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# Triterpene saponins from *Silphium radula*

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**Abstract**

Nine triterpene saponins (**1**–**9**) were isolated from leaves and stems of *Silphium radula* Nutt. (Asteraceae). Their structures were determined by extensive 1D ($^{13}C$, $^{1}H$, DEPT, TOCSY) and 2D NMR (NOESY, HSQC, HMBC) and ESI-MS studies. The compounds were identified as 3β,6β,16β-trihydroxyolean-12-en-23-al-3-*O*-β-glucopyranosyl-16-*O*-β-glucopyranoside (**1**), urs-12-ene-3β,6β,16β-triol-3-*O*-β-galactopyranosyl-(1 → 2)-β-glucopyranoside (**2**), 3β,6β,16β-trihydroxyolean-12-en-23-oic acid-3-*O*-β-glucopyranosyl-16-*O*-β-glucopyranoside (**3**), urs-12-ene-3β,6β,16β,21β-tetraol-3-*O*-β-glucopyranoside (**4**), olean-12-ene-3β,6β,16β,21β-tetraol-3-*O*-β-glucopyranoside (**5**), olean-12-ene-3β,6β,16β,21β,23-pentaol-3-*O*-β-glucopyranosyl-16-*O*-β-glucopyranoside (**6**), olean-12-ene-3β,6β,16β-triol-3-*O*-β-glucopyranosyl-16-*O*-α-arabinopyranosyl-(1 → 2)-β-glucopyranoside (**7**), olean-12-ene-3β,6β,16β,23-tetraol-3-*O*-β-glucopyranosyl-16-*O*-α-arabinopyranosyl-(1 → 2)-β-glucopyranoside (**8**), 3β,6β,16β,21β-tetrahydroxyolean-12-en-23-al-3-*O*-β-glucopyranoside (**9**). The presence of a 6β-hydroxyl function was not common in the oleanene or ursene class and the aglycones of these compounds were not found previously in the literature. Moreover, the cytotoxic activities of the isolated compounds were tested against human breast cancer cell line MDA-MB-231. Results showed that compound **2** decreased cell proliferation in a statistically significant manner at 25 μg/ml.


*Keywords:* *Silphium radula*; Asteraceae; Triterpene saponins; Anti-proliferative activity

## 1. Introduction

*Silphium* L. (Asteraceae) is a small genus of 11 species common to the prairies and woodlands of central North America (Clevinger and Panero, 2000). Native American tribes, including the Cherokee Indians, considered *Silphium* a medicinal herb and prepared extracts of the leaves and flowers for respiratory and kidney ailments and also applied the roots of *Silphium* as a poultice for bleeding wounds, backaches and hemorrhaging (Hamel and Chiltoskey, 1975). The potential of *Silphium* as a natural remedy is also well known in parts of Europe, where it is cultivated as a garden ornamental and herbal medicine. Several chemical investigations of European-grown *Silphium* species including *S. perfoliatum*, *S. integrifolium* and *S. trifoliatum* (now considered a subspecies of *S. asteriscus*, Clevinger and Panero, 2000) have appeared in the literature in recent years (Kowalski, 2004; Kowalski et al., 2005). For example, chemical analysis of *Silphium perfoliatium* resulted in the isolation of flavonoids (El-Sayed et al., 2002), phenolic acids (Kowalski and Wolski, 2003), essential oils (Kowalski and Wolski, 2005), sesquiterpenes (Bohlmann and Jakupovic, 1979, 1980), diterpenes (Pcolinski et al., 1994), and oleanolic-type triterpene saponins (Davidyants et al., 1984a,b,c; Davidyants et al., 1985; Davidyants et al., 1986). The saponin mixture showed blood cholesterol-lowering activity as well as fungicidal properties (Syrov et al.,

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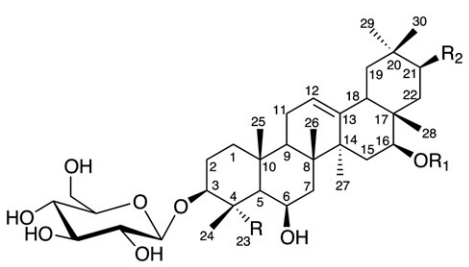
1992; Davidyants et al., 1997). In addition, there has been an increasing interest in European-cultivated species of *Silphium* as commercial sources for oleanolic and ursolic-type triterpenes (Kowalski, 2007).

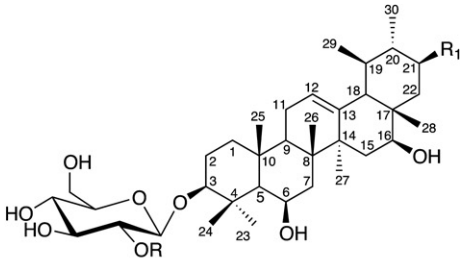
In an effort to further characterize triterpene saponins of chemosystematic and medicinal significance from *Silphium*, our research group has undertaken chemical investigations of all *Silphium* species harvested from their native habitats across North America. We report here the isolation and structural elucidation of nine new pentacyclic oleanene and ursene-type triterpene saponins (**1**–**9**) from leaf and stem methanolic extracts of *Silphium radula* Nutt., a species found in prairie remnants and rocky open forests of the southeastern United States (Rickett, 1967). To our knowledge, this is the first chemical report for this species.

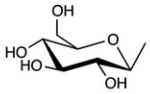
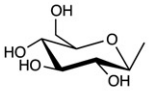
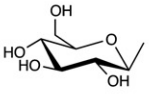
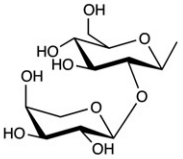
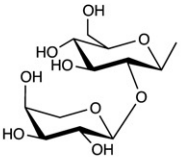
## 2. Results and discussion

The concentrated methanolic extract of a mixture of *S. radula* leaves and stems was subjected to solid phase extraction on reversed phase C-18, CC on silica gel and MPLC on reversed phase C-18, affording nine new saponins (**1**–**9**). Their structures were established by 1D and 2D NMR spectroscopy and ESI-MS analyses.

The HR-ESI-MS (negative-ion mode) of compound **1** exhibited a pseudomolecular ion peak at *m*/*z* 795.4531 $[M-H]^-$, ascribable to a molecular formula of $C_{42}H_{68}O_{14}$. The ESI-MS experiment gave (negative-ion mode) a quasi-molecular ion peak at *m*/*z* 795 $[M-H]^-$ indicating a molecular weight of 796 for compound **1**. Further fragment ion peaks in the ESI-MS-MS spectrum were observed





| | R | $R_1$ | $R_2$ |
|---|---|---|---|
| **1** | CHO |  | H |
| **3** | COOH |  | H |
| **5** | $CH_3$ | H | OH |
| **6** | $CH_2OH$ |  | OH |
| **7** | $CH_3$ |  | H |
| **8** | $CH_2OH$ |  | H |
| **9** | CHO | H | OH |

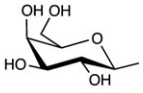
| | R | $R_1$ |
|---|---|---|
| **2** |  | H |
| **4** | H | OH |

