Steroidal Glycosides from the Leaves of *Cestrum nocturnum*

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Further phytochemical analysis aimed at the steroidal glycoside constituents of the leaves of *Cestrum nocturnum* has resulted in the isolation of eight new steroidal glycosides (**1**−**8**), which were classified into a spirostanol saponin (**1**), a furostanol saponin (**2**), a pseudo-furostanol saponin (**3**), two pregnane glycosides (**4**, **5**), two cholestane glycosides (**6**, **7**), and pregnane-carboxylic acid γ-lactone glycoside (**8**), and of two known spirostanol glycosides (**9**, **10**). The structures of the new compounds were elucidated on the basis of chemical and spectroscopic evidence.

Cestrum nocturnum L. (Solanaceae) is an evergreen shrub native to South America and the West Indies. The leaves of *C. nocturnum* have been used in Chinese folk medicine for the treatment of burns and swellings, being applied externally.[1] Our previous chemical study on the leaves of *C. nocturnum* led to the isolation of two new flavonol glycosides and seven steroidal saponins, including four new ones, some of which showed cytotoxic activity against cultured tumor cells.[2] Continuing investigation aimed at the steroidal glycoside constituents of the more polar fraction of the MeOH extract of the plant has resulted in the isolation of eight new steroidal glycosides (**1**−**8**), which were classified into a spirostanol saponin (**1**), a furostanol saponin (**2**), a pseudo-furostanol saponin (**3**), two pregnane glycosides (**4**, **5**), two cholestane glycosides (**6**, **7**), and a pregnane-carboxylic acid γ-lactone glycoside (**8**), and of two known spirostanol glycosides (**9**, **10**). In this paper, we report the structural determination of the new compounds based upon chemical and spectroscopic evidence, and also the results of the cytotoxic evaluation of the isolated compounds.

Results and Discussion

As described previously,[2] the glycoside-enriched fraction prepared from the MeOH extract of *C. nocturnum* leaves was further divided into 11 fractions (I−XI). Fractions VIII, IX, and X were subjected to Si gel and octadecylsilanized (ODS) Si gel column chromatography to give compounds **1**−**10**. Compounds **9** and **10** were identified as (25*R*)-2α-hydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside[2,3] and (25*R*)-2α,17α-dihydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside,[2] respectively.

Compound **1** was obtained as an amorphous solid with a molecular formula of $C_{62}H_{100}O_{34}$, as determined by data of the positive-ion HRTOFMS (*m*/*z* 1411.5925 $[M + Na]^+$), negative-ion HRTOFMS (*m*/*z* 1387.6050 $[M - H]^-$), ^{13}C NMR spectrum (62 carbon signals), and the results of the elemental analysis. The ^1H NMR spectrum of **1** showed two three-proton singlet signals at δ 0.94 and 0.74 and two three-proton doublet signals at δ 1.15 (*J* = 6.5 Hz) and 1.05 (*J* = 6.9 Hz), which were characteristic of the spirostanol skeleton, as well as signals for six anomeric protons at δ 5.57 (d, *J* = 7.3 Hz), 5.20 (d, *J* = 7.9 Hz), 5.17 (d, *J* = 7.7 Hz), 5.10 (d, *J* = 7.8 Hz), 4.93 (d, *J* = 7.7 Hz), and 4.92 (d, *J* = 7.7 Hz). Acid hydrolysis of **1** with 0.5 M HCl in dioxane−H_2O (1:1) gave D-galactose, D-glucose, and D-xylose as the carbohydrate moieties, while the labile aglycon was decomposed under acid conditions. The monosaccharides, including their absolute configurations, were identified by direct HPLC analysis of the hydrolysate, which was performed on an aminopropyl-bonded Si gel column using MeCN−H_2O (17:3) as solvent system, with detection being carried out by using an optical rotation (OR) detector. Comparison of the ^1H and ^{13}C NMR spectra of **1** with those of **9** revealed that the structures of the ring A−E portion and the pentaglycoside moiety attached at C-3 of the aglycon were identical to those of **9**. However, significant differences were recognized in the signals from the ring F portion (C-22−C-27) and the presence of the additional six carbon signals attributable to a terminal β-D-glucopyranosyl group. The $^1H-^1H$ COSY spectrum was carefully inspected to assign the structure of the ring F residue, with the methyl doublet signal due to Me-27 being used as the starting point of the analysis. The subsequent spin-coupling correlations led us to propose the ring F part of **1** as $-CH_2-CH(-O-)-CH(-Me)-CH_2-O-$. Evidence for the connectivities of the terminal free bonds of the fragment to C-22 was obtained by the observation of the $^1H/^{13}C$ long-range correlations from the methylene proton signals at δ 2.66 and 1.96 (H_2-23) and the oxymethylene proton signals at δ 3.64 and 3.57 (H_2-26) to C-22 at δ 111.6 in the HMBC spectrum. Thus, the presence of an oxygen atom at C-24 was evident. The proton multiplicities of H-24, with the *J* values of 12.1 Hz (H-24/H-25), 11.6 Hz (H-24/H-23ax), and 4.7 Hz (H-24/H-23eq), and NOE correlations from H-23ax to H-20 (δ 1.92), Me-21 (δ 1.05), and H-25 (δ 1.89), and from H-26ax to H-16 (δ 4.50) and H-24 in the phase-sensitive NOESY spectrum were consistent with the C-22α, C-24*S*, and C-25*S* configurations. The anomeric proton signal due to the additional glucosyl group at δ 4.93 showed an HMBC correlation with C-24 at δ 81.5. Accordingly, the structure of **1** was elucidated as (24*S*,25*S*)-24-[(β-D-glucopyranosyl)oxy]-2α-hydroxyspirost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

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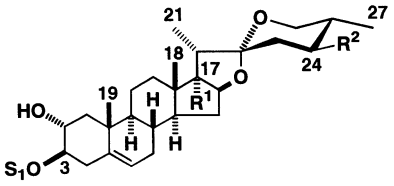
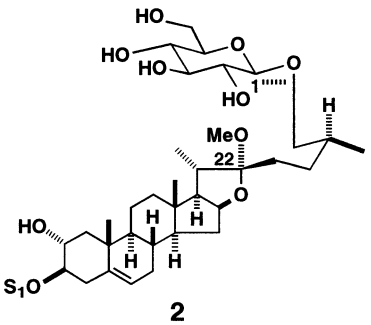


	R^1	R^2
1	H	O-β-D-Glc*p*
9	H	H
10	OH	H



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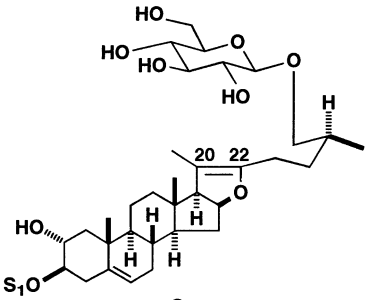

