Natural Product Sciences
24(1) : 54-58 (2018)
https://doi.org/10.20307/nps.2018.24.1.54

A New Steroidal Glycoside from *Allium macrostemon* Bunge

Yun Sik Kim, Joon Min Cha, Dong Hyun Kim, Tae Hyun Lee, and Kang Ro Lee*

Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Korea

Abstract – A phytochemical investigation of *Allium macrostemon* Bunge (Liliaceae) afforded the new pregnane steroidal glycoside, named allimacroside F (**1**), along with three known glycosides, benzyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**2**), phenylethyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**3**), (*Z*)-3-hexenyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**4**). The identification and structural elucidation of a new compound (**1**) was carried out based on spectral data analyses (^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY) and HR-FAB-MS.

Keywords – *Allium macrostemon*, Liliaceae, Steroidal glycoside, Allimacroside F.

Introduction

Allium macrostemon Bunge (Liliaceae), known as wild onion, is widely distributed in East Asian countries.[1] Its dried bulbs have been known as a traditional Chinese medicine “Xiebai”, and used for treatment of heart diseases such as thoracic pain, stenocardia, and heart asthma.[2] Various steroidal glycosides with medicinal properties have been reported from the genus *Allium*,[3] and previous phytochemical investigations on *A. macrostemon* demonstrated the presence of steroidal glycosides, including macrostemonosides A-S.[4-8] In the course of our search for the new steroidal glycosides from this plant, we reported the isolation of allimacrosides A–E.[9] In continuing study of this source, we isolated further a new pregnane-type steroidal glycoside, namely allimacroside F (**1**), together with three known compounds (**2** - **4**).

Experimental

General experimental procedures – Optical rotations were measured on a Jasco P-1020 polarimeter in MeOH. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. UV spectra were recorded using a Schimadzu UV-1601 UV-visible spectrophotometer. High resolution (HR)-fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including ^{1}H-^{1}H correlated spectroscopy (COSY), distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC) and nuclear overhauser effect spectroscopy (NOESY) experiments, were recorded on a Varian UNITY INOVA 700 NMR spectrometer operating at 700 MHz (^{1}H) and 175 MHz (^{13}C) with chemical shifts given in ppm (δ). Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector and Econosil RP-C$_{18}$ 10 µm column (250 × 10 mm). Silica gel 60 (Merck, 70 - 230 mesh and 230 - 400 mesh) and RP-C$_{18}$ silica gel (YMC GEL ODS-A, 12 nm, S-75 µm) were used for column chromatography. TLC was performed using percolated Silica gel F$_{254}$ plates and RP-18 F$_{254s}$ plates (Merck). Spots were detected by TLC under UV light or by heating after spraying with 10% H_2SO_4 in C_2H_5OH (v/v). A Hewlett-Packard (HP) GC system 6890 Series equipped with a 5973 Mass Selective Detector (MSD) system was controlled by the Enhanced ChemStation Version B.01.00 software. The capillary column used for GC was an Agilent J&W HP-5MS UI (30.0 m × 0.25 mm i.d., 0.25 µm film thickness coated 5% diphenyl 95% dimethylpolysiloxane).

Plant materials – *A. mactostemon* was collected in Taeback, Gangwon province, Korea in April, 2010, and the plant was identified by one of the authors (K. R. Lee). A voucher specimen (SKKU-NPL 1202) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation – Dried whole plants of *A. macrostemon* (1.5 kg) were extracted with 80% MeOH three times at room temperature and evaporated under

*Author for correspondence
Kang Ro Lee, Natural Products Laboratory, School of Pharmacy, Sungkyunkwan University, Suwon 16419, Korea
Tel: +82-31-290-7710; E-mail: krlee@skku.edu

