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FUROSTANOL GLYCOSIDES FROM BULBS OF *ALLIUM CHINENSE*

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Key Word Index—*Allium chinense*; Liliaceae; Xiebai; furostanol glycoside; chinenoside II; chinenoside III.

Abstract—Two new furostanol saponins, named chinenosides II and III, were isolated along with seven known compounds, from the bulbs of *Allium chinense* G. Don by a combination of silica gel, Diaion HP-20, and octadecylsilanized (ODS) silica gel column chromatographies and preparative HPLC. On the basis of chemical and spectroscopic evidence, the structures of chinenosides II and III were determined to be 26-*O*-*β*-glucopyranosyl 3*β*,26-dihydroxy-(25*R*)-5*α*-furost-20(22)-en-6-one 3-*O*-*β*-xylopyranosyl-(1 → 4)-[*α*-arbinopyranosyl(1 → 6)]-*β*-glucopyranoside and 26-*O*-*β*-glucopyranosyl 3*β*,26-dihydroxy-(25*R*)-5*α*-furost-20(22)-en-6-one 3-*O*-*α*-arabinopyranosyl(1 → 6)-*β*-glucopyranoside, respectively.

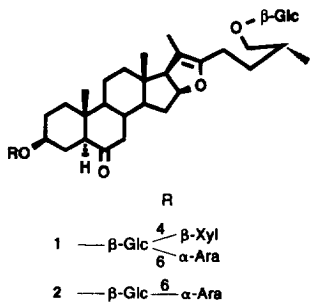
INTRODUCTION

The bulbs of *Allium chinense* G. Don are used as a Chinese crude drug 'Xiebai' for treatment of stenocardia, heart asthma, and so-called stagnant blood [1]. Okuyama *et al.* [2, 3] reported the isolation of several acid amides from the tubers of *A. bakeri* Reg. (*A. chinense*) which have a remarkable inhibitory activity against human blood-platelet aggregation. Also, Goda *et al.* [4] reported that *N*-coumaroyltyramines, lunularic acid, and *p*-coumaric acid, which had been isolated from the same source as inhibitors of prostaglandin and thromboxane synthetases, showed a significant inhibitory activity on platelet aggregation induced by arachidonic acid and collagen. Moreover, Matsuura [5] isolated a furostanol glycoside named chinenoside I from the same plant material.

Recently, we examined the constituents of *A. macrostemon* Bge. which is one of the original plants of 'Xiebai' [1] and isolated new furostanol saponins that have an inhibitory activity against ADP-induced human blood-platelet aggregation [6–8]. In continuation of this research, we investigated the chemical components of *A. chinense* collected at Hunan Province in China and isolated two new furostanol glycosides, named chinenosides II (**1**) and III (**2**), along with seven known compounds. Chinenosides II (**1**) and III (**2**) showed an inhibitory activity comparable to that of aspirin.

RESULTS AND DISCUSSION

The 75% ethanol extract of the dried bulbs of *A. chinense* was fractionated by a combination of silica gel, Diaion HP-20, and octadecylsilanized (ODS) silica gel column chromatographies and reversed-phase preparative HPLC procedures to yield compounds **1**–**9**.



Compounds **3**–**9** were known compounds and were identified by spectroscopic and chemical examination and comparison with the published data as tigogenin (**3**) [9], laxogenin (**4**) [5], laxogenin 3-*O*-*α*-arabinopyranosyl(1 → 6)-*β*-glucopyranoside (**5**) [5], laxogenin 3-*O*-*β*-xylopyranosyl(1 → 4)-[*α*-arabinopyranosyl(1 → 6)]-*β*-glucopyranoside (**6**) [5], 2,3,4,9-tetrahydro-1*H*-pyrido-[3,4-*b*]indole-3-carboxylic acid (**7**) [10], adenosine (**8**), and tryptophan (**9**), respectively.

Chinenoside II (**1**) was obtained as an amorphous powder and showed a purple colouration with Ehrlich reagent. On acid hydrolysis, compound **1** gave glucose, xylose, and arabinose in a ratio of 2:1:1, while, on enzymatic hydrolysis with *β*-glucosidase, **1** gave glucose and its corresponding spirostanol prosapogenin, which was identified as laxogenin 3-*O*-*β*-xylopyranosyl(1 → 4)-[*α*-arabinopyranosyl(1 → 6)]-*β*-glucopyranoside (**6**) [5].

