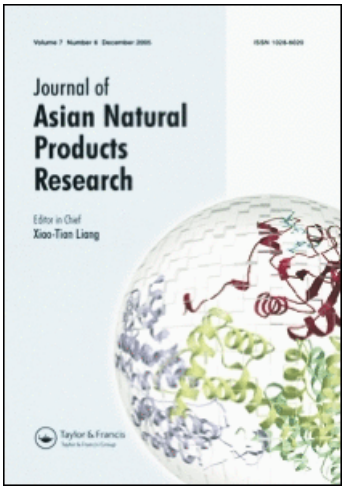

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Novel furostanol saponins from the bulbs of *Allium macrostemon* B. and their bioactivity on $[Ca^{2+}]_i$ increase induced by KCl

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Chemical reinvestigation of the ethanol extract of the dried bulbs of *Allium macrostemon* B. led to the isolation of two novel furostanol saponins, named macrostemonoside M (**1**) and macrostemonoside N (**2**), together with six known saponins. The structures of new compounds were elucidated on the basis of extensive spectroscopic analysis including 1D and 2D NMR as (25*R*)-22-hydroxy-5β-furostane-1β,2β,3β,6α-tetraol-26-*O*-β-D-glucopyranoside and 22-hydroxy-5β-furost-25-(27)-ene-1β,2β,3β,6α-tetraol-26-*O*-β-D-glucopyranoside, respectively. The pharmacological activities of all the saponins on $[Ca^{2+}]_i$ increase induced by KCl were evaluated.

Keywords: *Allium macrostemon* B; Novel furostanol saponins; Macrostemonoside M; Macrostemonoside N; $[Ca^{2+}]_i$ increase

1. Introduction

Allium macrostemon B. is widely distributed in the northeast of China. Its dried bulbs were popularly known as a traditional Chinese medicine "Xiebai" which can be used for treatment of some heart diseases such as throracic pain, stenocardia, heart asthma, etc. [1]. In our previous reports, we revealed 13 new active compounds from this plant by bioactivity-guided isolation, some of which presented strong inhibitory activity on ADP-induced human platelet aggregation [2–9]. The chemical reinvestigation of ethanol extract of *A. macrostemon* B. resulted in the isolation of two novel furostanol saponins (**1**, **2**), named as macrostemonoside M and N, and six known saponins. The structures (figure 1) were established by analysis of IR, ESI-MS, ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}HCOSY, TOCSY, NOESY, HMQC and HMBC

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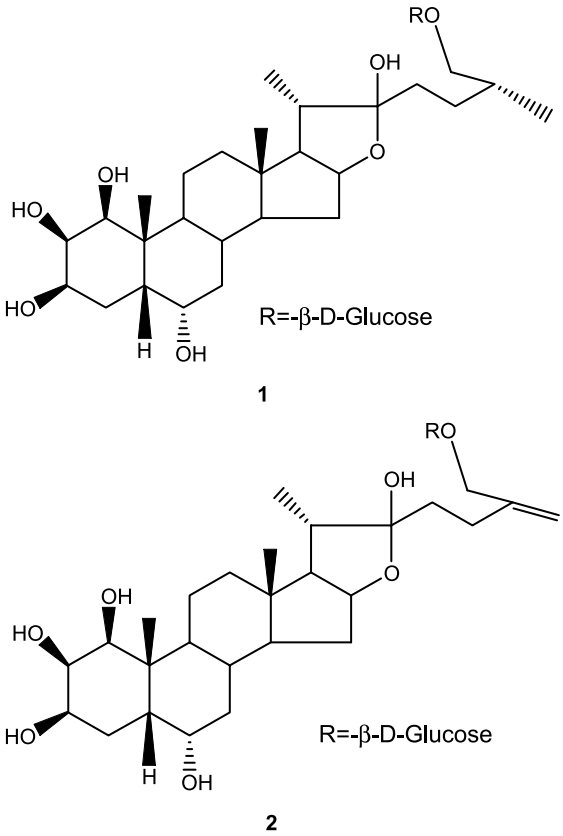

Figure 1. Structures of compounds **1** and **2**.

spectra. All compounds were tested for the effects on $[Ca^{2+}]_i$ increase induced by KCl in guinea-pig cardiomyocytes.