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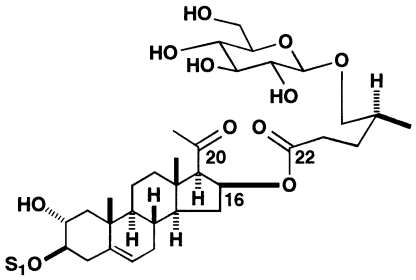

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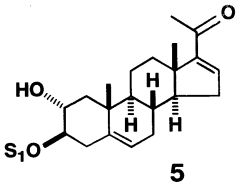

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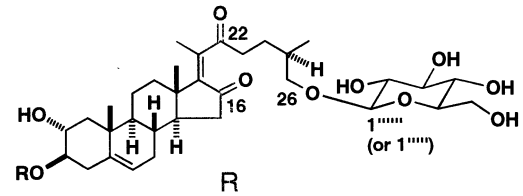
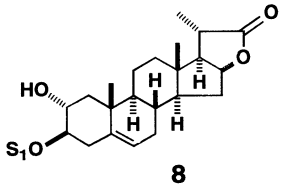


	R
6	S_1
7	S_2



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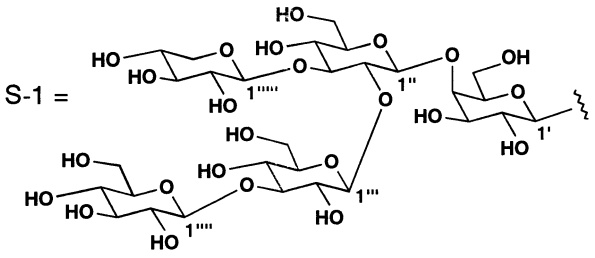
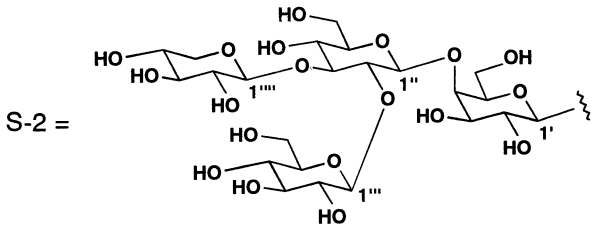


S-1 =



S-2 =

The ^{1}H NMR spectrum of **2** ($C_{63}H_{104}O_{34}$) showed siganls for four steroid methyl groups at δ 1.18 (3H, d, J = 6.9 Hz), 1.00 (3H, d, J = 6.6 Hz), 0.94 (3H, s), and 0.79 (3H, s), a methoxyl group at δ 3.26 (3H, s), an olefinic proton at δ 5.31 (br d, J = 4.3 Hz), and six anomeric protons at δ 5.57 (d, J = 7.3 Hz), 5.20 (d, J = 7.9 Hz), 5.17 (d, J = 7.7 Hz), 5.10 (d, J = 7.8 Hz), 4.92 (d, J = 7.7 Hz), and 4.85 (d, J = 7.8 Hz). These ^{1}H NMR data, together with an acetalic carbon signal at δ 112.6 in the ^{13}C NMR spectrum,[4] and a positive color reaction with Ehrlich's reagent,[5] suggested that **2** was the correspnding 22-methoxyfurostanol saponin of **9**. This was confirmed by enzymatic hydrolysis of **2** with β-D-glucosidase, giving **9** and D-glucose. The configuration of C-22 was determined to be α by an NOE correlation between the signals of the C-22 methoxyl protons and the H-16 proton. The structure of **2** was shown to be (25*R*)-26-[(β-D-glucopyranosyl)oxy]-2α-hydroxy-22α-methoxyfurost-5-en-3β-yl *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Compound **3** ($C_{62}H_{100}O_{33}$) was suggested to be a furostanol saponin closely related to **2** from its spectral data. It differed from **2** in the presence of one more olefinic functionality [δ_C 152.3 (C) and 103.5 (C)] in addition to the 5(6)-ene group. Furthermore, the Me-21 methyl doublet signal observed at δ 1.18 (J = 6.9 Hz) in the ^{1}H NMR spectrum of **2** was absent from **3**, but was replaced by a methyl singlet at δ 1.61. These data were suggestive of **3** being the corresponding $\Delta^{20(22)}$-pseudo-furostanol saponin of **2**. This was ascertained by the fact that the peracetate (**3a**) of **3** agreed with the product obtained by treatment of **2** with Ac_2O in pyridine at 110 °C for 3 h, during which dehydration at C-20 and C-22, as well as introduction of an acetyl group to all the hydroxyl groups of the molecule,

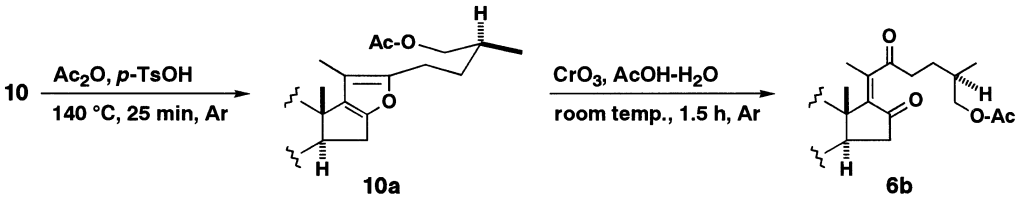


Figure 1.

occurred to **2**. The structure of **3** was established as (25*R*)-26-[(*β*-D-glucopyranosyl)oxy]-2α-hydroxyfurosta-5,20(22)-dien-3*β*-yl *O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside.

The ^{1}H and ^{13}C NMR spectra of **4** ($C_{62}H_{100}O_{35}$) revealed that it was a steroidal pentaglycoside essentially analogous to **3**. However, **4** was different from **3** 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* 205.4 and an ester carbonyl carbon signal at *δ* 173.2. The HMBC spectrum exhibited correlations of the ketone carbonyl carbon with H-17 at *δ* 2.49 (1H, d, *J* = 7.6 Hz) and Me-21 at *δ* 2.13 (3H, s) and of the ester carbonyl carbon with H-16 at *δ* 5.66 (1H, m) and H_2-23 at *δ* 2.42 and 2.38, indicating that C-20 and C-22 of **4** were the carbonyl groups, instead of the olefinic group in **3**. The structure of **4**, including the absolute configuration at C-26, was confirmed by the following chemical correlations. When the C-20 and C-22 bond of **3a** 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 (**4a**) of **4**. Accordingly, the structure of **4** was characterized as 3*β*-[(*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl)oxy]-16*β*-[[(4*R*)-5-(*β*-D-glucopyranosyloxy)-4-methyl-1-oxopentyl]oxy]-2α-hydroxypregn-5-en-20-one.