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Table 1. ^{13}C NMR data for chinenosides II (1) and III (2) in C_5D_5N

	Aglycone moiety					Sugar moiety			
	1		2			1		2	
C	δ_C	^1H LR coupled*	δ_C	^1H LR coupled*	C	δ_C	^1H LR coupled*	δ_C	^1H LR coupled*
1	36.5	19	36.8	19	Glc-1	101.8	Glc-2	102.0	Glc-2
2	29.2		29.5		2	74.5	Glc-3, Glc-4	75.1	Glc-3
3	76.8	Glc-1	76.6	4, Glc-1	3	75.9	Glc-2	78.5	Glc-2
4	26.7		26.9		4	79.6	Xyl-1	72.3	
5	56.3	1, 19	56.4	19	5	78.1		77.0	Glc-4
6	209.4	4, 5, 7	209.5		6	67.8	Ara-1	69.7	Ara-1
7	46.7				Xyl-1	104.8	Glc-4, Xyl-2, Xyl-5		
8	37.0	11	37.1		2	74.8			
9	53.4	19	53.6	19	3	78.1	Xyl-2, Xyl-4		
10	40.7	19	40.9	19	4	70.7			
11	21.5		21.6		5	66.9			
12	39.2	18	39.3	17, 18	Ara-1	105.3	Ara-2, Ara-5	105.4	Glc-6, Ara-2, Ara-5
13	43.8	11, 18	43.9	15, 17, 18	2	72.2	Ara-4	71.8	
14	54.6	18	54.7		3	74.2		74.4	Ara-2, Ara-4, Ara-5
15	33.9		34.0		4	69.5		69.1	
16	84.0		84.1		5	66.9		66.5	Ara-1
17	64.2	18, 21	64.3	18, 21	Glc'-1	104.5	Glc'-2	104.8	26
18	14.1		14.3	17	2	74.6	Glc'-3, Glc'-4	75.1	
19	12.9		13.0	5	3	78.2	Glc'-4	78.5	Glc'-2, Glc'-4
20	103.2	17, 21	103.4	17, 21	4	71.4	Glc'-3	71.7	
21	11.6		11.7		5	78.1	Glc'-3	78.4	
22	152.4	17, 21	152.5	17, 21	6	62.6		62.8	
23	23.4		23.6						
24	31.2	27	31.4	26, 27					
25	33.2	27	33.4	26, 27					
26	74.7	Glc'-1	74.9	27					
27	17.2	24	17.4	26					

*Long-range coupled protons observed in the long-range ^1H–^{13}C COSY and/or HMBC spectra.

The locations of glycosidic linkages were elucidated by analyses of the two-dimensional NMR spectra, especially the HMBC spectrum. Complete assignments of the ^1H and ^{13}C NMR signals of four sugars and the aglycone were achieved with the aid of the ^1H–^1H COSY, ^1H–^1H relay-COSY, and ^1H–^{13}C COSY spectra (Table 1).

The anomeric proton of the glucose residue at δ_H 4.87 (*d*, J = 7.6 Hz) exhibited a long-range correlation with C-3 of the aglycone (δ_C 76.8), while that of the other glucose unit (δ_H 4.77, *d*, J = 7.9 Hz) showed a correlation with C-26 of the aglycone (δ_C 74.7). Also, long-range correlations were observed between the anomeric proton of the xylose unit and C-4 of the inner glucose residue and between the anomeric proton of the arabinose unit and C-6 of the inner glucose residue.

Based on the data described above, chinenoside II was determined to be 26-*O*-β-glucopyranosyl 3β,26-dihydroxy-(25*R*)-5α-furost-20(22)-en-6-one 3-*O*-β-xylopyranosyl-(1 → 4)-[α-arabinopyranosyl(1 → 6)]-β-glucopyranoside (**1**).