2. Results and discussion

The structures of compounds **3**–**8** were determined by the comparison of their spectroscopic data with literature values of known compounds as (25*R*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-furost-3β,26β-diol-3-*O*-β-D-glucopyranosyl(1-2)-β-D-galactopyranoside, (25*S*)-26-*O*-β-D-glucopyranosyl-22-hydroxy-furost-3β,26β-diol-3-*O*-β-D-glucopyranosyl(1-2)-β-D-galactopyranoside, macrostemonoside E, macrostemonoside B, macrostemonoside F and macrostemonoside G [3,9,13,14].

Compound **1** was obtained as an amorphous powder, mp 154.5–155.5°C, $[\alpha]_D^{25}$ 47.5 (*c* 0.08, H_2O). Compound **1** was positive to the Ehrlich reagent reaction, suggesting a furostanol saponin structure for **1**. The IR spectrum gave a strong absorption attributable to hydroxyl groups (3418 cm^{-1}) and a glycosidic linkage (1034 cm^{-1}). Its molecular formula $C_{33}H_{56}O_{12}$ was determined from the HRESI-MS and NMR data. Positive and negative ESI-MS spectra of **1** revealed pseudo-molecular ion peaks and corresponding fragments at *m/z* 667 $[M + Na]^+$, 649 $[M + Na - H_2O]^+$, 487 $[M + Na–Glc–H_2O]^+$, and 643 $[M - H]^-$, 625 $[M - H–H_2O]^-$, 481 $[M - H–Glc]^-$. The ^1H NMR spectrum indicated four characteristic methyl proton signals

at δ 1.31 (d, $J = 6.8$ Hz), 0.97 (d, $J = 6.8$ Hz), 1.38 (s), and 0.89 (s) for the steroidal skeleton, and a sugar anomeric proton signal at δ 4.80. In its ^{13}C NMR spectrum, 33 carbon signals were detected, and 27 of them were assigned to aglycone moiety as four methyls, eight methylenes, 12 methines (including five oxygenated methines) and three quaternary carbons in combination with DEPT spectral analysis. The remaining six carbon signals were assigned to the sugar moiety that was determined to be glucose by comparing the NMR data with previously reported values. The β-configuration of anomeric proton was determined by the large coupling constant ($J_{1,2} = 8.0$).

Acid hydrolysis of compound **1** yielded aglycone (**1a**) and glucose. The ^{1}H–^{1}H COSY spectrum of **1** showed that the methine proton at δ 2.87 (H-5) was coupled to the methylene proton at δ 2.08 (H-4$_{ax}$) and oxymethine proton at δ 4.46 (H-6), and the methylene proton at δ 2.08 (H-4$_{ax}$) was coupled to the oxymethine proton at δ 4.65 (H-3), which confirmed the connectivity of protons for A ring. HMBC correlations also indicated the placement of four hydroxyl groups at C-1, C-2, C-3 and C-6. The HMBC correlation observed between the anomeric proton at δ 4.80 and carbon signal at δ 75.2 (C-26) determined the connection position of glucose on **1** (figure 2). ^{1}H and ^{13}C signals of **1** were definitely assigned by HMQC, ^{1}H–^{1}H COSY, TOCSY and HMBC experiments.

The (25*R*) configuration of **1** was deduced from the resonance of ^{1}H and ^{13}C signals (C-24, C-25, C-26 and C-27) around the C-25 centre as described in previous reports [10,11], which was also confirmed by the IR spectrum (intensity: 918 $<$ 889 cm^{-1}) of **1a** [12]. The relative stereochemistry of A and B ring in **1** was determined by analysis of the NOESY spectrum of **1a** (figure 3). In its NOESY spectrum, the NOE correlation between CH_3-19 and H-5 indicated the β-orientation of H-5, and therefore the α-orientation of the hydroxyl group at C-6 was judged due to the NOE correlation between H-5 and H-6. Furthermore, the NOES between the H-5β/H-4β, H-4α/H-3α, H-3α/H-2α, and H-2α/H-1α indicated that the hydroxyl groups at C-1, C-2, C-3 and C-6 were β-axial, β-equatorial, β-axial and α-equatorial, respectively. Thus, the structure of compound **1** was determined as (25*R*)-22-hydroxy-5β-furost-1β,2β,3β,6α-tetraol-26-*O*-β-D-glucopyranoside, named as macrostemonoside M.

