Chinese Journal of Natural Medicines

**Five new spirosterol saponins from Allii Macrostemonis Bulbus**

Rong WANG, Lulu WANG, Manli ZHANG, Yadi GUO, Jing ZHANG, Guoxu MA

**Citation:** Rong WANG, Lulu WANG, Manli ZHANG, Yadi GUO, Jing ZHANG, Guoxu MA, Five new spirosterol saponins from Allii Macrostemonis Bulbus, Chinese Journal of Natural Medicines, 2023, 21(3), 226−232. doi: 10.1016/S1875−5364(23)60423−6.

View online: https://doi.org/10.1016/S1875−5364(23)60423−6

Available online at www.sciencedirect.com
ScienceDirect
*Chinese Journal of Natural Medicines* 2023, **21**(3): 226-232
doi: 10.1016/S1875-5364(23)60423-6
Chinese Journal of Natural Medicines

•Original article•

# Five new spirosterol saponins from Allii Macrostemonis Bulbus

WANG Rong[1], WANG Lulu[2], ZHANG Manli[3], GUO Yadi[1], ZHANG Jing[1*], MA Guoxu[3*]

[1] *College of Chinese Medicinal Materials, Jilin Agricultural University, Changchun 130118, China;*
[2] *College of Medicine, Changchun University of Science and Technology, Changchun 130600, China;*
[3] *Key Laboratory of Bioactive Substances and Resource Utilization of Chinese Herbal Medicine, Institute of Medicinal Plant Development, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100193, China*

Available online 20 Mar., 2023

**[ABSTRACT]** Five new spirostanol saponins (**1**−**5**) and seven known compounds (**6**−**12**) were isolated from the *n*-butanol fraction of 75% ethanol extract of Allii Macrostemonis Bulbus. The identification and structural elucidation of all the isolates were performed through extensive 1D and 2D NMR experiments, HR-ESI-MS data analysis and comparisons with literature values. Antioxidant evaluation showed that compounds **6**−**11** exhibited certain scavenging effects on ABTS radical, where compounds **6**, **7** and **11** had $IC_{50}$ values of 0.208, 0.057 and 0.014 mg·mL$^{-1}$, respectively.

**[KEY WORDS]** Allii Macrostemonis Bulbus; Spirosterol saponin; Antioxidant
**[CLC Number]** R284.1 **[Document code]** A **[Article ID]** 2095-6975(2023)03-0226-07

## Introduction

Allii Macrostemonis Bulbus (Xiebai in Chinese) is the dried bulb of *Allium macrostemon* Bge. or *Allium chinense* G. Don which are perennial herbs of Liliaceae [1]. It is an important medicine for the treatment of "chest impediment". Pharmacological studies have revealed that Allii Macrostemonis Bulbus has antiplatelet aggregation, antitumor, anti-atherosclerosis, antioxidant, lipid-lowering, anti-spasmodic, antibacterial, and other bioactivities [2]. However, the pharmacodynamic material basis of this drug has not been clarified. Previous studies have reported that Allii Macrostemonis Bulbus contains abundant bioactive components, such as steroidal saponins [3-8], volatile oil [9-11], polysaccharides [12-14], and nitrogen-containing compounds [15, 16]. Among them, steroidal saponins are the main constitutes, and many saponins such as macrostemonosides A−S and chinenosides I−VII, have been isolated.

In this paper, continuing investigation of the *n*-butanol fraction of 75% ethanol extract through detailed chemical investigations by chromatography resulted in the isolation and identification of 12 compounds. Among them, compounds **1**−**5** are new compounds (Fig. 1), and compounds **6**−**8** are isolated from Allii Macrostemonis Bulbus for the first time. Moreover, the antioxidant activities of the isolated compounds were investigated. Herein, the detail of isolation, structural elucidation, and antioxidant evaluation of these compounds were reported.

## Results and Discussion

Compound **1** was isolated as white powder. Its reactions to Liebermann-Burchard and Molisch were positive, and to Ehrlich reagent was negative. After the thin layer of compound 1 was formed, the color developed with concentrated $H_2SO_4$/EtOH solution (1 : 9, *V*/*V*) was purplish red, which suggested that the compound may be a spirosterol saponin. The molecular formula $C_{51}H_{80}O_{24}$ was established from the HR-ESI-MS spectrum with a positive-ion peak at *m/z* 1099.4965 $[M + Na]^+$ (Calcd. 1099.4972). Analysis of its $^1$H NMR spectrum (Table 1) revealed the presence of three olefinic protons at $\delta_H$4.84 (1H, s, H-27), 4.80 (1H, s, H-27) and 5.19 (1H, m, H-6), three methyl protons at $\delta_H$0.88 (3H, s, Me-18), 0.83 (3H, s, Me-19), 1.11 (3H, d, *J* = 6.6 Hz, Me-21) of aglycone, and four anomeric protons at $\delta_H$5.62 (1H, d, *J* = 7.8 Hz, H-1'), 5.30 (1H, d, *J* = 7.8 Hz, H-1''), 5.34 (1H, d, *J* = 7.8 Hz, H-1'''), 4.92 (1H, d, *J* = 7.8 Hz, H-1'''') of four sugar moieties. The $^{13}$C NMR data (Table 2) showed a total of 51 carbon signals, where 27 carbons were assigned to the aglycone and the remaining 24 carbons to four sugar moieties. It revealed four terminal carbon signals at $\delta_C$103.63, 106.07, 105.49, 105.87 of four sugar moieties, while four character-

**[Received on]** 03-Aug.-2022
**[Research funding]** This work was supported by Jilin Science and Technology Development Program (No. 20200404023YY).
[*]**Corresponding author]** E-mails: zhjing0701@163.com (ZHANG Jing); mgxflfl8785@163.com (MA Guoxu)
These authors have no conflict of interest to declare.

