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Four new cytotoxic oligosaccharidic derivatives of 12-oleanene from *Lysimachia heterogenea* Klatt

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ABSTRACT

Cytotoxicity-guided phytochemical analysis on the extract of *Lysimachia heterogenea* Klatt led to the isolation of 3β,16β-12-oleanene-3,16,23,28-tetrol (**1**) and its four new oligosaccharidic derivatives heterogenosides A, B, C, and D (**2**–**5**). Their structural elucidation was mainly based on NMR and mass spectral data. The time course experimental results indicated that unlike the likely lysis activity of heterogenosides B–D, heterogenoside A showed a significantly time-dependent cytotoxicity.

The genus *Lysimachia*, traditionally classified in the family Primulaceae, was newly assigned into the Myrsinaceae on basis of molecular phylogenetic analysis.[1] The genus comprises more than 193 species, over 90% of which are endemic to China.[2] Over thirty new triterpenoid sapponins had been isolated from this genus in the last five years.[3] *Lysimachia heterogenea* Klatt, an endemic species, is a perennial herb, which was used as a folk medicine in subduing swelling and detoxicating in China.[4] As a part of the continuous investigation on the chemical components in this genus,[5] the human lung cancer (A549) cell line was used in cytotoxicity-guided phytochemical analysis, by which the effective fraction LH-1 of *L. heterogene* was found. The further investigation on the antitumor components of the fraction LH-1 led to the isolation of 3β,16β-12-oleanene-3,16,23,28-tetrol (**1**) and its four new oligosaccharidic derivatives heterogenosides A–D (**2**–**5**). In this Letter, the structure and the antitumor activity of these four new compounds was reported.

The dried *L. heterogenea* (1.70 kg) were powered, then extracted with 95% EtOH for 24 h at room temperature for three times to give the crude extract 180.0 g. The crude extract was sequentially partitioned with petroleum ether, EtOAc, and *n*-BuOH, respectively. Among them the *n*-BuOH fraction (100.2 g) exhibited cytotoxicity with IC_{50} value of 4.3 μg/mL, then it was chromatographed on a D101 resin column using 20% and 90% EtOH solvents as eluant, respectively. The latter eluate (90.6 g), with IC_{50} value of 4.0 μg/mL, was further chromatographed on a silica gel column to obtain fractions LH-1 and LH-2 using 20% and 70% MeOH/$CHCl_3$ solvents as eluant, respectively. Fractions LH-1 and LH-2 presented the cytotoxicity with the IC_{50} values of 2.5 and 45.0 μg/mL, respectively. Therefore, Fraction LH-1 was served as the effective antitumor fraction of *L. heterogenea*, and it was further purified by silica gel chromatography to yield 12-oleanene-3,16,23,28-tetrol (MeOH–$CHCl_3$, 5:95, v/v, 8 mg, **1**), heterogenoside A (MeOH–$CHCl_3$, 10:90, v/v, 15 mg, **2**), heterogenoside B (MeOH–$CHCl_3$, 15:85, v/v, 20 mg, **3**), heterogenoside C (MeOH–$CHCl_3$, 15:85, v/v, 40 mg, **4**), and heterogenoside D (MeOH–$CHCl_3$, 22:78, v/v, 13 g, **5**).

Heterogenoside A (**2**) was obtained as white powder (MeOH). The HRESIMS implied that **2** had the molecular $C_{41}H_{68}O_{13}$. The IR spectrum showed the presence of hydroxyl groups due to absorption bands at 3390, 1077, and 1043 cm^{-1}. The ^1H NMR spectrum displayed six quaternary methyl signals at δ 0.94, 0.99, 1.00, 1.02, 1.10, and 1.78, as well as an olefinic proton signal at δ 5.40 (Table 1). The HMBC correlations from H-24 to C-3, C-4, C-5, and C-23; H-25 to C-10; H-26 to C-7, C-8, C-9, and C-14; H-27 to C-14; H-29 to C-19, C-20, C-21, and C-30; H-30 to C-19, C-20, C-21, and C-29 established the aglycon to be 3β,16β-12-oleanene-3,16,23,28-tetrol. The sugar residues of **2** were determined to be arabinose and glucose after hydrolysis by co-TLC with authentic sugars. The coupling constant analysis

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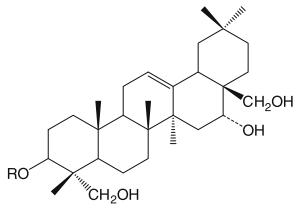
Table 1
The main ^{1}H NMR data for compounds **2–5** (500 MHz in pyridine-d_5)

