Steroidal Constituents from the Underground Parts of *Reineckea carnea* and Their Inhibitory Activity on cAMP Phosphodiesterase

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Phytochemical analysis of the underground parts of *Reineckea carnea* resulted in the finding of twelve steroidal constituents, five of which appeared to be new compounds and one to be the first isolation from a natural source. The identification and structural determination of the steroidal compounds were established by spectroscopic analysis and hydrolysis. The isolated saponins were evaluated for inhibitory activity on cAMP phosphodiesterase as a primary screening test to find new medicinal materials.

Keywords *Reineckea carnea*; Liliaceae; steroidal saponin; steroidal sapogenin; cAMP phosphodiesterase inhibition

The genus *Reineckea* (Liliaceae) is composed of the sole species, *Reineckea carnea*, which is indigenous to Japan and China.[1] The whole plant, including the underground parts, has been used as an antitussive, a hemostatic and an antidote in traditional Chinese medicine.[2] Several steroidal sapogenins and saponins have been already isolated from the aerial parts of the plant,[3] as well as sapogenins from the saponified methanolic extract of the whole plant.[4] We have now examined the chemical constituents of the underground parts of *R. carnea*, resulting in the isolation of twelve steroidal compounds, five of which are new compounds and one of which is the first isolation from a natural source. The identification and structural elucidation of the steroidal compounds and their inhibitory activity on cAMP phosphodiesterase are discussed in this paper.

Standard extraction and fractionation of the fresh underground parts of *R. carnea* eventually gave compounds **1**—**12**.

The structures of **1**—**8** were identified by spectroscopic analysis as (25*R*)-spirost-5-en-3β-ol (diosgenin) 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**1**),[5] 22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (**2**),[5] (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1-*O*-α-L-rhamnopyranoside 16-*O*-β-D-glucopyranoside (**3**),[6] (25*R*)-5β-spirostane-1β,3β,4β,5β-tetrol (kitigenin) (**4**),[3,4b–e,7] sodium 1β,3β,5β-trihydroxy-(25*R*)-5β-spirostan-4β-yl sulfate (**5**),[3] kitigenin 5-*O*-β-D-glucopyranoside (**6**), (25*R*)-5β-spirostane-1β,2β,3β,4β,5β-pentol (pentologenin) (**7**),[3,4b–d] and pentologenin 5-*O*-β-D-glucopyranoside (**8**),[3] respectively. Compound **3** has been previously obtained through partial acid hydrolysis of (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1-*O*-α-L-rhamnopyranoside 16-*O*-{*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside}, a cholestane bisdesmoside isolated from *Allium albopilosum*[6]; however, this is the first isolation from a natural source. Compound **6** is the C-25 isomer of (25*S*)-5β-spirostane-1β,3β,4β,5β-tetrol (convallagenin B)[8] 5-*O*-β-D-glucopyranoside, the corresponding furostanol saponin of which has been isolated from *Aspidistra elatior*.[9] To the best of our knowledge, **6** has not been reported previously. The detailed spectral data are shown in the Experimental section and Table I.

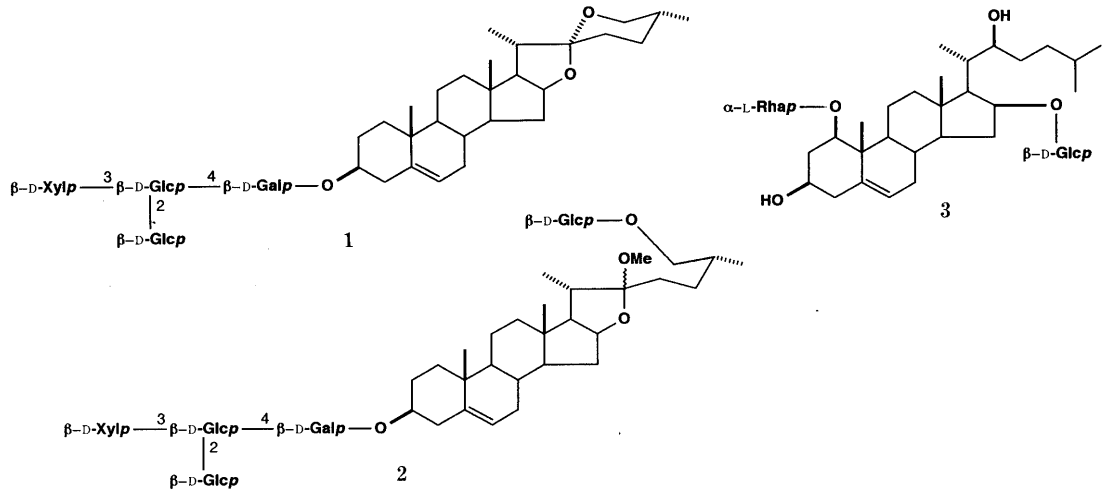


Chart 1

TABLE I. ^{13}C-NMR Spectral Data for Compounds **6**—**12**[a]

