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ARTICLE

Isolation and Structural Determination of Steroidal Glycosides from the Bulbs of Easter Lily (*Lilium longiflorum* Thunb.)

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The bulbs of the Easter lily (*Lilium longiflorum* Thunb.) are used as a food and medicine in several Asian cultures, and they are cultivated as an ornamental plant throughout the world. A new steroidal glycoalkaloid and two new furostanol saponins, along with two known steroidal glycosides, were isolated from the bulbs of *L. longiflorum*. The new steroidal glycoalkaloid was identified as (22*R*,25*R*)-spirosol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. The new furostanol saponins were identified as (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-glucopyranoside and (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-xylopyranosyl-(1→3)-β-D-glucopyranoside. The previously known steroidal glycosides, (22*R*,25*R*)-spirosol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside and (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside were identified in *L. longiflorum* for the first time. These new compounds from *L. longiflorum* and the isolation methodologies employed can be used for studies on the biological role of steroidal glycosides in plant development and plant−pathogen interactions, as well as for studies in food and human health, for which little is known.

KEYWORDS: *Lilium longiflorum* Thunb.; Liliaceae; Easter lily; steroidal glycoside; steroidal glycoalkaloid; furostanol saponin

INTRODUCTION

The Easter lily (*Lilium longiflorum* Thunb., family Liliaceae) has beautiful white flowers and a delicate aroma and is appreciated worldwide as an attractive ornamental. In addition to its economic importance and popularity in horticulture, lily bulbs are regularly consumed in Asia, as both food and medicine. The bulbs of several *Lilium* species, including *L. longiflorum*, *L. brownii* F. E. Br. ex Miellez var. *viridulum* Baker, *L. pensylvanicum* Ker Gawl., and *L. pumilum* DC, have been used traditionally in China as sedatives, anti-inflammatory and antitussive agents, and a general tonic (*1*, *2*). The crude drug "Bai-he", used in traditional Chinese medicine, is prepared from the bulbs of *Lilium* sp. and is regularly used for lung ailments in China today. Although the medicinal use of *L. longiflorum* is well documented, the compounds responsible for the reported properties are not known.

Bulbs of the genus *Lilium* are a rich source of secondary metabolites, including bitter phenylpropanoid glycosides identified in the bulbs of *L. speciosum* Thunb. (*3*), antitumor alkaloids identified in *L. hansonii* Leichtlin ex D. D. T. Moore (*4*), and steroidal glycoalkaloids identified in *L. brownii* var. *viridulum* (*5*). Extensive work has been done on the isolation and characterization of steroidal saponins in *Lilium*. Steroidal saponins have been reported in *L. auratum* Lindl. (*6*), *L. brownii* (*2*, *7*), *L. brownii* var. *viridulum* (*5*, *8*), *L. candidum* (*9*−*11*), *L. hansonii* (*12*), *L. henryi* Baker (*13*), *L. longiflorum* (*14*), *L. martagon* L. (*15*), *L. pardalinum* Kellogg (*16*), *L. pensylvanicum* (*1*), *L. pumilum* (*6*), *L. regale* E. H. Wilson (*13*, *17*, *18*), *L. speciosum* var. *speciosum* (*19*), and *L. speciosum* × *L. nobilissimum* (Makino) Makino (*20*). Steroidal saponins have been reported to exhibit a wide range of biological activities including antifungal (*21*), platelet aggregation inhibition (*22*, *23*), antidiabetic (*24*), cholesterol lowering (*25*), anti-inflammatory (*26*), antiviral (*27*), and anticancer (*28*−*30*). Although the putative biological activities of steroidal saponins are well documented, the biological role of these compounds within the plant is poorly understood.

The occurrence of steroidal glycoalkaloids in the Liliaceae family is also well documented (*31*). Steroidal alkaloids isolated from *Veratrum* and *Fritillaria* species show various biological activities including antihypertensive (*32*), anticholinergic (*33*), antifungal (*34*), and anticancer (*35*). In *Lilium*, steroidal glycoalkaloids have been identified in *L. philippinense* Baker (*36*), *L. candidum* L. (*37*), and *L. brownii* var. *viridulum* (*5*); however, no steroidal glycoalkaloids have been previously reported from *L. longiflorum*.

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With regard to the steroidal glycosides of *L. longiflorum* bulbs, six spirostanol saponins and three furostanol saponins with antitumor activity have been reported (*14*). To set the stage for biological activity studies on the role of steroidal glycosides in plant development and plant−pathogen interactions, as well as for studies in food and human health, this paper reports the isolation and structural determination of several new steroidal glycosides from the bulbs of *L. longiflorum*. The structures of the steroidal glycosides were elucidated by a combination of spectroscopic and chemical analysis.

MATERIALS AND METHODS

Plant Material. *L. longiflorum*, cultivar 7-4, bulbs were provided from the Rutgers University Easter lily breeding program. Bulbs were treated with Captan (Bayer CropScience AG, Monheim am Rhein, Germany) fungicide prior to planting. Bulbs were planted in beds containing Pro-Mix (Premier Horticulture Inc., Quakertown, PA) soil mix and were grown to mature plants under greenhouse conditions for 9 months prior to harvest. The greenhouse temperatures were set to provide a minimum day temperature of 24 °C and a minimum night temperature of 18 °C. Plants were fertilized biweekly with a 100 mg/L solution of NPK 15-15-15 fertilizer (J. R. Peters Inc., Allentown, PA). Each plant produced three to five new bulbs, which we used for extraction. The new bulbs were full-sized, fresh, and mature at harvest. Each plant was harvested by hand, and the bulbs were manually separated, immediately frozen under liquid nitrogen, lyophilized on a VirTis AdVantage laboratory freeze-dryer (SP Industries Inc.,Warminster, PA), and stored at −80 °C until analysis.

Chemicals. The following compounds were obtained commercially: (25*R*)-spirost-5-en-3β-ol, Sephadex LH-20, *N*,*O*-bis(trimethylsiyl)trifluoroacetamide with trimethylchlorosilane (99:1) silylating reagent, Dragendorff reageant, *p*-(dimethylamino)benzaldehyde, hydrochloric acid, sodium hydroxide, pyridine-d_5 (0.3% v/v TMS), D-(+)-glucose, L-(−)-glucose, D-(+)-rhamnose, L-(−)-rhamnose, D-(+)-arabinose, L-(−)-arabinose, D-(+)-xylose, and L-(−)-xylose (Sigma-Aldrich, St. Louis, MO); and (22*R*,25*R*)-spirosol-5-ene-3β-ol (Glycomix Ltd., Reading, U.K.). All solvents (acetonitrile, chloroform, ethanol, ethyl acetate, formic acid, *n*-butanol, and *n*-pentane) were of chromatographic grade (Thermo Fisher Scientific Inc., Fair Lawn, NJ). Water was deionized (18 MΩ cm) using a Milli-Q-water purification system (Millipore, Bedford, MA).