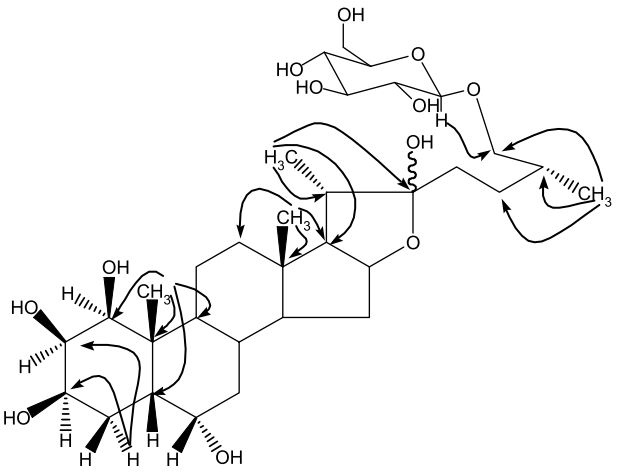

Figure 2. Selected HMBC correlations of compound **1**.

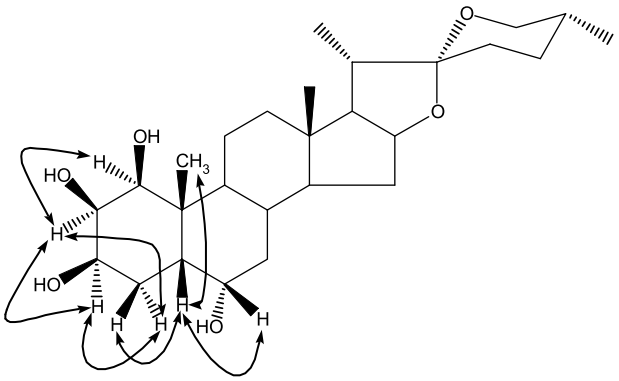

Figure 3. Key NOESY correlations of compound **1a**.

Compound **2** was isolated as a white amorphous powder, mp 151–153°, $[\alpha]_D^{25}$ −52.4 (*c* 0.07, H_2O), and showed positive reaction with Ehrlich reagent. The HRESI-MS showed the $[M + Na]^+$ ion at *m/z* 665.3480, consistent with the molecular formula of $C_{33}H_{54}O_{12}$. Combined with ^1H NMR and ^{13}C NMR spectral data, it was also judged by positive ESI-MS at *m/z* 665 $[M + Na]^+$, 647 $[M + Na–H_2O]^+$ and negative ESI-MS at *m/z* 641 $[M - H]^-$, 479 $[M - H–Glc]^-$, 461 $[M - H–Glc–H_2O]^-$. On acid hydrolysis of **2**, sugar components were identified as glucose by high-performance thin-layer chromatography (HPTLC) with authentic sample. Unambiguous complete assignment of the ^1H NMR and ^{13}C NMR signals for **2** was made by a combination of its DEPT, $^1H–^1H$ COSY, HMQC, HMBC, TOCSY and NOESY spectra. In ^1H NMR, three methyl proton signals at δ 1.37 (3H, s), 1.28 (3H, d, $J = 8.0$) and 0.89 (s) were observed and unsaturated methylene protons (δ 5.32, s; 5.04, s) in the downfield region revealed the presence of a double bond between C-25 and C-27. This was also supported by double bond carbon signals at δ 147.2 (C-25) and 110.6 (C-27) in the ^{13}C NMR spectrum. On comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1**, they were very similar to those of **1** with the exception of the signals due to C-25, C-26 and C-27. The signals for the $>CH–CH_3$ group at C-25 (27) in **1** were replaced by the signals assigned to the C-25 (27) exomethylene group at δ_H 5.32 (s) and 5.04 (s), and δ_C 147.2 (CH) and 110.6 (CH_3) in **2**. Furthermore, the significant downfield shift of about +3.2 ppm of the carbon signal due to C-26 was observed and the molecular formula of **1** was greater by 2 amu than that of **2**. These data strongly indicated that **2** was 25(27)-ene-macrostemonoside M. The relative stereochemistry of **2** was determined using the same way as **1** from the 1D and 2D NMR spectra. In view of the spectroscopic evidence, the structure of **2** was determined as 22-hydroxy-5β-furost-25(27)-ene-1β,2β,3β,6α-tetraol-26-*O*-β-D-glucopyranoside, named macrostemonoside N.