C	6	7	7[a]	8	9	10	10[a]	11	11[a]	12
1	73.4	78.2	78.2	77.8	77.8	77.7	77.8	87.3	86.7	79.6
2	33.9	67.5	67.4	68.1	68.1	67.0	67.0	68.3	68.1	67.2
3	71.9	75.7	75.6	76.3	76.2	73.8	73.7	76.0	75.7	75.5
4	68.0	68.3	68.2	67.6	67.6	74.8	75.1	68.2	68.0	69.6
5	87.9	78.0	78.0	87.5	87.5	78.1	78.2	77.7	77.6	78.7
6	24.8	30.4	30.4	24.9	25.0	30.5	30.6	29.8	29.8	69.6
7	28.5	28.4	28.5	28.5	28.5	28.2	28.3	28.3	28.4	35.4
8	34.9	34.9	35.0	34.7	34.7	34.7	34.9	35.0	35.1	30.1
9	46.7	45.4	45.5	46.3	46.3	45.4	45.5	45.8	45.8	45.5
10	47.3	45.1	45.2	46.7	46.7	45.5	45.7	45.9	46.0	45.4
11	21.6	21.7	21.8	21.7	21.7	21.6	21.7	22.0	22.1	21.5
12	40.1	40.1	40.2	40.0	39.8	39.6	39.8	40.1	40.2	40.0
13	40.6	40.7	40.8	40.6	40.9	40.5	40.7	40.7	40.8	40.7
14	56.1	56.3	56.4	56.0	56.0	55.8	56.0	56.2	56.3	56.1
15	32.2	32.2	32.3	32.2	32.2	32.1	32.2	32.2	32.3	32.3
16	81.1	81.1	81.2	81.1	81.3	81.0	81.2	81.1	81.2	81.1
17	63.0	63.1	63.2	63.0	64.4	62.9	63.0	63.1	63.1	63.1
18	16.6	16.6	16.7	16.5	16.3	16.5	16.6	16.6	16.6	16.5
19	13.7	13.8	13.8	13.7	13.7	13.7	13.7	14.2	14.1	16.2
20	42.1	42.0	42.2	42.0	40.6	42.0	42.1	42.1	42.2	42.1
21	15.0	15.0	15.0	15.0	16.4	15.0	15.0	15.0	15.0	15.0
22	109.3	109.3	109.4	109.3	112.7	109.1	109.3	109.3	109.4	109.3
23	31.9	31.8	32.0	31.9	30.9	31.8	31.9	31.9	32.0	31.8
24	29.3	29.3	29.4	29.3	28.2	29.2	29.3	29.3	29.4	29.2
25	30.6	30.6	30.8	30.6	34.2	30.6	30.7	30.6	30.7	30.6
26	66.9	66.9	67.1	66.9	75.2	66.8	67.0	66.9	67.1	66.9
27	17.3	17.3	17.4	17.3	17.1	17.3	17.3	17.3	17.4	17.3
OMe					47.3					
1′	97.5			97.5	97.5			107.1	106.8	
2′	75.9			75.9	75.9			76.0	75.7	
3′	78.6[b]			78.7[b]	78.7[b]			78.5	78.2	
4′	72.0			72.0	71.9			71.1	71.0	
5′	78.8[b]			78.8[b]	78.8[b]			67.6	67.5	
6′	62.9			62.9	62.9					
1″					105.0					
2″					75.2					
3″					78.5[c]					
4″					71.9					
5″					78.7[c]					
6″					63.0					

a) Spectra were measured in pyridine-d_5 except for **7**, **10** and **11** in pyridine-d_5–methanol-d_4 (11 : 1). *b*, *c*) Assignments with the same superscripts may be reversed in each vertical column.

Compound **9** presented a positive Ehrlich's reaction on TLC.[10] The structure of **9** based upon a 22-methoxy-furostanol saponin was suggested by a three-proton singlet signal at δ 3.27 in the ^{1}H-NMR spectrum, and a quaternary carbon signal at δ 112.7 and a methoxyl at δ 47.3 in the ^{13}C-NMR spectrum.[11] Enzymatic hydrolysis of **9** with β-glucosidase produced D-glucose and **8**. The structure of **9** was shown to be 22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5β-furostane-1β,2β,3β,4β,5β,22ξ,26-heptol 5-*O*-β-D-glucopyranoside.

The spectral features of **10**—**12** were essentially analogous to those of **7**, suggesting that they are pentologenin (**7**) derivatives.