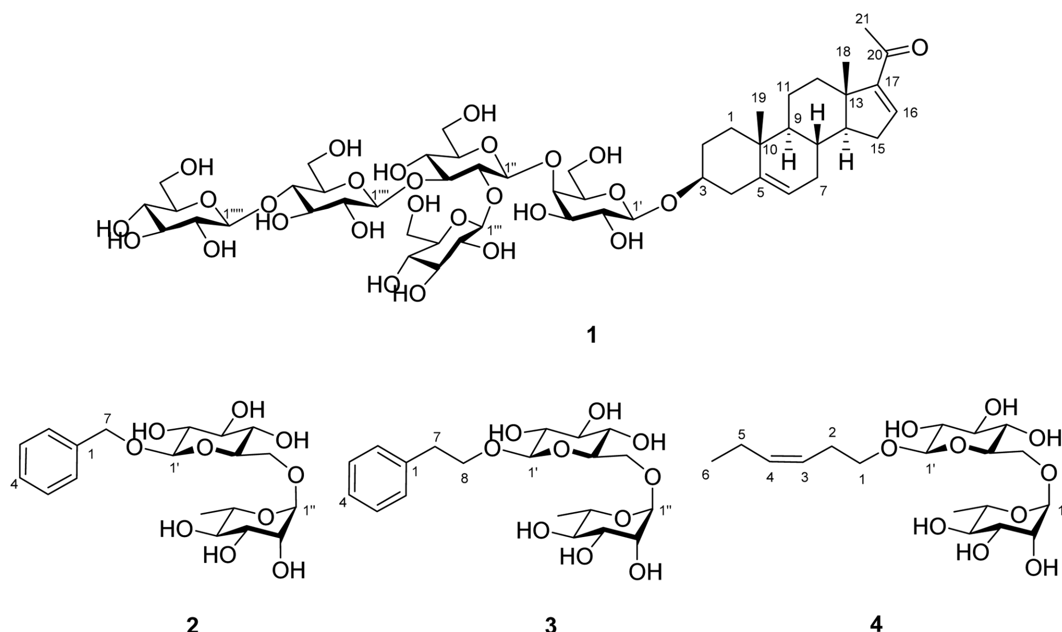

Fig. 1. The structures of **1** - **4** isolated from *A. macrostemon*.

reduced pressure to give a residue (210.0 g), which was dissolved in water (800 ml) and partitioned with solvents to give *n*-hexane (10.0 g), $CHCl_3$ (5.5 g), EtOAc (1.9 g), and *n*-BuOH (12.2 g) soluble layers. The *n*-BuOH-soluble layer (12.2 g) was chromatographed on a silica gel column (diameter × height: 5.5 × 35.0 cm, 300.0 g) with a $CHCl_3$-MeOH-H_2O (20:10:1 to 2:3:1) to give 10 fractions (B1-B10) based on a TLC analysis. Fraction B4 (1.3 g) was separated on a RP-C_{18} open column (2.5 × 30.0 cm, 60.0 g), eluting with 50% aqueous MeOH to give nine subfractions (B41-B49). Subfraction B42 (43 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 35% aqueous MeOH) to afford **2** (7 mg, t_R = 17.8 min). Subfraction B44 (27 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 35% aqueous MeOH) to afford **3** (6 mg, t_R = 33.7 min). Subfraction B45 (30 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 40% aqueous MeOH) to afford **4** (8 mg, t_R = 21.3 min). Fraction B7 (2.6 g) was separately chromatographed on a Diaion HP-20 column (2.5 × 35.0 cm, 80.0 g) eluting with a gradient solvent system of 100% H_2O and 100% MeOH, yielding subfractions B71 and B72. Subfraction B72 (1.5 g) separated on a RP-C_{18} silica gel open column (2.5 × 30.0 cm, 60 g), eluting with 40% aqueous MeOH to give six subfractions (B721-B726). Subfraction 726 (22 mg) was purified by an RP-C18 semi-prep. HPLC (2 mL/min, 36% aqueous MeCN) to afford **1** (3 mg, t_R = 15.2 min).

Allimacroside F (1) – White amorphous powder; $[\alpha]_D^{25}$ -59.3 (MeOH); UV (MeOH) λ_{max}: 239 nm; IR (KBr) ν_{max}: 3385, 2925, 1664, 1370, 1160, 1070 cm^{-1}; ^1H-NMR (Pyridine-d_5, 700 MHz) and ^{13}C-NMR (Pyridine-d_5, 175 MHz) see Table 1; HR-FAB-MS *m/z* 1147.4785 $[M+Na]^+$ (calcd. for $C_{51}H_{80}NaO_{27}$: 1147.4785).

