Triterpene Glucosides from the Leaves of *Aralia elata* and Their Cytotoxic Activities

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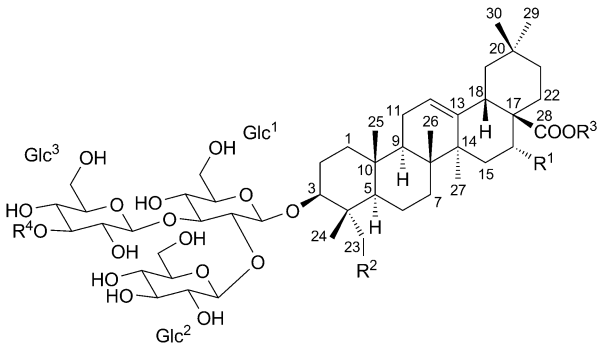
Three new triterpene glucosides, named congmuyenosides C–E (**1**–**3**, resp.), along with four known ones, were isolated from an EtOH extract of *Aralia elata* (MIQ.) SEEM. leaves. The structures of the new compounds were identified as 3-*O*-{*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl}caulophyllogenin (**1**), 3-*O*-{*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl}hederagenin 28-*O*-*β*-D-glucopyranosyl ester (**2**), 3-*O*-{*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl}echinocystic acid 28-*O*-*β*-D-glucopyranosyl ester (**3**) on the basis of spectral analyses, including MS, ^{1}H-NMR, ^{13}C-NMR, DEPT, HSQC, HMBC, NOESY, and HSQC-TOCSY experiments. All isolates obtained were evaluated for their cytotoxic activities against three human tumor cell lines (HepG2, SKOV3, and A549). Compound **3** showed significant cytotoxicity against A549 cell line (IC_{50} 9.9±1.5 μM).

Introduction. – The young leaves of *Aralia elata* (MIQ.) SEEM. (Araliaceae, *CiLaoYa* in Chinese) are cultivated as a vegetable crop in Northeast China. Additionally, the bark of its root cortex is widely used in folk medicine due to its tonic, antiarthritic, and antidiabetic effects [1]. Regarding the biologically active constituents of *A. elata*, many triterpenes were reported to have antidiabetic activities [2], cytoprotective effects on CCl_4-induced hepatic injury [3], EtOH absorption-inhibitory effects [4][5], and hypoglycemic activities [6–8]. Previously, our studies revealed that the growth of H_{22} tumor on mice and A549 transplantable tumor *in vitro* in nude mice could be inhibited by the total saponins of *A. elata* leaves [9][10]. To further investigate the constituents and to screen bioactive compounds from its leaves, a photochemical investigation on the leaves of this plant has now led to the isolation and structure elucidation of three new triterpenes, **1**–**3**, and four known triterpenes, **4**–**7**. Known compounds were identified by detailed 1D- and 2D-NMR analyses, comparison of their ESI-MS and spectral data with those reported in literature [11–13] as congmuyenoside A (**4**), congmuyenoside B (**5**), congmuyanoside B (**6**), and aralia-saponin VI (**7**). The cytotoxic effects of triterpenes **1**–**7** against human hepatoma (HepG2), human ovarian carcinoma (SKOV3), and human lung epithelial carcinoma cells (A549) were evaluated by means of the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. The results indicated that all the saponins inhibited the growth of HepG2, SKOV3, and A549 cells.



	R^1	R^2	R^3	R^4
1	OH	OH	H	β-D-Glc
2	H	OH	β-D-Glc	β-D-Glc
3	OH	H	β-D-Glc	β-D-Glc
4	H	OH	H	H
5	H	OH	H	β-D-Glc
6	OH	H	H	H
7	OH	H	β-D-Glc	H

Results and Discussion. – Compound **1**, named congmuyenoside C, was obtained as white amorphous powder (MeOH). The molecular formula was determined as $C_{54}H_{88}O_{25}$ from the $[M+Na]^+$ signal at *m/z* 1159.5515 by HR-FAB-MS. The IR spectrum indicated the presence of an ester C=O group (1740 cm^{-1}) and an olefinic moiety (1640 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table 1*) showed signals of six Me groups (*s* at δ(H) 0.89, 1.02 (6 H), 1.04, 1.15, and 1.77) and one olefinic H-atom (5.61 (br. *s*)). The four typical downfield CH *doublet*s at δ(H) 4.97 (*d*, *J* = 7.6), 5.63 (*d*, *J* = 7.9), 5.29 (*d*, *J* = 7.9), and 5.14 (*d*, *J* = 7.6) were assigned to the anomeric H-atoms of the β-D-glucopyranosyl moieties. The ^{13}C-NMR spectrum of **1** (*Table 2*) showed signals of a pair of olefinic C-atoms (δ(C) 122.4 and 145.2), four anomeric C-atoms (δ(C) 103.6, 103.8, 104.0, and 105.2), and a C=O group (δ(C) 180.1). On acid hydrolysis, **1** yielded only D-glucose as a sugar component by GC analysis using a hydrogen flame detector after treatment with L-cysteine methyl ester hydrochloride in pyridine [14], and cauiophyllogenin was obtained and identified as the aglycone on the basis of its NMR data and TLC by comparison with authentic samples.

