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Two New Steroidal Saponins from the Tubers of *Liriope spicata*

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Abstract□Two novel steroidal saponins designated as spicatosides A(**1**) and B(**2**) were isolated from the tubers of *Liriope spicata* and their structures were elucidated as 25(S)-ruscogenin-1-O-β-D-glucopyranosyl (1→2)-[β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside (**1**) and 26-O-β-D-glucopyranosyl 25(S)-22-O-methyl-furost-5-en-1β, 3β, 26-triol 1-O-β-D-glucopyranosyl (1→2)-[β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside (**2**), respectively.

Keywords□*Liriope spicata* Lour., Liliaceae, steroidal saponin, spicatoside A, spicatoside B.

The tubers of *Liriope spicata* Lour. (Liliaceae) are employed as those of *Ophiopogon japonicus* Ker-Gawler (Liliaceae) and are utilized locally in various parts of China and Korea for a nutrious tonic, antitussive and expectorant[1]. However, no detailed chemical investigation appears to have been done on this plant. The present communication reports the isolation and characterization of two new steroidal saponins named spicatosides A and B.

EXPERIMENTAL

Melting points are uncorrected. ^{1}H-NMR spectra were obtained on either a Varian FT-80A (80 MHz), a Bruker AM-300 (300MHz) or a Bruker AM-200 (200MHz) spectrometer using TMS as an internal standard. ^{13}C-NMR spectra were recorded with a Bruker AM-200 (50.3MHz) or a Bruker AM-300 (75.5MHz) instrument. EIMS were determined on a Hewlett-Packard 5985B GC/MS System equipped with direct inlet system.

Plant material

The tubers of *Liriope spicata* were collected in south Kyungnam Province in the spring season of 1986. Specimens have been deposited in College of Pharmacy, Yeungnam University.

Extraction and purification

The dried tubers of *Liriope spicata* (20kg) were refluxed with MeOH for 3 hr (5 times) and evaporated to dryness. The residue (2.97kg) was partitioned between ether and H_2O. The aqueous layer was then extracted with BuOH and concentrated in vacuo to afford residue (300g). A portion of BuOH extract was subjected to column chromatography over silica gel eluted with $CHCl_3$/MeOH/H_2O (75:25:3) to yield 4 fractions. Fractions 2 and 3 were separately purified by column chromatography on silica gel with EtOAc saturated with H_2O/MeOH (49:1) to yield pure spicatoside A (**1**) from fraction 2 and spicatoside B (**2**) from fraction 3, respectively.

Spicatoside A(1)

Colorless needles from MeOH; m.p. 243-245 °C; $[\alpha]_D^{21}$ = -85.9° (*c* 0.06, MeOH); IR ν_{max}^{KBr} cm^{-1} 3420, 1065, 1037, 987, 920, 895, 850 [intensity 920>895, 25(S)-spiroketal]; ^{1}H-NMR: see Table I; ^{13}C-NMR: see Table II; Anal. Clacd for $C_{44}H_{70}O_{17}\cdot 3H_2O$: C, 57.13; H, 7.63; Found: C, 56.90; H, 7.80.

Spicatoside B(2)

White amorphous powder from EtOH; m.p. 196-198 °C; $[\alpha]_D^{20}$ = -88.7° (*c* 0.13, MeOH); IR ν_{max}^{KBr} cm^{-1} 3420, 1070, 1050, 910, 840; ^{1}H-NMR: see Table I; ^{13}C-NMR: see Table II; Anal. Calcd for $C_{51}H_{84}O_{23}\cdot 5H_2O$: C, 53.02; H, 7.33; Found: C, 52.90; H, 7.41.

Acid hydrolysis of 1

A solution of **1** (50mg) in 4N-HCl/dioxane (1:1, 15 m*l*) was refluxed for 4 hr on a water bath. The reaction mixture was diluted with crushed ice and filtered. The residue was crystallized from MeOH to give an aglycone (**3**) as colorless needles; m.p.

