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STEROIDAL GLYCOSIDES FROM THE SUBTERRANEAN PARTS OF *LIRIOPE SPICATA* VAR. *PROLIFERA*

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Key Word Index—*Liriope spicata* var. *prolifera*; Liliaceae; underground organs; steroidal glycosides; lirioproliosides A, B, C and D; 25(*S*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl (1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-fucopyranoside; ophiopogonin.

Abstract—In a continuation of phytochemical studies on the underground organs of *Liriope spicata* var. *prolifera*, four new steroidal glycosides, lirioproliosides A–D, along with two known compounds, 25(*S*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl(1 → 2)[β-D-xylopyranosyl(1 → 3)]-β-D-fucopyranoside and ophiopogonin A, were identified. The structures of lirioproliosides A–D were established by a combination of spectroscopic and chemical methods as 25(*S*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-fucopyranoside-3-*O*-α-L-rhamnopyranoside, 25(*S*)-ruscogenin 1-*O*-[3-*O*-acetyl-α-L-rhamnopyranosyl(1 → 2)]-β-D-fucopyranoside, 25(*S*)-ruscogenin (1-*O*-[2-*O*-acetyl-α-L-rhamnopyranosyl(1 → 2)]-β-D-fucopyranoside and ruscogenin (1-*O*-[2-*O*-acetyl-α-L-rhamnopyranosyl(1 → 2)]-β-D-fucopyranoside, respectively. Among these steroidal glycosides, ophiopogonin A and lirioprolioside B, and lirioproliosides C and D, were isolated as epimeric pairs. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Liriope spicata (Thunb.) Lour. var. *prolifera* Y. T. Ma (Liliaceae) is a medicinal herb cultivated in Hubei Province, People's Republic of China. The tubers of the title plant are utilized locally in various parts of China as a tonic, antitussive and expectorant to replace the official crude drug Ophipogonis Tuber, the tubers of *Ophipogon japonica* (Thunb.) Ker.-Gawl. [1]. The isolation and structure elucidation of seven steroidal glycosides from the title plant were described [2]. Further phytochemical studies on this species have now resulted in the identification of four new steroidal glycosides, namely, lirioprolisosides A (**2**), B (**3**), C (**5**) and D (**6**) along with two known compounds, 25(*S*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-fucopyranoside (**1**) and ophiopogonin A (**4**). The isolation and structure determination of compounds **2**, **3**, **5**, and **6** are described herein, with the structures of the new compounds being assigned primarily by spectral data comparison with compound **1**.

RESULTS AND DISCUSSION

Compound **1** gave a positive (greenish) Liebermann–Burchard reaction, consistent with a steroidal glycoside. It exhibited a molecular formula of $C_{44}H_{70}O_{16}$ based on the negative FAB-mass spectrum, in which a quasi-molecular ion was observed at *m/z* 853 $[M-H]^-$, and on combusion analysis. Compound **1** showed strong hydroxyl group absorption and the characteristic bands (920 > 902 cm^{-1}) of a 25(*S*)-spiroketal moiety in its IR spectrum [3]. Upon hydrolysis with 2 M HCl in 50% dioxane, **1** afforded an aglycone, which was characterized as 25(*S*)-ruscogenin by direct comparison with an authentic sample (TLC, ^1H and ^{13}C NMR spectra) [4], along with D-fucose, L-rhamnose and D-xylose, which were identified by HPLC comparison with authentic samples. The ^1H NMR signals due to the anomeric protons at δ 6.39 (*br s*), 4.99 (*d*, *J* = 6.9 Hz), and 4.67 (*d*, *J* = 7.8 Hz), assignable to rhamnose H-1, xylose H-1 and fucose H-1, respectively, indicated the presence of one α- and two β-linked modes of glycosidation from

