Shenzhen Research Institute of Tsinghua University[1], Shenzhen; Shenzhen Key Lab for Research & Development of New Drugs[2], Shenzhen; Department of Natural Products Chemistry[3], Shenyang Pharmaceutical University, Shenyang; Department of Biology and Chemistry[4], City University of Hong Kong, Hong Kong SAR; Medical School[5], Tsinghua University, Beijing; Shenzhen Institute of Drug Control[6], Shenzhen, China

New furostanol saponins from the bulbs of *Allium macrostemon* Bunge and their cytotoxic activity

Haifeng Chen[1,2,5], Guanghui Wang[3,4], Naili Wang[1,2], Mengsu Yang[4], Zhao Wang[5] , Xiaowei Wang[6], Xinsheng Yao[3]

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Xinsheng Yao, Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang 110016, China
yaoxinsheng@tom.com

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Four new furostanol saponins, named as macrostemonoside O, macrostemonoside P, macrostemonoside Q and macrostemonoside R, along with five known compounds, were isolated from the dried bulbs of *Allium macrostemon* Bunge. The structures of these new compounds were established by the spectral data elucidation (IR, ESIMS, 1D and 2D NMR) as 26-*O*-β-D-glucopyranosyl-22-hydroxyl-5β-furost-25 (27)-ene-3β, 26-diol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside (macrostemonoside O), (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxyl-5β-furost-1β, 3β, 26-triol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside (macrostemonoside P), (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furost-1α, 2β, 3β, 26-tetraol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside (macrostemonoside Q) and (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxyl-5β-furost-2α, 3β, 26-triol-3-*O*-β-D-glucopyranosyl (1→2) [β-D-glucopyranosyl (1→3)]-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside (macrostemonoside R), respectively. Their cytotoxic activities on several cancer cell lines including solid tumor (HepG2, MCF-7, NCI-H460 and SF-268) and drug resistant tumor (R-HepG2) were investigated and five compounds showed diverse cytotoxity to these cancer cell lines which suggest that they might be used as potential leading compounds to cure cancer diseases.

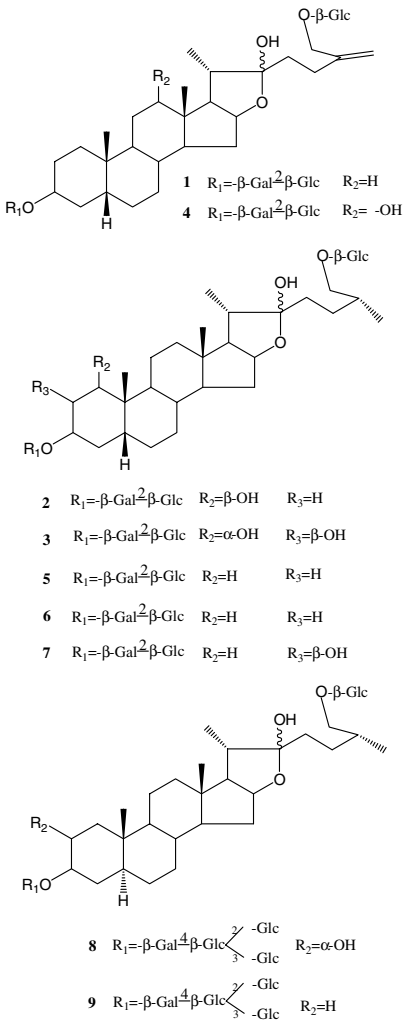
1. Introduction

Allium macrostemon Bunge is an herbaceous plant distributed in northeast China. Its dried bulbs are known as traditional Chinese medicine "Xie bai", which have various pharmacological effects on throracic pain and asthma (Jiang Su New Medicinal College 1977). A number of steroidal saponins with various biological activities, such as macrostemonoside A–H, have been isolated from the dried bulbs of *Allium macrostemon* Bunge (Jiang et al. 2000). In our present study, four new furostanol saponins (**1**, **2**, **3**, **8**), together with five known furostanol saponins (**4–7**, **9**) were obtained. This paper describes the isolation, structural elucidation of new compounds and their cytotoxic activities against five human cancer cell lines, HepG2 (liver), R-HepG2 (drug resistant), MCF-7 (breast), NCI-H460 (lung), and SF-268 (CNS).