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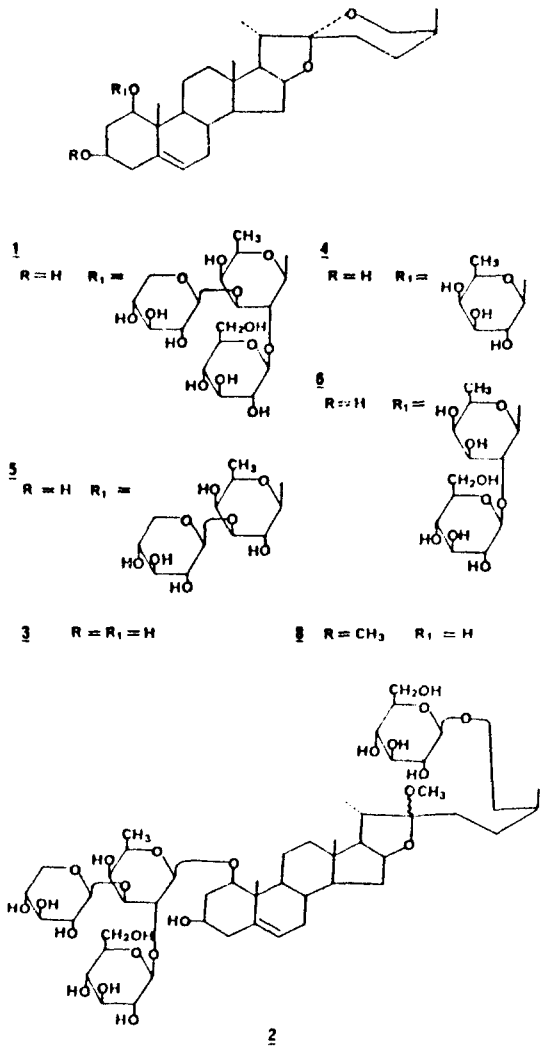

Table I. Partial ^{1}H-NMR spectral data for spicatosides A(1) and B(2) and prosapogenins I(4), II(5) and III(6) in pyridine-d_5[a]

Proton	Spicatoside[b]		Prosapogenin[c]		
	A(1)	B(2)	I(4)	II(5)	III(6)
18-CH_3	0.86, s	0.72, s	0.86, s	0.84, s	0.86, s
19-CH_3	1.35, s	1.22, s	1.33, s	1.33, s	1.36, s
21-CH_3	1.10, d	1.01, d	1.08, d	1.07, d	1.09, d
	(6.8)	(6.0)	(6.7)	(6.7)	(5.6)
27-CH_3	1.07, d	0.91, d	1.07, d	1.06, d	1.07, d
	(7.2)	(6.2)	(7.0)	(7.0)	(6.7)
H-6	5.56, d	5.45, d	5.59, d	5.58, d	5.55, d
	(5.2)	(5.3)	(5.3)	(5.4)	(5.2)
Anomeric	4.85, d	4.73, d	4.70, d	4.72, d	4.77, d
proton(s)	(7.6)	(7.4)	(7.5)	(7.7)	(7.5)
	5.28, d	5.15, d		5.21, d	5.10, d
	(7.6)	(7.5)		(7.4)	(7.7)
	5.45, d	5.31, d			
	(7.6)	(7.4)			
fucose CH_3	1.52, d	1.40, d	1.55, d	1.51, d	1.51, d
	(6.2)	(6.0)	(6.4)	(6.3)	(6.2)
OCH_3		3.12, s			

a) Data are δ(ppm), multiplicity, and J(in parentheses) in Hz.
b) measured with a Bruker AM-200 instrument.
c) measured with a Bruker AM-300 instrument.

