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STEROIDAL SAPONINS FROM *ALLIUM CHINENSE* AND THEIR INHIBITORY ACTIVITIES ON CYCLIC AMP PHOSPHODIESTERASE AND Na^+/K^+ ATPase

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Key Words Index—*Allium chinense*; Liliaceae; steroidal saponins; cyclic AMP phosphodiesterase inhibition; Na^+ K^+ ATPase inhibition.

Abstract—The saponin fraction prepared from the methanolic extract of *Allium chinense* bulbs exhibited inhibitory activities on cyclic AMP phosphodiesterase (cAMP PDE) (43.5%) and Na^+/K^+ ATPase (59.3%) at a sample concentration of 100 μg ml^{-1}, respectively. Attempted purification of the active fraction through column chromatography on silica gel and ODS silica gel resulted in the isolation of six steroidal saponins, one of which appeared to be a new compound and one to be the first isolation from a natural source. (25*R*,*S*)-5α-Spirostan-3β-ol tetrasaccharide showed inhibitory activities on both cAMP PDE and Na^+ K^+ ATPase, while (25*R*)-3β-hydroxy-5α-spirostan-6-one di- and tri-saccharides inhibited only cAMP PDE.

INTRODUCTION

The bulbs of *Allium chinense* are reputed to be effective as a heart failure cure in Chinese medicine and are included in some traditional Chinese preparations [1]. To identify the active constituents contained in the crude drug, we have carried out a phytochemical investigation of *A. chinense* bulbs by monitoring the inhibitory activities on cyclic AMP phosphodiesterase (cAMP PDE) [2–4] and Na^+/K^+ ATPase. This has resulted in the isolation of six steroidal saponins (**1**–**6**), one of which appeared to be a new compound (**2**) and one to be the first isolation from a natural source (**6**). This paper reports the identification and structural assignments of the isolated saponins and their inhibitory activities on cAMP PDE and Na^+/K^+ ATPase.

RESULTS AND DISCUSSION

Dried bulbs of *A. chinense* (3.0 kg) were refluxed with methanol and the solvent was removed under reduced pressure to give a crude extract (60 g). This extract showed 47.9% inhibition on cAMP PDE and 2.2% on Na^+/K^+ATPase at a sample concentration of 100 μg ml^{-1}. Fractionation of the extract was performed by Diaion HP-20 column chromatography, eluting with water–methanol (7:3; 1:1; 3:7), methanol–ethanol (1:1), and finally with ethyl acetate. The methanol–ethanol (1:1) eluate fraction (2.45 g) inhibited both cAMP PDE (43.5%) and Na^+/K^+ ATPase (59.3%) at 100 μg ml^{-1} (Table 1), and was subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography to give compounds **1**–**6**.

The ^{1}H NMR spectrum of **1** ($C_{38}H_{60}O_{13}$) showed typical signals of steroid methyl groups. Two appeared as singlets at δ0.78 and 0.65; the other two as doublets at δ1.15 (J = 6.9 Hz) and 0.70 (J = 5.7 Hz). The ^{13}C NMR spectrum exhibited signals for a carbonyl carbon at δ209.1, two anomeric carbons at δ105.5 and 102.1 and a ketal carbon at δ109.2 (Table 2). These spectral data and direct TLC comparison with an authentic sample allowed the identification of **1** as (25*R*)-3β-hydroxy-5α-spirostan-6-one (laxogenin) 3-*O*-{*O*-α-L-arabinopyranosyl-(1 → 6)-β-D-glucopyranoside}. This compound has been already isolated by us from *Smilax sieboldii* [5].