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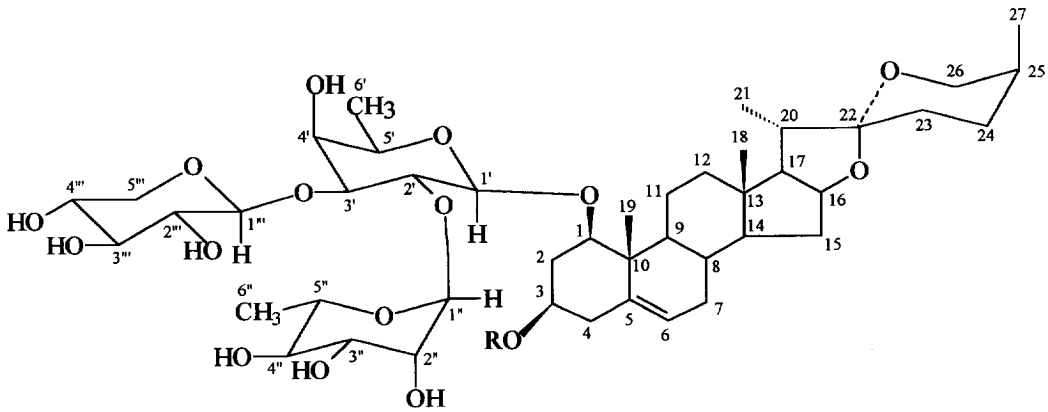

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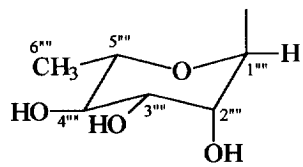
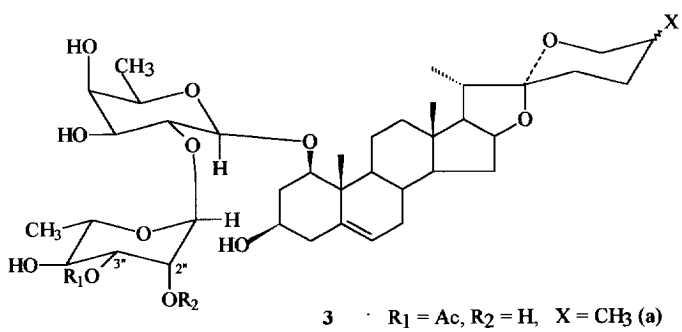
2 R = 



3 R_1 = Ac, R_2 = H, X = CH_3 (a)
4 R_1 = Ac, R_2 = H, X = CH_3 (e)
5 R_1 = H, R_2 = Ac, X = CH_3 (a)
6 R_1 = H, R_2 = Ac, X = CH_3 (e)

the coupling constants of these anomeric protons. This is in good agreement with the FAB-mass spectrum fragmentation pattern of **1** observed as follows: *m/z* (positive) 855 $[M+H]^+$, 709 $[M+H-146]^+$, 577 $[M+H-146-132]^+$, 431 $[M+H-146-132-146, \text{aglycone}+H]^+$. A terminal rhamnose in the sugar chain could be inferred from the diagnostic negative FAB-mass spectrum ion that occurred at *m/z* 721 $[M-H-132]^-$.

Comparison of the ^{13}C NMR data of **1** with those of 25(*S*)-ruscogenin [4] and its corresponding methyl α-L-rhamnopyranoside, β-D-xylopyranoside and β-D-fucopyranoside derivatives [5] revealed that rhamnose and xylose, respectively, were attached to the C-2′ and C-3′ positions of the inner fucose which in turn was attached to the C-3 position of the aglycone, 25(*S*)-ruscogenin, since glycosidation shifts were observed at the C-3 position (6.0 p.p.m.) of the aglycone and C-2′ (6.4 p.p.m.) and C-3′ (10.7 p.p.m.) of fucose as expected, but all the signals of rhamnose and xylose were virtually unchanged (Table 1). Moreover, the ^{13}C NMR data of the sugar chain of **1** were fully superimposable on those of ophiopogenin D [6], resulting in the identification of **1** as 25(*S*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl(1 → 2)][β-D-xylopyranosyl(1 → 3)]-β-D-fucopyranoside. Although **1** has been reported previously from *L. spicata* var. *prolifera* [7], no detailed ^1H NMR and FAB-mass sectrum data have been published so far for this compound.

