Antiviral Triterpenoid Saponins from the Roots of *Ilex asprella*

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Abstract

Two new sulfur-containing triterpenoid saponins, asprellanosides A (**1**) and B (**2**), were isolated from the roots of *Ilex asprella*, together with 10 known compounds (**3**–**12**). An *in vitro* anti-HSV-1 activity test of the isolates (**1**–**4**, **6**–**7**, and **9**–**12**) showed that only asprellanoside A (**1**) and oblonganoside H (**6**) exhibited anti-HSV-1 activity with TIC values of 0.14 and 0.18 mM, respectively.

Key words

Ilex asprella · Aquifoliaceae · sulfur-containing triterpenoid saponins · asprellanosides A and B · antiviral

Abbreviations

HSV-1:	herpes simplex virus type 1
TIC:	total inhibitory concentration

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Ilex asprella (Hook. f. et Arn.) Champ. ex Benth. (Aquifoliaceae), a deciduous shrub about 1–2 meters high, is growing in the thin forests on slopes in barren hill land or in the midst of the thicket in Guangdong, Guangxi, Hunan, and Jiangxi provinces, China. The roots and leaves have been used as a folk medicine for the treatment of viral and bacterial infectious diseases, such as influenza, tonsillitis, sphagitis, and trachitis [1]. In southern China, the root itself has also been an important ingredient to make herbal tea and beverages against viral and bacterial infections for hundreds of years. Several triterpenoids were reported from the roots and leaves of *I. asprella* [2–7]. However, little information about the pharmaceutical functions of its chemical compositions is available. As a part of our continuing research on antiviral compounds [8–17], the present paper describes the isolation and identification of two new sulfur-containing triterpenoid saponins from the roots of *I. asprella*. Most of the isolates (**1**–**4**, **6**–**7**, and **9**–**12**) were also evaluated for their anti-HSV-1 activity *in vitro*.

Compound **1**, a white amorphous powder, had a molecular formula of $C_{41}H_{66}O_{16}S$ as determined by negative ion HRFABMS (*m*/*z* 845.3982 $[M - H]^-$). The ^{13}C NMR and DEPT spectra (**Table 1**) showed 41 carbon signals, including 30 resonances assignable to a triterpene skeleton and the remaining 11 carbon resonances from one hexosyl and one pentosyl unit. The ^1H NMR spectrum (**Table 1**) displayed six methyl singlets (δ_H 0.80, 0.81, 0.82, 1.04, 1.20, and 1.36, each s, Me-24, 25, 26, 23, 29, 27), one methyl doublet (δ_H 0.91, d, J = 6.4 Hz, Me-30), an olefinic proton (δ_H 5.29, brs, H-12), and two anomeric protons [δ_H 4.42 (d, J = 7.6 Hz), 5.56 (d, J = 8.4 Hz)]. These data were similar to those of oblonganoside H (**6**) [18]. However, the molecular weight of **1** was 80 Da greater than that of **6**, corresponding to an SO_3H group. In the ^{13}C NMR spectrum, the xylosyl C-3′ of **1** was lower-field shifted by 6.6 ppm, while C-2′, C-4′, and C-5′ were upper- or lower-field shifted by − 1.8, − 1.2, and + 1.2 ppm, respectively, related to **6**. The aforementioned data indicated that the OH group at C-3′ of Xyl in **6** was sulfited with an SO_3H group in **1**. The HMBC correlations (**Fig. 3**) of Xyl H-1′ (δ_H 4.42) with δ_C 90.8 (Agly C-3), and Glc H-1″ (δ_H 5.56) with δ_C 178.6 (Agly C-28) further confirmed the xylosyl and glucosyl units located on C-3 and C-28 of **1**, respectively. Therefore, compound **1** was elucidated to be asprellanoside A.

