

Article

## Two New Steroidal Saponins from *Allium macrostemon* Bunge and Their Cytotoxicity on Different Cancer Cell Lines

Hai-Feng Chen <sup>1,\*</sup>, Guang-Hui Wang <sup>1</sup>, Qiang Luo <sup>1</sup>, Nai-Li Wang <sup>2</sup> and Xin-Sheng Yao <sup>2,\*</sup>

<sup>1</sup> Institute for Biomedical Research, Xiamen University, Xiamen 361005, China;

E-mails: guanghui@xmu.edu.cn (G.H.W.), luoqiang20032004@yahoo.com.cn (Q.L.)

<sup>2</sup> Department of Natural Products Chemistry, Shenyang Pharmaceutical University, Shenyang 110016, China; E-mail: wangnl@sz.tsinghua.edu.cn (N.L.W.)

\* Author to whom correspondence should be addressed; E-mails: haifeng@xmu.edu.cn (H.F.C.); yaoxinsheng@vip.tom.com (X.S.Y.); Tel.: +86-15985841835

Received: 22 April 2009; in revised form: 25 May 2009 / Accepted: 16 June 2009 /

Published: 19 June 2009

---

**Abstract:** Two new steroidal saponins (**1** and **2**) were isolated from the dried bulbs of *Allium macrostemon* Bunge. Their structures were elucidated by the spectral data as 26-*O*- $\beta$ -D-glucopyranosyl-5 $\alpha$ -furost-25 (27)-ene-3 $\beta$ , 12 $\beta$ , 22, 26-tetraol-3-*O*- $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 2) [ $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 4)- $\beta$ -D-galactopyranoside (**1**) and 26-*O*- $\beta$ -D-glucopyranosyl-5 $\beta$ -furost-20 (22)-25 (27)-dien-3 $\beta$ , 12 $\beta$ , 26-triol-3-*O*- $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 2)- $\beta$ -D-galactopyranoside (**2**), respectively. Their cytotoxic activities on several cancer cell lines (MCF-7, NCI-H460, SF-268 and HepG2) were tested. **1** showed special cytotoxicity on SF-268, while **2** showed cytotoxicity on NCI-H460 and SF-268 cell lines, respectively.

**Keywords:** *Allium macrostemon* Bunge; steroidal saponins; cytotoxic activity

---

### Introduction

The dried bulbs of *Allium macrostemon* Bunge are well known as the traditional Chinese medicine “Xie bai”, which is used for treatment of thoracic pain, stenocardia, heart asthma and diarrhea [1]. The main constituents of the ethanol extract of *Allium macrostemon* Bunge are steroidal saponins, which showed inhibitory effects on platelet aggregation [2] and degradation effects on blood serum

peroxides proton [3], as well as the cytotoxic activity on different cancer cell lines [4,5]. This paper describes the isolation and structural elucidation of two new steroid saponins from “Xie bai” ethanol extract. Their cytotoxic activities against several human cancer cell lines, including MCF-7 (breast), NCI-H460 (lung), SF-268 (CNS) and HepG2 (liver), were also tested.

## Results and Discussion

The dried bulbs of *Allium Macrotomon* Bunge were extracted with 60% ethanol. The concentrated ethanol extract was passed through a Diaion HP-20 column eluting with a EtOH-H<sub>2</sub>O gradient. The 60% ethanol eluting fraction was collected and further fractionated by silica gel and octadecylsilylated silica gel and repeated Prep-HPLC to yield compounds **1** and **2**.

