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Further Spirostanol Glycosides from the Tuber of *Liriope spicata*

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Abstract—Further studies have been done on the constituents of the tubers of *Liriope spicata* LOUR (Liliaceae). Four steroidal glycosides, tentatively designated as compounds **I**, **II**, **III** and **IV**, were isolated from the n-BuOH soluble fraction of this plant. The structures of these glycosides were established as β-sitosterol glucoside, prosapogenin **II** of spicatoside A, ophiopogonin B, and prosapogenin **III** of spicatoside A.

Keywords—*Liriope spicata* LOUR • Liliaceae • steroidal saponins • β-sitosterol glucoside

In our previous paper[1], it had been reported that the structures of two major new steroidal saponins of *Liriope spicata* tubers, namely spicatosides A and B, were established to be 25(S)-ruscogenin 1-O-β-D-glucopyranosyl(1→2)-[β-D-xylopyranosyl)1→3)]-β-D-fucopyranoside and 26-O-glucopyranosyl 25(S)-26-O-methyl-furost-5-en-1β, 3β-diol 1-O-β-D-glucopyransyl(1→2)-[β-D-glucopyranosyl (1→2)-[β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside, respectively.

The present paper reports the isolation and characterization of minor steroidal saponins together with β-sitosterol glucoside, tentatively designated as compounds **I**~**IV** in increasing order of polarities on silica gel TLC.

Compound **I**, m.p. 271~272°, is positive in the Lieberman-Burchard reaction. It shows a strong absorption of hydroxyl groups at 3,400 cm^{-1} in its IR spectrum. On acid hydrolysis, **I** gave β-sitosterol and glucose. The ^{1}H-NMR spectrum of **I** exhibited an anomeric proton signal at δ 5.01 (1H, *d*, *J*=7.7 Hz). Accordingly, **I** was considered to be β-sitosterol 3-O-β-D-glucopyranoside. The ^{13}C-NMR spectrum of **I** supported the above conclusion. However, mass spectral data of aglycon(**V**) suggested that **I** was concluded to be a mixture of β-sitosterol glucoside and small amounts of stigmasterol and campesterol glucosides.

Compound **II**, m.p. 192~194°, is positive in the Liebermann-Burchard reaction. It shows a strong absorption band of hydroxyl groups and characteristic absorption band of the 25(S)-spiroketal moiety at 988, 919, 897 and 851 cm^{-1} (intensity 919>897)[3] in the IR spectrum. On acidic hydrolysis, **II** yielded xylose and fucose together with an aglycon(**VI**), 25(S)-ruscogenin. The ^{1}H-NMR spectrum of **II** exhibited two anomeric signals at δ 4.71(1H, *d*, *J*=7.7 Hz) and 5.19 (1H, d, *J*=7.4 Hz) indicating all glycosidic linkages to be β. In the ^{13}C-NMR spectrum of **II**, the chemical shift of aglycon C-1 was deshieled by 5.5 ppm than that of 25(S)-ruscogenin indicating that the glycosidic linkage was attached at C-1 hydroxyl

group of the aglycon. In sugar moiety, two anomeric carbon signals were showed at δ 102.0, and 106.8. The significant downfield shift (+9.4 ppm) of inner fucose C-3 indicated that the terminal xylose was linked to C-3 of fucose. Consequently, the structure of **II** was determined as 25(S)-ruscogenin 1-O-β-D-xylopyranosyl (1→3)-β-D-fucopyranoside identical in all respects with prosapogenin **II** of spicatoside A.

Compound **III**, m.p. 269~271°, is positive in the Liebermann-Burchard reaction, it shows a strong absorption of hydroxyl groups and characteristic absorption band of the 25(R)-spiroketal moiety at 984, 920, 901 and 865 cm^{-1} (intensity 920<901) in its IR spectrum. On acid hydrolysis, **III** gave ruscogenin(**VII**), rhamnose and fucose. The ^{1}H-NMR spectrum of **III** showed two anomeric proton signals at δ 5.15 (1H, d, J=7.0 Hz) and δ 6.22 (1H, s) indicating the presence of one mole each of β-fucose and α-rhamnose. In the ^{13}C-NMR spectrum of **III**, the downfield shift of ruscogenin C-1 chemical shift as well as the presence of an upfield anomeric signal of fucose at δ 100.4 and the deshielded fucose C-2 signal at δ 76.7 indicated that the terminal rhamnosyl group was linked to fucose C-2 hydroxyl group which in turn attached at C-1 of ruscogenin. Therefore, **III** was determined as ruscogenin 1-O-α-L-rhamnopyranosyl (1→2)-β-D-fucopyranoside which was identical with ophiopogonin B isolated from *Ophiopogon japonicus* in all respects[4].

