Two New Triterpenoid Saponins from *Stauntonia obovatifoliola* HAYATA ssp. *intermedia*

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Two new hederagenin-type saponins, staunoside G (**1**) and staunoside H (**2**), along with twelve known triterpenoid saponins, were isolated from stems of *Stauntonia obovatifoliola* HAYATA ssp. *intermedia*. Their structures were determined by analysis of HR-EI-MS, and 1D- and 2D-NMR data, and comparison with those in literature. The two new compounds showed moderate cytotoxicities against three tumor cells, *i.e.*, A549 (lung carcinoma), 4T1 (mammary carcinoma), and HeLa (cervical carcinoma).

Introduction. – The plant *Stauntonia obovatifoliola* HAYATA ssp. *intermedia* (Lardizabalaceae) is called '*Wuzhinateng*' in Chinese [1]. Its stems and leaves are used as analgesic and for sedation in Chinese folk medicine [2]. The literature contains a fairly large number of articles dealing with plants of the genus *Stauntonia*, especially with chemical studies on *Stauntonia chinensis* and *S. hexaphylla* (*S. obovatifolia*) [3–5]. *S. chinensis* is known as '*Yemugua*', and recorded in the Pharmacoepia of P. R. China, as possessing similar efficacy as '*Wuzhinateng*' [6]. Recent research has revealed that it has pharmacological properties such as analgesic [7], antitumor [8], and antioxidant activities [9]. However, there are few publications on chemical constituents and pharmacological activities of *Wuzhinateng*, only one reporting anti-HIV-1 protease triterpenoids from its AcOEt-soluble fraction [10]. The present study was undertaken to find more bioactive compounds from the BuOH-soluble fraction. In total 14 compounds, **1**–**14**, including two new triterpenoid saponins, **1** and **2**, were isolated and identified by spectroscopic methods. Herein, we report the isolation, structure elucidation, and the anti-proliferative activities of the new compounds.

Results and Discussion. – The BuOH-soluble fraction of the extract of stems of *S. obovatifoliola* HAYATA ssp. *intermedia* was subjected to column chromatography (*D101* macroporous resin, silica gel, and octadecylsilanized silica gel (ODS)) and HPLC to yield two new compounds, **1** and **2**, and twelve known compounds: stauntoside A (**3**), 3-*O*-α-L-arabinopyranosyl 30-norhederagenin 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-

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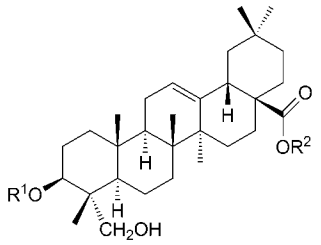
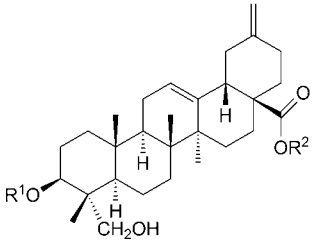
glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester (**4**), kizuta saponin K_{10} (**5**), kalopanax saponin B (Kizuta saponin K_{12}, **6**) [11], kalopanaxsaponin A (**7**), kalopanaxsaponin J (**8**), 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosylhederagenin 28-*O*-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester (**9**), kalopanaxsaponin H (sieboldianoside A, **10**), kalopanaxsaponin K (**11**) [12], 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*α*-L-arabinopyranosylhederagenin 28-*O*-*β*-D-xylopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester (**12**) [13], septemoside A (**13**) [14], and septemoside I (**14**) [15] (*Fig. 1*). All of these compounds were isolated from this plant for the first time, and compounds **8**, **9**, **11** – **14** were found in the genus *Stauntonia* for the first time. These compounds belong to hederagenin-type saponins, which are represented by kalopanaxsaponin A with its antitumor and anti-inflammatory properties [16][17].

