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Astersedifolioside A–C, three new oleane-type saponins with antiproliferative activity

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Abstract—A phytochemical analysis of *Aster sedifolius* has led to the isolation of three novel triterpenoid saponins, based on an oleane-type skeleton and named astersedifolioside A (**1**), B (**2**) and C (**3**). On the basis of chemical, and 2D NMR and mass spectrometry data, the structures of the new compounds were elucidated as 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside] (**1**), 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-β-D-xylopyranosyl (1→4)-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside] (**2**) and 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-β-D-xylopyranosyl (1→4)-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside] (**3**). The isolated compounds showed antiproliferative effect in KiMol, a transformed thyroid cell line.

1. Introduction

The genus *Aster s.l.* (fam. *Asteraceae;* tribe *Astereae*) includes more than 300 species, morphologically heterogeneous and geographically widespread, with centres of diversity in North America and Eurasia. Its wide geographical distribution, both in Old and New World, indicates a range of adaptation to various habitats, including harsh conditions. An example is given by sea aster, *A. tripolium*, a halophyte, which tolerates 2–6% NaCl. A large number of *Aster* species are grown as ornamentals for cut flowers, gardens and pot plants. *Aster* species have been also used in traditional Chinese medicine for the treatment of fever, cold, tonsillitis and snake bite and bee sting.[1]

An extremely rich chemical diversity characterizes *Aster* species as well as the other genera in the family. In particular, saponins are widely distributed and show remarkable structural varieties, as well as notable biological activities. In plants, the saponins play a role as preformed chemical barriers in defence mechanisms against insects,[2] and fungi.[3] Several triterpenoid saponins based on an oleane-type aglycon were isolated in some *Aster* spp.[4–7] In particular, two of these compounds, isolated from *A. lingulatus*, showed inhibitory activity on DNA synthesis in tumour leukaemia HL-60 cell line.[5]

The wild germplasm of *Aster*, demonstrated by molecular approaches to carry high genetic diversity,[8] could be a source of chemical diversity, as well. In this frame, a study on the bioactive constituents of *A. sedifolius* has been undertaken and resulted in the isolation of three new triterpenoid saponins, named astersedifolioside A–C (**1**–**3**). These compounds, tested for their antiproliferative effect against a transformed thyroid cell line, were

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able to arrest the cell growth dose dependently. The obtained data showed that astersedifolioside B and C are more efficient than astersedifolioside A.

2. Results and discussion

The butanol soluble part of the MeOH extract of the plant aerial parts of *A. sedifolius* was chromatographed on silica RP-18 by MPLC and then subjected to HPLC purification to afford astersedifolioside A (**1**), B (**2**) and C (**3**, Chart 1).

Astersedifolioside A (**1**) gave in the negative HRFABMS a pseudo-molecular ion peak at *m/z* 1056.5475 $[M-H]^-$, which indicated the molecular formula as $C_{53}H_{85}O_{21}$. The IR spectrum of **1** showed the presence of hydroxyl (3400 cm^{-1}), ester group (1750 cm^{-1}) and glycosidic linkage (1000–1100 cm^{-1}).

The ^1H NMR spectrum of **1** showed signals for seven tertiary methyl groups [δ 0.80, 0.84, 0.89, 0.95 (×2), 1.05 and 1.36], four anomeric sugar protons [δ 4.31 (d, $J=7.5$ Hz), 4.88 (b s), 5.19 (b s), 5.50 (d, $J=3.7$ Hz)] and a series of overlapped signals suggesting an olean-type triterpene glycoside. A further feature of the ^1H NMR spectrum was a signal at δ 5.31 (1H, b m) typical of H-12 of a Δ^{12} oleane skeleton, which was also indicated by the signals at δ 123.5 and 145.0, due to C-12 and C-13 in the ^{13}C NMR spectrum (Table 1).[9] In the same spectrum the presence of a carbonyl carbon at δ 179.7 and the carbon resonances of rings D and E suggested the occurrence of a glycosylated COOH group at C-28 (−3 ppm in comparison to models with free 28-COOH group).[9] A 2D COSY and HOHAHA allowed us to recognize the signal of H-3 at δ 3.40 (dd, $J=4.0$ and 11.5 Hz) that was connected by HSQC experiment with the relative carbon C-3 (δ 89.9). The J values of H-3 clearly indicated its axial position and the chemical shift value of C-3 (+10 ppm) in comparison to models[9] indicated C-3 as glycosylation site.

