Steroidal and Pregnane Glycosides from the Rhizomes of *Tacca chantrieri*

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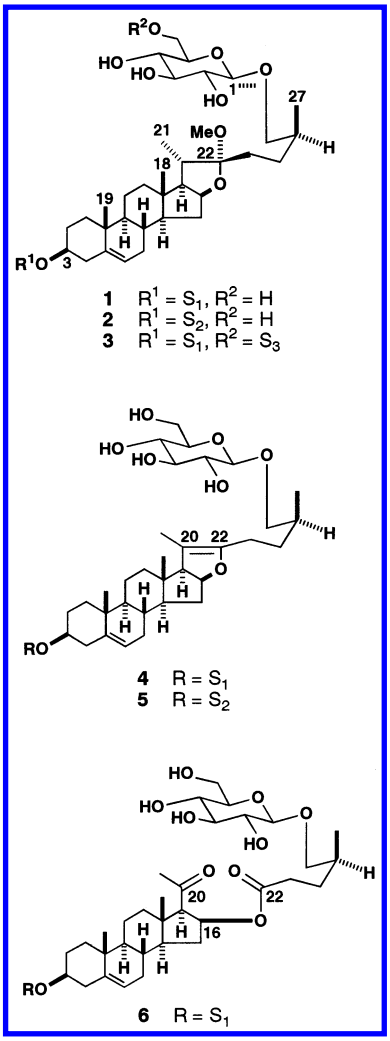
The rhizomes of *Tacca chantrieri* have been analyzed as part of a systematic study on saponin constituents of medicinal plants. This has resulted in the isolation of three new bisdesmosidic furostanol saponins (**1**−**3**), two new bisdesmosidic pseudofurostanol saponins (**4**, **5**), and two new pregnane glycosides (**6**, **7**). Their structures were determined on the basis of extensive spectroscopic studies and a few chemical transformations.

In a continuation of our study on saponin constituents of medicinal plants, we have examined the saponin-enriched fraction prepared from the MeOH extract of the rhizomes of *Tacca chantrieri* André (Taccaceae). This plant is indigenous to areas in southeast China and has been used in traditional Chinese medicine for the treatment of gastric ulcer, enteritis, and hepatitis.[1] Previously, we reported the structural characterization of two new diarylheptanoids and seven diarylheptanoid glucosides isolated from *T. chantrieri* rhizomes and their cytotoxic activities against HL-60 leukemia cells, HSC-2 cells, and HGF.[2] Further phytochemical analysis has been carried out on the rhizomes, with particular attention to the steroidal glycoside constituents, and has resulted in the isolation of three new bisdesmosidic furostanol saponins (**1**−**3**), two new bisdesmosidic pseudofurostanol saponins (**4**, **5**), and two new pregnane glycosides (**6**, **7**), among which **1** is the main secondary metabolite with a yield of 0.14% based on dry weight of the plant material. This paper reports the structural determination of the new glycosides.



Results and Discussion

The fractions enriched with steroidal glycosides, prepared by passing the MeOH extract of *T. chantrieri* rhizomes through a porous-polymer polystyrene resin (Diaion HP-20) column eluted with MeOH−H_2O mixtures followed by MeOH, were subjected to multiple chromatographic steps over silica gel and octadecylsilanized (ODS) silica gel, giving compounds **1** (0.14%), **2** (0.0027%), **3** (0.00090%), **4** (0.0018%), **5** (0.00029%), **6** (0.00082%), and **7** (0.00041%).

Compound **1** was obtained as an amorphous solid. The negative-ion FABMS of **1** showed an $[M - H]^-$ ion at *m/z* 1223, compatible with the molecular formula $C_{58}H_{96}O_{27}$, which was confirmed by data from the ^{13}C NMR spectrum, with a total of 58 carbon signals, and the results of elemental analysis. The ^1H NMR spectrum showed signals for four steroid methyl groups at δ 1.17 (3H, d, J = 6.9 Hz), 1.05 (3H, d, J = 6.7 Hz), 1.04 (3H, s), and 0.82 (3H, s), an olefinic proton at δ 5.33 (1H, br d, J = 4.6 Hz), and a methoxyl group at δ 3.26 (3H, s), as well as signals for five anomeric protons at δ 5.81 (1H, br s), 5.75 (1H, br s), 5.24 (1H, d, J = 7.8 Hz), 4.89 (1H, d, J = 7.8 Hz), and 4.85 (1H, d, J = 7.7 Hz). The two methyl carbon signals at δ 18.6 and 18.3 and their corresponding proton signals at δ 1.76 (3H, d, J = 6.2 Hz) and 1.69 (3H, d, J = 6.2 Hz) indicated that **1** had two deoxy sugars. When **1** was submitted to acid hydrolysis with 1 M HCl in dioxane−H_2O (1:1), it was hydrolyzed to yield a sapogenin identified as (25*S*)-spirost-5-en-3β-ol.[3] The monosaccharides of the acidic hydrolysate of **1** were identified as D-glucose and L-rhamnose on the basis of the HPLC analysis, which was performed on an aminopropyl-bonded silica gel column, with detection being carried out by using a combination of refractive index (RI) and optical rotation (OR) detectors. The above ^1H NMR and chemical data, together with an acetalic carbon signal at δ 112.7 in the ^{13}C NMR spectrum[3] and a positive coloration with Ehrlich's reagent,[4,5] indicated