Compound **IV**, m.p. 244~245°, is positive in the Liebermann-Burchard reaction. It gave quite similar IR spectrum to that of compound **II** suggesting to be a 25(S)-spirostanol derivative. Upon acidic hydrolysis, **IV** gave glucose, fucose and 25(S)-ruscogenin (**VI**). The ^{1}H-NMR spectrum of **IV** exhibited two anomeric doublets at δ 4.83 (1H, J=7.0 Hz) and δ 5.15 (1H, J=7.7 Hz) indicating that two glycosidic linkages are β. The ^{13}C-NMR spectral data of **IV** also showed that the sugar moiety was linked at C-1 hydroxyl group of 25(S)-rucogenin. The presence of an upfield anomeric signal of fucose at δ 99.8 and the deshielded fucose C-2 signal at δ 82.6 suggested that the terminal glucosyl group was linked to fucose C-2. Therefore, the structure of **IV** could be designated as 25(S)-ruscogenin 1-O-β-D-glucopyranosyl (1→2)-β-D-fucopyranoside, which was identified as prosapogenin **III** of spicatoside A.

We already reported the structures of compounds **II** and **IV** obtained by partial hydrolysis of spicatoside A in our previous paper[1]. However, this is the first report of the isolation from plant sources.

Experimental

All melting points were determined with Yanaco melting point apparatus and were uncorrected. The optical rotations were measured with Jasco DIP 360 automatic polarimeter. The IR spectra were recorded on a Perkin-Elmer 1310 or Mattson Polaris TM (FT-IR) spectrophotometer. ^{1}H-NMR spectra were obtained on a Bruker AM-300(300 MHz) instrument and ^{13}C-NMR spectra were recorded on a Bruker AM-300(75.5 MHz) spectrometer using TMS as an internal standard. Mass spectra were measured on a Hewlett-Packard HP-5985 B GC/MS spectrometer equipped with a direct inlet system. TLC chromatography was performed on precoated Kiseselgel 60 F_{254} plates (Merck, 5715) and sugars were run on precoated cellulose plates (Merck, 5552).

Plant material: see previous paper[1]

Extraction and purification: The dried tubers of *Liriope spicata*(15 kg) were refluxed with MeOH for 4 hr(4 times) and evaporated to dryness. The residue(2.23 kg) was suspended

in water and extracted with $CHCl_3$ and then with n-BuOH. The $CHCl_3$ solution was concentrated to give the residue (21 g), and the n-BuOH-soluble fraction was concentrated in vacuo to afford *n*-BuOH-soluble fraction (150 g). A portion of $CHCl_3$ extract was subjected to column chromatography over silica gel eluted with $CHCl_3$-MeOH (9 : 1) to afford compound **I** (2.1 g). A portion of BuOH extract was subjected to column chromatography over silica gel eluted with EtOAc saturated with H_2O-MeOH (gradient) to yield crude saponin fractions, which were separately purified by column chromatography on silical gel with EtOAc saturated with H_2O-MeOH (99 : 1 and then 98:2) to yield pure compounds **II** (60 mg), **III** (50 mg) and **IV** (45 mg).

Compound I (β-sitosterol glucoside)

White amorphous from MeOH; m.p. 271~273°; $[\alpha]_D^{23} = -41°$ (c 0.5, pyridine); IR ν_{Max}^{KBr} cm^{-1} 3400, 1645, 1070, 1020; ^{1}H-NMR (300 MHz, pyridine-d_5) δ : 0.68 (3 H, s, 18-CH_3), 0.83~0.90 (9 H, 26, 27, 29-CH_3), 0.94 (3 H, s, 19-CH_3), 1.00 (3 H, d, J=6.2 Hz, 21-CH_3), 5.01 (1H, d, J=7.7 Hz, anomeric H), 5.36 (1H, br.d, J=5.0 Hz, H-6); ^{13}C-NMR (75.5 MHz, pyridine-d_5) : 37.5, 30.3, 78.3[a], 40.0, 141.0, 121.9, 32.2, 32.1, 50.4, 37.0, 21.3, 39.4, 42.5, 56.9, 24.6, 28.6, 56.3, 12.0[b], 19.5, 36.4, 19.1[c], 34.3, 26.5, 46.1, 29.5, 19.3[c], 20.0, 23.4, 12.2[b] (aglycon signals C-1 to C-29), 102.6, 75.3, 78.5[a], 71.7, 78.4[a], 62.9 (glucose signals C-1′ to C-6′) [a,b,c]interchangeable.