193-196°C; $[\alpha]_D^{24} = -116°$ (c 0.06, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1} 3260, 985, 918, 880, 850 [intensity 918>890, 25(S)-spiroketal]; ^{1}H-NMR ($CDCl_3$, 80 MHz) δ 0.79 (3H, s, 18-CH_3), 0.99 (3H, d, J = 7.3 Hz, 21-CH_3), 1.04 (3H, s, 19-CH_3), 1.08 (3H, d, J = 5.7 Hz, 27-CH_3), 3.29 (1H, br.d, J = 11.0 Hz, H-26β), 3.96 (1H, dd, J = 11.0, 2.5 Hz, H-26α), 4.41 (1H, q, J = 6.2 Hz, H-16), 5.53 (1H, br. d, J = 4.3 Hz, H-6); MS m/z (rel. int.) 430 (M^+, 0.1), 415 (0.1), 412 (11.1), 394 (2.0), 379 (0.1), 371 (0.5), 361 (0.2), 358 (0.2), 316 (0.1), 301 (0.2), 287 (0.5), 280 (1.0), 269 (1.6), 265 (2.7), 139 (100), 115 (7.2). The aglycone (**3**) was identified as 25(S)-ruscogenin by direct comparison with an authentic sample. The filtrate was neutralized with Ag_2CO_3, filtered and concentrated to dryness in vacuo. The residue was examined by TLC (precoated cellulose, pyridine/EtOAc/HCOOH/H_2O = 36:36:7:21; Rf. 0.45: fucose, 0.43: xylose, 0.33: glucose).

Partial hydrolysis of 1 with alcoholic alkali metal solution

To a solution of **1** (400mg) in EtOH (40 m*l*) Na (4g) in EtOH (40 m*l*) was added and the reaction mixture kept at room temperature for 72 hr. The reaction mixture was poured into water and extracted with BuOH. After washing the extract with water, the reaction product was recovered in the usual way. TLC of the residue showed five spots, and the residue (200 mg) was subjected to column chromatography over silica gel with hexane/EtOAc (8:5) and then EtOAc saturated with H_2O/MeOH (gradient, 0 to 5%) to afford **3** (2 mg), prosapogenins **I**(**4**, 11 mg), **II**(**5**, 5 mg) and **III**(**6**, 30 mg), and then recovered **1**(60 mg) in the order of elution. **3** and recovered **1** were identified by comparison with the previously obtained samples of 25(S)-ruscoge-

Table II. ^{13}C-NMR spectral data for spicatosides A(1) and B(2) and related compounds[a)]

Carbon	3[b)]	1[c)]	2[c)]	4[d)]	5[d)]	6[d)]
C-1	78.2	83.4[a]	83.4	83.9	84.1	83.1
C-2	44.0	37.8	37.8	38.0	38.1	37.2
C-3	68.2	68.7	68.7	68.1	68.2	68.1
C-4	43.6	44.2	44.1	43.7	43.5	43.5
C-5	140.3	140.2	140.2	139.6	139.6	139.8
C-6	124.4	124.5	124.7	124.7	124.7	124.3
C-7	33.1	32.9	32.7	32.4	32.4	32.4
C-8	32.4	33.5	33.4	33.0	33.1	33.0
C-9	51.4	50.9	50.9	50.5	50.5	50.3
C-10	43.6	43.4	43.3	42.9	42.9	42.9
C-11	24.3	24.1	24.1	23.8	23.6	23.6
C-12	40.7	40.9	40.4	40.4	40.4	40.4
C-13	40.3	40.7	41.0	40.2	40.2	40.2
C-14	57.0	57.5	57.4	57.1	57.0	57.0
C-15	32.5	32.5	32.3	32.1	32.1	32.0
C-16	81.2	81.7	81.8	81.2	81.2	81.2
C-17	63.1	63.4	64.8	62.9	63.0	62.9
C-18	16.7	17.2	17.3	16.8	16.8	16.7
C-19	14.0	15.3	15.3	14.2	14.2	14.8
C-20	42.6	43.0	41.0	42.5	42.5	42.5
C-21	15.0	15.6	16.8	14.8	14.8	15.1
C-22	109.8	110.2	113.2	109.7	109.7	109.7
C-23	26.5	26.9	31.4	26.4	26.4	26.4
C-24	26.3	26.7	28.6	26.2	26.2	26.2
C-25	27.6	28.0	34.9	27.5	27.6	27.5
C-26	65.2	65.5	75.2	65.1	65.1	65.1
C-27	16.4	16.8	17.1	16.3	16.3	16.3
OCH_3			47.8			
Fuc C-1		101.0	100.9	102.5	102.2	99.8
C-2		79.3	79.3	72.1	72.0	82.4
C-3		83.6[a]	83.4	75.3	84.8	76.7
C-4		72.8[b]	72.7[a]	72.5	73.7	72.0
C-5		71.6	71.5	71.2	71.0	70.9
C-6		17.6	17.6	17.4	17.2	17.2
Xyl C-1		106.7	106.7		107.0	
C-2		75.6	75.6		75.4	
C-3		78.6[c]	78.7[b]		78.1	
C-4		71.2	71.1		71.0	
C-5		67.8	67.7		67.2	
Glc C-1		105.4	105.5			106.4
C-2		76.9	76.9			74.9
C-3	79.1	79.0				78.6
C-4	72.5[b]	72.5[a]				71.5
C-5	78.8[c]	78.9[b]				77.9
C-6	63.8	63.7				62.7
26-O-Glc						
C-1		105.5				
C-2		75.5				
C-3		78.9				
C-4		72.2				
C-5		78.5				
C-6		63.3				