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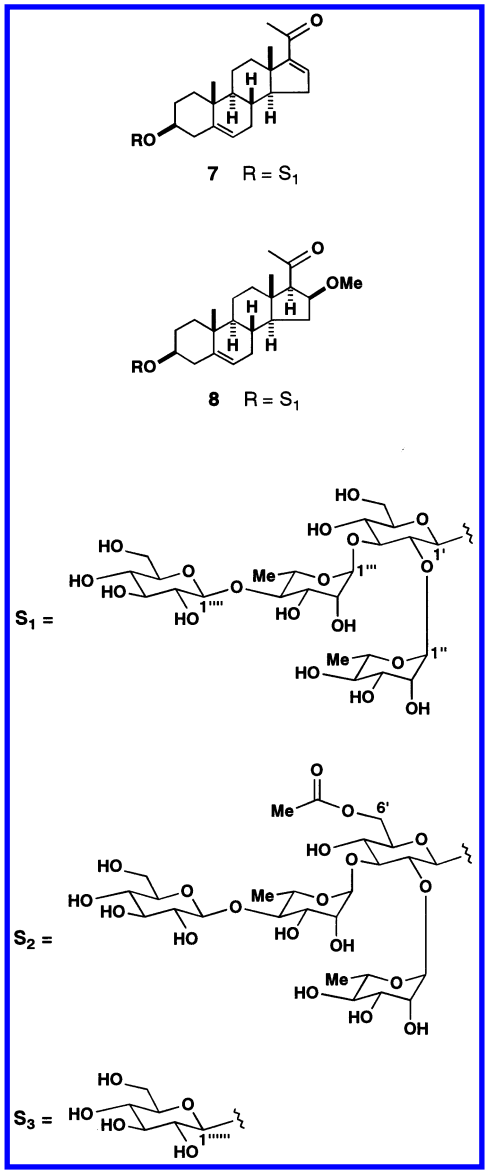

1 to be a saponin based upon (25*S*)-22-methoxyfurost-5-ene-3*β*,26-diol[6] with up to five monosaccharides. Sequential assignments of the signals from H-1 to H_2-6 or Me-6 of each monosaccharide, including their multiplet patterns and coupling constants, in the ^{1}H NMR spectrum of **1**, were established by analysis of the ^{1}H−^{1}H COSY and 2D HOHAHA spectra (Table 1). Then, the HMQC spectrum was applied to associate the protons with the corresponding one-bond coupled carbon resonances. Comparison of the carbon chemical shift thus assigned with those of the reference methyl glycosides,[3,7] taking into account the known effects of *O*-glycosylation, indicated that **1** contained two terminal *β*-D-glucopyranosyl units, a terminal α-L-rhamnopyranosyl unit, a C-4 substituted α-L-rhamnopyranosyl unit, and a C-2 and C-3 disubstituted *β*-D-glucopyranosyl unit. The *β*-orientations of the anomeric centers of the three glucosyl moieties were supported by the relatively large *J* values of their anomeric protons (*J* = 7.7−7.8 Hz). For the rhamnosyl moieties, the large $^1J_{C,H}$ values (166.1 and 172.1 Hz) and three-bond coupled strong HMBC correlations from the anomeric proton to the C-3 and C-5 carbons (the dihedral angles between H-1 and C-3 and between H-1 and C-5 about 180°) indicated that each anomeric proton was equatorial, thus possessing an α-pyranoid anomeric form.[8] In the HMBC spectrum, a correlation peak between the signals at δ_H 4.85 (H-1 of terminal glucosyl) and δ_C 74.9 (C-26 of aglycon) implied that one glucose unit was attached at C-26 of the aglycon, which is a structural feature most frequently encountered in the plant furostanol saponins.[3] Consequently, a tetraglycoside was assumed to be located at C-3 of the aglycon. Further HMBC correlations from *δ* 5.81 (H-1 of terminal rhamnosyl) to *δ* 78.6 (C-2 of 2,3-disubstituted glucosyl), *δ* 5.24 (H-1 of terminal glucosyl) to *δ* 84.4 (C-4 of substituted rhamnosyl), *δ* 5.75 (H-1 of substituted rhamnosyl) to *δ* 86.2 (C-3 of 2,3-disubstituted glucosyl), and *δ* 4.89 (H-1 of 2,3-disubstituted glucosyl) to *δ* 77.8 (C-3 of aglycon) confirmed the tetraglycoside sequence as rhamnosyl-(1→2)-[glucosyl-(1→4)-rhamnosyl-(1→3)]-glucosyl, which was attached at C-3 of the aglycon. An NOE correlation from the methoxyl proton signal at *δ* 3.26 to the H-16 proton signal at *δ* 4.45 was consistent with the C-22α configuration. Thus, **1** was determined to be (25*S*)-26-[(*β*-D-glucopyranosyl)oxy]-22α-methoxyfurost-5-en-3*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Table 1. ^{1}H NMR Chemical Shift Assignments for the Glycoside Moieties of Compound **1** in C_5D_5N

position		^{1}H	*J* (Hz)
Glc	1′	4.89, d	7.8
	2′	4.05, dd	8.7, 7.8
	3′	4.17, dd	9.1, 8.7
	4′	4.08, dd	9.1, 9.1
	5′	3.78, ddd	9.1, 4.5, 2.3
	6′a	4.44, dd	11.8, 2.3
	b	4.36, dd	11.8, 4.5
Rha	1″	5.81, br s	
	2″	4.73, br d	3.2
	3″	4.51, dd	9.3, 3.2
	4″	4.31, dd	9.7, 9.3
	5″	4.87, dq	9.7, 6.2
	6″	1.76, d	6.2
Rha	1‴	5.75, br s	
	2‴	4.80, br d	3.3
	3‴	4.57, dd	9.1, 3.3
	4‴	4.43, dd	9.1, 9.1
	5‴	4.83, dq	9.1, 6.2
	6‴	1.69, d	6.2
Glc	1⁗	5.24, d	7.8
	2⁗	4.09, dd	8.8, 7.8
	3⁗	4.21, dd	9.0, 8.8
	4⁗	4.27, dd	9.4, 9.0
	5⁗	3.78, ddd	9.4, 4.9, 2.3
	6⁗a	4.41, dd	11.5, 2.3
	b	4.34, dd	11.5, 4.9
Glc	1′′′′′	4.85, d	7.7
	2′′′′′	4.05, dd	8.7, 7.7
	3′′′′′	4.25, dd	9.1, 8.7
	4′′′′′	4.22, dd	9.1, 9.1
	5′′′′′	3.97, ddd	9.1, 5.3, 2.5
	6′′′′′a	4.57, dd	11.8, 2.5
	b	4.39, dd	11.8, 5.3