Benzyl-*O*-*α*-L-rhamnopyranosyl-(1 → 6)-*β*-D-glucopyranoside (2) – Colorless gum; IR (KBr) ν_{max}: 3385, 2923, 1049 cm^{-1}; ^1H-NMR (700 MHz, Pyridine-d_5): δ 7.53 (2H, d, J = 7.3 Hz, H-2, 6), 7.27 (2H, m, H-3, 5), 7.22 (1H, m, H-4), 5.16 (1H, d, J = 11.8 Hz, H-7a), 5.56 (1H, d, J = 1.2 Hz, H-1''), 4.91 (1H, d, J = 7.8 Hz, H-1'), 4.83 (1H, d, J = 11.8 Hz, H-7b), 1.63 (3H, d, J = 6.2 Hz, H-6''); ^{13}C-NMR (175 MHz, Pyridine-d_5): δ 138.5 (C-1), 128.4 (C-2, 6), 128.4 (C-3, 5), 127.6 (C-4), 103.5 (C-1'), 102.4 (C-1''), 78.3 (C-5'), 77.0 (C-3'), 74.9 (C-2'), 73.9 (C-4''), 72.6 (C-3''), 72.1 (C-2''), 71.7 (C-4'), 70.7 (C-1), 69.6 (C-5''), 68.2 (C-6'), 18.5 (C-6''); FAB-MS (positive mode) *m/z* = 417.23 $[M+H]^+$.

Phenylethyl-*O*-*α*-L-rhamnopyranosyl-(1 → 6)-*β*-D-glucopyranoside (3) – Colorless gum; UV (MeOH) λ_{max}: 254, 212 nm; IR (KBr) ν_{max}: 3385, 2925, 1047 cm^{-1}; ^1H-NMR (700 MHz, Pyridine-d_5): δ 7.30 (2H, d, J = 7.1 Hz, H-2, 6), 7.25 (2H, dd, J = 7.1, 7.4 Hz, H-3, 5), 7.19 (1H, dd, J = 7.4, 1.3 Hz, H-4), 5.52 (1H, d, J = 0.8 Hz, H-1''), 4.84 (1H, d, J = 7.8 Hz, H-1'), 4.18 (1H, m, H-8a), 3.93 (1H, dt, J = 9.8, 7.4 Hz, H-8b), 2.98 (2H, dd, J = 7.2, 7.2 Hz, H-7), 1.62 (3H, d, J = 6.2 Hz, H-6''); ^{13}C-NMR (175 MHz, Pyridine-d_5): δ 140.7 (C-1), 130.7 (C-3, 5), 130.0 (C-2, 6), 127.7 (C-4), 106.0 (C-1'), 103.8 (C-1''), 79.8 (C-3'), 78.4 (C-5'), 76.3 (C-2'), 75.3 (C-4''), 74.1 (C-3''), 73.6

(C-2''), 73.1 (C-8), 71.8 (C-4'), 71.1 (C-5''), 69.6 (C-6'), 37.9 (C-7), 20.0 (C-6''); FAB-MS (positive mode) m/z = 431.26 $[M+H]^+$.

(*Z*)-3-Hexenyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (4) – Colorless gum; IR (KBr) ν_{max}: 3385, 2933, 1048 cm^{-1}; ^{1}H-NMR (700 MHz, Pyridine-d_5): δ 5.51 (1H, d, J = 0.8 Hz, H-1''), 5.46 (1H, dtt, J = 10.8, 7.3, 1.5 Hz, H-3), 5.38 (1H, dtt, J = 10.8, 7.3, 1.5 Hz, H-4), 4.81 (1H, d, J = 7.7 Hz, H-1'), 4.12 (1H, dt , J = 9.5, 7.1 Hz, H-1a), 3.71 (1H, dt , J = 9.4, 7.1 Hz, H-1b), 2.41 (2H, q, J = 6.9 Hz, H-2), 1.92 (2H, quin, J = 7.2 Hz, H-5), 1.63 (3H, d, J = 6.2 Hz, H-6''), 0.82 (3H, t, J = 7.5 Hz, H-6); ^{13}C-NMR (175 MHz, Pyridine-d_5): δ 133.2 (C-4), 125.4 (C-3), 104.4 (C-1'), 102.4 (C-1''), 78.3 (C-3'), 76.9 (C-5'), 74.9 (C-2'), 73.8 (C-4''), 72.6 (C-3''), 72.1 (C-2''), 71.6 (C-4'), 69.6 (C-5''), 69.2 (C-1), 68.1 (C-6'), 28.1 (C-2), 20.6 (C-5), 18.4 (C-6''), 14.1 (C-6); FAB-MS (positive mode) m/z = 431.25 $[M+Na]^+$.