Table 1
$^{13}C$ and $^{1}H$ NMR spectroscopic data of compounds **1**–**5** (**1**, **2**, **4**, **5** in $C_5D_5N$;**3** in $CD_3OD$)

| Position | **1** | | **2** | | **3** | | **4** | | **5** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
| 1 | 40.5 | 1.54, 1.03, *m* | 41.4 | 1.53, 0.92, *m* | 42.0 | 1.63, 1.07, *m* | 41.4 | 1.57, 0.97, *m* | 41.2 | 1.57, 1.04, *m* |
| 2 | 25.6 | 2.30, 2.03, *m* | 27.0 | 2.30, 2.06, *m* | 26.7 | 1.93, 1.78, *m* | 27.0 | 2.37, 2.10, *m* | 26.9 | 2.36, 2.10, *m* |
| 3 | 82.3 | 4.22, *dd* (11.0, 4.5) | 89.4 | 3.38, *dd* (11.0, 4.5) | 87.1 | 4.00, *dd* (11.0, 4.5) | 89.3 | 3.47, *dd* (11.0, 4.5) | 89.2 | 3.45, *dd* (11.0, 4.5) |
| 4 | 56.4 | | 40.7 | | 55.3 | | 40.7 | | 40.7 | |
| 5 | 49.3 | 1.50, *br s* | 56.3 | 0.85, *br s* | 53.6 | 1.52, *br s* | 56.3 | 0.94, br s | 56.3 | 0.95, *br s* |
| 6 | 69.4 | 4.13, *br s* | 67.1 | 4.79, *br s* | 71.4 | 4.10, *br s* | 67.3 | 4.81, br s | 67.3 | 4.81, *br s* |
| 7 | 40.7 | 1.73, 1.66, *m* | 41.4 | 1.83, 1.53, *m* | 41.3 | 1.76, 1.53, *m* | 41.4 | 1.90, 1.87, *m* | 41.2 | 1.88, 1.87, *m* |
| 8 | 40.2 | | 39.9 | | 40.9 | | 39.9 | | 40.6 | |
| 9 | 47.6 | 1.80, *m* | 47.9 | 1.66, *m* | 48.9 | 1.65, *m* | 47.9 | 1.72, *m* | 47.8 | 1.80, *m* |
| 10 | 35.9 | | 36.5 | | 37.0 | | 36.6 | | 36.7 | |
| 11 | 23.8 | 2.11, *dd* (12.0, 3.5)<br>1.97, *dd* (12.0, 3.5) | 23.8 | 2.17, *dd* (12.0, 3.5)<br>2.02, *dd* (12.0, 3.5) | 24.7 | 2.06, *dd* (12.0, 3.5)<br>1.94, *dd* (12.0, 3.5) | 23.9 | 2.18, *dd* (12.0, 3.5)<br>2.03, *dd* (12.0, 3.5) | 24.0 | 2.20, *dd* (12.0, 3.5)<br>2.06, *dd* (12.0, 3.5) |
| 12 | 122.6 | 5.38, *t* (3.5) | 125.5 | 5.38, *t* (3.5) | 123.4 | 5.28, t (3.5) | 126.0 | 5.42, *t* (3.5) | 123.0 | 5.49, *t* (3.5) |
| 13 | 143.5 | | 138.5 | | 144.4 | | 138.6 | | 143.1 | |
| 14 | 44.5 | | 44.7 | | 45.3 | | 44.8 | | 44.5 | |
| 15 | 34.8 | 2.20, *dd* (12.0, 11.5)<br>2.03, *dd* (12.0, 4.5) | 36.5 | 2.24, *dd* (13.0, 11.5)<br>1.66, *dd* (13.0, 4.5) | 35.4 | 1.83, *dd* (12.0, 11.5)<br>1.57, *dd* (12.0, 4.5) | 37.1 | 2.29, 1.69, *m* | 36.6 | 2.25, *dd* (12.0, 11.5)<br>1.69, *dd* (12.0, 4.5) |
| 16 | 76.4 | 4.58, *dd* (11.5, 4.5) | 65.7 | 4.55, *dd* (11.5, 4.5) | 77.6 | 4.15, *dd* (11.5, 4.5) | 67.0 | 4.56, *dd* (11.5, 4.5) | 65.8 | 4.65, *dd* (11.5, 4.5) |
| 17 | 38.3 | | 39.3 | | 38.9 | | 40.8 | | 39.7 | |
| 18 | 49.9 | 2.31, *m* | 61.2 | 1.67, *d* (11.5) | 51.0 | 2.15, *m* | 61.0 | 1.83, *d* (11.5) | 49.3 | 2.51. m |
| 19 | 47.2 | 1.97, 1.20, *m* | 40.0 | 1.56, *m* | 48.0 | 1.77, 1.05, *m* | 39.0 | 1.82, m | 47.8 | 2.16, 1.42, *m* |
| 20 | 31.2 | | 39.9 | 0.98, *m* | 31.9 | | 48.2 | 1.29, *m* | 36.9 | |
| 21 | 35.2 | 1.80, 1.27, *m* | 31.3 | 1.54, 1.46, *m* | 35.8 | 1.43, 1.14, *m* | 70.5 | 3.97, *dd* (11.0, 4.0) | 72.7 | 4.12, *dd* (11.0, 4.0) |
| 22 | 30.7 | 2.80, 1.33, *m* | 36.0 | 2.60, 1.17, *m* | 31.2 | 2.17, 1.13, *m* | 45.9 | 3.21, *dd* (11.0, 4.0)<br>1.50, *dd* (11.0, 11.0) | 40.2 | 2.98, *dd* (11.0, 4.0)<br>1.63, *dd* (11.0, 11.0) |
| 23 | 207.6 | 9.87, *s* | 28.1 | 1.54, *s* | 183.1 | – | 28.2 | 1.54, *s* | 28.1 | 1.54, *s* |
| 24 | 11.9 | 2.00, *s* | 18.6 | 1.84, *s* | 13.9 | 1.52, *s* | 18.7 | 1.73, *s* | 18.7 | 1.74, *s* |
| 25 | 18.4 | 1.56, *s* | 17.4 | 1.62, *s* | 17.7 | 1.36, *s* | 17.4 | 1.62, *s* | 17.2 | 1.62, *s* |
| 26 | 17.3 | 1.51, *s* | 18.8 | 1.66, *s* | 18.8 | 1.27, *s* | 18.8 | 1.65, *s* | 18.6 | 1.64, *s* |
| 27 | 27.4 | 1.44, *s* | 25.0 | 1.33, *s* | 27.7 | 1.21, *s* | 25.0 | 1.35, *s* | 27.4 | 1.44, *s* |
| 28 | 23.2 | 1.15, *s* | 23.0 | 1.15, *s* | 23.3 | 0.87, *s* | 22.9 | 1.22, *s* | 22.6 | 1.23, *s* |
| 29 | 33.4 | 0.95, *s* | 18.0 | 0.99, *d* (6.5) | 33.8 | 0.91, *s* | 18.2 | 1.08, *d* (6.5) | 30.1 | 1.29, *s* |
| 30 | 24.2 | 0.98, *s* | 21.7 | 0.98, *d* (6.5) | 24.4 | 0.93, *s* | 16.8 | 1.41, *d* (6.5) | 18.0 | 1.26, *s* |
| | Glc at C-3 | | Glc at C-3 | | Glc at C-3 | | Glc at C-3 | | Glc at C-3 | |
| 1′ | 104.9 | 4.89, *d* (7.5) | 105.0 | 4.98, *d* (7.5) | 105.3 | 4.30, *d* (7.5) | 107.0 | 5.01, *d* (7.5) | 106.9 | 5.00, *d* (7.5) |
| 2′ | 75.4 | 3.95, *dd* (9.0, 7.5) | 84.6 | 4.24, *dd* (9.0, 7.5) | 75.5 | 3.14, *dd* (9.0, 7.5) | 75.9 | 4.10, *dd* (9.0, 7.5) | 75.9 | 4.10, *dd* (9.0, 7.5) |
| 3′ | 78.6 | 4.19, *dd* (9.0, 9.0) | 78.4 | 4.32, *dd* (9.0, 9.0) | 77.6 | 3.30, *dd* (9.0, 9.0) | 78.8 | 4.29, *dd* (9.0, 9.0) | 78.8 | 4.28, *dd* (9.0, 9.0) |
| 4′ | 71.6 | 4.18, *dd* (9.0, 9.0) | 71.6 | 4.19, *dd* (9.0, 9.0) | 71.6 | 3.30, *dd* (9.0, 9.0) | 72.0 | 4.26, *dd* (9.0, 9.0) | 71.9 | 4.27, *dd* (9.0, 9.0) |
| 5′ | 78.5 | 3.96, *m* | 78.0 | 3.95, *m* | 78.0 | 3.27, *m* | 78.4 | 4.06, *m* | 78.3 | 4.05, m |
| 6′ | 62.9 | 4.40, *dd* (12.0, 5.0)<br>4.58, *dd* (12.0, 2.5) | 62.9 | 4.38, *dd* (12.0, 5.0)<br>4.58, *dd* (12.0, 2.5) | 62.8 | 3.67, *dd* (12.0, 5.0)<br>3.82, *dd* (12.0, 2.5) | 63.2 | 4.47, *dd* (12.0, 5.0)<br>4.64, *dd* (12.0, 2.5) | 63.1 | 4.45, *dd* (12.0, 5.0)<br>4.3, *dd* (12.0, 2.5) |