Compound **2** was shown to have the molecular formula $C_{60}H_{98}O_{28}$ on the basis of the negative-ion FABMS (*m*/*z* 1265 [M − H]$^-$), ^{13}C NMR spectrum (60 signals), and elemental analysis. The ^{1}H and ^{13}C NMR spectra of **2** were quite similar to those of **1**. In addition, the presence of an acetyl group in **2** was shown by the IR (1730 cm^{-1}), ^{1}H NMR [*δ* 2.01 (3H, s)], and ^{13}C NMR [*δ* 170.8 (C=O) and 20.7 (Me)] spectra. Alkaline treatment of **2** with 3% NaOMe in MeOH furnished **1**, indicating that **2** was a monoacetate of **1**. In the HMBC spectrum, correlation peaks were observed from the H_2-6 protons due to the 2,3-disubstituted inner glucosyl moiety at *δ* 4.86 and 4.72 to the acetyl carbonyl carbon. The structure of **2** was assigned as (25*S*)-26-[(*β*-D-glucopyranosyl)oxy]-22α-methoxyfurost-5-en-3*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-

(1→4)-α-L-rhamnopyranosyl-(1→3)]-6-*O*-acetyl-*β*-D-glucopyranoside.

Compound **3** analyzed as $C_{64}H_{106}O_{32}$ by combined negative-ion FABMS (*m*/*z* 1385 $[M - H]^-$), ^{13}C NMR (64 signals), and elemental analysis. The deduced molecular formula was higher by $C_6H_{10}O_5$ than that of **1**, and the ^1H NMR spectrum showed signals for six anomeric protons at *δ* 5.80 (1H, br s), 5.74 (1H, br s), 5.23 (1H, d, *J* = 7.8 Hz), 5.11 (1H, d, *J* = 7.9 Hz), 4.88 (1H, d, *J* = 7.9 Hz), and 4.77 (1H, d, *J* = 7.8 Hz), along with signals for four steroid methyl protons at *δ* 1.18 (3H, d, *J* = 6.8 Hz), 1.04 (3H, d, *J* = 6.7 Hz), 1.03 (3H, s), and 0.83 (3H, s). Acid hydrolysis of **3** with 1 M HCl resulted in the production of (25*S*)-spirost-5-en-3*β*-ol, D-glucose, and L-rhamnose. On comparison of the whole ^{13}C NMR spectrum of **3** with that of **1**, a set of six signals corresponding to a terminal *β*-D-glucopyranosyl moiety were observed at *δ* 105.4 (CH), 75.1 (CH), 78.4 (CH), 71.5 (CH), 78.4 (CH), and 62.7 (CH_2), and the signals due to C-6 of the glucosyl moiety attached at C-26 of the aglycon and its neighboring carbons varied, while all other signals remained almost unaffected. The HMBC spectrum showed a long-range correlation peak from the anomeric proton of the terminal glucosyl group at *δ* 5.11 to C-6 of the inner glucosyl unit at *δ* 70.1, whose anomeric proton at *δ* 4.77, in turn, exhibited a correlation with C-26 of the aglycon at *δ* 74.8. The structure of **3** was thus formulated as (25*S*)-26-[(*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl)oxy]-22α-methoxyfurost-5-en-3*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Compound **4** was deduced as $C_{57}H_{92}O_{26}$ by the positive-ion FABMS (*m*/*z* 1215 $[M + Na]^+$), ^{13}C NMR (57 signals), and elemental analysis and was suggested to be a furostanol saponin closely related to **1** from its spectral data. It differed from **1** in the presence of one more olefinic functionality [δ_C 152.4 (C) and 103.5 (C)] in addition to the 5(6)-ene group. Furthermore, the Me-21 methyl doublet signal observed at *δ* 1.17 (*J* = 6.9 Hz) in the ^1H NMR spectrum of **1** was absent from **4**, but was replaced by a methyl singlet at *δ* 1.63. These data were suggestive of **4** being the corresponding $\Delta^{20(22)}$-furostanol saponin of **1**. This was confirmed by the fact that the peracetate (**4a**) of **4** agreed with the product obtained by treatment of **1** with Ac_2O in pyridine at 110 °C for 2.5 h, during which dehydration at C-20 and C-22, as well as the introduction of an acetyl group to all the hydroxyl groups of the sugar moiety, occurred to **1**. The structure of **4** was established as (25*S*)-26-[(*β*-D-glucopyranosyl)oxy]furosta-5,20(22)-dien-3*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranoside.

Compound **5** ($C_{59}H_{94}O_{27}$) was obtained as an amorphous solid. Its ^1H and ^{13}C NMR spectral properties were in good agreement with those of **4**, except for the presence of the signals for one acetyl group [δ_H 2.01 (3H, s); δ_C 170.8 (C=O) and 20.7 (Me)]. Alkaline treatment of **5** with 3% NaOMe in MeOH furnished **4**, and the HMBC spectrum showed correlation peaks between the signals of the H_2-6 protons due to the 2,3-disubstituted inner glucosyl moiety at *δ* 4.85 and 4.72 and the acetyl carbonyl carbon. The structure of **5** was shown to be (25*S*)-26-[(*β*-D-glucopyranosyl)oxy]-22α-methoxyfurosta-5,20(22)-dien-3*β*-yl *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-6-*O*-acetyl-*β*-D-glucopyranoside.