Compound **1** was obtained as white amorphous powder, which gave positive results for the *Liebermann–Burchard* reaction and with *Molish* reagent. Its molecular formula, $C_{69}H_{112}O_{34}$, was determined by the HR-ESI-MS spectra. The IR spectrum indicated that compound **1** possessed OH (3385 cm^{-1}) and ester C=O groups (1728 cm^{-1}), and a C=C bond (1639 cm^{-1}). Acid hydrolysis of **1** gave D-glucose, L-arabinose, D-xylose, and L-rhamnose, as confirmed by GC analysis of the respective trimethylsilyl derivatives.



	R^1	R^2
1	Glc-(1 → 4)-Xyl-(1 → 3)-Rha-(1 → 2)-Ara-	Rha-(1 → 4)-Xyl-(1 → 6)-Glc-
2	Xyl-(1 → 4)-Xyl-(1 → 3)-Rha-(1 → 2)-Ara-	Rha-(1 → 4)-Xyl-(1 → 6)-Glc-
5	Rha-(1 → 2)-Ara-	H
6	Glc-(1 → 4)-Xyl-(1 → 3)-Rha-(1 → 2)-Ara-	H
7	Ara-	Rha-(1 → 4)-Glc-(1 → 6)-Glc-
8	Rha-(1 → 2)-Ara-	Xyl-(1 → 6)-Glc-
9	Rha-(1 → 2)-Ara-	Rha-(1 → 4)-Glc-(1 → 6)-Glc-
10	Rha-(1 → 2)-Ara-	Glc-(1 → 6)-Glc-
11	Xyl-(1 → 3)-Rha-(1 → 2)-Ara-	Rha-(1 → 4)-Glc-(1 → 6)-Glc-
12	Xyl-(1 → 4)-Xyl-(1 → 3)-Rha-(1 → 2)-Ara-	Glc-(1 → 6)-Glc-
13	Glc-(1 → 4)-Xyl-(1 → 3)-Rha-(1 → 2)-Ara-	Rha-(1 → 4)-Glc-(1 → 6)-Glc-
14	Xyl-(1 → 4)-Xyl-(1 → 3)-Rha-(1 → 2)-Ara-	Rha-(1 → 4)-Glc-(1 → 6)-Glc-



	R^1	R^2
3	Ara-	Glc-(1 → 6)-Glc-
4	Ara-	Rha-(1 → 4)-Glc-(1 → 6)-Glc-

Fig. 1. *Structures of compounds* **1** – **14**

The hederagenin-type saponin nature of compound **1** was revealed by analysis of its NMR spectra (*Table*). Signals of six angular Me groups (δ(H) 0.82, 0.85, 0.94, 1.06, 1.08, and 1.13) and of one olefinic H-atom (δ(H) 5.35 (br. *s*)) of the aglycon were observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum exhibited signals of six aglycon Me groups (δ(H) 14.0,16.2, 17.6, 26.1, 33.1, and 23.7), two olefinic C-atoms (δ(C) 123.0 and 144.1), a CH_2OH group (δ(C) 64.1), a CH–O group (δ(C) 81.2), and one C=O group (δ(C) 176.4) (*Table*). Of 69 ^{13}C-NMR signals, 30 were assigned to a triterpenoid moiety and 39 to the saccharide portion. The downfield shift of C(3) (δ(C) 81.1) and the upfield shift of C(28) (δ(C) 176.4) indicated that the sugar moieties were attached to the aglycon at these two positions. The HMQC spectra of **1** exhibited seven anomeric H-atom signals (δ(H) 6.24 (br. *s*), 6.20 (*d*, $J = 8.0$), 5.46 (br. *s*), 5.21 (*d*, $J = 7.5$), 5.02 (*d*, $J = 6.5$), 4.95 (*d*, $J = 8.0$), and 4.86 (*d*, $J = 6.5$)) corresponding to the C-atom signals at δ(C) 101.3, 95.5, 99.8, 107.1, 104.4, 103.6 and 105.3, respectively (*Table*). In the ^{1}H-NMR spectrum, two Me signals at δ(H) 1.51 (*d*, $J = 6.0$, 3 H) and 1.59 (*d*, $J = 6.0$, 3 H) belonging to two rhamnoses were observed. The monosaccharides were identified as glucose, rhamnose, xylose, and arabinose by a combination of DEPT, HMQC, TOCSY, and HMBC experiments. In addition, the ^{1}H-, ^{13}C-, and 2D-NMR analysis indicated that all of the monosaccharides of **1** were in pyranose forms. The linkages between sugar moieties and C(3) of the aglycon were corroborated by following correlations: δ(H) 4.95 (H–C(1) of Glc^{I})/δ(C) 78.0 (C(4) of Xyl^{I}); δ(H) 5.21 (H–C(1) of Glc^{I})/δ(C) 83.2 (C(3) of Rha); δ(H) 6.24 (H–C(1) of Rha^{I})/δ(C) 75.3 (C(2) of Ara); and δ(H) 5.02 (H–C(1) of Ara)/δ(C) 81.2 (C(3)). The linkages of sugar moieties at C(28) were established based on HMBCs δ(H) 5.46 (H–C(1) of Rha^{II})/δ(C) 76.4 (C(4) of Xyl^{II}); δ(H) 4.86 (H–C(1) of Xyl^{II})/δ(C) 69.2 (H–C(6) of Glc^{II}); and δ(H) 6.20 (H–C(1) of Glc^{II})/δ(C) 176.4 (C(3)) (*Fig. 2*). Based on these evidences, **1** was identified as 6-deoxy-α-L-mannopyranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 6)-1-*O*-[(3β)-3-{[β-D-glucopyranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 3)-6-deoxy-α-L-manno-

