Triterpene Saponins from the Roots of *Ilex asprella*

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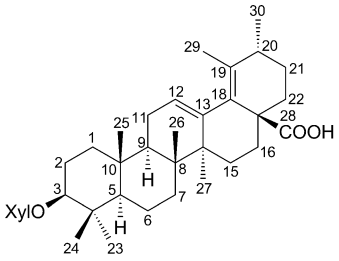
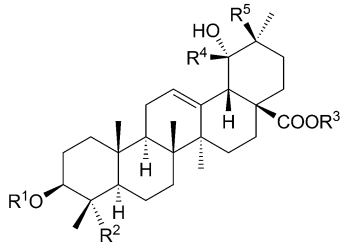
Six new triterpene saponins, ilexasprellanosides A–F (**1**–**6**, resp.), together with eleven known compounds were isolated from the roots of *Ilex asprella*. The new saponins were characterized as ursa-12,18-dien-28-oic acid 3-*O*-*β*-D-xylopyranoside (**1**), 19*α*-hydroxyursolic acid 3-*O*-*β*-D-(2′-*O*-acetylxylopyranoside) (**2**), 19*α*-hydroxyursolic acid 3-*O*-*β*-D-glucuronopyranoside (**3**), 3*β*,19*α*-dihydroxyolean-12-en-23,28-dioic acid 28-*O*-*β*-D-glucopyranoside (**4**), 19*α*-hydroxyoleanolic acid 3-*O*-*β*-D-(2′-*O*-acetylxylopyranoside) (**5**), 19*α*-hydroxyoleanolic acid 3-*O*-*β*-D-glucuronopyranoside (**6**). The structures of the new compounds were elucidated by analysis of their spectroscopic data and chemical degradation. Compounds **2**, **4**, oleanolic acid 3-*O*-*β*-D-glucuronopyranoside, 3-*β*-acetoxy-28-hydroxyurs-12-ene, and pomolic acid showed significant cytotoxic activities against human tumor cell line A549 (IC_{50} values of 1.87, 2.51, 1.41, 3.24, and 5.63 μM, resp.).

Introduction. – *Ilex asprella* (Aquifoliaceae) is mainly distributed in the south and east regions of the People's Republic of China. Its roots and leaves are widely used as a Traditional Chinese Medicine (TCM) for the treatment of headache, tussis, febris, dysentery, sore throat, *etc.* In addition, the leaves of *I. asprella* are also one of the raw materials of Cantonese herbal tea [1]. Previous investigations have resulted in the isolation of triterpenes and triterpene saponins from *I. asprella* [2–9]. Encouraged by the notable pharmacological properties of *I. asprella*, we have re-investigated the constituents of the roots and characterized six new triterpene saponins, **1**–**6**, together with eleven known compounds (*Fig. 1*). Herein, we report the isolation and structure elucidation of the new compounds, as well as their cytotoxic activities against A549 cell line.

Results and Discussion. – Compound **1** was obtained as amorphous powder, with a molecular formula of $C_{35}H_{54}O_7$ deduced from the $[M+Na]^+$ ion peak at m/z 609.3771 (calc. 609.3767) in the HR-ESI-MS and supported by the ^{13}C-NMR data (*Table 1*). The IR spectrum of **1** exhibited absorptions at 3445 (OH), 1697 (C=O), and 1647 cm^{-1} (C=C). The UV spectrum of **1** indicated the maximum absorption at 227 nm ($\log \varepsilon$ 3.79), attributed to a conjugated diene system. The ^{1}H-NMR spectrum (*Table 1*) of **1** revealed the presence of six tertiary Me groups resonating at δ(H) 1.84, 1.28, 1.13, 0.99, 0.96, and 0.84, a secondary Me group resonating at δ(H) 1.07 (d, $J=5.5$), an olefinic H-atom resonating at δ(H) 5.60 (t, $J=3.5$), and a H-atom due to an O-bearing CH group



1



	R^1	R^2	R^3	R^4	R^5
2	2'-*O*-Ac–Xyl	Me	H	Me	H
3	GluA	Me	H	Me	H
4	H	COOH	Glu	H	Me
5	2'-*O*-Ac–Xyl	Me	H	H	Me
6	GluA	Me	H	H	Me

