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SPICATOSIDE C, A NEW STEROIDAL SAPONIN FROM THE TUBERS OF *LIRIOPE SPICATA*

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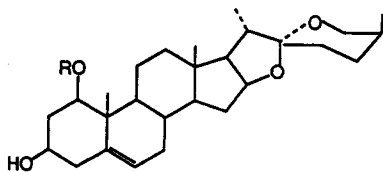
ABSTRACT.—A new steroidal saponin, spicatoside C [**1**], was isolated in the course of phytochemical investigation of the tubers of *Liriope spicata*. The structure of this compound was established as 25(*S*)-ruscogenin 1-*O*-β-D-fucopyranosyl(1→2)-[β-D-xylopyranosyl(1→4)]-β-D-fucopyranoside.

In previous papers, we have reported the isolation of several spirostanol glycosides, including spicatosides A and B (1,2). Recently, Yu *et al.* have isolated six 25(*S*)-ruscogenin and yamogenin glycosides from the roots of *Liriope spicata* (Thunb.) Lour var. *prolifera* Y.T. Ma (Liliaceae) (3). In our continuing research on *L. spicata*, we isolated a new spirostanol glycoside, named spicatoside C [**1**]. This paper describes the structure elucidation of this new compound.

The dried tubers of *L. spicata* were extracted with hot MeOH, and the dried MeOH extract was treated as described in the Experimental. A steroidal saponin, named spicatoside C [**1**], was isolated from the *n*-BuOH extract.

Compound **1** was positive in the Liebermann-Burchard reaction. It showed a strong hydroxyl group absorption band and characteristic absorption bands of a 25(*S*)-spiroketal moiety in the ir spectrum (4). On hydrolysis with acid, **1** gave



1 R=β-D-Fuc(1→2)[β-D-xyl(1→4)]-β-D-fuc
2 R=H
3 R=β-D-Fuc
4 R=β-D-Fuc(1→2)-β-D-fuc

D-fucose, D-xylose, and the aglycone **2**. Compound **2** was identified as 25(*S*)-ruscogenin on the basis of spectroscopic (eims, ^{1}H- and ^{13}C-nmr) evidence (5). The fabms of **1** exhibited a cationized molecular ion $[M+Na]^+$ at *m/z* 877 and a fragment ion $[genin+H]^+$ at *m/z* 431, suggesting that **1** is a 25(*S*)-ruscogenin trisaccharide. The remaining fragment ions at *m/z* 724 $[M+2H-132]^+$ and 710 $[M+2H-146]^+$, corresponding to the elimination of one mol of xylose and one mol of fucose from the $[M+2H]^+$ ion at *m/z* 856, respectively, showed the presence of two terminal sugar moieties. The ^{1}H-nmr spectrum of **1** exhibited three anomeric proton signals at δ 4.78 (1H, d, J=7.6 Hz), 5.10 (1H, d, J=7.5 Hz), and 5.16 (1H, d, J=7.8 Hz). The ^{13}C-nmr chemical shifts of the carbons of the A and B rings of the aglycone [**2**] suggested that the sugar moiety is linked only to the C-1 hydroxyl group of 25(*S*)-ruscogenin (5). Partial hydrolysis of **1** with alkali metal solution (6) afforded two prosapogenins, **3** and **4**, which yielded D-fucose as a common sugar component on acid hydrolysis. Based on analysis of the ^{1}H- and ^{13}C-nmr spectral data of **3**, the structure of **3** was determined as 25(*S*)-ruscogenin 1-*O*-β-D-fucopyranoside. The ^{1}H-nmr spectrum of **4** showed two anomeric proton signals at δ 4.74 (1H, d, J=7.5 Hz) and 4.80 (1H, d, J=7.2 Hz). Comparison