Isolation and Purification of Steroidal Glycosides 1−5 from *L. longiflorum*. *Sequential Solvent Extraction of Lyophilized L. longiflorum Bulbs*. Lyophilized lily bulbs (100 g) were frozen in liquid nitrogen, ground into a fine powder with a laboratory mill (IKA Labortechnik, Staufen, Germany), and extracted with *n*-pentane (3 × 100 mL) on a wrist-action autoshaker (Burrell Scientific, Pittsburgh, PA) at room temperature for 30 min. After centrifugation (5000 rpm for 10 min) (Sorvall RC-3C Plus, Thermo Fisher Scientific Inc.), the organic layers were discarded and the pellet was freed from residual solvent in a fume hood overnight. The residual defatted material was then extracted with a mixture of ethanol and water (7:3, v/v; 2 × 150 mL) on an autoshaker for 45 min at room temperature (25 °C). After centrifugation (5000 rpm for 10 min) and vacuum filtration through a Whatman 114 filter paper (Whatman International Ltd., Maidstone, U.K.), the supernatant was collected and the residue discarded. The supernatant was then evaporated under reduced pressure (30 °C; 1.0×10^{-3} bar) using a Laborota 4003 rotary evaporator (Heidolph Brinkman LLC, Elk Grove Village, IL) and lyophilized, yielding a crude bulb extract (13.7 g). The lyophilized crude bulb extract was then dissolved in deionized water (100 mL) and washed with ethyl acetate (5 × 100 mL), and the organic phase was discarded. The aqueous phase was then extracted with *n*-butanol (5 × 100 mL) and the aqueous phase discarded. The organic phase was then evaporated under reduced pressure (30 °C; 1.0×10^{-3} bar) and lyophilized, yielding a crude glycoside extract (2.42 g).

Gel Permeation Chromatography (*GPC*). Crude glycoside extract (1.0 g) was dissolved in a solution of ethanol and water (7:3, v/v; 5.0 mL), filtered with a 0.45 μm PTFE syringe filter (Thermo Fisher Scientific Inc.), and then applied onto a standard threaded 4.8 cm × 100 cm glass column (Kimble Chase Life Science and Research Products LLC, Vineland, NJ) packed with Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden) that was washed and conditioned in the same solvent mixture overnight. Chromatography was performed with isocratic ethanol/water (70:30, v/v) at a flow rate of 3.5 mL min^{-1}. The first 200 mL of effluent was discarded, and 30 fractions (25 mL each) were collected and analyzed by LC-MS. LC-MS analyses were performed on a HP 1100 series HPLC (Agilent Technologies Inc., Santa Clara, CA) equipped with an autoinjector, a quaternary pump, a column heater, and a diode array detector and interfaced to a Bruker 6300 series ion-trap mass spectrometer equipped with an electrospray ionization chamber. HP ChemStation and BrukerData Analysis software were used for data acquisition and data analysis. Reverse phase separations were performed using a Prodigy C18 column (250 mm × 4.6 mm i.d.; 5.0 μm particle size) (Phenomenex, Torrance, CA). The flow rate was set to 1.0 mL min^{-1}, and the column temperature was at 23 ± 2 °C. The binary mobile phase composition consisted of (A) 0.1% formic acid in deionized water and (B) 0.1% formic acid in acetonitrile. Chromatography was performed using a linear gradient of 15−43% B over 40 min and then to 95% B over 5 min; thereafter, elution with 95% B was performed for 10 min. The re-equilibration time was 10 min. All mass spectra were acquired in positive ion mode over a scan range of *m*/*z* 100−2000. Ionization parameters included capillary voltage, 3.5 kV; end plate offset, −500 V; nebulizer pressure, 50 psi; drying gas flow, 10 mL min^{-1}; and drying gas temperature, 360 °C. Trap parameters included ion current control, 30000; maximum accumulation time, 200 ms; trap drive, 61.2; and averages, 12 spectra. On the basis of the LC-MS profile, GPC fractions 6−17 were combined, evaporated under reduced pressure (30 °C; 1.0×10^{-3} bar), and lyophilized, yielding GPC fraction A (180 mg).

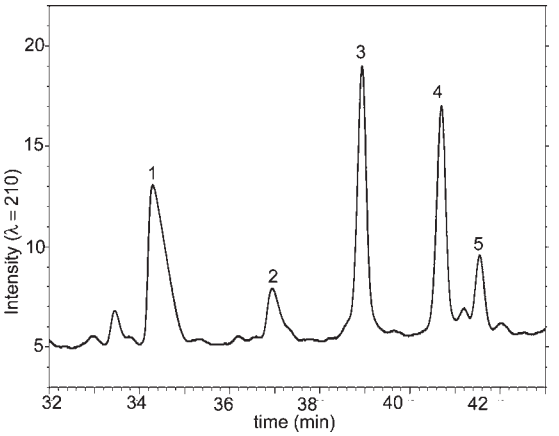

Figure 1. RP-HPLC chromatogram (λ = 210 nm) of **1**−**5** separated from *L. longiflorum n*-butanol extract fractionated by gel permeation chromatography (GPC).

Semipreparative Reverse-Phase High-Performance Liquid Chromatography (*RP-HPLC*). Fractionation of GPC fraction A was achieved by semipreparative RP-HPLC performed on a Luna C18 column (250 mm × 21.2 mm i.d.; 10 μm particle size) (Phenomenex) to afford **1**−**5** (**Figure 1**). Chromatography was performed on a Shimadzu LC-6AD liquid chromatograph (Shimadzu Scientific Instruments Inc., Columbia, MD) using a UV−vis detector and a 2 mL injection loop. Mixtures of (A) 0.1% formic acid in deionized water and (B) 0.1% formic acid in acetonitrile were used as the mobile phase. The flow rate was set to 20 mL min^{-1}, the column temperature was 23 ± 2 °C, and UV detection was recorded at λ = 210 nm. GPC fraction A was dissolved in a mixture of mobile phase A and mobile phase B (75:25, v/v) and filtered through a 0.45 μm PTFE syringe filter prior to injection. Chromatography was performed using a linear gradient of 5−30% B over 45 min and then to 90% B over 10 min; thereafter, elution with 90% B was performed for 10 min. The re-equilibration time was 10 min. The target compounds were collected, freed from solvent under reduced pressure (30 °C; 1.0×10^{-3} bar), and lyophilized. Final purification of **1** and **2** was performed with an isocratic separation using a mixture of 0.1% formic acid in deionized water and 0.1% formic acid in acetonitrile (80:20, v/v). The target compounds were collected, freed from solvent under reduced pressure (30 °C; 1.0×10^{-3} bar), and lyophilized, yielding **1** (15 mg) and **2** (7 mg) as white amorphous powders in high purity (>98%), as determined by LC-MS (**Figure 2**) and NMR. Final purification of **3**−**5** was performed with an isocratic separation using a mixture of 0.1%

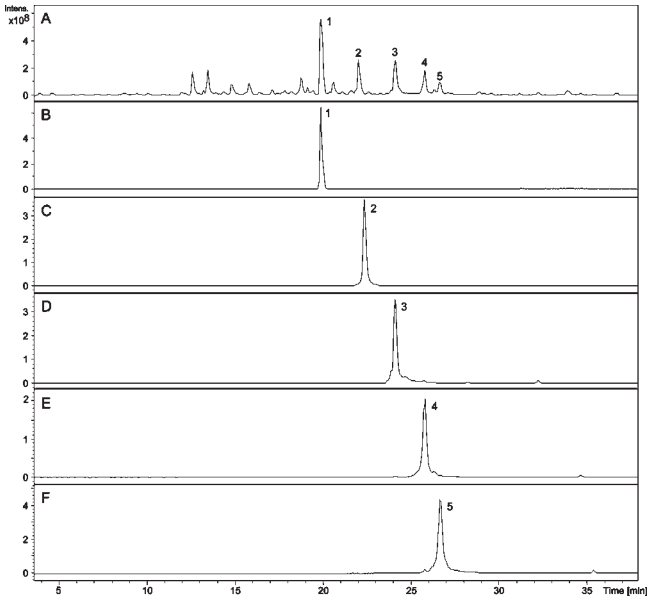

Figure 2. (**A**) Total ion chromatogram (TIC) of crude *L. longiflorum* *n*-butanol extract. (**B**−**F**) Total ion chromatograms (TIC) of **1**−**5** isolated by RP-HPLC.

formic acid in DI water and 0.1% formic acid in acetonitrile (75:25, v/v). The target compounds were collected, freed from solvent under reduced pressure (30 °C; 1.0×10^{-3} bar), and lyophilized, yielding **3** (25 mg), **4** (7 mg), and **5** (5 mg) as white amorphous powders in high purity (>98%), as determined by LC-MS (**Figure 2**) and NMR.