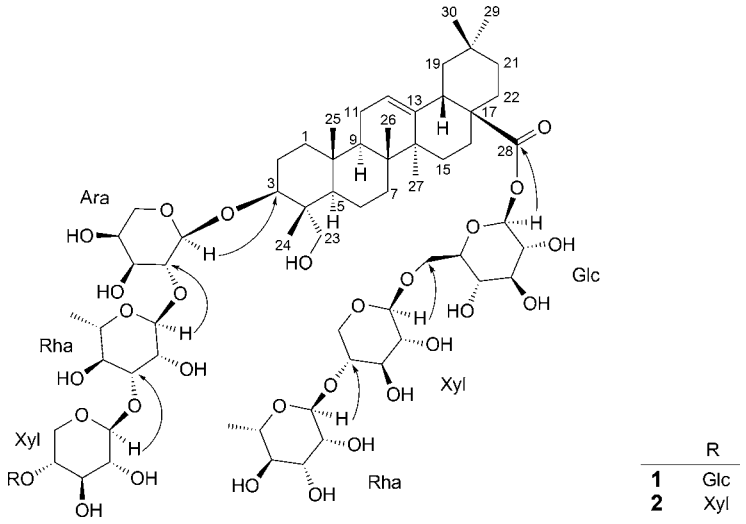


Fig. 2. *Key HMBCs* (H → C) of *compounds* **1** *and* **2**

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_5)pyridine) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**		**2**		Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)		δ(H)	δ(C)	δ(H)	δ(C)
1	0.97–1.04 (*m*),	39.1	1.04–1.11 (*m*),	39.1		3-*O*-Ara-2-*Rha*^{I}			
	1.47–1.54 (*m*)		1.54–1.61 (*m*)		1	6.24 (br. *s*)	101.3	6.30 (br. *s*)	101.4
2	1.93–2.20 (*m*),	26.3	2.19–2.26 (*m*),	26.3	2	4.77–4.84 (*m*)	71.9	4.26–4.33 (*m*)	71.9
	2.14–2.21 (*m*)		1.99–2.06 (*m*)		3	4.65 (br. *d*, *J* = 3.3)	83.2	4.68–4.75 (*m*)	83.1
3	4.19–4.27 (*m*)	81.1	4.27–4.34 (*m*)	81.1	4	4.41 (*d*, *J* = 2.8)	73.0	4.38–4.45 (*m*)	73.0
4	–	43.6	–	43.7	5	4.06–4.13 (*m*)	69.5	4.10–4.17 (*m*)	69.6
5	1.65–1.74 (*m*)	47.7	1.69–1.76 (*m*)	47.7	6	1.51 (*d*, *J* = 6.0)	18.3	1.59 (*d*, *J* = 6.0)	18.5
6	1.28–1.35 (*m*),	18.2	1.32–1.39 (*m*),	18.2					
	1.42–1.49 (*m*)		1.54–1.61 (*m*)			3-*O*-Ara-2-Rha^{I}-3-*Xyl*^{I}			
7	1.18–1.25 (*m*),	32.8	1.57–1.64 (*m*),	32.8	1	5.21 (*d*, *J* = 7.5)	107.1	5.25 (*d*, *J* = 7.5)	107.1
	1.51–1.58 (*m*)		1.23–1.30 (*m*)		2	3.95–4.02 (*m*)	75.3	3.98–4.05 (*m*)	75.3
8	–	40.0	–	39.9	3	4.03–4.10 (*m*)	75.8	3.96–4.03 (*m*)	75.5
9	1.65–1.73 (*m*)	48.2	1.72–1.79 (*m*)	48.2	4	4.04–4.11 (*m*)	78.0	4.07–4.14 (*m*)	76.1