(*continued on next page*)

Table 1 (*continued*)

| Position | **1** | | **2** | | **3** | | **4** | | **5** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| | Glc at C-16 | | Gal at C-2′glc | | Glc at C-16 | | | | | |
| 1″ | 106.3 | 5.06, *d* (7.5) | 107.3 | 5.25, *d* (7.5) | 106.1 | 4.30, *d* (7.5) | | | | |
| 2″ | 75.8 | 4.03, *dd* (9.0, 7.5) | 74.8 | 4.61, *dd* (8.6, 7.5) | 75.7 | 3.16, *dd* (9.0, 7.5) | | | | |
| 3″ | 78.8 | 4.26, *dd* (9.0, 9.0) | 75.0 | 4.18, *dd* (8.6, 4.0) | 77.9 | 3.30, *dd* (9.0, 9.0) | | | | |
| 4″ | 71.9 | 4.25, *dd* (9.0, 9.0) | 69.6 | 4.70, *dd* (4.0, 2.0) | 71.8 | 3.30, *dd* (9.0, 9.0) | | | | |
| 5″ | 78.1 | 3.99, *m* | 76.9 | 4.07, *m* | 78.3 | 3.34, *m* | | | | |
| 6″ | 63.0 | 4.36, *dd* (12.0, 5.0) | 61.4 | 4.41, *dd* (12.0, 4.5) | 62.9 | 3.68, *dd* (12.0, 5.0) | | | | |
| | | 4.48, *dd* (12.0, 2.5) | | 4.58, *dd* (12.0, 2.0) | | 3.82, *dd* (12.0, 2.5) | | | | |

at *m*/*z* 633 $[M-H-162]^-$ and *m*/*z* 472 $[M-H-162-162]^-$ corresponding to the successive loss of two hexosyl moieties. This result suggested that saponin **1** contained two sugar units. The $^{13}C$ NMR spectrum of compound **1** showed 42 signals, of which 30 were assigned to a triterpenoid moiety and 12 to the saccharide portion. The $^1H$ NMR spectrum of the aglycone moiety of **1** showed signals for seven tertiary methyl groups ($\delta$ 0.95, 0.98, 1.15, 1.44, 1.51, 1.56, 2.00), along with the resonances at $\delta$ 5.38 (1H, *t*, $J = 3.7$ Hz) and 9.87 (1H, *s*) ascribable, respectively, to olefinic and aldehydic protons. Further features were signals at $\delta$ 4.13 (1H, *br s*), 4.22 (1H, *dd*, $J = 11.0$, 4.5 Hz), and 4.58 (1H, *dd*, $J = 11.5$, 4.5 Hz), indicative of secondary alcoholic functions (Table 1).

In the $^{13}C$ NMR spectrum, the signals at $\delta$ 122.6 and 143.5 ascribable to C-12 and C-13 suggested a $\Delta^{12}$ oleanene skeleton (Mahato and Kundu, 1994). Full assignments of the proton and carbon resonances of the aglycone (Table 1) were secured by analyses of the $^1H$–$^1H$ DQF-COSY and HSQC spectra. The aldehyde function was located at C-23 on the basis of the downfield shift exhibited by C-4 ($\delta$ 56.4) and the highfield shifts exhibited, respectively, by C-3 ($\delta$ 82.3), C-5 ($\delta$ 49.3), and C-24 ($\delta$ 11.9) in comparison with the same carbon resonances in an oleanene skeleton bearing a Me-23 (Mahato and Kundu, 1994). Also, the chemical shift of Me-24 in the $^1H$ NMR spectrum ($\delta_H$ 2.00) was diagnostic for a 23-CHO (De Tommasi et al., 1998). The HMBC spectrum of **1** confirmed the position of the aldehyde function showing significant cross-peaks, due to $^2J_{C-H}$ and $^3J_{C-H}$ correlations between H-23 ($\delta$ 9.87) and C-3 ($\delta$ 82.3), C-4 ($\delta$ 56.4), and C-24 ($\delta$ 11.9). The signal at $\delta$ 4.22 (*dd*, $J = 11.0$, 4.5 Hz) attributed to H-3ax suggested the presence of a β-OH group at C-3. The location of a hydroxyl group at C-6 was determined by the correlation between H-6 ($\delta$ 4.13, *br s*) and C-5 ($\delta$ 56.4). H-5 α and H-6 resonated as broad singlets at $\delta$ 1.50 and 4.13, respectively, indicating an axial-equatorial relationship. Thus, the β-orientation of the C-6 hydroxyl group was defined (Wang et al., 2006a,b). Furthermore, a NOESY correlation between the signal at $\delta$ 4.13 (H-6) and the resonances 4.22 (H-3) and 9.87 (Me-23) confirmed the α-orientation of H-3 and H-6. The third secondary alcoholic function was located at C-16 on the basis of the carbon resonances of ring D and of the HMBC correlation between the signal at $\delta$ 1.15 (Me-28) and the carbon resonance at $\delta$ 76.4 (C-16). The coupling constants of H-16 ($\delta$ 4.58, *dd*, $J = 11.5$, 4.5 Hz) suggested the β-orientation of the C-16 hydroxyl group (Ukiya et al., 2001), which is in good agreement with the nOe effect between H-16 ($\delta$ 4.58) and Me-27 ($\delta$ 1.44) observed in the NOESY spectrum.

Thus, the aglycone of **1** was identified as 3β,6β,16β-trihydroxyolean-12-en-23-al. Glycosidation of the alcohol groups functions at C-3 and C-16 were indicated by the downfield shift (~10 ppm) observed for these carbon resonances in **1**, if compared to the corresponding signals in non-glycosidated model compounds (Mahato and Kundu, 1994).

Table 2
$^{13}C$ and $^{1}H$ NMR spectroscopic data of compounds **6**–**9** (**6**, **7** in $CD_3OD$; **8**, **9** in $C_5D_5N$)