The ^{1}H- and ^{13}C-NMR signals of the aglycone part were assigned by HSQC, ^{1}H,^{1}H-COSY, and HSQC-TOCSY experiments. The connectivities within the aglycone were deduced from the HMBCs (*Fig.*). In particular, the key long-range correlations Me(24)/C(3), C(4), C(5), and C(23), Me(25)/C(1), C(5), C(9), and C(10), Me(26)/C(7), C(8), C(9), and C(14), Me(27)/C(8), C(13), C(14), and C(15), and Me(29)/C(19), C(20), C(21), and C(30) evidenced that aglycone of **1** is the triterpene cauiophyllogenin.

The interglucosidic linkages were deduced from HMBC, HSQC-TOCSY, and ESI-MS results. In the HMBC spectrum, the cross-peaks H–C(1) of Glc^2/C(2) of Glc^1, H–C(1) of Glc^3/C(3) of Glc^1, and H–C(1) of Glc^4/C(3) of Glc^3 were observed. The correlations from the anomeric H-atom of the glucosyl moiety at δ(H) 4.97 to δ(C) 83.0 could also be observed, which confirmed this unit to be at C(3). Furthermore, the characteristic ion peaks at *m/z* 1135 ($[M-H]^-$), 973 ($[M-Glc-H]^-$), 811 ($[M-2Glc-H]^-$), 649 ($[M-3Glc-H]^-$), and 487 ($[M-4Glc-H]^-$) in the negative-ion-mode ESI-MS confirmed the above sugar linkages. In the HSQC-TOCSY spectrum, the key correlations H–C(1) of Glc^1/C(2), C(3), C(4), and C(5) of Glc^1, H–C(1) of Glc^2/C(2), C(3), C(4), and C(5) of Glc^2, H–C(1) of Glc^3/C(2), C(3), C(4), and C(5) of Glc^3, H–C(1) of Glc^4/C(2), C(3), C(4), and C(5) of Glc^4 established the interglucosidic linkages. Thus, the structure of **1** was established as 3-*O*-{β-D-glucopyranosyl-(1→3)-β-