10	–	36.9	–	36.9	5	4.24–4.31 (*m*),	64.8	4.30–4.37 (*m*),	64.9
11	1.81–1.89 (*m*)	23.8	1.91–1.98 (*m*)	23.8		3.58 (*d*, *J* = 10.7)		3.59 (br. *t*, *J* = 10.2)	
12	5.35 (br. *s*)	123.0	5.40 (br. *s*)	123.0					
13	–	144.1	–	144.1		3-*O*-Ara-2-Rha-3-Xyl^{I}-4-*Glc*^{I}		3-*O*-Ara-2-Rha^{I}-3-Xyl^{I}-4-*Xyl*^{II}	
14	–	42.2	–	42.1	1	4.95 (*d*, *J* = 8.0)	103.6	4.81 (*d*, *J* = 7.5)	103.6
15	0.97–1.04 (*m*),	28.3	1.03–1.10 (*m*),	28.36	2	3.93–4.00 (*m*)	74.3	4.08–4.15 (*m*)	74.3
	2.18–2.25 (*m*)		2.24–2.31 (*m*)		3	4.12–4.19 (*m*)	78.2	4.09–4.16 (*m*)	78.0
16	1.83–1.90 (*m*),	23.4	1.85–1.92 (*m*),	23.3	4	4.09–4.16 (*m*)	71.8	4.26–4.33 (*m*)	71.2
	1.93–2.00 (*m*)		1.97–2.04 (*m*)		5	3.90–3.97 (*m*)	78.8	4.28–4.35 (*m*),	67.3
17	–	47.1	–	47.0				3.74 (*d*, *J* = 11.5)	
18	3.13 (*dd*, *J* = 13.7, 3.5)	41.7	3.18 (*dd*, *J* = 13.8, 3.7)	41.7	6	4.26 (*d*, *J* = 4.6),	62.6		
19	1.14–1.21 (*m*),	46.2	1.19–1.26 (*m*),	46.2		4.47–4.54 (*m*)			
	1.62–1.69 (*m*)		1.68–1.75 (*m*)						
20	–	30.7	–	30.7		28-*O*-Glc^{II}		28-*O*-Glc	
21	1.02–1.09 (*m*),	34.0	1.08–1.15 (*m*),	34.0	1	6.20 (*d*, *J* = 8.0)	95.5	6.24 (*d*, *J* = 8.0)	95.6
	1.24–1.31 (*m*)		1.29–1.36 (*m*)		2	4.04–4.11 (*m*)	73.9	4.09–4.16 (*m*)	73.9
22	1.69–1.76 (*m*),	32.7	1.74–1.81 (*m*),	32.7	3	3.90–3.97 (*m*)	78.8	4.18–4.25 (*m*)	78.8
	1.82–1.89 (*m*)		1.90–1.97 (*m*)		4	4.21–4.28 (*m*)	71.1	4.25–4.32 (*m*)	71.0

Table (cont.)