Chinenoside III (**2**) also exhibited a purple colouration with Ehrlich reagent, and it provided glucose and arabinose in a ratio of 2:1 on acid hydroysis. The ^1H and ^{13}C NMR spectra of **2** were similar to those of chinenoside II (**1**), except for the absence of signals of a xylose residue and the significant upfield shift of the signal due to C-6 of a glucopyranosyl unit (δ_C 72.3, *t*). Thus, the structure of chinenoside III was determined to be 26-*O*-β-glucopyranosyl 3β,26-dihydroxy-(25*R*)-5α-furost-20(22)-en-6-one 3-*O*-α-arabinopyranosyl-(1 → 6)-β-glucopyranoside (**2**), which was confirmed by two dimensional NMR experiments (Table 1).

EXPERIMENTAL

General. Mp, uncorr.; Optical rotations: at 20°C; IR, KBr; ^1H and ^{13}C NMR, 400 and 100 MHz, respectively, with TMS as int. standard. The bulbs of *A. chinense* were collected at Huaihua Region on Hunan Province in China, and the herbarium sample is kept in Department of Phytochemistry, Shengyang Pharmaceutical University.

Extraction and fractionation. The dried bulbs of *A. chinense* (30 kg) were cut into small pieces and extracted with 75% EtOH (80 l × 3). The combined EtOH solns were concentrated *in vacuo* to remove the EtOH portion. The residue was suspended in H_2O and filtered. The filtrate was subjected to Diaion HP-20 (1.2 kg) CC and

eluted with H_2O and then with MeOH. The fractions eluted with MeOH (178 g) were combined and separated again by silica gel (1.2 kg) CC using $CHCl_3$–MeOH–H_2O gradient mixtures to give 11 fractions (fr. 1 to fr. 11).

Fr. 2 (4.0 g) eluted with $CHCl_3$:MeOH:H_2O (100:20:5) was further separated by silica gel with $CHCl_3$:MeOH (70:1) to give tigogenin (**3**, 20 mg) and laxogenin (**4**, 70 mg), while fr. 3 (6.0 g) eluted with $CHCl_3$:MeOH:H_2O (100:20:10), on silica gel CC with $CHCl_3$:MeOH (20:1), gave laxogenin 3-*O*-α-arabinopyranosyl(1 → 6)-β-glucopyranoside (**5**, 465 mg) and adenosine (**8**, 225 mg). Fr. 5 (1.3 g) eluted with $CHCl_3$:MeOH:H_2O (80:20:10) gave laxogenin 3-*O*-β-xylopyranosyl(1 → 4)-[α-arabinopyranosyl(1 → 6)]-β-glucopyranoside (**6**, 1.3 g).

Fr. 6 (12.0 g) eluted with $CHCl_3$:MeOH:H_2O (80:30:10) was subjected to silica gel CC with $CHCl_3$:MeOH:H_2O (100:20:5) to yield an additional crop of laxogenin 3-*O*-β-xylopyranosyl(1 → 4)-[α-arabinopyranosyl(1 → 6)]-β-glucopyranoside (**6**, 1.8 g) and an unidentified compound U-1 (70 mg).

Fr. 7 (34.0 g) eluted with $CHCl_3$:MeOH:H_2O (70:30:10) was also subjected to silica CC with $CHCl_3$:MeOH:H_2O (80:20:5) to give 2,3,4,9-tetrahydro-1*H*-pyrido[3,4-*b*]indole-3-carboxylic acid (**7**, 230 mg) and a saponin mixture (4.6 g). The latter was further separated by Diaion HP-20 column chromatography with H_2O–MeOH solvent system to give two saponin mixtures, MS-1 (H_2O eluate, 1.2 g) and MS-2 (60% MeOH–H_2O eluate, 466 mg), along with tryptophan (**9**, 40% MeOH–H_2O eluate, 160 mg).

Parts of the saponin mixtures MS-1 (156 mg) and MS-2 (466 mg) were purified by reversed-phase prep. HPLC (column, $5C_{18}$ 10 × 250 mm; solvent, 45% H_2O–MeOH; flow rate, 2.0 ml min^{-1}). Chinenoside II (**1**, 53 mg) was obtained from MS-1, together with two unidentified saponins, U-4 (22 mg) and U-5 (41 mg), while chinenoside III (**2**, 127 mg) was obtained from MS-2 along with two unidentified saponins, U-6 (63 mg) and U-7 (141 mg).