Acid hydrolysis of 1 and sugar determination – **1** (2.0 mg) was dissolved in 2 mL of 15% HCl. The solution was heated at 80 ºC for 2 h. The hydrolysate was extracted with CH_2Cl_2, and the aqueous layer was neutralized using an Amberlite IRA-67 column to yield the sugars. The sugar acquired from the hydrolysis was dissolved in

Table 1. ^{1}H and ^{13}C NMR data of **1** in Pyridine-d_5. (δ in ppm, 700 MHz for ^{1}H and 175 MHz for ^{13}C)[a]

Position	1		Position	1	
	δ_H	δ_C		δ_H	δ_C
1	1.59 m, 0.90 m	38.6	Gal 1'	4.87 d (7.7)	104.0
2	2.06 m, 1.66 m	31.4	2'	4.41 m	74.5
3	3.85 m	79.4	3'	4.07 m	76.4
4	2.65 dd (13.3, 2.4), 2.40 t-like (12.2)	40.5	4'	4.57 m	81.4
5	-	142.8	5'	3.94 m	76.6
6	5.29 br d (5.2)	122.6	6'	4.65 m, 4.15 m	61.8
7	1.83 m, 1.52 m	33.0	Glc 1''	5.12 d (7.8)	106.4
8	1.49 m	31.6	2''	4.37 m	82.7
9	0.89 m	52.0	3''	4.13 m	89.2
10	-	38.3	4''	3.77 m	72.0
11	1.45 m, 1.45 m	22.2	5''	3.82 m	78.8
12	2.58 m, 1.34 m	36.4	6''	4.45 m, 3.98 m	64.3
13	-	47.5	Glc 1'''	5.55 d (7.7)	106.2
14	1.26 m	57.7	2'''	4.03 m	77.5
15	2.12 ddd (16.9, 6.5, 3.3) 1.85 m	33.6	3'''	4.18 m	79.5
16	6.58 dd (2.9, 1.8)	146.0	4'''	4.13 m	72.9
17	-	156.5	5'''	3.82 m	79.8
18	0.90 s	17.2	6'''	4.55 m, 4.27 m	63.8
19	0.86 s	20.5	Glc 1''''	5.25 d (7.7)	105.3
20	-	197.6	2''''	4.02 m	76.0
21	2.23 s	28.4	3''''	4.18 m	78.0
			4''''	4.21 m	82.7
			5''''	3.99 m	77.9
			6''''	4.51 m, 4.22 m	63.0
			Glc 1'''''	5.11 d, (7.5)	106.3
			2'''''	4.04 m	76.1
			3'''''	3.89 m	80.0
			4'''''	4.22 m	72.2
			5'''''	4.10 m	79.1
			6'''''	4.55 m, 4.36 m	63.7

[a] J values are in parentheses and reported in Hz; the assignments were based on ^{1}H-^{1}H COSY, HMQC, and HMBC experiments.

anhydrous pyridine (0.1 mL), and 2.0 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 1.5 h and trimethylsilylated through adding 0.1 mL of 1-trimethylsilylimidazole for 2 h. The mixture was partitioned with *n*-hexane and H_2O (0.3 mL each), and the *n*-hexane layer (1.0 μL) was analyzed through GC/MS. Identification of D-galactose (20.093 min) and D-glucose (22.103 min) were detected in each case by co-injection of the hydrolysate with standard silylated sugars.

Result and Discussion

Structures of **2 - 4** were identified by comparing ^{1}H-, ^{13}C-NMR, and MS spectral data with those in the literatures to be benzyl-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**2**),[10] phenylethyl-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**3**),[11,12] (*Z*)-3-hexenyl-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**4**).[13] Compounds **2 - 4** were isolated from this source for the first time.