Compound **6** was deduced as $C_{57}H_{92}O_{28}$ from its positive-ion FABMS (*m*/*z* 1247 $[M + Na]^+$), ^{13}C NMR (57 signals), and elemental analysis data. The ^1H and ^{13}C NMR spectra of **6** revealed the presence of four methyl groups on the aglycon and five monosaccharides and were essentially analogous to those of **4**. However, **6** was different from **4** in the lack of the signals assignable to the tetrasubstituted olefinic group forming the bond between C-20 and C-22 and in the presence of a ketone carbonyl carbon signal at *δ* 205.5 and an ester carbonyl carbon signal at *δ* 173.3. The HMBC spectrum exhibited correlations of the ketone carbonyl carbon with H-17 at *δ* 2.50 (1H, d, *J* = 7.6 Hz) and Me-21 at *δ* 2.14 (3H, s) and of the ester carbonyl carbon with H-16 at *δ* 5.67 (1H, m) and H_2-23 at *δ* 2.44 and 2.36, indicating that C-20 and C-22 of **6** were the carbonyl groups, instead of the olefinic group in **4**. The structure of **6**, including the absolute configuration at C-25, was confirmed by the following chemical conversion. When the C-20 and C-22 bond of **4a** was oxidatively cleaved by treating it with CrO_3 in AcOH at room temperature for 2 h, the resultant product was completely consistent with the peracetyl derivative of **6** (**6a**). Accordingly, **6** was characterized as 16*β*-[[(4*S*)-5-(*β*-D-glucopyranosyloxy)-4-methyl-1-oxopentyl]oxy]-3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranosyl)oxy]pregn-5-en-20-one.

Compound **7** had a molecular formula of $C_{45}H_{70}O_{20}$, established by analysis of the positive-ion FABMS (*m*/*z* 953 $[M + Na]^+$) and ^{13}C NMR spectrum (45 signals) and by elemental analysis. The ^1H NMR spectrum of **7** displayed two three-proton singlet signals at *δ* 1.04 and 0.93, indicating the presence of two angular methyl groups, and a methyl singlet at *δ* 2.25 attached to a deshielding moiety, as well as four anomeric proton signals at *δ* 5.80 (1H, br s), 5.74 (1H, br s), 5.24 (1H, d, *J* = 7.8 Hz), and 4.88 (1H, d, *J* = 7.8 Hz). The existence of an α,*β*-unsaturated carbonyl group was verified by the IR (1660 cm^{-1}), UV [239.8 nm (log *ε* 3.84)], and ^{13}C NMR [*δ* 196.3 (C=O), 155.2 (C), and 144.7 (CH)] spectra. These spectral data and comparison with those of previously reported compounds allowed identification of the aglycon of **7** as 3*β*-hydroxypregna-5,16-dien-20-one.[9] The ^1H and ^{13}C NMR shifts of the tetraglycoside moiety linked to C-3 of the pregnane were superimposable on those of **1**, **3**, **4**, and **6**. Furthermore, alkaline treatment of **6** afforded **7** and 16-methoxy-3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranosyl)oxy]pregn-5-en-20-one (**8**). All of these data were consistent with the structure 3*β*-[(*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[*O*-*β*-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)]-*β*-D-glucopyranosyl)oxy]pregna-5,16-dien-20-one, which was assigned to **7**.

Compounds **1**−**7** are new steroidal glycosides. The known naturally occurring 22,26-hydroxyfurostanol saponins exclusively exist in the form of glycoside, bearing a monosaccharide at C-26.[3] The monosaccharide in the furostanol saponins reported thus far is limited to be *β*-D-glucopyranose except for one furostanol saponin from *Dracaena afromontana*, which has an α-L-rhamnopyranosyl group at C-26.[10] Compound **3** is distinctive in carrying a diglucosyl group, *O*-glucosyl-(1→6)-glucosyl, in place of a monoglucosyl unit at C-26. A few compounds related to **6** have been isolated;[11−13] however, the C-25 configuration of them is not clearly presented in all the reports. In this investigation, we unequivocally determined the C-25 configuration of **6** as *S* by a chemical correlation method. Compound **6** could be defined as a pregnane glycoside rather than a furostanol saponin.[11−13] Compounds **1**−**7** did not show apparent cytotoxic activity against HL-60 cells at a sample concentration of 10 *μ*g/mL, although some furostanol sa-

ponins and pregnane glycosides have been reported to be cytotoxic.[11−14]

Experimental Section

General Experimental Procedures. The instruments and experimental conditions, as well as the plant material used, were the same as described in the previous paper.[2]

Extraction and Isolation. The plant material (dry wt, 7.3 kg) was extracted with hot MeOH (3 L × 2). The MeOH extract (630 g) was passed through a Diaion HP-20 (2.2 kg, Mitsubishi-Kasei, Tokyo, Japan) column, eluting with 30% MeOH, 50% MeOH, MeOH, EtOH, and EtOAc (4 L of each). The 50% MeOH eluate portion (70 g) was chromatographed on a silica gel (1.2 kg, 200−400 mesh, Fuji-Silysia Chemical, Aichi, Japan) column, eluting with a stepwise gradient mixture of $CHCl_3$−MeOH (9:1, 4:1, 3:1, 2:1, and 1:1; 4 L of each) and finally with MeOH (4 L). The $CHCl_3$−MeOH (2:1) eluate portion (15 g) was subjected to column chromatography on silica gel (400 g), eluting with $CHCl_3$−MeOH−H_2O (20:10:1, 2 L) to give **5** (21 mg, 0.00029%). The $CHCl_3$−MeOH (1:1) and MeOH eluate portions were combined (22 g), which was then chromatographed on ODS silica gel (500 g, 75 μm, Nacalai Tesque, Kyoto, Japan) with MeOH−H_2O (2:1, 2 L) and on ODS silica gel (400 g) with MeCN−H_2O (2:5, 1.5 L; 1:2, 2 L) to give **4** (130 mg, 0.0018%). The MeOH eluate portion (115 g) was chromatographed on silica gel (1.2 kg), eluting with a stepwise gradient mixture of $CHCl_3$−MeOH (9:1, 4:1, 3:1, 2:1, and 1:1; 4 L of each) and finally with MeOH (4 L). The $CHCl_3$−MeOH (1:1) and MeOH eluate portions were combined (40 g), which was further subjected to column chromatography on silica gel (800 g) eluting with $CHCl_3$−MeOH−H_2O (30:10:1; 20:10:1, 3 L of each) and on ODS silica gel (500 g) with MeOH−H_2O (2:1, 2 L; 4:1, 3 L) and MeCN−H_2O (1:2, 1.5 L) to give **1** (10 g, 0.14%), **2** (200 mg, 0.0027%), **3** (66 mg, 0.00090%), **6** (60 mg, 0.00082%), and **7** (30 mg, 0.00041%).