2. Investigations, results and discussion

The bulbs of *Allium macrotemon* Bunge were dried and extracted with 60% ethanol. The concentrated ethanol extract was passed through a Diaion HP-20 column eluting with EtOH-H_2O and then the 60% ethanol eluting fraction was collected and further isolated by silica gel, octadecylsilanized (ODS) silica gel, and repeated Prep-HPLC to yield nine compounds. Compound **1**, **2**, **3**, **8** were characterized as new furostanol saponins by NMR data analysis. Compound **4–7**, **9** were determined as known saponins by comparison with reported data.

Compound **1** was obtained as an amorphous powder, which exhibited a positive reaction to Ehrlich reagent. The molecular formula was determined as $C_{45}H_{74}O_{19}$ by the HR-ESIMS at m/z 901.4872 $[M + H - H_2O]^+$ (calcd. 901.4797). The ^{1}H NMR of **1** showed three methyl signals at δ 0.86 s and 0.97 s, and δ 1.38 d (J = 6.8 Hz). Of all 45 carbon signals observed in ^{13}C NMR spectrum, 27 carbon signals (Me × 3, CH_2 × 12, CH × 8, C × 4), including a double bond carbon signals at δ 147.2 and 110.6 (C-25, C-27), were belonged to the aglycone moiety with the analysis of their chemical shift. The ^{1}H NMR and ^{13}C NMR data indicated that **1** had the same sugar moiety as that of macrostemonoside G (**4**) but a different aglycone. Comparison of ^{13}C NMR data of **1** with those for **4** suggested the absence of a hydroxyl group in **1** at C-12. In HMBC spectrum, the long-range correlations from δ 5.32, 5.64 (CH_2-27) to δ 72.0 (C-26), 147.2 (C-25), 28.4 (C-24); δ 1.38 (Me-21), to δ 64.0 (C-17), 40.6 (C-20), 110.3 (C-22); δ 0.86 (Me-18) to δ 40.3 (C-12), 41.2 (C-13), 56.4 (C-14), 64.0 (C-17) and δ 0.97 (Me-19) to δ 30.9 (C-1), 35.2 (C-10), 36.9 (C-5), 40.2 (C-9) suggested the presence of C_{25}-C_{27} double bond and the structure of aglycone.



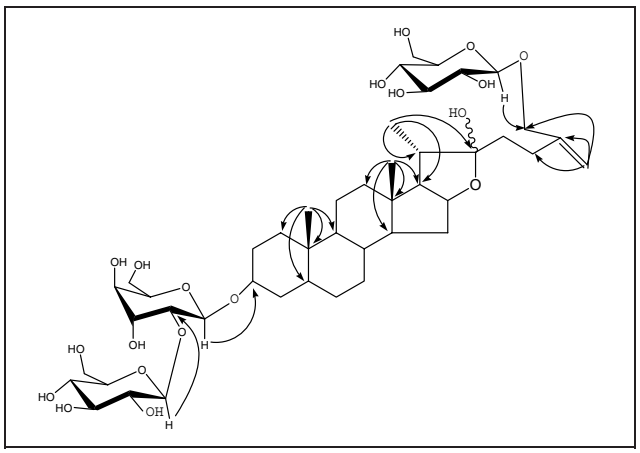


Fig.: Selected HMBC correlations of compound **1**

On acid hydrolysis, **1** yielded glucose and galactose in a ratio of 2 : 1. Three anomeric protons were observed at δ 4.88 (1 H, d, J = 7.6 Hz), 4.90 (1 H, d, J = 7.6 Hz) and 5.28 (1 H, d, J = 7.6 Hz) in the ^{1}H NMR spectrum. Starting from this three anomeric protons, the exact identity of the monosaccharides and the sequence of the oligosaccharide chain were also determined by the analysis of a combination of DEPT, ^{1}H-^{1}H COSY, HMQC, HMBC, and TOCSY spectra. In HMBC spectrum, the anomeric proton signals at δ 4.90 (H-1 of the inner galactose attached to C-3 of the aglycone), 4.88 (H-1 of the terminal glucose attached to C-26 of the aglycone) and 5.28 (H-1 of the glucose attached to C-2 of the inner galactose) showed long-range correlations with carbon signals at δ 75.5 (C-3 of the aglycone), 72.0 (C-26 of the aglycone) and 81.8 (C-2 of the galactose attached to the C-3 of the aglycone) respectively which provided ample evidence to determine the way of linkages by which the sugar were connected and the connection from sugar chain to the aglycone. The large $^3J_{1,2}$ coupling constants of anomeric protons (7.6, 7.6, 7.6 Hz) indicated that all sugar linkage were β-glycosyl configuration.