Lirioplioside A (**2**) exhibited a molecular formula of $C_{50}H_{80}O_{20}$, based on a combination of the negative FAB-mass spectrum, in which the quasi-molecular ion was detected at *m/z* 1000, and elemental analysis. The characteristic absorptions attributable to a 25(*S*)-spiroketal unit were apparent in the IR spectrum, which, when coupled with the positive coloration (greenish) obtained in the Liebermann–Burchard reaction, suggested that **2** was a steroidal glycoside. Upon

Table 1. ^{13}C NMR data of steroidal glycosides **1–6**

	1	**2**	**3**	**4**	**5**	**6**
Aglycone						
1	84.5	84.4	84.6	84.6	84.2	84.2
2	38.6	36.3	38.5	38.5	38.4	38.4
3	68.5	74.4	68.7	68.7	68.6	68.6
4	44.3	40.2	44.2	44.2	44.4	44.4
5	139.7	138.6	139.7	139.7	139.7	139.7
6	124.8	125.6	124.7	124.7	124.8	124.8
7	33.7	33.5	33.7	33.7	33.7	33.7
8	32.7	32.6	32.6	32.6	32.7	32.7
9	51.2	51.2	51.2	51.2	51.2	51.2
10	43.4	43.4	43.4	43.4	43.4	43.4
11	24.6	24.5	24.6	24.6	24.5	24.5
12	41.0	41.0	41.0	41.0	41.0	41.0
13	40.8	40.7	40.8	40.8	40.8	40.8
14	57.6	57.7	57.6	57.6	57.7	57.7
15	33.0	33.0	33.0	33.0	32.9	32.9
16	81.6	81.6	81.5	81.5	81.5	81.5
17	63.5	63.5	63.7	63.7	63.6	63.6
18	17.4	17.4	17.4	17.4	17.4	17.4
19	15.3	15.5	15.5	15.5	15.4	15.4
20	43.0	43.1	43.0	42.5	43.1	42.6
21	15.3	15.5	15.3	15.6	15.3	15.6
22	109.9	109.9	109.9	109.4	109.9	109.4
23	27.1	27.1	27.0	32.4	27.1	32.4
24	26.8	26.8	26.8	29.8	26.8	29.9
25	28.1	28.2	28.1	31.1	28.2	31.2
26	65.5	65.5	65.5	67.2	65.5	67.3
27	16.9	16.9	16.9	17.8	16.9	17.9
Fucose						
1′	100.5	100.5	100.5	100.5	100.2	100.2
2′	73.9	73.9	75.3	75.3	75.0	75.0
3′	85.9	86.1	76.9	76.9	76.6	76.6
4′	72.9	73.1	73.5	73.5	73.6	73.6
5′	71.2	71.2	71.4	71.4	71.4	71.4
6′	17.6	17.6	17.7	17.7	17.7	17.7
Rhamnose						
1″	101.8	101.8	101.7	101.7	98.8	98.8
2″	72.7	72.9	70.4	70.4	74.6	74.6
3″	72.9	72.8	76.9	76.9	70.8	70.8
4″	74.6	74.5	71.3	71.3	74.4	74.4
5″	69.6	69.7	69.9	69.9	69.7	69.7
6″	19.6	19.5	19.4	19.4	19.4	19.4
Xylose			3″-OAc	3″-OAc	2″-OAc	2″-OAc
1‴	106.5	106.7	21.7	21.7	21.6	21.6
2‴	78.4	78.5	170.8	170.8	170.3	170.3
3‴	75.0	75.0				
4‴	71.1	71.1				
5‴	67.3	67.4				
Rhamnose						
1⁗	100.7					
2⁗	72.8					
3⁗	73.1					
4⁗	74.4					
5⁗	70.2					
6⁗	19.2					