The molecular formula of **10** was estimated as $C_{27}H_{43}NaO_{10}S$ by elemental analysis, negative-ion FAB-MS, ^{13}C-NMR spectrum and quantitative atomic flame photometry analysis. The IR spectrum of **10** showed a characteristic absorption band at 1245 cm^{-1} attributable to an S–O stretching vibration of the sulfate group, as well as a band at 3425 cm^{-1} due to hydroxyl groups. Acetylation of **10** with acetic anhydride in pyridine introduced three acetyl groups into **10** (**10a**). Acid hydrolysis of **10** with 2 N hydrochloric acid gave **7** and sulfuric acid,[12] indicating that **10** is a sulfate of **7**. To clarify the linkage position of sulfuric acid, precise assignments of the ^{1}H and ^{13}C signals of **7** and **10** had to be established. In the ^{1}H-NMR spectrum of **7**, a three-proton singlet signal at δ 1.59 was assigned to the H-19 methyl group, which was shifted to a lower field by *ca.* 0.5 ppm compared to that of (25*S*)-5β-spirostan-3β-ol (sarsasapogenin)[13] by interaction with the 1β- and 5β-hydroxyl groups. In the proton detected heteronuclear multiple-bond connectivity (HMBC) spectrum of **7**, the H-19 signal showed $^2J_{C-H}$ and $^3J_{C-H}$ correlations with the ^{13}C signals at δ 78.0 (C–OH), 78.2 (CH–OH), 45.2 (C) and 45.5 (CH), leading to their assignments as C-5, C-1, C-10 and C-9, respectively. The signal at δ 78.2 (C-1) was correlated to the ^{1}H signal at δ 4.30 (d, J = 3.3 Hz) in the proton detected heteronuclear multiple quantum coherence (HMQC) spectrum. By tracing out the $^3J_{H-H}$ correlations, starting from the signal at δ 4.30 in the ^{1}H–^{1}H shift correlation spectroscopy (COSY) spectrum,

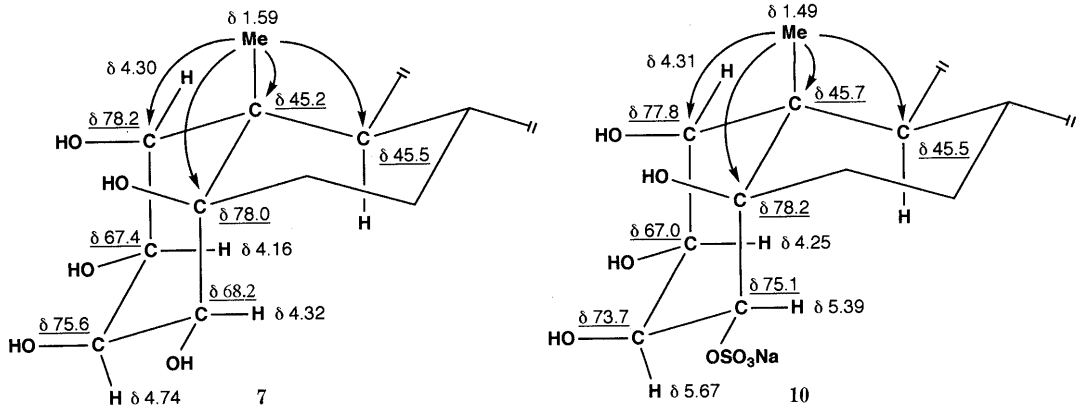
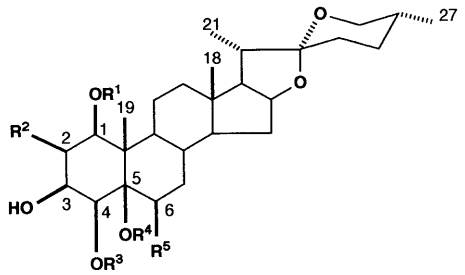
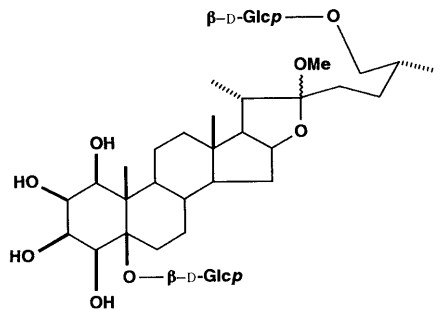


Fig. 1. ^{1}H- and ^{13}C-NMR (Underlined Figures) Assignments of **7** and **10** in Pyridine-d_5–Methanol-d_4 (11 : 1)
The arrows indicated ^{1}H–^{13}C long-range correlations observed in the HMBC spectrum.



	R^1	R^2	R^3	R^4	R^5
4	H	H	H	H	H
5	H	H	SO_3Na	H	H
6	H	H	H	β-D-Glc*p*	H
7	H	HO	H	H	H
8	H	HO	H	β-D-Glc*p*	H
10	H	HO	SO_3Na	H	H
11	β-D-Xyl*p*	HO	H	H	H
12	H	HO	H	H	OH



9

Chart 2

the resonances at δ 4.16 (dd, J=3.3, 3.3 Hz), 4.74 (dd, J=3.3, 3.3 Hz) and 4.32 (d, J=3.3 Hz) were assigned to H-2, H-3 and H-4, which were correlated to the ^{13}C signals at δ 67.4 (C-2), 75.6 (C-3) and 68.2 (C-4) in the HMQC spectrum. The ^{1}H and ^{13}C signals of **10** were also assigned by the same procedures [^{1}H-NMR: δ 4.31, 4.25, 5.67 and 5.39 (H-1—H-4); ^{13}C-NMR: δ 77.8, 67.0, 73.7 and 75.1 (C-1—C-4)] (Fig. 1). On comparison of the assignments of **10** with those of **7**, the H-4 proton and C-4 carbon signals appeared at lower fields by 1.07 and 6.9 ppm, respectively, accounting for the sulfate group linkage to the C-4 hydroxy position (Fig. 1). From the data presented above, the structure of **10** was determined to be sodium 1β,2β,3β,5β-tetrahydroxy-(25*R*)-5β-spirostan-4β-yl sulfate.