According to the results described, the structure of **1** was determined as 26-*O*-β-D-glucopyranosyl-22-hydroxy-5-β-furost-25 (27)-ene-3β, 26-diol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside, named macrostemonoside O.

Compound **2** was isolated as an amorphous powder. Its molecular formula of $C_{45}H_{76}O_{20}$ was deduced by positive-ion HR-ESIMS at m/z 959.4843 $[M + Na]^+$ (calcd 959.4828). The ^{1}H NMR spectrum contained four steroid methyl proton signals at δ 1.32 (3 H, d, J = 6.8 Hz), 1.22 (3 H, s), 0.97 (3 H, d, J = 6.8 Hz), 0.84 (3 H, s), and three anomeric protons at δ 5.34 (1 H, d, J = 7.6 Hz), 4.93 (1 H, d, J = 7.6 Hz), 4.82 (1 H, d, J = 7.6 Hz). Comparison of the ^{13}C NMR spectrum of **2** with that of **6** showed considerable structural similarity. However, the molecular formula of **2** was higher by one oxygen atom than that of **6** and difference were recognized in the carbon signals from the ring A/B portion which suggested the presence of an additional hydroxyl group in ring A/B portion of **2**. The ^{13}C NMR spectrum showed that the carbon signals due to C-1, C-2, C-9 and C-10 of **2** shift downfield by approximately +42.2, +3.7, +1.8 and +5.2 ppm, respectively, while the carbon signal due to C-3, C-4 and C-5 shift to higher field by −3.3, −2.3 and −6.3 ppm comparing with those of **6**. In the HMBC spectrum, the long-range correlation between the methyl proton at δ 1.22 (CH_3-19) and carbon signal at δ 73.1, 30.7, 42.0, 40.4 indicated the additional hydroxyl group at C-1. In NOESY spectrum, the NOE correlation between proton signals at δ 4.93 (H-1 of Gal) and 3.80 suggested the β-orientation of C-1 hydroxyl group. The triglycoside moiety of **2** was shown to be the same as that in **6** and the structure of **2** was assigned as (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furostane-1β, 3β, 26-triol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside, named macrostemonoside P.

Compound **3** was shown to have the molecular formula $C_{45}H_{76}O_{21}$ was confirmed by HR-ESIMS at m/z 975.4755 $[M + Na]^+$ (calcd 975.4777). The ^{1}H NMR spectrum showed three anomeric protons at δ 5.29 (d, J = 6.8), 4.96 (o), 4.80 (d, J = 7.6), together with those for four steroid methyl groups at δ 0.83 (3 H, s), 1.26 (3 H, s), 1.30 (3 H, d, J = 6.8 Hz), 0.97 (3 H, d, J = 6.8 Hz). The ^{1}H NMR and ^{13}C NMR spectral data of **3** were similar to those of **5** except for two oxygenated methines at δ 78.3 and δ 67.3 in the ^{13}C NMR spectrum and δ 4.05 and δ 3.97 in the ^{1}H NMR spectrum. The HMBC spectrum showed the long-range correlations between proton signals at δ 1.26 (H-19), 3.97 (H-2) and carbon signal at δ 78.3 (C-1) which revealed the presence of hydroxyl groups at C-1 and C-2. In NOESY spectrum, the NOE correlation between H-2 (δ 3.97) and H-3 (δ 4.87) indicated the β-orientation of hydroxyl group at C-2, therefore the α-orientation of hydroxyl group at C-1 was judged due to NOE observed between H-1 (δ 4.05) and H-19 (δ 1.26). On the basis of above spectroscopic evidence, the structure of **3** was (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furost-1α, 2β, 3β, 26-tetraol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside, named macrostemonoside Q.