Fig. 1. *Structures of compounds* **1**–**6**

at δ(H) 3.36 (*dd*, J = 10.5, 5.5). The ^{13}C-NMR spectrum (*Table 2*) of **1** exhibited signals of four olefinic C-atoms at δ(C) 139.4, 134.7, 126.3, and 123.6, of one C=O group at δ(C) 178.7, and of one O-bearing CH group at δ(C) 88.5. In addition, the ^{1}H- and ^{13}C-NMR spectra of **1** indicated the presence of a pentose moiety with the anomeric H-atom resonating at δ(H) 4.80 (*d*, J = 7.0). Acid hydrolysis and GC analysis evidenced the occurrence of a D-xylosyl moiety in **1**. The coupling constant (J = 7.0) of the anomeric H-atom suggested that the anomeric C-atom of the D-xylosyl moiety was β-configured. Comparison of the NMR data of **1** with those of ursa-12,18-dien-28-oic acid 3-*O*-β-D-arabinopyranoside revealed that the structures of both compounds were very similar except for the sugar moiety [10]. Thus, the structure of **1** (*Fig. 1*) was proposed to be ursa-12,18-dien-28-oic acid 3-*O*-β-D-xylopyranoside, which was further confirmed by the following HMBCs: H–C(12) (δ(H) 5.60 (*t*, J = 3.5)) to C(13) (δ(C) 139.4) and C(18) (δ(C) 123.6); Me(30) (δ(H) 1.07 (*d*, J = 5.5)) to C(19) (δ(C) 134.7); Me(29) (δ(H) 1.84 (*s*)) to C(19); and the anomeric H-atom (δ(H) 4.80 (*d*, J = 7.0)) to C(3) (δ(C) 88.5) of the aglycone (*Fig. 2*). Accordingly, the structure of **1** was unambiguously established as ursa-12,18-dien-28-oic acid 3-*O*-β-D-xylopyranoside, named ilexasprellanoside A.

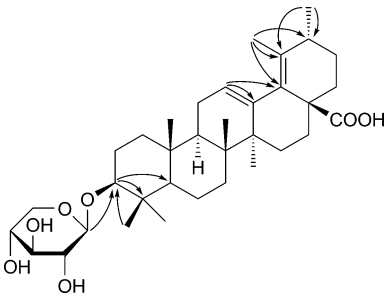


Fig. 2. *Key HMBCs* (H→C) *of compound* **1**

Table 1. *^{1}H-NMR Data* (500 MHz; (D_5) pyridine) *of* **1**–**3**. δ in ppm, J in Hz.

Position	**1**	**2**	**3**
Aglycone			
1	0.91–1.03 (*m*), 1.51–1.62 (*m*)	0.83–0.98 (*m*), 1.46–1.55 (*m*)	0.83–0.86 (*m*), 1.31–1.35 (*m*)
2	1.86–1.88 (*m*), 2.12–2.17 (*m*)	1.99–2.04 (*m*), 2.05–2.09 (*m*)	1.85–1.90 (*m*), 2.00–2.07 (*m*)
3	3.36 (*dd*, J=10.5, 5.5)	3.19 (*dd*, J=11.5, 4.0)	3.37 (*dd*, J=12.0, 4.5)
5	0.81–0.84 (*m*)	0.73–0.79 (*m*)	0.80–0.86 (*m*)
6	1.22–1.25 (*m*), 1.38–1.49 (*m*)	1.01–1.06 (*m*), 1.09–1.14 (*m*)	1.24–1.30 (*m*), 1.47–1.49 (*m*)
7	1.38–1.46 (*m*), 1.51–1.60 (*m*)	1.27–1.37 (*m*), 1.99–2.07 (*m*)	1.31–1.35 (*m*), 1.55–1.62 (*m*)
9	1.45–1.49 (*m*)	1.80–1.88 (*m*)	1.75–1.79 (*m*)
11	1.92–1.97 (*m*)	1.94–1.96 (*m*)	1.94–1.96 (*m*)
12	5.60 (*t*, J=3.5)	5.52 (*t*, J=3.5)	5.56 (br. *s*)
15	1.51–1.57 (*m*), 1.67–1.74 (*m*)	1.27–1.32 (*m*), 1.49–1.55 (*m*)	0.95–1.00 (*m*), 2.05–2.10 (*m*)
16	1.84–1.87 (*m*), 2.12–2.15 (*m*)	1.49–1.56 (*m*), 2.00–2.06 (*m*)	1.82–1.87 (*m*), 2.00–2.05 (*m*)
18	–	3.61 (br. *s*)	3.03 (*s*)
20	2.43–2.45 (*m*)	1.98–2.01 (*m*)	1.47–1.49 (*m*)
21	1.37–1.41 (*m*), 1.48–1.54 (*m*)	1.84–1.88 (*m*), 1.99–2.05 (*m*)	1.85–1.93 (*m*), 1.99–2.05 (*m*)
22	1.42–1.47 (*m*), 2.30–2.33 (*m*)	1.94–1.96 (*m*), 2.01–2.03 (*m*)	2.01–2.05 (*m*), 2.08–2.15 (*m*)
23	1.28 (*s*)	1.18 (*s*)	1.29 (*s*)
24	0.96 (*s*)	1.01 (*s*)	0.98 (*s*)
25	0.84 (*s*)	0.81 (*s*)	0.80 (*s*)
26	0.99 (*s*)	1.11 (*s*)	1.09 (*s*)
27	1.13 (*s*)	1.77 (*s*)	1.76 (*s*)
29	1.84 (*s*)	1.47 (*s*)	1.42 (*s*)
30	1.07 (*d*, J=5.5)	1.05 (*d*, J=6.5)	1.10 (*d*, J=6.5)
3-*O*-Sugar			
1′	4.80 (*d*, J=7.0)	4.78 (*d*, J=8.0)	5.02 (*d*, J=7.5)
2′	3.95 (*t*, J=7.5)	4.16 (*t*, J=7.0)	4.11 (*t*, J=6.0)
3′	4.10 (*t*, J=9.0)	4.20 (*t*, J=9.0)	4.31 (*t*, J=8.5)
4′	4.29–4.34 (*m*)	4.18–4.22 (*m*)	4.58 (*t*, J=8.5)
5′	4.33 (*dd*, J=11.5, 6.0), 3.72 (*dd*, 11.5, 6.0)	4.33 (*dd*, J=11.0, 4.5), 3.74 (*dd*, J=11.5, 6.0)	4.68 (*d*, J=7.5)
Ac	–	2.12 (*s*)	–