The ^{1}H NMR spectrum of **5** ($C_{50}H_{78}O_{27}$) displayed two three-proton singlet signals at *δ* 0.95 and 0.91, indicating the presence of two angular methyl groups, and a methyl singlet at *δ* 2.24 attached to a deshielding moiety, as well as five anomeric proton signals as observed in the above-mentioned compounds. The existence of an α,*β*-unsaturated carbonyl group was verified by the IR (1651 cm^{-1}), UV [238 nm (log *ϵ* 3.81)], and ^{13}C NMR [*δ* 196.2 (C=O), 155.2 (C), and 144.6 (CH)] spectra. These spectral data and comparison with those of previous reported compounds[6,7] suggested that the aglycon of **5** was 2α,3*β*-dihydroxypregna-5,16-dien-20-one. Alkaline treatment of **4** with 7% NaOMe in MeOH afforded **5**. All of these data were consistent with the structure 3*β*-[(*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl)oxy]-2α-hydroxypregna-5,16-dien-20-one, which was assigned to **5**.

The ^{1}H and ^{13}C NMR spectra of **6** ($C_{62}H_{98}O_{34}$) suggested that it was a C_{27} steroidal bisdesmoside on the basis of the cholestane skeleton and identified two carbonyl [δ_C 210.4 (C) and 205.6 (C)], an oxymethylene [δ_H 3.95 and 3.59; δ_C 75.1 (CH_2)], a methyl on a double bond [δ_H 1.97 (3H, s)], and a tetrasubstituted olefinic [δ_C 145.7 (C) and 142.5 (C)] groups as well as the 2α-hydroxyl, 3*β*-pentaglycosyloxy, C-18 and C-19 angular methyl, C-27 methyl, and 5(6)-olefinic functionalities. In addition, signals for one more terminal *β*-D-glucopyranosyl group were assigned. In the HMBC spectrum, correlation peaks from the methylene proton signals due to H_2-15 at *δ* 2.13 and 1.95 to the carbonyl carbon signal at *δ* 205.6 and from the methyl at *δ* 1.97 exclusively assigned to Me-21 and two consecutive methylenes at *δ* 2.79 (2H, H_2-23) and 2.11 and 1.84 (H_2-24) to the *δ* 210.4 resonance gave ample evidence for the presence of two carbonyl groups at C-16 and C-22. Further HMBC correlations from Me-18 to *δ* 142.5 and from Me-21 to *δ* 142.5 and 145.7 were indicative of a double bond between C-17 and C-20. The location of the one more *β*-D-glucopyranosyl group at C-26 was shown by a $^3J_{C,H}$ correlation between the signals of the anomeric proton at *δ* 4.83 (d, *J* = 7.8 Hz) and the C-26 methylene carbon at *δ* 75.1. Finally, the gross structure of **6** was determined by the following chemical correlation. Compound **6** was treated with *β*-D-glucosidase to give the 26-*O*-deglucosyl derivative (**6a**), which was acetylated with Ac_2O in pyridine to furnish **6a** heptadecaacetate (**6b**). On the other hand, a mixture of **10** in *p*-TsOH in Ac_2O was heated at 140 °C for 25 min to give an acetate of (25*R*)-2α,3*β*,26-trihydroxyfurosta-5,16,-20(22)-triene 3-*O*-pentaglycoside (**10a**). The acetate was then oxidized with CrO_3 in AcOH, and the resultant product was coincident with **6b**. Thus, the structure of **6** was characterized as (25*R*)-3*β*-[(*O*-*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl)-oxy]-26-[(*β*-D-glucopyranosyl)oxy]-2α-hydroxycholesta-5,17-diene-16,22-dione.

The spectral data of **7** ($C_{56}H_{88}O_{29}$) were very similar to those of **6** and allowed the identification of the aglycon moiety of **7** as being the same as that of **6**. The structural difference between **6** and **7** was considered to lie in the sugar moiety attached at C-3 of the aglycon. When the ^{13}C NMR spectrum of **7** was compared with that of **6**, the signals due to the terminal glucosyl residue linked to C-3 of the inner glucosyl moiety could not be observed in **7**. Furthermore, the ^{13}C NMR assignments of the sugar were in good agreement with those of lycotetrasyl, a tetraglycoside often encountered in the plant saponins.[2,8] The structure of **7** was shown to be (25*R*)-3*β*-[(*O*-*β*-D-glucopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranosyl)oxy]-26-[(*β*-D-glucopyranosyl)oxy]-2α-hydroxycholesta-5,17-diene-16,22-dione.

The ^{1}H NMR spectrum of **8** ($C_{51}H_{80}O_{28}$) exhibited five anomeric proton signals, and the ^{13}C NMR spectrum confirmed that the pentaglycoside linked to the aglycon was identical to that of the concomitantly isolated glycosides, except for **7**. Consequently, the aglycon of **8** led to a $C_{22}H_{32}O_4$ composition. Analysis of the ^{1}H and ^{13}C NMR spectra revealed that **8** included the 2α-hydroxyl, 3*β*-pentaglycosyloxy, C-18 and C-19 angular methyl, and 5(6)-olefinic groups. In addition, the presence of a *γ*-lactone ring in the molecule was suggested by the IR (1747 cm^{-1}) and ^{13}C NMR (*δ* 181.1) spectra. The signals at *δ* 2.65 (1H, m) and 1.24 (3H, d, *J* = 5.4 Hz) assignable to H-20 and Me-21, respectively, showed long-range correlations with the δ_C 181.1 resonance, and the H-16 proton and C-16 carbon signals were observed at the downfield-shifted positions at δ_H 4.89 (td, *J* = 7.7, 4.6 Hz) and δ_C 82.6. This indicated a *γ*-lactone ring formation between C-16 and C-22.[9,10] The

NOE correlations from H-14α (δ 0.89) to H-15α (δ 2.10) and H-17 (δ 1.76), from H-16 to H-15α and H-17, from H-17 to Me-21, and from Me-18 (δ 0.69) to H-20 made the relative stereochemistry assignable as 16β, 17β, and 20α. The structure of **8** was defined as 3β-[(*O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)-oxy]-2α,16β-dihydroxypregn-5-ene-20-carboxylic acid γ-lactone.

Compounds **1**−**8** are newly described compounds, and the leaves of *C. nocturnum* were found to contain a variety of steroidal derivatives such as the spirostan, furostan, pseudo-furostan, pregnane, cholestane, and pregnane-carboxylic acid γ-lactone glycosides. The isolated compounds were evaluated for their cytotoxic activity against human oral squamous cell carcinoma (HSC-2) cells. Compounds **2**, **9**, and **10** showed cytotoxic activity as potent as doxorubicin used as a positive control (Table 2).

Experimental Section

General Experimental Procedures. The instruments and experimental conditions were the same as described in the previous paper,[2] with the exception of that for the measurements of HRTOFMS, which were recorded on a JASCO Q-TOF Ultime API + LockSpray (Tokyo, Japan) mass spectrometer.