The ^1H NMR spectrum showed a further signal at δ 4.48 (1H, b m) in the region of proton linked to oxygen-bearing carbons thus suggesting an additional hydroxyl group on the aglycon. This hypothesis was confirmed by 2D HMBC spectrum, which showed cross-peaks between this proton signal with C-14, C-15, C-17, C-18 and C-22, thus unambiguously locating the hydroxyl group at C-16. The 16α-configuration was evident from the small J values of H-16, resonating as a broad multiplet at δ 4.48 in the ^1H NMR spectrum, characteristic of an equatorial proton.

All these data pointed to a 3β,16α-dihydroxyolean-12-en-28-oic acid (skeleton), commonly known as echinocystic acid. Its 18β-series have been recognized by the chemical shift values of C-12, C-13, C-18 and C-29.[10]

On acid hydrolysis, followed by trimethylsilylation and by GLC analysis of the released monosaccharides on a chiral column, compound **1** afforded D-glucose, L-rhamnose and L-arabinose in the molar ratio of 1:2:1.

The presence of four sugar residues were in good agreement with four anomeric proton signals resonating at δ 4.31 (d, $J=7.5$ Hz), 4.88 (b s), 5.19 (b s) and 5.50 (d, $J=3.7$ Hz). Starting from the anomeric proton signals, the proton resonances of each sugar could be assigned

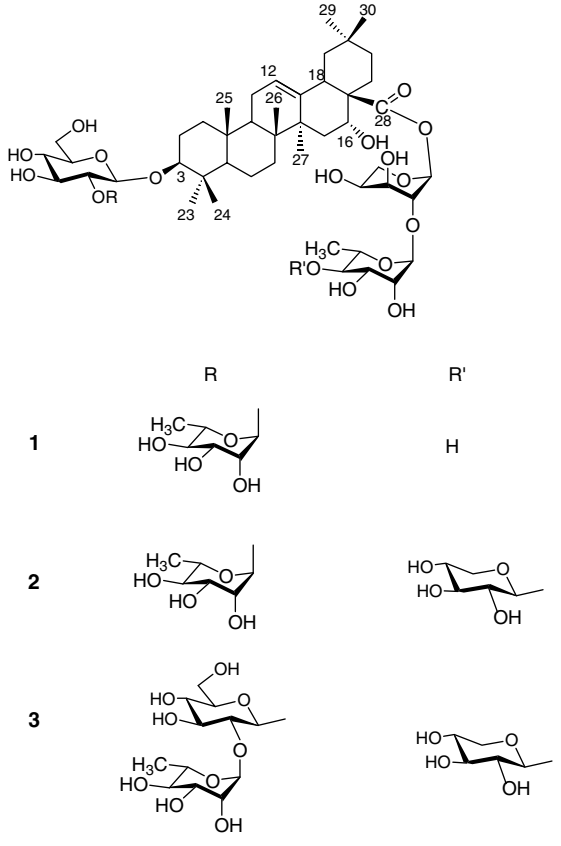

Chart 1. Chemical structures of astersedifoliosides A–C (**1**–**3**).