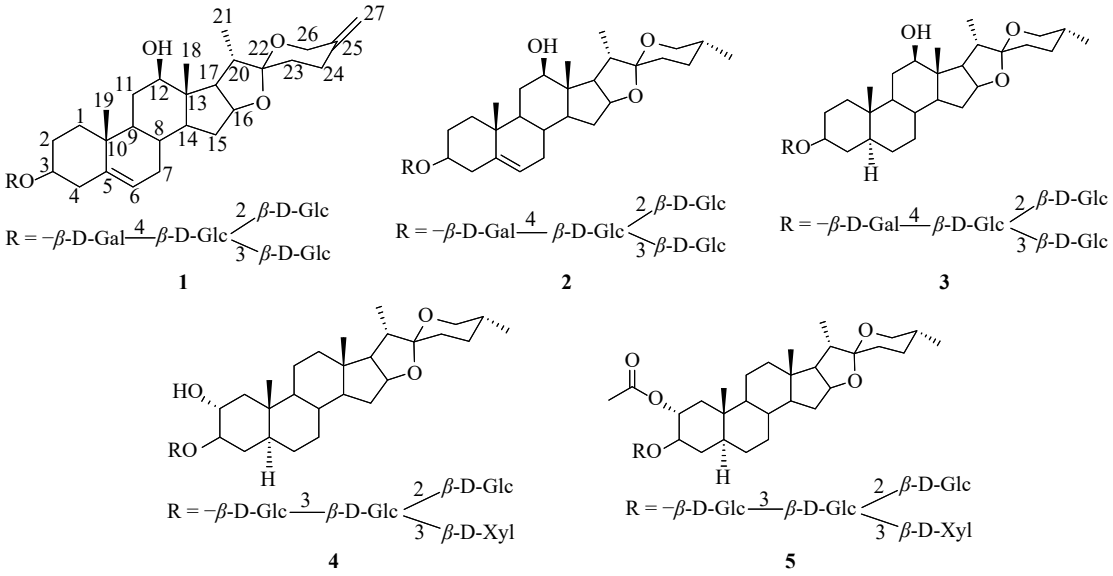


**Fig. 1 Structures of compounds 1–5**

istic alkene carbon signals at $\delta_C$145.33 (C-25), 141.88 (C-5), 122.54 (C-6), 109.69 (C-27) implied double bonds at C-5/C-6 and C-25/C-27. The proton signals were assigned to the corresponding carbons through direct $^1$H and $^{13}$C correlations in the HMQC spectrum. In the HMBC spectrum (Fig. 2), there were the correlations from H-19 ($\delta_H$0.83, 3H, s) to C-1 ($\delta_C$37.93), C-10 ($\delta_C$38.35), C-9 ($\delta_C$42.74), C-5 ($\delta_C$141.88), from H-18 ($\delta_H$0.88, 3H, s) to C-13 ($\delta_C$41.36), C-14 ($\delta_C$51.11), C-17 ($\delta_C$57.49), C-12 ($\delta_C$77.75). Meanwhile, the HMBC correlations from H-21 ($\delta_H$1.11, 3H, d, $J$ = 6.6 Hz) to C-17 ($\delta_C$57.49), C-20 ($\delta_C$40.68), C-22 ($\delta_C$110.36), and from $H_2$-27 to C-25 ($\delta_C$145.33), C-26 ($\delta_C$65.78), C-24 ($\delta_C$34.09) suggested that compound **1** existed a spirostenoside skeleton. From the $^1$H–$^1$H COSY analysis, the correlations between $H_2$-1/$H_2$-2/H-3 and $H_2$-11/H-12 revealed that $\delta_C$81.19 was attributed to C-3 and C-3 was connected with glycosyl. The HMBC correlations from $\delta_H$4.07 (1H, m) to C-12 ($\delta_C$77.75) indicated that the hydroxyl group was attached to C-12, and the hydroxyl group was *β*-oriented based on the NOESY cross-peak of H-12 with H-17 [5]. Comparison of the $^{13}$C NMR spectroscopic signals of the aglycone moiety of **1** with literature values [3] and an extensive HMQC and HMBC data analysis, showed that the aglycone of **1** was spirostane-5,25(27)-dien-3*β*,12*β*-diol. Acid hydrolysis of **1** gave D-galactose and D-glucose in a ratio of 1∶3, and the connectivity of the four sugars was mainly based on the HMBC correlations: H-1' ($\delta_H$5.62, d, $J$ = 7.8 Hz) with C-3 ($\delta_C$81.19) of the aglycone; H-1" ($\delta_H$5.30, d, $J$ = 7.8 Hz) with C-4' ($\delta_C$82.34), H-1'" ($\delta_H$5.34, d, $J$ = 7.8 Hz) with C-3" ($\delta_C$89.40), and H-1"" ($\delta_H$4.92, d, $J$ = 7.8 Hz) with C-2" ($\delta_C$79.59), and all the sugars were determined to be *β*-oriented according to the $J$ values. Comparing the NMR data of the sugar moieties of compound **1** and compound odospiroside [17], they were basically consistent, indicated that they had the same sugar chain. Therefore, compound **1** was identified as spirostane-5,25(27)-dien-3*β*,12*β*-diol-3-*O*-*β*-D-glucopyranosyl(1→2)[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranosid.

Compound **2** was obtained as white powder. It has a molecular formula of $C_{51}H_{82}O_{24}$ deduced by HR-ESI-MS {*m/z* 1101.5834 $[M + Na]^+$ (Calcd. 1101.5837)}. The $^1$H NMR spectrum (Table 1) displayed four methyl singlet signals at $\delta_H$ 0.83 (3H, s, Me-18), $\delta_H$ 0.87 (3H, s, Me-19), $\delta_H$ 1.15 (3H, d, $J$ = 6.9 Hz, Me-21) and δ 0.69 (3H, s, Me-27) of aglycone. Moremore, the $^{13}$C NMR spectrum (Table 2) revealed a total of 51 carbon signals, where 27 carbons were assigned to the aglycone and the remaining 24 carbons to the sugar moieties. In fact, the $^1$H and $^{13}$C NMR spectroscopic data of **2** were similar to those of compound **1**, except for the absence of one double bond at C-25/C-27 in **2**, which was further confirmed by the H-H COSY, HMBC, HMQC and NOESY spectra (Fig. 2). Furthermore, according to the chemical shifts of C-24 ($\delta_C$ 30.17), C-25 ($\delta_C$31.51) and C-26 ($\delta_C$67.76), C-25 was determined to be *β*-oriented [3]. Therefore, compound **2** was established as 25*R*-spirostane-5(6)-en-3*β*,12*β*-diol-3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl (1→4)-*β*-D-galactopyranosid.