No.	**2**	**3**	**4**	**5**
1	1.09[a]	1.12[a]	1.04[a]	1.11[a]
	1.64 (m)	1.64 (m)	1.59[a]	1.58[a]
2	2.02 (m)	2.02 (m)	1.95[a]	1.95[a]
	2.23[a]	2.22[a]	2.09 (m)	2.09 (m)
3	4.24[a]	4.25[a]	4.11 (m)	4.10 (m)
5	1.68[a]	1.69[a]	1.60[a]	1.60[a]
6	1.38[a]	1.38[a]	1.35[a]	1.35[a]
	1.69[a]	1.70[a]	1.69[a]	1.69[a]
7	1.32[a]	1.35[a]	1.30[a]	1.31[a]
	1.78[a]	1.78[a]	1.72[a]	1.74[a]
9	1.89 (br s)	1.91 (br s)	1.86 (m)	1.88 (br s)
11	1.95 (m)	1.95 (m)	1.90 (m)	1.92[a]
12	5.39 (br s)	5.39 (br s)	5.39 (br s)	5.38 (br s)
15	1.58 (m)	1.59 (m)	1.58[a]	1.58[a]
	2.19[a]	2.20[a]	2.19 (m)	2.18 (m)
16	4.60[a]	4.61[a]	4.59[a]	4.59[a]
18	2.49 (dd, 15.0, 1.5 Hz)	2.49 (dd, 15.0, 1.5 Hz)	2.49 (dd, 15.0, 1.5 Hz)	2.48 (dd, 15.0, 1.5 Hz)
19	1.32[a]	1.32[a]	1.31[a]	1.30[a]
	2.72 (t, 15.0 Hz)	2.72 (t, 15 .0 Hz)	2.71 (t, 15 .0 Hz)	2.70 (t, 15 .0 Hz)
21	1.43 (m)	1.43 (m)	1.42 (m)	1.42 (m)
	2.38 (td, 12.5, 4.5 Hz)	2.38 (td, 12.5, 4.5 Hz)	2.38 (td, 12.5, 4.5 Hz)	2.38 (td, 12.5, 4.5 Hz)
22	1.38[a]	1.38[a]	1.35[a]	1.35[a]
	2.28 [a]	2.28 [a]	2.25 (td, 12.5, 4.5 Hz)	2.25 (td, 12.5, 4.5 Hz)
23	3.68[a]	3.71[a]	3.71[a]	3.70 (d, 11.5 Hz)
	4.27[a]	4.35[a]	4.18 (d, 11.0 Hz)	4.28[a]
24	0.94 (s)	1.00 (s)	1.02 (s)	1.08 (s)
25	1.00 (s)	1.00 (s)	0.98 (s)	0.98 (s)
26	0.99 (s)	1.00 (s)	0.96 (s)	0.96 (s)
27	1.77 (s)	1.77 (s)	1.74 (s)	1.75 (s)
28	3.62 (d, 11 Hz)	3.61 (d, 11.5 Hz)	3.61 (d, 11.5 Hz)	3.61 (d, 11.5 Hz)
	3.74 (d, 11 Hz)	3.73 (d, 11.5 Hz)	3.73 (d, 11.5 Hz)	3.73 (d, 11.5 Hz)
29	1.02 (s)	1.03 (s)	1.02 (s)	1.03 (s)
30	1.11 (s)	1.12 (s)	1.12 (s)	1.11 (s)
Arabinose				
1	4.92 (d, 7.5 Hz)	4.86 (d, 7.5 Hz)	5.12 (d, 8.0 Hz)	5.00 (d, 5.5 Hz)
2	4.30[a]	4.07 (br s)	4.54 (m)	4.57[a]
3	4.40[a]	4.32[a]	4.34 (m)	4.18 (m)
4	4.22[a]	4.21[a]	4.42[a]	4.21[a]
5	3.67 (dd, 10.0, 0.5 Hz)	3.63 (dd, 10.0, 0.5 Hz)	3.72[a]	3.72[a]
	4.40[a]	4.61[a]	4.57[a]	4.57[a]
Glucose (at C-2 of arabinose)				
1	5.24 (d, 8.0 Hz)	5.01 (d, 7.5 Hz)	5.14 (d, 7.5 Hz)	5.49 (d, 7.0 Hz)
2	4.16 (t, 7.5 Hz)	3.97[a]	4.05[a]	4.05[a]
3	4.24[a]	4.25[a]	4.22 (m)	4.22 (m)
4	4.25[a]	4.27[a]	4.22[a]	4.22[a]
5	3.91 (br s)	3.82 (br s)	3.87[a]	3.78[a]
6	4.29[a]	4.34[a]	4.35[a]	4.35[a]
	4.51 (dd,11.0, 1.0 Hz)	4.47 (d,11.0 Hz)	4.47(d,11.0 Hz)	4.41[a]
Glucose (at C-4 of arabinose)				
1			5.14 (d, 7.5 Hz)	5.00 (d, 7.5 Hz)
2			4.05[a]	3.94 (m)
3			4.22 (m)	4.19[a]
4			4.21[a]	4.22[a]
5			3.87[a]	3.87[a]
6			4.35[a]	4.35[a]
			4.47 (d,11.0 Hz)	4.41[a]
Xylose				
1		4.90 (d, 6.5 Hz)		4.96 (d, 6.0 Hz)
2		3.98[a]		4.11 (m)
3		3.97[a]		4.00[a]
4		3.98[a]		4.17[a]
5		3.48 (m)		3.72[a]
		4.28[a]		4.58[a]