Table 1: *In vitro* cytotoxic activity of compound 1–9 on tumor cell lines[a]

Compd.	Cell lines IC_{50} (μM)				
	NCI-H460	SF-268	MCF-7	HEPG2	R-HepG2
1	13.62 ± 0.52	8.71 ± 0.67	5.45 ± 0.33	16.34 ± 2.12	9.15 ± 1.12
2	*	*	*	*	*
3	48.51 ± 3.21	*	304.43 ± 4.52	36.21 ± 2.52	*
4	*	*	*	*	*
5	10.87 ± 1.08	8.15 ± 1.03	12.17 ± 2.10	27.17 ± 1.98	11.41 ± 1.21
6	26.09 ± 2.33	11.74 ± 2.12	24.46 ± 2.45	22.28 ± 2.36	8.15 ± 1.21
7	70.63 ± 2.62	*	*	48.75 ± 2.11	*
8	*	*	*	*	*
9	*	*	*	*	*

[a] Data shown are the mean IC_{50} of three independent experiments ± SD
* No cytotoxic activity was observed when the tumor cells was treated with compound

Compound **8** was isolated as an amorphous powder. The molecular formula $C_{57}H_{96}O_{30}$ was determined by HR-ESIMS at m/z 1283.5859 $[M + Na]^+$ (calcd 1283.5884) as well as from analysis of its ^1H NMR, ^{13}C NMR and DEPT spectra. In the ^1H NMR spectrum, **8** showed four methyl groups on the aglycone at δ 1.30 (3 H, d, J = 6.8 Hz), 0.96 (3 H, d, J = 6.8 Hz), 0.84 (3 H, s) and 0.69 (3 H, s). Five anomeric proton signals could be observed at δ 5.57 (1 H, d, J = 7.6 Hz), 5.28 (1 H, d, J = 7.6 Hz), 5.15 (1 H, d, J = 7.6 Hz), 4.90 (1 H, d, J = 7.6 Hz) and 4.79 (1 H, d, J = 7.7 Hz). The $^3J_{1,2}$ coupling constants of the anomeric proton signals (J = 7.6, 7.6, 7.6, 7.6, 7.7 Hz) exhibited the β-configuration of all sugars. In the ^{13}C NMR spectrum, of all the 57 carbon signals, 27 were assigned to the aglycone moiety and 30 to sugar moiety. Analysis of the ^{13}C NMR spectrum of **8** and comparison with that of **9** implied that the sugar moiety of **8** was identical to that of **9**, but differed from **9** in terms of the aglycone structure. In HMBC spectrum, proton signals at δ 2.19 (H-1) and 1.83 (H-4) showed long-range correlations with carbon signal at δ 70.4 which suggested the presence of hydroxyl group at C-2. The α-configuration of 2-OH was judged by the NOE correlation between H-2 (δ 3.95) and H-19 (δ 0.69) in NOESY spectrum. The 25*R*-configuration of **8** was deduced by the IR spectrum (intensity: $918 < 889\ cm^{-1}$) of aglycone of **8** (Kudo et al. 1984). Thus, the structure of compound **8** was determined as (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-furostane-2α, 3β, 26-triol-3-*O*-β-D-glucopyranosyl (1→2)-[β-D-glucopyranosyl (1→3)]-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside, named macrostemonoside R.

The structures of compounds **4–7**, **9** were determined as macrostemonoside G, (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furostane-3β, 26-diol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside, (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-5β-furostane-3β, 26-diol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-galactopyranoside, macrostemonoside J and macrostemonoside B by the comparison of their spectroscopic data with literature values (Peng et al. 1994, 1995; Janeczko et al. 1987; Ma et al. 1996).

The *in vitro* cytotoxicity of compound **1–9** against various cancer cell lines was evaluated by the MTT assay. IC_{50} values were calculated by LOGIT method (Table 1). Compounds **1**, **5**, and **6** showed considerable cytotoxity especially to the cell line SF-268 with respective IC_{50} values of 8.71, 8.15 and 11.74 μM. And the IC_{50} values of each compound tested on R-HepG2 were lower than that on HepG2 which suggested their strong effectiveness on drug resistant cells as well as on parental cancer cells. Compounds **3** and **7** showed diverse cytotoxic activities which demonstrating their selective effect on different cell lines. Compounds **2**, **4**, **8**, and **9** did not show any cytotoxic activities suggesting that the absence of a hydroxyl group in the aglycone contributes to the cytotoxicity.