Compound **1** was isolated as a white amorphous powder. The molecular formula was determined to be $C_{51}H_{80}O_{27}$ from the molecular ion peak $[M+Na]^+$ at *m/z* 1147.4785 (calcd. for $C_{51}H_{80}NaO_{27}$: 1147.4785) in the positive-ion HR-FAB-MS. The IR spectrum showed characteristic absorptions for α,β-unsaturated ketone (1664 cm^{-1}), hydroxyl (3385 cm^{-1}), and glycosidic linkage (1000 - 1160 cm^{-1}).[14] The ^{1}H-NMR spectrum of **1** (Table 1) displayed the signals of two olefinic protons at δ_H 6.58 (dd, J = 2.9, 1.8 Hz, H-16) and 5.29 (br d, J = 5.2 Hz, H-6), an oxygenated methine proton at δ_H 3.85 (m, H-3), and three methyl singlet signals at δ_H 2.23 (s, H-21), 0.90 (s, H-18), and 0.86 (s, H-19) of aglycone, and five anomeric protons at 4.87 (d, J = 7.7 Hz, H-1'), 5.12 (d, J = 7.8 Hz, H-1''), 5.55 (d, J = 7.7 Hz, H-1'''), 5.25 (d, J = 7.7 Hz, H-1''''), and 5.11 (d, J = 7.5 Hz, H-1''''') of five sugar moieties. The ^{13}C-NMR spectrum (Table 1) showed a total of 51 carbon signals, of which 21 carbons were assigned to the aglycone and the remaining 30 carbons to five hexoses. The ^{13}C-NMR and DEPT spectra displayed 21 signals for the aglycone, which are composed of one ketone carbon at δ_C 197.6, four olefinic carbons at δ_C 156.5, 146.0, 142.8 and 122.6, one oxygenated methine carbon at δ_C 79.4, two quaternary carbons at δ_C 47.5 and 38.3, three methine carbons at δ_C 57.7, 52.0, and 31.6, seven methylene carbons at δ_C 40.5, 38.6, 36.4, 33.6, 33.0, 31.4 and 22.2, and three methyl carbons at δ_C 28.4, 20.5 and 17.2. A comparison of the NMR spectral findings of **1** with literature data revealed that the aglycone pair of **1** was identical to that of allimacroside A.[9] Detailed comparison of ^{13}C-NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC spectra of **1** with those of allimacroside A suggested that the sugar moiety of **1** was similar to that of allimacroside A with the exception of presence of an additional glucopyranose [δ_H 5.11 (d, J = 7.5, H-1''''') and δ_c 106.3, 76.1, 80.0, 72.2, 79.1, and 63.7]. In the HMBC

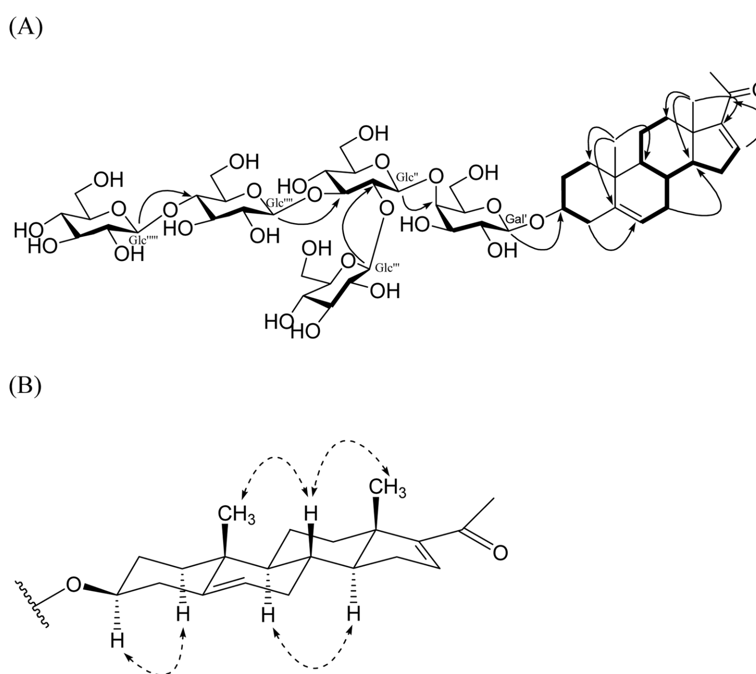

Fig. 2. Key HMBC (HC), ^{1}H-^{1}H COSY (—) correlations **1** (A), and NOESY (◄--►) correlations of **1** (B).

