Agapanthussaponins A—D, New Potent cAMP Phosphodiesterase Inhibitors from the Underground Parts of *Agapanthus inapertus*

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The saponin fraction prepared from the methanolic extract of the underground parts of *Agapanthus inapertus* was found to exhibit inhibitory activity on cAMP phosphodiesterase (55.2%) at a concentration of 100 μg/ml. From this fraction, four new steroidal saponins, designated as agapanthussaponin A (1), B (2), C (3) and D (4), were isolated as the active principles, along with an inactive new furostanol saponin (5). The structures of 1—5 were determined by spectroscopic data and hydrolysis. The IC_{50} of 1—4 on cAMP phosphodiesterase were 0.7, 1.2, 1.1 and 2.0 ($\times 10^{-5}$ M), respectively, which are more potent than that of papaverine (IC_{50} 3.0×10^{-5} M).

Keywords *Agapanthus inapertus*; Liliaceae; steroidal saponin; agapanthussaponin; cAMP phosphodiesterase; inhibitory activity

Cyclic adenosine 3′,5′-monophosphate (cAMP) plays an important role as a "second messenger" in various biological processes. As we pointed out in the preceding papers,[1] the cAMP phosphodiesterase inhibition test provides a useful tool for the screening of biologically active compounds present in natural sources. Recently, we reported a number of new steroidal glycosides, including steroidal alkaloids, from plants of the Liliaceae family, some of which exhibited potent inhibitory activity on cAMP phosphodiesterase.[2] During the course of our systematic survey of the bioactive constituents from the Liliaceae plants, four new steroidal saponins, named agapanthussaponin A (**1**), B (**2**), C (**3**) and D (**4**), were isolated from the methanolic extract of the underground parts of *Agapanthus inapertus* as potent cAMP phosphodiesterase inhibitors, together with an inactive new furostanol saponin (**5**). Plants of the genus *Agapanthus*, with about 20 species, are distributed mainly in South Africa and are thought to be taxonomically related to the genus *Allium*.[3] No chemical work appears to have been done on *A. inapertus* until now. This paper describes the isolation and structural elucidation of the new saponins and their inhibitory activity on cAMP phosphodiesterase.

The underground parts of *A. inapertus* were extracted with hot methanol, and the extract showed considerable inhibitory activity on cAMP phosphodiesterase (77.0%) at a concentration of 100 μg/ml. The methanolic extract was partitioned between 1-butanol and H_2O. The saponin fraction prepared by the chromatographic fractionation of the 1-butanol-soluble phase showed 55.2% inhibition of phosphodiesterase. A series of chromatographic separations of the saponin fraction gave five compounds (**1**—**5**).

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D$ −62.0° (methanol). It formed a soapy lather when shaken with water and gave a positive coloration in the Liebermann–Burchard reaction, suggesting **1** to be a steroidal glycoside. The molecular formula, $C_{45}H_{74}O_{19}$, was determined by elemental analysis, negative-ion FAB-MS (*m/z* 917 $[M-H]^-$) and ^{13}C-NMR spectrum. The IR spectrum featured a strong absorption at 3390 cm^{-1} due to hydroxyl groups, and characteristic absorptions at 975, 915, 895 and 860 cm^{-1}, with the absorption at 895 cm^{-1} being of greater intensity than that at 915 cm^{-1}, implying the presence of a (25*R*)-spiroacetal moiety in the molecule.[4] The ^1H-NMR spectrum of **1** showed signals for two tertiary methyl groups at δ 1.18 and 0.87 (each s), three secondary methyl groups at δ 1.72 (d, *J*=6.2 Hz), 1.12 (d, *J*=7.0 Hz) and 0.69 (d, *J*=5.4 Hz), and three anomeric protons at δ 6.33 (br s), 4.97 (d, *J*=7.8 Hz) and 4.85 (d, *J*=7.2 Hz). The signal at δ 1.72 was due to the methyl group of 6-deoxyhexopyranose.[2a] The ^{13}C-NMR spectrum showed