Compound **2**, a white amorphous powder, possessed a molecular formula of $C_{35}H_{56}O_{11}S$ as deduced by negative ion HRFABMS (*m*/*z* 683.3460 $[M - H]^-$). The ^1H and ^{13}C NMR spectra of **2** (**Table 1**), showing six methyl singlets, one methyl doublet, an olefinic proton, one anomeric proton signal, 35 carbon signals arising from a triterpene skeleton, and one pentosyl unit, were similar to those of ziyu glycoside II [19], a triterpenoid arabinopyranoside from *I. cornuta* [20]. However, compared to ziyu glycoside II, the molecular weight of **2** was 80 Da greater, and C-18 and C-22 of **2** were upper-field shifted by 7.5 and 6.0 ppm, respectively, while C-29 was lower-field shifted by 2.9 ppm. The big NMR data differences revealed that the α-orientated C-29 methyl in ziyu glycoside II was changed to a β orientation in **2**, which was the same as that of ilexgenin B [21]. In addition, the arabinosyl C-3′ of **2** was downfield shifted by 6.8 ppm, and C-2′, C-4′, and C-5′ were upper- or lower-field shifted by − 1.3, − 0.8, + 2.0 ppm, respectively, in relation to ziyu glycoside II [19]. This indicated that **2** had an additional SO_3H group attached to the C-3′ position of the arabinosyl unit. In the HMBC spectrum of **2** (**Fig. 3**), correlations of Ara H-1′ (δ_H 4.77) with δc 89.0 (Agly C-3) confirmed the arabinosyl unit located at the C-3 position, and other HMBC correlations (**Fig. 3**) supported the structure of **2** as shown in **Fig. 1**. Therefore, compound **2** was determined to be asprellanoside B.

Ten isolated compounds (**1**–**4**, **6**–**7**, and **9**–**12**) were tested for their *in vitro* anti-HSV-1 activity and cytotoxicity on Vero cells (African green monkey kidney cells) (**Table 2**) [13]. Only asprellanoside A (**1**) and oblonganoside H (**6**) showed anti-HSV-1 activities with TIC values of 0.14 and 0.18 mM, respectively, while their maximal noncytotoxic concentrations (MNCC) against Vero cells were higher than 1.00 mM. These triterpenoids appear to be associated with the anti-HSV-1 activity for *I. asprella*. The structure-activity relationship of these triterpenoids indicated that the C-3 linked xylosyl unit and the C-23/C-24 methyls might be important to the anti-HSV-1 activity. When C-3 was linked with an arabinosyl (**2**) or other sugar units such as in **4** and **9**, these compounds lost their anti-HSV-1 activities. If the C-23 or C-24 methyls were oxidized as in **3** and **7**, their activities were also lost. The present study may provide scientific support for the use of *I. asprella* roots as a main ingredient in some commercial herbal tea beverages against viral and bacterial infections.

* These authors contributed equally to this paper.

Position	1[a]		2[b]	
	^{13}C	^{1}H	^{13}C	^{1}H
1	39.8	1.50 (m); 0.85 (m)	38.8	1.52 (m); 0.92 (m)
2	27.1	1.80 (m); 1.64 (m)	27.1	2.07 (m); 1.86 (m)
3	90.8	3.12 (dd, 11.6, 4.0)	89.0	3.31 (dd, 11.5, 4.5)
4	40.1		39.7	
5	57.0	0.71 (m)	56.0	0.84 (m)
6	19.4	1.41 (m); 1.25 (m)	18.7	1.52 (m); 1.32 (m)
7	34.1	1.45 (m); 1.26 (m)	33.6	1.59 (m); 1.36 (m)
8	41.2		40.3	
9	49.0	1.61 (m)	47.8	1.81 (m)
10	37.8		37.1	
11	24.7	1.87 (m); 1.32 (m)	25.0	2.70 (m); 1.68 (m)
12	129.7	5.29 (brs)	127.3	5.56 (brs)
13	139.6		139.6	
14	42.6		42.2	
15	29.7	1.95 (m); 0.99 (m)	29.4	2.29 (m); 2.13 (m)
16	26.5	2.67 (m); 1.60 (m)	26.7	3.22 (m); 2.10 (m)
17	48.7		48.1	
18	55.0	2.56 (s)	47.5	3.28 (s)
19	73.6		73.6	
20	42.6	1.24 (m)	43.0	2.05 (m)
21	27.2	2.21 (m); 1.84 (m)	24.1	2.01 (m); 1.75 (m)
22	38.3	1.78 (m); 1.59 (m)	32.3	2.23 (m); 1.95 (m)
23	28.5	1.04 (s)	28.3	1.30 (s)
24	17.0	0.80 (s)	17.1	0.99 (s)
25	16.0	0.81 (s)	15.7	0.87 (s)
26	17.6	0.82 (s)	17.4	1.05 (s)
27	24.7	1.36 (s)	24.6	1.75 (s)
28	178.6		181.3	
29	27.1	1.20 (s)	30.0	1.47 (s)
30	16.6	0.91 (d, 6.4)	17.1	1.13 (d, 6.5)
1′	107.0	4.42 (d, 7.6)	107.5	4.77 (d, 7.2)
2′	73.8	3.56 (m)	71.3	4.63 (brt, 8.5)
3′	85.2	4.38 (t, 8.8)	81.1	5.20 (dd, 3.0, 9.0)
4′	70.1	3.77 (m)	68.4	4.90 (brs)
5′	65.9	3.95 (m); 3.32 (m)	67.0	4.25 (m); 3.77 (m)
1′′	95.8	5.56 (d, 8.4)		
2′′	73.8	3.54 (m)		
3′′	78.3	3.62 (m)		
4′′	71.1	3.59 (m)		
5′′	78.6	3.48 (m)		
6′′	62.4	3.95 (m); 3.82 (m)		