Table 1. ^{13}C NMR data of the aglycone portion of astersedifolioside A (**1**), B (**2**) and C (**3**)[a]

	1		
Position	δ_C (mult.)		
1	39.7 (CH_2)	16	74.0 (CH)
2	27.0 (CH_2)	17	48.8 (C)
3	89.9 (CH)	18	41.0 (CH)
4	38.6 (C)	19	47.1 (CH_2)
5	55.9 (CH)	20	31.2 (C)
6	18.7 (CH_2)	21	36.3 (CH_2)
7	33.6 (CH_2)	22	32.9 (CH_2)
8	40.5 (C)	23	29.6 (CH_3)
9	47.8 (CH)	24	17.4 (CH_3)
10	37.2 (C)	25	16.6 (CH_3)
11	24.8 (CH_2)	26	17.9 (CH_3)
12	123.5 (CH)	27	27.3 (CH_3)
13	145.0 (C)	28	179.7 (C)
14	42.7 (CH)	29	33.3 (CH_3)
15	36.0 (CH_2)	30	24.6 (CH_3)

Data extracted from A (**1**). Data for B (**2**) and C (**3**) exactly agree with those of A (**1**).

[a] The spectra were measured in CD_3OD.

as reported in Table 2 by 2D COSY and HOHAHA experiments. A further HSQC experiment correlated the proton resonances with the relevant carbons (Table 2). The anomeric configuration of glucopyranosyl unit has been determined as β on the basis of the large value of the coupling constant ($J_{1,2}$ = 7.5 Hz). The anomeric configuration of both rhamnopyranoses has been indicated by the ^{13}C NMR chemical shift values of C-3 and C-5.[11] The arabinose was suggested to be in the α-configuration by the $J_{1,2}$ value of 3 Hz. However, a small value of H-1/H-2 is not diagnostic on its own due to a rapid conformational mobility ($^4C_1 \leftrightarrow {}^1C_4$)[12] and we obtained further information by a ROESY spectrum. Thus, dipolar correlations of H-1 Ara with H-2, H-3 and H-5 Ara were indicative of an α-arabinopyranose in a rapid $^4C_1 \leftrightarrow {}^1C_4$ conformational exchange.

Once the ^1H and ^{13}C resonance assignments of the single monosaccharides have been completed, we determined the interglycosidic linkages by an HMBC experiment (Fig. 1). In particular, long-range correlations were observed between the anomeric proton at δ 4.31 (d, J = 7.5 Hz, Glu) with the carbon at δ 89.9 ppm (C-3 aglycon), and between the anomeric proton at δ 5.5 (d J = 3.7 Hz, Ara) with the carbon at δ 179.7 ppm (C-28 aglycon). A diagnostic HMBC (Fig. 1) of C-2 Glu (δ

Table 2. ^{13}C and ^1H NMR data of the sugar portion of astersedifolioside A (**1**), B (**2**) and C (**3**)[a]