Table 2. *^{1}C-NMR Data* (125 MHz; (D_5)pyridine) *of* **1**–**6**. δ in ppm.

Position	**1**	**2**	**3**	**4**	**5**	**6**
1	39.1	38.4	38.4	38.6	38.4	38.4
2	26.7	26.4	26.9	27.6	26.5	26.3
3	88.5	88.9	89.1	75.3	88.9	89.1
4	39.4	40.2	40.3	51.8	39.9	39.9
5	55.9	55.7	54.7	54.2	55.8	55.8
6	18.3	18.6	18.5	21.6	18.6	18.5
7	34.8	33.5	33.5	32.7	33.2	33.2
8	39.1	40.3	39.9	40.3	40.0	40.2
9	48.1	47.9	47.6	48.3	48.2	48.1
10	36.7	37.0	36.8	36.7	37.1	37.0
11	23.4	24.0	23.9	23.9	24.1	24.0
12	126.3	128.0	128.0	123.3	123.3	123.3
13	139.4	139.9	139.8	144.2	144.8	144.8
14	44.7	42.3	42.3	41.8	42.1	42.1
15	29.1	29.3	29.1	28.5	28.8	28.8
16	26.6	26.4	26.3	28.6	29.1	29.2
17	50.1	48.2	48.2	46.2	46.0	46.0
18	123.6	54.6	54.6	44.3	44.8	44.7
19	134.7	72.7	72.6	80.8	81.2	81.1
20	34.8	42.1	42.1	35.3	35.7	35.7
21	29.1	26.9	26.9	28.7	29.2	29.2
22	35.5	38.4	38.4	32.7	33.2	33.2
23	28.1	28.3	28.3	180.6	28.3	28.1
24	16.9	17.4	17.4	11.9	16.7	16.7
25	16.1	15.4	15.4	15.7	15.3	15.3
26	18.0	16.7	16.7	17.3	17.4	17.1
27	22.2	24.8	24.8	24.3	24.8	24.7
28	178.7	180.6	180.9	177.2	180.9	180.6
29	20.2	26.5	27.1	28.7	28.8	28.9
30	20.4	16.6	16.8	24.6	24.9	24.8
1′	107.5	104.8	107.3	95.6	104.8	107.3
2′	75.3	75.6	75.5	73.8	75.6	75.5
3′	78.4	76.2	78.1	79.0	76.2	78.1
4′	71.0	71.3	73.4	70.8	71.3	73.4
5′	66.9	67.1	77.8	78.6	67.1	77.8
6′			172.9	61.9		172.9
Ac	–	170.0, 21.2	–	–	170.0, 21.2	–

For compound **2**, the molecular formula was determined as $C_{37}H_{58}O_9$ based on the $[M+Na]^+$ ion peak at *m*/*z* 669.3967 (calc. 669.3979) in the HR-ESI-MS and supported by the ^{13}C-NMR data (*Table 2*). Acid hydrolysis of **2** gave a known compound, pomolic acid [11] and D-xylose, suggesting **2** to be a pomolic acid xyloside. Comparison of the ^{13}C-NMR data of **2** with those of pomolic acid disclosed that the chemical shift of C(3) of **2** was shifted downfield to δ(C) 88.9, revealing the position of attachment of the xylosyl residue. This was confirmed by the HMBC between the anomeric H-atom resonating at δ(H) 4.78 (*d*, $J=8.0$) and C(3) (δ(C) 88.9) of the aglycone. The coupling constant ($J=8.0$) of the anomeric H-atom indicated that the anomeric C-atom of the