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
23	3.80–3.87 (*m*), 4.26–4.33 (*m*)	64.1	3.86–3.93 (*m*), 4.26–4.33 (*m*)	64.0
24	1.08 (*s*)	14.0	1.13 (*s*)	14.1
25	0.94 (*s*)	16.2	0.99 (*s*)	16.2
26	1.06 (*s*)	17.6	1.11 (*s*)	17.6
27	1.13 (*s*)	26.1	1.18 (*s*)	26.1
28	–	176.4	–	176.6
29	0.82 (*s*)	33.1	0.86 (*s*)	33.1
30	0.85 (*s*)	23.7	0.89 (*s*)	23.7
	3-*O*-Ara			
1	5.02 (*d*, *J* = 6.5)	104.4	5.06 (*d*, *J* = 6.5)	104.1
2	4.50–4.57 (*m*)	75.3	4.26–4.33 (*m*)	76.0
3	3.90–3.97 (*m*)	74.9	3.94–4.01 (*m*)	75.0
4	4.56–4.63 (*m*)	69.4	4.30–4.37 (*m*)	69.4
5	3.64 (*d*, *J* = 11.3), 4.19–4.26 (*m*)	65.9	3.69 (*d*, *J* = 11.0), 4.22–4.29 (*m*)	65.9

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
5	4.03–4.10 (*m*)	78.0	4.09–4.16 (*m*)	78.0
6	4.25–4.32 (*m*), 4.59–4.66 (*m*)	69.2	4.27–4.34 (*m*), 4.65–4.72 (*m*)	69.2
	28-*O*-Glc^{II}-6-Xyl^{II}		28-*O*-Glc-6-Xyl^{III}	
1	4.86 (*d*, *J* = 6.5)	105.3	4.91 (*d*, *J* = 6.5)	105.4
2	3.89–3.96 (*m*)	75.0	3.95–4.02 (*m*)	75.3
3	4.24–4.31 (*m*)	75.3	4.26–4.33 (*m*)	75.4
4	4.05–4.12 (*m*)	76.4	4.08–4.15 (*m*)	76.0
5	3.45 (*t*, *J* = 10.0), 4.21–4.28 (*m*)	63.8	3.50 (*t*, *J* = 10.5), 4.24–4.31 (*m*)	63.8
	28-*O*-Glc^{I}-6-Xyl^{II}-4-Rha^{II}		28-*O*-Glc-6-Xyl^{III}-4-Rha^{II}	
1	5.46 (br. *s*)	99.8	5.51 (br. *s*)	99.8
2	4.44–4.51 (*m*)	72.5	4.48–4.55 (*m*)	72.5
3	4.45–4.52 (*m*)	72.6	4.47–4.54 (*m*)	72.6
4	4.04–4.11 (*m*)	73.9	4.07–4.14 (*m*)	74.0
5	4.78–4.85 (*m*)	69.8	4.82–4.89 (*m*)	69.9
6	1.59 (*d*, *J* = 6.0)	18.4	1.51 (*d*, *J* = 6.0)	18.4

pyranosyl-(1 → 2)-α-L-arabinopyranosyl]oxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose.

Compound **2** was also obtained as white amorphous powder, which gave positive results for the *Liebermann–Burchard* reaction and with *Molish* reagent. Its molecular formula, $C_{68}H_{110}O_{33}$, was deduced from its HR-ESI-MS. The IR and NMR spectra indicated that the structure of compound **2** was similar to that of **1**, the only difference being that the glucose in the *C(3)–O*-sugar chain was displaced by a xylose (*Table* and *Fig. 2*). Therefore, by analysis of 1D- and 2D-NMR data and comparison with those of compound **1**, **2** was identified as 6-deoxy-α-L-mannopyranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 6)-1-*O*-[(3β)-23-hydroxy-28-oxo-3-{[β-D-xylopyranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 3)-6-deoxy-α-L-mannopyranosyl-(1 → 2)-α-L-arabinopyranosyl]oxy}-olean-12-en-28-yl]-β-D-glucopyranose.