Compound **1** was obtained as an amorphous powder. The molecular formula was determined as C<sub>57</sub>H<sub>94</sub>O<sub>30</sub> by the HR-ESIMS at *m/z* 1281.5723 [M+Na]<sup>+</sup> (calcd. 1281.5728). The <sup>1</sup>H-NMR of **1** showed three methyl signals at δ 0.63 (s), 1.36 (s) and δ 1.61 (d, *J* = 6.8 Hz). Of all 57 carbon signals observed in <sup>13</sup>C-NMR spectrum, 27 carbon signals (Me × 3, CH<sub>2</sub> × 12, CH × 8, C × 4), including a characteristic C<sub>25</sub>-C<sub>27</sub> double bond carbon signals at δ 147.2 and 110.4. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data indicated that **1** had the same sugar moiety as that of Macrostemonoside E [6] but a different aglycone. Comparison of the <sup>13</sup>C-NMR data of the aglycone of **1** with those for Macrostemonoside E suggested differences at C-12, C-20, C-22, C-25 and C-27. The absence of characteristic C<sub>20</sub>-C<sub>22</sub> double bond carbon at δ 152.4 and 103.7 and the presence of carbon signal at δ 110.9 revealed the presence of a hydroxyl group at C-22. In HMBC spectrum, the long-range correlations from δ 1.36 (H-18) to δ 79.3 (C-12) suggested the presence of hydroxyl at C-12 and correlations from olefinic proton signals at δ 5.34, 5.70 (H-27) to δ 72.0 (C-26), 147.2 (C-25), 28.4 (C-24) suggested the presence of a C<sub>25</sub>-C<sub>27</sub> double bond in the aglycone. In the NOESY spectrum, the absence of NOE correlation between proton signals at d 1.36 (H-18) and 3.58 (H-12) suggested the β-orientation of the C-12 hydroxyl and this was also confirmed by the comparison of C-12 carbon signal at δ 79.3 with Macrostemonoside G [7] which has the β-orientation of the C-12 hydroxyl at δ 79.6.

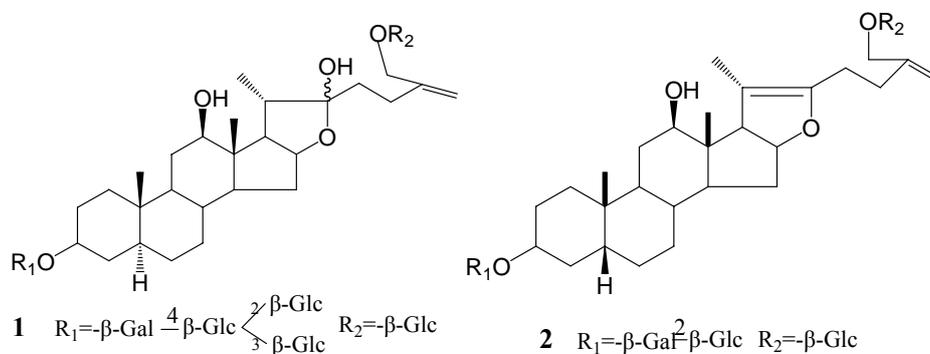
With the same NMR data of the sugar parts compared with Macrostemonoside E, the sugar moiety was finally unambiguously determined by acid hydrolysis and analysis of a combination of DEPT, <sup>1</sup>H-<sup>1</sup>H COSY, HMQC, HMBC and TOCSY spectra. Then, the structure of **1** was determined as 6-*O*-β-D-glucopyranosyl-5α-furost-25(27)-ene-3β,12β,22,26-tetraol-3-*O*-β-D-glucopyranosyl (1→2) [β-D-glucopyranosyl (1→3)]-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside.

Compound **2** was also isolated as an amorphous powder. Its molecular formula of C<sub>45</sub>H<sub>72</sub>O<sub>19</sub> was deduced by positive-ion HR-ESIMS at *m/z* 939.4572 [M+Na]<sup>+</sup> (calcd. 939.4566). The <sup>1</sup>H-NMR spectrum contained three steroid methyl groups at δ 0.92 (3H, s), 0.99 (3H, s) and 2.00 (3H, s). These three single peak methyl proton signals, especially the signal shift downfield to δ 2.00 compared with that of signal at H-21 of **1**, revealed the presence of double bonds at C<sub>25</sub>-C<sub>27</sub> and C<sub>20</sub>-C<sub>22</sub>. Comparison of the <sup>13</sup>C-NMR spectrum of **2** with that of Macrostemonoside G [7] showed considerable structural similarity. However, the molecular formula of **2** was lower by 18 units (one H<sub>2</sub>O) than that of Macrostemonoside G and the difference were recognized in the carbon signals from the ring E portion. The <sup>13</sup>C-NMR spectrum showed that the carbon signals of C-16, C-17, C-20, C-22 and C-24 of **2** were shifted downfield by approximately + 3.3, + 0.9, + 63.2, + 31.1 and + 2.8 ppm, respectively, while the