hydrolysis with 2 M HCl in 50% dioxane, **2** afforded the known aglycone, 25(*S*)-ruscogenin (TLC, ^{1}H and ^{13}C NMR spectra), and the monosaccharides D-fucose, L-rhamnose and D-xylose, which were identified in a similar manner to that described above. The ^{1}H NMR signals arising from the anomeric protons at δ 6.48 (*br*

s), 5.59 (*br s*), 5.06 (*d*, $J = 7.5$ Hz), and 4.59 (*d*, $J = 7.6$ Hz) and the relevant anomeric carbons at δ 101.8, 100.7, 106.7, and 100.5, recognized by HETCOR NMR, suggested the presence of two α- and two β-glycosidic bonds, which was consistent with the observed FAB-mass spectrum fragmentation patterns of **2** at *m/z* 1000 $[M_{calcd} - H]^-$, 854 $[M_{calcd} - H - 146]^-$, 721 $[M - H - 146 - 132]^-$, 575 $[M - H - 146 - 132 - 146]^-$ (negative) and at *m/z* 1002 $[M_{calcd} + H]^+$, 577 $[M + H - 146 - 132 - 146]^+$, and 431 $[M + H - 146 - 132 - 146 - 146,$ aglycone $+ H]^+$ (positive). Permethylation of **2** by Hakomori's method [8] afforded a deca-*O*-methyl derivative, which was further methanolysed to give methyl 3,4-di-*O*-methylxylopyranoside and 2,3,4-tri-*O*-methylrhamnopyranoside by GLC. In addition, methyl 2,3-diacetyl-4-*O*-methylfucopyranoside was also identified by GLC after further acetylation of the methanolysed residue of the permethylation derivatives of **2**. These findings allowed the conclusion to be made that in **2** both the C-1 and C-3 hydroxy groups of 25(*S*)-ruscogenin were glycosylated by either rhamnose or fucose bearing either rhamnose and xylose at the C-2′ and C-3′ positions. Upon partial hydrolysis, **2** afforded 25(*S*)-ruscogenin, and the prosapogenins 25(*S*)-ruscogenin 1-*O*-β-D-fucopyranoside-3-α-L-rhamnopyranoside [6] and **1**, identified by direct comparison with authentic samples, which led to the determination of the sequences of the sugar chains and the binding sites at the aglycone residue. As a consequence, the structure of **2** was elucidated as 25(*S*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl($1 \rightarrow 2$)][β-D-xylopyranosyl($1 \rightarrow 3$)]-β-D-fucopyranoside-3-*O*-α-L-rhamnopyranoside.

Liriopliosides B (**3**) was obtained as an inseparable epimeric mixture with the known compound ophiopogonin A {ruscogenin 1-*O*-[3-*O*-acetyl-α-L-rhamnopyranosyl($1 \rightarrow 2$)]-β-D-fucopyranoside} (**4**) [9]. The common molecular formula of **3** and **4** was determined as $C_{41}H_{64}O_{13}$ by means of the FAB-mass spectrum and elemental analysis. The compound mixture gave a positive Liebermann–Burchard reaction and showed a strong hydroxyl group absorption band (ν_{max} 3600–3200 cm^{-1}), as well as ester group absorption (ν_{max} 1734 cm^{-1}) and the characteristic absorption bands of a spiroketal moiety (982, 922, 900, 860 cm^{-1}) in the IR spectrum. The ^{13}C NMR data for the sugar moieties of the mixture of **3** and **4** were superimposable and most of the signals for the aglycone were coincidental except for the observed difference in the ring-E carbons due to the epimeric centre at C-25 (Table 1). A diagnostic character of the sugar chain was the rather low-field ^1H NMR signals at δ 5.91 (*dd*, $J = 3.1$ and 9.8 Hz) arising from H-3″ of the terminal rhamnose caused by the introduction of an acetyl group to the C-3″ hydroxyl group substituent. Thus, the structure of **3** was deduced as 25(*S*)-ruscogenin 1-*O*-[3-*O*-acetyl-α-L-rhamnopyranosyl($1 \rightarrow 2$)]-β-D-fucopyranoside.