The glycosidic nature of **11** was indicated by strong absorption bands at 3400 and 1045 cm^{-1} in the IR spectrum of **11**. The presence of a β-xylopyranosyl moiety was demonstrated by the appearance of an anomeric proton signal at δ 5.45 (d, J=7.7 Hz) in the ^{1}H-NMR spectrum, and also by five characteristic ^{13}C signals at δ 106.8, 75.7, 78.2, 71.0 and 67.5. Acid hydrolysis of **11** with 1 N hydrochloric acid gave **7** and D-xylose. Confirmative assignments of the NMR signals of **11** were performed by a combined use of 2D-NMR spectra, as in the case of **7** and **10**. The ^{13}C signal due to C-1 was shifted to a lower field by 8.5 ppm as compared with that of **7**. Thus, the xylopyranosyl moiety was concluded to be linked at the C-1 hydroxy position, and the full structure of **11** was determined to be (25*R*)-5β-spirostane-1β,2β,3β,4β,5β-pentol 1-*O*-β-D-xylopyranoside.

Compound **12**, which has the molecular formula, $C_{27}H_{44}O_8$, deduced from high-resolution positive-ion FAB-MS, has one more oxygen atom than **7**. In the ^{1}H-NMR spectrum of **12**, the signal attributable to the H-19 methyl protons appeared at δ 1.95, which was shifted downfield by 0.30 ppm as compared with that of **7**, whereas the signal due to the C-18 methyl remained almost unaffected, accounting for the introduction of a 6β-axial hydroxyl group. Furthermore, on comparison of the ^{13}C-NMR spectrum with that of **7**, the signal due to the C-6 methylene carbon, which was observed at δ 30.4 in **7**, was displaced by the signal due to a hydroxymethine carbon at δ 69.6 in **12**, accompanied by downfield shifts of the signals due to C-5 (+ 0.7 ppm), C-7 (+7.0 ppm) and C-19 (+2.3 ppm) and by an upfield shift of C-8 (−4.8 ppm). Accordingly, the structure of **12** was formulated as (25*R*)-5β-spirostane-1β,2β,3β,4β,5β,6β-hexol.

Compounds **6** and **9**—**12** have not been reported previously.

The isolated steroidal compounds were evaluated for inhibitory activity on cAMP phosphodiesterase as a

TABLE II. Inhibitory Activity on cAMP Phosphodiesterase of the Isolated Steroidal Saponins

Compounds	IC_{50} ($\times 10^{-5}$ M)
1	11.7
2	32.2
3	27.8
4	17.9
5	157
6	32.5
7	33.3
8	500<
9	37.6
10	211
11	2.7
12	10.4
Papaverine	3.0

primary screening test to find new medicinal materials.[14] The IC_{50} values are listed in Table II. Compounds **4**, **7** and **12**, 5β-spirostans with several hydroxyl groups attached at the A and B rings, exhibited relatively potent inhibitory activity. Introduction of a glucosyl group to the C-5 hydroxyl group and a sulfonyl group to C-4 in **4** and **7** markedly reduced the activity. Compound **11**, which was the C-1 xyloside of **7**, was a potent inhibitor, showing almost equal IC_{50} values compared to papaverine, which was used as a positive control. In our previous papers,[15] we reported that several steroidal saponins exhibited potent inhibitory activity on phosphodiesterase, but that the corresponding 22-hydroxy-26-glucosyloxyfurostanol saponins were poor inhibitors; **2** was less potent than **1**, which was an example in point. Inconsistently, **9**, a furostanol saponin with several hydroxyl groups attached at the A ring, exhibited medium activity while the corresponding spirostanol saponin (**8**) showed no activity. Detailed structure-inhibition relationships will be reported in the near future.

Experimental

Optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 instrument and MS on a VG AutoSpec E machine. Atomic flame photometry was performed on a Hitachi Z-8100 polarized Zeeman atomic absorption spectrophotometer. Elemental analysis was carried out with a Perkin-Elmer 240B elemental analyzer for C and H analysis, and with a Dionex QIC ion-chromatography system for S analysis. NMR spectra were taken with a Bruker AM-400 spectrometer for 1D-NMR and a JEOL EX-400 or a Bruker AM-500 for 2D-NMR. Chemical shifts are given in δ-value, referring to tetramethylsilane (TMS) as the internal standard. Silica-gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-Kasei), Sephadex LH-20 (Pharmacia) and octadecylsilanized (ODS) silica-gel (Nacalai Tesque) were used for column chromatographies. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick for analytical TLC and 0.5 mm thick for preparative TLC, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck), and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed on a Tosoh HPLC system (Tosoh: pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010 or UV-8000) equipped with a Kaseisorb LC-120-5 column (Tokyo-Kasei-Kogyo: 10 mm i.d. × 250 mm, ODS, 5 μm) for preparative HPLC and a TSK-gel Silica-60 column (Tosoh: 4.6 mm i.d. × 250 mm, silica-gel, 5 μm) for analysis of the sugar derivative. The liquid scintillation counter used was an Aloka LSC-903 instrument. Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase and cAMP were obtained from Sigma, and [^{3}H]cAMP from Radiochemical Center.