D-xylosyl moiety was β-configured. Additionally, the ^{1}H- and ^{13}C-NMR spectra of **2** evidenced the presence of an Ac group (δ(H) 2.12 (*s*), δ(C) 21.2, 170.0). The HMBC between H–C(2′) of the xylose (δ(H) 4.16 (*t*, *J* = 7.0)) and the C=O C-atom of the Ac group indicated that the Ac group was located at C(2′) of the xylose [12]. Therefore, the structure of **2** was established as 19α-hydroxyursolic acid-3-*O*-β-D-(2′-*O*-acetylxylopyranoside), named ilexasprellanoside B.

Compound **3** was obtained as an amorphous powder, with a molecular formula $C_{36}H_{56}O_{10}$ deduced from the $[M+Na]^+$ ion peak at *m*/*z* 671.3765 (calc. 671.3771) in the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra of **3** displayed many similarities with those of **2** except for the sugar moiety, suggesting that **2** and **3** possessed the same aglycone. The sugar moiety was elucidated as D-glucuronic acid (GluA) by comparison of the NMR data of **3** with those of ilexpernoside D, a known saponin previously isolated from *I. asprella* [13]. The β-linkage of the GluA moiety was determined by the *J* value (7.5 Hz) of the anomeric H-atom. In the HMBC spectrum of **3**, the correlation between the anomeric H-atom signal at δ(H) 5.02 (*d*, *J* = 7.5) and C(3) (δ(C) 89.1) indicated that the GluA moiety was linked to C(3) of the agylcone. Accordingly, the structure of **3** was established as 19α-hydroxyursolic acid 3-*O*-β-D-glucuronopyranoside, named ilexasprellanoside C.

Compound **4** was obtained as an amorphous powder. The HR-ESI mass spectrum showed a $[M+Na]^+$ ion peak at *m*/*z* 687.3713 corresponding to the molecular formula $C_{36}H_{56}O_{11}$ (calc. 687.3720). The ^{1}H-NMR spectrum of **4** (*Table 3*) exhibited six Me *singlet*s at δ(H) 1.59, 1.52, 1.11, 1.09, 0.96, and 0.93, and the signal of an olefinic H-atom at δ(H) 5.51 (br. *s*). The ^{13}C-NMR spectrum of **4** displayed the signals attributed to two C=O groups (δ(C) 180.6 and 177.2), two O-bearing CH groups (δ(C) 75.3 and 80.8), and two olefinic C-atoms (δ(C) 123.3 and 144.2). The above evidences suggested that **4** possessed an olean-12-ene skeleton. The cross-peak Me(25) (δ(H) 0.96 (*s*))/Me(24) (δ(H) 1.59 (*s*)) in the NOESY spectrum and the HMBC between H–C(3) (δ(H) 4.62 (*dd*, *J* = 10.0, 6.0)) and the C=O C-atom (δ(C) 180.6) suggested that the COOH group was at C(23). Additionally, the NMR spectra of **4** revealed the presence of a glucosyl moiety, which was confirmed by acid hydrolysis to yield glucose and ilexolic acid B [14]. The coupling constant of the anomeric H-atom (δ(H) 6.28 (*d*, *J* = 7.5)) evidenced β-configuration of the glucosyl moiety. In the HMBC spectrum, the correlation between the anomeric H-atom and C(28) (δ(C) 177.2) of the aglycone indicated that the glucosyl moiety was attached to C(28). From the evidences mentioned above, the structure of **4** was established as 3β,19α-dihydroxyolean-12-en-23,28-dioic acid 28-*O*-β-D-glucopyranoside, named ilexasprellanoside D.

Compound **5** was obtained as an amorphous powder. Its molecular formula was determined as $C_{37}H_{58}O_9$ by the presence of $[M+Na]^+$ ion peak at *m*/*z* 669.3957 (calc. 669.3979) in its HR-ESI-MS. The ^{1}H- and ^{13}C-NMR data of the aglycon moiety of **5** were superimposable with those of the aglycon part of the known compound siaresinolic acid [15]. Comparison of the NMR data of **5** with those of **2** suggested that a 2′-*O*-acetylxylosyl moiety was also present in **5**. The linkage of the Ac group was confirmed by the HMBC between H–C(2′) (δ(H) 4.16 (*t*, *J* = 7.0)) and the C=O C-atom of the Ac group (δ(C) 170.0). Acid hydrolysis yielded D-xylose and siaresinolic acid. The HMBC between the anomeric H-atom at δ(H) 4.78 (*d*, *J* = 8.0) and C(3) (δ(C) 88.9) of the aglycone indicated that the xylosyl moiety was at C(3). The β-linkage of the

Table 3. *^{1}H-NMR Data* (500 MHz; (D_5)pyridine) *of* **4**–**6**. δ in ppm, J in Hz.