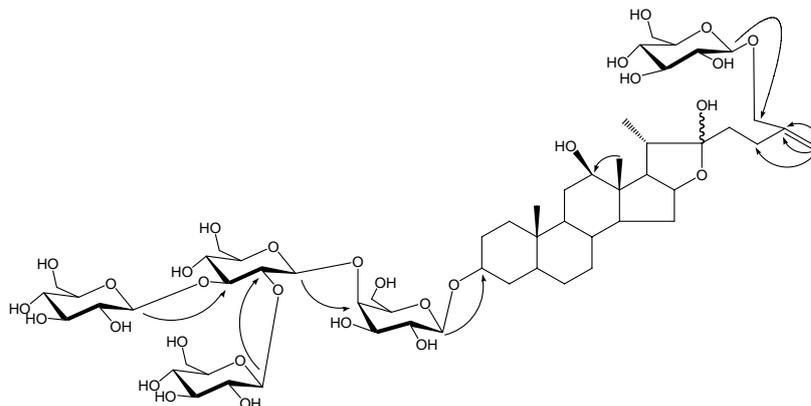
carbon signal of C-21 and C-23 shifted to higher field by - 3.3 and -13.1 ppm comparing with those of Macrostemonoside G, which suggested the presence of a double bond between C-20 and C-22. This was also confirmed by long-range correlations between the proton signal at  $\delta$  0.92 (H-21) and carbon signals at  $\delta$  104.8 (C-20) and 151.7 (C-22) in the HMBC spectrum. The HMBC correlation between the proton signal at  $\delta$  0.92 (CH<sub>3</sub>-18) and the carbon signal at  $\delta$  78.7 (C-12) indicated a hydroxyl group at C-12. In the NOESY spectrum, the  $\beta$ -orientation of the C-12 hydroxyl group was inferred due to the absence of NOE correlation between the proton signals at  $\delta$  0.92 (H-18) and 3.53 (H-12). The triglycoside moiety of **2** was shown to be the same as that Macrostemonoside G and the structure of **2** was assigned as 26-*O*- $\beta$ -D-glucopyranosyl-5 $\beta$ -furost-20 (22)-25 (27)-dien-3 $\beta$ ,12 $\beta$ ,26-triol-3-*O*- $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 2)- $\beta$ -D-galactopyranoside.

The *in vitro* cytotoxicity of compounds **1** and **2** against various cancer cell lines was evaluated by MTT assay. IC<sub>50</sub> values were calculated by the LOGIT method (Table 1). Compound **1** showed mild cytotoxicity, especially towards the SF-268 cell line with IC<sub>50</sub> values of 35.2  $\mu$ M and compound **2** showed mild cytotoxicity, especially to the SF-268 and NCI-H460 cell lines, with IC<sub>50</sub> values of 25.7 and 35.4  $\mu$ M, respectively. Comparison of the structure and the cytotoxic activity of **1** and **2** with those steroidal saponins reported previously [5] suggests that the presence of a C<sub>25</sub>-C<sub>27</sub> double bond and C-12 hydroxyl in the steroidal pentaglycoside aglycone contribute to the cytotoxicity in the SF-268 cell line and a C<sub>20</sub>-C<sub>22</sub> double bond in the steroidal triglycoside aglycone containing a C-12 hydroxyl contribute to the selective cytotoxicity to the SF-268 and NCI-H460 cell lines, respectively.

**Figure 1.** The structures of compounds **1** and **2**.



**Figure 2.** The key HMBC of compound **1**.



**Table 1.** *In vitro* cytotoxic activity of compounds 1 and 2 on cancer cell lines<sup>a</sup>.

Compounds	Cell lines IC <sub>50</sub> (μM)			
	NCI-H460	SF-268	MCF-7	HEPG2
1	>100	35.2±1.02	>100	>100
2	25.7±0.62	35.4±0.71	>100	>100

<sup>a</sup> Data shown are the mean IC<sub>50</sub> of three independent experiments ±SD.

**Table 2.** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data of compound 1 (C<sub>5</sub>D<sub>5</sub>N)<sup>a</sup>.