Compound **2** ($C_{40}H_{62}O_{14}$) was obtained as an amorphous solid, $[\alpha]_D$ −36.0° (methanol). The spectral features of **2** were quite similar to those of **1**. The presence of an acetyl group in the molecule was shown by the IR (ν_{max} 1725 cm^{-1}), ^{1}H NMR (δ2.12, 3H, *s*) and ^{13}C NMR (δ170.1 and 21.2) spectra. When **2** was treated with 4% potassium hydroxide in ethanol, it was hydrolysed to yield **1**. Therefore, compound **2** must be monoacetate of **1**. In the ^{13}C NMR spectrum of **2**, the signal due to the arabinose C-2 carbon was shifted to lower field by 1.4 ppm, whereas the signals due to C-1 and C-3 to upper fields by 3.4 and 2.4 ppm, respectively, as compared with those of **1**. Furthermore, the downfield-shifted ^{1}H signal at δ5.88 (*dd*, J = 7.9, 6.7 Hz) was assigned to the ara-

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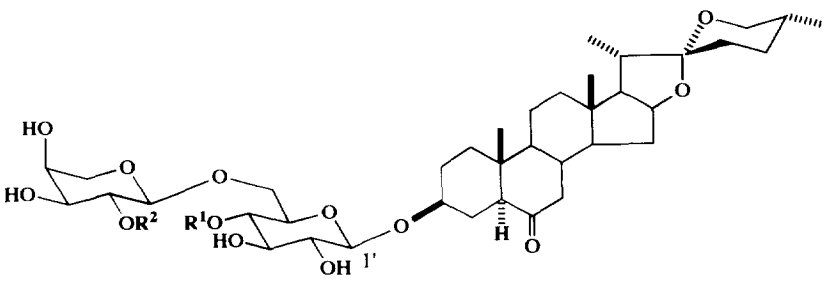
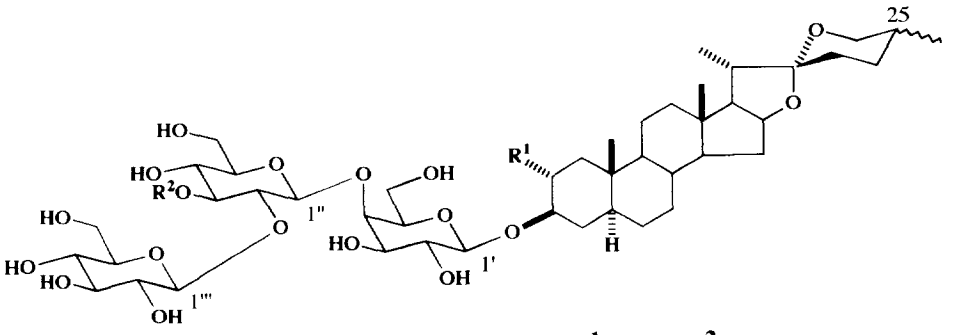


	R^1	R^2
1	H	H
2	H	Ac
3	β-D-Xyl*p*	H



		R^1	R^2
4	(25*R*, *S*)	H	β-D-Glc*p*
5	(25*R*, *S*)	OH	β-D-Glc*p*
6	(25*R*, *S*)	OH	H

Table 1. Inhibitory activities on cAMP PDE and Na^+/K^+ ATPase of the MeOH extract of *A. chinense* bulbs and the fractions obtained by dividing the extract through a Diaion HP-20 column

	Inhibition (%)	
	cAMP PDE	Na^+/K^+ ATPase
MeOH extract	47.9	2.2
30% MeOH eluate fr.	26.6	1.7
50% MeOH eluate fr.	29.4	4.6
70% MeOH eluate fr.	66.2	8.0
MeOH–EtOH eluate fr.	43.5	59.3
EtOAc eluate fr.	23.1	28.7

Data are mean values of duplicate or triplicate experiments, and are expressed as percentage of inibition at a sample concentration of 100 μg ml^{-1}.

binose 2-H proton. Thus, the acetyl moiety was shown to be linked to the arabinose C-2 hydroxyl position, and the structure of **2** was determined to be laxogenin 3-*O*-{*O*-(2-*O*-acetyl-α-L-arabinopyranosyl)-(1 → 6)-β-D-glucopyranoside}.

On comparison of the ^{13}C NMR spectrum of **3** ($C_{43}H_{68}O_{17}$) with that of **1**, five additional signals corresponding to a terminal β-D-xylopyranosyl unit appeared and the signal due to C-4 of the inner glucose was shifted downfield by 7.9 ppm. Therefore, the structure of **3** was identified as laxogenin 3-*O*-{*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside} [6].