Position	**4**	**5**	**6**
Aglycone moiety			
1	1.09–1.11 (*m*), 1.49–1.55 (*m*)	0.84–1.01 (*m*), 1.47–1.55 (*m*)	0.83–0.88 (*m*), 1.29–1.35 (*m*)
2	1.87–1.90(*m*), 2.02–2.08 (*m*)	1.98–2.07 (*m*), 2.05–2.09 (*m*)	1.85–1.91 (*m*), 2.01–2.07 (*m*)
3	4.62 (*dd*, *J*=10.0, 6.0)	3.19 (*dd*, *J*=12.0, 4.5)	3.37 (*dd*, *J*=12.0, 4.5)
5	1.94–1.99 (*m*)	0.73–0.79 (*m*)	0.79–0.86 (*m*)
6	1.49–1.52 (*m*), 1.63–1.69 (*m*)	1.02–1.06 (*m*), 1.09–1.15 (*m*)	1.27–1.30 (*m*), 1.46–1.49 (*m*)
7	1.36–1.39 (*m*), 1.63–1.70 (*m*)	1.26–1.32 (*m*), 1.99–2.07 (*m*)	1.30–1.35 (*m*), 1.56–1.62 (*m*)
9	1.90–1.93 (*m*)	1.82–1.88 (*m*)	1.74–1.80 (*m*)
11	1.97–1.99 (*m*)	1.94–1.96 (*m*)	1.90–1.94 (*m*)
12	5.51 (br. *s*)	5.53 (*t*, *J*=4.5)	5.51 (br. *s*)
15	1.08–1.11 (*m*), 2.24–2.29 (*m*)	1.18–1.23 (*m*), 2.09–2.11 (*m*)	1.10–1.13 (*m*), 2.02–2.07 (*m*)
16	1.95–1.98 (*m*), 2.25–2.29 (*m*)	1.29–1.32 (*m*), 1.47–1.55 (*m*)	2.12–2.17 (*m*), 2.27–2.33 (*m*)
18	3.47 (br. *s*)	3.61 (br. *s*)	3.59 (br. *s*)
19	3.54 (br. *s*)	3.58 (br. *s*)	3.58 (*d*, *J*=4.5)
21	0.98–1.05 (*m*), 1.12–1.15 (*m*)	1.27–1.35 (*m*), 1.47–1.49 (*m*)	1.15–1.18 (*m*), 1.23–1.28 (*m*)
22	1.87–1.90 (*m*), 1.98–2.02 (*m*)	1.37–1.41 (*m*), 2.01–2.03 (*m*)	1.31–1.35 (*m*), 2.01–2.07 (*m*)
23	–	1.07 (*s*)	1.28 (*s*)
24	1.59 (*s*)	0.87 (*s*)	0.96 (*s*)
25	0.96 (*s*)	0.81 (*s*)	0.80 (*s*)
26	1.11 (*s*)	1.01 (*s*)	1.04 (*s*)
27	1.52 (*s*)	1.65 (*s*)	1.66 (*s*)
29	1.09 (*s*)	1.10 (*s*)	1.00 (*s*)
30	0.93(*s*)	1.18 (*s*)	1.17 (*s*)
3-*O*-Sugar			
1′	–	4.78 (*d*, *J*=8.0)	5.01 (*d*, *J*=7.0)
2′	–	4.16 (*t*, *J*=7.0)	4.12 (*t*, *J*=6.0)
3′	–	4.20 (*t*, *J*=9.0)	4.32 (*t*, *J*=8.5)
4′	–	4.19–4.23 (*m*)	4.59 (*t*, *J*=8.5)
5′	–	4.28 (*dd*, *J*=13.0, 5.5), 3.73 (*dd*, 13.0, 5.5)	4.68 (*d*, *J*=7.5)
28-*O*-Sugar			
1′	6.28 (*d*, *J*=7.5)	–	
2′	4.16 (*t*, *J*=8.5)	–	
3′	3.98–4.01 (*m*)	–	
4′	4.27–4.28 (*m*)	–	
5′	4.32–4.39 (*m*)	–	
6′	4.33 (*dd*, *J*=8.5, 4.5), 3.40 (*dd*, 8.5, 4.5)	–	
Ac	–	2.12 (*s*)	–

xylosyl moiety was established by the J value of the anomeric H-atom (J=8.0). Thus, the structure of **5** was established as 19α-hydroxyoleanolic acid 3-*O*-β-D-(2′-*O*-acetylxylopyranoside), named ilexasprellanoside E.