3. Experimental

3.1. General

Melting points were determined with a Yanaco MP-S_3 micro-melting point apparatus and are uncorrected. Optical rotations were obtained on a P-1020 digital polarimeter (JASCO Corporation). IR spectra were measured on a SHIMADZU FT/IR-8400 spectrometer. 1D and 2D NMR spectra were taken on a Bruker AV-400 (400 MHz for ^1H NMR) spectrometer in C_5D_5N solution. ESIMS spectra were acquired using a Bruker esquire 2000 mass spectrometer. Column chromatography was carried out on Diaion HP-20 (Mitsubishi Kasei), silica gel (200–300 mesh, Qingdao Factory of Marine Chemical Industry, Qingdao, China) and ODS (40–63 μm, Merck). TLC analyses were taken on Silica gel $60F_{254}$ (Qingdao Factory of Marine Chemical Industry, Qingdao, China) and the spots were detected spraying with Ehrlich reagent and heating. Preparative HPLC was performed using an ODS column (250 × 20 mm, 10 μm, SHIMADZU Pak; Detector: RID). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), was purchased from Sigma (St. Louis, MO, USA). RPMI-1640 medium, fetal bovine serum (FBS) and trypsin-EDTA solution (1X) were obtained from GIBCO-BRL (Grand Island, NY, USA).

3.2. Plant material

The bulbs of *Allium macrostemon* Bunge were purchased from Liaoning Province of China and identified by Professor QiShi Sun (Department of Pharmacognosy, Shenyang Pharmaceutical University). A voucher specimen (No. 203554) was deposited at the Department of Natural Product Chemistry, Shenyang Pharmaceutical University, Shenyang (110016), China.

3.3. Extraction and isolation

The dried bulbs of *Allium macrotemon* Bunge (5 kg) were extracted with 60% ethanol twice for 2 h each. The alcoholic extract was concentrated under reduced pressure, suspended in water and then passed through a Diaion HP-20 column using an EtOH–H_2O gradient system (0–100%). The 60% EtOH eluate fraction (90 g), which was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1; 8 : 2 : 0.2; 7 : 3 : 0.5; 6 : 4 : 0.8) and MeOH finally, gave 9 fractions. Fraction 3 was further purified by ODS column chromatography eluting with MeOH–H_2O (3 : 7; 4 : 4; 5 : 5) and repeated Rp-18 HPLC preparation to yield compounds **1** (35.1 mg), **2** (41.5 mg), **3** (8.6 mg), **4** (26.2 mg), **5** (149.0 mg), **6** (35.4 mg), **7** (10.2 mg). Fraction 5 was also further purified by ODS column chromatography eluting with MeOH–H_2O (5 : 5; 6 : 4; 7 : 3) and repeated Rp-18 HPLC preparation to yield compounds **8** (56.2 mg), **9** (532.7 mg), **10** (120.1 mg), **11** (170.3 mg).

3.4. Compound 1

An amorphous powder, m.p. 168–170 °C, $[\alpha]_D^{29}$ −45.5° (H_2O, c 0.06). HR-ESIMS (positive mode) at m/z 901.4872 $[M + H-H_2O]^+$ (calcd. 901.4797). ESIMS (positive mode) at m/z 941 $[M + Na]^+$, 901 $[M+H-H_2O]^+$, 739 $[M+H-H_2O\text{-Glc}]^+$, 577 $[M+H-H_2O\text{-Glc}\times 2]^+$, 415 $[M + H-H_2O\text{-Glc}\times 2\text{-Gal}]^+$, 397 $[M + H-H_2O\times 2\text{-Glc}\times 2\text{-Gal}]^+$; IR ν_{max} (KBr) cm^{-1}: 3417 (OH), 2934 (CH), 1000–1100; ^1H NMR (C_5D_5N) data see Table 2; ^{13}C NMR, see Table 4.

Table 2: ^{1}H NMR data of compounds 1 and 2 (C_5D_5N)[a,b]