of the ^{13}C-nmr spectrum of **4** with that of **3** revealed that the C-2 signal of the inner fucopyranosyl moiety was deshielded (+10.2 ppm). In addition, the signals of C-1 and C-3 were shifted by −3.6 ppm and −0.5 ppm, respectively, indicating that the terminal fucose unit was attached at C-2 of the inner fucose moiety. Thus, **4** was 25(*S*)-ruscogenin 1-*O*-β-D-fucopyranosyl(1→2)-β-D-fucopyranoside. On comparison of the ^{13}C-nmr spectrum of **1** with that of **4**, the C-3, C-4, and C-5 chemical shifts of the inner fucopyranosyl moiety of **1** revealed glycosidation shifts at C-3 (−1.9 ppm), C-4 (+4.8 ppm), and C-5 (+1.5 ppm), indicating that the terminal xylose unit was attached at C-4 of the inner fucose moiety. Additional confirmation of the structure of **1** was achieved through detailed analysis of the ^1H-^1H COSY, ^1H-^{13}C HETCOR, and ^{13}C-^1H long range COSY nmr spectra of **1**. The proton signal at δ 4.78 (H-1′) correlated with the proton at δ 4.57 (H-2′) in the ^1H-^1H COSY spectrum, and the signal at δ 4.57 correlated with the anomeric carbon of fucose at δ 105.4 (C-1″) in the ^{13}C-^1H long-range COSY nmr spectrum of **1**. Based on the above evidence, the structure of **1** was determined as 25(*S*)-ruscogenin 1-*O*-β-D-fucopyranosyl(1→2)-[β-D-xylopyranosyl(1→4)]-β-D-fucopyranoside, named spicatoside C [**1**], which is a new natural product.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured on a Jasco DIP-360 polarimeter. Ir spectra were determined in KBr on a Mattson Polaris TM (Ft-ir) spectrophotometer. Nmr spectra were recorded on either a Bruker AM-300 (300 MHz for ^1H-nmr and 75.5 MHz for ^{13}C-nmr) or Varian Unity (500 MHz for 2D nmr) spectrometer using TMS as an internal standard, and were measured at room temperature. Chemical shifts are given in ppm. Fabms and eims spectra were recorded on Hewlett-Packard 5985B and Kratos MS 25 RFA mass spectrometers. Tlc was carried out on precoated Si gel 60 F_{254} sheets (Merck) and detection was achieved by spraying with 10% H_2SO_4 followed by heating. Sugars were run on precoated cellulose plates (Merck) and detected by aniline phthalate. Cc utilized Merck Si gel.

PLANT MATERIAL.—*Liriope spicata* plants used in this study were collected in South Kyungnam Province, Korea, in the spring of 1986. A voucher specimen is deposited in the College of Pharmacy, Yeungnam University.

EXTRACTION AND ISOLATION.—The dried tubers of *L. spicata* (15 kg) were extracted with hot MeOH (5 times, 3 h for each extraction) and evaporated to dryness. The residue (2.23 kg) was suspended in H_2O and extracted with $CHCl_3$, and then with *n*-BuOH. A portion of the *n*-BuOH-soluble fraction (150 g) was subjected to repeated

TABLE 1. Partial ^1H-Nmr Spectral Data for **1–4** in Pyridine-d_5.[a]

Proton	Compound			
	1	**2**	**3**	**4**
H-6	5.56 br d (4.7)	5.58 br d (5.0)	5.59 br d (5.3)	5.59 br d (4.2)
Me-18	0.86 s	0.81 s	0.86 s	0.88 s
Me-19	1.34 s	1.07 s	1.33 s	1.36 s
Me-21	1.09 d (7.3)	0.98 d (6.0)	1.08 d (6.7)	1.08 d (6.7)
H-26α		3.98 dd (11.0, 2.5)		
H-26β		3.33 br d (11.0)		
Me-27	1.06 d (7.1)	1.09 d (6.5)	1.07 d (7.0)	1.07 d (6.9)
Fuc-Me	1.47 d (6.4)		1.55 d (6.4)	1.48 d (6.3)
	1.62 d (6.3)			1.57 d (6.3)
Anomeric protons	4.78 d (7.6)		4.70 d (7.5)	4.74 d (7.5)
	5.10 d (7.5)			4.80 d (7.2)
	5.16 d (7.8)			

[a]Chemical shifts are expressed in δ values, with multiplicities indicated, and *J* values in Hz in parentheses.

cc over Si gel eluted with $CHCl_3$-MeOH-H_2O (7:3:1, lower layer) to obtain a pure compound, spicatoside C [**1**].

Spicatoside C [**1**].—White amorphous powder from MeOH: mp 224–226°; $[\alpha]^{23}D$ −55° (c=0.5, MeOH); ir ν max (KBr) 3414, 1066, 988, 919, 897, 851 cm^{-1} [919>897, 25(*S*)-spiroketal]; fabms *m/z* 877 $[M+Na]^+$, 856 $[M+2H]^+$, 724 $[M+2H-132]^+$, 710 $[M+2H-146]^+$, 577 $[genin+H+146]^+$, 431 $[genin+H]^+$; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2; *anal.* calcd for $C_{44}H_{70}O_{16}\cdot3H_2O$, C 58.13, H 8.43, found C 58.01, H 8.50.