Compound II (prosapogenin II of spicatoside A)

Colorless needles from aqueous MeOH; m.p. 192~194°; $[\alpha]_D^{24} = -72°$ (c 0.5, MeOH); IR ν_{Max}^{KBr} cm^{-1} 3414, 1066, 988, 919, 897, 851 [919 >897, 25(S)-spiroketal]; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.84 (3 H, s, 18-CH_3), 1.07 (6 H, each d, J=5.9 Hz, 21, 27-CH_3), 1.21 (3 H, s, 19-CH_3) 1.50 (3 H, d, J=6.3 Hz, fucose-CH_3), 4.71 (1 H, d, J=7.7 Hz, anomeric H), 5.19 (1 H, d, J=7.4 Hz, anomeric H), 5.57 (1 H, br.d, J=5.2 Hz, H-6); ^{13}C-NMR (75.5 MHz, pyridine-d_5) : see Table. I.

Compound III (ophiopogonin B)

Colorless needles from aqueous MeOH; m.p. 269~271°; $[\alpha]_D^{25} = -98°$ (c 0.5, MeOH); IR ν_{Max}^{KBr} cm^{-1} 3409, 1065, 984, 920, 901, 865 [901>920, 25(R)-spiroketal]; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.70 (3 H, d, J=5.3 Hz, 27-CH_3), 0.84 (3 H, s, 18-CH_3), 1.04 (3 H, d, J=6.6 Hz, 21-CH_3), 1.36 (3 H, s, 19-CH_3), 1.47 (3 H, d, J=6.4 Hz, fucose-CH_3), 1.67 (3 H, d, J=6.2 Hz, rhamnose-CH_3), 5.15 (1 H, d, J=7.0 Hz, anomeric H), 5.57 (1 H, br.d, J=5.2 Hz, H-6), 6.22 (1H, s, anomeric H); ^{13}C-NMR (75.5 MHz, pyridine-d_5) : see Table I.

Compound IV (prosapogenin III of spicatoside A)

Colorless needles from aqueous MeOH; m.p. 244~245°; $[\alpha]_D^{25} = -84°$ (c 0.3, MeOH); IR ν_{Max}^{KBr} cm^{-1} 3410, 1067, 987, 919, 897, 850 [919>897, 25(S)-spiroketal]; ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.87 (3 H, s, 18-CH_3), 1.07 (3 H, d, J=7.4 Hz, 27-CH_3), 1.09 (3 H, d, J=7.4 Hz, 21-CH_3), 1.38 (3 H, s, 19-CH_3), 1.54 (3 H, d, J=6.3 Hz, fucose-CH_3), 4.83 (1H, d, J=7.8 Hz, anomeric H), 5.15 (1 H, d, J=7.6 Hz, anomeric H), 5.56 (1 H, br.d, J=5.0 Hz, H-6); ^{13}C-NMR (75.5 MHz, pyridine-d_5) : see Table. I.

Acid hydrolysis of compounds I, II, III and IV

A solution of **I** (50 mg), **II** (30 mg), **III** (30 mg) and **IV** (30 mg) in 4 N-HCl-dioxane (1 : 1, 3 ml per 10 mg of glycosides) was separately refluxed for 4 hr on a water bath. Each reaction mixture was poured into crushed ice and filtered. The residue from **I** was crystallized from MeOH to give an aglycon (**V**) as colorless

Table I. ^{13}C-NMR chemical shifts of Liriope glycosides in pyridine-d_5 (75.5 MHz)