[a,b,c]Assignments may be reversed in each column.

a) Spectra were recorded in pyridine-d_5 and assignments were made by DEPT spectra.

b) Watanabe, Y., Sanada, S., Ida, Y., and Shoji, J.: *Chem. Pharm. Bull.* **31**, 1980-1990 (1983).

c) Measured with a Bruker AM-200 instrument at 50.3 MHz.

d) Measured with a Bruker AM-300 instrument at 75.5 MHz.

nin and spicatoside A. **4** and **5** were insufficient for purification and used for NMR characterization without further purification (see Table I and II). After measurements of NMR, the recovered samples of **4** and **5** were separately refluxed with acid in the same manner as described above. **3** was identified by TLC in each case. Fucose from **4** and fucose and xylose from **5** were detected by TLC as described above.

Prosapogenin III(6)

Colorless needles from MeOH; m.p. 245-246 °C; $[\alpha]_D^{25} = -90.9°$ (*c* 0.11, MeOH); IR ν_{max}^{KBr} cm^{-1} 3380, 1060, 984, 915, 890, 845, 835 [intensity 915 >890, 25(S)-spiroketal]; ^{1}H-NMR: see Table I; ^{13}C-NMR: see Table II. Acid hydrolysis of **6** was performed in the same manner as described above to give **3**, fucose and glucose which were identified by TLC comparison with authentic samples.

Permethylation of 1

1(50 mg) was permethylated with NaH (100 mg) and CH_3I (6 m*l*) by the Hakomori's method.[3)] The product was purified by column chromatography with hexane/EtOAc (1:1) to afford nona-O-methyl spicatoside A(**7**, 30 mg), which was crystallized from MeOH as colorless plates; m.p. 98-100 °C; $[\alpha]_D^{24} = -36.3$ °C (*c* 0.17, MeOH); IR ν_{max}^{KBr} cm^{-1} 1095, 1072, 985, 920, 890, 850 [intensity 920 >890, 25(S)-spiroketal]; ^{1}H-NMR ($CDCl_3$, 80 MHz) 0.78 (3H,

s, 18-CH_3), 0.99 (3H, d, J = 6.4 Hz, 21-CH_3), 1.03 (3H, s, 19-CH_3), 1.07 (3H, d, J = 6.6 Hz, 27-CH_3), 1.20 (3H, d, J = 6.3 Hz, fucose CH_3), 3.33, 3.37, 3.47, 3.51, 3.55 (3H each, s, 5 × OCH_3), 3.53, 3.59 (6H each, s, 4 × OCH_3), 4.22 (1H, d, J = 6.3 Hz, anomeric H), 4.81 (2H, d, J = 7.2 Hz, 2 × anomeric H), 5.52 (1H, br.d, J = 4.8 Hz, H-6); MS, *m/z* (rel. int.) 821 [(M-175)$^+$, 0.3], 777 [(M-219)$^+$, 0.1], 553 (1.1), 427 (47.2), 219 (7.4), 187 (100), 175 (32.7), 143 (46.8), 111 (75.9).