Compounds **1** and **2** were evaluated for their antitumor activities against A549, 4T1, and HeLa cell lines, using the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay *in vitro* [18]. Kalopanaxsaponin A with a noncytotoxic δ-hederin moiety has been found as a basic saponin structure for the antitumor activity of hederagenin monodesmosides [19][20]. Therefore, we used it as a positive drug. Both compounds **1** and **2** exhibited low cytotoxicities against three tumor cells mentioned above that IC_{50} values were $\geq$240 μg/ml, while the values of the positive drug were 11.6, 11.1, and 7.7 μg/ml, respectively. In addition, the 70% EtOH fraction eluted from a *D101* macroporous resin column showed significant cytotoxic activities against the three tumor cells with IC_{50} values of 19.46, 19.49, and 13.98 μg/ml, respectively.

In summary, two new hederagenin-type triterpenoid saponins, together with twelve known saponins, with low antitumor activities were isolated from the stems of *S. obovatifoliola* HAYATA ssp. *intermedia*. All of these compounds were isolated from this plant for the first time, and **8**, **9**, **11** – **14** were found in the genus *Stauntonia* for the first time. The 70% EtOH fraction eluted from a *D101* macroporous resin column contained triterpenoid monodesmosides just like kalopanaxsaponin A; therefore, it had antitumor activity. The cytotoxicity assay of **1** and **2** confirmed that hederagenin-type triterpenoid saponins with sugars linked at both C(3) and C(28) hardly have obvious antiproliferative properties against tumor cells, in agreement with previously published results [19][20].

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Experimental Part

General. TLC: Silica gel *G* (*Qingdao Marine Chemical Factory*, Qingdao, P. R. China). Anal. TLC: *RP-18 F_{254}* plates (*Merck*). Column chromatography (CC): silica gel (SiO_2, 200 – 300 mesh; *Qingdao Marine Chemical Factory*, Qingdao, P. R. China) and *D101* macroporous resin column (*Tianjin Haiguang Chemical Co., Ltd.*, Tianjin, P. R. China). Prep. HPLC: *Shimadzu LC-20AT* with a DAD detector, monitored at 210 nm, with a *C18* column (*Agilent Eclipse XDB-C18* Semi-Prep., 9.4 × 250 mm, 5 μm). A microplate reader (*Multiskan GO*, *Thermo Fisher*) was used to determine the absorbance at 570 nm. Optical rotation: *JASCOP-1000* Polarimeter. IR Spectra: *Bruker Tensor 27*/*Hyperion 1000* system (*Bruker Optics*, Billerica, MA); $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker Avance DRX 500* NMR; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Agilent 1260* series *LC*/*MSD Trap SL* mass spectrometer; in

m/*z*. HR-ESI-MS (pos. and neg.-ion modes): *Bruker FT-ICR-MS solarix Maldi/ESI 9.4T* spectrometer; in *m*/*z*.

Plant Material. The stems of *S. obovatifoliola* HAYATA ssp. *intermedia* were collected from Long'an, Guangxi Zhuang Autonomous Region, P. R. China, in October 2011, and were identified by Prof. *Bin Dai*, Guangxi Institute of Nationality Medical Research. A voucher specimen (No. 20111030) has been deposited with the Herbarium of School of Traditional Chinese Medicine, Capital Medical University.

Extraction and Isolation. Dry stems of the plant (14.8 kg), cut into small pieces, were refluxed with 90 l of 60% EtOH (2 ×), 4 h each time. Extracts were concentrated by a rotary evaporator, suspended in H_2O, and sequentially partitioned with AcOEt and BuOH saturated with H_2O. The BuOH-soluble fraction was subjected to CC (*D101*; sequentially with H_2O, 30, 50 (SiO_2), and 70% EtOH). Elution with $CHCl_3$/MeOH/H_2O, afforded three fractions, *Frs. A – C*. *Fr. A* was chromatographed on a reversed-phase column and purified by HPLC to furnish compounds **1** (28 mg), **2** (58 mg), **11** (91 mg), **13** (52 mg), and **14** (520 mg). *Fr. B* and *Fr. C* gave **3** (26 mg), **4** (10 mg), **5** (90 mg), **6** (100 mg), **9** (52 mg), **10** (600 mg), and **12** (10 mg). The 70% EtOH elutate was chromatographed to afford compounds **7** (22 mg) and **8** (16 mg).