No.	1	2	No.	1	2
1	1.84; 1.46 (o)	3.80 (o)	C-3 Gal-1	4.90 (d, 7.6)	4.93 (d, 7.6)
2	1.97 (o)	2.13; 1.79 (o)	2	4.66 (o)	4.60 (o)
3	4.32 (o)	4.66 (o)	3	4.26 (o)	4.04 (o)
4	1.81 (o)	1.78 (o)	4	4.56 (o)	4.51 (o)
5	2.16 (o)	2.35 (o)	5	4.02 (o)	4.24 (o)
6	0.92 (o)	1.09; 0.95 (o)	6	4.10 (o)	4.52; 4.38 (o)
7	1.21 (o)	1.65; 1.24 (o)	Gal-Glc-1	5.28 (d, 7.6)	5.34 (d, 7.6)
8	1.47 (o)	1.51 (o)	2	4.08 (o)	4.06 (o)
9	1.25 (o)	1.15 (o)	3	4.17 (o)	3.48 (o)
10	–	–	4	4.31 (o)	4.29 (o)
11	1.30; 1.23 (o)	1.18 (o)	5	3.84 (m)	4.14 (o)
12	1.72; 1.09 (o)	1.69; 1.02 (o)	6	4.49 (o)	4.42 (o)
13	–	–	C-26 Glc-1	4.88 (d, 7.6)	4.82 (d, 7.6)
14	1.04 (o)	1.07 (o)	2	4.05 (o)	4.01 (o)
15	1.99; 1.39 (o)	1.99; 1.40 (o)	3	3.91 (m)	4.22 (o)
16	4.98	4.95 (o)	4	4.22 (o)	4.21 (o)
17	1.96 (o)	1.92 (o)	5	4.21 (o)	3.93 (o)
18	0.86 (s)	0.84 (s)	6	4.38 (o)	4.28 (o)
19	0.97 (s)	1.22 (s)	20	2.26 (o)	2.20 (o)
21	1.38 (d, 6.8)	1.32 (d, 6.8)	22	–	–
23	2.41 (o)	2.03 (o)	24	2.71 (m)	2.03; 1.67 (o)
25	–	1.90 (o)	26	4.60 (o)	3.93; 3.61 (o)
27	5.32 (s); 5.64 (s)	0.97 (d, 6.8)			

[a] Recorded on a Bruker-400 NMR spectrometer
[b] Multiplicities and coupling constants are in parentheses

3.5. Compound 2

An amorphous powder, m.p. 197–199 °C, $[\alpha]_D^{25}$ −51.4° (H_2O, c 0.07). HR-ESIMS (positive mode) at m/z 959.4843 $[M + Na]^+$ (calcd 959.4828). ESIMS (positive mode) at m/z 959 $[M + Na]^+$, 941 $[M + Na-H_2O]^+$, 797 $[M + Na\text{-}162]^+$, 779 $[M + Na\text{-}162 - H_2O]^+$, 617 $[M + Na\text{-}162\times2 - H_2O]^+$, 599 $[M + Na\text{-}162\times2 - H_2O\times2]^+$; IR ν_{max} (KBr) cm^{-1}: 3400 (OH), 2927 (CH), 1050; ^{1}H NMR (C_5D_5N) data see Table 2; ^{13}C NMR, see Table 4.

3.6. Compound 3

An amorphous powder, m.p. 189–191 °C, $[\alpha]_D^{25}$ −36.9° (H_2O, c 0.07). HR-ESIMS (positive mode) at m/z 975.4755 $[M + Na]^+$ (calcd 975.4777) ESIMS (positive mode) at m/z 975 $[M + Na]^+$, 957 $[M + Na-H_2O]^+$, 935 $[M + H-H_2O]^+$, 813 $[M + Na\text{-}162]^+$, 773 $[M + H-H_2O\text{-}162]^+$, 633 $[M + Na-H_2O\text{-}162\times2]^+$, 611 $[M + H-H_2O\text{-}162\times2]^+$, 449 $[M + H-H_2O\text{-}162\times3]^+$; ESIMS (negative mode) at m/z 951 $[M - H]^-$, 789 $[M - H\text{-}162]^-$, 627 $[M - H\text{-}162\times2]^-$, 447 $[M - H-H_2O\text{-}162\times3]^-$; IR ν_{max} (KBr) cm^{-1}: 3423 (OH), 2930 (CH), 1038; ^{1}H NMR (C_5D_5N) data see Table 3; ^{13}C NMR, see Table 4.