Isolation Fresh underground parts of *R. carnea* (10 kg) collected at Minami-izu, Shizuoka Prefecture, Japan, were extracted with hot MeOH. After removing the solvent under reduced pressure, the MeOH extract was diluted with H_2O and extracted with *n*-BuOH. The *n*-BuOH-soluble phase was chromatographed on silica-gel, using CH_2Cl_2–MeOH mixtures of increasing polarity (9 : 1, 4 : 1, 2 : 1, 1 : 1) and finally, MeOH, to give seven fractions (I—VII).

Fraction II was subjected to silica-gel column chromatography with $CHCl_3$–MeOH–H_2O–25% NH_3 solution (450 : 50 : 3 : 2) and ODS silica-gel with MeOH–H_2O (4 : 1) to give compound **7** (54.6 mg).

Chromatography of fraction III on silica-gel with $CHCl_3$–MeOH–H_2O (70 : 10 : 1, 60 : 10 : 1) and $CHCl_3$–MeOH–H_2O–25% NH_3 solution (250 : 50 : 3 : 2, 200 : 50 : 3 : 2), and on ODS silica-gel with MeOH–H_2O (17 : 3) gave compounds **4** (8.0 mg), **5** (878 mg) and **12** (56.7 mg), respectively.

Fraction IV was passed through a Diaion HP-20 column with H_2O gradually enriched with MeOH. The MeOH eluate portion was further fractionated by silica-gel column chromatography with $CHCl_3$–MeOH–H_2O–25% NH_3 solution (300 : 100 : 1 : 3), giving four fractions (IVa—d). Fraction IVb was subjected to column chromatography on ODS silica-gel with MeOH–H_2O (4 : 1) and silica-gel with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) to yield compound **8** (1.14 g) and a mixture of compounds **6** and **11**, which were separated by preparative TLC with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) to give **6** (30.4 mg) and **11** (20.6 mg) as the pure compounds. Fraction IVd was purified by passing it through a Sephadex LH-20 column with MeOH to yield compound **10** (320 mg).

Fraction V was passed through a Diaion HP-20 column and the MeOH eluate fraction was chromatographed on silica-gel with $CHCl_3$–MeOH–H_2O (30 : 10 : 1), ODS silica-gel with MeOH–H_2O (4 : 1) and on Sephadex LH-20 with MeOH, and finally subjected to preparative HPLC with MeOH–H_2O (4 : 1) to give compound **3** (218 mg).

Fraction VI was passed through a Diaion HP-20 column and the MeOH eluate fraction was purified by silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (30 : 10 : 1, 7 : 4 : 1) to give compound **1** (1.38 g).

Fraction VII was subjected to silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (30 : 10 : 1, 7 : 4 : 1) and ODS silica-gel with MeOH–H_2O (4 : 1) to give compounds **2** (5.03 g) and **9**; the latter contained a few impurities and was further purified by preparative HPLC with MeOH–H_2O (1 : 1) to give **9** (620 mg) as the pure compound.

Compound 6 A white amorphous powder, $[\alpha]_D^{25}$ −38.0° (c=0.10, MeOH). Negative-ion FAB-MS m/z: 625 [M−H]$^-$, 463 [M−glucosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2940 (CH), 1445, 1375, 1240, 1200, 1145, 1100, 1070, 1040, 980, 955, 915, 895, 875, 860, 800 (intensity 915<895, (25*R*)-spiroacetal). ^{1}H-NMR (pyridine-d_5) δ: 5.17 (1H, d, J=7.7 Hz, H-1′), 4.54 (1H, q-like, J=7.3 Hz, H-16), 4.42 (1H, q-like, J=3.8 Hz, H-3), 4.37 (1H, dd, J=11.7, 2.2 Hz, H-6′a), 4.26 (1H, d, J=3.8 Hz, H-4), 4.14 (1H, dd, J=11.7, 5.4 Hz, H-6′b), 4.09 (1H, dd, J=8.6, 8.6 Hz, H-3′), 4.07 (1H, dd, J=3.8, 3.8 Hz, H-1), 3.94 (1H, dd, J=8.6, 8.6 Hz, H-4′), 3.90 (1H, ddd, J=8.6, 5.4, 2.2 Hz, H-5′), 3.84 (1H, dd, J=8.6, 7.7 Hz, H-2′), 3.56 (1H, dd, J=10.6, 3.2 Hz, H-26a), 3.47 (1H, dd, J=10.6, 10.6 Hz, H-26b), 1.61 (3H, s, H-19), 1.11 (3H, d, J=6.9 Hz, H-21), 0.84 (3H, s, H-18), 0.73 (3H, d, J=5.7 Hz, H-27).