Position	δ <sub>C</sub>	δ <sub>H</sub>	Position	δ <sub>C</sub>	δ <sub>H</sub>
1	37.1(t)	1.54, 0.79(o)	C3 Gal-1	102.4(d)	4.81(d, <i>J</i> = 7.8)
2	29.8(t)	1.96, 1.22(o)	2	73.1(d)	4.44(o)
3	75.1(d)	4.07(o)	3	75.5(d)	4.23(o)
4	34.7(t)	1.78, 1.31(o)	4	80.1(d)	4.62(m)
5	44.6(d)	0.83(o)	5	76.1(d)	4.14(o)
6	28.9(t)	1.14, 1.09(o)	6	60.5(t)	4.65,4.23(o)
7	32.2(t)	1.49, 0.48(o)	(Inner)Glc-1	105.0(d)	5.51(d, <i>J</i> = 7.8)
8	34.3(d)	1.46(o)	2	81.4(d)	4.35(o)
9	53.5(d)	0.63(o)	3	88.5(d)	4.21(o)
10	35.7(s)	----	4	70.7(d)	3.81(o)
11	31.6(t)	1.83, 1.46(o)	5	77.8(d)	4.16(o)
12	79.3(d)	3.58(m)	6	62.3(t)	4.56(o)
13	46.7(s)	----	3-Glc-1	104.5(d)	5.26 (d, <i>J</i> = 7.8)
14	55.0(d)	1.14(o)	2	75.2(d)	4.05(o)
15	32.4(t)	2.06, 1.55(o)	3	78.6(d)	3.81(o)
16	81.2(d)	5.07(o)	4	70.8(d)	4.26(o)
17	63.7(d)	2.33 (t, <i>J</i> = 8.2)	5	77.5(d)	3.82(o)
18	11.3(q)	1.36(s)	6	62.7(t)	4.14,4.28(o)
19	12.2(q)	0.63(s)	2-Glc-1	104.8(d)	4.75 (d, <i>J</i> = 7.8)
20	41.6(d)	2.41(m)	2	75.1(d)	4.10(o)
21	15.6(q)	1.61 (d, <i>J</i> = 6.8)	3	78.6(d)	4.24(o)
22	110.9(s)	----	4	71.6(d)	4.20(o)
23	38.0(t)	2.45(o)	5	78.4(d)	3.95(o)
24	28.4(t)	2.76(m)	6	62.8(t)	4.18,4.05(o)
25	147.2(s)	-----	C-26 Glc-1	103.9(d)	5.15 (d, <i>J</i> = 7.8)
26	72.0(t)	4.52(o)	2	75.2(d)	4.03(o)
27	110.4(t)	5.34(s); 5.70(s)	3	78.6(d)	4.05(o)
			4	71.5(d)	4.21(o)
			5	78.5(d)	4.18(o)
			6	63.0(t)	4.52,4.23(o)

<sup>a</sup>Recorded on a Bruker-400 NMR spectrometer (100 MHz for <sup>13</sup>C).

**Table 3.**  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR data of compound **2** ( $\text{C}_5\text{D}_5\text{N}$ )<sup>a</sup>.

Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$	Position	$\delta_{\text{C}}$	$\delta_{\text{H}}$
1	31.0(t)	1.79, 1.95 (o)	C3 Gal-1	102.5(d)	4.90 (d, $J=7.6$ )
2	26.8(t)	2.00, 1.49 (o)	2	81.8(d)	4.65 (o)
3	75.5(d)	4.33 (o)	3	75.2(d)	4.23 (o)
4	31.0(t)	1.79, 1.98 (o)	4	69.9(d)	4.56 (o)
5	36.8(d)	2.17 (o)	5	76.6(d)	4.00 (o)
6	27.1(t)	1.21, 1.91 (o)	6	62.2(t)	4.35, 4.42 (o)
7	26.8(t)	1.99, 1.51 (o)	Glc-1	106.0(d)	5.29 (d, $J=7.6$ )
8	34.3(d)	1.45 (o)	2	76.9(d)	4.05 (o)
9	39.5(d)	1.46 (o)	3	78.1(d)	4.15 (o)
10	35.3(s)	-----	4	71.8(d)	4.35 (o)
11	31.6(t)	2.50 (o)	5	78.4(d)	3.83 (o)
12	78.7(d)	3.53 (m)	6	62.9(t)	4.32, 4.51 (o)
13	50.0(s)	-----	C26 Glc-1	103.8(d)	4.93 (d, $J=7.6$ )
14	53.5(d)	3.24 (m)	2	75.2(d)	4.00 (o)
15	31.2(t)	1.87 (o)	3	78.6(d)	4.21 (o)
16	84.6(d)	4.92 (o)	4	71.9(d)	4.22 (o)
17	64.6(d)	2.92 (d, $J=10.4$ )	5	78.5(d)	3.90 (o)
18	9.5(q)	0.92 (s)	6	62.9(t)	4.37, 4.57 (o)
19	23.9(q)	0.99 (s)			
20	104.8(s)	-----			
	)				
21	12.3(q)	2.00 (s)			
22	151.7(s)	-----			
	)				
23	24.9(t)	2.48 (o)			
24	31.2(t)	2.54 (o)			
25	146.3(s)	-----			
	)				
26	71.8(t)	4.43, 4.00 (o)			
27	111.6(t)	5.10, 5.39 (o)			