Compound **3** was isolated as white powder. Its physicochemical properties were same with those of compounds **1** and **2**. A comparison of the NMR data (Table 2) of **3** and **2** showed that there were no characteristic alkene carbon signals at C-5/C-6 in **3**. The hydrogen signal of Me-18 ($\delta_H$0.83) was in the relative low field region compared with Me-19 ($\delta_H$0.63), so H-5 was determined to be *α*-oriented [9]. On the basis of the $^1$H-$^1$H COSY, HMBC, HMQC and NOESY spectra (Fig. 2), compound **3** was demonstrated as 25*R*-5*α*-spirostane-3*β*,12*β*-diol-3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranosid.

Compound **4** was isolated as white powder and its molecular formula was determined to be $C_{50}H_{82}O_{23}$ based on its

**Table 1 $^{1}$H NMR data (600 MHz) for the aglycone moieties of compounds 1–5[a] (Pyridine-$d_5$, $J$ in Hz)**

| No. | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| 1 | 1.68, m; 0.93, m | 1.70, m; 0.99, m | 1.63, m; 1.00, m | 2.20, m; 1.19, m | 2.15, m; 1.08, m |
| 2 | 2.25, m; 2.73, m | 1.69, m; 1.37, m | 1.34, m; 1.10, m | 4.04, m | 4.01, m |
| 3 | 4.56, m | 4.71, m | 4.57, m | 4.37, m | 4.52, m |
| 4 | 2.43, m; 2.66, m | 2.42, m; 2.68, m | 1.51, m; 0.79, m | 1.66, m; 1.39, m | 1.27, m; 1.32, m |
| 5 | - | - | 1.96, t (6.4, 8.6) | 1.00, m | 1.04, m |
| 6 | 5.19, m | 5.29, m | 0.66, m | 1.61, m; 1.36, m | 1.60, m; 1.31, m |
| 7 | 1.27, m; 1.50, m | 2.09, m; 1.26, m | 1.50, m; 1.08, m | 2.02, m; 1.44, m | 1.74, m; 1.41, m |
| 8 | 1.66, m | 1.67, m | 1.65, m | 1.37, m | 1.39, m |
| 9 | 2.11, m | 1.65, m | 2.01, m | 0.57, m | 0.49, m |
| 10 | - | - | - | - | - |
| 11 | 1.22, m; 0.97, m | 1.40, m; 1.81, m | 1.03, m; 0.89, m | 1.17, m; 1.40, m | 1.39, m; 1.18, m |
| 12 | 4.07, m | 3.90, m | 4.02, m | 1.61, m; 0.77, m | 1.42, m; 0.79, m |
| 13 | - | - | - | - | - |
| 14 | 0.95, m | 0.63, s | 0.49, t (7.3, 14.5) | 0.97, m | 1.04, t (8.2, 6.8) |
| 15 | 1.40, m; 1.42, m | 1.41, m; 1.51, m | 1.66, m; 1.52, m | 1.86, m; 1.57, m | 1.82, m; 1.59, m |
| 16 | 4.32, m | 4.46, m | 4.43, m | 4.50, m | 4.45, m |
| 17 | 1.02, m | 1.10, m | 1.05, m | 1.79, dt (7.3, 8.4) | 1.72, m |
| 18 | 0.88, s | 0.83, s | 0.83, s | 0.81, s | 0.83, s |
| 19 | 0.83, s | 0.87, s | 0.63, s | 0.70, s | 0.66, s |
| 20 | 1.70, m | 1.95, m | 2.04, m | 1.94, m | 1.80, m |
| 21 | 1.11, d (6.6) | 1.15, d (6.9) | 1.15, d (6.6) | 1.14, d (7.0) | 1.15, d (6.9) |
| 22 | - | - | - | - | - |
| 23 | 1.37, m; 1.32, m | 2.02, m; 1.49, m | 1.36, m; 1.34, m | 1.53, m | 1.57, m |
| 24 | 1.83, m; 1.78, m | 1.84, m; 1.79, m | 0.90, m; 1.19, m | 1.49, m | 1.52, m |
| 25 | - | 1.57, m | 1.01, m | 0.94, m | 1.02, m |
| 26 | 4.44, m; 4.02, m | 3.60, m; 3.51, m | 4.24, m; 3.88, m | 3.61, m; 3.51, m | 3.62, m; 3.52, m |
| 27 | 4.84, s; 4.80, s | 0.69, s | 0.70, s | 0.69, s | 0.70, s |
| Ac-$CH_3$ | - | - | - | - | 2.05, s |

[a] Assignments are based on APT, HMQC, COSY, HMBC, and NOESY experiments.