Table 3: ^{1}H NMR data of compounds 3 and 8 (C_5D_5N)[a,b]

No.	3	8	No.	3	8
1	4.05 (o)	2.19; 1.15 (o)	C-3 Gal-1	4.96 (o)	4.90 (d, 7.6)
2	3.97 (o)	3.95 (o)	2	4.51 (o)	4.53 (o)
3	4.87 (brs.)	3.86 (o)	3	4.21 (o)	4.12 (o)
4	1.88, 1.84 (o)	1.83; 1.46 (o)	4	4.50 (o)	4.56 (o)
5	2.34 (m)	0.98 (o)	5	4.03 (o)	4.02 (o)
6	1.62, 1.14 (o)	1.12; 0.99 (o)	6	4.53, 4.38 (o)	4.60; 4.18 (o)
7	1.23, 0.91 (o)	2.01; 1.39 (o)	Gal-Glc-1	5.29 (d, 6.8)	5.15 (d, 7.6)
8	1.52 (o)	1.36 (o)	2	4.01 (o)	4.30 (o)
9	1.20 (o)	0.57 (m)	3	4.13 (o)	4.16 (o)
10	–	–	4	4.25 (o)	3.81 (o)
11	1.33, 1.20 (o)	1.45; 1.19 (o)	5	3.47 (m)	4.15 (o)
12	1.64, 0.95 (o)	1.67; 1.03 (o)	6	4.29, 4.20 (o)	4.52; 4.23 (o)
13	–	–	3-Glc-1		5.28 (d, 7.6)
14	1.05 (o)	1.01 (o)	2		4.02 (o)
15	1.96, 1.38 (o)	1.48; 0.71 (o)	3		4.01 (o)
16	4.94 (o)	4.94 (o)	4		4.10 (o)
17	1.90 (o)	1.91 (o)	5		3.82 (o)
18	0.83 (s)	0.84 (s)	6		4.52; 4.37 (o)
19	1.26 (s)	0.69 (s)	2-Glc-1		5.57 (d, 7.6)
20	2.21 (t, 6.8)	2.22 (o)	2		4.02 (o)
21	1.30 (d, 6.8)	1.30 (d, 6.8)	3		4.02 (o)
22	–	–	4		4.22 (o)
23	2.03, 1.99 (o)	2.03; 2.01 (o)	5		3.85 (o)
24	2.03, 1.66	2.03; 1.65 (o)	6		4.45; 4.37 (o)
25	1.91 (o)	1.90 (o)	C-26 Glc-1	4.80 (d, 7.6)	4.79 (d, 7.7)
26	3.94 (o); 3.61 (d, 5.6, 9.4)	3.92; 3.60 (o)	2	4.01 (o)	4.01 (o)
27	0.97 (d, 6.8)	0.96 (d, 6.8)	3	3.93 (o)	4.21 (o)
			4	4.22 (o)	4.03 (o)
			5	4.21 (o)	3.91 (o)
			6	4.44, 4.40 (o)	4.52; 4.00 (o)

[a] Recorded on a Bruker-400 NMR spectrometer
[b] Multiplicities and coupling constants are in parentheses

Table 4: ^{13}C NMR data of compounds 1, 2, 3 and 8 (C_5D_5N)[a]

No.	1	2	3	8	No.	1	2	3	8
1	30.9	73.1	78.3	45.5	C-3 Gal-1	102.5	97.2	97.6	103.2
2	26.7	31.7	67.3	70.4	2	81.8	80.1	79.7	72.5
3	75.5	72.2	77.5	84.0	3	75.2	77.2	75.7	75.4
4	27.0	27.6	28.2	34.0	4	69.8	70.2	70.3	79.7
5	36.9	30.7	30.3	44.6	5	76.6	75.7	77.3	75.9
6	26.7	26.3	25.6	28.1	6	62.1	62.8	62.8	60.5
7	26.7	26.3	26.3	32.3	Gal-Glc-1	106.1	105.5	105.4	104.6
8	35.4	35.7	35.6	34.5	2	76.9	75.9	75.1	81.2
9	40.2	42.0	42.1	54.3	3	78.0	78.8	78.6	88.6
10	35.2	40.4	41.6	36.8	4	71.7	70.3	70.5	70.7
11	21.1	20.9	21.1	21.4	5	78.3	78.6	78.9	78.2
12	40.3	40.2	40.1	40.1	6	62.8	62.2	62.0	62.3
13	41.2	40.9	40.9	41.0	3-Glc-1				104.5
14	56.4	56.1	56.1	56.2	2				75.1
15	32.3	32.3	32.3	32.2	3				78.5
16	81.3	81.0	81.0	81.1	4				71.2
17	64.0	64.0	64.0	63.9	5				77.5
18	16.7	16.6	16.6	16.7	6				62.5
19	24.0	19.4	19.6	13.4	2-Glc-1				104.8
20	40.6	40.6	40.6	40.6	2				75.6
21	16.3	16.4	16.4	16.4	3				78.5
22	110.3	110.6	110.6	110.6	4				71.7
23	38.0	37.1	37.1	37.1	5				78.4
24	28.4	28.3	28.3	28.3	6				62.8
25	147.2	34.2	34.2	34.2	C-26 Glc-1	103.9	104.9	104.9	104.9
26	72.0	75.2	75.2	75.2	2	75.1	75.2	75.1	75.3
27	110.6	17.4	17.4	17.4	3	78.5	78.6	78.4	78.6
					4	71.6	71.7	71.7	71.5
					5	78.5	78.4	78.6	78.4
					6	62.7	61.8	62.2	62.9