spectrum, the key correlations from δ_H 4.87 (H-1') to δ_C 79.4 (C-3), from δ_H 5.12 (H-1'') to δ_C 81.4 (C-4'), from δ_H 5.55 (H-1''') to δ_C 82.7 (C-2''), from δ_H 5.25 (H-1'''') to δ_C 89.2 (C-3'') and δ_H 5.11 (H-1''''') to δ_C 82.7 (C-4'''') suggested that the sequence of sugar moieties and the linkage position between the sugar unit and aglycone was the C-3 hydroxyl group (Fig. 2 A). Large coupling constants ($^3J_{H1, H2} \geq 7.5$ Hz) for anomeric protons revealed the β-configuration of all sugars. The relative stereochemistry of the aglycone was corroborated by NOESY cross-peaks of H_{ax}-1/H-3, H-19/H-8/H-18, and H-9/H-14 (Fig. 2 B). On acid hydrolysis, **1** yielded D-galactose and D-glucose in a ratio of 1:4 by GC analysis after derivatization.[15] Thus, the structure of **1** was established as pregna-5,16-dien-3β-ol-20-one 3-*O*-β-D-glucopyranosyl (1→4)-β-D-glucopyranosyl (1→3)-[β-D-glucopyranosyl (1→2)]-β-D-glucopyranosyl (1→4)-β-D-galactopyranoside, named allimacroside F.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A5A2A 28671860). We thank the Korea Basic Science Institute (KBSI) for the MS spectral measurements.

References

(1) Usui, A.; Matsuo, Y.; Tanaka, T.; Ohshima, K.; Fukuda, S.; Mine, T.; Nakayama, H.; Ishimaru, K. *Nat. Prod. Commun.* **2017**, *12*, 89-91.

(2) Jiangsu New Medical College. Dictionary of Chinese Drugs; Shanghai Science and Technological Publisher: China, **2001**, p 2642.

(3) Sobolewska, D.; Michalska, K.; Podolak, I.; Grabowska, K. *Phytochem. Rev.* **2016**, *15*, 1-35.

(4) Xie, W.; Zhang, Y.; Wang, N.; Zhou, H.; Du, L.; Ma, X.; Shi, X.; Cai, G. *Eur. J. Pharmacol.* **2008**, *599*, 159-165.

(5) Peng, J.; Yao, X.; Okada, Y.; Okuyama T. *Chem. Pharm. Bull.* **1994**, *42*, 2180-2182.

(6) Chen, H. -F.; Wang, N. -L.; Sun, H. -L.; Yang, B. -F.; Yao, X. -S. *J. Asian Nat. Prod. Res.* **2006**, *8*, 21-28.

(7) Chen, H.; Wang, G.; Wang, N.; Yang, M.; Wang, Z.; Wang, X.; Yao, X. *Pharmazie* **2007**, *62*, 544-548.

(8) Cheng, S. -B.; Wang, Y.; Zhang, Y. -F.; Wang, Y. *Zhong cao yao* **2013**, *44*, 1078-1081.

(9) Kim, Y. S.; Suh, W. S.; Park, K. J.; Choi, S. U.; Lee, K. R. *Steroids* **2017**, *118*, 41-46.

(10) Hamerski, L.; Bomm, M. D.; Silva, D. H. S.; Young, M. C. M.; Furlan, M.; Eberlin, M. N.; Castro-Gamboa, I.; Cavalheiro, A. J.; da Silva Bolzani, V. *Phytochemistry* **2005**, *66*, 1927-1932.

(11) Umehara, K.; Hattori, I.; Miyase, T.; Ueno, A.; Hara, S.; Kageyama, C. *Chem. Pharm. Bull.* **1988**, *36*, 5004-5008.

(12) Inagaki, J.; Watanabe, N.; Moon, J. -H.; Yagi, A.; Sakata, K.; Ina, K.; Luo, S. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 738-739.

(13) Kishida, M.; Fujii, M.; Ida, Y. *Heterocycles* **2005**, *65*, 2127-2137.

(14) Yokosuka, A.; Mimaki, Y.; Sashida, Y. *J. Nat. Prod.* **2000**, *63*, 1239-1243.

(15) Temraz, A.; El Gindi, O. D.; Kadry, H. A.; De Tommasi, N.; Braca, A. *Phytochemistry* **2006**, *67*, 1011-1018.

Received August 17, 2017
Revised October 30, 2017
Accepted October 31, 2017