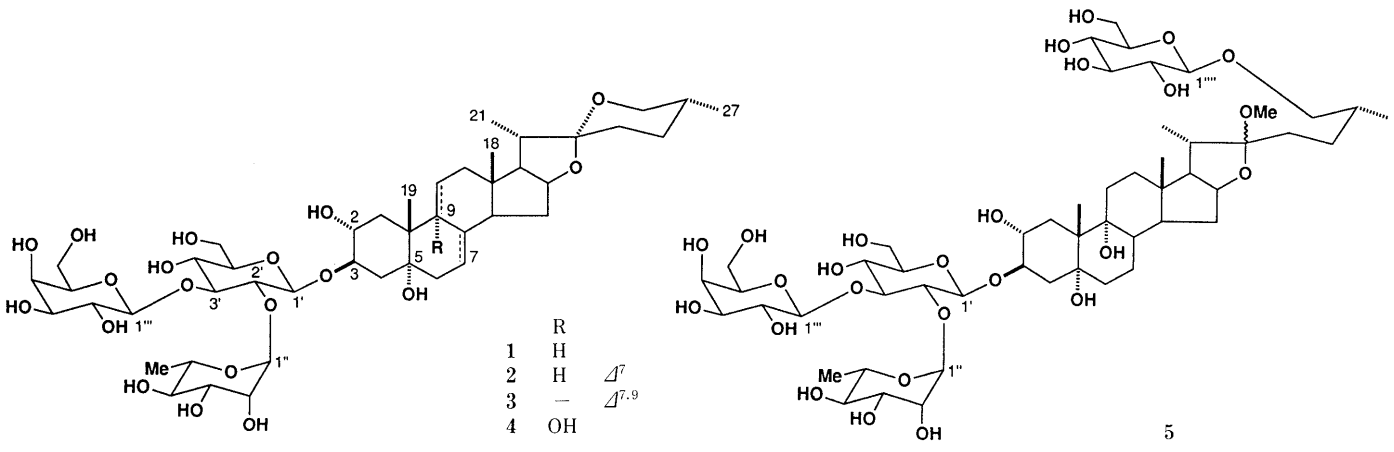

Chart 1

four signals in the field lower than 100 ppm; the signals at δ 105.1, 102.2 and 100.8 were due to the anomeric carbons, and the signal at δ 109.2 was assignable to the C-22 carbon of the spirostan skeleton.[5] The above data were consistent with **1** being a (25*R*)-spirostanol trisaccharide.

Acid hydrolysis of **1** with 1 N hydrochloric acid in dioxane–H_2O (1:1) at 80 °C for 1.5 h gave a steroidal sapogenin ($C_{27}H_{44}O_5$) (**1a**) together with D-glucose, D-galactose and L-rhamnose. The ^{13}C-NMR spectrum of **1a** showed seven signals between 60—110 ppm; the signals at δ 109.2 (C), 81.3 (CH), 66.9 (CH_2), 63.1 (CH) were readily assigned to the C-22, C-16, C-26 and C-17 carbons of the spirostan skeleton,[5] and the signals at δ 73.9 (C), 73.7 (CH) and 73.3 (CH) were presumed to be due to the carbons bearing hydroxyl groups. The presence of three hydroxyl groups in **1a** was confirmed by the ^{1}H-NMR signals at δ 6.12 (br), 6.02 (br) and 5.25 (br s) in pyridine-d_5, which disappeared upon the addition of methanol-d_4, accompanied by the signals at δ 4.75 and 4.29 being simplified. The above data indicated **1a** to be a (25*R*)-spirostan bearing two secondary hydroxyl groups and a tertiary hydroxyl group. Double resonance experiments in the ^{1}H-NMR spectrum of **1a** verified the presence of a $-CH_2-CH(OH)-CH(OH)-CH_2-$ group in **1a** (Fig. 1). Acid hydrolysis of **1** with 1 N hydrochloric acid at 100 °C for 3 h gave a less polar sapogenin (**1b**) than **1a**, which must have been an artifact produced through the dehydration of **1a** and was identified as (25*R*)-spirost-5-ene-2α,3β-diol (yuccagenin) on the basis of spectroscopic evidence.[6] These spectral data indicated a substitution by the hydroxyl groups at the C-2, C-3 and C-5 positions in **1a**. It was evident from the *J* values of the H-2 and H-3 protons ($J_{H2-Hlaxial}$ = 11.5 Hz, J_{H2-H3} = 9.1 Hz and $J_{H3-H4axial}$ = 11.3 Hz in pyridine-d_5–methanol-d_4, 10:1) that the C-2 and C-3 hydroxyl groups have α-equatorial and β-equatorial configurations, respectively. Comparison of the ^{13}C-NMR spectrum of **1a** with that of (25*R*)-5α-spirostane-2α,3β-diol (gitogenin)[5,7] revealed the presence of a 5α-hydroxyl group, which caused marked upfield shifts of the signals due to C-1, C-3, C-7 and C-9 by 5.8, 3.0, 5.8 and 8.9 ppm, respectively, in **1a**. Thus, the structure of **1a** turned out to be (25*R*)-5α-spirostane-2α,3β,5α-triol.