	1		**2**		**3**	
	δ_C (mult.)	δ_H (mult., J, Hz)	δ_C (mult.)	δ_H (mult., J, Hz)	δ_C (mult.)	δ_H (mult., J, Hz)
3-O-Sugars						
Glu I						
1	104.2 (CH)	4.31 (d, 7.5)	104.1 (CH)	4.32 (d, 7.5)	104.4 (CH)	4.31 (d, 7.5)
2	79.0 (CH)	3.43	78.8 (CH)	3.45	79.0 (CH)	3.43
3	78.7 (CH)	3.34	78.7 (CH)	3.31	78.8 (CH)	3.32
4	71.1 (CH)	3.58	71.0 (CH)	3.57	71.2 (CH)	3.57
5	77.5 (CH)	3.22	77.6 (CH)	3.22	77.5 (CH)	3.21
6	62.3 (CH_2)	3.53, 3.58	62.4 (CH_2)	3.52, 3.57	62.3 (CH_2)	3.53, 3.59
Glu II						
1					102.2 (CH)	4.75 (d, 7.5)
2					80.0 (CH)	3.50
3					78.6 (CH)	3.37
4					71.4 (CH)	3.59
5					77.4 (CH)	3.23
6					62.6 (CH_2)	3.54, 3.60
Rha I						
1	102.1 (CH)	5.19 (b s)	102.0 (CH)	5.21 (b s)	102.3 (CH)	5.24 (b s)
2	72.1 (CH)	3.89	72.1 (CH)	3.89	72.2 (CH)	3.90
3	72.1 (CH)	3.65	72.1 (CH)	3.66	72.2(CH)	3.66
4	73.9 (CH)	3.36	73.8 (CH)	3.36	74.0 (CH)	3.37
5	68.7 (CH)	3.98	68.8 (CH)	3.98	69.6 (CH)	3.99
6	17.8 (CH_3)	1.28	17.9 (CH_3)	1.28	17.9 (CH_3)	1.29
26-O-sugars						
Ara						
1	94.0 (CH)	5.50 (d, 3.7)	93.8 (CH)	5.47 (d, 3.7)	93.9 (CH)	5.42 (d, 3.5)
2	75.6 (CH)	3.58	75.5 (CH)	3.57	75.8 (CH)	3.56
3	71.0 (CH)	3.94	70.8 (CH)	3.92	71.1 (CH)	3.94
4	67.1 (CH)	3.32	66.9 (CH)	3.33	66.8 (CH)	3.32
5	63.5 (CH_2)	3.30, 3.60	63.4 (CH_2)	3.30, 3.59	63.5 (CH_2)	3.29, 3.59
Rha II						
1	101.2 (CH)	4.88 (b s)	101.4 (CH)	4.91 (b s)	101.2 (CH)	4.93 (b s)
2	73.0 (CH)	3.88	73.0 (CH)	3.82	71.9 (CH)	3.82
3	73.1 (CH)	3.68	72.4 (CH)	3.93	72.3 (CH)	3.93
4	75.2 (CH)	3.40	80.4 (CH)	3.61	80.5 (CH)	3.61
5	70.4 (CH)	3.60	69.8 (CH)	3.70	69.9 (CH)	3.70
6	18.8 (CH_3)	1.27	17.9 (CH_3)	1.32	17.8 (CH_3)	1.32
Xyl						
1			107.6 (CH)	4.50 (d, 7.4)	107.5 (CH)	4.49 (d, 7.4)
2			74.5 (CH)	3.28	74.5 (CH)	3.28
3			78.3 (CH)	3.55	78.3 (CH)	3.55
4			73.0 (CH)	3.61	73.0 (CH)	3.61
5			69.0 (CH_2)	3.45, 3.92	69.0 (CH_2)	3.45, 3.92

[a] The spectra were measured in CD_3OD.

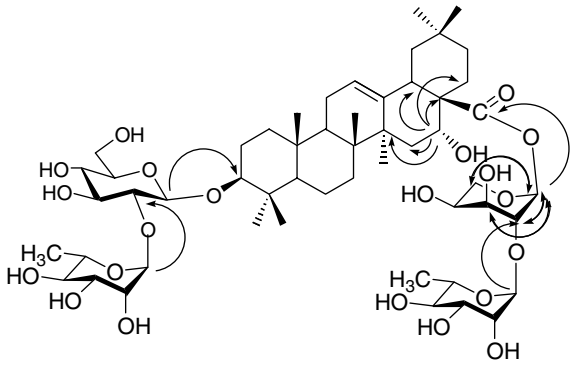

Figure 1. Selected HMBC (H→C) and ROESY (H↔H) correlations exhibited by compound **1**.

79.0) with the low-field H-1 Rha I (δ 5.19) indicated position 2 of the glucose to be involved in the glycosidic linkage with the rhamnose. This sugar residue appeared terminal on the basis both of the ^{13}C NMR chemical shift values[13] and on the absence of further HMBC correlations. With regard to the glycosidic chain at C-28, an HMBC cross-peak of C-2 Ara (δ 75.6) with the anomeric proton of the remaining rhamnose residue (δ 4.88, Rha II) indicated position 2 of the arabinose as a glycosidic site to which the second rhamnose unit should be linked. This evidence thus clarified the disaccharide chain at C–28.

On the basis of these data, astersedifolioside A (**1**, Chart 1) has been established as 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside].