Table 1. *^{1}H- and ^{13}C-NMR* (at 500 and 125 MHz, resp.) *Data of the Aglycone Part of* **1**–**3**. In (D_5)pyridine; δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	0.90–1.04 (*m*), 1.44–1.47 (*m*)	38.7 (*t*)	0.85–0.90 (*m*), 1.36–1.40 (*m*)	38.7 (*t*)	0.88–0.94 (*m*), 1.40–1.48 (*m*)	38.8 (*t*)
2	1.86–1.94 (*m*), 2.18–2.26 (*m*)	26.0 (*t*)	1.84–1.90 (*m*), 2.14–2.19 (*m*)	25.9 (*t*)	1.70–1.76 (*m*), 2.08–2.15 (*m*)	26.5 (*t*)
3	4.14 (*dd*, *J*=4.0, 8.2)	83.0 (*d*)	4.18 (*dd*, *J*=4.0, 10.0)	82.9 (*d*)	3.29 (*dd*, *J*=4.1, 11.5)	89.3 (*d*)
4	–	43.5 (*s*)	–	43.5 (*s*)	–	39.5 (*s*)
5	1.59 (*d*, *J* =11.6)	48.1 (*d*)	1.65–1.71 (*m*)	48.0 (*d*)	0.78 (*d*, *J*=11.6)	55.9 (*d*)
6	1.31–1.38 (*m*), 1.66–1.71 (*m*)	18.3 (*t*)	1.28–1.35 (*m*), 1.62–1.68 (*m*)	18.2 (*t*)	1.28–1.33 (*m*), 1.48–1.51 (*m*)	18.5 (*t*)
7	1.30–1.36 (*m*), 1.65–1.71 (*m*)	33.2 (*t*)	1.02–1.08 (*m*), 1.26–1.34 (*m*)	33.9 (*t*)	1.33–1.39 (*m*), 1.56–1.62 (*m*)	33.4 (*t*)
8	–	39.9 (*s*)	–	39.8 (*s*)	–	40.1 (*s*)
9	2.79 (*t*, *J*=13.4)	47.3 (*d*)	1.54 (*d*, *J*=12)	47.8 (*d*)	2.80 (*t*, *J*=13.5)	47.1 (*d*)
10	–	36.9 (*s*)	–	36.8 (*s*)	–	37.0 (*s*)
11	1.90–2.00 (*m*)	23.8 (*t*)	1.78–1.88 (*m*)	23.6 (*t*)	1.90–2.00 (*m*)	23.8 (*t*)
12	5.61 (br. *s*)	122.4 (*d*)	5.39 (br. *s*)	122.8 (*d*)	5.61 (br. *s*)	122.7 (*d*)
13	–	145.2 (*s*)	–	144.1 (*s*)	–	144.5 (*s*)
14	–	42.1 (*s*)	–	42.1 (*s*)	–	42.1 (*s*)
15	2.34–2.40 (*m*), 2.40–2.48 (*m*)	36.2 (*t*)	1.05–1.11 (*m*), 2.24–2.30 (*m*)	28.2 (*t*)	1.78 (*d*, *J*=12.5), 2.56 (*d*, *J*=12.5)	36.1 (*t*)
16	5.21 (br. *s*)	74.8 (*d*)	1.80–1.86 (*m*), 1.98–2.04 (*m*)	23.3 (*t*)	5.31 (br. *s*)	74.4 (*d*)
17	–	48.9 (*s*)	–	46.9 (*s*)	–	49.1 (*s*)
18	3.60–3.66 (*m*)	41.5 (*d*)	3.15 (*dd*, *J*=4.0, 13.0)	41.7 (*d*)	3.53 (*dd*, *J*=4.0, 11.3)	41.3 (*d*)
19	1.33–1.44 (*m*), 1.85–1.91 (*m*)	47.3 (*t*)	1.16–1.21(*m*), 1.65–1.73 (*m*)	46.1 (*t*)	1.34–1.40 (*m*), 1.71–1.77 (*m*)	47.2 (*t*)
20	–	31.0 (*s*)	–	30.7 (*s*)	–	30.8 (*s*)
21	1.29–1.37 (*m*), 1.66–1.73 (*m*)	36.2 (*t*)	1.25–1.33 (*m*), 1.52–1.60 (*m*)	32.8 (*t*)	1.23–1.29 (*m*), 2.40–2.46 (*m*)	36.0 (*t*)
22	2.18–2.30 (*m*), 2.37–2.43 (*m*)	32.8 (*t*)	1.66–1.72 (*m*), 1.72–1.78 (*m*)	32.5 (*t*)	2.04–2.09 (*m*), 2.38–2.44 (*m*)	32.2 (*t*)
23	3.68 (*d*, *J*=10.7), 4.29 (*d*, *J*=10.7)	64.8 (*t*)	3.64 (*d*, *J*=10.9), 4.27 (*d*, *J*=10.9)	64.7 (*t*)	1.23 (*s*)	28.0 (*q*)
24	1.04 (*s*)	13.4 (*q*)	1.03 (*s*)	13.3 (*q*)	1.08 (*s*)	16.8 (*q*)
25	0.89 (*s*)	16.1 (*q*)	0.88 (*s*)	16.0 (*q*)	0.86 (*s*)	15.6 (*q*)
26	1.02 (*s*)	17.5 (*q*)	1.08 (*s*)	17.5 (*q*)	1.12 (*s*)	17.5 (*q*)
27	1.77 (*s*)	27.2 (*q*)	1.16 (*s*)	26.0 (*q*)	1.84 (*s*)	27.3 (*q*)
28	–	180.1 (*s*)	–	176.4 (*s*)	–	175.9 (*s*)
29	1.02 (*s*)	33.3 (*q*)	0.87 (*s*)	33.0 (*q*)	1.01 (*s*)	33.2 (*q*)
30	1.15 (*s*)	24.8 (*q*)	0.86 (*s*)	23.6 (*q*)	1.05 (*s*)	24.6 (*q*)