Assignments of the ^{13}C-NMR signals of the saccharide moiety of **1** were performed by comparison with those of authentic methyl glycosides, taking into account the downfield shifts due to *O*-glycosylation,[5,8] which indicated the existence of a terminal α-L-rhamnopyranosyl unit (δ 102.2, 72.4, 72.8, 74.1, 69.6 and 18.6), a terminal β-D-galactopyranosyl unit (δ 105.1, 72.4, 75.2, 70.1, 77.4 and 62.1) and a 2,3-disubstituted β-D-glucopyranosyl unit (δ 100.8, 77.2, 89.3, 69.6, 77.8 and 62.2) in **1**. The ^{1}H-NMR spectrum of the decaacetate derivative (**1c**) of **1**, which was prepared with acetic anhydride in pyridine, offered further support for the above findings. The H-2 and H-3 methine protons of the inner glucose moiety of **1c** appeared at δ 4.04 (dd, *J* = 9.8, 7.9 Hz) and 4.36 (dd, *J* = 9.8, 9.8 Hz), whereas the other hydroxymethine and hydroxymethylene protons appeared downfield by *O*-acetylation; the assignments were carried out through double resonance experiments, starting with the anomeric protons. Mild hydrolysis of **1** with 0.2 N hydrochloric acid at 100 °C for 30 min gave L-rhamnose and a partial hydrolysate (**1d**), the ^{13}C-NMR spectrum of which showed the presence of a terminal β-D-galactopyranosyl unit (δ 106.4, 73.0, 75.1, 70.2, 77.4 and 62.1) and a 3-substituted β-D-glucopyranosyl unit (δ 103.7, 73.7, 88.6, 69.9, 78.0 and 62.3) in **1d**. Thus, the structure of the saccharide moiety was shown to be *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranose. The saccharide group was concluded to be linked to the C-3 hydroxy position of the aglycon because in the

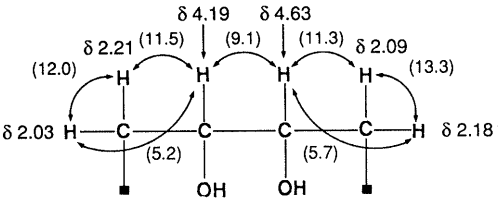

Fig. 1. Partial Structure of **1a** Shown by the ^{1}H-NMR Spectrum in Pyridine-d_5–Methanol-d_4 (10:1)

J values (Hz) are given in parentheses.

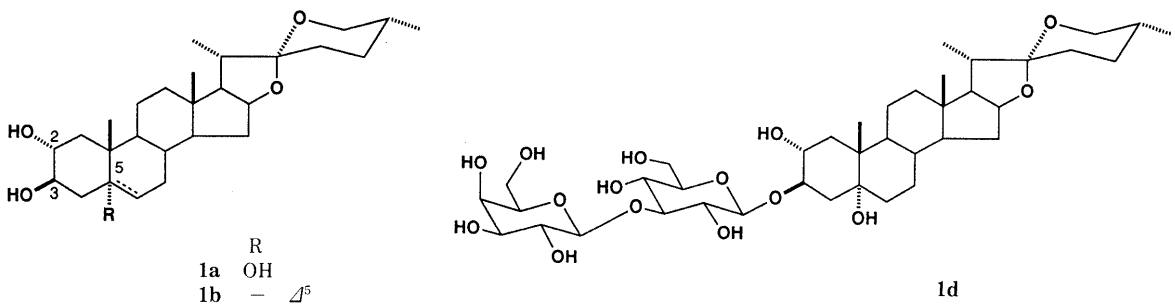

Chart 2

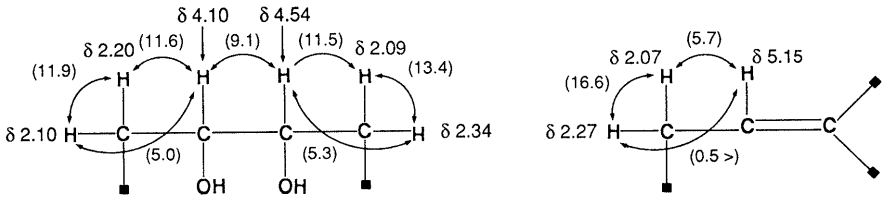

Fig. 2. Partial Structure of **2a** Shown by the ^{1}H-NMR Spectrum in Pyridine-d_5–Methanol-d_4

^{13}C-NMR spectrum of **1**, the signal due to C-3 shifted to a lower field by 9.2 ppm, whereas the signals due to C-2 and C-4 moved to upper fields by 2.3 and 3.6 ppm, as compared with those of **1a**. From the data presented above, the full structure of **1** was established as (25*R*)-5α-spirostane-2α,3β,5α-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside}, named agapanthussaponin A.