Identification of Compounds 1−5. Compounds **1**−**5** were identified by a combination of spectroscopic data (^{1}H NMR, ^{13}C NMR, HMBC, HMQC, MS, IR), chromatographic data, and chemical analysis. Melting points were obtained using a Thomas-Hoover Capillary Melting Point Apparatus (Arthur H. Thomas Co., Philadelphia, PA) and by differential scanning calorimetry using a Perkin-Elmer Diamond DSC (Perkin-Elmer, Waltham, MA). IR spectra were recorded on a Nexus 670 FT-IR spectrophotometer. Observed rotations were obtained on a Perkin-Elmer model 341LC polarimeter. High -resolution mass spectra (HRMS) were recorded on a BioTOF II ESI under the following conditions: source temperature, 150 °C; acceleration voltage, 8500; mass resolution, 10000 fwhm; scan range, *m*/*z* 100−1000; drying gas, N_2. ESI$^+$-MS spectra were recorded on a Bruker 6300 series ion-trap mass spectrometer under the conditions reported above. 1D ^{1}H NMR and ^{13}C NMR spectra and 2D heteronuclear multiple bond coherence (HMBC) and heteronuclear multiple quantum coherence (HMQC) spectra were acquired on an AMX-400 spectrometer and an AMX-500 spectrometer (Bruker, Rheinstetten, Germany). Samples for NMR analysis were dissolved in pyridine-d_5, and chemical shifts are given as δ values with reference to tetramethylsilane (TMS).

Acid Hydrolysis of Compounds 1−5. A solution of each compound (1 mg) in 1 N HCl in methanol (0.5 mL) was refluxed at 80 °C for 2 h. After hydrolysis, the solution was adjusted to pH 7 with NaOH (4 N) and evaporated to dryness under reduced pressure (30 °C; 1.0×10^{-3} bar). The residue was dissolved in water (1 mL) and extracted with *n*-pentane (2 mL). The *n*-pentane phase was used for aglycone analysis, and the aqueous phase was used for sugar analysis.

Aglycone Analysis. The *n*-pentane phase obtained after hydrolysis was evaporated to dryness under reduced pressure (30 °C; 1.0×10^{-3} bar), dissolved in a mixture of pyridine and BSTFA with TMCS (99:1) silylating reagent (1:1, v/v, 100 μL), and refluxed in a sealed tube (60 °C for 1 h). After cooling to room temperature, the solution was analyzed by GC-MS. An Agilent 6890 series GC system coupled to an Agilent 5973 mass spec detector (Santa Clara, CA) was used for GC-MS analysis. A capillary column (HP-5, 5% phenyl, 95% dimethyl polysiloxane stationary phase, 30 m × 0.25 mm i.d. × 0.25 mm film thickness) was used for the chromatographic separation. The temperature program was as follows: 70 °C for 2 min, then increased 8 °C/min to 240 °C, and held for 10 min. The other parameters used were splitless injector heated at 250 °C and helium as the carrier gas with a constant flow of 1 mL min^{-1}. MS parameters were as follows: operated in electron impact (EI) ionization mode at 70 eV; scan range, *m*/*z* 50−550. The transfer line was maintained at 250 °C. Comparisons were made with retention times and mass spectra of aglycone reference standards prepared according to the same procedure.

Sugar Composition Analysis. The aqueous fraction obtained after hydrolysis was evaporated to dryness under reduced pressure (30 °C; 1.0×10^{-3} bar), dissolved in a mixture of pyridine and BSTFA with TMCS (99:1) silylating reagent (1:1, v/v, 100 μL), and refluxed in a sealed tube (60 °C for 1 h). After cooling to room temperature, the solution was analyzed by GC-MS. The temperature program was as follows: 50 °C for 6 min, then increased at 4 °C/min to 160 °C, and held for 5 min. The other parameters used were a splitless injector heated to 200 °C and helium as the carrier gas with a constant flow of 1 mL min^{-1}. MS parameters were the same as reported above. Identifications were made based on retention times and mass spectra of sugar standards prepared according to the same procedure.

Determination of Sugar Absolute Configurations. Absolute configuration of sugars was determined by enantioselective GC-FID. A chiral RT-BetaDEXsm capillary column (30 m × 0.25 mm × 0.25 μm; Restek Corp., State College, PA) was used for chromatographic separation. The temperature program was as follows: 60 °C for 0 min, then increased at 4 °C/min to 160 °C, then increased at 15 °C/min to a final temperature 230 °C, and maintained for 15 min. The other parameters used were as follows: FID detector heated to 230 °C; split injector with a 10:1 split ratio maintained at 230 °C; helium as the carrier gas with a constant flow of 1 mL min^{-1}. Comparisons were made with retention times of optically pure sugar standards prepared following the same procedure.

Thin Layer Chromatography (TLC). Each compound (1 mg) was dissolved in methanol (0.5 mL), spotted on a 20 cm ×20 cm silica gel 60 F254 TLC plate (Merck & Co., Inc., Whitehouse Station, NJ), and developed with chloroform/methanol/water (8:4:1, v/v/v). To detect furostanols, TLC plates were developed with Ehrlich's reagent [3.2 g of *p*-(dimethylamino)benzaldehyde in 60 mL of 95% ethanol and 60 mL of 12 N HCl] and heated to 110 °C for 5 min. Bright red spots were indicative of a positive reaction. To detect alkaloids, the TLC plates were developed with Dragendorff's reagent and heated to 110 °C for 5 min. Orange spots were indicative of a positive reaction.