Liriopliosides C (**5**) and D (**6**) were also isolated as an inseparable epimeric mixture. The common formula of **5** and **6** was determined as $C_{14}H_{64}O_{13}$ after FAB-mass spectrum and elemental analysis. Since the ^{13}C NMR data of the aglycone moiety of **5** and **6** were not distinguishable from those of **3** and **4**, respectively, and also because they shared the same fragmentation pattern of the sugar chains, it was reasonable to conclude that the structures of **5** and **6** differ from those of **3** and **4** only in the acetylation site at the terminal rhamnose. This was clarified by the low-field ^1H NMR signal observed at δ 6.05 (*t*-like, $J = 2.1$ Hz) for the mixture of **5** and **6**, assignable to H-2″ of rhamnose from the diagnostic small coupling constant, instead of the doublet doublet signal at δ 5.91 (*dd*, $J = 3.1$ and 9.8 Hz) that was observed for **3** and **4**.

Since acyl migration between the C-2 and C-3 hydroxyl groups of rhamnose is a well-known phenomenon [10], a chemical reaction was carried out to further confirm the acyl relationship of compounds **3** (**4**) and **5** (**6**). As anticipated, **3** and **4** were proven by TLC to be partially converted to **5** and **6**, respectively, in pyridine solution after being heated at 80° for 70 hours; compounds **5** and **6** were in turn partially transformed to **3** and **4** under identical conditions.

Thus, the structures of **5** and **6** were established as 25(*S*)-ruscogenin 1-*O*-[2-*O*-acetyl-α-L-rhamnopyranosyl($1 \rightarrow 2$)]-β-D-fucopyranoside and ruscogenin 1-*O*-[2-*O*-acetyl-α-L-rhamnopyranosyl($1 \rightarrow 2$)]-β-D-fucopyranoside, respectively.

It is notable that *Ophipogon japonica* has been reported to contain both steroidal glycosides [11–13] and homoisoflavonoids [14–16], but *Liriope spicata* var. *prolifera* only contains the former group of compounds and not homoisoflavonoids. From the phytochemical point of view, these findings partially explain why *Liriope spicata* var. *prolifera* can be sometimes used as a substitute for, but not a replacement of, the crude drug Radix Ophiopogonis. Pharmacological comparison studies on these two plants are in progress.

EXPERIMENTAL

General. Mps (uncorr.): Yanagimoto micro-melting point apparatus. Optical rotations: Jasco DIP-140 polarimeter. IR spectra: Hitachi EPI-2 instrument. NMR spectra: JEOL GX-400 spectrometer or Varian XL-300 spectrometer (δ (p.p.m.), TMS). FAB-MS: direct-inlet on a VG ZAB-HS mass spectrometer (glycerol). Gas-liquid chromatography (GLC): Shimizu GC-6A unit (FID), as follows. (a) For *O*-methylated sugars: column, 5% neopentylglycol succinate (NPGS) on Shimalite 3 mm × 2 m; column temp., 145°; injection temp., 165°; carrier gas N_2, 1.0 kg/cm^2. (b) For *O*-acetyl-*O*-methyl sugars: column, 5% NPGS on shimalite 3 mm × 2 m; column temp., 180°; injection temp., 200°; carrier gas N_2, 1.5 kg/cm^2. TLC: precoated Kieselgel 60 F_{254} plates (Merck) with: (a) hexane–Me_2CO (2:1); (b) $CHCl_3$–MeOH–H_2O (80:20:2); (c) $CHCl_3$–MeOH–AcOEt–H_2O (2:2:4:1, lower phase); 10% H_2SO_4 followed by heating.