The spectral data of **2**—**4** showed that they possess a saccharide structure identical to **1**, but differ slightly from it in the aglycon structures.

Acid hydrolysis of **2** ($C_{45}H_{72}O_{19}$) with 1 N hydrochloric acid yielded an aglycon ($C_{27}H_{42}O_5$) (**2a**). The ^{1}H-NMR spectrum of **2a** displayed signals for two tertiary methyl groups at δ 1.09 and 0.79 (each s), and two secondary methyl groups at δ 1.12 (d, J=6.8 Hz) and 0.69 (d, J=5.5 Hz), similarly to **1a**. The ^{1}H- and ^{13}C-NMR spectra of **2a** showed the existence of a trisubstituted olefinic group in **2a** [^{1}H-NMR: δ 5.15 (1H, br); ^{13}C-NMR: δ 139.3 (C) and 116.0 (CH)]. Furthermore, double resonance experiments in the ^{1}H-NMR spectrum of **2a** verified that a $-CH_2-CH(OH)-CH(OH)-CH_2-$ group and a $-CH_2-CH=C-$ group are present in **2a** (Fig. 2), indicating **2a** to be a 7,8-dehydro (Δ^7) or 9,11-dihydro (Δ^9) derivative of **1a**; the latter was readily ruled out by the ^{13}C-NMR data. In the ^{13}C-NMR spectrum of **2a**, the signals at δ 40.5 (C), 41.1 (CH_2) and 21.8 (CH_2) were assigned to C-10, C-12 and C-11, respectively, without any conflict, and the differences in shift values from those of **1a** were less than 0.7 ppm. The signals at δ 55.1 (CH), 43.7 (CH) and 37.3 (CH_2) were assigned to C-14, C-9 and C-6, which moved by −1.3, −2.1 and +2.8 ppm, in comparison with those of **1a**. Accordingly, the structure of **2a** was estabilised as (25*R*)-5α-spirost-7-ene-2α,3β,5α-triol, and the structure of **2** as (25*R*)-5α-spirost-7-ene-2α,3β,5α-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside}, named agapanthussaponin B.

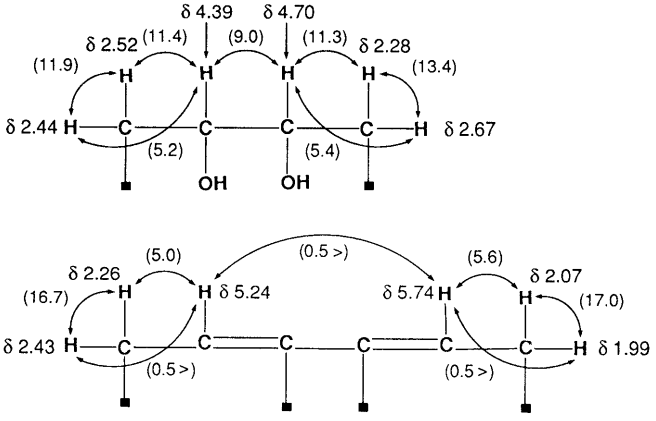

Fig. 3. Partial Structure of **3** Shown by the ^{1}H-NMR Spectrum in Pyridine-d_5

Acid hydrolysis of **3** ($C_{45}H_{70}O_{19}$) gave several unidentified artifactual sapogenols; no genuine aglycon could be obtained. The UV and ^{13}C-NMR spectra of **3** indicated that **3** has a conjugated tetrasubstituted olefinic group [UV: λ_{max} 237 shoulder (sh) (log ε 4.07), 243 (log ε 4.11) and 251 sh (log ε 3.93); ^{13}C-NMR: δ 142.6 (C), 135.6 (C), 121.2 (CH) and 117.5 (CH)]. Detailed analysis of the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) spectrum of **3** revealed two structural fragments composed of **3**, $-CH_2-CH(OH)-CH(OH)-CH_2-$ and $-CH_2-CH=C-C=CH-CH_2-$ (Fig. 3), leading to the structure of the aglycon moiety of **3** as (25*R*)-5α-spirosta-7,9-diene-2α,3β,5α-triol. The UV spectrum of **3** agreed well with that of a reported steroidal compound with a $\Delta^{7,9}$ system [λ_{max} 236 (log ε 4.11), 243 (log ε 4.16) and 251 (log ε 4.00)].[9] Thus, the structure of **3** was established as (25*R*)-5α-spirosta-7,9-diene-2α,3β,5α-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside}, named agapanthussaponin C.