Compound 1: amorphous solid; $[\alpha]_D^{25}$ −82.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3400 (OH), 2930 (CH), 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.81 (1H, br s, H-1″), 5.75 (1H, br s, H-1‴), 5.33 (1H, br d, J = 4.6 Hz, H-6), 5.24 (1H, d, J = 7.8 Hz, H-1⁗), 4.89 (1H, d, J = 7.8 Hz, H-1′), 4.85 (1H, d, J = 7.7 Hz, H-1⁗′), 4.45 (1H, m, H-16), 3.91 (1H, m, $W_{1/2}$ = 26.3 Hz, H-3), 3.26 (3H, s, OMe), 1.76 (3H, d, J = 6.2 Hz, Me-6″), 1.69 (3H, d, J = 6.2 Hz, Me-6‴), 1.17 (3H, d, J = 6.9 Hz, Me-21), 1.05 (3H, d, J = 6.7 Hz, Me-27), 1.04 (3H, s, Me-19), 0.82 (3H, s, Me-18); ^{13}C NMR, see Table 2; FABMS (negative mode) *m*/*z* 1223 $[M - H]^-$; *anal*. C 55.60%, H 8.14%, calcd for $C_{58}H_{96}O_{27}$·3/2H_2O, C 55.62%, H 7.97%.

Acid Hydrolysis of 1. A solution of **1** (10 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 95 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and chromatographed on silica gel (15 g) eluting with $CHCl_3$−MeOH (9:1 to 1:1, 100 mL of each) to give an aglycon [(25*S*)-spirost-5-en-3β-ol] (3.0 mg) and a sugar fraction (2.7 mg). The sugar fraction was dissolved in H_2O (1 mL) and passed through a Sep-pak C_{18} cartridge (Waters, Milford, MA), which was then analyzed by HPLC under the following conditions: column, Kaseisorb LC NH_2-60-5 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Tokyo-Kasei, Tokyo, Japan); solvent, MeCN−H_2O (3:1); flow rate, 0.8 mL/min; detection, RI and OR. Identification of D-glucose and L-rhamnose present in the sugar fraction was carried out by comparing their retention times and optical rotations with those of authentic samples: t_R (min) 8.1 (L-rhamnose, negative optical rotation); 12.6 (D-glucose, positive optical rotation).

Compound 2: amorphous solid; $[\alpha]_D^{25}$ −106.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (KBr) ν_{max} 3400 (OH), 2930 (CH), 1730 (C=O), 1040 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.82 (1H, br s, H-1″), 5.76 (1H, br s, H-1‴), 5.32 (1H, br d, J = 4.7 Hz, H-6), 5.28 (1H, d, J = 7.8 Hz, H-1⁗), 4.87 (1H, d, J = 7.6 Hz, H-1′), 4.86 and 4.72 (each 1H, m, H_2-6′), 4.85 (1H, d, J = 7.8 Hz, H-1⁗′), 4.46 (1H, m, H-16), 3.89 (1H, m, $W_{1/2}$ = 24.6 Hz, H-3), 3.26 (3H, s, OMe), 2.01 (3H, s, Ac), 1.75 (3H, d, J = 6.2 Hz, Me-6″), 1.67 (3H, d, J = 6.2 Hz, Me-6‴), 1.17 (3H, d, J = 6.9 Hz, Me-21), 1.05 (3H, d, J = 6.7 Hz, Me-27), 1.02 (3H, s, Me-19), 0.81 (3H, s, Me-18); ^{13}C NMR, see Table 1; FABMS (negative mode) *m*/*z* 1265 $[M - H]^-$; *anal*. C 55.87%, H 8.20%, calcd for $C_{60}H_{98}O_{28}$·H_2O, C 56.06%, H 7.86%.

Table 2. ^{13}C NMR Chemical Shift Assignments of Compounds **1**−**7** in C_5D_5N