$[Ca^{2+}]_i$ was elevated gradually after application of KCl (60 mmol/L) in the presence of Ca^{2+}(1.8 mmol/L) and the FI value was increased from (200.01 ± 9.13) (resting) to (767.70 ± 78.20) (peak). After pre-treatment with sample (10 μmol/L) for 5 min, the results of compounds **1**, **2**, **3** and **6** showed that the increase of $[Ca^{2+}]_i$ mobilized by KCl was further increased. The magnitude of enhancement was much higher than that in the KCl group, which suggesting these compounds may increase the function of cardiac muscle. Compound **5** inhibited the increase of $[Ca^{2+}]_i$ mobilized by KCl during 50–200 s, indicating its potential for the treatment of heart failure (figure 4).

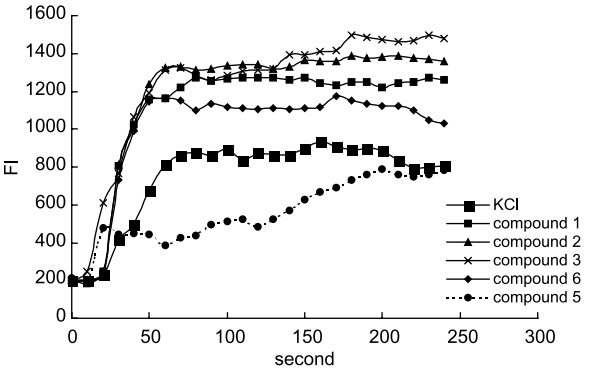

Figure 4. Effects of compounds **1**, **2**, **3**, **5**, **6** on $[Ca^{2+}]_i$ increase induced by KCl in guinea-pig cardiomyocytes.

3. Experimental

3.1 General experimental procedures

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 Corp.). 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 ^{1}H NMR) spectrometer in C_5D_5N solution. ESI-MS 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) and the spots were detected by spraying with Ehrlich reagent and heating. Preparative HPLC was performed using an ODS column (250 × 20 mm, 10 μm, Shimadzu Pak; Detector: RID). Fluorescence intensity (FI) of $[Ca^{2+}]_i$ was measured on a laser scanning confocal microscope (Insight Plus-IQ, Meridian, USA).

3.2 Plant material

The bulbs of *Allium macrostemon* B. were purchased from Liaoning Province of China and were 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, Shenyang, China.

3.3 Extraction and isolation

The dried bulbs (5 kg) of *A. macrostemon* B. were refluxed with 60% ethanol, and the concentrated ethanol extract was dissolved in water and filtered. The filtrate was subjected to column chromatography on Diaion HP-20 (1 kg) and eluted with H_2O, 60% EtOH and 95% EtOH, respectively. The fraction eluted with 60% EtOH (130 g) was evaporated and then dissolved in MeOH. The MeOH-soluble fraction was further separated on silica gel column (600 g) using $CHCl_3$/MeOH/H_2O in a gradient way to give nine fractions (fr.1 to fr.9).

Fr.2 (2.0 g) eluted with $CHCl_3$/MeOH/H_2O (8:2:0.2) was subjected to ODS column by eluting with MeOH/H_2O (3:7) and finally purified by preparative HPLC to afford compounds **1** (42 mg) and **2** (46 mg). Fr.5 eluted with $CHCl_3$/MeOH/H_2O (7:3:0.5) was further separated by ODS (50 g) and preparative HPLC with MeOH/H_2O (7:3) as eluent to give compounds **3** (35.4 mg), **4** (219.2 mg) and **5** (74.6 mg). Compounds **6** (120.1 mg), **7** (60.0 mg) and **8** (25.3 mg) were also purified from fr.5 by Rp-18 HPLC with MeOH/H_2O (5:5).