HR-ESI-MS {*m/z* 1068.4167 $[M + NH_4]^+$ (Calcd. 1068.4170)}. Analysis of its $^{1}$H NMR spectrum (Table 1) displayed the signals of four anomeric protons at $\delta_H$4.95 (1H, d, $J$ = 7.8 Hz), 5.62 (1H, d, $J$ = 7.9 Hz), 5.21 (1H, d, $J$ = 7.9 Hz), 5.34 (1H, d, $J$ = 7.9 Hz) of four sugar moieties, and four methyl singlet signals at $\delta_H$0.81 (3H, s, Me-18), 0.70 (3H, s, Me-19), 1.14 (3H, d, $J$ = 7.0 Hz, Me-21) and 0.69 (3H, s, Me-27) of aglycone. The $^{13}$C NMR spectrum (Table 2) showed a total of 50 carbon signals, where 27 carbons were assigned to the aglycone and the remaining 23 carbons to the sugar moieties. Actually, the $^{1}$H and $^{13}$C NMR spectroscopic data of aglycone in **4** were similar to those of compound **3**, except for the hydroxyl position in the structure. The position of the hydroxyl group at C-2 in compound **4** was confirmed by the HMBC correlations of C-2 to H-1 ($\delta_H$ 1.19, 2.20) and H-4 ($\delta_H$ 1.66, 1.39), and the hydroxyl group was $\alpha$-oriented based on the NOESY cross-peak of H-2 with H-19 [5]. The 25*R* form was determined by the chemical shifts of C-24 ($\delta_C$28.99), C-25 ($\delta_C$ 31.51) and C-26 ($\delta_C$67.76) [3]. The 23 carbon signals were supposed to be four sugars, namely one D-xylose and three D-glucose moieties. The connectivity of the sugars was mainly based on the HMBC correlations: H-1' ($\delta_H$4.95, d, $J$ = 7.8 Hz) with C-3 ($\delta_C$82.28) of the aglycone; H-1" ($\delta_H$5.62, d, $J$ = 7.9 Hz) with C-3' ($\delta_C$84.97); H-1'" ($\delta_H$5.21, d, $J$ = 7.9 Hz) with C-3" ($\delta_C$89.54) and H-1"" ($\delta_H$5.34, d, $J$ = 7.9 Hz) with C-2" ($\delta_C$80.69). All the sugars were determined to be $\beta$-oriented

**Table 2 $^{13}$C NMR data (150 MHz) for compounds 1–5[a] (Pyridine-$d_5$)**

| No. | 1 | 2 | 3 | 4 | 5 | No. | 1 | 2 | 3 | No. | 4 | 5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 37.93 | 37.92 | 36.69 | 46.50 | 41.67 | Gal-1' | 103.63 | 103.62 | 103.29 | Glc-1' | 104.13 | 103.56 |
| 2 | 29.89 | 30.89 | 22.16 | 71.34 | 71.54 | 2' | 76.46 | 76.45 | 76.50 | 2' | 76.25 | 76.27 |
| 3 | 81.19 | 81.18 | 81.22 | 82.28 | 82.00 | 3' | 74.13 | 74.13 | 74.14 | 3' | 84.97 | 82.05 |
| 4 | 40.12 | 40.11 | 35.69 | 33.02 | 33.03 | 4' | 82.34 | 82.01 | 82.05 | 4' | 71.70 | 71.76 |
| 5 | 141.88 | 141.86 | 41.02 | 45.43 | 45.51 | 5' | 78.49 | 78.48 | 78.48 | 5' | 79.39 | 78.59 |
| 6 | 122.54 | 122.55 | 29.81 | 30.17 | 30.17 | 6' | 63.97 | 63.97 | 63.97 | 6' | 63.47 | 64.02 |
| 7 | 33.05 | 32.70 | 32.70 | 33.09 | 33.29 | Glc-1" | 106.07 | 106.07 | 106.06 | Glc-1" | 105.82 | 106.27 |
| 8 | 32.51 | 32.51 | 36.12 | 35.44 | 36.12 | 2" | 79.59 | 79.59 | 79.59 | 2" | 80.69 | 79.60 |
| 9 | 42.74 | 45.50 | 45.50 | 55.20 | 55.27 | 3" | 89.40 | 89.37 | 89.39 | 3" | 89.54 | 89.11 |
| 10 | 38.35 | 38.35 | 38.07 | 37.75 | 36.69 | 4" | 71.75 | 71.75 | 71.75 | 4" | 72.14 | 72.44 |
| 11 | 31.05 | 31.04 | 30.80 | 22.31 | 22.17 | 5" | 79.64 | 79.63 | 79.63 | 5" | 79.60 | 79.54 |
| 12 | 77.75 | 78.19 | 78.19 | 41.66 | 41.03 | 6" | 61.41 | 61.41 | 61.50 | 6" | 61.46 | 61.35 |
| 13 | 41.36 | 41.34 | 41.67 | 40.92 | 38.07 | Glc-1"' | 105.49 | 105.48 | 105.48 | Xyl-1"' | 105.48 | 105.48 |
| 14 | 51.11 | 55.23 | 55.24 | 57.19 | 57.33 | 2"' | 76.18 | 76.17 | 75.84 | 2"' | 76.40 | 76.31 |
| 15 | 33.14 | 33.14 | 33.28 | 34.98 | 35.59 | 3"' | 77.13 | 77.13 | 77.13 | 3"' | 78.46 | 78.29 |
| 16 | 82.42 | 82.42 | 82.43 | 82.03 | 81.50 | 4"' | 63.90 | 63.90 | 63.91 | 4"' | 73.47 | 76.19 |
| 17 | 57.49 | 57.50 | 57.31 | 63.85 | 63.90 | 5"' | 78.78 | 78.77 | 78.79 | 5"' | 63.91 | 65.18 |
| 18 | 17.25 | 17.27 | 17.54 | 17.52 | 17.54 | 6"' | 63.21 | 63.22 | 63.22 | - | - | - |
| 19 | 20.30 | 20.29 | 13.20 | 14.31 | 13.18 | Glc-1"" | 105.87 | 105.86 | 105.86 | Glc-1"" | 105.62 | 105.63 |
| 20 | 40.68 | 42.86 | 42.88 | 42.87 | 42.88 | 2"" | 76.23 | 76.22 | 76.22 | 2"" | 76.6 | 76.75 |
| 21 | 15.94 | 15.98 | 15.98 | 15.96 | 15.98 | 3"" | 72.47 | 72.48 | 72.48 | 3"" | 76.97 | 77.08 |
| 22 | 110.36 | 110.19 | 110.15 | 110.15 | 110.15 | 4"" | 63.76 | 63.75 | 63.72 | 4"" | 72.48 | 74.27 |
| 23 | 21.98 | 33.09 | 33.03 | 32.69 | 32.70 | 5"" | 79.01 | 79.01 | 78.92 | 5"" | 79.14 | 78.48 |
| 24 | 34.09 | 30.17 | 30.17 | 28.99 | 29.80 | 6"" | 63.18 | 63.17 | 63.16 | 6"" | 63.20 | 63.17 |
| 25 | 145.33 | 31.51 | 31.51 | 31.51 | 31.51 | | | | | | | |
| 26 | 65.78 | 67.76 | 67.76 | 67.76 | 67.76 | | | | | | | |
| 27 | 109.69 | 18.26 | 18.26 | 18.26 | 18.26 | | | | | | | |
| Ac-CO | - | - | - | - | 172.14 | | | | | | | |
| Ac-$CH_3$ | - | - | - | - | 22.04 | | | | | | | |