Compounds **4** ($C_{51}H_{84}O_{23}$) and **5** ($C_{51}H_{84}O_{24}$) were identified by their IR, ^1H and ^{13}C NMR spectra as (25*R*, *S*)-5α-spirostan-3β-ol 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} and (25*R*,*S*)-5α-spirostane-2α,3β-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} [7–10], respectively.

Compound **6** ($C_{45}H_{74}O_{19}$) was immediately indicated to have the same aglycone as **5** by the ^1H and ^{13}C NMR spectra. Acid hydrolysis of **6** gave a mixture of (25*R*)-5α-spirostane-2α,3β-diol (gitogenin) and its C-25 isomer, and D-glucose and D-galactose in a ratio of 2:1. Lack of the terminal β-D-glucose moiety attached to C-3 of the inner D-glucose, as compared with **5**, was the only difference recognized in the spectral data for **6**. The structure

Table 2. ^{13}C NMR spectral data for compounds **1**–**6***

				4		**5**		**6**	
C	**1**	**2**	**3**	25*R*	(25*S*)	25*R*	(25*S*)	25*R*	(25*S*)
1	36.8	36.8	36.7	37.2		45.6		45.6	
2	29.6	29.5	29.4	29.9		70.4		70.5	
3	76.8	76.8	77.0	77.4		84.1		84.7	
4	27.0	27.0	26.9	34.9		34.1		34.1	
5	56.4	56.4	56.4	44.7		44.6		44.7	
6	209.1	209.6	209.6	28.9		28.1		28.1	
7	46.7	46.7	46.7	32.4		32.1		32.1	
8	37.3	37.4	37.3	35.3		34.6		34.6	
9	53.6	53.7	53.7	54.4		54.3		54.4	
10	40.8	40.9	40.8	35.8		36.8		36.9	
11	21.5	21.5	21.5	21.3		21.4		21.4	
12	39.6	39.6	39.6	40.2		40.0		40.0	
13	41.1	41.1	41.0	40.8		40.7		40.7	
14	56.4	56.4	56.4	56.5		56.3		56.3	
15	31.8	31.8	31.7	32.1		32.2		32.2	
16	80.8	80.8	80.8	81.1	(81.2)	81.1	(81.2)	81.1	(81.2)
17	62.8	62.8	62.8	63.1		63.0	(62.8)	63.0	(62.8)
18	16.4	16.4	16.4	16.6		16.6		16.6	
19	13.1	13.1	13.0	12.3		13.4		13.4	
20	42.0	41.9	41.9	41.9	(42.5)	42.0	(42.5)	42.0	(42.4)
21	15.0	14.9	14.9	15.0	(14.9)	15.0	(14.8)	15.0	(14.8)
22	109.2	109.2	109.2	109.2	(109.7)	109.2	(109.7)	109.2	(109.7)
23	31.8	31.7	31.7	31.9	(26.2)†	31.8	(26.2)†	31.8	(26.2)†
24	29.2	29.2	29.2	29.3	(26.4)†	29.3	(26.4)†	29.2	(26.4)†
25	30.6	30.6	30.5	30.6	(27.6)	30.6	(27.5)	30.6	(27.5)
26	66.9	66.9	66.9	66.9	(65.1)	66.9	(65.1)	66.8	(65.1)
27	17.3	17.3	17.3	17.3	(16.3)	17.3	(16.3)	17.3	(16.3)
1′	102.1	102.1	102.0	102.5		103.2		105.1	
2′	75.2	75.2	74.9†	73.2		72.5		72.7	
3′	78.5	78.5	78.4	75.6		75.7		75.5	
4′	71.9	72.1	79.8	80.2		79.7		80.9	
5′	77.0	76.9	74.8†	76.1		76.0		75.5	
6′	69.7	69.7	68.1	60.6		60.6		60.4	
1″			105.1	105.1‡		104.8‡		103.4	
2″			74.8†	81.5		81.3		85.9	
3″			76.3	88.6		88.6		77.7	
4″			71.0	70.9§		70.7		70.5	
5″			67.3	77.6		77.5		79.0	
6″				62.4		62.6		61.8	
1‴	105.5	102.1	105.6	104.9‡		104.6‡		106.8	
2‴	72.3	73.7	72.5	75.3		75.3		76.6	
3‴	74.5	72.1	74.5	78.7		78.6		78.5‡	
4‴	69.1	69.0	69.8	71.0§		71.3		71.7	
5‴	66.5	66.1	67.3	77.9		78.2		78.3‡	
6‴				63.1		63.0		63.2	
1⁗				104.6		104.5			
2⁗				75.3		75.5			
3⁗				78.7		78.6			
4⁗				71.6		71.6			
5⁗				78.7		78.4			
6⁗				62.4		62.3			
Ac		170.1							
		21.2							