Compound 1, (22*R*,25*R*)-spirosol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside: amorphous solid; $[\alpha]^{28}{}_D$ −76.9 (*c* 0.02; MeOH); mp 303 °C (dec); IR ν_{max} (film) cm^{-1} 3336 (OH), 2930 (CH), 1585, 1450, 1347, 1255, 1029, 984, 897, 811; HRESI-TOFMS, *m*/*z* 884.5028 $[M + H]^+$ (calculated for $C_{45}H_{74}NO_{16}$, 884.5002); ESI$^+$-MS, *m*/*z* 884.7 (100, $[M + H]^+$), 738.5 (3, $[M - Rha + H]^+$), 576.4 (19, $[M - Glu - Rha + H]^+$), 453.8 (1.6, $[M + H + Na]^{+2}$), 414.6 (15, $[M - 2Glu - Rha + H]^+$) (**Figure 3**); ^{1}H NMR (400 MHz) δ_H 0.82 [d, 3H, *J* = 5.2 Hz, 27-H], 0.88 [s, 3H, 18-H], 1.06 [s, 3H, 19-H], 1.09 [d, 3H, *J* = 6.8 Hz, 21-H], 1.78 [d, 3H, *J* = 6.4 Hz, 6″-H], 2.75 [m, 2H, 26-H], 3.88 [m, 1H, 3-H], 3.90 [m, 1H, 2′-H], 4.33 [1H, 6b‴-H], 4.56−4.40 [1H, 6a‴-H], 4.48 [m, 2H, 6′-H], 4.61 [dd, 1H, *J* = 9.2, 3.2, 3″-H], 4.97 [d, 1H, *J* = 7.2 Hz, 1′-H], 5.15 [d, 1H, *J* = 7.6 Hz, 1‴-H], 5.30 [d, 1H, *J* = 4.8 Hz, 6-H], 6.27 [s, 1H, 1″-H]; ^{13}C NMR (400 MHz, pyridine-d_5) δ 37.5 [C-1], 30.2 [C-2], 78.1 [C-3], 38.9 [C-4], 140.8 [C-5], 121.8 [C-6], 32.4 [C-7], 31.6 [C-8], 50.3 [C-9], 37.2 [C-10], 21.2 [C-11], 40.1 [C-12], 40.7 [C-13], 56.7 [C-14], 32.6 [C-15], 78.8 [C-16], 63.6 [C-17], 16.5 [C-18], 19.4 [C-19], 41.6 [C-20], 15.7 [C-21], 98.4 [C-22], 34.7 [C-23], 31.1 [C-24], 31.7 [C-25], 48.1 [C-26], 19.9 [C-27], 100.0 [C-1′], 77.8 [C-2′], 76.2 [C-3′], 82.1 [C-4′], 77.3 [C-5′], 62.0 [C-6′], 101.8 [C-1″], 72.5 [C-2″], 72.8 [C-3″], 74.2 [C-4″], 69.5 [C-5″], 18.7 [C-6″], 105.3 [C-1‴], 75.0 [C-2‴], 78.3 [C-3‴], 71.2 [C-4‴], 78.5 [C-5‴], 62.1 [C-6‴]; ^{1}H NMR and ^{13}C NMR are consistent with the literature (*5*).

Compound 2, (22*R*,25*R*)-spirosol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside: amorphous solid; $[\alpha]_D{}^{28}$ −32.0° (MeOH; *c* 0.05); mp 285 °C (dec); IR ν_{max} (film) cm^{-1} 3353 (OH), 2932 (CH), 1732 (C=O), 1588, 1451, 1370, 1252, 1033, 983, 897, 813; HRESI-TOFMS *m*/*z* 926.5085 $[M + H]^+$ (calculated for $C_{47}H_{76}NO_{17}$, 926.5108); ESI$^+$-MS, *m*/*z* 926.6 (100, $[M + H]^+$), 780.5 (14, $[M - Rha + H]^+$), 576.4 (15, $[M - Glu - Ac - Rha + H]^+$), 474.8 (3, $[M + H + Na]^{2+}$), 414.3 (30, $[M - 2Glu - Ac - Rha + H]^+$) (**Figure 3**); ^{1}H NMR (400 MHz) δ_H 0.82 [d, 3H, *J* = 4.8 Hz, 27-H], 0.88 [s, 3H, 18-H], 1.06 [s, 3H, 19-H], 1.11 [d, 3H, *J* = 6.4 Hz, 21-H], 1.78 [d, 3H, *J* = 6 Hz, 6″-H], 2.06 [s, 3H, CO−CH_3], 4.35 [t, 1H, *J* = 9.4, 4″-H], 4.60 [dd, 1H,

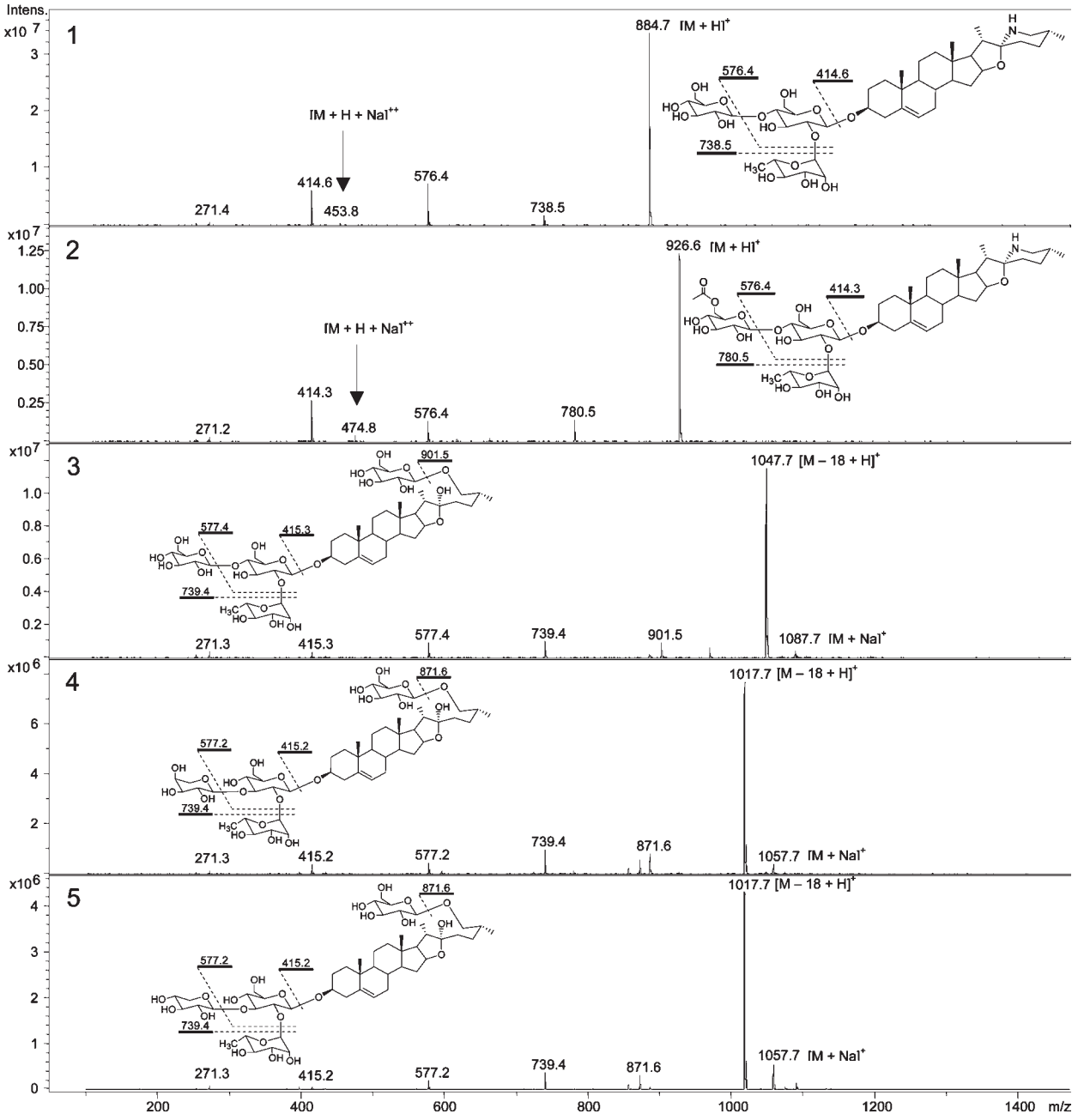

Figure 3. ESI$^+$-MS mass spectra of **1**−**5**.