[a] Assignments are based on APT, HMQC and HMBC experiments.

according to the *J* values. To sum up, compound **4** was identified as 25*R*-5*α*-spirostane-2*α*,3*β*-diol-3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-xylopyranosyl (1→3)]-*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranoside.

Compound **5** was obtained as white powder. Its physicochemical properties were the same with that of compound **4**, and its molecular formula was assigned as $C_{52}H_{84}O_{24}$ according to HR-ESI-MS. A comparison of the $^1$H and $^{13}$C NMR spectroscopic data of **5** and **4** (Tables 1 and 2) suggested that they were similar except for the presence of acetoxyl group at $\delta_C$ 172.14 and 22.04 in **5**. The position of the acetoxyl group at C-2 was confirmed by the HMBC correlations between C-Ac and H-2 ($\delta_H$4.01), and the acetoxyl group was *α*-orientated based on the NOESY cross-peak of H-2 with H-19 [5]. Comparison of NMR spectrum for the sugar moieties of **5** with those of **4** suggested the same sugar chains, and the difference was mainly the substituents at C-2. Therefore, compound **5** was established as 25*R*-5*α*-spirostane-2*α*-*O*-Ac-3-*O*-*β*-D-glucopyranosyl(1→2)-[*β*-D-xylopyranosyl(1→3)]-*β*-D-glucopyranosyl (1→3)-*β*-D-glucopyranoside.

Additionally, seven known compounds were isolated. By comparing their NMR spectroscopic data with literature values, their structures were determined to be coniferin (**6**) [18], naphthisoxazol A (**7**) [19], stagonoculiazin (**8**) [20], adenosine

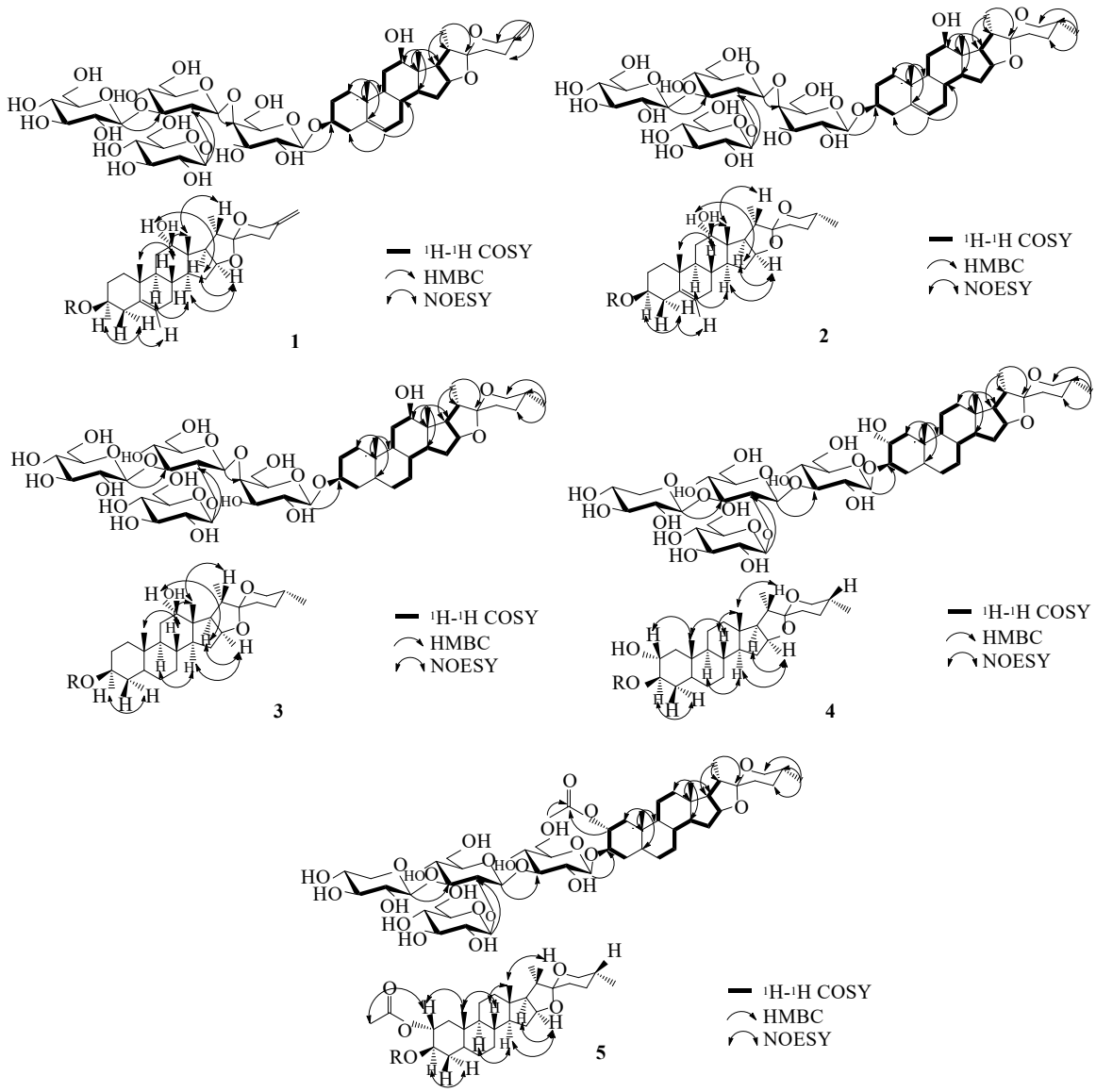


**Fig. 2 Key $^1$H-$^1$H COSY, HMBC, and NOESY correlations of compounds 1–5**

(**9**) [21], syringin (**10**) [22], tyramine (**11**) and 3-indolebutyric acid (**12**).