*Spectra were measured in pyridine-d_5.
†‡§Assignments may be interchanged.

of **6** was identified as (25*R*,*S*)-5α-spirostane-2α,3β-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1 → 2)-*O*-β-D-glucopyranosyl-(1 → 4)-β-D-galactopyranoside} [9, 10].

Compound **2** is a new steroidal saponin. Compound **6** has been already reported as the corresponding spriostanol saponin of a new furostanol saponin from *Capsicum annuum* [9] and a partial hydrolysate of a spriostanol saponin from *Tribulus cistoides* [10]. However, this is the first isolation of **6** from a natural source.

The inhibitory activities of the isolated saponins on cAMP PDE and Na^+/K^+ ATPase are listed in Tables 3 and 4. The laxogenin glycosides (**1**–**3**) exhibited considerable inhibitory activity on cAMP PDE, among which **2**, embracing an acetyl group at the saccharide moiety, was the most potent inhibitor, showing an IC_{50} value of 3.3×10^{-5} M. This is almost as potent as papaverine, used as a positive control. Compounds **1**–**3**, however, showed no inhibitory activity on Na^+/K^+ ATPase. Compound **4**, (25*R*,*S*)-5α-spirostan-3β-ol tetrasaccharide, inhibited both cAMP PDE and Na^+/K^+ ATPase. Analysis of the constituents with cAMP PDE inhibitory activity in the 70% methanol eluate fraction, and evaluation for the cardeotonic effect of the isolated saponins, are in progress.

Table. 3. Inhibitory activity on cAMP PDE of the isolated saponins

Compounds	$IC_{50}(\times 10^{-5}$ M)
1	11.2
2	3.3
3	12.3
4	7.0
5	36.9
6	42.1
Papaverine	3.0

Table. 4. Inhibitory activity on Na^+/K^+ ATPase of the isolated saponins

Compounds	$IC_{50}(\times 10^{-5}$ M)
1	—*
2	—
3	—
4	4.0
5	—
6	—
Ouabain	0.1

* $< 1.0 \times 10^{-4}$ M.

EXPERIMENTAL

General. NMR: Bruker AM-400 (ppm, *J* Hz); CC: silica gel (Fuji-Silysia Chemical), ODS silica gel (Nacalai Tesque) and Diaion HP-20 (Mitsubishi-Kasei); TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck); HPLC: Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, UV-8000) equipped with a TSK-gel ODS-Prep column (Tosoh, 250 × 4.6 mm i.d., ODS, 5 μm).

Chemicals. Beef heart PDE, snake venom nucleotidase, cAMP PDE and ouabain sensitive dog kidney Na^+/K^+ ATPase: Sigma. [^{3}H] cAMP: Radiochemical Center. All other chemicals used were of biochemical-reagent grade.

Plant materials. Dried bulbs of *A. chinense* were purchased from Uchida-Wakanyaku (Japan).