The molecular formula of compound **6** was $C_{36}H_{56}O_{10}$ as deduced from the $[M+Na]^+$ ion peak at m/z 671.3765 (calc. 671.3771) in the HR-ESI-MS. Comparison of the NMR data of **6** with those of **5** and **3** allowed elucidation of the structure of **6** as 19α-

hydroxyoleanolic acid 3-*O*-*β*-D-glucuronopyranoside, named ilexasprellanoside F. The linkage between the GluA moiety and the aglycone was confirmed by the HMBC between the anomeric H-atom at δ(H) 5.01 (*d*, $J = 7.0$) and C(3) (δ(C) 89.1) of the aglycone.

The other eleven known compounds were identified as ursa-12,18-dien-28-oic acid 3-*O*-*β*-D-arabinopyranoside [10], suavissimoside R1 [16], 3*β*-[(*α*-L-arabinopyranosyl)-oxy]-19*α*-hydroxyurs-12-en-28-oic acid 28-*β*-D-glucopyranosyl ester [10], oleanolic acid 3-*O*-*β*-D-glucuronopyranoside [17], 3-*β*-acetoxy-28-hydroxyurs-12-ene [18], pomolic acid [11], 2*β*,3*α*-dihydroxyurs-12-en-28-oate [19], 3-*O*-[6′-*O*-palmitoyl-*β*-D-glucosyl]-stigmasta-5,25(27)-diene [20], 3-*O*-[6′-*O*-stearoyl-*β*-D-glucosyl]stigmasta-5,25(27)-diene [20], 3-*O*-*β*-D-glucosylstigmasta-5,25(27)-diene [8], and syringic acid [21], respectively. They were isolated for the first time from this plant except 3-*O*-*β*-D-glucosylstigmasta-5,25(27)-diene.

All the compounds obtained were evaluated for their inhibitory activities against human tumor cell A549 with fluorouracil (5-FU) as a positive control. Compounds **2**, **4**, oleanolic acid 3-*O*-*β*-D-glucuronopyranoside, 3-*β*-acetoxy-28-hydroxyurs-12-ene, pomolic acid, and 5-Fu showed notable cytotoxic activities against human tumor cell line A549 (IC_{50} values 1.87 ± 0.13, 2.51 ± 0.29, 1.41 ± 0.16, 3.24 ± 0.18, 5.63 ± 0.76, and 28.96 ± 1.13 μM, resp.).

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Experimental Part

General. Column chromatography (CC): silica gel *H* (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Industry*) and *Sephadex LH-20* gel (*Pharmacia*). Semi-prep. HPLC: *Waters 600* pump with *600* controller, *Waters C18 Nova-Pak* column (300 × 7.8 mm, 5 μm), with *ELSD* detector (*Alltech*; split ratio, 10%); flow rate, 2.5 ml/min. GC: *Agilent 6890 N* gas chromatograph; cap. column (28 m × 0.32 mm i.d.; *HP-5*); FID detector, operated at 260° (column temp. 180°); N_2 as carrier gas (40 ml/min). Optical rotations: *Perkin-Elmer 243 B* digital polarimeter. UV Spectra: *TU-1901* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *NEXUS-470 FTIR* (*Nicolet*) spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Varian Inova-500* and *Varian Unity-500* instruments; at 500 (^1H) or 125 MHz (^{13}C) in C_5D_5N at r.t.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS (pos.): *QSTAR* (*ABI*, *USA*) mass spectrometer; in *m*/*z*. HR-ESI-MS (pos.): *Bruker APEX II FT-ICRMS* mass spectrometer; in *m*/*z*.

Plant Material. The roots of *Ilex asprella* (Hook et Arn.) Champ. ex Benth were collected in August 2006 from Guangdong Province, P. R. China. The plant was identified by one of the authors (*P.-F. T.*). A voucher specimen (CM200608) was deposited with the Herbarium of Peking University, Modern Research Center for Traditional Chinese Medicine.