| Position | **6** | | **7** | | **8** | | **9** | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) | $\delta_C$ | $\delta_H$ ($J$ in Hz) |
| 1 | 41.8 | 1.57, 0.96, *m* | 41.5 | 1.75, 1.57, *m* | 40.7 | 1.80, 1.97, m | 40.6 | 1.58, 1.05, *m* |
| 2 | 26.4 | 1.94, 1.83, *m* | 27.2 | 1.84, 1.78, *m* | 25.7 | 2.35, 2.16, *m* | 25.5 | 2.33, 2.05, *m* |
| 3 | 83.3 | 3.59, *dd* (11.0, 4.5) | 90.5 | 3.13, *dd* (11.0, 4.5) | 81.8 | 4.29, *dd* (11.0, 4.5) | 82.5 | 4.24, *dd* (11.0, 4.5) |
| 4 | 44.8 | | 40.3 | – | 44.2 | | 56.3 | |
| 5 | 48.2 | 1.23, *br s* | 57.3 | 0.76, *br s* | 47.9 | 1.83, *br s* | 49.3 | 1.59, *br s* |
| 6 | 68.4 | 4.45, *br s* | 68.5 | 4.51, *br s* | 66.7 | 4.99, *br s* | 69.6 | 4.27, *br s* |
| 7 | 41.0 | 1.83, 1.54, *m* | 42.1 | 1.57, 0.97, *m* | 40.4 | 1.59, 1.09, *m* | 40.4 | 1.83, 1.70, *m* |
| 8 | 41.2 | | 41.0 | – | 39.4 | | 40.5 | |
| 9 | 47.8 | 1.63, *m* | 49.0 | 1.58, *m* | 47.4 | 1.74, *m* | 47.6 | 1.84, *m* |
| 10 | 37.0 | | 37.2 | – | 36.1 | | 35.8 | |
| 11 | 24.6 | 2.04, *dd* (12.0, 3.5)<br>1.93, *dd* (12.0, 3.5) | 24.6 | 2.05, *dd* (12.0, 3.5)<br>1.89, *dd* (12.0, 3.5) | 23.6 | 2.17, *dd* (12.0, 3.5)<br>2.01, *dd* (12.0, 3.5) | 23.9 | 2.15, *dd* (12.0, 3.5)<br>2.03, *dd* (12.0, 3.5) |
| 12 | 124.2 | 5.29, *t* (3.5) | 123.4 | 5.25, *t* (3.5) | 122.3 | 5.38, *t* (3.5) | 123.8 | 5.47 t (3.5) |
| 13 | 143.2 | | 144.3 | – | 143.2 | | 143.1 | |
| 14 | 45.3 | | 45.2 | – | 44.2 | | 44.5 | |
| 15 | 35.2 | 1.83, *dd* (12.0, 11.5)<br>1.56 *dd* (12.0, 4.5) | 35.5 | 1.84, *dd* (12.0, 11.5)<br>1.57, *dd* (12.0, 4.5) | 34.7 | 2.23, *dd* (12.0, 11.5)<br>1.96, *dd* (12.0, 4.5) | 36.3 | 2.20, *dd* (12.0, 11.5)<br>1.62, *dd* (12.0, 4.5) |
| 16 | 78.0 | 4.10, *dd* (12.0, 4.0) | 77.2 | 4.16, *dd* (11.5, 4.5) | 75.9 | 4.54, *dd* (11.5, 4.5) | 65.7 | 4.61, *dd* (11.5, 4.5) |
| 17 | 40.3 | | 38.9 | – | 37.9 | | 40.0 | |
| 18 | 50.2 | 2.18, *m* | 51.0 | 2.14, *m* | 49.6 | 2.32, *m* | 49.2 | 2.50, *m* |
| 19 | 47.4 | 1.84, 1.14, *m* | 48.2 | 1.76, 1.04, *m* | 46.9 | 1.59, 1.16, *m* | 47.7 | 2.14, 1.39, *m* |
| 20 | 37.0 | | 31.8 | | 30.8 | | 36.8 | |
| 21 | 74.2 | 3.55, *dd* (11.8, 4.5) | 35.9 | 1.38, 1.13, *m* | 34.9 | 1.66, 1.27, m | 72.6 | 4.10, *dd* (11.8, 4.5) |
| 22 | 40.2 | 1.82, *dd* (11.0, 4.0)<br>1.53, *dd* (11.0, 4.0) | 30.8 | 2.28, 1.06, *m* | 29.9 | 2.84, *dd* (11.0, 4.0)<br>1.34, *dd* (11.0, 4.0) | 40.7 | 2.95, *dd* (11.0, 4.0)<br>1.64, *dd* (11.0, 4.0) |
| 23 | 64.6 | 3.48, *dd* (12.0)<br>3.74, *dd* (12.0) | 28.2 | 1.13, *s* | 64.0 | 4.00, *dd* (12.0)<br>4.50, *dd* (12.0) | 207.7 | 9.91, *s* |
| 24 | 15.0 | 1.09, *s* | 18.5 | 1.23, *s* | 15.0 | 1.62, *s* | 11.9 | 2.01, *s* |
| 25 | 18.0 | 1.27, *s* | 17.5 | 1.33, *s* | 17.4 | 1.64, *s* | 17.2 | 1.59, *s* |
| 26 | 18.8 | 1.35, *s* | 18.8 | 1.27, *s* | 18.0 | 1.54, *s* | 18.5 | 1.62, *s* |
| 27 | 27.5 | 1.19, *s* | 27.5 | 1.18, *s* | 27.0 | 1.38, *s* | 27.4 | 1.39, *s* |
| 28 | 23.3 | 0.89, *s* | 23.2 | 0.86, *s* | 22.8 | 1.22, *s* | 22.5 | 1.20, *s* |
| 29 | 29.8 | 0.97, *s* | 31.8 | 0.90, *s* | 33.0 | 0.86, *s* | 30.0 | 1.27, *s* |
| 30 | 17.9 | 0.88, *s* | 24.4 | 0.91, *s* | 23.9 | 0.94, *s* | 17.9 | 1.26, *s* |
| | Glc at C-3 | | Glc at C-3 | | Glc at C-3 | | Glc at C-3 | |
| 1′ | 105.7 | 4.40, *d* (7.5) | 106.6 | 4.32, *d* (7.5) | 105.2 | 5.17, *d* (7.5) | 104.8 | 4.90, *d* (7.5) |
| 2′ | 75.6 | 3.20, *dd* (9.0, 7.5) | 75.6 | 3.20, *dd* (9.0, 7.5) | 75.4 | 4.05, *dd* (9.0, 7.5) | 75.3 | 3.95, *dd* (9.0, 7.5) |
| 3′ | 78.3 | 3.35, *dd* (9.0, 9.0) | 78.2 | 3.33, *dd* (9.0, 9.0) | 78.2 | 4.19, *dd* (9.0, 9.0) | 78.5 | 4.21, *dd* (9.0, 9.0) |
| 4′ | 71.7 | 3.33, *dd* (9.0, 9.0) | 71.6 | 3.31, *dd* (9.0, 9.0) | 71.1 | 4.20, *dd* (9.0, 9.0) | 71.5 | 4.27, *dd* (9.0, 9.0) |
| 5′ | 77.5 | 3.27, *m* | 77.6 | 3.26, *m* | 77.8 | 3.90, *m* | 78.5 | 3.96, *m* |
| 6′ | 62.8 | 3.85, *dd* (12.0, 2.5)<br>3.67, *dd* (12.0, 5.0) | 62.8 | 3.86, *dd* (12.0, 2.5)<br>3.65, *dd* (12.0, 5.0) | 62.3 | 4.50, *dd* (12.0, 2.5)<br>4.33, *dd* (12.0, 5.0) | 62.8 | 4.56, *dd* (12.0, 2.5)<br>4.40, *dd* (12.0, 5.0) |

(*continued on next page*)

Table 2 (*continued*)

| Position | 6 | | 7 | | 8 | | 9 | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) | $\delta_C$ | $\delta_H$ (*J* in Hz) |
| | Glc at C-16 | | Glc at C-16 | | Glc at C-16 | | | |
| 1″ | 105.5 | 4.37, *d* (7.5) | 104.5 | 4.43, *d* (7.5) | 103.9 | 4.99, *d* (7.5) | | |
| 2″ | 75.6 | 3.20, *dd* (9.0, 7.5) | 83.5 | 3.40, *dd* (9.0, 7.5) | 82.9 | 4.09, *dd* (9.0, 7.5) | | |
| 3″ | 78.3 | 3.35, *dd* (9.0, 9.0) | 78.0 | 3.55, *dd* (9.0, 9.0) | 77.8 | 4.28, *dd* (9.0, 9.0) | | |
| 4″ | 71.5 | 3.27, *dd* (9.0, 9.0) | 71.6 | 3.29, *dd* (9.0, 9.0) | 71.2 | 4.20, *dd* (9.0, 9.0) | | |
| 5″ | 77.6 | 3.33, *m* | 77.3 | 3.26, *m* | 77.2 | 3.87, *m* | | |
| 6″ | 62.7 | 3.85, *dd* (12.0, 2.5) | 62.7 | 3.86, *dd* (12.0, 2.5) | 62.3 | 4.50, *dd* (12.0, 2.5) | | |
| | | 3.67, *dd* (12.0, 5.0) | | 3.65, *dd* (12.0, 5.0) | | 4.33, *dd* (12.0, 5.0) | | |
| | | | Ara at C-2glcI′ | | Ara at C-2glcI | | | |
| 1‴ | | | 106.4 | 4.48, *d* (6.5) | 106.0 | 5.18, *d* (6.5) | | |
| 2‴ | | | 73.6 | 3.65, *dd* (8.5, 6.5) | 73.2 | 4.55, *dd* (8.5, 6.5) | | |
| 3‴ | | | 74.0 | 3.55, *dd* (8.5, 3.0) | 73.8 | 4.19, *dd* (8.5, 3.0) | | |
| 4‴ | | | 69.5 | 3.80, *m* | 68.7 | 4.32, *m* | | |
| 5‴ | | | 67.3 | 3.84, *dd* (12.0, 2.0) | 66.6 | 4.02, *dd* (12.0, 2.0) | | |
| | | | | 3.50, *dd* (12.0, 3.0) | | 3.74, *dd* (12.0, 3.0) | | |