Table 2. *^{1}H- and ^{13}C-NMR* (at 500 and 125 MHz, resp.) *Data of the Sugar Part of* **1**–**3**. In (D_5)pyridine; δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Glc[1]						
1	4.97 (*d*, *J* = 7.6)	103.8 (*d*)	4.97 (*d*, *J* = 7.9)	103.7 (*d*)	4.82 (*d*, *J* = 7.6)	104.9 (*d*)
2	4.24–4.32 (*m*)	79.2 (*d*)	3.95–4.04 (*m*)	79.2 (*d*)	4.32–4.40 (*m*)	79.0 (*d*)
3	3.99–4.13 (*m*)	88.7 (*d*)	3.97–4.05 (*m*)	88.7 (*d*)	4.20–4.31 (*m*)	88.5 (*d*)
4	3.93–4.13 (*m*)	69.9 (*d*)	3.94–4.06 (*m*)	69.8 (*d*)	3.96–4.08 (*m*)	69.9 (*d*)
5	3.61–3.66 (*m*)	77.6 (*d*)	3.58–3.68 (*m*)	77.6 (*d*)	3.92–4.05 (*m*)	77.7 (*d*)
6	4.41 (*dd*, *J* = 2.4, 12.5), 4.16 (*dd*, *J* = 5.2, 12.5)	62.0 (*t*)	4.44–4.38 (*m*), 4.30–4.25 (*m*)	62.2 (*t*)	4.46–4.40 (*m*), 4.28–4.21 (*m*)	62.2 (*t*)
Glc[2]						
1	5.63 (*d*, *J* = 7.9)	103.6 (*d*)	5.63 (*d*, *J* = 7.7)	103.5 (*d*)	5.64 (*d*, *J* = 7.6)	103.6 (*d*)
2	4.02–4.14 (*m*)	76.3 (*d*)	4.03–4.12 (*m*)	76.2 (*d*)	4.01–4.12 (*m*)	76.4 (*d*)
3	4.18–4.25 (*m*)	78.5 (*d*)	3.89–4.02 (*m*)	78.5 (*d*)	3.91–3.96 (*m*)	78.4 (*d*)
4	4.15–4.24 (*m*)	72.1 (*d*)	4.07–4.22 (*m*)	72.1 (*d*)	4.08–4.16 (*m*)	72.5 (*d*)
5	3.79–3.83 (*m*)	77.7 (*d*)	4.13–4.26 (*m*)	77.5 (*d*)	3.80–3.92 (*m*)	77.6 (*d*)
6	4.37 (*dd*, *J* = 2.0, 11.6), 4.20 (*dd*, *J* = 5.5, 11.6)	62.4 (*t*)	4.40–4.36 (*m*), 4.20–4.14 (*m*)	61.7 (*t*)	4.26–4.20 (*m*)	63.3 (*t*)
Glc[3]						
1	5.29 (*d*, *J* = 7.9)	104.0 (*d*)	5.28 (*d*, *J* = 7.9)	103.9 (*d*)	5.36 (*d*, *J* = 7.6)	104.1 (*d*)
2	3.96–4.06 (*m*)	74.1 (*d*)	3.94–4.06 (*m*)	74.1 (*d*)	4.08–4.17 (*m*)	74.0 (*d*)
3	4.19–4.27 (*m*)	87.7 (*d*)	4.15–4.27 (*m*)	87.6 (*d*)	4.12–4.28 (*m*)	87.6 (*d*)
4	3.93–4.13 (*m*)	69.9 (*d*)	3.94–4.06 (*m*)	69.8 (*d*)	4.08–4.14 (*m*)	69.8 (*d*)
5	3.93–4.01 (*m*)	78.0 (*d*)	3.78–3.89 (*m*)	77.9 (*d*)	4.08–4.20 (*m*)	77.9 (*d*)
6	4.43 (*dd*, *J* = 2.4, 11.6), 4.30 (*dd*, *J* = 5.5, 11.6)	63.1 (*t*)	4.42–4.37 (*m*), 4.30–4.24 (*m*)	63.1 (*t*)	4.34–4.29 (*m*), 4.20–4.14 (*m*)	62.0 (*t*)
Glc[4]						
1	5.14 (*d*, *J* = 7.6)	105.2 (*d*)	5.12 (*d*, *J* = 7.9)	105.1 (*d*)	5.14 (*d*, *J* = 7.6)	105.1 (*d*)
2	4.03–4.14 (*m*)	75.1 (*d*)	3.96–4.06 (*m*)	75.1 (*d*)	3.97–4.06 (*m*)	75.0 (*d*)
3	3.94–4.03 (*m*)	78.5 (*d*)	4.07–4.17 (*m*)	77.9 (*d*)	4.08–4.15 (*m*)	78.5 (*d*)
4	4.09–4.16 (*m*)	71.5 (*d*)	4.07–4.15 (*m*)	71.4 (*d*)	4.08–4.16 (*m*)	71.4 (*d*)
5	4.15–4.24 (*m*)	78.0 (*d*)	3.89–4.02 (*m*)	78.5 (*d*)	4.12–4.22 (*m*)	77.9 (*d*)
6	4.48 (*dd*, *J* = 2.4, 11.6), 4.23 (*dd*, *J* = 5.0, 11.6)	62.4 (*t*)	4.48 (*dd*, *J* = 2.0, 11.3), 4.23 (*dd*, *J* = 5.5, 11.3)	62.0 (*t*)	4.50–4.45 (*m*), 4.27–4.22 (*m*)	62.5 (*t*)
28-*O*-Glc						
1			6.29 (*d*, *J* = 7.9)	95.7 (*d*)	6.32 (*d*, *J* = 7.6)	95.9 (*d*)
2			3.94–4.06 (*m*)	74.1 (*d*)	3.97–4.06 (*m*)	74.2 (*d*)
3			3.89–3.95 (*m*)	78.8 (*d*)	4.20–4.31 (*m*)	78.9 (*d*)
4			4.26–4.36 (*m*)	71.1 (*d*)	4.22–4.36 (*m*)	71.1 (*d*)
5			3.95–4.04 (*m*)	79.1 (*d*)	3.96–4.08 (*m*)	79.4 (*d*)
6			4.45–4.40 (*m*),4.35–4.30 (*m*)	62.3 (*t*)	4.47–4.42 (*m*),4.32–4.26 (*m*)	62.3 (*t*)