TABLE 2. ^{13}C-Nmr Chemical Shifts of Compounds **1**–**4** in Pyridine-d_5.[a]

Carbon	Compound			
	1	**2**	**3**	**4**
C-1	83.3	78.2	83.9	83.1
C-2	37.1	43.9	38.0	37.1
C-3	68.2	68.4	68.1	68.2
C-4	43.7	43.6	43.7	43.8
C-5	139.9	140.2	139.6	139.7
C-6	124.4	124.3	124.7	124.7
C-7	32.4	33.1	32.4	32.5
C-8	33.0	32.4	33.0	33.1
C-9	50.3	51.2	50.5	50.6
C-10	42.9	43.6	42.9	43.0
C-11	23.7	24.3	23.8	23.9
C-12	40.4	40.7	40.4	40.5
C-13	40.2	40.3	40.2	40.2
C-14	56.9	57.1	57.1	56.9
C-15	32.1	32.4	32.1	32.1
C-16	81.2	81.2	81.2	81.2
C-17	62.9	63.1	62.9	62.9
C-18	16.7	16.8	16.8	16.8
C-19	14.8	13.9	14.2	14.8
C-20	42.5	42.6	42.5	42.5
C-21	15.0	14.9	14.8	14.8
C-22	109.7	109.8	109.7	109.7
C-23	26.4	26.4	26.4	26.4
C-24	26.2	26.3	26.2	26.2
C-25	27.5	27.6	27.5	27.6
C-26	65.1	65.2	65.1	65.1
C-27	16.3	16.3	16.3	16.4
Fuc C-1	99.8		102.5	98.9
C-2	82.0		72.1	82.3
C-3	72.9		75.3	74.8
C-4	78.7		72.5	73.9
C-5	73.5		71.2	72.0
C-6	17.1		17.4	17.3
Fuc C-1	105.4			107.4
(→^{2}Xyl) C-2	71.7			72.2
C-3	75.0			75.1
C-4	72.2			72.8
C-5	70.6			70.9
C-6	17.4			17.3
Xyl C-1	106.1			
(→^{4}Fuc) C-2	75.1			
C-3	78.5			
C-4	71.0			
C-5	67.2			

[a]Assignment was assisted with ^{13}C-^{1}H HETCOR nmr experiment.

ACID HYDROLYSIS OF **1**.—A solution of **1** (30 mg) in 4 N HCl-dioxane (1:1) (9 ml) was refluxed for 1 h on a waterbath. The reaction mixture was poured into crushed ice and filtered. The residue from **1** was purified by recrystallization from MeOH to afford an aglycone [**2**] as colorless needles: mp 190–191°; $[\alpha]^{20}D -110°$ (c=0.2, $CHCl_3$); ir ν max (KBr) 3414, 989, 920, 896, 852 cm^{-1} [920>896, 25(*S*)-spiroketal]; eims *m/z* 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); ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2. The filtrate was adjusted to pH 7 with Ag_2CO_3 and filtered. The filtrate was concentrated and examined by tlc with pyridine-EtOAc-HOAc-H_2O (36:36:7:21). Fucose (R_f 0.66) and xylose (R_f 0.60) from **1** were identified by comparison with authentic samples.

PARTIAL HYDROLYSIS OF **1**.—To a solution of **1** (400 mg) in EtOH (40 ml), Na (4 g) in EtOH (40 ml) was added, and the reaction mixture was kept at room temperature for 72 h. The reaction mixture was poured into H_2O and extracted with *n*-BuOH. After washing the extract with H_2O, the product was recovered in the usual way. Tlc of the product showed four spots and the product (180 mg) was subjected to cc over Si gel with *n*-hexane-EtOAc (8:5), and then EtOAc saturated with H_2O/MeOH (gradient, 0 to 5%) to afford 25(*S*)-ruscogenin [**2**], prosapogenins **3** and **4**, and recovered **1**, in order of elution. Compound **2** was identified by direct comparison with a previously obtained sample of 25(*S*)-ruscogenin. The prosapogenins **3** and **4** were refluxed with acid in the same manner as described above. In both hydrolysates, fucose was detected by tlc as the common sugar component. Compound **3** was recrystallized from aqueous MeOH as white needles: mp 221–223°; $[\alpha]^{20}D -97.6°$ (c=0.5, pyridine); ir ν max (KBr) 3414, 1066, 988, 919, 897, 851 cm^{-1} [919>897, 25 (*S*)-spiroketal]; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2. Compound **4** was recrystallized from MeOH as white amorphous material: mp 183–185°; $[\alpha]^{20}D -74.3°$ (c=0.5, pyridine); ir ν max (KBr) 3416, 1065, 986, 920, 900, 857 cm^{-1} [920>900, 25 (*S*)-spiroketal]; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 2.

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