Astersedifolioside B (**2**) had a molecular formula of $C_{58}H_{93}O_{25}$, established by HRFABMS. Its molecular weight, 132 mass units higher than that of **1**, indicated the presence of an additional pentose. The presence of five sugars in **2** was apparent from the five anomeric proton signals at δ_H 4.32 (d, J= 7.5), 4.50 (d, J=7.4), 4.91 (b s), 5.21 (b s), 5.47 (d, J=3.7), which correlated with the corresponding carbon signals at δ_C 104.1, 107.6, 101.4, 102.0, 93.8, respectively, in the HSQC experiment. A comparative analysis of ^1H and ^{13}C NMR, and 2D COSY spectra (Tables 1 and 2 and Materials and methods) of **2** with those of **1,** showed a good coincidence in the chemical shift of both compounds and some slight modifications among the resonances of the rhamnose unit included in the ester glycosidic chain linked at C-28. In particular, signals of C-4 Rha II (80.4) and H-4 Rha II (δ 3.61) were downfield shifted (+5.2 and +0.21 ppm, respectively), accompanied by a slight upfield shift of C-3 Rha II (−0.7 ppm) as compared with those of **1**. These data suggested C-4 Rha II as the glycosylated position to which the additional monosaccharide is linked. This was also confirmed by the ROESY cross-peak of the additional anomeric proton signal resonating at 4.50 with H-4 Rha II (δ 3.61). The same anomeric proton at δ 4.50 constituted the starting point to deduce through 2D COSY and HOHAHA the sequence of the further pentose unit in the pyranose form. Unfortunately, it was impossible to determine all the coupling constants between the protons belonging to this sugar residue because of their overlapping NMR resonances. However, the structure of this sugar moiety was clarified by chemical analysis. Thus **2** was submitted to an acid hydrolysis, followed by trimethylsilylation. GLC analysis on a chiral column of the released monosaccharides afforded D-glucose, L-rhamnose, L-arabinose and D-xylose, in the molar ratio of 1:2:1:1. The β-configuration at C-1 Xyl was assigned on the basis of the vicinal coupling constant of 7.4 Hz, observed in the ^1H NMR spectrum of **2** for the anomeric proton resonating at δ 4.50 (Table 2). Analysis of ROESY and HMBC spectra of **2**, revealed in addition to the correlations showed in Figure 1 for compound **1**, the cross-peaks relative to the additional β-D-xylopyranosyl unit. Thus, a dipolar interaction of H-4 Rha II with H-1 Xyl and an HMBC correlation of this last proton with C-4 Rha II indicated position 4 of the rhamnose II as the linkage site of the additional xylose. Consequently, the structure of **2** was formulated as 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-β-D-xylopyranosyl (1→4)-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside].

Astersedifolioside C (**3**) was isolated as an amorphous solid, with the molecular formula $C_{64}H_{103}O_{30}$, deduced by negative ion HRFABMS. The presence of six sugars in **3** was evident from six anomeric proton signals at δ 4.31, 4.49, 4.75, 4.93, 5.24 and 5.42, associated with the relevant carbon atoms in the ^{13}C NMR (δ 104.4, 107.5, 102.2, 101.2, 102.3, 93.9, respectively), through the HSQC spectrum. Comparison of the molecular formula and ^1H and ^{13}C NMR spectra of **3** (Tables 1 and 2) with parallel data arising from **2**, evidenced almost identical resonances for both compounds with the presence of an additional hexose monosaccharide in **3**. The D-glucopyranoside nature of this monosaccharide has been determined by chemical methods. Thus compound **3**, subjected to hydrolysis (2 N HCl), trimethylsilylation and GLC analysis on a chiral column of the released monosaccharides, afforded D-glucose, L-rhamnose, L-arabinose and D-xylose in the molar ratio 2:2:1:1.