<sup>a</sup> Recorded on a Bruker-400 NMR spectrometer (100 MHz for  $^{13}\text{C}$ ).

## Experimental

### General

Melting points were determined with a Yanaco MP-S<sub>3</sub> 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 spectrometer in  $\text{C}_5\text{D}_5\text{N}$  solution (at 400 MHz for  $^1\text{H}$ -NMR). 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 $\mu\text{m}$ , Merck). TLC analyses were taken on Silica gel 60F<sub>254</sub>

(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× 20mm, 10µm, SHIMADZU Pak; Detector: RID). 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), were 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).

#### Plant material

The bulbs of *Allium Macrostemon* Bunge were purchased from Liaoning Province, P. R. China, and identified by Professor Qishi Sun (Department of Pharmacognosy, Shenyang Pharmaceutical University). The voucher specimen (No.203554) has been deposited at the Department of Natural Product Chemistry, Shenyang Pharmaceutical University, P. R. China.

#### Extraction and isolation

The dried bulbs of *Allium Macrostemon* Bunge (6 kg) were extracted twice with 60% ethanol for 2 hours each. The alcoholic extract was concentrated under reduced pressure, suspended in water and then passed through Diaion HP-20 column using EtOH-H<sub>2</sub>O gradient system (0-100%). The 60% EtOH eluate fraction (100 g), which was subjected to silica gel column chromatography with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (9:1:0.1; 8:2:0.2; 7:3:0.5; 6:4:0.8) and MeOH finally, gave nine fractions. Fraction 6 was further purified by ODS column chromatography eluting with MeOH-H<sub>2</sub>O (3:7; 4:6; 5:5) and repeated Rp-18 HPLC preparation to yield **1** (10.2 mg) and **2** (15.5 mg).

**Compound 1:** An amorphous powder, mp 205-206°C;  $[\alpha]_D^{21} -27.4^\circ$  (H<sub>2</sub>O, *c* 0.10); HR-ESIMS (positive mode) at *m/z* 1281.5723 [M+Na]<sup>+</sup> (calcd. 1281.5728); ESIMS (positive mode) at *m/z* 1,281 [M+ Na]<sup>+</sup>, 1,263 [M+ Na- H<sub>2</sub>O]<sup>+</sup>, 1,119 [M+ Na- 162]<sup>+</sup>, 1,101 [M+ Na- H<sub>2</sub>O- 162]<sup>+</sup>, 939 [M+ Na- H<sub>2</sub>O-162× 2]<sup>+</sup>, 777 [M+ Na- H<sub>2</sub>O- 162× 3]<sup>+</sup>; ESIMS (negative mode) at *m/z* 1,257 [M- H]<sup>-</sup>, 1,095 [M- H- 162]<sup>-</sup>, 933 [M-H-162×2]<sup>-</sup>, 771 [M- H- 162×3]<sup>-</sup>, 609 [M- H- 162× 4]<sup>-</sup>; IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3,420 (OH), 2,938 (CH), 1,000-1,100; <sup>1</sup>H-NMR (C<sub>5</sub>D<sub>5</sub>N) and <sup>13</sup>C-NMR data see Table 2.