For the sugar portion, the $^1$H-NMR spectrum of **1** (Table 1), showed two anomeric proton signals ($\delta$ 4.89, *d*, $J = 7.5$ Hz; $\delta$ 5.06, *d*, $J = 7.5$ Hz). 1D-TOCSY spectra obtained by selectively irradiating anomeric resonances gave in both cases the spin-system of a β-glucopyranosyl. HSQC experiments, which correlated all the proton resonances with those of each corresponding carbon, showed no glycosidation shifts, suggesting that the two sugars were terminal units. The location of the sugar residues at C-3 and C-16 were unambiguously defined by the HMBC experiment, which showed long-range correlations between C-3 ($\delta$ 82.3) and H-1glcI ($\delta$ 4.89) and C-16 ($\delta$ 76.4) and H-1glcII ($\delta$ 5.06) (Table 1). On the basis of the above data, compound **1** was identified as 3β,6β,16β-trihydroxyolean-12-en-23-al-3 -*O*-β-glucopyranosyl-16-*O*-β-glucopyranoside.

The negative HR-ESI-MS of compound **2** showed a molecular ion peak at *m*/*z* 781.4752 $[M-H]^-$, in accordance with an empirical molecular formula of $C_{32}H_{70}O_{13}$. The negative ESI-MS spectrum showed a psuedomolecular ion peak at 781 $[M-H]^-$ indicating a molecular weight of 782 for compound **2**. ESI-MS-MS experiments showed further fragment ions at 619 $[M-H-162]^-$ and 457$[M-H-162-162]^-$, suggesting the presence of two hexosyl moieties. Compound **2** displayed 42 carbon resonances in its $^{13}$C NMR spectrum (Table 1), of which 30 could be assigned to the aglycone and 12 to the sugar moiety. The $^{13}$C NMR spectrum of the aglycone portion exhibited eight methyl resonances at $\delta$ 17.4, 18.0, 18.6, 18.8, 21.7, 23.0, 25.0, 28.1, two sp$^2$-hybridized carbons at $\delta$ 125.5 and 138.5, resonances for three hydroxymethine groups ($\delta$ 65.7, 67.1 and 89.4). These data, when correlated with information from the $^1$H NMR spectrum (Table 1) [six methyl singlets at $\delta$ 1.33, 1.45, 1.54, 1.62, 1.66, 1.84, two methyl doublets at $\delta$ 0.98 ($J = 6.5$ Hz) and 0.99 ($J = 6.5$ Hz) one olefinic proton at $\delta$ 5.38, and three oxymethyne protons at $\delta$ 3.38, 4.55 and 4.79], indicated that the aglycone of **2** is based on a olean-12-ene-3,6,16-tetraol skeleton (Table 1). The orientations of the hydroxy groups were assigned as 3β,6β,16β on the basis of $^1$H NMR coupling constants and by comparison with compound **1**. Assignments of all chemical shifts of protons and carbons of the aglycone portion were ascertained from a combination of 1D-TOCSY, DQF-COSY, HSQC and HMBC analysis. The $^1$H NMR spectrum for the sugar portion of compound **2** showed two anomeric proton signals at $\delta$ 4.98 (1H, *d*, $J = 7.5$ Hz) and 5.25 (1H, *d*, $J = 7.5$ Hz). The chemical shifts of all the individual protons of the two sugar units were attributed on the basis of 2D-TOCSY and DQF-COSY spectral analysis, and the $^{13}$C chemical shifts of their relative attached carbons were clearly assigned from the HSQC spectrum (Table 2). These data showed the presence of a terminal β-galactopyranosyl ($\delta$ 5.25) and a 2-substituted β-glucopyranosyl ($\delta$ 4.98) as indicated by the downfield shift of C-2$_{glc}$ ($\delta$ 84.6) signal. An unambiguous determination of the linkage site was obtained from the HMBC spectrum, which showed key correlation peaks

between the proton signal at $\delta$ 4.98 (H-$1_{glc}$) and the carbon resonance at $\delta$ 89.4 (C-3), and between the proton signal at $\delta$ 5.25 (H-$1_{gal}$) and the carbon resonance at $\delta$ 84.6 (C-$3_{glc}$). Thus, compound **2** was defined as urs-12-ene-3β,6β,16β-triol-3-*O*-β-galactopyranosyl-(1 → 2)-β-glucopyranoside.

Compound **3** exhibited a quasi-molecular ion peak at *m/z* 811.4463 $[M-H]^-$ in the HR-ESI-MS-spectrum (negative mode), in accordance with an empirical molecular formula of $C_{42}H_{68}O_{15}$. The negative ESI-MS spectrum showed a psuedomolecular ion peak at *m/z* 811 $[M-H]^-$ indicating a molecular weight of 812 for compound **3**. A detailed analysis of the NMR spectroscopic data ($^1H$, $^{13}C$, 2D-TOCSY, DQF-COSY, HSQC) of compound **3** in comparison with those of **1** (Table 1) showed a difference in the replacement of the 23-CHO function in **1** by a 23-COOH group in **3**. In particular, the HMBC correlations between the proton signal at $\delta$ 1.52 (Me-24) and the carbon resonances at $\delta$ 53.6 (C-5), 55.3 (C-4), 87.1 (C-3) and 183.1 (C-23) supported the occurrence of a 23-COOH group (Mahato and Kundu, 1994). From these data, the structure of **3** was determined as 3β,6β,16β-trihydroxyolean-12-en-23-oic acid-3-*O*-β-glucopyranosyl-16-*O*-β -glucopyranoside.

The negative HR-ESI-MS of compound **4** gave a psuedomolecular ion peak at *m/z* 635.4173 $[M-H]^-$, in agreement with a molecular formula of $C_{36}H_{60}O_9$. Compound **4** displayed a molecular peak at *m/z* 635 $[M-H]^-$ in ESI-MS negative mode indicating a molecular weight of 636, as well as a fragment ion of *m/z* 473 $[M-H-162]^-$ corresponding to the loss of a hexosyl moiety. Compound **4** displayed 36 carbon resonances in its $^{13}C$ NMR spectrum (Table 1). For the aglycone portion along with eight methyl resonances ($\delta$ 16.8, 17.4, 18.2, 18.7, 18.8, 22.9 and 25.0) and two $sp^2$-hybridized carbon signals ($\delta$ 126.0 and 138.6), signals for four hydroxymethine groups ($\delta$ 67.0, 67.3, 70.5 and 89.3) could be observed. A detailed analysis of the NMR spectroscopic data ($^1H$, $^{13}C$, 2D-TOCSY, DQF-COSY, HSQC) of the aglycone moiety of compound **4** in comparison with those of **2** showed differences in the carbon resonances of ring E. In the HMBC spectrum, the methyl signal at $\delta$ 1.41 (Me-30) showed a long-range correlation with the carbon resonance at $\delta$ 70.5, allowing us to deduce the occurrence of an additional secondary alcoholic function at C-21 (Mimaki et al., 2004). The coupling constants of H-21 ($\delta$ 3.97, *dd*, $J = 11.0$, 4.0 Hz) and the NOESY correlation between H-21 and Me-29 suggested the β-orientation of the C-21 hydroxyl group (Mimaki et al., 2004). The sugar unit, identified as β-glucopyranosyl, was placed at C-3 of the aglycone on the basis of the HMBC correlation between the anomeric proton at $\delta$ 5.01 and the carbon resonance at $\delta$ 89.3 (C-3). Thus, compound **4** was defined as urs-12-ene-3β,6β,16β,21β-tetraol-3-*O*-β-glucopyranoside.