Compound **4**, composed of the molecular formula $C_{45}H_{74}O_{20}$, which was confirmed by elemental analysis, negative-ion FAB-MS and ^{13}C-NMR spectrum, has one more oxygen atom than does **1**. The ^{13}C-NMR spectrum of **4** showed a close similarity to that of **1** except for a missing CH carbon signal and the appearance of a new oxygen-bearing quaternary carbon resonanced at δ 77.4, accounting for the presence of one more tertiary hydroxyl group in addition to the C-5 hydroxyl group in **4**. In the proton detected heteronuclear multiple-bond connectivity (HMBC) spectrum of **4**, a quaternary carbon signal at δ 76.7 showed $^2J_{C-H}$ and $^3J_{C-H}$ correlations with the H-4 equatorial proton signal at δ 2.35 (dd, J=13.6, 5.9 Hz), the H-1 equatorial at δ 2.09 (dd, J=12.1, 5.3 Hz) and the H-19 methyl at δ 1.23 (s), resulting in the assignment of the signal to C-5. Another quaternary carbon signal at δ 77.4 showed correlations with an H-12 equatorial at δ 1.48 (1H, br d, J=12.8 Hz) and an H-19 methyl (Fig. 4). Furthermore, in the ^{13}C-NMR spectrum of **4**, the signals at δ 48.9, 35.4, 35.1, and 21.9 were assigned to C-14, C-12, C-1 and C-7, respectively, by means of the combined use of ^{1}H–^{1}H COSY and proton detected heteronuclear multiple quantum coherence (HMQC) spectra, which were shifted upfield by 7.4, 5.0, 4.9 and 4.7 ppm as compared with those of **1**. Thus, the presence of a 9α-hydroxyl group was evident, and the structure of **4** was established as (25*R*)-5α-spirostane-2α,3β,5α,9α-tetrol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside}, named agapanthussaponin D.

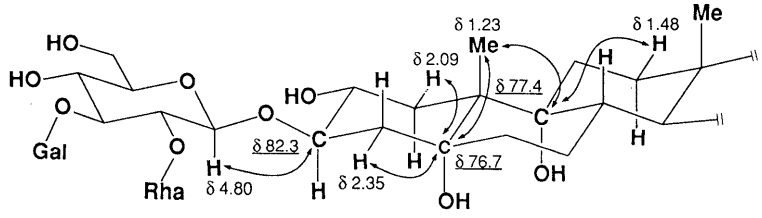

Fig. 4. ^{1}H–^{13}C Long-Range Correlations of **4** in Pyridine-d_5–Methanol-d_4 (10 : 1)
Underlined figures indicate ^{13}C-NMR chemical shifts.

TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**, **1a**, Gitogenin, **1b**, **1d**, **2**, **2a** and **3**—**5**[a]