Plant Material. The fresh leaves of *C. nocturnum* cultivated in the Tokyo Metropolitan Medicinal Plant Garden (Tokyo, Japan) were collected in October 1998 and identified by Y. Sashida. A voucher of the plant is on file in our laboratory (voucher No. CN-98-007., Lab. of Med. Plant Sci.).

Extraction and Isolation. The MeOH extract (77 g) prepared from the plant material (fresh weight, 1.2 kg) was divided into 11 fractions (I−XI).[2] Fraction VIII was further separated by ODS Si gel (75 μm, Nacalai Tesque, Kyoto, Japan) column chromatography eluting with MeCN−H_2O (1:3) into three subfractions (VIIIa−VIIIc). Each subfraction was repeatedly chromatographed on ODS Si gel eluting with MeOH−H_2O solvent systems (4:3; 1:1; 4:5) to give **2** (317 mg), **4** (31.1 mg), **6** (106 mg), **7** (47.7 mg), **9** (17.0 mg), and **10** (9.2 mg). Fraction IX was subjected to column chromatography on Si gel (200−400 mesh, Fuji-Silysia Chemical, Aichi, Japan) eluting with $CHCl_3$−MeOH−H_2O (20:10:1) and ODS Si gel with MeOH−H_2O (4:3) and MeCN−H_2O (1:3) to afford **1** (71.6 mg). Compounds **3** (39.8 mg), **5** (98.5 mg), and **8** (16.9 mg) were isolated from fraction X, which was established by subjecting fraction X to Si gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (25:10:1; 14:8:1) and ODS Si gel column chromatography with MeOH−H_2O (8:5; 4:3) and MeCN−H_2O (1:3). Compound **2** was obtained as a mixture of the C-22 hydroxyl and C-22 methoxyl forms, which could not be separated because of its readily convertible nature in dissolved solvent. It is presumed to exist in the C-22 hydroxyl form, and the C-22 methoxyl derivative may be produced through the extraction and isolation procedures. The C-22 hydroxyl form present in the mixture was completely converted to the C-22 methoxyl form by treatment with hot MeOH, and the structural elucidation of **2** was carried out with the methoxyl form.

Compound 1: amorphous powder; $[\alpha]^{28}_D$ −48.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3417 (OH), 2925 (CH), 1453, 1434, 1373, 1255, 1160, 1075, 1040, 894 cm^{-1}; ^1H NMR (C_5D_5N) δ 5.57 (1H, d, *J* = 7.3 Hz, H-1‴), 5.29 (1H, br d, *J* = 4.2 Hz, H-6), 5.20 (1H, d, *J* = 7.9 Hz, H-1″), 5.17 (1H, d, *J* = 7.7 Hz, H-1⁗), 5.10 (1H, d, *J* = 7.8 Hz, H-1‴), 4.93 (1H, d, *J* = 7.7 Hz, H-1⁗), 4.92 (1H, d, *J* = 7.7 Hz, H-1′), 4.50 (1H, m, H-16), 4.04 (1H, ddd, *J* = 12.1, 11.6, 4.7 Hz, H-24), 3.64 (1H, dd, *J* = 11.5, 5.6 Hz, H-26eq), 3.57 (1H, dd, *J* = 11.5, 11.5 Hz, H-26ax), 2.66 (1H, dd, *J* = 12.9, 4.7 Hz, H-23eq), 1.96 (1H, dd, *J* = 12.9, 11.6 Hz, H-23ax), 1.92 (1H, m, H-20), 1.89 (1H, m, H-25), 1.15 (3H, d, *J* = 6.5 Hz, Me-27), 1.05 (3H, d, *J* = 6.9 Hz, Me-21), 0.94 (3H, s, Me-19), 0.74 (3H, s, Me-18); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m*/*z* 1411.5925 $[M + Na]^+$ (calcd for $C_{62}H_{100}O_{34}Na$, 1411.5994); HRTOFMS (negative mode) *m*/*z* 1387.6050 $[M - H]^-$ (calcd for $C_{62}H_{99}O_{34}$, 1387.6018); *anal.* C 49.63%, H 7.65%, calcd for $C_{62}H_{100}O_{34}\cdot 6H_2O$, C 49.73%, H 7.54%.

Table 1. ^{13}C NMR Spectral Data for Compounds **1**−**8** in C_5D_5N

C	1	2	3	4	5	6	7	8
1	45.7	45.7	45.7	45.7	45.6	45.3	45.3	45.7
2	70.0	70.0	70.0	70.0	70.0	69.9	69.9	69.9
3	84.6	84.6	84.4	84.3	84.4	84.3	84.1	84.3
4	37.6	37.6	37.5	37.7	37.6	37.6	37.6	37.6
5	140.1	140.1	140.0	140.2	140.5	140.2	140.1	140.1
6	121.9	121.9	121.9	121.7	121.6	121.5	121.4	121.6
7	32.1	32.1	32.2	31.8	32.2	31.6	31.5	31.9
8	31.0	31.0	30.8	30.4	29.8	30.2	30.1	30.7
9	50.1	50.2	50.1	50.3	50.6	49.7	49.7	50.2
10	37.9	37.9	37.8	37.8	37.9	37.9	37.8	37.8
11	21.1	21.1	21.3	20.7	21.0	20.9	20.8	20.6
12	39.7	39.6	39.5	38.0	31.7	35.9	35.9	37.9
13	40.4	40.8	43.3	42.3	46.2	43.4	43.3	41.4
14	56.5	56.4	54.7	53.9	56.3	50.3	50.3	54.5
15	32.0	32.2	34.4	35.4	35.0	37.8	37.8	33.2
16	81.5	81.3	84.4	75.2	144.6	205.6	205.6	82.6
17	62.3	64.1	64.4	66.6	155.2	142.5	142.5	58.8
18	16.3	16.2	14.1	13.8	15.9	16.7	16.7	13.5
19	20.4	20.4	20.4	20.4	20.3	20.3	20.3	20.4
20	42.1	40.5	103.5	205.4	196.2	145.7	145.6	36.2
21	14.8	16.3	11.7	30.4	27.1	15.7	15.7	17.9
22	111.6	112.6	152.3	173.2		210.4	210.5	181.1
23	40.8	30.8	23.6	32.2		38.7	38.7	
24	81.5	28.2	31.4	29.0		28.0	27.9	
25	38.2	34.2	33.4	33.4		33.4	33.3	
26	65.1	75.2	74.9	74.7		75.1	75.0	
27	13.5	17.1	17.3	16.9		17.4	17.4	
OMe		47.3						
1′	103.3	103.3	103.2	103.3	103.3	103.3	103.2	103.3
2′	72.6	72.6	72.6	72.5	72.6	72.6	72.5	72.6
3′	75.4	75.4	75.3	75.4	75.4	75.4	75.4	75.4
4′	79.0	79.0	79.1	79.0	79.1	79.0	79.4	79.0
5′	75.7	75.7	75.6	75.7	75.7	75.7	75.6	75.7
6′	60.6	60.6	60.6	60.6	60.6	60.6	60.5	60.6
1″	104.4	104.4	104.3	104.4	104.3	104.3	104.6	104.3
2″	80.6	80.6	80.6	80.6	80.6	80.6	81.1	80.6
3″	87.0	87.0	86.9	86.9	87.0	87.0	86.9	86.9
4″	70.4	70.4	70.3	70.4	70.3	70.4	70.3	70.3
5″	77.5	77.5	77.4	77.5	77.5	77.5	77.5	77.5
6″	62.8	62.9	62.8	62.8	62.8	62.9	62.8	62.8
1‴	104.0	103.9	103.8	104.0	103.9	104.0	104.7	103.9
2‴	74.7	74.7	74.6	74.7	74.6	74.7	76.0	74.6
3‴	87.7	87.7	87.7	87.7	87.7	87.7	78.3	87.7
4‴	69.7	69.7	69.6	69.7	69.6	69.7	71.3	69.7
5‴	77.7	77.7	77.7	77.8	77.7	77.7	78.3	78.4
6‴	62.3	62.3	62.1	62.3	62.3	62.4	62.6	62.3
1⁗	105.4	105.4	105.3	105.5	105.4	105.4	104.8	105.4
2⁗	75.6	75.6	75.5	75.6	75.5	75.6	75.0	75.6
3⁗	78.0	78.0	77.9	78.0	78.0	78.0	78.6	78.0
4⁗	71.5	71.5	71.5	71.5	71.5	71.5	70.8	71.5
5⁗	78.5	78.4	78.4	78.4	78.4	78.4	67.2	78.4
6⁗	62.5	62.5	62.4	62.5	62.4	62.5		62.5
1⁗′	104.9	104.9	104.8	104.9	104.8	104.8	104.8	104.8
2⁗′	75.2	75.2	75.1	75.2	75.2	75.2	75.1	74.5
3⁗′	78.4	78.5	78.4	78.6	78.4	78.6	78.5	78.4
4⁗′	70.6	70.6	70.6	70.6	70.6	70.6	71.6	70.6
5⁗′	67.2	67.2	67.1	67.2	67.2	67.2	78.0	67.2
6⁗′							62.7	
1⁗″	106.4	105.0	104.8	104.9		104.8		
2⁗″	75.7	75.2	75.1	75.2		75.2		
3⁗″	78.6	78.4	78.5	78.3		78.5		
4⁗″	71.7	71.8	71.7	71.7		71.7		
5⁗″	78.0	78.6	78.3	78.5		78.5		
6⁗″	62.9	62.9	62.8	62.8		62.8		