Compound **5** showed the same molecular formula as compound **4** ($C_{36}H_{60}O_9$), indicated by both HR-ESI-MS (*m/z* 635.4163 $[M-H]^-$) and ESI-MS analysis (*m/z* 635 $[M-H]^-$). Eight tertiary methyl signals ($\delta$ 1.23, 1.26, 1.29, 1.44, 1.54, 1.62, 1.64, 1.74) in the $^1H$ NMR spectrum along with resonances of ring E in the $^{13}C$ NMR spectrum showed that compound **5** differed from **4** only in the olean-12-ene skeleton instead of an urs-12-ene skeleton, being identical for the other structural features (Table 1). Thus, compound **5** was defined as olean-12-ene-3β,6β,16β,21β-tetraol-3-*O*-β-glucopyranoside.

The molecular formula for compound **6** was established as $C_{42}H_{70}O_{15}$ by the negative-ion mode HR-ESI-MS showing a pseudo-molecular ion peak at *m/z* 813.4649 $[M-H]^-$. Compound **6** also exhibited a quasi-molecular ion peak in negative-ion mode ESI-MS at 813 $[M-H]^-$, indicating a molecular weight of 814. Data from the $^{13}C$ NMR spectrum (Table 2) of **6** suggested also in this case a polyhydroxylated-olean-12-ene glycoside structure. The $^{13}C$ NMR spectrum showed 42 resonances, of which 30 were assigned to a triterpenoid moiety and 12 to the glycosyl portion. The following NMR data exhibited the structural features of olean-12-en-3β,6β,16β,21β,23-pentaol as the aglycone: seven tertiary methyl resonances ($\delta$ 0.88, 0.89, 0.97, 1.09, 1.19, 1.27 and 1.35), resonances at $\delta$ 3.55 (*dd*, $J = 12.0$, 4.0 Hz) 3.59 (*dd*, $J = 11.0$, 4.5 Hz), 4.10 (*dd*, $J = 11.5$, 4.5 Hz), 4.45 (*br s*) ascribable, respectively, to the 21β,3β,16β,6β-protons on hydroxymethine carbons, one hydroxymethylene ($\delta$ 3.48 and 3.78, each a doublet, $J = 12.0$ Hz), and the resonance of H-12 at $\delta$ 5.29 (*t*, $J = 3.5$ Hz). On the basis of the HMBC experiment that provided unambiguous correlations between the methyl signal at $\delta$ 1.09 (Me-24) and the carbon resonances at $\delta$ 44.8 (C-4), 48.2 (C-5), 83.3 (C-3) and 64.6 (C-23), the primary alcoholic function was located at C-23 (Mahato and Kundu, 1994). Analysis of the NMR spectroscopic data of the sugar portion indicated the occurrence of two β-glucopyranosyl units linked at C-3 and C-16, as in compound **1**. Thus the structure of **6** was defined as olean-12-ene-3β,6β,16β,21β,23-pentaol-3-*O*-β-glucopyranosyl-16-*O*-β-glucopyranoside.

The negative HR-ESI-MS of compound **7** showed a molecular ion peak at *m/z* 913.5189 $[M-H]^-$, in accordance with an empirical molecular formula of $C_{47}H_{78}O_{17}$. Compound **7** displayed a molecular peak at *m/z* 913 $[M-H]^-$ in ESI-MS negative mode indicating a molecular weight of 914. The $^{13}C$ NMR spectrum of compound **7** showed 47 signals, of which 30 were assigned to the aglycone and 17 to the saccharide portion. Analysis of the $^1H$ and $^{13}C$ NMR spectra of the aglycone moiety (Table 2) clearly showed that it differed from that of compound **5** for the absence of the secondary alcoholic function at C-21. The sugar portion of **1** exhibited, in the $^1H$ NMR spectrum (Table 2), three anomeric proton resonances ($\delta$ 4.32, *d*, $J = 7.5$ Hz; 4.43, *d*, $J = 7.5$ Hz; 4.48, *d*, $J = 6.5$ Hz). The structures of the oligosaccharide moieties were deduced using 1D-TOCSY and 2D NMR experiments, which indicated that two β-glucopyranosyl units and one α-arabinopyranosyl unit were present (Table 2). The absence of any $^{13}C$ NMR glycosidation shift for one glucopyranosyl residue ($\delta$ 4.32) and the arabinopyranosyl unit suggested that these sugars were terminal units. Gly-

cosidation shifts were observed for C-$2_{glcI}$ ($\delta$ 83.5) (Table 2). Direct evidence for the sugar sequence and their linkage sites to the aglycone was derived from the results of the HMBC experiment that showed unequivocal correlations between resonances at $\delta$ 4.32 and 90.5 (H-$1_{glc}$-C-3), $\delta$ 4.43 and 77.2 (H-$1_{glc}$-C-16), and $\delta$ 4.48 and 83.5 (H-$1_{ara}$-C-$2_{glcI}$). Thus, compound **7** was identified as olean-12-ene-3β,6β,16β-triol-3-*O*-β-glucopyranosyl-16-*O*-α-arabinopyranosyl-(1 → 2)-β-glucopyranoside.

The HR-ESI-MS spectrum (negative-ion mode) of compound **8** exhibited a pseudomolecular ion peak at *m/z* 929.5114 $[M-H]^-$, ascribable to a molecular formula of $C_{47}H_{77}O_{18}$. The ESI-MS experiment (negative-ion mode) also showed a quasi-molecular ion peak at *m/z* 929 $[M-H]^-$ indicating a molecular weight of 796. Analysis of the $^1H$ and $^{13}C$ NMR spectra of the aglycone moiety of compound **8** (Table 2) clearly showed that it differed from that of compound **6** for the absence of the secondary alcoholic function at C-21. Analysis of the glycosidic NMR data demonstrated that they were superimposable with those of **7**. Thus, compound **8** was defined as olean-12-ene-3β,6β,16β,23-tetraol-3-*O*-β-glucopyranosyl-16-*O*-α-arabinopyranosyl-(1 → 2)-β-glucopyranoside.

The molecular formula for compound **9** was established as $C_{36}H_{58}O_{10}$ by the negative-ion mode HR-ESI-MS spectrum showing a pseudo-molecular ion peak at *m/z* 649.3945 $[M-H]^-$. Compound **9** also exhibited a quasi-molecular ion peak in negative-ion mode ESI-MS at 649 $[M-H]^-$, indicating a molecular weight of 650. Analysis of NMR spectroscopic data of compound **9** (Table 2) in comparison with those of **5** clearly indicated that **9** differed from **5** (Table 1) only for the replacement of the 23-Me group with an aldehydic function. On the basis of the foregoing data the structure 3β,6β,16β,21β-tetrahydroxyolean-12-en-23-al-3-*O*-β-glucopyranoside was assigned to compound **9**.

To the best of our knowledge, the aglycones of compounds **1**–**9** are reported here for the first time. Most unusual is the co-occurrence of 6β-OH and 16β-OH functions in an olean-12-ene or an urs-12-ene series. The only other compound reported in the literature from this rare class of 6β-hydroxylated β-amryin derivatives is daturadiol, isolated first from *Datura innoxia* (Kocor and St. Pyrek, 1973), and more recently from *Terminalia brasiliensis* (Araujo and Chaves, 2005). However, daturadiol does not possess a 16β-OH.