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	37.5	37.5	37.4	37.5	37.6	37.4	37.3
2	30.0	30.1	30.0	30.0	30.1	30.0	30.0
3	77.8	78.4	77.8	77.7	78.4	77.8	77.8
4	38.6	38.7	38.6	38.6	38.7	38.6	38.7
5	140.8	140.8	140.7	140.7	140.8	140.9	141.2
6	121.8	121.8	121.8	121.8	121.8	121.6	121.6
7	32.2	32.2	32.2	32.4	32.4	31.9	31.8
8	31.6	31.6	31.6	31.4	31.4	31.0	30.3
9	50.2	50.3	50.2	50.2	50.3	50.4	50.7
10	37.1	37.1	37.1	37.1	37.1	37.0	37.1
11	21.0	21.0	21.0	21.2	21.2	20.6	20.9
12	39.7	39.7	39.7	39.6	39.6	38.1	35.1
13	40.8	40.8	40.8	43.4	43.4	42.3	46.3
14	56.5	56.5	56.5	54.9	54.9	54.0	56.4
15	32.3	32.3	32.3	34.5	34.5	35.5	32.3
16	81.3	81.3	81.3	84.5	84.5	74.7	144.7
17	64.1	64.1	64.2	64.5	64.5	66.6	155.2
18	16.3	16.3	16.4	14.1	14.1	13.7	15.9
19	19.3	19.3	19.3	19.4	19.4	19.4	19.2
20	40.4	40.4	40.5	103.5	103.5	205.5	196.3
21	16.2	16.2	16.2	11.8	11.8	30.5	27.1
22	112.7	112.7	112.7	152.4	152.4	173.3	
23	30.9	30.9	31.0	31.4	31.4	32.2	
24	28.1	28.1	28.1	23.6	23.6	29.0	
25	34.4	34.4	34.3	33.7	33.7	33.5	
26	74.9	74.9	74.8	75.2	75.2	74.7	
27	17.5	17.5	17.5	17.1	17.1	16.9	
OMe	47.3	47.3	47.4				
1′	99.8	100.1	99.8	99.8	100.1	99.8	99.8
2′	78.6	78.6	78.6	78.6	78.6	78.6	78.6
3′	86.2	85.6	86.2	86.2	85.6	86.3	86.2
4′	69.7	69.9	69.7	69.7	69.9	69.7	69.7
5′	78.0	74.7	78.0	78.1	74.7	78.1	78.0
6′	62.2	64.1	62.1	62.1	64.2	62.2	62.2
1″	102.6	102.7	102.6	102.6	102.7	102.6	102.6
2″	72.5	72.4	72.3	72.5	72.4	72.5	72.5
3″	72.8	72.7	72.7	72.8	72.7	72.8	72.8
4″	73.8	73.7	73.7	73.8	73.8	73.8	73.8
5″	69.9	70.0	69.9	69.9	70.0	69.9	69.9
6″	18.6	18.6	18.6	18.7	18.6	18.7	18.6
1‴	103.1	103.2	103.1	103.1	103.2	103.1	103.1
2‴	72.0	72.0	72.0	72.0	72.0	72.1	72.0
3‴	72.3	72.4	72.4	72.4	72.4	72.4	72.4
4‴	84.4	84.4	84.3	84.5	84.5	84.4	84.4
5‴	68.7	68.7	68.7	68.7	68.7	68.7	68.7
6‴	18.3	18.2	18.2	18.3	18.2	18.3	18.3
1⁗	106.5	106.5	106.4	106.5	106.5	106.5	106.5
2⁗	76.4	76.4	76.4	76.4	76.4	76.4	76.4
3⁗	78.5	78.6	78.5	78.6	78.6	78.5	78.6
4⁗	71.4	71.4	71.4	71.4	71.4	71.4	71.4
5⁗	78.4	78.4	78.4	78.4	78.4	78.4	78.4
6⁗	62.5	62.5	62.5	62.5	62.5	62.6	62.6
1⁗′	105.0	105.1	104.8	105.2	105.1	105.0	
2⁗′	75.2	75.2	75.0	75.2	75.2	75.2	
3⁗′	78.6	78.6	78.5	78.6	78.6	78.6	
4⁗′	71.7	71.7	71.6	71.7	71.7	71.7	
5⁗′	78.5	78.5	77.2	78.5	78.4	78.6	
6⁗′	62.9	62.9	70.1	62.8	62.8	62.8	
1⁗″			105.4				
2⁗″			75.1				
3⁗″			78.4				
4⁗″			71.5				
5⁗″			78.4				
6⁗″			62.7				
Ac		170.8			170.8		
		20.7			20.7		

Alkaline Methanolysis of 2. Compound **2** (12.0 mg) was treated with 3% NaOMe in MeOH (5 mL) at room temperature for 1 h. The reaction mixture was neutralized by passage

through an Amberlite IR-120B (Organo, Tokyo, Japan) column and purified by silica gel (15 g) column chromatography eluting with $CHCl_3$−MeOH−H_2O (20:10:1, 100 mL) to give **1** (9.0 mg).

Compound 3: amorphous solid; $[\alpha]_D^{25}$ −54.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3388 (OH), 2934 (CH), 1047 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.80 (1H, br s, H-1″), 5.74 (1H, br s, H-1‴), 5.32 (1H, br d, J = 4.0 Hz, H-6), 5.23 (1H, d, J = 7.8 Hz, H-1⁗), 5.11 (1H, d, J = 7.9 Hz, H-1⁗′), 4.88 (1H, d, J = 7.9 Hz, H-1′), 4.77 (1H, d, J = 7.8 Hz, H-1⁗″), 4.45 (1H, m, H-16), 3.90 (1H, m, $W_{1/2}$ = 24.3 Hz, H-3), 3.28 (3H, s, OMe), 1.75 (3H, d, J = 6.2 Hz, Me-6″), 1.69 (3H, d, J = 6.2 Hz, Me-6‴), 1.18 (3H, d, J = 6.8 Hz, Me-21), 1.04 (3H, d, J = 6.7 Hz, Me-27), 1.03 (3H, s, Me-19), 0.83 (3H, s, Me-18); ^{13}C NMR, see Table 1; FABMS (negative mode) *m*/*z* 1385 $[M - H]^-$; *anal*. C 50.27%, H 7.72%, calcd for $C_{64}H_{106}O_{32}\cdot 8H_2O$, C 50.19%, H 8.03%.