Extraction and isolation. Dried bulbs of *A. chinense* (3.0 kg) were extracted with hot MeOH. The MeOH extract (60 g), after removal of the solvent under red. press., was partitioned by passing it through Diaion HP-20 CC, eluting with H_2O–MeOH gradients (7:3; 1:1; 3:7), MeOH–EtOH (1:1) and finally with EtOAc. The MeOH–EtOH (1:1) eluate fr. (2.45 g) was divided into six frs (I–VI) by silica gel CC, eluting with $CHCl_3$–MeOH–H_2O (40:10:1; 20:10:1) and finally with MeOH. Fr. I was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (60:10:1) as solvent system to give **2** (33.6 mg). Frs II and III were chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (50:10:1) and ODS silica gel using MeOH–H_2O (4:1) to give **1** (52.3 mg) and **3** (131 mg), respectively. Frs IV and V were chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (70:10:1; 60:10:1; 40:10:1; 30:10:1) to give **6** (36.6 mg) and **4** (44.8 mg), respectively. The most polar fr. VI was subjected to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (20:10:1) and ODS silica gel CC with MeOH–H_2O (9:1; 7:3) to give **5** (26.3 mg).

Compound **1**. Amorphous solid, $[\alpha]_D^{30}$ −98.7° (MeOH: *c* 0.25). SIMS *m/z* 747 $[M + Na]^+$; IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1705 (C=O). ^{1}H NMR (pyridine-d_5): δ4.98 (1H, *dd*, *J* = 6.6 Hz, 1‴-H), 4.94 (overlapping with H_2O signals, 1′-H), 1.15 (3H, *d*, *J* = 6.9 Hz, 21-Me), 0.78 (3H, *s*, 18-Me), 0.70 (3H, *d*, *J* = 5.7 Hz, 27-Me), 0.65 (3H, *s*, 19-Me).

Compound **2**. Amorphous solid, $[\alpha]_D^{30}$ −62.9° (MeOH; *c* 0.10). Anal. calcd. for $C_{40}H_{62}O_{14}$: C, 62.63; H, 8.15%. Found: C, 62.79; H, 8.67%. Negative-ion FABMS *m/z* 765 $[M - H]^-$, 723 $[M - acetyl]^-$. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2920 and 2850 (CH), 1725 and 1705 (C=O), 1455, 1375, 1235, 1170, 1045, 1010, 980, 960, 915, 895, 865, 770 (intensity 915 < 895, 25*R*-spiroacetal). ^{1}H NMR (pyridine-d_5): δ5.88 (1H, *dd*, *J* = 7.9, 6.7 Hz, 2‴-H), 5.00 (1H, *d*, *J* = 6.7 Hz, 1‴-H), 4.98 (1H, *d*, *J* = 8.5 Hz, 1′-H), 4.54 (1H, *q*-like, *J* = 6.7 Hz, 16-H), 3.59 (1H, *dd*, *J* = 10.6, 3.4 Hz, 26a-H), 3.49 (1H, *dd*, *J* = 10.6, 10.6 Hz, 26b-H), 2.12 (3H, *s*, Ac), 1.15 (3H, *d*, *J* = 6.9 Hz, 21-Me), 0.79 (3H, *s*, 18-Me), 0.70 (3H, *d*, *J* = 5.5 Hz, 27-Me), 0.68 (3H, *s*, 19-Me).

Alkaline hydrolysis of **2**. Compound **2** (2 mg) was treated with 4% KOH (2 ml) for 2 hr at room temp., and the reaction mixt. was passed through an Amberlite IR-120B (Organo) column to yield **1** (1.2 mg).

Compound **3**. Amorphous solid, $[\alpha]_D^{27}$ −71.0 (MeOH: *c* 0.12). Negative-ion FABMS *m/z* 856 $[M]^-$; IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 1705 (C=O). ^1H NMR (pyridine-d_5): δ5.46 (1H, *d*, *J* = 7.8 Hz, 1″-H), 5.06 (1H, *d*, *J* = 7.4 Hz, 1‴-H), 4.94 (1H, *d*, *J* = 7.8 Hz, 1′-H), 1.15 (3H, *d*, *J* = 6.8 Hz, 21-Me), 0.79 (3H, *s*, 18-Me), 0.70 (3H, *d*, *J* = 5.7 Hz, 27-Me), 0.64 (3H, *s*, 19-Me).