Acid Hydrolysis of 1a. A solution of **1** (12.6 mg) in 0.5 M HCl (dioxane−H_2O, 1:1, 2 mL) was heated at 95 °C for 1 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 Diaion HP-20 (Mitsubishi-Kasei, Tokyo, Japan) eluting with H_2O−MeOH (3:2), followed by Me_2CO−EtOH (1:1) to give a sugar fraction (3.5 mg). The sugar fraction was passed through a Sep-Pak C_{18} cartridge (Waters, Milford, MA) and a Toyopak

Table 2. Cytotoxic Activities of Compounds **1**−**10** and Doxorubicin against HSC-2 Cells

compound	LD_{50} (μg/mL)
1	>300
2	6.1
3	>300
4	>300
5	192
6	281
7	>300
8	>300
9	2.7
10	4.4
doxorubicin	2.5

IC-SP M cartridge (Tosoh, Tokyo, Japan), which was then analyzed by HPLC under the following conditions: column, Capcell Pak NH_2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido, Tokyo, Japan); solvent, MeCN−H_2O (17:3); flow rate, 0.9 mL/min; detection, RI and OR. The identification of D-xylose, D-galactose, and D-glucose present in the sugar fraction was carried out by comparison of their retention times and polarities with those of authentic samples. t_R (min) 9.61 (D-xylose, positive polarity), 13.53 (D-galactose, positive polarity), 14.73 (D-glucose, positive polarity).

Compound 2: amorphous powder; $[\alpha]^{27}_D$ −60.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3417 (OH), 2935 and 2903 (CH), 1453, 1433, 1376, 1259, 1159, 1077, 1040, 892 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.57 (1H, d, *J* = 7.3 Hz, H-1‴), 5.31 (1H, br d, *J* = 4.3 Hz, H-6), 5.20 (1H, d, *J* = 7.9 Hz, H-1″), 5.17 (1H, d, *J* = 7.7 Hz, H-1⁗), 5.10 (1H, d, *J* = 7.8 Hz, H-1‴), 4.92 (1H, d, *J* = 7.7 Hz, H-1′), 4.85 (1H, d, *J* = 7.8 Hz, H-1⁗′), 3.26 (3H, s, OMe), 1.18 (3H, d, *J* = 6.9 Hz, Me-21), 1.00 (3H, d, *J* = 6.6 Hz, Me-27), 0.94 (3H, s, Me-19), 0.79 (3H, s, Me-18); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m*/*z* 1427.6340 [M + Na]$^+$ (calcd for $C_{63}H_{104}O_{34}Na$, 1427.6307); HRTOFMS (negative mode) *m*/*z* 1403.6366 [M − H]$^-$ (calcd for $C_{63}H_{103}O_{34}$, 1403.6331); *anal*. C 50.32%, H 7.83%, calcd for $C_{63}H_{104}O_{34}\cdot 5H_2O$, C 50.29%, H 7.70%.

Enzymatic Hydrolysis of 2. Compound **2** (15.2 mg) was treated with β-D-glucosidase (7.2 mg, Sigma, St. Louis, MO) in HOAc−NaOAc buffer (pH 5.0, 3.0 mL) at room temperature for 15 h. The crude hydrolysate was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (30:10:1) to give **9** (9.6 mg) and D-glucose (3.2 mg). D-Glucose was identified by direct TLC comparison with an authentic sample; R_f 0.40 (*n*-BuOH−Me_2CO−H_2O, 4:5:1).

Compound 3: amorphous powder; $[\alpha]^{28}_D$ −46.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3442 (OH), 2925 (CH), 1650, 1453, 1434, 1376, 1159, 1077, 1039, 894 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.57 (1H, d, *J* = 7.7 Hz, H-1‴), 5.30 (1H, br d, *J* = 4.1 Hz, H-6), 5.17 (1H, d, *J* = 7.8 Hz, H-1″), 5.16 (1H, d, *J* = 7.7 Hz, H-1⁗), 5.12 (1H, d, *J* = 7.8 Hz, H-1‴), 4.92 (1H, d, *J* = 7.7 Hz, H-1′), 4.83 (1H, d, *J* = 7.8 Hz, H-1⁗′), 1.61 (3H, s, Me-21), 1.01 (3H, d, *J* = 6.6 Hz, Me-27), 0.94 (3H, s, Me-19), 0.69 (3H, s, Me-18); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m*/*z* 1395.6025 [M + Na]$^+$ (calcd for $C_{62}H_{100}O_{33}Na$, 1395.6045); HRTOFMS (negative mode) *m*/*z* 1371.6121 [M − H]$^-$ (calcd for $C_{62}H_{99}O_{33}$, 1371.6069); *anal*. C 50.48%, H 7.73%, calcd for $C_{62}H_{100}O_{33}\cdot 11/2H_2O$, C 50.57%, H 7.60%.