J = 8.8, 3.2, 3″-H], 4.64 [d, 1H, J = 8.4 Hz, 6b‴-H], 4.92 [dd, 1H, J = 11.6, 2, 6a‴-H], 4.98 [d, 1H, J = 7.2 Hz, 1′-H], 5.09 [d, 1H, J = 8.1‴-H], 5.29 [d, 1H, J = 4.4 Hz, 6-H], 6.24 [br s, 1H, 1″-H]; for ^{13}C NMR (500 MHz, pyridine-d_5) spectroscopic data, see **Table 1**.

Compound ***3****,* (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside: amorphous solid; $[\alpha]^{28}_{D}$ −60.0 (*c* 0.05; MeOH); mp 207 °C (dec); IR ν_{max} (film) cm^{-1} 3347 (OH), 2893 (CH), 1662, 1377, 1256, 1027, 909, 839, 812; HRESI-TOFMS *m*/*z* 1087.5296 [M + Na]$^+$ (calculated for $C_{51}H_{84}O_{23}Na$, 1087.5307); ESI$^+$-MS, *m*/*z* 1087.7 (20, [M + Na]$^+$), 1047.7 (100, [M − 18 + H]$^+$), 901.5 (9, [M − 18 − Rha + H]$^+$), 739.4 (17, [M − 18 − Glu − Rha + H]$^+$), 577.4 (11, [M − 18 − 2Glu − Rha + H]$^+$), 415.3 (9, [M − 18 − 3Glu − Rha + H]$^+$) (**Figure 3**); ^{1}H NMR (500 MHz) δ_H 0.91 [s, 3H, 18-H], 1.00 [d, 3H, J = 6.4 Hz, 27-H], 1.07 [s, 3H, 19-H], 1.35 [d, 3H, J = 6.8 Hz, 21-H], 1.76 [d, 3H, J = 6.5 Hz, 6″-H], 4.56 [dd, 1H, J = 12, 2.5, 6‴′-H], 4.60 [dd, 1H, J = 9.5, 3.5, 3″-H], 4.83 [d, 1H, J = 8 Hz, 1‴′-H], 4.96 [d, 1H, J = 6.5 Hz, 1′-H], 5.14 [d, 1H, J = 8, 1‴-H], 5.29 [d, 1H, J = 4 Hz, 6-H], 6.26 [br s, 1H, 1″-H]; ^{13}C NMR (400 MHz, pyridine-d_5) δ 37.5 [C-1], 30.2 [C-2], 78.2 [C-3], 39.0 [C-4], 140.8 [C-5], 121.9 [C-6], 32.5 [C-7], 31.7 [C-8], 50.4 [C-9], 37.2 [C-10], 21.2 [C-11], 39.9 [C-12], 40.8 [C-13], 56.6 [C-14], 32.4 [C-15], 81.1 [C-16], 63.9 [C-17], 16.5 [C-18], 19.4 [C-19], 40.7 [C-20], 16.5 [C-21], 110.7 [C-22], 37.1 [C-23], 28.4 [C-24], 34.3 [C-25], 75.3 [C-26], 17.5 [C-27], 100.0 [C-1′], 77.8 [C-2′], 76.2 [C-3′], 82.1 [C-4′], 77.3 [C-5′], 62.1 [C-6′], 101.8 [C-1′], 72.5 [C-2″], 72.8 [C-3″], 74.2 [C-4″], 69.5 [C-5″], 18.7 [C-6″], 105.2 [C-1‴], 75.0 [C-2‴], 78.5 [C-3‴], 71.2 [C-4‴], 78.3 [C-5‴], 61.9 [C-6‴], 105.0 [C-1‴′], 75.2 [C-2‴′], 78.6 [C-3‴′], 71.7 [C-4‴′], 78.2 [C-5‴′], 62.8 [C-6‴′]; ^{1}H NMR and ^{13}C NMR are consistent with the literature (*12*).

Compound ***4****,* (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-glucopyranoside: amorphous solid; $[\alpha]_{D}^{28}$ −48.6° (MeOH; *c* 0.07); mp 200 °C (dec); IR ν_{max} (film) cm^{-1} 3367 (OH), 2899 (CH), 1699, 1377, 1256, 1040, 912, 840, 811, 780; HRESI-TOFMS *m*/*z* 1057.5211 [M + Na]$^+$ (calculated for $C_{50}H_{82}O_{22}Na$, 1057.5190); ESI$^+$-MS, *m*/*z* 1057.7 (5, [M + Na]$^+$), 1017.7 (100, [M − 18 + H]$^+$), 871.6 (7, [M − 18 − Rha + H]$^+$), 739.4 (13, [M − 18 − Ara − Rha + H]$^+$), 577.2 (7, [M − 18 − Ara − Rha − Glu + H]$^+$), 415.2 (4, [M − 18 − 2Glu − Ara − Rha + H]$^+$) (**Figure 3**); ^{1}H NMR (400 MHz) δ_H 0.91 [s, 3H, 18-H], 1.00 [d, 3H, J = 6.4 Hz, 27-H],

Table 1. ^{13}C NMR Chemical Shift Assignments for **2**, **4**, and **5** in Pyridine-d_5

	compound		
carbon	**2**	**4**	**5**
C-1	37.5	37.5	37.5
C-2	30.2	30.1	30.1
C-3	78.1[a]	77.7[a]	77.7[a]
C-4	39.0	38.7	38.7
C-5	140.8	140.7	140.7
C-6	121.9	121.9	121.9
C-7	32.4	32.4	32.4
C-8	31.6[b]	31.7	31.7
C-9	50.4	50.3	50.3
C-10	37.2	37.2	37.2
C-11	21.2	21.1	21.1
C-12	40.1	39.9	39.9
C-13	40.7	40.8[b]	40.8[b]
C-14	56.7	56.6	56.6
C-15	32.6	32.5	32.5
C-16	79.0	81.1	81.1
C-17	63.5	63.9	63.9
C-18	16.5	16.5	16.5
C-19	19.4	19.4	19.4
C-20	41.7	40.7[b]	40.7[b]
C-21	15.7	16.5	16.5
C-22	98.4	110.7	110.7
C-23	34.7	37.2	37.2
C-24	31.0	28.4	28.4
C-25	31.7[b]	34.3	34.3
C-26	48.0	75.3[c]	75.3[c]
C-27	19.8	17.5	17.5
C-1′	99.9	99.9	100.0
C-2′	77.6	78.0	78.0
C-3′	76.1	88.0	88.2
C-4′	83.3	69.7	69.7
C-5′	77.4	77.6[a]	77.7[a]
C-6′	62.0	62.4	62.4
C-1″	102.0	102.5	102.4
C-2″	72.5	72.5	72.5
C-3″	72.8	72.9	72.9
C-4″	74.2	74.1	74.1
C-5″	69.6	69.5	69.5
C-6″	18.7	18.7	18.7
C-1‴	105.6	105.6	105.5
C-2‴	75.1	72.3	74.7
C-3‴	78.2[a]	74.6	78.4
C-4‴	71.9	69.7	70.7
C-5‴	74.9	67.8	67.3
C-6‴	64.8		
C-1⁗		105.0	105.0
C-2⁗		75.2[c]	75.2[c]
C-3⁗		78.6[d]	78.6[d]
C-4⁗		71.7	71.7
C-5⁗		78.5[d]	78.5[d]
C-6⁗		62.8	62.8
Ac—CH_3	20.8		
Ac—C=O	170.9		

[a] Assignments may be interchanged in each column. [b] Assignments may be interchanged in each column. [c] Assignments may be interchanged in each column. [d] Assignments may be interchanged in each column.