The antioxidant activities of these isolated monomer compounds were evaluated *in vitro* against DPPH radical, ABTS radical and OH radical (Fig. 3). Compounds **6**–**11** showed certain scavenging capacity for ABTS radical in a dose-dependent manner. The $IC_{50}$ values of compounds **8**–**10** for ABTS radical scavenging were 1.367, 1.169 and 1.423 mg·mL$^{-1}$, respectively. Compounds **6**, **7** and **11** had enhanced ABTS radical scavenging capacity, with $IC_{50}$ values

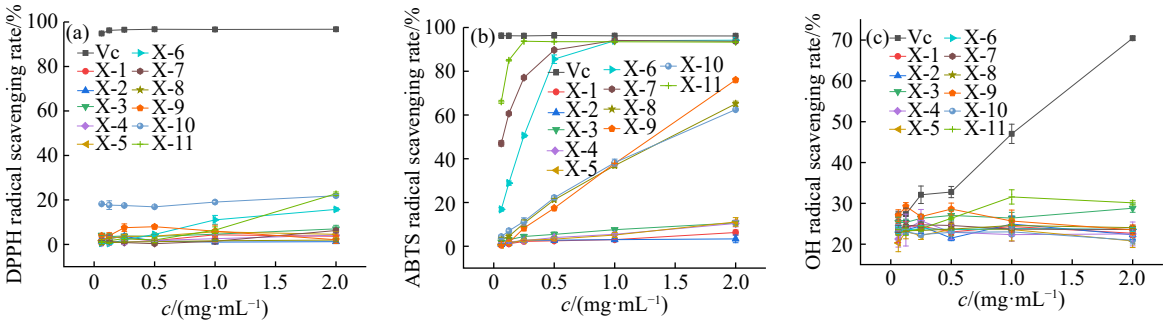


**Fig. 3 Antioxidant activities of compounds 1-11/Vc**

of 0.208, 0.057 and 0.014 mg·mL$^{-1}$, respectively.

## Experimental

### *General experimental procedures*

NMR spectra were obtained using a Bruker AV III 600 NMR spectrometer with chemical shift values presented as δ values, with TMS as the internal standard (samples dissolved in an appropriate amount of deuterated pyridine-$d_5$ and deuterated methanol). HR-ESI-MS was performed using an LTQ-Orbitrap XL spectrometer (Thermo Fisher Scientific, Boston, MA, USA). Acchrom S6000 high-performance liquid chromatograph [Acchrom Tech (Beijing) Technology Co., Ltd., Beijing, China] equipped with ELSD-UM 5800 Plus [Unimicro (Shanghai) Technologies Co., Ltd., Shanghai, China] was used for analysis. Colurmn chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Plant, Qingdao, China), inverted $C_{18}$ filler (50 μm, Nantong Hairuo Chemical Co., Ltd., Nantong, China) and macroporous resin D101 (Tianjin Haoju Resin Technology Co., Ltd., Tianjin, China). All solvents used (petroleum ether, ethanol, methanol, *n*-butanol, dichloromethane, deuterated pyridine-$d_5$ and deuterated methanol) were of analytical grade (Beijing Chemical Plant, Beijing, China).

### *Plant material*

Fresh Allii Macrostemonis Bulbus was purchased from Changchun, and dried in an oven at 50 °C. The dried samples were crushed, sieved through 40 mesh sieve, sealed and stored for later use.

### *Extraction and isolation*

The dried powder of Allii Macrostemonis Bulbus (4.0 kg) was extracted by petroleum ether under reflux (3 times, 2 h each time) at a solid-liquid ratio of 1 ∶ 5 (*W*/*V*) to degrease. According to the solid-liquid ratio of 1 ∶ 10 (*W*/*V*), the filter residue was extracted with 75% ethanol solution (*V*/*V*, 1 h each time) under reflux for three times. After removal of the solvent, extraction was performed using saturated *n*-butanol for three times, and the resultant extract was subjected to D-101 macroporous resin column using a gradient of EtOH−$H_2O$ to give four fractions (30%, 50%, 70% and 90%).