Fr. 8 (27.0 g) eluted with $CHCl_3$:MeOH:H_2O (65:35:10) was subjected to Diaion HP-20 column chromatography with H_2O–MeOH solvent system. The eluate with 60% MeOH–H_2O (8.2 g) was further separated by medium-pressure CC (column, RP-8; solvent, MeOH:H_2O (70:30)) to give an additional sample of chinenoside II (**1**, 715 mg) and a saponin mixture MS-3 (2.9 g).

Chinenoside II (**1**). Amorphous powder, $[\alpha]_D$ −63.1° (pyridine; *c* 0.43). IR ν_{max} cm^{-1}: 3400 (OH), 1700 (C=O), 1000–1100 (glycosyl C–O). FD-MS *m/z* 1041 $[M + Na]^+$. Anal. calc for $C_{49}H_{78}O_{22}\cdot 3H_2O$: C, 54.85; H, 7.84; found, C, 54.67; H, 7.91%. ^{1}H NMR: δ0.64 (3H, *s*, 19-H_3), 0.65 (3H, *s*, 18-H_3), 1.01 (3H, *d*, *J* = 6.4 Hz, 27-H_3), 1.63 (3H, *s*, 21-H_3), 2.45 (1H, *d*, *J* = 10.0 Hz, 17-H), 3.59 (1H, *dd*, *J* = 8.8, 4.9 Hz, 26-H), 3.90 (1H, *m*, 26-H), 3.90 (1H, *m*, 3-H), 4.75 (1H, *m*, 16-H), 4.77 (1H, *d*, *J* = 7.9 Hz, Glc'-1), 4.87 (1H, *d*, *J* = 7.6 Hz, Glc-1), 4.98 (1H, *d*, *J* = 7.3 Hz, Ara-1), 5.36 (1H, *d*, *J* = 7.9 Hz, Xyl-1). ^{13}C NMR: Table 1.

Chinenoside III (**2**). Amorphous powder, $[\alpha]_D$ −42.8° (pyridine; *c*. 0.59). IR ν_{max} cm^{-1}: 3400 (OH), 1700 (C=O), 1000–1100 (glycosyl C–O). FD-MS *m/z* 909 $[M + Na]^+$. ^{1}H NMR: 0.65 (6H, *s*, 18-H_3, 19-H_3), 1.03 (3H, *d*, *J* = 6.7 Hz, 27-H_3), 1.64 (3H, *s*, 21-H_3), 2.45 (1H, *d*, *J* = 10.1 Hz, 17-H), 3.61 (1H, *dd*, *J* = 9.5, 5.5 Hz, 26-H), 3.94 (1H, *m*, 26-H), 4.02 (1H, *m*, 3-H), 4.75 (1H, *ddd*, *J* = 9.4, 7.9, 6.0 Hz, 16-H), 4.82 (1H, *d*, *J* = 7.6 Hz, Glc'-1), 4.959 (1H, *d*, *J* = 6.7 Hz, Ara-1), 4.964 (1H, *d*, *J* = 7.6 Hz, Glc-1). ^{13}C NMR: Table 1.

Acid hydrolysis of glycosides. Each glycoside (2 mg) was heated with 2 N HCl–dioxane (1:1, 2 ml) in a sealed tube at 100°C for 4 hr. The reaction mixture was concentrated to dryness by blowing N_2 gas at room temp. The residue was trimethylsilylated with hexamethyldisilazane:trimethylchlorosilane (2:1) mixture [11] at room temp. GC: 3 m × 3 mm packed with 10% SE-30; detection, FID; column temperature, 195°; carrier gas, N_2 (16 ml min^{-1}). Retention times (min): arabinose (12.5, 13.1), xylose (18.2, 19.3), glucose (36.2, 38.5).

Enzymatic Hydrolysis of **1**. A mixture of **1** (100 mg) and β-glucosidase (from almond, 150 mg) in an acetate buffer (pH 4.1, 30 ml) was incubated at 37° for 24 hr. After dilution with H_2O, reaction mixture applied to a column of MCI gel CHP-20P (40 g) and eluted with H_2O and then MeOH–EtOAc mixture. An eluate with MeOH–AcOEt (1:1) gave laxogenin 3-*O*-β-xylopyranosyl(1 → 4)-[α-arabinopyranosyl(1 → 6)]-β-glucopyranoside (**6**, 57 mg).

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