[a] The signals were overlapped.

of the anomeric protons at δ 4.92 (d, J = 7.5 Hz) and 5.24 (d, J = 7.5 Hz) supported the α-L-configuration of arabinose and β-D-configuration of glucose. The HMBC correlations from H′-1 to C-3 and H″-1 to C′-2 were used to determine the oligosaccharide as 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl moiety. On the basis of above findings, the structure of **2** was concluded to be 3-*O*-[β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-3β,16β-12-oleanene-3,16,23,28-tetrol (Fig. 1).

Heterogenoside B (**3**) was isolated as white powder (MeOH). The HRESIMS revealed its molecular formula to be $C_{46}H_{76}O_{17}$. The ^{13}C NMR data of **3** were similar to that of **2**, except **3** contained an additional pentose unit (Table 2). The hydrolysis analysis and



1 R = H-

2 R = *β*-*D*-Glu''-(1→2)-*α*-*L*-Ara'-

3 R = *β*-*D*-Xyl'''-(1→2)-*β*-*D*-Glu''-(1→2)-*α*-*L*-Ara'-

4 R = *β*-*D*-Glu'''-(1→4)-[*β*-*D*-Glu''-(1→2)]-*α*-*L*-Ara'-

5 R = *β*-*D*-Xyl''''-(1→2)-*β*-*D*-Glu'''-(1→4)-[*β*-*D*-Glu''-(1→2)]-*α*-*L*-Ara'-

Figure 1. The structures of compounds **1**–**5**.

the coupling constant of the anomeric proton at δ 4.90 (d, J = 6.5 Hz) confirmed the pentose as β-D-xylose (Table 1). The HMBC correlations from H′-1 to C-3, H″-1 to C′-2, H‴-1 to C″-2, H″-2 to C″-1 and C‴-1 established the 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl moiety. Hence, the structure of **3** was identified as 3-*O*-[β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl]-3β, 16β-12-oleanene-3,16,23,28-tetrol (Fig. 1).

Heterogenoside C (**4**) appeared as white powder (MeOH). The HRESIMS determined its molecular formula to be $C_{47}H_{78}O_{18}$. Compared with the ^{13}C NMR and HRESIMS spectrum of **2**, **4** differed in the additional hexose unit (Table 2). The analysis of the hydrolysis products and the coupling constant of the anomeric proton at δ 5.14 (d, J = 7.5 Hz) inferred the hexose sugar was β-D-glucose (Table 1). The HMBC correlations from H-3 to C′-1, H′-1 to C-3, C′-2 and C′-5, H′-2 to C′-1, C′-3 and C″-1, H″-1 to C′-2, H‴-1 to C′-4 deduced that C-3 was linked with β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl moiety. Thus, **4** was elucidated as 3-*O*-{β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β,16β-12-oleanene-3,16,23,28-tetrol (Fig. 1).