The 90% fraction of the above-mentioned (12.795 g) was subjected to a silica gel (100–200 mesh) column, eluting with a stepwise gradient of $CH_2Cl_2$/MeOH (from 9 ∶ 1 to 0 ∶ 1, *V*/*V*) to give seven fractions (Frs. A–G) by TLC analysis. Fr.C (2.32 g) was loaded onto a silica gel column (200–300 mesh) and eluted with $CH_2Cl_2$/MeOH/$H_2O$ (stepwise, from 9 ∶ 3 ∶ 1 to 7 ∶ 4 ∶ 1, *V*/*V*/*V*) to yield five fractions (Frs. C-1–5). Fr. C-3 (0.87 g) was separated over an ODS (50 μm) column, eluting with MeOH/$H_2O$ (stepwise, from 3 ∶ 2 to 9 ∶ 1, *V*/*V*) to give compound **2** (134.81 mg) and compound **3** (145.64 mg). Fr. C-5 (0.51 g) was subjected to an ODS (50 μm) column and eluted with MeOH/$H_2O$ (stepwise, from 3 ∶ 2 to 9 ∶ 1, *V*/*V*) to yield compound **1** (86.68 mg). Fr. E (1.12 g) was applied onto a silica gel (200–300 mesh) column, eluting with $CH_2Cl_2$/MeOH/$H_2O$ (stepwise, from 8 ∶ 3 ∶ 1 to 5 ∶ 3 ∶ 1, *V*/*V*/*V*) to obtain four fractions (Frs. E-1–4). Fr. E-2 was further subjected to an ODS (50 μm) column and eluted with MeOH/$H_2O$ (stepwise, from 4 ∶ 1 to 9 ∶ 1, *V*/*V*) to give compound **5** (11.18 mg). Fr. E-3 was subjected to an ODS (50 μm) column and eluted with MeOH/$H_2O$ (stepwise, from 4 ∶ 1 to 9 ∶ 1, *V*/*V*) to yield compound **4** (9.85 mg). The 30% fraction (16.007 g) was subjected to a silica gel (100–200 mesh) column, eluting with a stepwise gradient of $CH_2Cl_2$/MeOH (from 7 ∶ 1 to 0 ∶ 1, *V*/*V*) to give nine fractions (Frs. H–P) by TLC analysis. Fr. I (0.42 g) was separated over a silica gel (200–300 mesh) column, eluting with $CH_2Cl_2$/MeOH/$H_2O$ (stepwise, from 11 ∶ 4 ∶ 2 to 6.5 ∶ 2.5 ∶ 1, *V*/*V*/*V*) to yield five fractions (Frs. I-1–5). Fr. I-1 was loaded onto an ODS (50 μm) column and eluted with ACN/$H_2O$ (1 ∶ 9, *V*/*V*) to obtain compound **6** (10.74 mg) Fr. I-3 was subjected to an ODS (50 μm) column and eluted with ACN/$H_2O$ (1 ∶ 9, *V*/*V*) to yield compound **10** (22.90 mg) and compound **8** (10.14 mg). Fr. K was recrystallized to give compound **9** (123.55 mg). Fr. O (1.13 g) was chromatographed over a silica gel column (200–300 mesh), eluting with $CH_2Cl_2$/MeOH/$H_2O$ (stepwise, from 10 ∶ 4 ∶ 2 to 13 ∶ 8 ∶ 1, *V*/*V*/*V*) to obtain eight fractions (Frs. O-1–8). Fr. O-5 was applied onto an ODS (50 μm) column and eluted with MeOH/$H_2O$ (stepwise, from 1 ∶ 5 to 3 ∶ 7, *V*/*V*) to yield compound **7** (73.75 mg). Fr. O-6 was subjected to an ODS (50 μm) column, eluting with MeOH/$H_2O$ (stepwise, from 1 ∶ 5 to 3 ∶ 7, *V*/*V*) to give compound **11** (13.33 mg) and compound **12** (7.07 mg).

### *Antioxidant assay*

#### *2,2-Diphenyl-1-picrylhydrazylradical (DPPH) scavenging assay*

The DPPH radical scavenging capacity was determined as described by Om P *et al*. [23]. Compounds **1**–**11** and Vc (50 μL, 0–2 mg·mL$^{-1}$) were separately mixed with fresh DPPH ethanol solution (150 μL, 0.4 mmol·L$^{-1}$), and then allowed to react in the darkness for 30 min. $V_C$ was used as a positive control. The absorbance was measured at 517 nm. The DPPH radical scavenging rate was calculated according to formula (1):

$$\text{Scavenging rate}\ (\%) = [1-(A_1-A_2)/A_0] \times 100 \quad (1)$$

Where $A_0$ was the absorbance of DPPH solution (150 μL) with distilled water (50 μL); $A_1$ was the absorbance of compounds **1**–**11**/Vc (50 μL, 0–2 mg·mL$^{-1}$) and DPPH solution (150 μL); and $A_2$ was the absorbance of absolute ethanol instead of DPPH solution.

#### *2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS) scavenging assay*

Herein, 204 mg of ABTS and 35.14 mg of $K_2S_2O_8$ were separately diluted to 50 mL with water, mixed and shaked well, avoiding light reaction at room temperature for 16 h to yield ABTS mother liquor. The above solution was diluted with ethanol until its absorbance was 0.7 at 734 nm. Samples and Vc (80 μL, 0–2 mg·mL$^{-1}$) were separately mixed with ABTS solution (140 μL), and then allowed to react in the darkness at room temperature for 30 min. The absorbance

was measured at 734 nm. The ABTS radical scavenging rate was calculated according to formula (2):

$$\text{Scavenging rate}\ (\%) = (1 - A_1/A_0) \times 100 \quad (2)$$

Where $A_0$ was the absorbance of ABTS solution (140 μL) with distilled water (80 μL); $A_1$ was the absorbance of compounds **1**–**11**/Vc (80 μL, 0–2 mg·mL$^{-1}$) and ABTS solution (140 μL) [24].

*Hydroxyl radical (•OH) scavenging assay*

Salicylic acid ethanol solution (50 μL, 10 mmol·L$^{-1}$), $FeSO_4$ solution (50 μL, 10 mmol·L$^{-1}$) and compound **1**–**11**/Vc (50 μL, 0–2 mg·mL$^{-1}$) were absorbed to be tested respectively in 96-well plate, mixed well and reacted at 37 °C for 30 min. $H_2O_2$ solution (50 μL, 100 mmol·L$^{-1}$) was added to terminate the reaction. The absorbance was measured at 510 nm. The OH radical scavenging rate was calculated according to formula (3):

$$\text{Scavenging rate}\ (\%) = [1 - (A_1 - A_2)/A_0] \times 100 \quad (3)$$

Where $A_0$ was the absorbance of salicylic acid ethanol solution (50 μL), $FeSO_4$ solution (50 μL), distilled water (50 μL) and $H_2O_2$ solution (50 μL); $A_1$ was the absorbance of compounds **1**–**11**/Vc (50 μL, 0–2 mg·mL$^{-1}$), salicylic acid ethanol solution (50 μL), $FeSO_4$ solution (50 μL) and $H_2O_2$ solution (50 μL); and $A_2$ was the absorbance of distilled water instead of $H_2O_2$ solution [25].

## Supporting Information

The NMR spectra of compounds **1**–**12** are available as Supporting Information, and can be requested by sending E-mail to the corresponding author.