C	**1**	**1a**	Gitogenin	**1b**	**1d**	**2**	**2a**	**3**	**4**	**5**
1	40.0	40.7	46.5	46.6	39.9	39.9[b]	39.8	39.2	35.1	35.0
2	71.0	73.3[b]	73.1	72.7	71.1	70.5	73.1	70.8	70.8	70.9
3	82.9	73.7[b]	76.7	76.8	83.8	82.7	73.1	83.0	82.3	82.7
4	40.4	44.0	37.2	40.9	41.1	39.7[b]	43.4	38.6	40.6	40.7
5	73.6	73.9	45.3	141.3	73.6	73.1	73.3	73.0	76.7	76.8
6	34.4	34.5	28.4	121.3	34.3	37.2	37.3	37.6	34.8	34.8
7	26.6	26.6	32.4[b]	32.3[b]	26.5	115.9	116.0	117.5	21.9	21.8
8	34.3	34.3	34.7	31.2	34.3	139.1	139.3	135.6	37.4	37.4
9	45.6	45.8	54.7	50.4	45.6	43.5	43.7	142.6	77.4	77.5
10	40.6	41.2	37.6	38.6	40.6	39.9	40.5	43.0	43.2	43.2
11	21.7	21.8	21.6	21.3	21.7	21.7	21.8	121.2	28.1	28.1
12	40.4	40.5	40.2	39.9	40.4	40.4	41.1	42.4	35.4	35.3
13	41.0	41.0	40.9	40.5	41.0	41.7	41.8	40.7	41.0	41.4
14	56.3	56.4	56.5	56.7	56.3	55.0	55.1	51.7	48.9	48.8
15	32.2	32.2	32.2[b]	32.2[b]	32.2	31.5	31.5	31.6	32.1	32.0
16	81.3	81.3	81.2	81.1	81.2	80.9	80.9	81.4	81.4	81.6
17	63.1	63.1	63.1	62.9	63.2	62.8	62.8	62.3	63.1	64.4
18	16.7	16.8	16.7	16.4	16.7	16.5	16.5	16.0	16.0	16.2
19	17.3	17.4	13.8	20.7	17.3	19.1	19.3	26.3	20.3	20.2
20	42.0	42.0	42.0	42.0	42.0	42.5	42.5	42.6	42.0	40.6
21	15.0	15.0	15.0	15.0	15.0	14.9	14.9	14.6	15.0	15.9
22	109.2	109.2	109.2	109.3	109.3	109.3	109.3	109.3	109.3	112.7
23	31.9	31.9	31.9	31.8	31.8	31.8	31.8	31.8	31.9	30.9
24	29.3	29.3	29.3	29.3	29.3	29.3	29.3	29.2	29.3	28.3
25	30.6	30.6	30.6	30.6	30.6	30.6	30.6	30.6	30.6	34.3
26	66.9	66.9	66.9	66.9	66.9	66.9	66.9	67.0	66.9	75.2
27	17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.3	17.2
OMe										47.3
1′	100.8				103.7	101.0		101.2	100.7	101.0
2′	77.2				73.7	77.1		77.1	77.2	77.2
3′	89.3				88.6	89.2		89.3	89.3	89.2
4′	69.6				69.9	69.5		69.5	69.6	69.7
5′	77.8				78.0	77.7		77.7	77.8	77.8
6′	62.2				62.3	62.1		62.1	62.2	62.3
1″	102.2					102.2		102.2	102.2	102.2
2″	72.4					72.4		72.4	72.4	72.4[b]
3″	72.8					72.7		72.7	72.8	72.7
4″	74.1					74.1		74.2	74.1	74.1
5″	69.6					69.5		69.5	69.6	69.5
6″	18.6					18.6		18.6	18.6	18.5
1‴	105.1				106.4	105.1		105.1	105.2	105.1
2‴	72.4				73.0	72.4		72.4	72.4	72.5[b]
3‴	75.2				75.1	75.2		75.3	75.3	75.2
4‴	70.1				70.2	70.1		70.1	70.1	70.1
5‴	77.4				77.4	77.4		77.5	77.4	77.4
6‴	62.1				62.1	62.1		62.1	62.1	62.1
1⁗										105.0
2⁗										75.2
3⁗										78.4[c]
4⁗										71.9
5⁗										78.6[c]
6⁗										63.0

a) Spectra were measured in pyridine-d_5. b, c) Assignments may be interchangeable in each column.

Compound **5** ($C_{52}H_{88}O_{26}$) presented a positive Ehrlich's test,[10] and the ^{1}H- and ^{13}C-NMR spectra indicated **5** to be a 22-methoxyfurostanol saponin.[5] Enzymatic hydrolysis of **5** with β-glucosidase in an acetic acid/sodium acetate buffer (pH 5) gave D-glucose and the corresponding spirostanol saponin, which was identified as agapanthussaponin D (**4**). Thus, the structure of **5** was shown to be 22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-2α,3β,5α,9α,22ξ,26-hexol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-galactopyranosyl-(1→3)]-β-D-glucopyranoside}.

The IC_{50} of the isolated saponins and their derivatives

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

Compounds	IC_{50} ($\times 10^{-5}$ M)
1	0.7
1a	—
1d	44.7
2	1.2
2a	—
3	1.1
4	2.0
5	—
Papaverine	3.0

on cAMP phosphodiesterase are listed in Table II. Compounds **1**—**4** exhibited more potent activity than that of a well-known phosphodiesterase inhibitor, papaverine. Recently, several new positive inotropic drugs, non-catechol and non-cardenoglycoside type agents, have been developed,[11] which act through an increase in intracellular cyclic AMP content. Further biological tests which could be used to develop new positive inotropic agents are in progress.