The β-configuration at C-1 Glu II was assigned on the basis of the vicinal coupling constant of 7.5 Hz. The sequences of the oligoglycoside chains were pursued by HMBC. With regard to the C-28 sugar chain, it was found for **3** an identical sequence of **2** on the basis of the following cross-peaks between H-1 Ara (δ 5.42) and C-28 (δ 179.7), H-1 Rha II (δ 4.93) and C-4 Ara (δ 66.8) and H-1 Xyl (δ 4.49) and C-4 Rha II (δ 80.5). Concerning the ring A glycosylation site (**3**, Chart 1), a cross-peak of H-1 Glu I (δ 4.31, d, 7.5) with C-3 of the aglycon (δ 89.9) positioned the first β-glucose residue. Then the observation of a correlation between H-1 Glu II (δ 4.75, d, 7.5) and C-2 Glu I (δ 79.0) linked the second β-glucopyranose at position 2 of the first glucose residue. Finally, the correlation H-1 Rha I (δ 5.24)/C-2 Glu II (δ 80.0) identified position 2 of the second glucose unit as interested in the glycosidic linkage with the terminal rhamnose (Rha I).

Thus, astersedifolioside C (**3**) has been established as 3-O-[α-L-rhamnopyranosyl (1→2)-β-D-glucopyrano-

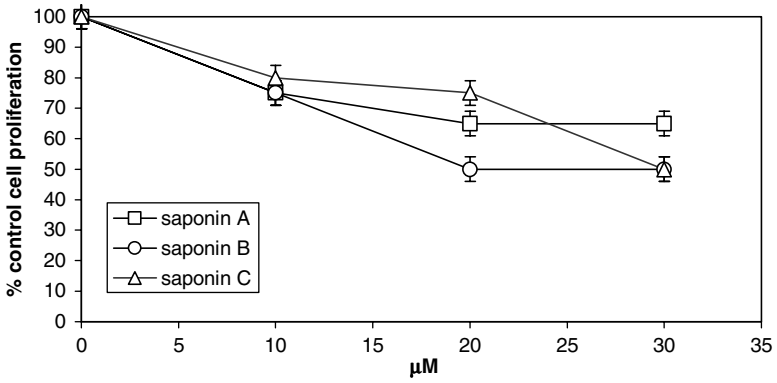

Figure 2. Effect of increasing concentrations (10, 20 and 30 M) of astersedifolioside A, B, C on transformed thyroid cell line, KiMol.

syl(1 → 2)-. β-D-glucopyranosyl] echinocystic acid 28-[O-β-D-xylopyranosyl (1 → 4)-O-α-L-rhamnopyranosyl (1 → 2)-α-L-arabinopyranoside]. Astersedifolioside A–C, tested on transformed thyroid cell line (KiMol), at concentration ranging from 10 to 30 μM, showed an inhibitory effect on cell proliferation dose dependently (Fig. 2). The inhibition was measurable at the concentration as low as 10 μM and became maximal at 30 μM. Addition of these compounds led to the arrest of the cell growth after 24 h of incubation. In particular, astersedifolioside B and C were able to block the cell proliferation more efficiently than astersedifolioside A at high concentration (30 μM). Comparison of the sugar composition of astersedifolioside A and B shows that a further xylose significantly increases the activity (Fig. 2). Similar results have been reported for related compounds from *A. ligulatus*,[5] where a xylose and a rhamnose increase and decrease, respectively, the cytotoxic activity on human leukaemia HL-60 cell line. On the other hand, an additional glucose, as found in astersedifolioside C, does not affect the inhibitory effect on cell proliferation at high concentration (30 μM), but seems to reduce it at intermediate concentration (20 μM).

3. Experimental

3.1. General experimental procedures

Low and high resolution FAB mass spectra (glycerol matrix) were measured on a Prospect Fisons mass spectrometer. Optical rotations were determined on a Perkin Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and 10-cm microcell. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker AMX-500 spectrometer. Chemical shifts were referred to the residual solvent signal (CD_3OD: δ_H 3.34, δ_C 49.0). The multiplicities of ^{13}C NMR resonances were determined by DEPT experiments. ^{1}H connectivities were determined by using COSY and HOHAHA experiments; the 2D HOHAHA experiments were performed in the phase-sensitive mode (TPPI) using the MLEV-17 (mixing time 125 ms) sequence for mixing.[14] One-bond heteronuclear ^{1}H–^{13}C connectivities were determined with 2D HSQC[15] pulse sequence with an interpulse delay set for $^{1}J_{CH}$ of 130 Hz. Two and three bond heteronuclear ^{1}H–^{13}C connectivities were determined with 2D HMBC experiments,[16] optimized for $^{2-3}J_{CH}$ of 8 Hz. Nuclear Overhauser effect (NOE) measurements were performed by 2D ROESY experiments.[17] Medium pressure liquid chromatography (MPLC) was performed on a Büchi 861 apparatus using LiChroprep RP-18 (40–63 μm) columns. HPLC in isocratic mode was performed on a Varian apparatus equipped with a RI-3 refractive index detector.