Extraction and Isolation. The dried roots (20 kg) of *I. asprella* were extracted with 70% EtOH (160 l, 2 h, 78°) for three times. After removal of the solvent under reduced pressure at 60°, the residue (1.7 kg) was suspended in H_2O, and defatted with petroleum ether (PE). The aq. layer was further extracted with AcOEt (3 × 1 l) and BuOH (3 × 1 l), successively, to provide the AcOEt (170 g) and BuOH extract (700 g). BuOH extract (500 g) was subjected to CC (*D101* porous polymer resin; H_2O and 10, 30, 50, 70, 95% aq. EtOH, resp.). The fractions eluted with 50% EtOH (90 g) and 70% EtOH (65 g) had similar compositions. They were pooled, and a portion (100 g) of the combined fraction was subjected to CC (SiO_2; $CHCl_3$/MeOH/H_2O 8:1:0 → 1:1:0.1) to afford four subfractions, *Frs. 1–4*. *Fr. 1* (8 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 10:1) to afford 2*β*,3*α*-dihydroxyurs-12-en-28-oate (9 mg). *Fr. 2*

(25 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 10:1) to afford *Frs. 2.1* and *2.2*. *Fr. 2.2* was purified by CC (*ODS*; MeOH/H_2O 1:3→3:1) to afford *Frs. 2.2.1* and *2.2.2*. *Fr. 2.2.1* was subjected to semiprep. HPLC (MeOH/H_2O 55:45) to yield compound **1** (t_R 20.5 min, 9.0 mg) and ursa-12,18-dien-28-oic acid 3-*O*-*β*-D-arabinopyranoside (t_R 18.5 min; 5.0 mg). *Fr. 2.2.2* was subjected to semiprep. HPLC (MeOH/H_2O 45:55) to yield compounds **2** (t_R 11.5 min; 6.0 mg) and **5** (t_R 16 min; 5 mg). *Fr. 3* (10 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:1→100:5) to afford pomolic acid (23 mg). A portion of the AcOEt extract (130 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:0→100:15) to afford six subfractions, *Frs. 1–6*. *Fr. 1* (5 g) was submitted to CC (SiO_2; $CHCl_3$/MeOH 100:10→100:20) to afford 3*β*-acetoxy-28-hydroxyurs-12-ene (9 mg). *Fr. 2* (5 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:1) and semiprep. HPLC (MeOH/H_2O 1:1) to afford syringic acid (t_R 11.5 min; 7 mg). *Fr. 3* (10 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:10→100:20) to afford *Frs. 3.1* and *3.2*. *Fr. 3.2* was separated by CC (*Sephadex LH-20*; H_2O) and semiprep. HPLC (MeOH/H_2O 1:1) to afford 3-*O*-[6′-*O*-palmitoyl-*β*-D-glucosyl]stigmasta-5,25(27)-diene (t_R 15 min; 10.0 mg) and 3-*O*-[6′-*O*-stearoyl-*β*-D-glucosyl]stigmasta-5,25(27)-diene (t_R 17 min; 10.0 mg). *Fr. 4* (13 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:10) to afford 3-*O*-*β*-D-glucosylstigmasta-5,25(27)-diene (32 mg). *Fr. 5* (15 g) was subjected to CC (*ODS*; MeOH/H_2O 2:1) to afford oleanolic acid 3-*O*-*β*-D-glucuronopyranoside (5 mg), 3*β*-[(*α*-L-arabinopyranosyl)oxy]-19*α*-hydroxyurs-12-en-28-oic acid 28-*β*-D-glucopyranosyl ester (8 mg) and *Fr. 5.1*. *Fr. 5.1* was purified by CC (*Sephadex LH-20*; MeOH/H_2O 1:1) and semiprep. HPLC (MeOH/H_2O 43:57) to afford compounds **3** (t_R 8 min; 6.0 mg) and **6** (t_R 13 min; 5.0 mg). *Fr. 6* (18 g) was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:3→100:15) to afford *Frs. 6.1 – 6.3*. *Fr. 6.1* was separated by CC (SiO_2; $CHCl_3$/MeOH 100:8) to afford compound **4** (15.0 mg) and suavissimoside R1 (18 mg).

Ilexasprellanoside A (= *3-(β-D-Xylopyranosyloxy)ursa-12,18-dien-28-oic Acid*; **1**). Amorphous powder. $[\alpha]_D^{26} = +110.0$ ($c = 0.96$, MeOH). UV (MeOH): 227 (3.79). IR: 3445, 2935, 2874, 1697,1647, 1454, 1388, 1372, 1275, 1203, 1166, 1074, 1044. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 609.3771 ($[M+Na]^+$, $C_{35}H_{54}NaO_7^+$; calc. 609.3762).