Compound 7 A white amorphous powder, $[\alpha]_D^{25}$ −59.2° (c=0.84, $CHCl_3$–MeOH (1 : 1)). Negative-ion FAB-MS m/z: 479 [M−H]$^-$, 153. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2930 (CH), 1435, 1380, 1340, 1245, 1200, 1175, 1115, 1055, 1005, 980, 960, 920, 900, 865, 805 (intensity 920<900, (25*R*)-spiroacetal). ^{1}H-NMR (pyridine-d_5) δ: 4.82 (1H, dd, J=3.2, 3.2 Hz, H-3), 4.59 (1H, q-like, J=7.1 Hz, H-16), 4.38—4.37 (2H, overlapping, H-1 and H-4), 4.22 (1H, dd, J=3.2, 3.2 Hz, H-2), 3.60 (1H, dd, J=10.5, 3.5 Hz, H-26a), 3.52 (1H, dd, J=10.5, 10.5 Hz, H-26b), 1.65 (3H, s, H-19), 1.13 (3H, d, J=6.9 Hz, H-21), 0.87 (3H, s, H-18), 0.70 (3H, d, J=5.6 Hz, H-27). ^{1}H-NMR (pyridine-d_5–methanol-d_4, 11 : 1) δ: 4.74 (1H, dd, J=3.3, 3.3 Hz, H-3), 4.55 (1H, q-like, J=6.9 Hz, H-16), 4.32 (1H, d, J=3.3 Hz, H-4), 4.30 (1H, d, J=3.3 Hz, H-1), 4.16 (1H, dd, J=3.3, 3.3 Hz, H-2), 3.57 (1H, dd, J=10.6, 2.7 Hz, H-26a), 3.48 (1H, dd, J=10.6, 10.6 Hz, H-26b), 1.59 (3H, s, H-19), 1.12 (3H, d, J=6.9 Hz, H-21), 0.85 (3H, s, H-18), 0.71 (3H, d, J=5.8 Hz, H-27).

Compound 9 A white amorphous powder, $[\alpha]_D^{25}$ −51.0° (c=0.10, MeOH). Negative-ion FAB-MS m/z: 836 [M]$^-$, 673 [M−glucosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3405 (OH), 2930 (CH), 1450, 1375, 1305, 1255, 1050, 885, 800, 700. ^{1}H-NMR (pyridine-d_5) δ: 5.32 (1H, d, J=7.7 Hz, H-1′), 4.85 (1H, d, J=7.7 Hz, H-1″), 4.50 (1H, q-like, J=7.0 Hz, H-16), 3.27 (3H, s, OMe), 1.73 (3H, s, H-19), 1.18 (3H, d, J=6.8 Hz, H-21), 1.01 (3H, d, J=6.6 Hz, H-27), 0.82 (3H, s, H-18).

Enzymatic Hydrolysis of 9 Compound **9** (20 mg) was dissolved in an

AcOH/AcONa buffer (pH 5) with β-glucosidase (Tokyo-Kasei-Kogyo) (20 mg) and the mixture was incubated at room temperature for 5 h. The reaction mixture was subjected to silica-gel column chromatography, eluted first with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and then with MeOH to give compound **8** (11.5 mg) and D-glucose. D-Glucose was identified by direct TLC comparison with an authentic sample: *Rf* 0.42 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1).

Compound 10 A white amorphous powder, $[\alpha]_D^{25}$ −52.0° (*c* = 0.10, $CHCl_3$–MeOH (1 : 1)). *Anal.* Calcd for $C_{27}H_{43}O_{10}NaS$: C, 55.56; H, 7.60; S, 5.49. Found: C, 54.72; H, 7.67; S, 5.56. Negative-ion FAB-MS *m/z*: 559 $[M-Na]^-$, 479 $[M-SO_3Na]^-$. IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2940 (CH), 1450, 1400, 1245, 1180, 1150, 1105, 1060, 1030, 990, 960, 900, 880, 850, 785, 760, 705. ^{1}H-NMR (pyridine-d_5) δ: 5.69 (1H, dd, *J* = 3.5, 3.5 Hz, H-3), 5.44 (1H, d, *J* = 3.5 Hz, H-4), 4.55 (1H, q-like, *J* = 7.4 Hz, H-16), 4.33 (1H, d, *J* = 3.5 Hz, H-1), 4.20 (1H, dd, *J* = 3.5, 3.5 Hz, H-2), 3.59 (1H, dd, *J* = 10.5, 2.8 Hz, H-26a), 3.51 (1H, dd, *J* = 10.5, 10.5 Hz, H-26b), 1.59 (3H, s, H-19), 1.13 (3H, d, *J* = 6.9 Hz, H-21), 0.81 (3H, s, H-18), 0.68 (3H, d, *J* = 5.2 Hz, H-27). ^{1}H-NMR (pyridine-d_5–methanol-d_4, 11 : 1) δ: 5.67 (1H, dd, *J* = 3.5, 3.5 Hz, H-3), 5.39 (1H, d, *J* = 3.5 Hz, H-4), 4.54 (1H, q-like, *J* = 7.4 Hz, H-16), 4.31 (1H, d, *J* = 3.5 Hz, H-1), 4.25 (1H, dd, *J* = 3.5, 3.5 Hz, H-2), 3.59 (1H, br d, *J* = 10.3 Hz, H-26a), 3.51 (1H, dd, *J* = 10.3, 10.3 Hz, H-26b), 1.49 (3H, s, H-19), 1.12 (3H, d, *J* = 6.8 Hz, H-21), 0.79 (3H, s, H-18), 0.69 (3H, d, *J* = 5.2 Hz, H-27).