1.07 [s, 3H, 19-H], 1.35 [d, 3H, J = 6.8 Hz, 21-H], 1.76 [d, 3H, J = 6 Hz, 6″-H], 3.08 [d, 1H, J = 11.6, 5‴-H], 4.05 [m, 1H, Hz, 2⁗-H], 3.90−3.85 [m, 1H, 2′-H], 4.84 [d, 1H, J = 7.6 Hz, 1⁗-H], 4.91 [m, 1H, 16-H], 4.92 [d, 1H, J = 7.6, 1‴-H], 4.99 [d, 1H, J = 7.2 Hz, 1′-H], 5.33 [d, 1H, J = 4 Hz, 6-H], 6.30 [br s, 1H, 1″-H]; for ^{13}C NMR (400 MHz, pyridine-d_5) spectroscopic data, see **Table 1**.

Compound **5**, (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-xylopyranosyl-(1→3)-β-D-glucopyranoside: amorphous solid; $[\alpha]_D^{28}$ −46.4° (MeOH; *c* 0.03); mp 200 °C (dec); IR ν_{max} (film) cm^{-1} 3362 (OH), 2898 (CH), 1636, 1377, 1256, 1035, 912, 838, 811; HRESI-TOFMS *m*/*z* 1057.5242 $[M + Na]^+$ (calculated for $C_{50}H_{82}O_{22}Na$, 1057.5190); ESI$^+$-MS, *m*/*z* 1057.7 (2, $[M + Na]^+$), 1017.7 (100, $[M - 18 + H]^+$), 871.6 (7, $[M - 18 - Rha + H]^+$), 739.4 (9, $[M - 18 - Xyl - Rha + H]^+$), 577.2 (3, $[M - 18 - Xyl - Rha - Glu + H]^+$), 415.2 (2, $[M - 18 - 2Glu - Xyl - Rha + H]^+$) (**Figure 3**); ^{1}H NMR (500 MHz) δ_H 0.91 [s, 3H, 18-H], 1.00 [d, 3H, J = 6.5 Hz, 27-H], 1.08 [s, 3H, 19-H], 1.35 [d, 3H, J = 7 Hz, 21-H], 1.77 [d, 3H, J = 6.5 Hz, 6″-H], 4.83 [d, 1H, J = 8 Hz, 1⁗-H], 4.89−4.88 [m, 1H, 16-H], 4.99 [d, 1H, J = 7 Hz, 1′-H], 5.01 [d, 1H, J = 5.5, 1‴-H], 5.33 [d, 1H, J = 4 Hz, 6-H], 6.34 [br s, 1H, 1″-H]; for ^{13}C NMR (400 MHz, pyridine-d_5) spectroscopic data, see **Table 1**.

RESULTS AND DISCUSSION

Structure Elucidation. Lyophilized lily bulbs were washed with *n*-pentane and extracted with ethanol and water. After the removal of the solvent, the extract was dissolved in deionized water, washed with ethyl acetate, and extracted with *n*-butanol. The organic phase was evaporated under reduced pressure and lyophilized, yielding a crude steroidal glycoside extract. The crude glycoside extract was fractionated by GPC (Sephadex LH-20) and repeated semipreparative RP-HPLC to yield compounds **1**−**5** (**Figure 2**). On the basis of ^{1}H NMR, ^{13}C NMR, 2D NMR (HMQC and HMBC), HRESI-TOFMS, and chemical analysis, including GC-MS analysis of the sugar and aglycone TMSi derivatives after acid hydrolysis, **1** and **3** were identified as (22*R*,25*R*)-spirosol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside, previously isolated from *L. brownii* var. *viridulum* (*5*), and (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside, previously isolated from *Dioscorea deltoidea* Wall. ex Griseb., *Ophiopogon planiscapus* Nakai, *L. hansonii*, and *Allium nutans* L. (*38*, *39*, *12*, *40*) (**Figure 5**). This is the first report of these compounds isolated from the bulbs of *L. longiflorum*.

Compound **2** was obtained as a white amorphous powder. The compound was positive to the Dragendorff reaction, indicative of an alkaloid (*5*). The IR spectrum showed absorption at 1732 and 3353 cm^{-1} due to the presence of an acetyl group and hydroxyl groups. HRESI-TOFMS showed a $[M + H]^+$ ion at *m*/*z* 926.5085 (calculated for $C_{47}H_{76}NO_{17}$, 926.5108). Additionally, a $[M + H + Na]^{2+}$ ion was observed at *m*/*z* 474.7520 (calculated for $C_{47}H_{76}NO_{17}Na$, 474.7500). Thus, the molecular formula was calculated as $C_{47}H_{75}NO_{17}$, suggestive of an acetylated steroidal glycoalkaloid. The aglycone was readily deduced from ^{1}H NMR, ^{13}C NMR, ESI$^+$-MS, and chemical analysis. The ^{1}H NMR spectrum showed two singlets at δ_H 1.06 and 0.88, which are indicative of tertiary methyl groups of the spirosolane skeleton. Furthermore, two doublet signals at δ_H 1.11 and 0.82 were assignable to secondary methyl groups. A quaternary carbon signal at δ_H 98.4 and olefinic carbon signals at δ_C 140.8 and 121.9 in the ^{13}C NMR spectrum are consistent with a Δ^5 spirosolane aglycone. The unsaturation between C-5 and C-6 was further substantiated by a doublet at δ_H 5.29 in the ^{1}H NMR spectrum for the H-6 signal. Upon acid hydrolysis, derivatization, and GC-MS analysis, the retention time and mass spectrum of the TMSi derivative of the aglycone were consistent with those of (22*R*,25*R*)-spirosol-5-en-3β-ol, which was prepared following the same procedure. The structure of the oligosaccharide moiety was readily deduced from ^{1}H NMR, ^{13}C NMR, HMBC, ESI$^+$-MS, and chemical analysis. Three anomeric protons were observed at δ_H 4.98, 6.24, and 5.09, which implied the presence of three saccharide residues. Coupling constants for the anomeric proton resonances suggested β-interglycosidic linkages. Additionally, the ^{13}C NMR spectrum contained three anomeric carbon signals observed at δ_C 105.6, 102.0, and 99.9, consistent with the presence of three saccharide residues. The HMBC experiment showed long-range correlations between