Methanolysis of 7

7(20 mg) was refluxed with 2% methanolic HCl (20 m*l*) for 4 hr. The reaction mixture was concentrated to a half volume, then added to crushed ice and filtered. The filtrate was neutralized with Ag_2CO_3. The concentrated hydrolysate was examined by GLC and identified as methyl 2,3,4-tri-O-methyl xylopyranoside (Rt: 2.35, 2.90), methyl 2,3,4,6-tetra-O-methyl glucopyranoside (Rt: 4.48, 6.29) and methyl 4-O-methyl fucopyranoside (Rt: 9.03, 12.90) [column: 10% DEGS chromosorb WHP 100-200 mesh, 2.2 mm × 6 ft; column temp.: 180 °C; flow rate (N_2): 41.3 m*l*/min]. The residue was recrystallized from MeOH to yield 3-O-methyl 25(S)-ruscogenin (**8**) as colorless fine needles; m.p. 216-217 °C; $[\alpha]_D^{20} = -128°$ (*c* 0.09, $CHCl_3$); IR ν_{max}^{KBr} cm^{-1} 3500, 1070, 1035, 985, 910, 897, 848 [intensity 910 > 897, 25(S)-spiroketal]; ^{1}H-NMR ($CDCl_3$, 80 MHz) δ 0.79 (3H, s, 18-CH_3), 0.99 (3H, d, J = 6.5 Hz, 21-CH_3), 1.03 (3H, s, 19-CH_3), 1.08 (3H, d, J = 6.5Hz, 27-CH_3), 3.29 (1H, br.d, J = 11.0, d, 26β), 3.34 (3H, s, OCH_3), 3.95 (1H, dd, J = 11.0, 2.4 Hz, H-26 α), 5.55 (1H, br.d, J = 5.1 Hz, H-6); MS, *m/z* (rel. int.) 444 (M^+, 0.3), 426 (54.4), 412 (4.9), 394 (10.1), 385 (1.0), 379 (3.3), 372 (0.3), 354 (2.1), 340 (0.2), 330 (0.3), 322 (0.6), 315 (0.4), 312 (2.7), 307 (0.5), 301 (1.3), 298 (2.7), 283 (3.7), 280 (6.3), 265 (5.8), 251 (4.3), 139 (100).

Enzymatic hydrolysis of 2

A mixture of **2**(200 mg) and β-glucosidase (Sigma Co., 50 mg) in water (40 m*l*) was incubated at 37-40 °C for 72 hr, then MeOH was added and filtered. The filtrate was evaporated in vacuo to give a residue, which was chromatographed over silica gel with EtOAc saturated with H_2O/MeOH (93:7) to give a spirostanol glycoside, colorless needles from MeOH, m.p. 244-245 °C; $[\alpha]_D^{24} = -85.3°$ (*c* 0.07, MeOH) and glucose. The former showed to be identical to spicatoside A(**1**) in every respect (mmp, TLC, IR, ^{1}H- and ^{13}C-NMR).

RESULTS AND DISCUSSION

The MeOH extract of the tubers of *Liriope spicata,* on repeated chromatographic separation, gave two new steroidal saponins designated spicatosides A(**1**) and B(**2**).