Heterogenoside D (**5**) presented as white powder (MeOH). The HRESIMS gave its molecular formula to be $C_{52}H_{86}O_{22}$. The ^{13}C NMR data of the aglycon of **5** was identical to that of **2**, **3**, and **4** (Table 2). Compared with the HRESIMS data of **4**, **5** comprised an additional pentose unit which was ascertained to be β-D-xylose by comparing the hydrolysis products with the authentic sugar and analyzing the coupling constant of the anomeric proton at δ 4.96 (d, J = 6.0 Hz) (Table 1). The glycosidic linkages between monosaccharide residues were defined as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl due to the long-range correlations from H′-1 to C-3 and C′-5, H″-1 to C′-2, H‴-1 to C′-4, H‴-2 to C‴-1 and C‴-3, H⁗-1 to C‴-2, H⁗-5 to C⁗-1, C⁗-3 and C⁗-4. Therefore, **5** was structurally explained to be 3-*O*-{β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→2)]-α-L-arabinopyranosyl}-3β, 16β-12-oleanene-3,16,23,28-tetrol (Fig. 1).

In addition to the above four new compounds, the known compound of 3β,16β-12-oleanene-3,16,23,28-tetrol was identified by comparing its spectral data (Table 2) with the literature.[6]

The half-inhibitory concentration (IC_{50}) values of heterogenosides A–D were measured after 2, 4, 9, 18, 36, and 72 h of incubation with the A549 cells, respectively. The activity of heterogenosides B–D slightly increased during continuous incubation, their IC_{50} values were calculated to be 26.4, 23.6, and 20.2 μM at the end of 2 h of incubation, and 19.1, 16.3, and 14.5 μM at the end of 72 h of incubation, respectively. Unlike heterogenosides B–D, heterogenoside A showed weaker activity with IC_{50} value of more than 100.0 μM even after 9 h of incubation, and 24.5 μM at the end of 72 h of incubation. The time course experiment indicated that the activity of heterogenoside A was significantly time-dependent, while the activity of heterogenosides B–D was likely due to lysis.

Table 2
^{13}C NMR data for compounds **1–5** (125 MHz in pyridine-d_5)

No.	1	2	3	4	5	No.	2	3	4	5
1	39.0	38.9	38.9	38.9	38.9	*Arabinose*				
2	27.7	26.1	26.0	25.9	25.8	1	106.3	106.5	103.5	104.1
3	73.6	82.2	82.2	82.3	82.5	2	79.7	81.2	81.2	80.3
4	42.8	43.5	43.5	43.5	43.6	3	73.7	73.9	72.7	73.4
5	48.8	47.7	47.8	48.0	48.1	4	74.6	74.5	77.2	78.4
6	18.6	18.2	18.2	18.2	18.2	5	66.3	66.5	63.7	64.3
7	33.0	32.9	32.9	32.9	32.9	*Glucose (at C-2 of arabinose)*				
8	40.1	40.1	40.1	40.1	40.1	1	106.7	105.3	105.6	105.1
9	47.2	47.2	47.2	47.2	47.2	2	75.8	86.2	75.7	76.1
10	37.1	36.9	36.9	36.8	36.9	3	78.7	77.6	78.3	78.3
11	23.9	23.9	23.9	23.9	23.9	4	71.3	71.0	71.3	71.5
12	121.2	122.3	122.3	122.3	122.3	5	78.4	78.3	78.1	78.3
13	148.2	145.2	145.2	145.2	145.2	6	62.6	62.3	62.5	62.7
14	42.0	42.0	42.0	42.0	42.0	*Glucose (at C-4 of arabinose)*				
15	34.8	34.8	34.8	34.8	34.8	1			105.7	103.7
16	74.2	74.2	74.2	74.2	74.2	2			76.1	85.3
17	40.9	41.0	40.9	40.9	40.9	3			78.6	77.6
18	42.5	42.6	42.5	42.5	42.5	4			71.4	71.1
19	48.3	48.4	48.2	48.3	48.3	5			78.1	77.8
20	31.2	31.2	31.2	31.2	31.2	6			62.6	62.3
21	37.1	37.1	37.1	37.1	37.1	*Xylose*				
22	30.4	30.5	30.4	30.4	30.4	1		108.0		107.6
23	68.3	64.6	64.7	64.9	65.0	2		76.3		76.2
24	13.1	13.6	13.6	13.4	13.4	3		78.0		78.1
25	16.2	16.4	16.4	16.4	16.3	4		70.4		70.7
26	17.1	17.1	17.1	17.1	17.1	5		67.2		67.4
27	27.3	27.3	27.3	27.3	27.3					
28	70.1	70.2	70.1	70.1	70.1					
29	33.4	33.4	33.4	33.4	33.4					
30	24.8	24.9	24.8	24.8	24.9					

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.056.

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