Experimental

Optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 instrument, UV on a Hitachi 557 spectrometer and MS on a Hitachi M-80 or a VG AutoSpec E machine. Elemental analysis was carried out with Perkin-Elmer 240B elemental analyzer. NMR spectra were taken with a Bruker AM-400 or AM-500 spectrometer. Chemical shifts are given in δ-values referring to tetramethylsilane (TMS) as the internal standard. Assignments of the ^{13}C-NMR spectra were achieved on the basis of the various distortionless enhancement by polarization transfer (DEPT) spectra, and by correlation with previously reported compounds. Silica-gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-kasei) 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, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed with a Tosoh HPLC system (Tosoh: pump, CCPM; controller, CCP controller PX-8010; detector, RI-8010 or UV-8000) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-kasei-kogyo, 10 mm i.d. × 250 mm or 4.6 mm i.d. × 250 mm, ODS, 5 μm) or a TSK-gel Silica-60 column (Tosoh, 4.6 mm i.d. × 250 mm, silica-gel, 5 μm). The liquid scintillation counter used was an Aloka LSC-903 instrument. Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase and cyclic AMP were obtained from Sigma, and [^{3}H]cAMP from Radiochemical Center.

Isolation Fresh underground parts of *A. inapertus* (3.4 kg), purchased from Heiwaen, Japan, were extracted with hot MeOH. After removing of the solvent under reduced pressure, the concentrated material was suspended in H_2O and extracted with *n*-BuOH. The *n*-BuOH-soluble phase was chromatographed on silica-gel with CH_2Cl_2–MeOH with an increasing proportion of MeOH (6 : 1; 4 : 1; 2 : 1), and finally with MeOH. The MeOH eluate fraction was subjected to a Diaion HP-20 column with H_2O gradually enriched with MeOH. The 80% MeOH and MeOH eluate fractions containing abundant saponins were combined and subjected to silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (30 : 10 : 1; 20 : 10 : 1; 7 : 4 : 1) and ODS silica-gel with MeOH–H_2O (4 : 1; 1 : 1) to give **1** (1.29 g), a mixture of **2** and **4**, as was shown by the ^{1}H-NMR spectrum, **3** (1.20 g), and **5** with a few impurities. Separation of the mixture of **2** and **4** was carried out by the following procedures. To a pyridine solution of the mixture was added an excess amount of Ac_2O, and the solution was left standing overnight. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed with H_2O and dried over anhydrous Na_2SO_4. The acetate was chromatographed on silica-gel with hexane–Me_2CO (3 : 2) and hexane–$CHCl_3$–EtOAc (1 : 1 : 2) to give **2** decaacetate and **4** decaacetate. Each acetate was hydrolyzed with 4% KOH in EtOH at room temperature for 3 h and neutralized by passage through an Amberlite IR-120B column (Organo), followed by purification by silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 7 : 4 : 1) to yield **2** (1.24 g) and **4** (1.08 g). Compound **5** with a few impurities was subjected to preparative HPLC with (MeOH–H_2O, 3 : 2) to give **5** (544 mg) as a pure compound.

Compound 1 A white amorphous powder, $[\alpha]_D^{23}$ −62.0° (c=0.10, MeOH). *Anal.* Calcd for $C_{45}H_{74}O_{19}\cdot 3/2H_2O$: C, 57.13; H, 8.20. Found: C, 57.01; H, 8.07. Negative-ion FAB-MS *m/z*: 917 [M−H]$^-$, 772 [M−rhamnosyl]$^-$, 756 [M−galactosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2930 (CH), 1445, 1375, 1240, 1205, 1040, 975, 940, 915, 895, 860, 830, 810, 780. ^{1}H-NMR (pyridine-d_5) δ: 6.33 (1H, br s, H-1″), 4.97 (1H, d, J=7.8 Hz, H-1‴), 4.85 (1H, d, J=7.2 Hz, H-1′), 3.57 (1H, dd, J=10.5, 2.6 Hz, H-26a), 3.49 (1H, dd, J=10.5, 10.5 Hz, H-26b), 1.72 (3H, d, J=6.2 Hz, H-6″), 1.18 (3H, s, H-19), 1.12 (3H, d, J=7.0 Hz, H-21), 0.87 (3H, s, H-18), 0.69 (3H, d, J=5.4 Hz, H-27).