Acid Hydrolysis of 3. A solution of **3** (10 mg) in 1 M HCl (dioxane−H_2O, 1:1, 2 mL) was subjected to acid hydrolysis as described for **1** to give an aglycon [(25*S*)-spirost-5-en-3β-ol] (2.0 mg) and a sugar fraction (5.0 mg). HPLC analysis of the sugar fraction under the same conditions as for **1** showed the presence of D-glucose and L-rhamnose; t_R (min) 8.1 (L-rhamnose, negative optical rotation); 12.6 (D-glucose, positive optical rotation).

Compound 4: amorphous solid; $[\alpha]_D^{25}$ −60.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3389 (OH), 2932 (CH), 1041 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.83 (1H, br s, H-1″), 5.77 (1H, br s, H-1‴), 5.32 (1H, br d, J = 4.3 Hz, H-6), 5.25 (1H, d, J = 7.8 Hz, H-1⁗), 4.91 (1H, d, J = 7.8 Hz, H-1′), 4.84 (1H, d, J = 7.8 Hz, H-1⁗′), 4.81 (1H, m, H-16), 3.91 (1H, m, $W_{1/2}$ = 27.0 Hz, H-3), 1.76 (3H, d, J = 6.2 Hz, Me-6″), 1.69 (3H, d, J = 6.1 Hz, Me-6‴), 1.63 (3H, s, Me-21), 1.03 (3H, d, J = 6.6 Hz, Me-27), 1.06 (3H, s, Me-19), 0.71 (3H, s, Me-18); ^{13}C NMR, see Table 1; FABMS (positive mode) *m*/*z* 1215 $[M + Na]^+$; *anal*. C 54.99%, H 7.67%, calcd for $C_{57}H_{92}O_{26}\cdot 3H_2O$, C 54.88%, H 7.92%.

Acetylation of 4. Compound **4** (10 mg) was acetylated with Ac_2O (2 mL) in pyridine (2 mL) at room temperature for 12 h. The reaction mixture was chromatographed on silica gel (20 g) eluting with hexane−Me_2CO (1:1, 200 mL) to give peracetate **4a** (9 mg).

Preparation of 4a (Peracetate of 4) from 1. Compound **1** (2.5 g) was treated with a mixture of Ac_2O (15 mL) and pyridine (15 mL) at 110 °C for 2.5 h. After addition of H_2O (20 mL) into the reaction mixture followed by evaporation to dryness, it was chromatographed on silica gel (95 g) eluting with hexane−Me_2CO (1:1, 800 mL) to give **4a** (2.0 g).

Compound 4a: amorphous solid; $[\alpha]_D^{25}$ −52.0° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 2950 (CH), 1750 (C=O), 1035 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.37 (1H, br d, J = 4.8 Hz, H-6), 2.16−1.94 (Ac × 15), 1.56 (3H, s, Me-21), 1.23 (3H, d, J = 6.1 Hz, Me-6‴), 1.18 (3H, d, J = 6.2 Hz, Me-6″), 1.00 (3H, s, Me-19), 0.89 (3H, d, J = 6.7 Hz, Me-27), 0.66 (3H, s, Me-18).

Compound 5: amorphous solid; $[\alpha]_D^{25}$ −42.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3389 (OH), 2933 (CH), 1739 (C=O), 1044 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.82 (1H, br s, H-1″), 5.76 (1H, br s, H-1‴), 5.32 (1H, br d, J = 4.5 Hz, H-6), 5.23 (1H, d, J = 7.8 Hz, H-1⁗′), 4.87 (1H, d, J = 7.8 Hz, H-1′), 4.85 and 4.72 (each 1H, m, H_2-6′), 4.83 (1H, d, J = 7.7 Hz, H-1⁗″), 4.82 (1H, m, H-16), 3.90 (1H, m, $W_{1/2}$ = 20.7 Hz, H-3), 2.01 (3H, s, Ac), 1.75 (3H, d, J = 6.1 Hz, Me-6″), 1.67 (3H, d, J = 6.2 Hz, Me-6‴), 1.63 (3H, s, Me-21), 1.05 (3H, s, Me-19), 1.04 (3H, d, J = 6.8 Hz, Me-27), 0.72 (3H, s, Me-18); ^{13}C NMR, see Table 1; FABMS (positive mode) *m*/*z* 1257 $[M + Na]^+$; HR-MALDI-TOFMS *m*/*z* 1257.5891 $[M + Na]^+$ (calcd for $C_{59}H_{94}O_{27}$-Na, 1257.5880).

Alkaline Methanolysis of 5. Compound **5** (8.0 mg) was treated with 3% NaOMe in MeOH (5 mL) at room temperature for 1 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and purified by silica gel (15 g) column chromatography eluting with $CHCl_3$−MeOH−H_2O (20:10:1, 100 mL) to give **4** (6.0 mg).

Compound 6: amorphous solid; $[\alpha]_D^{25}$ −22.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); IR (film) ν_{max} 3389 (OH), 2933 (CH), 1712 (C=O), 1044 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.81 (1H, br s, H-1″), 5.75 (1H, br s, H-1‴), 5.67 (1H, m, H-16), 5.32 (1H, br d, J = 4.5 Hz, H-6), 5.24 (1H, d, J = 7.8 Hz, H-1⁗″), 4.90 (1H, d, J = 7.7 Hz, H-1′), 4.80 (1H, d, J = 7.8 Hz, H-1⁗‴), 3.91 (1H, m, $W_{1/2}$ = 29.4 Hz, H-3), 2.50 (1H, d, J = 7.6 Hz, H-17), 2.44 and 2.36 (each 1H, m, H_2-23), 2.14 (3H, s, Me-21), 1.75 (3H, d, J = 6.2 Hz, Me-6″), 1.69 (3H, d, J = 6.1 Hz, Me-6‴), 1.22 (3H, s, Me-18), 1.05 (3H, s, Me-19), 0.92 (3H, d, J = 6.7 Hz, Me-27); ^{13}C NMR, see Table 1; FABMS (positive mode) *m*/*z* 1247 $[M + Na]^+$; *anal*. C 53.18%, H 7.73%, calcd for $C_{57}H_{92}O_{28}\cdot 3H_2O$, C 53.51%, H 7.72%.