Plant material. The subterranean parts of *L. spicata* (Thunb.) Lour. var. *prolifera* Y. T. Ma, harvested in Xiangyang County, Hubei Province, China, were col-

lected in September 1988 and identified by one of us (G.-J. X.), and a voucher specimen has been deposited at the herbarium of China Pharmaceutical University, Nanjing, China.

Extraction and isolation. The concd hot MeOH (3 l × 3) extract (1.3 kg) from the subterranean parts (10 kg) of the title plant was combined and evapd to dryness *in vacuo*. The residue was dissolved in H_2O and extracted with Et_2O. The aq. layer was further extracted with BuOH satd with H_2O, and the BuOH-soluble fr. was concd *in vacuo* to afford a brown residue (169 g). The BuOH extract was subjected to CC on Sephadex LH-20 with MeOH to afford 5 frs. Fr. 2 was subjected to rechromatography on silica gel with $CHCl_3$ and increasing percentages of MeOH and H_2O (lower phase) to afford compounds **1** (80 mg), **2** (55 mg), **3** and **4** (25 mg), and **5** and **6** (40 mg).

Compound **1**. Needles (MeOH), mp 225–228°; $[\alpha]_D^{19}$ − 85.3° (pyridine, *c* 0.7) {lit. mp 240–242°, $[\alpha]_D$ − 100.9° (pyridine, *c* 0.45) [7]}. IR ν_{max} cm^{-1}: 3600–3200 (OH), 980, 920, 902, 856 [920 > 902, 25(*S*)-spiroketal]. Anal. calcd for $C_{44}H_{70}O_{16}$ $2H_2O$: C, 59.30, H, 8.37, found: C, 59.74, H, 8.27. ^1H NMR (C_5D_5N): δ 0.86 (3H, *s*, CH_3-18), 1.06 (3H, *d*, *J* = 6.7 Hz, CH_3-27), 1.08 (3H, *d*, *J* = 5.8 Hz, CH_3-21), 1.42 (3H, *s*, CH_3-19), 1.51 (3H, *d*, *J* = 6.5 Hz, CH_3-6′), 1.75 (3H, *d*, *J* = 5.8 Hz, CH_3-6″), 4.67 (1H, *d*, *J* = 7.8 Hz, H-1′), 4.99 (1H, *J* = 6.9 Hz, H-1‴), 5.60 (1H, *d*, *J* = 4.6 Hz, H-6), 6.34 (1H, *br s*, H-1″). ^{13}C NMR data are shown in Table 1. FAB-MS *m/z* (positive): 855 $[M+H]^+$, 709 $[M+H-146]^+$, 577 $[M+H-146-132]^+$, 431 $[M+H-146-132-146$, aglycone + H$]^+$; (negative) 853 $[M-H]^-$, 721 $[M-H-132]^-$.