Spicatoside A(**1**), m.p. 243-5 °C, showed a strong absorption band of hydroxy groups and characteristic absorption bands of a 25(S)-spiroketal moiety in the IR spectrum[2]. On acidic hydrolysis of **1** with 2N-HCl-dioxane, **1** gave glucose, xylose, fucose and an aglycone (**3**), m.p. 193-6 °, which was identified as 25(S)-ruscogenin by direct comparison with an authentic sample. **1** showed $(M+Li)^+$ at *m/z* 877 and $(M+Na)^+$ at *m/z* 893 in the FAB mass spectra and exhibited in the ^{1}H-NMR spectrum three anomeric proton doublets with J = 7.6 Hz at δ4.85, 5.28 and 5.45 indicating all glycosidic linkages are β. These results suggested that **1** was considered to be a triglycoside of 25(S)-ruscogenin possessing one mole each of glucose, xylose and fucose. The permethylether of **1** prepared by Hakomori's method[3] showed terminal permethylated pentosyl and hexosyl cations at *m/z* 175 and 219, respectively, together with $(M-175)^+$ at *m/z* 821, and $(M-219)^+$ at *m/z* 777 in its mass spectrum, suggesting the sugar residue was a branched chain trisaccharide rather than a linear one. Partial hydrolysis of **1** with alcoholic sodium metal solution[4] afforded three prosapogenins **I**(**4**), **II**(**5**), and **III**(**6**) with the aglycone, 25(S)-ruscogenin. On acid hydrolysis, prosapogenin **I**(**4**) gave fucose, and prosapogenin **II**(**5**) afforded fucose and xylose whereas prosapogenin **III**(**6**) yielded fucose and glucose. Based on the analysis of the ^{1}H- and ^{13}C-NMR spectral data of **4**, the structure of **4** was determined to be 25(S)-ruscogenin 1-O-β-D-fucopyranoside. On comparison of the ^{13}C-NMR spectrum of **5** with that of **4**, the C-3 signal of fucopyranosyl moiety was deshielded (9.5 ppm) than **4**. Consequently, the structure of **5** was assigned to be 25(S)-ruscogenin 1-O-β-D-xylopyranosyl (1→3)-β-D-fucopyranoside. Similarly, the presence of significant glycosidation shift of C-2 signal of the fucopyranosyl residue (10.3 ppm) in the ^{13}C-NMR spectrum of **6** indicated that the terminal glucose moiety was bound to C-2 hydroxyl group of fucopyranose unit. Therefore, the structure of **6** was determined as 25(S)-ruscogenin 1-O-β-D-glucopyranosyl (1→2)-β-D-fucopyranoside. On the basis of the above observations, the structure of spicatoside A(**1**) was determined as formulation **1**, which was further supported by the

following evidence. The ^{1}H-NMR spectrum of its nona-O-methylether (**7**) showed three anomeric proton signals at δ 4.22 (1H, d, J = 6.3 Hz) and 4.81 (2H, d, J = 7.2 Hz) with nine O-methyl signals supporting all glycosidic linkages are β. Methanolysis of **7** afforded methyl 2,3,4-tri-O-methyl xylopyranoside, methyl 2,3,4,6-tetra-O-methyl glucopyranoside, methyl 4-O-methylfucopyranoside and 25 (S)-ruscogenin 3-O-methylether (**8**), m.p. 216-217 °C. Based on the above results, the structure of spicatoside A(**1**) was established to be 25(S)-ruscogenin 1-O-β-D-glucopyranosyl (1→2)-[β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside.

Spicatoside B(**2**), m.p. 196-198 °C, gave positive Ehrlich reaction[5] and the ^{13}C-NMR spectrum showed characteristic furostanol carbon signals. On enzymatic hydrolysis with almond emulsin, **2** afforded glucose and a prosapogenin, m.p. 244-245 °C, which was identified as spicatoside A(**1**) by direct comparison with the isolated sample of spicatoside A and NMR spectral data. In the light of the above observations, the structure of spicatoside B(**2**) was characterized to be 26-O-β-D-glucopyranosyl 25 (S)-22-O-methyl-furost-5-en-1β, 3β, 26-triol 1-O-β-D-glucopyranosyl (1→2)-[β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside (=methyl proto-spicatoside A).

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