Compound **4**. Amorphous solid, $[\alpha]_D^{28}$ −50.0° (pyridine: *c* 0.10). Negative-ion FABMS *m/z* 1063 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH). ^1H NMR (pyridine-d_5): δ 5.58 (1H, *d*, *J* = 7.6 Hz, 1‴-H), 5.30 (1H, *d*, *J* = 7.9 Hz, 1⁗-H), 5.14 (1H, *d*, *J* = 7.9 Hz, 1″-H), 4.88 (1H, *d*, *J* = 7.5 Hz, 1′-H), 1.15 (3H, *d*, *J* = 6.9 Hz, 21-Me), 1.08 (*d*, *J* = 7.3 Hz, 27-Me of 25*S*-isomer), 0.83 (*s*, 18-Me of 25*R*-isomer), 0.82 (*s*, 18-Me of 25*S*-isomer), 0.70 (*d*, *J* = 5.0 Hz, 27-Me of 25*R*-isomer), 0.65 (3H, *s*, 19-Me).

Compound **5**. Amorphous solid, $[\alpha]_D^{26}$ −42.0° (pyridine: *c* 0.10). Negative-ion FABMS *m/z* 1079 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH). ^1H NMR (pyridine-d_5): δ5.59 (1H, *d*, *J* = 8.0 Hz, 1‴-H), 5.30 (1H, *d*, *J* = 7.7 Hz, 1⁗-H), 5.17 (1H, *d*, *J* = 7.7 Hz, 1″-H), 4.92 (1H, *d*, *J* = 7.7 Hz, 1′-H), 1.13 (3H, *d*, *J* = 6.9 Hz, 21-Me), 1.08 (*d*, *J* = 7.0 Hz, 27-Me of 25*S*-isomer), 0.81 (*s*, 18-Me of 25*R*-isomer), 0.80 (*s*, 18-Me of 25*S*-isomer), 0.71 (3H, *s*, 19-Me), 0.70 (*d*, *J* = 5.1 Hz, 27-Me of 25*R*-isomer).

Compound **6**. Amorphous solid, $[\alpha]_D^{28}$ −52.0° (pyridine: *c* 0.10). Anal. calcd. for $C_{45}H_{74}O_{19}\cdot 3/2H_2O$: C, 57.11; H, 8.21%. Found: C, 57.29; H, 8.10%. Negative-ion FABMS *m/z* 917 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2930 (CH), 1450, 1375, 1255, 1240, 1170, 1065, 985, 920, 895, 870, 850, 700. ^1H NMR (pyridine-d_5): δ5.27 (1H, *d*, *J* = 7.5 Hz, 1″-H), 5.14 (1H, *d*, *J* = 7.8 Hz, 1‴-H), 4.93 (1H, *d*, *J* = 7.8 Hz, 1′-H), 1.14 (*d*, *J* = 6.9 Hz, 21-Me of 25*S*-isomer), 1.13 (*d*, *J* = 7.0 Hz, 21-Me of 25*R*-isomer), 1.08 (*d*, *J* = 7.1 Hz, 27-Me of 25*S*-isomer), 0.81 (*s*, 18-Me of 25*R*-isomer), 0.80 (*s*, 18-Me of 25*S*-isomer), 0.72 (3H, *s*, 19-Me), 0.70 (*d*, *J* = 5.7 Hz, 27-Me of 25*R*-isomer).

Acid hydrolysis of **6**. A soln of **6** (5 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 4 ml) was heated at 100° for 2 hr under an Ar atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column, and fractionated by Sep-Pak C_{18} cartridge (Waters), eluting with H_2O (20 ml) followed by MeOH (20 ml) to give the sugar fr. (1.9 mg) and the sapogenin fr. From the sapogenin fr., a mixt. of (25*R*)-5α-spirostane-2α,3β-diol (gitogenin) and its C-25 isomer (1.7 mg) was obtained. The sugar fr. (1.5 mg) was diluted with H_2O (1 ml) and treated with (−)-α-methyl benzylamine (5 mg) and $Na(BH_3CN)$ (8 mg) in EtOH (1 ml) at 40° for 4 hr, followed by acetylation with Ac_2O (0.3 ml) in pyridine (0.3 ml). The reaction mixt. was passed through a Sep-Pak C_{18} cartridge with H_2O–MeCN (4:1, 10 ml) and then MeCN (10 ml). The MeCN eluate fr. was further passed through a TOYOPAK IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a mixt. of 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which were then analysed by HPLC [11, 12]. Derivatives of D-glucose and D-galactose were detected in a ratio of 2:1.