[a] Recorded on a Bruker-400 (100 MHz for ^{13}C) NMR spectrometer

3.7. Compound 8

An amorphous powder, m.p. 216-218 °C, $[\alpha]_D^{25}$ −25.2° (H_2O, c 0.08). HR-ESIMS (positive mode) at m/z 1283.5859 $[M + Na]^+$ (calcd 1283.5884). ESIMS (positive mode) at m/z 1283 $[M + Na]^+$, 1121 $[M + Na\text{-}162]^+$, 959 $[M + Na\text{-}162\times2]^+$, 797 $[M + Na\text{-}162\times3]^+$, 779 $[M + Na\text{-}162\times3\text{-}H_2O]^+$, 617 $[M + Na\text{-}162\times4\text{-}H_2O]^+$; ESIMS (negative mode) at m/z 1259 $[M - H]^-$, 1097 $[M - H\text{-}162]^-$, 935 $[M - H\text{-}162\times2]^-$, 773 $[M - H\text{-}162\times3]^-$, 611 $[M - H\text{-}162\times4]^-$, 449 $[M - H\text{-}162\times5]^-$; IR ν_{max} (KBr) cm^{-1}: 3418 (OH), 2928 (CH), 1042; ^1H NMR (C_5D_5N) data see Table 3; ^{13}C NMR, see Table 4.

3.8. Acid hydrolysis of saponins

Each saponin (5 mg) was heated in an ampoule with 5 mL aq. 15% HCl at 110 °C for 2 h. The aglycon was extracted with dichloromethane three times and the aqueous residue was evaporated under reduced pressure. Then, 1 mL of pyridine and 2 mg of $NH_2OH \cdot HCl$ were added to the residue, and the mixture was heated at 100 °C for 1 h. After cooling, Ac_2O (0.5 mL) was added and the mixtures were heated at 100 °C for 1 h. The reaction mixtures were evaporated under reduced pressure, and the resulting aldononitrile peracetates were analyzed by GC-MS using standard aldononitrile peracetates as reference samples.

3.9. Cell culture

HepG2, MCF-7, NCI-H460, SF-268 and 293 cells were maintained in RPMI 1640 (Gibco BRL) containing 10% FBS (Gibco), 2 mg/ml sodium bicarbonate, 100 μg/ml penicillin sodium salt and 100 μg/ml streptomycin sulfate. R-HepG2 cells (Lo Elka et al. 2002) were maintained in the presence of 1.2 μM doxorubicin (Sigma). Cells were grown to 70% confluence, trypsinized with 0.05% trypsin-2 mM EDTA, and plated for experimental use. In all experiments, cells were grown in RPMI-1640 medium with 10% FBS for 24 h prior to treatment.

3.10. Cytotoxicity assay

1.0×10^4 HepG2, R-HepG2, MCF-7, NCI-H460, SF-268 cells were seeded in 96 well tissue culture plates and treated with the tested compounds (100–3.125 μM) or vehicle (0.1% DMSO) at various concentrations and incubated for 48hr followed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay at 570 nm (Carmichael et al. 1987). IC_{50} value is the concentration of test drug where $100\times(C - T)/(C - B) = 50$, C is the OD (optical density) of control, T is the OD of treated sample, B is blank. Briefly, the results of the ten compounds on different cell lines are the mean of the $IC_{50} \pm SD$ (standard deviation) of three independent experiments calculated with LOGIT method (Boyd et al. 1995).

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