Acetylation of 10 Compound **10** (20 mg) was acetylated with Ac_2O in pyridine and the crude acetate was purified by silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) to give the corresponding triacetate (**10a**) of **10** (15.5 mg) as a white amorphous powder. Compound **10a**: Negative-ion FAB-MS *m/z*: 685 $[M-Na]^-$, 643 $[M-Na-Ac]^-$. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2940 (CH), 1740 (C=O), 1445, 1365, 1230, 1170, 1140, 1095, 1045, 1025, 1000, 970, 890, 830, 740, 695. ^{1}H-NMR (pyridine-d_5) δ: 6.90 (1H, dd, *J* = 3.7, 3.7 Hz, H-3), 5.98 (1H, d, *J* = 3.7 Hz, H-1), 5.68 (1H, d, *J* = 3.7, 3.7 Hz, H-2), 5.62 (1H, d, *J* = 3.7 Hz, H-4), 4.51 (1H, q-like, *J* = 7.1 Hz, H-16), 3.58 (1H, dd, *J* = 10.6, 3.0 Hz, H-26a), 3.53 (1H, dd, *J* = 10.6, 10.6 Hz, H-26b), 2.18, 2.01, 1.96 (each 3H, s, Ac), 1.33 (3H, s, H-19), 1.12 (3H, d, *J* = 6.9 Hz, H-21), 0.80 (3H, s, H-18), 0.68 (3H, d, *J* = 5.3 Hz, H-27).

Acid Hydrolysis of 10 Compound **10** (20 mg) was treated with 2 N HCl in dioxane–H_2O (1 : 1) at 100 °C for 2 h. The reaction mixture was passed through a Sep-Pak C_{18} cartridge (Waters), and eluted successively with H_2O (20 ml) and MeOH (20 ml). The MeOH eluate fraction was chromatographed on silica-gel with $CHCl_3$–MeOH (4 : 1) to give compound **7** (9.6 mg). The H_2O phase was examined by paper chromatography (Toyo Roshi, No 50) employing *n*-BuOH–MeOH–H_2O (1 : 3 : 1). H_2SO_4 was detected as a light yellow spot when spraying the paper with a solution of $BaCl_2$ (100 mg/50 ml in 70% MeOH) followed by spraying with a solution of potassium rhodizonate (10 mg/50 ml in 50% MeOH).

Quantitative Analysis of Na of 10 Compound **10** (0.8 mg, 1.3×10^{-6} mol) was dissolved in 0.2 ml of concentrated HCl, to which was added H_2O to make a volume of 10 ml. The Na content of the sample solution was determined by atomic flame photometry analysis. The lamp was filled with Ar gas and the Na element was ionized in an air-acetylene flame (wavelength: 589 nm; slit width: 0.4 nm). The calibration curve of Na was obtained from the known concentrations of standard Na solutions (Wako Pure Chemical Industries). The sample solution was shown to contain 3.53 ppm of Na (1.5×10^{-6} mol in 10 ml).