3.2. Plant material

Aster sedifolius, accession no 151, coming from Botanical Garden of Stuttgart (Germany) was used in this work. Seeds were germinated in vitro on January 1998 in sterile water after a sterilization with 80% (v/v) ethanol for 6 min, 3% sodium hypochlorite solution for 20 min and final rinses with sterile water. Potted plants, which are perennial, were grown in greenhouse under natural daylight. Aerial parts were collected on October 2001 from three years-old plants pollarded in June after flowering.

3.3. Extraction and isolation

Fresh aerial parts (172 g, dry weight) were air dried under controlled temperature (22 °C) and without exposure of light. They were chopped and then exhaustively extracted at room temperature with the following solvents in the order: hexane, $CHCl_3$, $CHCl_3$/MeOH (9:1) and MeOH. Each solvent extraction takes two days and was repeated three times using 2 L of solvent. The MeOH extract (12 g) was partitioned between BuOH and water and the organic layer was concentrated in vacuo to afford a crude extract (7.3 g), which was chromatographed by MPLC on RP-18 column using a linear gradient solvent system from H_2O to MeOH.

Fractions eluted with H_2O/MeOH 1:9 (268.1 mg) and MeOH (1.702 g) were added and rechromatographed on RP-18 column using a linear gradient system from H_2O/MeOH (6:4) to MeOH. Fraction (137.9 mg), eluted with H_2O/MeOH (1:9), was further purified first on semi-preparative C-18 HPLC column with a mobile phase of H_2O/MeOH (3:7), and after on analytic C-18 HPLC column with a mobile phase of H_2O/MeOH (1:1), yielding

the three new compounds: astersedifolioside A (9.8 mg), astersedifolioside B (6.6 mg) and astersedifolioside C (7.4 mg).

Astersedifolioside A (**1**). 3-O-[α-L-Rhamnopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside]: $[\alpha]_D^{25}$ −19.1 (*c* 0.1 MeOH); HRFABMS (negative ion): found *m/z* 1056.5475 $[M-H]^-$; calculated for $C_{53}H_{85}O_{21}$ *m/z* 1057.5559; ^1H NMR data: δ 0.80 (3H, s, Me-26), 0.84 (3H, s, Me-25), 0.89 (3H, s, Me-29), 0.95 (6H, s, Me-23 and Me-30), 1.05 (3H, s, Me-24), 1.36 (3H, s, Me-27), 2.98 (1H, dd, J=4.5, 13.6 Hz, H-18), 3.40 (1H, dd, J=4.0, 11.5 Hz, H-3), 4.48 (1H, b m, H-16), 5.31 (1H, b m, H-12), for sugars see Table 2; ^{13}C NMR data: Tables 1 and 2.

Astersedifolioside B (**2**). 3-O-[α-L-Rhamnopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-β-D-xylopyranosyl (1→4)-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside]: $[\alpha]_D^{25}$ −21.2 (*c* 0.1 MeOH); HRFABMS (negative ion): found *m/z* 1188.5909 $[M-H]^-$; calculated for $C_{58}H_{93}O_{25}$ *m/z* 1189.5979; ^1H NMR data: δ 0.80 (3H, s, Me-26), 0.84 (3H, s, Me-25), 0.88 (3H, s, Me-29), 0.95 (3H, s, Me-23), 0.96 (3H, s, Me-30), 1.05 (3H, s, Me-24), 1.36 (3H, s, Me-27), 2.96 (1H, dd, J=4.5, 13.6 Hz, H-18), 3.40 (1H, dd, J=4.0, 11.5 Hz, H-3), 4.48 (1H, b m, H-16), 5.31 (1H, b m, H-12), for sugars see Table 2; ^{13}C NMR data: Tables 1 and 2.