3.3.1 Compound 1. Amorphous powder, mp 154.5–155.5°C; $[\alpha]_D^{25}$ −47.5 (*c* 0.008, H_2O); IR (KBr) ν_{max} 3418 (OH), 2936 (CH), 1647, 1454, 1381, 1258, 1034, 910, 876, 799, 532, 417 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) data: see table 1; ^{13}C NMR (C_5D_5N, 100 MHz) data: see table 2; ESI-MS (positive ion mode) *m/z* 667 $[M + Na]^+$, 649 $[M + Na–H_2O]^+$, 487$[M + Na–Glc–H_2O]^+$; ESI-MS (negative ion mode) 643 $[M - H]^-$, 625 $[M - H–H_2O]^-$, 481 $[M - H–Glc]^-$; HRESI-MS *m/z* 667.3676 $[M + Na]^+$ (calcd for $C_{33}H_{56}O_{12}Na$, 667.3669).

3.3.2 Compound (1a). Amorphous powder, $[\alpha]_D^{25}$ −69.8 (*c* 0.007, $CHCl_3$); IR (KBr) ν_{max} 3348 (OH), 2935 (CH), 1454, 1377, 1242, 1180, 1084, 1053, 1010, 980, 918, 889, 846, 791, 523 cm^{-1}; ^1H NMR (C_5D_5N) data: see table 1; ^{13}C NMR data: see table 2.

3.3.3 Compound (2). Amorphous powder, mp 151–153°C; $[\alpha]_D^{25}$ −52.4 (*c* 0.007, H_2O); IR (KBr) ν_{max} 3418 (OH), 2936 (CH), 1651, 1454, 1381, 1273, 1161, 1041,926, 876, 795, 486,

Table 1. ^1H NMR data of compounds **1**, **1a** and **2** (C_5D_5N).[a,b]

No.	***1***	***1a***	***2***	*No.*	***1***	***1a***	***2***
1	4.22 (m)	4.20 (s)	4.21 (m)	19	1.38 (s)	1.37 (s)	1.37 (s)
2	4.05 (m)	4.05 (t, 2.8)	4.05 (m)	20	2.22 (m)	1.96 (m)	2.25 (m)
3	4.65 (m)	4.63 (br s)	4.63 (m)	21	1.31 (d, 7.2)	1.12 (d, 6.8)	1.28 (d, 8.0)
4	2.72 (m)	2.72 (dt, 12.8, 3.6)	2.72 (m)	23	2.03 (m)	1.66 (m)	2.23 (m)
	2.08 (m)	2.06 (m)	2.06 (m)		2.02 (m)		
5	2.85 (m)	2.87 (dt, 13.2, 4.4)	2.87 (m)	24	2.04 (m)	1.56 (m)	2.72 (m)
					1.66 (m)		
6	4.46 (m)	4.43 (m)	4.45 (m)	25	1.91 (m)	1.59 (m)	––-
7	2.08 (m)	2.10 (m)	2.08 (m)	26	3.93 (m)	3.57 (m)	4.59 (m)
	1.47 (m)	1.46 (m)	1.45 (m)		3.61 (m)	3.49 (m)	4.33 (m)
8	1.79 (m)	1.80 (m)	1.78 (m)	27	0.97 (d, 7.2)	0.68 (d, 6.8)	5.32 (s)
							5.04 (s)
9	1.36 (m)	1.40 (m)	1.35 (m)	C-26 Glc			
11	1.42 (m)	1.42 (m)	1.43 (m)	1	4.80 (d, 8.0)		4.88 (d, 7.6)
	1.32 (m)	1.33 (m)	1.34 (m)				
12	1.69 (m)	1.65 (m)	1.68 (m)	2	4.01 (m)		4.06 (m)
	1.09 (m)	1.08 (m)	1.09 (m)				
14	1.23 (m)	1.26 (m)	1.24 (m)	3	4.22 (m)		4.21 (m)
15	1.94 (m)	1.94 (m)	1.94 (m)	4	4.21 (m)		4.23 (m)
	1.46 (m)	1.49 (m)	1.44 (m)				
16	4.96 (m)	4.59 (dd, 7.6, 6.8)	4.98 (m)	5	3.92 (m)		3.92 (m)
17	1.96 (m)	1.87 (m)	1.98 (m)	6	4.54 (m)		4.52 (m)
					4.38 (m)		4.37 (m)
18	0.89 (s)	0.84 (s)	0.89 (s)				