Assay of cAMP PDE activity. The PDE activity was assayed by the modified method described previously [13, 14]. The assay was a two-step isotopic procedure. Tritium-labelled cAMP was hydrolysed to 5′-AMP by PDE, and the 5′-AMP was then further hydrolysed to adenosine by snake venom nucleotidase. The hydrolysate was treated with an anion-exchange resin (Dowex AG1-X8; BIO-RAD) to adsorb all charged nucleotides, leaving $[^3H]$ adenosine as the only labelled compound to be counted.

Assay of Na^+/K^+ ATPase activity. The Na^+/K^+ ATPase activity was assayed according to the reported method [15] with some modification. The reaction mixt. composed of 50 mM Tris–HCl (pH 7.3, 37°), 3 mM ATP, 4 mM Mg^{2+}, 130 mM Na^+, 20 mM K^+, 0.02 units of Na^+/K^+ ATPase, with and without test compound dissolved in DMSO, was incubated for 15 min at 37°. The concn of DMSO in the mixt. was held at 5%. The reaction was terminated by addition of 50% CCl_3COOH. The released inorganic phosphate was determined by a modification of the method of ref. [16]. To the test soln was added 0.5% Na dodecyl sulphate, 0.1% 2,4-diaminophenol·2HCl in 1% Na_2SO_3 and 1% ammonium heptamolybdate in 1 M H_2SO_4. After 20 min, the absorbance at 660 nm was recorded.

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REFERENCES

1. Jiang Su New Medical College (ed.) (1977) *Dictionary of Traditional Chinese Crude Drugs*, Vol. 2, pp. 2642–2643. Shanghai Scientific Technologic, Shanghai.
2. Endoh, M., Yamashita, S. and Taira, N. (1982) *J. Pharmacol. Exp. Ther.* **221**, 775.
3. Yoshioka, K., Takayanagi, I., Hisayama, T., Furukawa, A. (1989) *J. Pharmacobio-Dyn.* **12**, 653.
4. Yoshioka, K., Takayanagi, I. and Hisayama, T. (1989) *J. Pharmacobio-Dyn.* **12**, 660.
5. Kubo, S., Mimaki, Y., Sashida, Y., Nikaido, T. and Ohmoto, T. (1992) *Phytochemistry* **31**, 2445.
6. Matsuura, H., Ushiroguchi, T., Itakura, Y. and Fuwa, T. (1989) *Chem. Pharm. Bull.* **37**, 1390.
7. Ding, Y., Chen, Y.-Y., Wang, D.-Z. and Yang, C.-R. (1989) *Phytochemistry* **28**, 2787.
8. Peng, J. P., Wu, Y., Yao, X. S., Okuyama, T. and Narui, T. (1992) *Yaoxue Xuebao* **27**, 918.
9. Yahara, S., Ura, T., Sakamoto, C. and Nohara, T. (1994) *Phytochemistry* **37**, 831.
10. Achenbach, H., Hübner, H., Brandt, W. and Reiter, M. (1994) *Phytochemistry* **35**, 1527.
11. Oshima, R. and Kumanotani, J. (1981) *Chem. Letters* 943.

12. Oshima, R., Yamauchi, Y. and Kumanotani, J. (1982) *Carbohydr. Res.* **107**, 169.
13. Nikaido, T., Ohmoto, T., Noguchi, H., Kinoshita, T., Saitoh, H. and Sankawa, U. (1981) *Planta Med.* **43**, 18.
14. Nikaido, T., Ohmoto, T., Sankawa, U., Tomimori, T., Miyaichi, Y. and Imoto, Y. (1988) *Chem. Pharm. Bull.* **36**, 654.
15. Esmann, H. (1988) *Meth. Enzymol.* **156,** 105.
16. Fiske, C. H. and Subbarow, Y. (1925) *J. Biol. Chem.* **66,** 375.