Acetylation of 6. Compound **6** (10 mg) was acetylated with Ac_2O (2 mL) in pyridine (2 mL) at room temperature for 12 h. The reaction mixture was chromatographed on silica gel (20 g) eluting with hexane−Me_2CO (1:1, 200 mL) to give peracetate **6a** (10 mg).

Preparation of 6a (Peracetate of 6) from 4a (Peracetate of 4). The CrO_3 (160 mg) solution in AcOH−H_2O (19:1, 10 mL) was added to **4a** (1.5 g) dissolved in AcOH−H_2O (19:1, 20 mL), and it was stirred at room temperature for 2 h. After the excess CrO_3 was decomposed by MeOH (20 mL), the reaction mixture was diluted with H_2O (100 mL) and extracted with Et_2O (40 mL × 4). The Et_2O extract was chromatographed on silica gel (50 g) eluting with hexane−Me_2O (1:1, 500 mL) to give **6a** (200 mg).

Compound 6a: amorphous solid; $[\alpha]_D^{25}$ −50.0° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 2940 (CH), 1750 (C=O), 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.48 (1H, ddd, J = 7.8, 7.7, 4.5 Hz, H-16), 5.35 (1H, br d, J = 5.2 Hz, H-6), 2.38 (1H, d, J = 7.6 Hz, H-17), 2.15 (3H, s, Me-21), 2.09−1.93 (Ac × 15), 1.22 (3H, d, J = 6.1 Hz, Me-6‴), 1.18 (3H, d, J = 6.1 Hz, Me-6″), 1.02 (3H, s, Me-19), 1.00 (3H, s, Me-18), 0.87 (3H, d, J = 6.7 Hz, Me-27).

Compound 7: amorphous solid; $[\alpha]_D^{25}$ −44.0° (*c* 0.10, $CHCl_3$−MeOH, 1:1); UV (MeOH) λ_{max} 239.8 nm (log ϵ 3.84); IR (film) ν_{max} 3417 (OH), 2932 (CH), 1660 (C=O), 1028 cm^{-1}; ^1H NMR (C_5D_5N) δ 6.62 (1H, dd, J = 3.1, 2.0 Hz, H-16), 5.80 (1H, br s, H-1″), 5.74 (1H, br s, H-1‴), 5.34 (1H, br d, J = 5.0 Hz, H-6), 5.24 (1H, d, J = 7.8 Hz, H-1⁗′), 4.88 (1H, d, J = 7.8 Hz, H-1′), 3.89 (1H, m, $W_{1/2}$ = 22.5 Hz, H-3), 2.25 (3H, s, Me-21), 1.75 (3H, d, J = 6.2 Hz, Me-6″), 1.68 (3H, d, J = 6.2 Hz, Me-6‴), 1.04 (3H, s, Me-19), 0.93 (3H, s, Me-18); ^{13}C NMR, see Table 1; FABMS (positive mode) *m*/*z* 953 $[M + Na]^+$; *anal*. C 52.13%, H 7.71%, calcd for $C_{45}H_{70}O_{20}\cdot 6H_2O$, C 52.01%, H 7.95%.

Preparation of 7 from 6. Compound **6** (40 mg) was treated with 7% NaOMe in MeOH (5 mL) at room temperature for 1.5 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B column and purified by ODS silica gel (15 g) column chromatography eluting with MeOH−H_2O (8:5, 300 mL) to give **7** (4.0 mg) and **8** (5.0 mg).

Compound 8: amorphous solid; $[\alpha]_D^{25}$ −46.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3388 (OH), 2933 (CH), 1048 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.82 (1H, br s, H-1″), 5.76 (1H, br s, H-1‴), 5.31 (1H, br d, J = 4.6 Hz, H-6), 5.24 (1H, d, J = 7.8 Hz, H-1⁗′), 4.90 (1H, d, J = 7.7 Hz, H-1′), 4.53 (1H, m, H-16), 3.90 (1H, m, $W_{1/2}$ = 23.8 Hz, H-3), 3.24 (3H, s, OMe), 2.67 (1H, d, J = 6.4 Hz, H-17), 2.17 (3H, s, Me-21), 1.76 (3H, d, J = 6.2 Hz, Me-6″), 1.69 (3H, d, J = 6.1 Hz, Me-6‴), 1.02 (3H, s, Me-19), 0.64 (3H, s, Me-18); ^{13}C NMR (C_5D_5N) δ 37.4 (C-1), 30.0 (C-2), 77.8 (C-3), 38.7 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7), 31.6 (C-8), 50.1 (C-9), 37.0 (C-10), 20.9 (C-11), 38.6 (C-12), 44.4 (C-13), 54.6 (C-14), 32.1 (C-15), 81.8 (C-16), 71.5 (C-17), 14.5 (C-18), 19.3 (C-19), 207.5 (C-20), 31.5 (C-21), 57.0 (OMe), 99.9 (C-1′), 37.4 (C-2′), 37.4 (C-3′), 37.4 (C-4′), 37.4 (C-5′), 37.4 (C-6′), 102.6 (C-1″), 72.5 (C-2″), 72.8 (C-3″), 73.8 (C-4″), 69.9 (C-5″), 18.7 (C-6″), 103.2 (C-1‴), 72.1 (C-2‴), 72.4 (C-3‴), 71.4 (C-4‴), 68.7 (C-5‴), 18.3 (C-6‴), 106.6 (C-1⁗), 76.5 (C-2⁗), 78.6 (C-3⁗), 71.4 (C-4⁗), 78.4 (C-5⁗), 62.6 (C-6⁗).

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