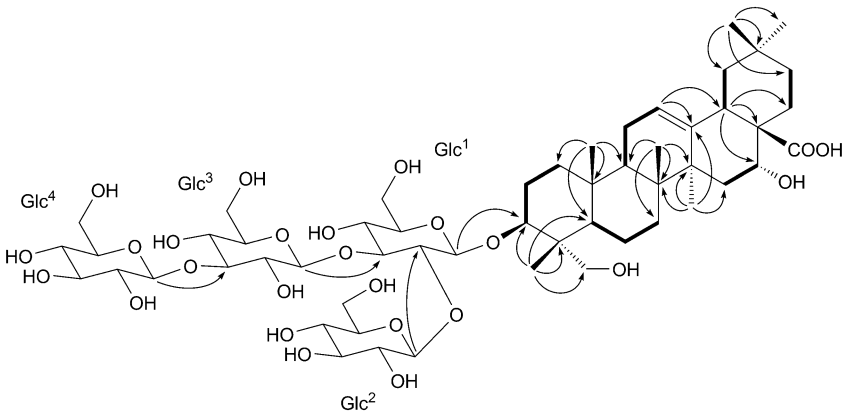


Figure. *Key HMB* (H→C) *and* $^1H,^1H$*-COSY* (—) *correlations of* **1**

D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}caulophyllogenin.

Compound **2**, named congmuyenoside D, was obtained as white amorphous powder with a molecular formula $C_{60}H_{98}O_{29}$, based on the HR-FAB-MS (*m/z* 1305.6095 ([*M*+Na]$^+$)). The ^{1}H-NMR spectrum of **1** (*Tables 1* and *2*) showed signals of six Me groups (*s* at δ(H) 0.86, 0.87, 0.88, 1.03, 1.08, and 1.16), an olefinic H-atom (δ(H) 5.39 (br. *s*)), and five anomeric CH *doublet*s (δ(H) 4.97 (*d*, *J*=7.9), 5.12 (*d*, *J*=7.9), 5.28 (*d*, *J*=7.9), 5.63 (*d*, *J*=7.7), and 6.29 (*d*, *J*=7.9)). The ^{13}C-NMR spectrum (*Table 1*) exhibited 60 C-atom signals that were attributed by a DEPT experiment to six Me, 15 CH_2, and 31 CH groups, as well as eight quaternary C-atoms, including two olefinic C-atoms (δ(C) 122.8 and 144.1), five anomeric C-atoms (δ(C) 103.7, 103.5, 103.9, 105.1, and 95.7), and an ester C=O group (δ(C) 176.4).