Carbon	II	IV	III	Carbon	II	IV	III
C-1	83.7	83.0	84.3	Fuc C-1	102.0	99.8	100.4
C-2	38.0	37.3	37.9	C-2	71.9	82.6	76.7
C-3	68.1	68.2	68.2	C-3	84.7	76.8	74.2
C-4	43.6	43.6	43.7	C-4	73.6	72.0	73.2
C-5	139.6	139.7	139.5	C-5	70.9	71.0	71.0
C-6	124.7	124.4	124.7	C-6	17.2	17.3	17.2
C-7	32.4	32.4	32.4	Xyl C-1	106.8		
C-8	33.0	33.0	33.1	C-2	75.3		
C-9	50.5	50.4	50.6	C-3	78.0		
C-10	42.9	42.9	42.8	C-4	70.9		
C-11	23.7	23.7	24.0	C-5	67-1		
C-12	40.4	40.4	40.4	Glc C-1		106.5	
C-13	40.2	40.2	40.2	C-2		74.9	
C-14	57.1	56.9	57.2	C-3		78.7	
C-15	32.0	32.0	32.0	C-4		71.5	
C-16	81.2	81.2	81.1	C-5		78.0	
C-17	62.9	62.9	63.0	C-6		62.7	
C-18	16-7	16-7	16.9	Rha C-1			101.4
C-19	14.7[a]	14.8[a]	14.8[a]	C-2			72.4
C-20	42.5	42.4	42.0	C-3			72.4
C-21	14.8[a]	15.1[a]	15.0[a]	C-4			74.4
C-22	109.7	109.7	109.3	C-5			69.1
C-23	26.4	26.4	31.8	C-6			18.9
C-24	26.2	26.2	29.2				
C-25	27.5	27.5	30.6				
C-26	65.1	65.0	66.9				
C-27	16.3	16.3	17.4				

[a] Assignment may be reversed in each column.

needles; m.p. 131~132°; IR ν^{KBr}_{Max} cm^{-1} 3400, 1650, 1050, 1020; MS *m/z* (rel. int.). 414(M^+, 100.0), 412(M^+, 31.2), 400(M^+, 22.9). The aglycon(**V**) was identified as β-sitosterol by direct comparison with an authentic sample. The residues from **II** and **IV** were separately crystallized from MeOH to yield the same aglycon(**VI**) as colorless needles; m.p. 190~191°; IR ν^{KBr}_{Max} cm^{-1}; 3414, 989, 920, 896, 852 [920>896, 25(S)-spiroketal]; MS *m/z* (rel. int.): 430(M^+, 0.1), 415(M-CH_3, 0.1), 412(M-H_2O, 11.1), 394(M-2H_2O, 2.0), 379 (M-CH_3-H_2O, 0.1), 139(100.0) ; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.79(3 H, s, 18-CH_3), 1.00(3 H, d, J=6.7 Hz, 21-CH_3), 1.05(3 H, s, 19-CH_3), 1.08(3 H, d, J=7.1 Hz, 27-CH_3), 3.30(1 H, br.d, J=11.0 Hz, H-26 β), 3.46 (1 H, dd, J=11.6 and 4.4 Hz, H-1), 3.56 (1 H, m, H-3), 3.95(1 H, dd, J=11.0 and 2.7 Hz, H-26 α), 5.54(1 H, br.d, J=5.7 Hz, H-6); The aglycon(**VI**) was identified as 25 (S)-ruscogenin by direct comparison with an authentic sample. After recrystallization from MeOH the residue from **III** gave **VII** as colorless needles. 207~209°; IR ν^{KBr}_{Max} cm^{-1} ; 3410, 980, 918, 896, 857[918<896, 25(R)-spiroketal] ; MS *m/z* (rel. int.): 430(M^+, 0.1), 412(M-H_2O, 30.0), 394(M-2H_2O, 6.4), 139(100.0);

^{1}H-NMR(300 MHz, $CDCl_3$) δ: 0.80(3H, d, *J* =5.5 Hz, 27-CH_3), 0.81(3H, s, 18-CH_3), 0.97(3H, d, *J*=6.4 Hz, 21-CH_3), 1.09(3H, s, 19-CH_3), 3.42~3.68(3H, m, H-26, -1, and -3), 5.55(1 H, br.d, *J*=5.2 Hz, H-6). The aglycon(**VII**) was concluded to be ruscogenin according to literature data[4].

The filtrates were neutralized with Ag_2CO_3 separately and filtered. The solution was evaporated to dryness under reduced pressure. Each residue was examined by TLC using a precoated cellulose plate developed in pyridine-EtOAc-HOAc-H_2O(36 : 36 : 7 : 21). Compound **I** : Rf, 0.33 : glucose. Compound **II** : Rf, 0.46 : fucose, 0.43 : xylose. Compound **III** : Rf, 0.44 : fucose, 0.53 : rhamnose. Compound **IV** : Rf, 0.45 : fucose, 0.32 : glucose.

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