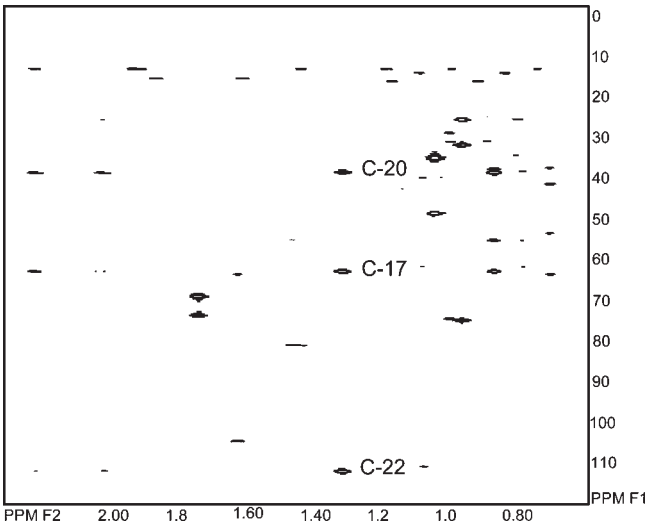

Figure 4. Partial HMBC spectrum of **4**, showing the correlation between H-21 and the carbon signals of C-20, C17, and C-22.

the anomeric proton signal at δ_H 4.98 [H-1′] and the carbon signal at δ_C 78.1 [C-3], between the anomeric proton signal at δ_H 5.09 [H-1‴] and the carbon signal at δ_C 83.3 [C-4′], and the between anomeric proton signal at δ_H 6.24 [H-1″] and the carbon signal at δ_C 77.6 [C-2′]. Upon acid hydrolysis, derivatization, and GC-MS/enantioselective GC-FID analysis, the sugars of the trisaccharide moiety were identified as D-(+)-glucose and L-(−)-rhamnose in a 2:1 ratio. The ^{1}H NMR spectrum showed a doublet integrating for three protons at δ_H 1.78, indicative of the methyl group of rhamnose. The ESI$^+$-MS mass spectrum showed the protonated molecular ion 926.6 $[M + H]^+$ and the protonated double charged sodium adduct at 474.8 $[M + H + Na]^{2+}$. Additionally, ion fragments at m/z 780.5 $[M - Rha + H]^+$, 576.4 $[M - Glu - Ac - Rha + H]^+$, and 414.3 $[M - 2Glu - Ac - Rha + H]^+$ were observed and were consistent with a trisaccharide moiety containing D-(+)-glucose, an acetylated D-(+)-glucose, and a L-(−)-rhamnose moiety (**Figure 3**). The presence of an acetyl group was shown by the IR absorption at 1732 cm^{-1}, ^{1}H NMR δ_H 2.06 (s, 3H, CO−CH$_3$), and ^{13}C NMR δ_C 170.9 and 20.8. The carbon signals corresponding to the saccharide moiety of **2** were similar to those reported for (25*R*,26*R*)-26-methoxyspirost-5-en-3*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucopyranoside, isolated from *L. speciosum* × *L. nobilissimum* (*20*). Alkaline hydrolysis of **2** with 1 N sodium hydroxide yielded **1**. Upon comparison of the ^{13}C NMR spectra of **1** and **2**, the C-6‴ and C-4‴ signals were shifted from δ_C 62.1 and 71.2 to δ_C 64.8 and 71.9, respectively. The signal for C-5‴ was shifted from δ_C 78.5 to 74.9, and all other carbon signals were similar. Upon comparison of the ^{1}H NMR spectra of **1** and **2**, the signals assignable to H$_2$-6‴ methylene protons of the terminal glucose were shifted to a lower field as compared to those of **2**. Thus, the acetyl moiety was linked to the C-6 hydroxy position of the terminal glucose unit. Accordingly, the structure of **2** was determined to be (22*R*,25*R*)-spirosol-5-en-3*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→4)]-*β*-D-glucopyranoside (**Figure 5**).

Compound **4** was obtained as a white amorphous powder. The compound was positive to Ehrlich's reaction, indicative of a furostanol saponin (*39*). The IR spectrum showed absorption at 3367 cm^{-1} due to the presence of hydroxyl groups. HRESI-TOFMS showed a $[M + Na]^+$ ion at m/z 1057.5211 (calculated for $C_{50}H_{82}O_{22}Na$, 1057.5190). Additionally, a $[M - H]^-$ ion was observed at m/z 1033.6. Thus, the molecular formula was calculated as

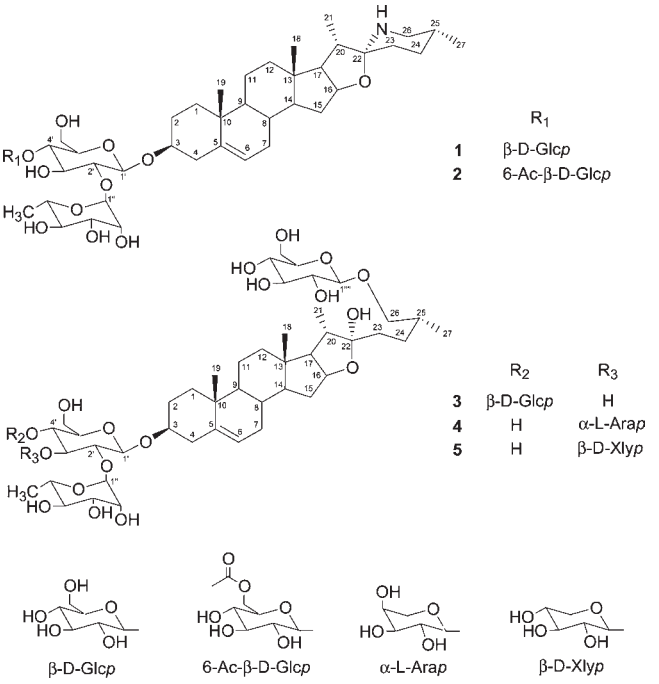

Figure 5. Structures of steroidal glycosides prepared from *L. longiflorum* bulbs.

$C_{50}H_{82}O_{22}$, consistent with a furostanol saponin. The aglycone was readily deduced from ^{1}H NMR, ^{13}C NMR, HMBC, ESI-MS, and chemical analysis. The ^{1}H NMR spectrum showed two singlets at δ_H 1.07 and 0.91, which are indicative of the tertiary methyl groups of the furostane skeleton. Furthermore, two doublet signals at δ_H 1.35 and 1.0 were assignable to secondary methyl groups. (25*R*)-26-*O*-*β*-D-glucopyranosyl-22*ξ*-methoxyfurost-5-en-3*β*,26-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-*β*-D-glucopyranoside, previously identified in *L. longiflorum* possessing antitumor activity, contains a methoxy group at the C-22 position with a −OCH$_3$ signal at δ_C 47.73, which is missing in **4**. Also, the quaternary carbon signal for C-22 had a minor upfield shift to δ_C 110.7 instead of the reported value of δ_C 112.7 for the C-22 methoxy derivative (*14*). Long-range coupling was observed between the methyl proton signal at δ_H 1.35 [H-21] and the carbon signals at δ_C 40.7 [C-20], 63.9 [C-17], and 110.7 [C-22], supporting that the shift seen in this region is consistent with a C-22 hydroxy compared to the C-22 methoxy derivative with the reported values of δ_C 40.5 [C-20], 64.2 [C-17], and 112.7 [C-22] (*14*) (**Figure 4**). The carbon peaks from C-5 to C-27 were similar to those reported for (25*R*,*S*)-26-*O*-*β*-D-glucopyranosyl-furost-5-en-3*β*,22α,26-triol 3-*O*-*β*-D-galactopyranosyl-(1→2)-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside, isolated from *Tribulus terrestris* L., which suggests the presence of an α-hydroxy group at the C-22 position of the furostane skeleton (*41*). Consistent with (25*R*)-26-*O*-*β*-D-glucopyranosyl-furost-5-en-3*β*,22α-triol 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranoside, isolated from the rhizomes of *Tupistra chinensis* Bak., the peak of C-25 at δ_C 34.3 as compared to δ_C 34.42 suggests an *R* configuration (*42*). Olefinic carbon signals at δ_C 140.7 and 121.9 in the ^{13}C NMR spectrum were consistent with a Δ^5 furostane skeleton. The unsaturation between C-5 and C-6 is further substantiated by an olefinic proton signal at δ_H 5.33 in the ^{1}H NMR spectrum for H-6. Upon acid hydrolysis, derivatization, and GC-MS analysis, the retention time and mass spectrum of the TMSi derivative of the aglycone were consistent with that of (25*R*)-spirost-5-en-3*β*-ol, which was prepared following the same procedure. The structure