Ilexasprellanoside B (= *3-[(2-O-Acetyl-β-D-xylopyranosyl)oxy]-19-hydroxyurs-12-en-28-oic Acid*; **2**). Amorphous powder. $[\alpha]_D^{26} = +1.67$ ($c = 0.12$, MeOH). IR: 3419, 2927, 2874, 1697, 1730, 1631, 1453, 1384, 1318, 1261, 1111, 1072. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 669.3967 ($[M+Na]^+$, $C_{37}H_{58}NaO_9^+$; calc. 669.3979).

Ilexasprellanoside C (= *(3β)-19,28-Dihydroxy-28-oxours-12-en-3-yl β-D-Glucopyranosiduronic Acid*; **3**). Amorphous powder. $[\alpha]_D^{26} = +4.1$ ($c = 0.48$, MeOH). IR: 3444, 2937, 2877, 1693, 1455, 1388, 1205, 1165, 1090. ^{1}H- and ^{13}C-NMR: see *Tables 1* and *2*, resp. HR-ESI-MS: 671.3765 ($[M+Na]^+$, $C_{36}H_{56}NaO_{10}^+$; calc. 671.3771).

Ilexasprellanoside D (= *1-O-[(19α)-3,19,23-Trihydroxy-23,28-dioxoolean-12-en-28-yl]-β-D-glucopyranose*; **4**). Amorphous powder. $[\alpha]_D^{26} = +45.0$ ($c = 0.24$, MeOH). IR: 3426, 2933, 2877, 1709, 1622, 1452, 1388, 1262, 1231, 1165, 1075, 1030. ^{1}H- and ^{13}C-NMR: see *Tables 3* and *2*, resp. HR-ESI-MS: 687.3713 ($[M+Na]^+$, $C_{36}H_{56}NaO_{11}^+$; calc. 687.3720).

Ilexasprellanoside E (= *(19α)-3-[(2-O-Acetyl-β-D-xylopyranosyl)oxy]-19-hydroxyolean-12-en-28-oic Acid*; **5**). Amorphous powder. $[\alpha]_D^{26} = +110.7$ ($c = 0.94$, MeOH). IR: 3419, 2927, 2874, 1697,1730, 1631, 1453, 1384, 1318, 1261, 1111, 1072. ^{1}H- and ^{13}C-NMR: see *Tables 3* and *2*, resp. HR-ESI-MS: 669.3967 ($[M+Na]^+$, $C_{37}H_{58}NaO_9^+$; calc. 669.3979).

Ilexasprellanoside F (= *(3β,19α)-19,28-Dihydroxy-28-oxoolean-12-en-3-yl β-D-Glucopyranosiduronic Acid*; **6**). Amorphous powder. $[\alpha]_D^{26} = +4.1$ ($c = 0.24$, MeOH). IR: 3444, 2937, 2877, 1693, 1455, 1388, 1205, 1165, 1090. ^{1}H- and ^{13}C-NMR: see *Tables 3* and *2*, resp. HR-ESI-MS: 671.3765 ($[M+Na]^+$, $C_{36}H_{56}NaO_{10}^+$; calc. 671.3771).

Acid Hydrolysis and Determination of Sugar Components. Compounds **1**, **2**, **4**, and **5** (2 mg) were hydrolyzed with 2N aq. CF_3COOH (10 ml) at 110° for 8 h in a sealed tube. The mixture was diluted with H_2O (20 ml) and extracted with AcOEt (3 × 10 ml).

The aq. layer was concentrated under reduced pressure and analyzed by TLC ($CHCl_3$/MeOH/H_2O 8:5:1). Spots were visualized by spraying with 95% EtOH/H_2SO_4/anisaldehyde 9:0.5:0.5, then heated at 120° for 10 min. The R_f values of glucose and xylose were 0.40 and 0.56, resp. For the determination of the absolute configuration, the aq. layer was completely dried and dissolved in anh. pyridine (100 l), L-cysteine methyl ester hydrochloride (0.1M, 200 µl) was added, and the mixture was warmed at 60° for 1 h.

The trimethysilylation reagent HMDS (hexamethyldisilazane)/TMCS (Me_3SiCl)/pyridine 2:1:10 (*Acros Organics*, Belgium) was then added, and the soln. was incubated at 60° for further 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification. D-Glucose (t_R 11.25 min) and D-xylose (t_R 5.32 min).

Cytotoxicity Assay. Human tumor cell line A549 was maintained in *RPMI 1640* medium (*Thermo Fisher*) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, and 100 IU/ml streptomycin. Cells were cultured in 96-well microtiter plates for the assay. Appropriate dilutions (10^{-2} to 10^2 μM) of the test compounds were added to the cultures. After 72 h incubation in humidified air containing 5% CO_2 at 37°, growth inhibition of cancer cells was evaluated by the MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) method [22]. Results are expressed as the mean value of triplicate data points. 5-FU was used as the positive control.

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