## References


[1] China Pharmacopoeia Commission. *Pharmacopoeia of the people's Republic of China* (2020) [S]. Vol 1. Beijing: China Medical Science and Technology Press, 2020: 392.

[2] Yao ZH, Qin ZF, Dai Y, *et al*. Phytochemistry and pharmacology of Allii Macrostemonis Bulbus, a traditional Chinese medicine [J]. *Chin J Nat Med*, 2016, **14**(7): 481-498.

[3] Cheng SB. *Study on Steroidal Saponins from the Bulbs of Allium macrostemonis Bunge* [D]. Hangzhou: Zhejiang University, 2013, 118.

[4] Kuroda M, Mimaki Y, Kameyama A, *et al*. Steroidal saponins from *Allium chinense* and their inhibitory activities on cyclic AMP phosphodiesterase and $Na^+/K^+$ ATPase [J]. *Phytochemistry*, 1995, **40**(4): 1071-1076.

[5] Kim YS, Suh WS, Park KJ, *et al*. Allimacrosides A−E, new steroidal glycosides from *Allium macrostemon* Bunge [J]. *Steroids*, 2017, **118**(8): 41-46.

[6] Peng JP, Wu Y, Yao XS, *et al*. Two new steroidal saponins from *Allium macrostemon* Bunge [J]. *Acta Pharm Sin*, 1992, **27**(12): 918-922.

[7] Chen HF, Wang GH, Wang NL, *et al*. New furostanol saponins from the bulbs of *Allium macrostemon* Bunge and their cytotoxic activity [J]. *Pharmazie*, 2007, **62**(7): 544-548.

[8] Ou WC, Chen HF, Zhong Y, *et al*. Inhibition of platelet activation and aggregation by furostanol saponins isolated from the bulbs of *Allium macrostemon* Bunge [J]. *Am J Med Sci*, 2012, **344**(4): 261-267.

[9] Wang T. *Study on Chemical Constituents from Allii Macrostemonis Bulbus* [D]. Changchun: Jilin University, 2017, 160

[10] Huang F, Zhou H, Yu SS. Optimization of extraction of volatile oil from *Allium macrostemon* Bunge and chemical composition analysis by gas chromatography-mass spectrometry [J]. *Food Sci*, 2014, **35**(8): 80-84.

[11] Han CH, Gao SN, Bai YH, *et al*. Analysis of volatile oil from bulb and leaf of *Allium macrostemon* Bge. before and after processing by gas chromatography-mass spectrometry [J]. *Lishizhen Med Mater Med Res*, 2017, **28**(1): 111-113.

[12] Gan YZ, Zhong KY, Huang L. Study on extraction, isolation and purification of polysaccharides from *Allium macrostemon* Bunge and its interaction with DNA [J]. *Biochem*, 2019, **5**(2): 82-84.

[13] Zhang ZJ, Wang FH, Ge H, *et al*. Structural characterization and *in vitro* antioxidant activities of a neutral polysaccharide from *Allium macrostemon* Bunge [J]. *Sci Technol Food Ind*, 2017, **38**(8): 77-81, 86

[14] Zhang ZJ, Wang FH, Wang MC, *et al*. A comparative study of the neutral and acidic polysaccharides from *Allium macrostemon* Bunge [J]. *Carbohydr Polym*, 2015, **117**(24): 980-987.

[15] Wang YH, Yi XM, Rao ZH, *et al*. Study on chemical constituents from the bulbs of *Allium chinense* G. Don [J]. *J Guangdong Pharm Univ*, 2017, **33**(4): 453-456.

[16] Peng JP, Qiao YQ, Yao XS. Nitrogen-containing compounds from *Allium macrostemon* Bunge and *Allium chinense* G. Don [J]. *Chin J Med Chem*, 1995, **5**(2): 134-139.

[17] Janeczko Z, Jansson P E, Sendra J. A new stereoidal saponin from *Polygonatum officinale* [J]. *Planta Med*, 1987, **53**(1): 52-54.

[18] Sun H, Zhang YF, Huo HX, *et al*. Chemical constituents from pericarps of *Aquilaria yunnanensis* [J]. *Chin Tradit Herbal Drugs*, 2020, **51**(1): 9-15.

[19] Li GQ, Zhang YB, Guan HS. A new isoxazol from *Glehnia littoralis* [J]. *Fitoterapia*, 2008, **79**(3): 238-239.

[20] Yu Y, Ma BJ, Liu JS, *et al*. Two new alkaloid metabolites produced by endophytic fungus *Stagonosporopsis oculihominis* isolated from *Dendrobium huoshanense* [J]. *Phytochem Lett*, 2017, **19**(6): 266-270.

[21] Ai FW, Zhang S, Li YF, *et al*. Study on the chemical constituents of *Typhonium giganteum* Engl [J]. *Chin Tradit Herb Drugs*, 2010, **41**(2): 201-203.

[22] Zhang DD, Li JY, Shi YH, *et al*. Glycosides from roots of *Isatis indigotica* [J]. *Chin Tradit Herb Drugs*, 2019, **50**(15): 3575-3580.

[23] Sharma OP, Bhat TK. DPPH antioxidant assay revisited [J]. *Food Chem*, 2009, **113**(4): 1202-1205.

[24] Zhang DY, Luo M, Wang W, *et al*. Variation of active constituents and antioxidant activity in pyrola [*P. incarnata* Fisch. ] from different sites in Northeast China [J]. *Food Chem*, 2013, **141**(3): 213-2219.

[25] Zhu YL, Yu XF, Ge Q, *et al*. Antioxidant and anti-aging activities of polysaccharides from *Cordyceps cicadae* [J]. *Int J Biol Macromol*, 2020, **157**(9): 394-400.


**Cite this article as:** WANG Rong, WANG Lulu, ZHANG Manli, GUO Yadi, ZHANG Jing, MA Guoxu. Five new spirosterol saponins from Allii Macrostemonis Bulbus [J]. *Chin J Nat Med*, 2023, **21**(3): 226-232.