Several studies have demonstrated that polyhydroxylated triterpenes isolated from members of the Asteraceae exhibit potent anti-inflammatory effects, as well as, anti-tumor and cytotoxic activities (Akihisa et al., 1996; Ukiya et al., 2001; Ukiya et al., 2002; Neukirch et al., 2005). Moreover, recent studies on an oleanane triterpenoid possessing both 3-β and 6-α hydroxyl groups have exhibited anti-tumor activity against a diverse panel of tumor cell lines (Wang et al., 2006a). Taking into account the unusual structural features of *Silphium* saponins to determine their possible functional role as cancer preventives, we tested their effect on breast cancer cell proliferation. Initially, we tested the effect of 25 μg/ml of compounds **1**–**9** on growth of MDA-MB-231 human breast cancer cells for 96 h with fresh compounds added every 48 h. At the concentration tested, only one of the saponins, compound **2** (urs-12-ene-3β,6β,16β-triol-3-*O*-β-galactopyranosyl-(1 → 2)-β-glucopyranoside) reduced cell number compared to the vehicle alone (DMSO). The effective concentration of compound **2** was determined in Fig. 1A and B, where we show that compared to the vehicle control, there was an 80% reduction in cell number at 25 μg/ml. This effect was almost 100% at 50 and 100 μg/ml. Since the observed decrease in cell number appears to be due to decreased cell proliferation (i.e. reduced number of viable nuclei) and not due to increased membrane permeability, saponins may induce inhibitory effects in cell cycle progression. This result indicates *Silphium* as a potential source for anti-breast cancer compounds.

Compound **2** was the only saponin tested in this study that possesses a galactopyranosyl residue, suggesting that this sugar residue may be involved in the cytotoxic activity. Structure-activity studies of saponins have demonstrated that the nature of the sugar moiety is very important for cytotoxic activity (Bader et al., 1996). Compound **2** also differs from all other saponins tested here by the presence of a monodesmosidic disaccharide chain attached to the C-3 position, while all other saponins tested are either bidesmosidic or monodesmosidic with a single glucopyranosyl residue attached to the C-3 position, indicating that the length and linkage of the sugar chain may also influence cytotoxicity. A survey of 16 triterpenoid saponins with different sugar linkages found monodesmosides more active than bidesmosides in killing cancer cells (Quetin-Leclercq et al., 1992).

Triterpenoid saponins from other plant sources have also been shown to activate estrogen receptors (Lee et al., 2003) as well as inhibit breast cancer cell proliferation via suppression of cell cycle progression and cell survival signaling and promotion of apoptosis (Mujoo et al., 2001; Chen et al., 2003). Future studies will focus on the exact mechanism by which compound **2** exerts its anti-proliferative effects on breast cancer cells.

## 3. Experimental

### 3.1. General experimental procedure

Optical rotations were measured in MeOH with a Perkin–Elmer 241 polarimeter equipped with a sodium lamp (589 nm) and a 1 dm microcell. ESI-MS was performed on a Finnigan LCQ Ion Trap mass spectrometer and HR-ESI-MS experiments were performed on Fourier transform ion cyclotron mass spectrometer (Ion Spec, Varian). The spectra were recorded by infusion into the ESI (electrospray ionization) source using MeOH as a solvent. $^1H$ and $^{13}C$ NMR spectra were recorded in either pyridine-$d_5$ or $CD_3OD$ on a Varian Inova 500 Mz spectrometer. All

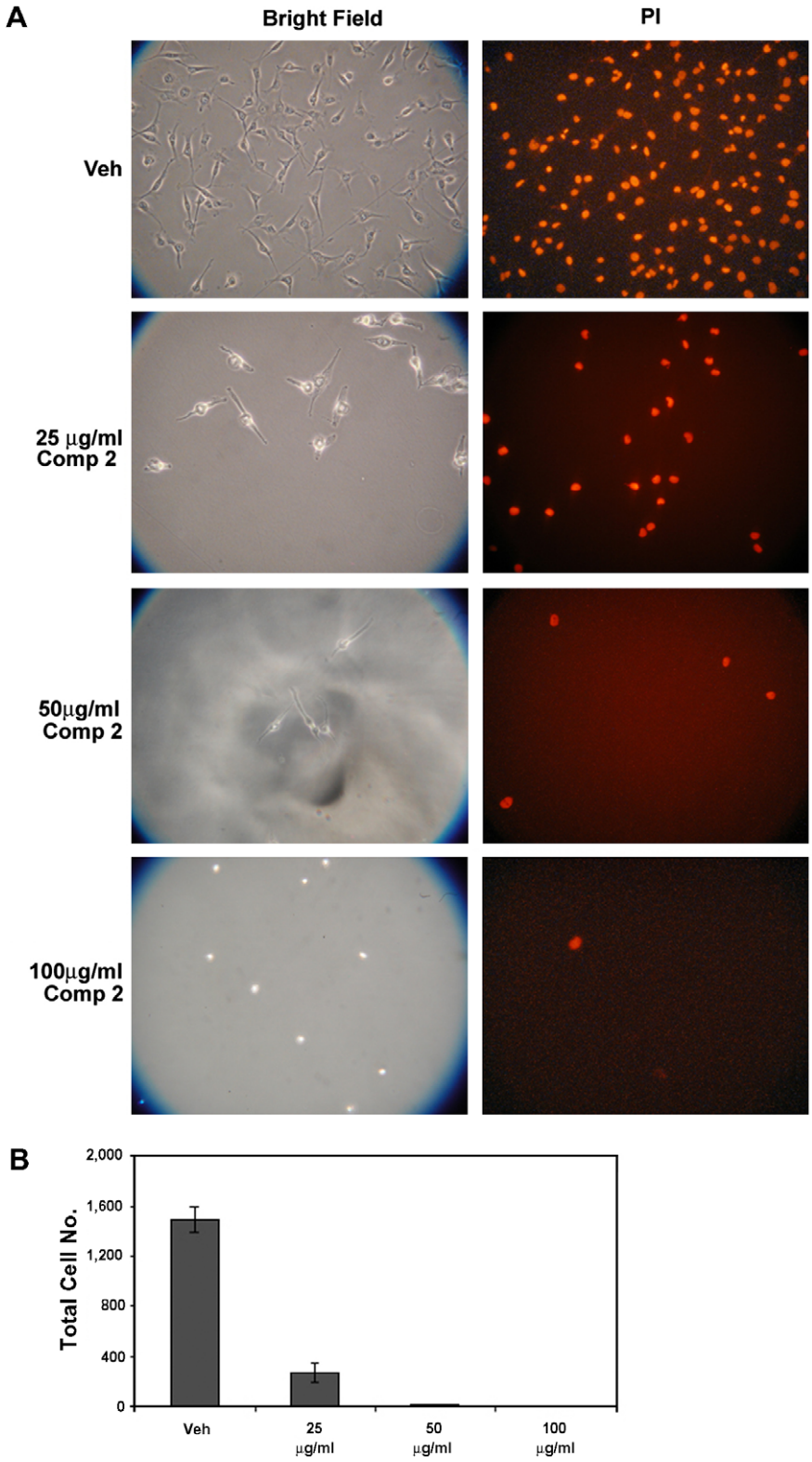



Fig. 1. Effect of compound **2** on breast cancer cell proliferation. Semi-confluent MDA-MB-231 cells were treated with vehicle (0.1% DMSO) or compound **2** at 25, 50, or 100 µg/ml every 48 h for 96 h. (A) Representative cells from either bright field (left) or fluorescence microscopy following propidium iodide (PI) staining to visualize nuclei (right). (B) Total number of cells with intact, viable nuclei following vehicle or compound **2** at 25, 50, or 100 µg/ml. Cell number was quantified from 30 microscopic fields/well. Error bars are standard deviations for $N = 3$.

chemical shifts ($\delta$) are given in ppm units with reference to TMS as an internal standard and the coupling constants ($J$) are given in Hz.