On acid hydrolysis, **2** only yielded hederagenin as the aglycone and D-glucose as sugar component, which were identified by comparison with authentic samples. On the basis of the 2D-NMR (^{1}H,^{1}H-COSY, HMQC, and HSQC-TOCSY) experiments, the spin systems of the five sugar moieties were assigned as compiled in *Table 2*. In the HMBC spectrum, cross-peaks H–C(1) of Glc^2/C(2) of Glc^1, H–C(1) of Glc^3/C(3) of Glc^1, H–C(1) of Glc^3/C(3) of Glc^1, H–C(1) of Glc^4/C(3) of Glc^3, and H–C(1) of Glc^5/C(28) were observed. Moreover, the significant fragment ions observed at *m/z* 1281 ([*M*−H]$^-$), 1119 ([*M*−Glc−H]$^-$), 957 ([*M*−2Glc−H]$^-$), 795 ([*M*−3Glc−H]$^-$), 633 ([*M*−4Glc−H]$^-$) and 471 ([*M*−5Glc−H]$^-$) in the negative-ion-mode ESI-MS, confirmed the linkages of the sugar units. Hence, **2** was determined as 3-*O*-{β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}hederagenin 28-*O*-β-D-glucopyranosyl ester.

Congmuyenoside E (**3**) was obtained as white amorphous powder. The molecular formula was determined as $C_{60}H_{98}O_{29}$ by positive-ion-mode HR-FAB-MS (*m/z* 1305.6090 ([*M*+Na]$^+$)). An acid hydrolysis of **3** only liberated echinocystic acid [12][13] as the aglycone and D-glucose, which was identified by GC analysis [14]. The ^{1}H-NMR spectrum revealed the presence of seven tertiary Me groups (δ(H) 0.86, 1.01, 1.05, 1.08, 1.12, 1.23, and 1.84) and an olefinic H-atom (δ(H) 5.61 (br. *s*)). The ^{13}C-NMR

spectrum (*Tables 1* and *2*) showed 60 C-atom signals that were assigned to seven Me, 13 CH_2, 32 CH groups, and eight quaternary C-atoms by a DEPT experiment. In addition to the above-mentioned signals, the ^{1}H-NMR spectrum of **3** exhibited resonances in the downfield region due to the five anomeric H-atoms at δ(H) 4.82, 5.64, 5.36, 5.14, and 6.32 that correlated in the HMQC experiment with the corresponding C-atoms at δ(C) 104.9, 103.6, 104.1, 105.1, and 95.9, respectively. Along with mass spectra, these data revealed the presence of five monosaccharide residues in **3**, and the anomeric configurations of the sugar moieties were determined as β on the basis of the J(H,H) values (7.0–8.0 Hz). In the HMBC spectrum, the cross-peaks H–C(1) of Glc^2/C(2) of Glc^1, H–C(1) of Glc^3/C(3) of Glc^1, H–C(1) of Glc^3/C(3) of Glc^1, H–C(1) of Glc^4/C(3) of Glc^3, and H–C(1) of Glc^5/C(28) confirmed the interglucosidic linkages. Moreover, the significant fragment-ion peaks observed at m/z 1281 ($[M-H]^-$), 1119 ($[M-Glc-H]^-$), 957 ($[M-2Glc-H]^-$), 795 ($[M-3Glc-H]^-$), 633 ($[M-4Glc-H]^-$), and 471 ($[M-5Glc-H]^-$ in the negative-ion-mode ESI-MS confirmed the sugar linkages. Accordingly, compound **3** was identified as 3-*O*-{β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-echinocystic acid 28-*O*-β-D-glucopyranosyl ester.

The cytotoxic activities of compounds **1**–**7** against HepG2, SKOV3, and A549 cell lines were evaluated by MTT method [15–17]. As showed in *Table 3*, these compounds showed only marginal cytotoxic activities against all three tested cell lines.

Table 3. *Cytotoxicities of Compounds* **1**–**7**

Compound	IC_{50} [μM][a])		
	HepG-2	Skov3	A549
1	39.7±7.9	92.1±8.4	40.2±10.0
2	62.4±12.8	120.2±13.0	187.3±22.7
3	29.4±8.3	55.4±5.5	9.9±1.5
4	82.4±8.5	150.2±7.5	55.1±9.0
5	128.4±28.1	165.4±10.2	96.5±5.7
6	17.4±3.7	100.3±10.8	25.4±5.9
7	25.2±3.4	80.2±7.0	27.9±4.6
5-Fluorouracil[b])	6.8±0.6	16.1±4.6	11.7±3.6

[a]) Mean±SD, $n=3$. [b]) The positive control, 5-fluorouracil, was purchased from *Shanghai Parmaceutical Industry Co. Ltd*.