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

Table 2. ^{13}C NMR data of compounds **1**, **1a** and **2** (C_5D_5N).[a]

No.	***1***	***1a***	***2***	*No.*	***1***	***1a***	***2***
1	78.5	78.5	78.5	19	19.9	19.9	19.9
2	67.9	67.9	67.9	20	40.6	42.0	40.6
3	72.5	72.4	72.4	21	16.4	14.9	16.3
4	27.7	27.7	27.7	22	110.6	109.2	110.3
5	38.0	38.0	38.0	23	37.2	31.8	37.9
6	66.9	66.8	66.8	24	28.3	29.2	28.3
7	32.4	32.2	32.4	25	34.2	30.5	147.2
8	35.1	35.1	35.1	26	75.2	66.8	72.0
9	41.7	41.7	41.7	27	17.4	17.2	110.6
10	43.2	43.1	43.1	C-26 Glc			
11	21.3	21.3	21.3	1	104.9		103.9
12	40.1	40.1	40.1	2	75.1		75.1
13	41.1	40.8	41.1	3	78.5		78.5
14	56.0	56.1	56.0	4	71.7		71.6
15	35.7	35.7	35.7	5	78.4		78.5
16	81.0	81.0	81.1	6	62.8		62.7
17	64.0	63.1	63.9				
18	16.7	16.5	16.7				

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

463 cm^{-1}; ^1H NMR (C_5D_5N, 400 MHz) data: see table 1; ^{13}C NMR (C_5D_5N, 100 MHz) data: see table 2; ESI-MS (positive ion mode) *m/z* 665 $[M + Na]^+$, 647 $[M + Na–H_2O]^+$; ESI-MS (negative ion mode) 641 $[M - H]^-$, 479 $[M - H–Glc]^-$, 461 $[M - H–Glc–H_2O]^-$; HRESI-MS *m/z* 665.3480 $[M + Na]^+$ (calcd for $C_{33}H_{54}O_{12}Na$, 665.3513).

3.4 Acid hydrolysis of 1 and 2

Compound **1** (5 mg) was refluxed with 7% HCl (3 mL) for 3 h. The reaction mixture was partitioned with EtOAC and H_2O. The H_2O layer was concentrated and the monosaccharide was identified as glucose by HPTLC (silica gel), together with the authentic sample ($CHCl_3$/MeOH/H_2O, 10:5:1) [14,15].

Compound **2** (5 mg) was subjected to acid hydrolysis as described for **1**. The monosaccharide was also identified as glucose by HPTLC with the same chromatographic condition.

3.5 Cell isolation

Guinea-pigs were killed by cervical dislocation, then the heart was quickly removed and cannulated on a Langendorff apparatus and retrogradely perfused through the aorta with Ca^{2+}-containing standard Tyrode solution (mmol/L: NaCl 126, KCl 5.4, HEPES 10, NaH_2PO_4. $2H_2O$ 0.33, $MgCl_2 \cdot 6H_2O$ 1.0, $CaCl_2$ 1.8 and glucose 10; pH was adjusted to 7.40 ± 0.05 with NaOH) for 5 min. After the blood was washed out, the heart was perfused with Ca^{2+}-free standard Tyrode solution until it stopped beating. Thereafter, the heart was enzymatically digested with Ca^{2+}-free Tyrode solution containing collagenase II (8 mg/50 mL) and BSA (8 mg/50 mL). The ventricular tissue was minced after it softened and placed in KB medium (mmol/L: glutamic acid 70, taurine 15, KCl 30, KH_2PO_4 10, HEPES 10, $MgCl_2 \cdot 6H_2O$ 0.5, Glucose 10 and EGTA 0.5; pH was adjusted to 7.30–7.40 with KOH). Single cell was obtained by gentle pipetting and stored at 4°C for 1–2 h.