of the oligosaccharide moiety was readily deduced from ^{1}H NMR, ^{13}C NMR, HMBC, ESI$^+$-MS, and chemical analysis. All four sugars of **4** have carbon NMR values similar to those reported for (25*R*)-26-*O*-β-D-glucopyranosyl-22ξ-methoxy-furost-5-en-3β,26-diol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-glucopyranoside (*14*). The ^{1}H NMR spectrum contained four anomeric proton signals observed at δ_H 6.30, 4.99, 4.92, and 4.84. The coupling constants of the anomeric proton resonances suggested β-interglycosidic linkages. The carbon signals were at δ_C 105.6, 105.0, 102.5, and 99.9, which is consistent with the presence of four saccharide residues. The HMBC experiment showed long-range correlations between the anomeric proton signal at δ_H 4.99 [H-1′] and the carbon signal at δ_C 77.7 [C-3], between the anomeric proton signal at δ_H 4.92 [H-1‴] and the carbon signal at δ_C 88 [C-3′], and between the anomeric proton signal at δ_H 6.30 [H-1″] and the carbon signal at δ_C 78 [C-2′]. Upon acid hydrolysis, derivatization, and GC-MS/enantioselective GC-FID analysis, the sugars were identified as D-(+)-glucose, L-(−)-rhamnose, and L-(−)-arabinose in a 2:1:1 ratio. The ^{1}H NMR spectrum showed a doublet integrating for three protons at δ_H 1.76, indicative of the methyl group of rhamnose. The ESI$^+$-MS mass spectrum showed a base ion peak at 1017.7 [M − 18 + H]$^+$ and the sodium adduct at 1057.7 [M + Na]$^+$. Additionally, ion fragments at *m*/*z* 871.6 [M − 18 − Rha + H]$^+$, 739.4 [M − 18 − Ara − Rha + H]$^+$, 577.2 [M − 18 − Ara − Rha − Glu + H]$^+$, and 415.2 [M − 18 − 2Glu − Ara − Rha + H]$^+$ were observed and were consistent with **4** being bidesmodic with the trisaccharide moiety at the C-3 position containing D-(+)-glucose, L-(−)-arabinose, and L-(−)-rhamnose, and a D-(+)-glucose moiety at the C-26 position, indicative of a furostanol saponin (**Figure 3**). Accordingly, the structure of **4** was determined to be (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β, 22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-glucopyranoside (**Figure 5**).

Compound **5** was determined to be an isomer of compound **4**. The structure of the oligosaccharide moiety was readily deduced from ^{1}H NMR, ^{13}C NMR, HMBC, ESI$^+$-MS, and chemical analysis. **5** showed carbon NMR peaks similar to those of compounds **3** and **4**, except for the 3‴ sugar peaks. The ^{1}H NMR spectrum contained four anomeric proton signals observed at δ_H 6.34, 5.01, 4.99, and 4.89. The ^{13}C NMR spectrum contained four anomeric carbon signals observed at δ_C 105.4, 105.0, 102.4, and 100.0. The HMBC experiment showed long-range correlations between the anomeric proton signal at δ_H 4.99 [H-1′] and the carbon signal at δ_C 77.4 [C-3], between the anomeric proton signal at δ_H 5.01 [H-1‴] and the carbon signal at δ_C 88.2 [C-3′], and between the anomeric proton signal at δ_H 6.34 [H-1″] and the carbon signal at δ_C 78 [C-2′]. Upon acid hydrolysis, derivatization, and GC-MS/enantioselective GC-FID analysis, the sugars were identified as D-(+)-glucose, L-(−)-rhamnose, and L-(−)-xylose in a 2:1:1 ratio. Accordingly, the structure of **5** was determined to be (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-xylopyranosyl-(1→3)-β-D-glucopyranoside (**Figure 5**).

Although saponins are widely distributed secondary metabolites and have been identified in over 100 plant families and in marine organisms such as starfish and sea cucumber (*43*), thus far, steroidal glycoalkaloids are limited to members of the families Solanaceae and Liliaceae (*44*). Steroidal saponins with acetylation of saccharide residues have been identified; however, the biological significance of acetylation of these compounds is unclear. Some genotypes of *Solanum chacoense*, a wild potato species that is resistant to *Leptinotarsa decemlineata*, contain glycoalkaloids that are acetylated at the C-23 position of the steroid aglycone (*45*), but we are unaware of any steroidal glycoalkaloids that contain naturally occurring acetylated saccharides. Interestingly, the presence or absence of the acetyl moiety of the *S. chacoense* glycoalkaloids markedly affected resistance to foliar feeding of both adults and larvae of *L. decemlineata*. Differences in acetylation of the terminal glucose of the trisaccharide moiety of **1** and **2** may also play a biological role in *L. longiflorum*. Work is currently underway to compare the antifungal activity of these compounds against common lily pathogens by quantifying the ability of each pathogen to cleave the sugar residues of **1** and **2**.

The furostanol saponins **3**−**5** are similar in structure except for the terminal monosaccharide residues and interglycosidic linkages. **3** has a hexose as the terminal sugar linked via the C-4′ carbon of the inner glucose, whereas both **4** and **5** contain a pentose as the terminal sugar linked via the C-3′ carbon of the inner glucose. In fact, differences in oligosaccharide composition and interglycosidic linkages have been shown to affect the biological activity of steroidal saponins possessing the same aglycone moiety (*46*). Differences in oligosaccharide composition and interglycosidic linkages in **3**−**5** may also play a role in the biology of these compounds in *L. longiflorum*.

In the present work, a new acetylated steroidal glycoalkaloid, (22*R*,25*R*)-spirosol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside, and two new furostanol saponins, (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→3)-β-D-glucopyranoside and (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-xylopyranosyl-(1→3)-β-D-glucopyranoside, from the bulbs of *L. longiflorum* have been isolated and their structures elucidated. Additionally, a known steroidal glycoalkaloid, (22*R*,25*R*)-spirosol-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside, and a known furostanol saponin, (25*R*)-26-*O*-(β-D-glucopyranosyl)-furost-5-en-3β,22α,26-triol 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside, were isolated for the first time from the bulbs of *L. longiflorum*. On the basis of our identification of several new steroidal glycosides of *L. longiflorum*, we are studying the distribution of these compounds in all of the organs of the plant and their potential roles in plant−pathogen interactions. The extraction and purification techniques reported above are also currently being used for the production of sufficient quantities of pure compounds to study the effects of steroidal glycosides on human health and nutrition.

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