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Expermental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; 200–300 mesh; *Merck*, Germany). HPLC: *Waters Delta-600* pump and *Waters 2414* refractive-index detector; *TSK-gel ODS-120T* column (10 μm, 40×300 mm, *Tosoh*, Japan). GC: *Fuli-9790* hydrogen flame detector; *DM-5* (0.25 μm, 30 m× 0.25 mm; *Dikma*, China). IR Spectra: *Jasco FT/IR-230* infrared spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *JNM LA-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Perkin-Elmer*

SCIEX API III biomolecular mass analyzer; in *m/z*. HR-FAB-MS: *JEOL JMS DX-302* mass spectrometer; in *m/z*.

Plant Materials. The leaves of *A. elata* were collected in Daxing'anling, Heilongjiang Province, P. R. China, in April 1998 and identified by Prof. *Zhenyue Wang* at Heilongjiang University of Chinese Medicine, P. R. China. A voucher specimen (No. 19980006) is deposited with the Herbarium of Heilongjiang University of Chinese Medicine, P. R. China.

Extraction and Isolation. The fresh leaves of *A. elata* (7.8 kg) were extracted with 70% EtOH under reflux (3 × 30 l) for 2 h, and the combined extracts were filtered and evaporated *in vacuo* to a syrup, which was suspended in H_2O (5 l), and extracted with petroleum ether (3 × 3 l). The aq. layer was evaporated. The residue (830 g) was suspended in H_2O (2 l), and the suspension was subjected to *AB-8* cross-linked polystyrene CC, sequentially eluted with H_2O, 30% EtOH, and 70% EtOH. The 70% EtOH elution fraction was concentrated under vacuum to yield a syrup residue, and this crude residue (170 g) was then subjected to CC (SiO_2; $CHCl_3$/MeOH 10:1 → 1:1 gradient) to give five fractions, *Frs. 1–5*.

CC (SiO_2; $CHCl_3$/MeOH 10:1 → 2:1; and *ODS*; MeOH/H_2O 1:1 → 8:2) of *Fr. 2* (22 g) yielded three yellow fractions, *Frs. A_1–A_3* (385, 385, and 430 mg, resp.), which were purified by prep. HPLC (*TSK-gel ODS-120T* column (10 μm, 40 × 300 mm, flow rate 8 ml/min; MeOH/H_2O 4:6) to afford **1** (t_R 32.5 min; 95 mg) from *Fr. A_1*, **2** (t_R 36.8 min; 82 mg) from *Fr. A_2*, and **3** (t_R 40.3 min; 79 mg) from *Fr. A_3*. Compound **4** (37.1 mg) was obtained from *Fr. 3* after CC (*ODS*; MeOH/H_2O 4:6 → 9:1) and HPLC (MeOH/H_2O 45:55; t_R 25.5 min). *Fr. 4* was separated by HPLC (MeOH/H_2O 45:55) to give compounds **5** (t_R 27.4 min; 37.5 mg) and **7** (t_R 30.4 min; 26.6 mg). *Fr. 5* was separated by CC (*ODS*; MeOH/H_2O 3:7 → 9:1) and HPLC (MeOH/H_2O 45:55) to furnish compounds **6** (t_R 24.5 min; 25.1 mg) and **7** (t_R 30.9 min; 10.6 mg).

Acid Hydrolysis of **1**–**3**. Compounds **1**–**3** (each 3 mg) were hydrolyzed in 7% aq. H_2SO_4 (1.0 ml) for 12 h at 80°. The mixtures were cooled and partitioned between $CHCl_3$ (2.0 ml) and H_2O (2.0 ml). The aq. layers were washed with $CHCl_3$ (3 × 3.0 ml), neutralized with $Ba(OH)_2$, filtered, and evaporated under reduced pressure. The residues were dissolved in pyridine (1.0 ml), and L-cysteine methyl ester hydrochloride in pyridine (0.1M, 2.0 ml) was added. The mixtures were heated at 60° for 1 h. An equal volume of Ac_2O was added under heating, and incubation was continued for 1 h. The acetylated thiazolidine derivatives were analyzed by GC with a *DM-5* column (30 m × 0.25 mm, 0.25 μm) using authentic samples as standards. Temps. of both injector and detector were 280°. A temp. gradient system was used for the oven, starting at 160° and increasing up to 195° at a rate of 5°/min. The determination of the sugar units in compounds **1**–**3** were achieved by comparison of t_R values of peaks between the hydrolysate and authentic sample of D-glucose (t_R 18.7 min).