Compound 11 A white amorphous powder, $[\alpha]_D^{25}$ −29.0° (*c* = 0.10, $CHCl_3$–MeOH (1 : 1)). Negative-ion FAB-MS *m/z*: 612 $[M]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2940 (CH), 1410, 1240, 1045, 975, 955, 915, 895, 860 (intensity 915 < 895, (25*R*)-spiroacetal). ^{1}H-NMR (pyridine-d_5) δ: 5.44 (1H, d, *J* = 7.7 Hz, H-1′), 4.81 (1H, dd, *J* = 3.2, 3.2 Hz, H-3), 4.58 (1H, q-like, *J* = 7.5 Hz, H-16), 4.53 (1H, d, *J* = 3.2 Hz, H-1), 4.39 (1H, dd, *J* = 10.6, 3.9 Hz, H-5′a), 4.34 (1H, dd, *J* = 3.2, 3.2 Hz, H-2), 4.29 (1H, d, *J* = 3.2 Hz, H-4), 4.22—4.13 (2H, overlapping, H-3′ and H-4′), 4.02 (1H, dd, *J* = 8.4, 7.7 Hz, H-2′), 3.73 (1H, dd, *J* = 10.6, 10.6 Hz, H-5′b), 3.59 (1H, dd, *J* = 10.4, 3.0 Hz, H-26a), 3.51 (1H, dd, *J* = 10.4, 10.4 Hz, H-26b), 1.68 (3H, s, H-19), 1.12 (3H, d, *J* = 6.9 Hz, H-21), 0.87 (3H, s, H-18), 0.70 (3H, d, *J* = 5.3 Hz, H-27). ^{1}H-NMR (pyridine-d_5–methanol-d_4, 11 : 1) δ: 5.45 (1H, d, *J* = 7.7 Hz, H-1′), 4.81 (1H, dd, *J* = 2.9, 2.9 Hz, H-3), 4.58 (1H, q-like, *J* = 7.3 Hz, H-16), 4.56 (1H, d, *J* = 2.9 Hz, H-1), 4.39 (1H, dd, *J* = 10.5, 3.2 Hz, H-5′a), 4.34 (1H, dd, *J* = 2.9, 2.9 Hz, H-2), 4.30 (1H, d, *J* = 2.9 Hz, H-4), 4.22—4.13 (2H, overlapping, H-3′ and H-4′), 4.02 (1H, dd, *J* = 7.9, 7.7 Hz, H-2′), 3.73 (1H, dd, *J* = 10.5, 10.5 Hz, H-5′b), 3.58 (1H, dd, *J* = 10.2, 3.3 Hz, H-26a), 3.51 (1H, dd, *J* = 10.2, 10.2 Hz, H-26b), 1.69 (3H, s, H-19), 1.12 (3H, d, *J* = 6.9 Hz, H-21), 0.87 (3H, s, H-18), 0.70 (3H, d, *J* = 5.3 Hz, H-27).

Acid Hydrolysis of 11 A solution of **11** (5 mg) in 1 N HCl (dioxane–H_2O, 1 : 1) was heated at 100 °C for 2 h. The reaction mixture was neutralized by an Amberlite IRA-93ZU (Organo) column and separated into sapogenin fraction and sugar fraction by passing it through Sep-Pak C_{18} cartridge eluted successively with H_2O (10 ml) and MeOH (10 ml). The sapogenin fraction was purified by silica-gel column chromatography with $CHCl_3$–MeOH (4 : 1) to give compound **7** (2.2 mg). The sugar fraction was diluted with H_2O (1 ml) and treated with (−)-α-methylbenzylamine (5 mg) and $Na[BH_3CN]$ (8 mg) in EtOH (1 ml) at 40 °C for 4 h, followed by acetylation with Ac_2O (0.3 ml) in pyridine (0.3 ml). The reaction mixture was passed through a Sep-Pak C_{18} cartridge, initially with H_2O–MeCN (4 : 1) (10 ml), and then with MeCN (10 ml). The MeCN eluate fraction was further passed through a TOYOPAK IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative of the monosaccharide, which was analyzed by HPLC under the following conditions: solvent, hexane–EtOH (19 : 1); flow rate, 0.8 ml/min; detection, UV 230 nm. The derivative of D-xylose was detected (t_R 21.45 min).

Compound 12 A white amorphous powder, $[\alpha]_D^{25}$ −58.4° (*c* = 0.25, $CHCl_3$–MeOH (2 : 1)). Positive-ion FAB-MS *m/z*: 497.3089 ($[M+H]^+$, Calcd for $C_{27}H_{45}O_8$: 497.3114), 153. IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 2940 and 2870 (CH), 1445, 1375, 1335, 1315, 1240, 1200, 1175, 1145, 1100, 1050, 1005, 980, 965, 915, 895, 875, 860, 800 (intensity 915 < 895, (25*R*)-spiroacetal). ^{1}H-NMR (pyridine-d_5) δ: 4.90 (1H, dd, *J* = 3.0, 3.0 Hz, H-6), 4.78 (1H, dd, *J* = 3.9, 3.2 Hz, H-3), 4.59 (1H, q-like, *J* = 7.0 Hz, H-16), 4.33 (1H, d, *J* = 3.2 Hz, H-1), 4.30 (1H, d, *J* = 3.9 Hz, H-4), 4.24 (1H, dd, *J* = 3.2, 3.2 Hz, H-2), 3.58 (1H, dd, *J* = 10.6, 3.8 Hz, H-26a), 3.50 (1H, dd, *J* = 10.6, 10.6 Hz, H-26b), 1.95 (3H, s, H-19), 1.13 (3H, d, *J* = 6.9 Hz, H-21), 0.88 (3H, s, H-18), 0.69 (3H, d, *J* = 5.7 Hz, H-27).

Assay of cAMP Phosphodiesterase Activity The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously.[14b,c] The assay consisted of a two-step isotopic procedure. Tritium-labelled cAMP was hydrolyzed to 5′-AMP by phosphodiesterase, and the 5′-AMP was then further hydrolyzed to adenosine by snake venom nucleotidase. The hydrolysate was treated with an anion-exchange resin (Dowex AG1-X8; Bio-Rad) to adsorb all charged nucleotides and to leave [^{3}H]adenosine as the only labelled compound to be counted.

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