Acetylation of 3. Compound **3** (11.7 mg) was acetylated with Ac_2O (1 mL) in C_5H_5N (1 mL) at 40 °C for 20 h. The crude acetate was chromatographed on Si gel eluting with hexane−Me_2CO (1:1) to give **3a** (8.5 mg).

Compound 3a: amorphous powder; $[\alpha]^{24}_D$ −26.0° (*c* 0.10, MeOH); IR (film) ν_{max} 2917 and 2850 (CH), 1746 (C=O), 1434, 1370, 1227, 1039, 903, 840 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.48 (1H, d, *J* = 8.0 Hz, H-1‴), 5.44 (1H, br d, *J* = 5.3 Hz, H-6), 5.34 (1H, br s, H-1⁗′), 5.33 (1H, d, *J* = 8.2 Hz, H-1⁗), 4.97 (1H, d, *J* = 7.6 Hz, H-1″), 4.92 (1H, d, *J* = 8.0 Hz, H-1⁗′), 4.92 (1H, d, *J* = 8.0 Hz, H-1′), 2.51, 2.44, 2.41, 2.27, 2.23, 2.16 × 3, 2.14, 2.13, 2.11, 2.10 × 2, 2.05, 2.03, 2.02, 2.01, 1.99 × 2 and 1.98 (each 3H, s, Ac), 1.64 (3H, s, Me-21), 1.16 (3H, s, Me-19), 0.95 (3H, d, *J* = 6.6 Hz, Me-27), 0.73 (3H, s, Me-18); FABMS (positive mode) *m*/*z* 2236.8 [M + Na]$^+$.

Preparation of 3a from 2. Compound **2** (223 mg) was treated with a mixture of Ac_2O (10 mL) and C_5H_5N (5 mL) at 110 °C for 3 h under an Ar atmosphere. After addition of H_2O (10 mL) into the reaction mixture, it was extracted with Et_2O (15 mL × 3). The Et_2O extract was chromatographed on Si gel eluting with hexane−Me_2CO (1:1) to give **3a** (242 mg).

Compound 4: amorphous powder; $[\alpha]^{29}_D$ −50.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3389 (OH), 2928 (CH), 1709 (C=O), 1423, 1372, 1257, 1159, 1076, 1040, 893 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.66 (1H, m, H-16), 5.57 (1H, d, *J* = 7.4 Hz, H-1‴), 5.30 (1H, br d, *J* = 4.4 Hz, H-6), 5.20 (1H, d, *J* = 7.8 Hz, H-1″), 5.17 (1H, d, *J* = 7.7 Hz, H-1⁗′), 5.10 (1H, d, *J* = 7.8 Hz, H-1⁗), 4.93 (1H, d, *J* = 7.8 Hz, H-1′), 4.79 (1H, d, *J* = 7.7 Hz, H-1⁗′), 2.49 (1H, d, *J* = 7.6 Hz, H-17), 2.42 and 2.38 (each 1H, m, H_2-23), 2.13 (3H, s, Me-21), 1.20 (3H, s, Me-19), 0.95 (3H, s, Me-18), 0.92 (3H, d, *J* = 6.7 Hz, Me-27); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m*/*z* 1427.5897 [M + Na]$^+$ (calcd for $C_{62}H_{100}O_{35}Na$, 1427.5943); HRTOFMS (negative mode) *m*/*z* 1403.6022 [M − H]$^-$ (calcd for $C_{62}H_{99}O_{35}$, 1403.5967); *anal*. C 49.47%, H 7.40%, calcd for $C_{62}H_{100}O_{35}\cdot 4H_2O$, C 50.40%, H 7.37%.

Acetylation of 4. Compound **4** (10.3 mg) was acetylated with Ac_2O (1 mL) in C_5H_5N (1 mL) at 40 °C for 20 h. The crude acetate was chromatographed on Si gel eluting with hexane−Me_2CO (1:1) to give **4a** (7.4 mg).

Compound 4a: amorphous powder; $[\alpha]^{25}_D$ −38.0° (*c* 0.10, MeOH); IR (film) ν_{max} 2957, 2939 and 2918 (CH), 1746 (C=O), 1434, 1370, 1228, 1039, 904 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.45 (1H, d, *J* = 8.1 Hz, H-1‴), 5.43 (1H, br d, *J* = 5.7 Hz, H-6), 5.34 (1H, br s, H-1⁗′), 5.33 (1H, d, *J* = 8.0 Hz, H-1⁗), 5.03 (1H, d, *J* = 8.0 Hz, H-1″), 4.92 (1H, d, *J* = 8.2 Hz, H-1′), 4.90 (1H, d, *J* = 8.2 Hz, H-1⁗′), 2.50, 2.45, 2.40, 2.26, 2.22, 2.15 × 2, 2.12, 2.11, 2.10, 2.09 × 2, 2.04, 2.03, 2.01, 2.00 × 2, 1.99 and 1.97 (each 3H, s, Ac), 2.15 (3H, s, Me-21), 1.20 (3H, s, Me-19), 1.12 (3H, s, Me-18), 0.85 (3H, d, *J* = 6.7 Hz, Me-27); FABMS (positive mode) *m*/*z* 2268.3 [M + Na]$^+$.

Preparation of 4a from 3a. The CrO_3 (17.5 mg) solution in AcOH−H_2O (19:1, 7 mL) was added to **3a** (218 mg) dissolved in AcOH−H_2O (19:1, 7 mL), and it was stirred at room temperature for 2 h under an Ar atmosphere. After the excess CrO_3 was decomposed by MeOH (3 mL), the reaction mixture was diluted with H_2O (30 mL) and extracted with Et_2O (30 mL × 3). The Et_2O extract was chromatographed on Si gel eluting with hexane−Me_2CO (1:1) to yield **4a** (40.6 mg).