Liriopliosides A (**2**). Needles (MeOH), mp 208–210°; $[\alpha]_D^{19}$ − 67.7° (pyridine, *c* 0.50). IR ν_{max} cm^{-1}: 3600–3200 (OH), 980, 920, 902, 856 [920 > 902, 25(*S*)-spiroketal]. Anal. calcd for $C_{50}H_{80}O_{20}$ $2H_2O$: C, 57.90, H, 8.16; found: C, 57.90, H, 8.14. ^1H NMR (C_5D_5N): δ 0.86 (3H, *s*, CH_3-18), 1.06 (3H, *d*, *J* = 6.7 Hz, CH_3-27), 1.08 (3H, *d*, *J* = 6.2 Hz, CH_3-21), 1.42 (3H, *s*, CH_3-19), 1.51 (3H, *d*, *J* = 6.4 Hz, CH_3-6′), 1.73 (3H, *d*, *J* = 5.8 Hz, CH_3-6″), 1.75 (3H, *d*, *J* = 5.8 Hz, CH_3-6⁗), 4.59 (1H, *d*, *J* = 7.6 Hz, H-1′), 5.06 (1H, *J* = 7.5 Hz, H-1‴), 5.53 (1H, *d*, *J* = 4.3 Hz, H-6), 5.59 (1H, *br s*, H-1⁗), 6.48 (1H, *br s*, H-1″). ^{13}C NMR data are shown in Table 1. FAB-MS *m/z*: 1000 $[M_{calcd}-H]^-$, 854 $[M_{calcd}-H-146]^-$, 721 $[M-H-146-132]^-$, 575 $[M-H-146-132-146]^-$ (negative) and at *m/z* 1002 $[M_{calcd}+H]^+$, 577 $[M+H-146-132-146]^+$, and 431 $[M+H-146-132-146-146$, aglycone + H$]^+$ (positive).

Liriopliosides B (**3**) *and ophiopogonin A* (**4**). White powder (MeOH–H_2O), mp 185–188°; $[\alpha]_D^{19}$ − 64.4° (pyridine, *c* 0.51). IR ν_{max} cm^{-1}: 3600–3200 (OH), 1734 (CO), 982, 922, 900, 860 (spiroketal). Anal. calcd for $C_{41}H_{64}O_{13}$ $2H_2O$: C, 61.48, H, 8.56; found: C, 61.63, H, 8.47. ^1H NMR (C_5D_5N): δ 0.69 (trace but diagnostic, *s*, CH_3(e)-27), 0.88 (3H, *s*, CH_3-18), 1.06 (3H, *d*, *J* = 6.7 Hz, CH_3(a)-27), 1.08 (3H, *d*, *J* = 6.2 Hz, CH_3-21), 1.44 (3H, *s*, CH_3-19), 1.66 (3H, *d*, *J* = 6.4 Hz, CH_3-6′), 1.78 (3H, *d*, *J* = 5.9 Hz, CH_3-6″), 2.51 (3H, *s*, $\underline{CH_3}$-CO-), 4.67 (1H, *d*, *J* = 7.8 Hz, H-1′), 5.59 (1H, *d*, *J* = 5.4 Hz, H-6), 5.91 (1H, *dd*, *J* = 3.1 and 9.8 Hz, H-3″), 6.38 (1H, *br s*, H-1″). ^{13}C NMR data are shown in Table 1. FAB-MS (negative) *m/z*: 763 $[M-H]^-$, 721 $[M-H-Ac]^-$; (positive) *m/z*: 765 $[M+H]^+$, 723 $[M+H-Ac]^+$, 577 $[M+H-Ac-146]^+$, 431 $[M+H-Ac-146-146$, aglycone + H$]^+$, 413 [aglycone + H − $H_2O]^+$.

Liriopliosides C (**5**) *and liriopliosides D* (**6**). White powder (MeOH–H_2O), mp 203–207°; $[\alpha]_D^{19}$ − 101.6° (pyridine, *c* 0.50). IR ν_{max} (cm^{-1}): 3600–3200 (OH), 1734 (CO), 982, 922, 900, 860 (spiroketal). Anal. calcd for $C_{41}H_{64}O_{13}H_2O$: C, 62.89, H, 8.50; found: C, 62.81, H, 8.62. ^1H NMR (C_5D_5N): δ 0.69 (trace but diagnostic, CH_3-27(e)), 0.89 (3H, *s*, CH_3-18), 1.07 (3H, *d*, *J* = 6.7 Hz, CH_3-27(a)), 1.08 (3H, *d*, *J* = 6.2 Hz, CH_3-21), 1.45 (3H, *s*, CH_3-19), 1.58 (3H, *d*, *J* = 6.4 Hz, CH_3-6′), 1.78 (3H, *d*, *J* = 5.9 Hz, CH_3-6″), 2.51 (3H, *s*, $\underline{CH_3}$-CO-), 4.68 (1H, *d*, *J* = 7.8 Hz, H-1′), 5.60 (1H, *d*, *J* = 4.7 Hz, H-6), 6.05 (1H, *t*, *J* = 2.1 Hz, H-2″), 6.27 (1H, *br s*, H-1″). ^{13}C NMR data are shown in Table 1. FAB-MS (negative) *m/z*: 763 $[M-H]^-$, 721 $[M-H-Ac]^-$; (positive) *m/z*: 765 $[M+H]^+$, 723 $[M+H-Ac]^+$, 577 $[M+H-Ac-146]^+$, 432 $[M+2H-Ac-146-146$, aglycone + 2H$]^+$, 414 [aglycone + H − $H_2O]^+$.