Congmuyenoside C (*=(3β,16α)-3-{[β-D-Glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]oxy}-16,23-dihydroxyolean-12-en-28-oic Acid*; *3-O-{β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}-caulophyllogenin*; **1**). White amorphous powder. $[\alpha]_D^{20} = +23.1$ ($c = 0.3$, pyridine). IR (KBr): 3400, 2920, 1740, 1640, 1550, 1460, 1394, 1300, 1260, 1080, 1035, 995. ^{1}H- and ^{13}C- NMR: *Tables 1* and *2*. ESI-MS (neg.): 1135 ($[M-H]^-$), 973 ($[M-Glc-H]^-$), 811 ($[M-2Glc-H]^-$), 649 ($[M-3Glc-H]^-$), 487 ($[M-4Glc-H]^-$). HR-FAB-MS (pos.): 1159.5515 ($[M+Na]^+$, $C_{54}H_{88}NaO_{25}^+$; calc. 1159.5512)

Congmuyenoside D (*=1-O-[(3β)-3-{[β-D-Glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]oxy}-23-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose*; *3-O-{β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}hederagenin-28-O-β-D-glucopyranosyl Ester*; **2**). White amorphous powder. $[\alpha]_D^{20} = +28.1$ ($c = 0.3$, pyridine). IR (KBr): 3400, 2920, 2850, 1740, 1660, 1550, 1460, 1380, 1080, 1030. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 1281 ($[M-H]^-$), 1119 ($[M-Glc-H]^-$), 957 ($[M-2Glc-H]^-$), 795 ($[M-3Glc-H]^-$), 633 ($[M-4Glc-H]^-$), 471 ($[M-5Glc-H]^-$). HR-FAB-MS (pos.): 1305.6095 ($[M+Na]^+$, $C_{54}H_{88}NaO_{25}^+$; calc. 1305.6091).

Congmuyenoside E (*=1-O-[(3β,16α)-3-{[β-D-Glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl]oxy}-16-hydroxy-28-oxoolean-12-en-28-yl]-β-D-glucopyranose*; *3-O-{β-D-Glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl}echinocystic Acid 28-O-β-D-Glucopyranosyl Ester*; **3**). White amorphous powder. $[\alpha]_D^{20} = +18.0$ ($c = 0.25$, pyridine). IR (KBr): 3400, 2860, 1738, 1628, 1450, 1394, 1310, 1210,

1040, 890. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. ESI-MS (neg.): 1281 ($[M-H]^-$), 1119 ($[M-Glc-H]^-$), 957 ($[M-2Glc-H]^-$), 795 ($[M-3Glc-H]^-$), 633 ($[M-4Glc-H]^-$), 471 ($[M-5Glc-H]^-$). HR-FAB-MS (pos.): 1305.6090 ($[M+Na]^+$, $C_{54}H_{88}NaO_{25}^+$; calc. 1305.6091).

Cytotoxicity Assays. Test compounds were dissolved in DMSO, diluted to 2 mg/ml in *RPMI 1640* medium with free fetal bovine serum (FBS), and stored at $-20°$. The human tumor cell lines used in the present study were supplied from the Tumor Hospital of Harbin Medical University. They were maintained as adherent-type cultures under standard conditions. HepG-2, Skov3, and A549 cells were cultured at 37° under a humidified atmosphere of 5% CO_2 in *RPMI 1640* medium supplemented with 10% FBS and subculture twice weekly to maintain continuous logarithmic growth. Cells were seeded into (aliquots/well) at a density of 8×10^3 cells/ml for 24 h. Serial drug dilutions were prepared in medium immediately prior to each assay. Thereafter, aliquots of serial dilution of each test compound were added (parallel triplicate wells were set), and then the cells were incubated for 48 h in 5% CO_2 incubator at 37°. The cell viability was assessed through a MTT conversion assay. Aliquots of MTT soln. (5 mg/ml in PBS) were added to every well. Then, the plates were incubated for 4 h at 37°. The medium was then completely removed from the wells and replaced with 100 µl of DMSO. The OD of the dissolved formazan dye was recorded at 540 nm using a microplate spectrophotometer (*BMG Labtechnologies, Fluostar Optima*). The data were expressed as mean ± standard deviation. The standard *Microsoft* Excel program was used for the calculation of the IC_{50} values.

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