Column chromatography was carried out on Kieselgel 60 (60–200 µm, Merck) or LiChroprep RP-18 (40–60 µm, Merck). MPLC was performed on a Beckman 120B pump

using Millipore Vantage-L (16 × 250, 32 × 250 mm) columns with a flow rate of 2 ml $min^{-1}$. Fractions were monitored by TLC on silica gel plates (Merck precoated silica gel 60 $F_{254}$) and developed in the solvent system, EtOAc:HOAc:$H_2O$ (9:2:2). Spots were visualized by heating after spraying with 10% $H_2SO_4$.

### 3.2. Plant material

Aerial parts of *S. radula* were collected by Dr. Jeffery Williams in Lee County, Texas in July 2002 near a rest area on Hwy 290 west of Giddings, Texas. Samples of *S. radula* were identified by Prof. Mark Bierner and a voucher specimen was deposited at the University of Texas at Austin Plant Resource Center (No. JW2002/7/3).

### 3.3. Extraction and isolation

The dried mixture of leaves and stems of *S. radula* (500 g) was successively extracted for 48 h with $CH_2Cl_2$–MeOH (1:1), MeOH and MeOH–$H_2O$ (1:1) at room temperature to give three separate crude residues. The conc. MeOH and MeOH–$H_2O$ extracts were combined and dried under vacuum and the concentrate (70 g) was dissolved in $H_2O$ and loaded onto a 7.5 × 10 cm C-18 column. The column was washed with water to remove sugars and then with MeOH–$H_2O$ (2:3, v/v) to remove phenolics. Saponins were removed with MeOH–$H_2O$ (85:15, v/v) and the crude saponin fraction was subjected to silica gel CC eluted with a gradient of $CHCl_3$:MeOH:$H_2O$ (9:1:0.1 to 6:4:0.4) to give seven major fractions (I–VII). Fraction I was subjected to reversed phase C-18 MPLC with a gradient of $CH_3CN$:$H_2O$ with 1% $H_3PO_4$ affording compound **1** (128.6 mg). Fraction II was subjected to reversed phase C-18 MPLC using a MeOH:$H_2O$ gradient giving compound **2** (23.4 mg). Remaining saponin mixtures III–VII obtained from the above processing were repeatedly subjected to MPLC purification on silica RP-18 using a MeOH–$H_2O$ gradient, affording compounds **3** (15.6 mg), **4** (2.7 mg), **5** (4.6 mg), **6** (115.9 mg), **7** (42.9 mg), **8** (263 mg) and **9** (96.4 mg).

### 3.4. 3β,6β,16β-Trihydroxyolean-12-en-23-al-3-O-β-glucopyranosyl-16-O-β-glucopyranoside (*1*)

White powder; $[\alpha]_D^{20}$ +23.46 (MeOH; *c* 0.24); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1; ESI-MS (negative ion mode): *m/z* 795 $[M-H]^-$; ESI-MS-MS (795) *m/z* 633 $[M-H-162]^-$, 472 $[M-H-162-162]^-$; HR-ESI-MS (negative ion mode) *m/z* 795.45363 (calcd. For $C_{42}H_{70}O_{14}$).

### 3.5. Urs-12-ene-3β,6β,16β-triol-3-O-β-galactopyranosyl-(1 → 2)-β-glucopyranoside (*2*)

White powder; $[\alpha]_D^{20}$ +21.78 (MeOH; *c* 0.24); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1; ESI-MS (negative ion mode): *m/z* 781 $[M-H]^-$; ESI-MS-MS (781) *m/z* 619 $[M-H-162]^-$, 457 $[M-H-162-162]^-$; HR-ESI-MS (negative ion mode) *m/z* 781.47437 (calcd. For $C_{42}H_{70}O_{13}$).

### 3.6. 3β,6β,16β-Trihydroxyolean-12-en-23-oic acid-3-O-β-glucopyranosyl-16-O-β-glucopyranoside (*3*)

White powder; $[\alpha]_D^{20}$ +12.67 (MeOH; *c* 0.26); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1; ESI-MS (negative ion mode): *m/z* 811 $[M-H]^-$; HR-ESI-MS (negative ion mode) *m/z* 811.44855 (calcd. For $C_{42}H_{68}O_{15}$).

### 3.7. Urs-12-ene-3β,6β,16β,21β-tetraol-3-O-β-glucopyranoside (*4*)

White powder; $[\alpha]_D^{20}$ +10.90 (MeOH; *c* 0.35); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1; ESI-MS (negative ion mode) 635 $[M-H]^-$: *m/z*; HR-ESI-MS (negative ion mode) *m/z* 635.41646 (calcd. For $C_{36}H_{60}O_9$).

### 3.8. Olean-12-ene-3β,6β,16β,21β-tetraol-3-O-β-glucopyranoside (*5*)

White powder; $[\alpha]_D^{20}$ +7.88 (MeOH; *c* 0.24); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1; ESI-MS (negative ion mode): *m/z* 635 $[M-H]^-$; HR-ESI-MS (negative ion mode) *m/z* 635.41646 (calcd. For $C_{36}H_{60}O_9$).

### 3.9. Olean-12-ene-3β,6β,16β,21β,23-pentaol-3-O-β-glucopyranosyl-16-O-β-glucopyranoside (*6*)

White powder; $[\alpha]_D^{20}$ +16.76 (MeOH; *c* 0.28); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 2; ESI-MS (negative ion mode): *m/z* 813 $[M-H]^-$; HR-ESI-MS (negative ion mode) *m/z* 813.46420 (calcd. For $C_{42}H_{70}O_{15}$).

### 3.10. Olean-12-ene-3β,6β,16β-triol-3-O-β-glucopyranosyl-16-O-α-arabinopyranosyl-(1 → 2)-β-glucopyranoside (*7*)

White powder; $[\alpha]_D^{20}$ +14.28 (MeOH; *c* 0.27); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 2; ESI-MS (negative ion mode): *m/z* 913 $[M-H]^-$; HR-ESI-MS (negative ion mode) *m/z* 913.51663 (calcd. For $C_{47}H_{78}O_{17}$).

### 3.11. Olean-12-ene-3β,6β,16β,23-tetraol-3-O-β-glucopyranosyl-16-O-α-arabinopyranosyl-(1 → 2)-β-glucopyranoside (*8*)

White powder; $[\alpha]_D^{20}$ +17.74 (MeOH; *c* 0.28); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 2; ESI-MS (negative ion mode): *m/z* 929 $[M-H]^-$; HR-ESI-MS (negative ion mode) *m/z* 929.51154 (calcd. For $C_{47}H_{78}O_{18}$).

### 3.12. 3β,6β,16β,21β-Tetrahydroxyolean-12-en-23-al-3-O-β-glucopyranoside (*9*)

White powder; $[\alpha]_D^{20}$ +10.26 (MeOH; *c* 0.23); For $^1H$ and $^{13}C$ NMR spectroscopic data, see Table 1; ESI-MS (nega-

tive ion mode): $m/z$ 649 $[M-H]^-$; HR-ESI-MS (negative ion mode) $m/z$ 649.39572 (calcd. For $C_{36}H_{58}O_{10}$).

### 3.13. Biological assays

#### 3.13.1. Cell culture

MDA-MB-231 human breast cancer cells (from the American type culture collection (ATCC)) were cultured in a Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37.9702 °C in 5% $CO_2$.

#### 3.13.2. Cell proliferation

Semi-confluent cells seeded on six well plates in phenol-red free DMEM and 5% FBS (charcoal-stripped) were treated with vehicle or compounds **1**–**9** every 48 h for 96 h. Stock solutions were in 100% DMSO and were diluted in DMEM to a final concentration of 0.1% (v/v) DMSO prior to treatment. Cells were fixed in MeOH, stained with propidium iodide (PI), and intact nuclei were counted from digital images acquired by an Olympus CKX41 microscope. Total number of cells was quantified from 30 microscopic fields/well from experiments carried out in triplicate.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2007.10.017.

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