Compound 5: amorphous powder; $[\alpha]^{28}_D$ −42.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 238 nm (log ϵ 3.81); IR (film) ν_{max} 3417 (OH), 2938 and 2837 (CH), 1651 (C=O), 1587, 1454, 1416, 1373, 1239, 1158, 1078, 1038, 893 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 6.59 (1H, dd, *J* = 2.9, 1.9 Hz, H-16), 5.57 (1H, d, *J* = 7.5 Hz, H-1‴), 5.33 (1H, br d, *J* = 5.0 Hz, H-6), 5.20 (1H, d, *J* = 7.9 Hz, H-1″), 5.16 (1H, d, *J* = 7.7 Hz, H-1⁗′), 5.10 (1H, d, *J* = 7.8 Hz, H-1‴), 4.92 (1H, d, *J* = 7.8 Hz, H-1′), 2.24 (3H, s, Me-21), 0.95 (3H, s, Me-19), 0.91 (3H, s, Me-18); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m*/*z* 1133.4626 [M + Na]$^+$ (calcd for $C_{50}H_{78}O_{27}Na$, 1133.4628); HRTOFMS (negative mode) *m*/*z* 1109.4690 [M − H]$^-$ (calcd for $C_{50}H_{77}O_{27}$, 1109.4652); *anal*. C 50.29%, H 7.39%, calcd for $C_{50}H_{78}O_{27}\cdot 9/2H_2O$, C 50.37%, H 7.36%.

Preparation of 5 from 4. Compound **4** (12.0 mg) was treated with 7% NaOMe in MeOH (3 mL) at room temperature for 2 h. The reaction mixture was neutralized by passage through an Amberlite IR-120B (Organo) column and purified by Si gel column chromatography eluting with $CHCl_3$−MeOH−H_2O (20:10:1) to afford **5** (5.8 mg).

Compound 6: amorphous powder; $[\alpha]^{29}_D$ −76.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 245 nm (log ϵ 3.75); IR (film) ν_{max} 3389 (OH), 2925 (CH), 1712 and 1632 (C=O), 1416, 1377, 1158, 1075, 1039, 893 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.57 (1H, d, *J* = 7.3 Hz, H-1‴), 5.27 (1H, br d, *J* = 4.3 Hz, H-6), 5.21 (1H, d, *J* = 7.8 Hz, H-1″), 5.17 (1H, d, *J* = 7.7 Hz, H-1⁗′), 5.11 (1H, d, *J* = 7.8 Hz, H-1‴), 4.93 (1H, d, *J* = 7.7 Hz, H-1′), 4.83 (1H, d, *J* = 7.8 Hz, H-1⁗′), 3.95 and 3.59 (each 1H, m, H_2-26), 2.79 (2H, m, H_2-23), 2.13 and 1.95 (each 1H, m, H_2-15), 2.11 and

1.84 (each 1H, m, H_2-24), 1.97 (3H, s, Me-21), 0.99 (3H, d, J = 6.7 Hz, Me-27), 0.95 (3H, s, Me-19), 0.92 (3H, s, Me-18); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m/z* 1409.5808 $[M + Na]^+$ (calcd for $C_{62}H_{98}O_{34}Na$, 1409.5837); HRTOFMS (negative mode) *m/z* 1385.5923 $[M - H]^-$ (calcd for $C_{62}H_{97}O_{34}$, 1385.6861); *anal*. C 49.79%, H 7.51%, calcd for $C_{62}H_{98}O_{34}\cdot 6H_2O$, C 49.79%, H, 7.41%.

Enzymatic Hydrolysis of 6. Compound **6** (21.7 mg) was treated with β-D-glucosidase (15.2 mg, Sigma) in HOAc−NaOAc buffer (pH 5.0, 4 mL) at room temperature for 30 min. The crude hydrolysate was chromatographed on Si gel eluting with $CHCl_3$−MeOH−H_2O (20:15:1) to give **6a** (15.4 mg) and D-glucose (4.8 mg).

Compound 6a: amorphous powder; $[\alpha]^{24}_D$ −92.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 245 nm (log ϵ 3.84); IR (film) ν_{max} 3377 (OH), 2919 (CH), 1713 and 1631 (C=O), 1434, 1416, 1376, 1158, 1074, 1039, 891 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.58 (1H, d, J = 7.8 Hz, H-1‴), 5.27 (1H, br d, J = 4.4 Hz, H-6), 5.16 (1H, d, J = 7.9 Hz, H-1″), 5.15 (1H, d, J = 7.9 Hz, H-1⁗), 5.13 (1H, d, J = 7.9 Hz, H-1‴), 4.92 (1H, d, J = 7.7 Hz, H-1′), 1.96 (3H, s, Me-21), 1.07 (3H, d, J = 6.6 Hz, Me-27), 0.93 (3H, s, Me-18), 0.91 (3H, s, Me-19); ^{13}C NMR (C_5D_5N) δ 45.3, 69.6, 84.1, 37.4, 140.1, 121.4, 31.5, 30.1, 49.6, 37.8, 20.8, 35.9, 43.3, 50.3, 37.8, 205.5, 142.5, 16.6, 20.2, 145.6, 15.7, 210.7, 39.0, 27.8, 36.0, 67.4, 17.2 (C-1−C-27), 103.0, 72.6, 75.3, 79.2, 75.6, 60.6 (C-1′−C-6′), 104.3, 80.5, 86.9, 70.2, 77.4, 62.7 (C-1″−C-6″), 103.7, 74.6, 87.6, 69.6, 77.6, 62.2 (C-1‴−C-6‴), 105.2, 75.4, 77.9, 71.5, 78.3, 62.3 (C-1⁗−C-6⁗), 104.7, 75.1, 78.3, 70.6, 67.1 (C-1⁗′−C-5⁗′); FABMS (positive mode) *m/z* 1247 $[M + Na]^+$; *anal*. C 51.39%, H 7.48%, calcd for $C_{56}H_{88}O_{29}\cdot 9/2H_2O$, C 51.49%, H 7.48%.

Acetylation of 6a. Compound **6a** (13.2 mg) was acetylated with Ac_2O (1 mL) in C_5H_5N (1 mL) at room temperature for 20 h. The crude acetate was chromatographed on Si gel eluting with hexane−EtOAc (1:2) to give **6b** (14.9 mg).

Compound 6b: amorphous powder; $[\alpha]^{26}_D$ −70.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 247 nm (log ϵ 3.84); IR (film) ν_{max} 2959 and 2941 (CH), 1747 and 1632 (C=O), 1434, 1370, 1230, 1040, 903 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.45 (1H, d, J = 7.5 Hz, H-1‴), 5.38 (1H, br d, J = 4.4 Hz, H-6), 5.34 (1H, br s, H-1⁗′), 5.32 (1H, d, J = 7.9 Hz, H-1⁗), 5.09 (1H, d, J = 7.5 Hz, H-1″), 4.92 (1H, d, J = 7.9 Hz, H-1′), 2.49, 2.46, 2.39, 2.24, 2.22, 2.13 × 2, 2.11, 2.10, 2.08, 2.07 × 2, 2.00, 1.99, 1.98 × 2, 1.96 (each 3H, s, Ac), 1.97 (3H, s, Me-21), 1.10 (3H, s, Me-19), 0.92 (3H, s, Me-18), 0.91 (3H, d, J = 7.0 Hz, Me-27); FABMS (positive mode) *m/z* 1962.4 $[M + Na]^+$; *anal*. C 54.89%, H 6.48%, calcd for $C_{90}H_{122}O_{46}\cdot 3/2H_2O$, C 54.96%, H 6.41%.