[a] CD_3OD; [b] C_5D_5N

Table 1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR for compounds **1** and **2** (δ values; J values in Hz are in parentheses).

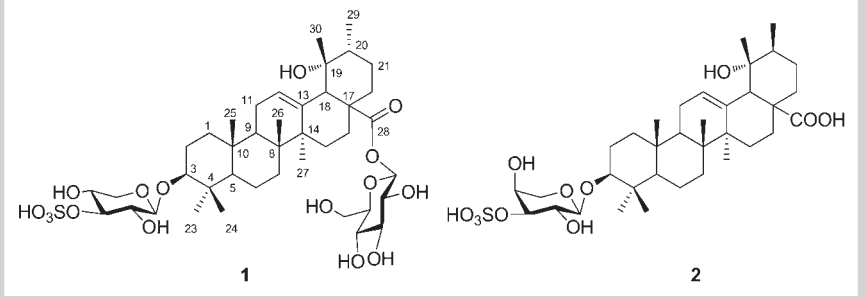

Fig. 1 Chemical structures of compounds **1** and **2** isolated from *I. asprella*.

Materials and Methods

Air-dried roots of *Ilex asprella* were obtained from the Pharmaceutical Material and Drug Co., Ltd. of Guangzhou University of Chinese Medicine, Guangzhou, China. The plant materials were identified by Mr. Zhi-Ping Wang, from Guangdong Medical College, Guangzhou, China. A vouch specimen (200701) was deposited in the Guangzhou Jinan Biomedicine Research & Development Center.

The known compounds were identified as oblonganoside I (**3**) [18], ilexsaponin B_2 (**4**) [22], ziyu glycoside I (**5**) [23], oblonganoside H (**6**) [18], ilexsaponin A_1 (**7**) [24], ilexgenin A (**8**) [24], ilex-

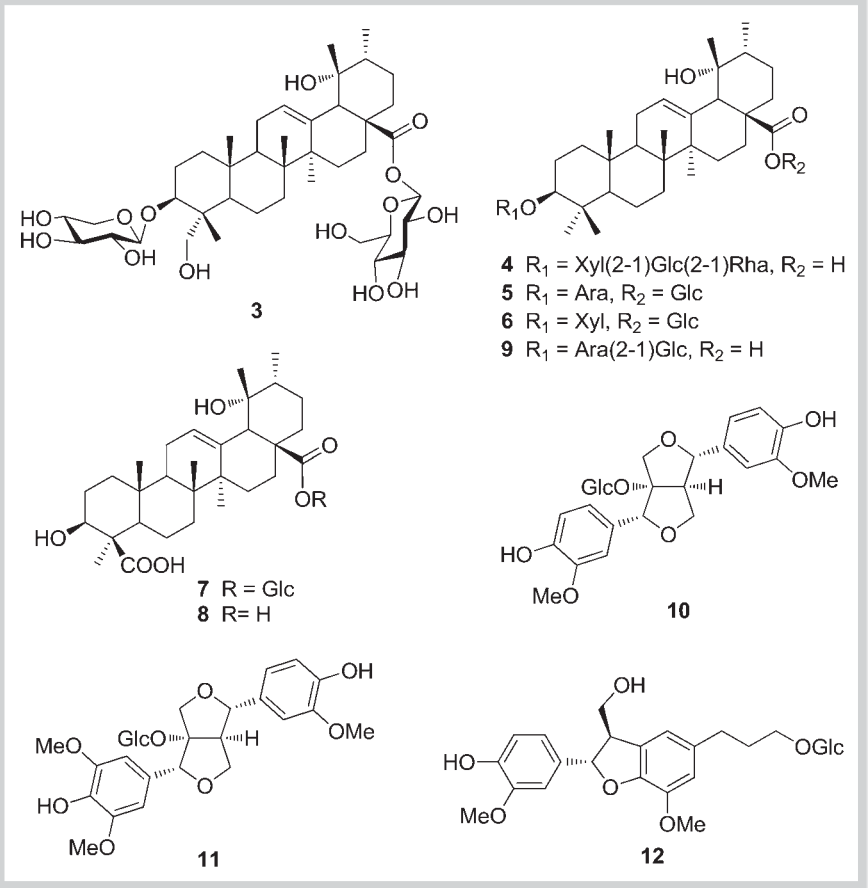

Fig. 2 Chemical structures of known compounds **3–12** from *I. asprella*.