Astersedifolioside C (**3**). 3-O-[α-L-Rhamnopyranosyl (1→2)-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl] echinocystic acid 28-[O-β-D-xylopyranosyl (1→4)-O-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside]: $[\alpha]_D^{25}$ −19.3 (*c* 0.1 MeOH); HRFABMS (negative ion): found *m/z* 1350.6420 $[M-H]^-$; calculated for $C_{64}H_{103}O_{30}$ *m/z* 1351.6504; ^1H NMR data: δ 0.80 (3H, s, Me-26), 0.84 (3H, s, Me-25), 0.88 (3H, s, Me-29), 0.95 (3H, s, Me-23), 0.96 (3H, s, Me-30), 1.05 (3H, s, Me-24), 1.36 (3H, s, Me-27), 2.97 (1H, dd, J=4.5, 13.6 Hz, H-18), 3.40 (1H, dd, J=4.0, 11.5 Hz, H-3), 4.48 (1H, b m, H-16), 5.31 (1H, b m, H-12), for sugars see Table 2; ^{13}C NMR data: Tables 1 and 2.

3.4. Determination of the absolute configuration of sugars

A solution of each isolated compound (1 mg) in 1 N HCl (0.25 mL) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated by blowing with N_2. The residue was dissolved in 1-(trimethylsilyl) imidazole (Trisil-Z) and pyridine (0.1 mL) and the solution was stirred at 60 °C for 5 min. After drying the solution with a stream of N_2, the residue was separated by water and CH_2Cl_2 (1 mL, v:v = 1:1). The CH_2Cl_2 layer was analyzed by GC using a L-Chirasil-Val column (0.32 mm×25 m). Temperatures of injector and detector were 200 °C for both. A temperature gradient system was used for the oven; the initial temperature was maintained at 100 °C for 1 min and then raised to 180 °C at the rate of 5 °C/min. Peaks of the idrolysate of **1** were detected at 8.90 (L-arabinose), 12.89 (L-rhamnose) and 14.66 (D-glucose) in the ratio of 1:2:1. Peaks of the idrolysate of **2** were detected at 8.90 (L-arabinose), 10.98 (D-xylose), 12.89 (L-rhamnose) and 14.66 (D-glucose) in the ratio of 1:1:2:1. Peaks of the idrolysate of **3** were detected at 8.90 (L-arabinose), 10.98 (D-xylose), 12.89 (L-rhamnose) and 14.66 (D-glucose) in the ratio of 1:1:2:2. Retention times for authentic samples after being treated simultaneously with Trisil-Z were 8.82 (D-arabinose), 8.90 (L-arabinose), 10.98 (D-xylose), 11.05 (L-xylose), 12.78 (D-rhamnose), 12.89 (L-rhamnose), 14.66 (D-glucose) and 14.73 (L-glucose). Coinjection of hydrolysate of **1** with standards D-arabinose, L-rhamnose and D-glucose gave single peaks. Coinjection of hydrolysate of **2** and **3** with standards D-arabinose, D-xylose, L-rhamnose and D-glucose gave single peaks.

3.5. Biological assay

Cell proliferation assay was carried out in triplicate in a 24-well dishes containing subconfluent cells at a density of 20,000 cells/well. Test substances were introduced after the cells had attached.[18] Saponins were added at several concentrations for 24 h, after which cells were counted. Means were compared using the impaired Student's *t*-test and $p<0.05$ as the threshold for statistical significance.

3.6. Cells and culture

KiMol cells were derived from FRTL-5 cells on infection and transformation with a wild-type strain of KiMSV-MolMuLV. The cells were grown in Coon's modified Ham's F-12 medium supplemented with 5% calf serum.[19]

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