All solutions were gassed with 95% oxygen and 5% carbon dioxide and warmed to (37 ± 0.5)°C. Only rod-shaped myocytes with clear cross-striations were studied [16].

3.6 Fluo-3/AM loading

Isolated single cardiomyocytes were adhered to the coverslips of the chamber. Then cells were rinsed once with standard Tyrode solution and incubated with Fluo-3/AM 20 μmol/L working solution containing 0.03% Pluronic F-127 at 37°C for 45 min. After loading, cells were washed once with Tyrode solution to remove the extracellular Fluo-3/AM [17].

3.7 $[Ca^{2+}]_i$ measurement

The fluorescent change of the Fluo-3/AM loaded cell was detected by a laser scanning confocal microscope with 488 nm for excitation from an argon ion laser and 530 nm for emission, and an inverted microscope with 40 × objective. $[Ca^{2+}]_i$ was represented by fluorescent intensity (FI). The samples were added between the second and third scans and the images were stored on disk.

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References

[1] Jiangsu New Medical College, *Dictionary of Chinese Drugs*, pp. 2642–2643, Shanghai Science and Technological Publisher (2001).
[2] J.P. Peng, X.S. Yao, Y. Okada, T. Okuyama. *Chem. Pharm. Bull.*, **42**, 2180 (1994).
[3] J.P. Peng, X.S. Yao, H. Kobayashi, C.Y. Ma. *Planta Med.*, **61**, 58 (1995).
[4] J.P. Peng, X.S. Yao, Y. Okada, T. Okyuama. *Acta Pharm. Sin.*, **29**, 526 (1994).
[5] J.P. Peng, X. Wang, X.S. Yao. *Chin. Chem. Lett.*, **4**, 141 (1993).
[6] J.P. Peng, Y. Wu, X.S. Yao, T. Okuyama, T. Narui. *Acta Pharm. Sin.*, **27**, 918 (1992).
[7] Y. Wu, J.P. Peng, X.S. Yao, T. Okuyama, T. Narui. *Acta Pharm. Sin.*, **9**, 69 (1992).
[8] J.P. Peng, Y. Wu, X.S. Yao, T. Okuyama, T. Narui. *Chin. Chem. Lett.*, **3**, 285 (1992).
[9] J.P. Peng, X. Wang, X.S. Yao. *Acta Pharm. Sin.*, **28**, 526 (1993).
[10] Z.Z. Liang, R. Aquino, F.D. Simone, A. Dini, O. Schettino, C. Pizza. *Planta Med.*, **54**, 344 (1988).
[11] N. Fukuda, N. Imamura, E. Saito, T. Nohara, T. Kawasaki. *Chem. Pharm. Bull.*, **29**, 325 (1981).
[12] K. Keito, M. Kazumoto, M. Nobuhiro, T. Kawasaki. *Chem. Pharm. Bull.*, **32**, 4229 (1984).
[13] S. Nagumo, S. Kishi, T. Inoue, M. Nagai. *Yakugaku Zasshi*, **111**, 306 (1991).
[14] Z. Jiang, J.F. Gallard, M.T. Adeline, V. Dumontet, M.V. Tri, T. Sevenet, M. Pais. *J. Nat. Prod.*, **62**, 873 (1999).
[15] K. Koike, M. Kudo, Z.H. Jia, T. Nikaido, Y. Ide, T. Sakura. *J. Nat. Prod.*, **62**, 228 (1999).
[16] B.F. Yang, G.R. Li, C.Q. Xu. *Acta Pharmacol. Sin.*, **20**, 961 (1999).
[17] J. Ai, H.H. Gao, S.Z. He, L. Wang, D.L. Luo, B.F. Yang. *Acta Pharmacol. Sin.*, **22**, 512 (2001).