**Compound 2:** An amorphous powder, mp 175-177°C;  $[\alpha]_D^{21} 4.8^\circ$  (H<sub>2</sub>O, *c* 0.08); HR-ESIMS (positive mode) at *m/z* 939.4572 [M+Na]<sup>+</sup> (calcd. 939.4566). FABMS (*n*= 1) at *m/z* 917 [M+ H]<sup>+</sup>, 755 [M+ H- 162]<sup>+</sup>, 737 [M+ H- 162- H<sub>2</sub>O]<sup>+</sup>, 593 [M+ H- 162× 2]<sup>+</sup>, 575 [M+ H- 162× 2- H<sub>2</sub>O]<sup>+</sup>, 431 [M+ H- 162×2- 162]<sup>+</sup>; IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3,410 (OH), 2,924 (CH), 1,010-1,120; <sup>1</sup>H-NMR (C<sub>5</sub>D<sub>5</sub>N) and <sup>13</sup>C-NMR data see Table 3.

#### Acid hydrolysis of saponins

Each saponin (5 mg) was heated in an ampoule with aq. 15% HCl (5 mL) at 110 °C for 2 h. The aglycon was extracted with dichloromethane three times and the aqueous residue was evaporated under reduced pressure. Then, pyridine (1 mL) and NH<sub>2</sub>OH·HCl (2 mg) were added to the residue, and the mixture was heated at 100 °C for 1 h. After cooling, Ac<sub>2</sub>O (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 aldonitrile peracetates were analyzed by GC-MS using standard aldonitrile peracetates as reference samples.

### Cell culture

MCF-7, NCI-H460, SF-268 and HepG2 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. 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 hr prior to treatment.

### Cytotoxicity assay

$1.0 \times 10^4$  MCF-7, NCI-H460, SF-268 and HepG2 cells were seeded in 96 well tissue culture plates and treated with the two compounds on different concentration for 48 h. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) reagent (5 mg/mL in PBS, 10 µL) was added to each well and incubated for 4 h. After that, the suspended liquid was poured out and DMSO (100 µL) added to each well and swirled gently. Finally, the plate cover was removed and the absorbance in each well measured at 570 nm in a micro titer plate reader [8].

### Acknowledgements

The authors are grateful to Mr. Jinghui Huang (Shenzhen Research Center of Traditional Chinese Medicines and Natural Products, Shenzhen, P.R. China) for all the NMR and ESIMS measurements.

### References

1. Jiangsu New Medical College. *Dictionary of Chinese Drugs*; Shanghai Science and Technological Publisher: Shanghai, China, 2001; pp. 2642-2643.
2. Peng, J.P.; Wang, X.; Yao, X.S. Study on two new furostanol glycosides from *Allium macrostemon* Bunge. *Chin. Chem. Lett.* **1998**, *33*, 355-361.
3. Jiang, Y.; Wang, N.L.; Yao, X.S. Progress studying traditional Chinese medicine “Xiebai”. *Nat. Prod. Res. Dev.* **2000**, *12*, 74-79.
4. Chen, H.F.; Wang, N.L.; Yao, X.S. Study on bioactive steroidal saponins of *Allium Macrostemon* Bunge. *Chin. J. Med. Chem.* **2005**, *15*, 142-146.
5. Chen, H.F.; Wang, G.H.; Wang, N.L.; Yang, M.S.; Wang, Z.; Wang, X.W.; Yao, X.S. New furostanol saponins from the bulbs of *Allium Macrostemon* Bunge and their Cytotoxic activity. *Pharmazie* **2007**, *62*, 544-548.
6. Peng, J.P.; Wang, X.; Yao, X.S. Studies on two new furostanol glycosides from *Allium Macrostemon* Bunge. *Yao Xue Xue Bao* **1993**, *28*, 526-531.
7. Peng, J.P.; Yao, X.S.; Kobayashi, H.; Ma, C.Y. Novel furostanol glycosides from *Allium macrostemon*. *Planta Med.* **1995**, *61*, 58-61.

8. Carmichael, J.; DeGraff, W.G.; Gazdar, A.F.; Minna, J.D.; Mitchell, J. Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity. *Cancer Res.* **1987**, *47*, 936-942.

*Sample Availability:* Samples of the compound **1** and **2** are available from the authors.

© 2009 by the authors; licensee Molecular Diversity Preservation International, Basel, Switzerland. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).