Hydrolysis of **1** *and* **2**. Separate solutions of **1** and **2** (each 35 mg) in 2 M HCl–50% dioxane were refluxed for 3 hr. Each resultant mixture was diluted with H_2O and then extracted with $CHCl_3$ and the $CHCl_3$ layer was washed with H_2O and dried over Na_2SO_4. The $CHCl_3$ soln was filtered and the filtrate was evaporated to dryness under red. press. The residues from **1** and **2** were crystallized from MeOH to give the same aglycone which was identified as 25(*S*)-ruscogenin by co-TLC with an authentic sample [R_f 0.31 with solvent system (a)] and by ^1H and ^{13}C NMR spectral data comparison with literature values [4]. The absolute configurations of sugars were determined by high-performance liquid chromatography (HPLC) as described in ref. [2].

Methylation and methanolysis of **2**. Methylation of **2** (10 mg) by Hakomori's method as described in ref. [8] afforded per-*O*-methyl ether **2**: liquid, ^1H NMR ($CDCl_3$): δ 3.38–3.62 (30H, *s*, OCH_3 × 10), 4.19 (1H, *d*, *J* = 7.5 Hz, H-1′, fucose), 4.42 (1H, *d*, *J* = 7.5 Hz, H-1‴, xylose), 5.04 (2H, *br s*, H-1″ and 1⁗, rhamnose). The per-*O*-methyl ether of **2** was refluxed with methanolic 5% HCl (3 ml) for 2 hr, then the reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was dissolved in Me_2CO and examined by GLC for sugar derivatives as follows, R_t (min): 3.1 (per-*O*-methylrhamnopyranoside), 3.8 (per-*O*-methylxylopyranoside) with GLC condition (a); and R_t (min) 12.7, 20.5 (methyl 2,3-di-*O*-acetyl-4-*O*-methylfucopyranoside) after acetylation by Ac_2O under condition (b).

Partial hydrolysis of **2**. A soln of **2** (5 mg) in 0.05 N HCl in MeOH (0.5 ml) was heated at 80° for 30 min. The reaction mixture was diluted with H_2O, neutralized

with $NaHCO_3$ and concentrated to dryness *in vacuo*. The residue was then dissolved in MeOH and filtered, and from the filtrate 25(*S*)-ruscogenin [4], 25(*S*)-ruscogenin 1-*O*-β-D-fucopyranoside-3-α-L-rhamnopyranoside [6] and **1** were detected by TLC [solvent system (b)] by direct comparison with authentic samples with R_f 0.48, 0.31 and 0.15, respectively.

Interconversion of **3** *and* **5** *and of* **4** *and* **6**. A soln of a mixture of compounds **3** and **4** (2 mg) in pyridine (0.5 ml each) was heated in a H_2O bath at 80° for 70 hr; these compounds were proved by TLC to be partially converted to **5** and **6** (R_f 0.70 and 0.80 with solvent system (b), respectively). Compounds **5** and **6** underwent a similar transformation to **3** and **4** under identical conditions.

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