)	5	65.25	4.40#, 3.56(d, 1H, *J*=10.2Hz)
26	17.5	17.57	0.91(s, 3H)	xyl-1	103.83	4.91(d, 1H, *J*=7.8Hz)
27	26.2	26.30	1.11(s, 3H)	2	73.37	3.99(m, 1H)
28	180.4	176.63		3	77.76	4.09(d, 1H, *J*=9.0Hz)
29	33.3	33.29	0.79(s, 3H)	4	71.03	4.12#
30	23.8	23.84	0.82(s, 3H)	5	67.91	4.28(d, 1H, *J*=7.8Hz) , 3.80 (d,1H, *J*=10.2Hz)
				glc-1	105.03	4.87(d, 1H, *J*=7.8Hz)
C-3				2	75.40	3.85(d, 1H, *J*=8.4, 8.4Hz)
Ara-1		104.66	5.02(d, 1H, *J*=6.0Hz)	3	77.32	4.06#
2		75.86	4.46(m, 1H)	4	78.58	4.21#
3		75.13	4.06#	5	76.97	3.51(dd, 1H, *J*=9.0, 9.0Hz)
4		69.64	4.15#	6	61.42	4.08#, 3.97(d, 1H, *J*= 8.4Hz)
5		66.15	4.18#, 3.64(br d, 1H)	rha-1	102.88	5.66(br s, 1H)
rha-1		101.63	6.08(br s, 1H)	2	72.73	4.57(br s, 1H)
2		71.98	4.79#	3	72.92	4.44(d, 1H, *J*=3.6Hz)
3		83.25	4.60(br s, 1H)	4	74.03	4.24#
4		73.16	4.35(dd, 1H, *J*=9.0, 9.0Hz)	5	70.58	4.78(d, 1H, *J*=3.0Hz)
				6	18.69	1.58(d, 3H, *J*=6.0Hz)

Note: 1. All spectra were recorded on Bruker AVANCE-600MHz NMR spectrometer in pyridine-d_5; 2. # Overlapped signals; 3. The signals of carbon and proton were unambiguously assigned through HMQC, COSY, TOCSY and HMBC.

Figure 1 Structure of compound **1**



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