Preparation of (25*R*)-2α,3*β*,26-Trihydroxyfurosta-5,16,20(22)-triene 3-*O*-Pentaglycoside Peracetate (10a) from 10. Compound **10** (59.9 mg) was treated with a mixture of Ac_2O (9 mL) and *p*-TsOH (8.8 mg) at 140 °C for 25 min under an Ar atmosphere. After addition of H_2O (10 mL) into the reaction mixture, it was extracted with Et_2O (20 mL × 3). The Et_2O extract was chromatographed on Si gel eluting with hexane−EtOAc (2:5) to give **10a** (56.0 mg).

Compound 10a: amorphous powder; $[\alpha]^{24}_D$ −38.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 226 nm (log ϵ 3.92); IR (film) ν_{max} 2960 (CH), 1750 (C=O), 1434, 1371, 1229, 1042, 903 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.45 (1H, d, J = 7.9 Hz, H-1‴), 5.42 (1H, br d, J = 5.7 Hz, H-6), 5.34 (1H, br s, H-1⁗′), 5.33 (1H, d, J = 8.2 Hz, H-1⁗), 5.05 (1H, d, J = 7.7 Hz, H-1″), 4.93 (1H, d, J = 7.9 Hz, H-1′), 2.50, 2.45, 2.40, 2.25, 2.23, 2.14 × 2, 2.12, 2.11, 2.09 × 2, 2.08, 2.01, 2.00, 1.98 × 2, 1.97 (each 3H, s, Ac), 1.97 (3H, s, Me-21), 1.14 (3H, s, Me-19), 0.91 (3H, d, J = 6.7 Hz, Me-27), 0.86 (3H, s, Me-18); FABMS (positive mode) *m/z* 1946.9 $[M + Na]^+$; *anal*. C 55.26%, H 6.48%, calcd for $C_{90}H_{122}O_{45}\cdot 2H_2O$, C 55.15%, H 6.48%.

Preparation of 6b from 10a. The CrO_3 (9.2 mg) solution in AcOH−H_2O (19:1, 8 mL) was added to **10a** (50.0 mg) dissolved in AcOH−H_2O (19:1, 2 mL), and it was stirred at room temperature for 1.5 h under an Ar atmosphere. After the excess CrO_3 was decomposed by MeOH (3 mL), the reaction mixture was diluted with H_2O (30 mL) and extracted with Et_2O (30 mL × 3). The Et_2O extract was chromatographed on Si gel eluting with hexane−EtOAc (1:4) to yield **6a** (26.6 mg).

Compound 7: amorphous powder; $[\alpha]^{29}_D$ −104.0° (*c* 0.10, MeOH); UV (MeOH) λ_{max} 247 nm (log ϵ 3.80); IR (film) ν_{max} 3417 (OH), 2918 (CH), 1712 and 1633 (C=O), 1433, 1416, 1377, 1159, 1074, 1039, 892 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.57 (1H, d, J = 7.9 Hz, H-1″), 5.26 (1H, br d, J = 4.7 Hz, H-6), 5.23 (1H, d, J = 7.8 Hz, H-1⁗), 5.19 (1H, d, J = 7.9 Hz, H-1‴), 4.93 (1H, d, J = 7.7 Hz, H-1′), 4.81 (1H, d, J = 7.8 Hz, H-1⁗′), 1.96 (3H, s, Me-21), 0.98 (3H, d, J = 6.7 Hz, Me-27), 0.94 (3H, s, Me-19), 0.91 (3H, s, Me-18); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m/z* 1247.5264 $[M + Na]^+$ (calcd for $C_{56}H_{88}O_{29}Na$, 1247.5309); HRTOFMS (negative mode) *m/z* 1223.5375 $[M - H]^-$ (calcd for $C_{56}H_{87}O_{29}$, 1223.5333); *anal*. C 51.52%, H 7.55%, calcd for $C_{56}H_{88}O_{29}\cdot 9/2H_2O$, C 51.49%, H 7.48%.

Compound 8: amorphous powder; $[\alpha]^{28}_D$ −54.0° (*c* 0.10, MeOH); IR (film) ν_{max} 3417 (OH), 2919 and 2851 (CH), 1747 (C=O), 1463, 1372, 1310, 1159, 1071, 1038, 893 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 5.57 (1H, d, J = 7.4 Hz, H-1″), 5.30 (1H, br d, J = 4.6 Hz, H-6), 5.21 (1H, d, J = 7.9 Hz, H-1‴), 5.16 (1H, d, J = 7.7 Hz, H-1⁗′), 5.10 (1H, d, J = 7.8 Hz, H-1⁗), 4.92 (1H, d, J = 7.9 Hz, H-1′), 4.89 (1H, td, J = 7.7, 4.6 Hz, H-16), 2.65 (1H, m, H-20), 2.10 and 1.40 (each 1H, m, H_2-15), 1.76 (1H, dd, J = 7.7, 6.5 Hz, H-17), 1.24 (3H, d, J = 5.4 Hz, Me-21), 0.90 (3H, s, Me-19), 0.89 (1H, m, H-14), 0.69 (3H, s, Me-18); ^{13}C NMR, see Table 1; HRTOFMS (positive mode) *m/z* 1163.4719 $[M + Na]^+$ (calcd for $C_{51}H_{80}O_{28}Na$, 1163.4734); HRTOFMS (negative mode) *m/z* 1139.4797 $[M - H]^-$ (calcd for $C_{51}H_{79}O_{28}$, 1139.4758); *anal*. C 49.78%, H 7.51%, calcd for $C_{51}H_{80}O_{28}\cdot 5H_2O$, C 49.75%, H 7.37%.

HSC-2 Cell Culture Assay. HSC-2 cells were maintained as monolayer cultures at 37 °C in Dulbecco's modified Eagle medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated FBS (JRH Biosciences, Lenexa, KS) in a humidified 5% CO_2 atmosphere. Cells were trypsinized and inoculated at 6×10^3 per each 96-microwell plate (Falcon, flat bottom, treated polystyrene, Becton Dickinson, San Jose, CA) and incubated for 24 h. After washing once with phosphate-buffered saline (PBS, 0.01 M phosphate buffer, 0.15 M NaCl, pH 7.4) supplemented with 100 U/mL penicillin (Sigma) and 100 μg/mL streptomycin (Sigma), they were treated for 24 h without or with test compounds. They were washed once with PBS and incubated for 4 h with 0.2 mg/mL MTT (Sigma) in DMEM supplemented with 10% FBS. After the medium was removed, the cells were lysed with 0.1 mL of DMSO and the relative viable cell number was determined by measuring the absorbance at 540 nm of the cell lysate, using Labsystems Multiskan (Biochromatic, Helsinki, Finland) connected to a Star/DOT Matrix printer JL-10.[11,12] The LD_{50} value, which reduces the viable cell number by 50%, was determined from the dose−response curve.

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