Staunoside G (=*6-Deoxy-α-*L*-mannopyranosyl-(1 → 4)-β-*D*-xylopyranosyl-(1 → 6)-1*-O-*[(3β)-3-{[β-*D*-glucopyranosyl-(1 → 4)-β-*D*-xylopyranosyl-(1 → 3)-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl]oxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-β-*D*-glucopyranose*; **1**). White amorphous powder. $[\alpha]_D^{20} = -21.2$ ($c = 0.42$, MeOH). IR: 3385, 2929, 1728, 1639, 1454, 1387, 1048. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 1483.70249 ($[M-H]^-$, $C_{69}H_{111}O_{34}^-$; calc. 1483.69622).

Staunoside H (=*6-Deoxy-α-*L*-mannopyranosyl-(1 → 4)-β-*D*-xylopyranosyl-(1 → 6)-1*-O-*[(3β)-23-hydroxy-28-oxo-3-{[β-*D*-xylopyranosyl-(1 → 4)-β-*D*-xylopyranosyl-(1 → 3)-6-deoxy-α-*L*-mannopyranosyl-(1 → 2)-α-*L*-arabinopyranosyl]oxy}olean-12-en-28-yl]-β-*D*-glucopyranose*; **2**). White amorphous powder. $[\alpha]_D^{20} = -26.8$ ($c = 0.39$, MeOH). IR: 3386, 2927, 1730, 1643, 1453, 1386, 1050. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 1477.68746 ($[M+Na]^+$, $C_{68}H_{110}NaO_{33}^+$; calc. 1477.68271).

Acid Hydrolysis of **1** *and* **2**. Compounds **1** and **2** (7.0 mg) were dissolved in 2M CF_3COOH (2.5 ml) and heated at 100° for 2 h. After removal of solvent under reduced pressure, the acidic soln. was evaporated again after addition of H_2O to remove acid. This procedure was repeated until a neutral soln. was obtained, which was finally evaporated and dried *in vacuo* to furnish a monosaccharide residue. The residue was dissolved in pyridine (0.5 ml), to which 2 mg of L-cysteine methyl ester hydrochloride was added. The mixture was kept at 60° for 2 h and evaporated under N_2 and dried *in vacuo*. Next, 0.2 ml of 1-(trimethylsilyl)-1*H*-imidazole were added. The resulting mixture was kept at 60° for 1 h. The mixture was partitioned between hexane and H_2O (each 2 ml), and the hexane extract was analyzed by GC under the following conditions: cap. column, *DB-5* (60 m × 0.25 mm × 0.25 μm); detection, FID; detector temp., 280°; injection temp., 250°; initial temp. was maintained at 160° for 2 min and then raised to 280° at the rate of 10°/min, and final temp. was maintained for 10 min; carrier, N_2 gas. The same procedure was carried out to analyze the acid hydrolysates of **1** and **2**. The absolute configurations of the sugars were determined by comparing the retention times (t_R) of derivatives of sugars with those of authentic sugars prepared in a similar way. The t_R values of derivatives were: D-glucose, 21.4; D-xylose, 19.0; L-rhamnose, 18.1; and L-arabinose, 14.4 min.

Cytotoxicity Assays. A549, 4T1, and HeLa cells were used for cytotoxicity assays. Cells were maintained in RPMI 1640 medium (*Gibco*) supplemented with 10% heat-inactivated fetal bovine serum (*Gibco*), and 100 U/ml penicillin/streptomycin at 37° under a humidified atmosphere of 95% air and 5% CO_2. For the assay, cells were planted into 96-well plate with a density of 1×10^3 cells per well. Twenty-four h later, cells were treated with compounds **1** and **2** (0 – 240 μg/ml), or kalopanaxsaponin A (7.5 – 80 μg/ml) for 24, 48, and 72 h, resp., with six replicates for each treatment. Then, 15 μl of MTT (5 mg/ml) were added into the culture system. Four h later, the supernatant was discarded, and 100 μl of DMSO were added. Cell viability was determined by measuring the optical density at 570 nm using a microplate reader. Untreated cells in medium were used as control. Corresponding groups without cells were used as blanks. The concentration required to reduce absorbance by 50% (IC_{50}) *vs.* the control was determined.

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