Table 2 Anti-HSV-1 activities of compounds **1–4**, **6–7**, and **9–12**.

Samples	TIC (mM)	MNCC (mM)
1	0.14	> 1.00
2	N	> 1.00
3	N	> 1.00
4	N	> 1.00
6	0.18	> 1.00
7	N	> 1.00
9	N	> 1.00
10	N	> 1.00
11	N	> 1.00
12	N	> 1.00
Aciclovir	0.0043[a]	1.11

[a]: IC_{50} value (concentration required to reduce 50% of cytopathic effect). N: no activity

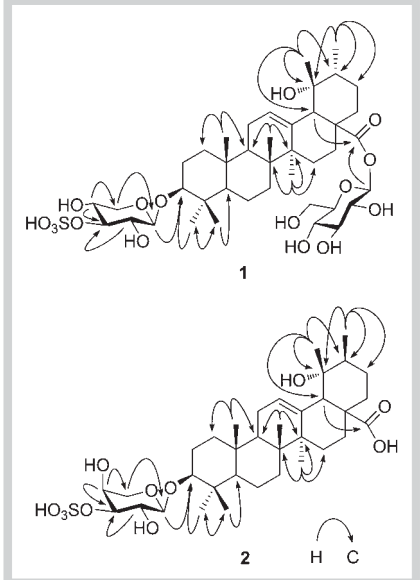

Fig. 3 The key HMBC correlations (H→C) of compounds **1** and **2**.

side I (**9**) [25], (+)-1-hydroxypinoresinol-1-*O*-*β*-D-glucopyranoside (**10**) [26], (+)-fraxinresinol-1-*O*-*β*-D-glucopyranoside (**11**) [27], and (7*S*,8*R*)-dihydrodehydrodiconifery lalcohol-9′-*β*-D-glucopyranoside (**12**) [28] (**Fig. 2**) on the basis of FAB-MS and NMR spectra data and comparison with those reported in the literature.

Asprellanoside A {3-O-β-D-3-O-sulphonylxylopyranosyl-pomolic acid-28-O-β-D- glucopyranosyl ester, 1}: white amorphous powder; $[\alpha]_D^{26}$ −2.15 (*c* 0.792, MeOH); IR (KBr) ν_{max} = 3442, 2927, 2877, 1736, 1640, 1463, 1387, 1258, 1228, 1130, 1074, 998, 882 cm^{-1}; FABMS (neg. ion mode) *m/z* 845 $[M - H]^-$, HRESIMS *m/z* 845.3982 $[M - H]^-$ (calcd. for $C_{41}H_{65}O_{16}S$, 845.3993); ^1H and ^{13}C NMR: **Table 1**.

Asprellanoside B {ilexgenin B 3-O-α-L-3-O-sulphonylarabinopyranoside, 2}: white amorphous powder; $[\alpha]_D^{26}$ −3.42 (*c* 0.785, Pyridine); IR (KBr) ν_{max} 3440, 2938, 2877, 1688, 1640, 1460, 1236, 1141, 1022 cm^{-1}; FABMS (neg. ion mode) *m/z* 683 $[M - H]^-$, HRESIMS: *m/z* 683.3460 $[M - H]^-$ (calcd. for $C_{35}H_{55}O_{11}S$, 683.3465); ^1H and ^{13}C NMR: **Table 1**.

Supporting information

Detailed protocols for extraction and isolation, the cell lines and biochemicals, cytotoxicity assay, anti-HSV-1 assay, and acid hydrolysis of compounds **1** and **2** are available as Supporting Information. In addition, the ^{1}H, ^{13}C NMR, HSQC, HMBC, and ^{1}H-^{1}H COSY spectra of compounds **1** (**Fig. 1S–5S**) and **2** (**Fig. 6S–10S**) are available as Supporting Information.

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Conflict of Interest

There are no conflicts of interest among all authors with respect to this work.

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