Acid Hydrolysis of 1 A solution of **1** (80 mg) in 1 N HCl (dioxane–H_2O, 1 : 1, 6 ml) was heated at 80 °C for 1 h. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column, and was purified by silica-gel column chromatography with $CHCl_3$–MeOH (12 : 1), and then with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) to furnish an aglycon (**1a**) (5 mg) and a mixture of monosaccharides (15 mg). When a solution of **1** in 1 N HCl was heated at 100 °C for 3 h, **1** was hydrolyzed and dehydrated to yield an artificial sapogenol (**1b**) (26 mg), identified as yuccagenin.[6] Compound **1a**: a white amorphous powder, $[\alpha]_D^{23}$ −54.0° (c=0.10, MeOH). EI-MS *m/z* (%): 448.3190 [M$^+$, Calcd for $C_{27}H_{44}O_5$: 488.3189] (4), 428 (2), 375 (11), 316 (22), 301 (12), 287 (13), 139 (100), 115 (19). IR ν_{max}^{KBr} cm^{-1}: 3405 (OH), 2925 (CH), 1445, 1375, 1255, 1240, 1205, 1175, 1095, 1045, 1000, 975, 955, 935, 915, 895, 860, 795. ^{1}H-NMR (pyridine-d_5) δ: 6.12 (1H, br, OH), 6.02 (1H, br, OH), 5.25 (1H, br s, OH), 4.75 (1H, m, H-3), 4.55 (1H, q-like, J=6.9 Hz, H-16), 4.29 (1H, m, H-2), 3.57 (1H, dd, J=10.4, 3.3 Hz, H-26a), 3.49 (1H, dd, J=10.4, 10.4 Hz, H-26b), 1.12 (3H, d, J=6.6 Hz, H-21), 1.11 (3H, s, H-19), 0.88 (3H, s, H-18), 0.69 (3H, d, J=5.4 Hz, H-27). ^{1}H-NMR (pyridine-d_5–methanol-d_4, 10 : 1) δ: 4.63 (1H, ddd, J=11.3, 9.1, 5.7 Hz, H-3), 4.51 (1H, q-like, J=6.9 Hz, H-16), 4.19 (1H, ddd, J=11.5, 9.1, 5.2 Hz, H-2), 3.55 (1H, dd, J=10.5, 3.3 Hz, H-26a), 3.46 (1H, dd, J=10.5, 10.5 Hz, H-26b), 2.21 (1H, dd, J=12.0, 11.5 Hz, H-1 axial), 2.18 (1H, dd, J=13.3, 5.7 Hz, H-4 equatorial), 2.09 (1H, dd, J=13.3, 11.3 Hz, H-4 axial), 2.03 (1H, dd, J=12.0, 5.2 Hz, H-1 equatorial), 1.11 (3H, d, J=6.6 Hz, H-21), 1.10 (3H, s, H-19), 0.87 (3H, s, H-18), 0.70 (3H, d, J=5.4 Hz, H-27).

The monosaccharide mixture of (2 mg) 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 (Waters), 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 mixture of 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which was then analyzed by HPLC.[12] Derivatives of D-glucose, D-galactose and L-rhamnose were detected.

Acetylation of 1 Compound **1** (52 mg) was acetylated with Ac_2O in pyridine and the crude acetate was purified by silica-gel column chromatography with hexane–$CHCl_3$–EtOAc (1 : 1 : 2) to yield the corresponding decaacetate (**1c**) (36.5 mg) as a white amorphous powder. Compound **1c**: IR ν_{max}^{KBr} cm^{-1}: 3510 (OH), 2940 (CH), 1740 (C=O), 1435, 1365, 1220, 1170, 1125, 1040, 975, 945, 910, 890, 860, 830, 795. ^{1}H-NMR (pyridine-d_5) δ: 5.86 (1H, dd, J=10.4, 3.6 Hz, H-3″), 5.77 (1H, br d, J=3.5 Hz, H-4‴), 5.72 (1H, br s, H-1″), 5.71 (1H, dd, J=10.3, 3.5 Hz, H-3‴), 5.69 (1H, br d, J=3.6 Hz, H-2″), 5.64 (1H, dd, J=10.4, 10.4 Hz, H-4″), 5.63 (1H, m, H-2), 5.58 (1H, dd, J=10.3, 7.9 Hz, H-2‴), 5.26 (1H, dd, J=9.8, 9.8 Hz, H-4′), 5.10 (1H, d, J=7.9 Hz, H-1‴), 4.90 (1H, dq, J=10.4, 6.2 Hz, H-5″), 4.89 (1H, m, H-3), 4.77 (1H, d, J=7.9 Hz, H-1′), 4.58 (1H, dd, J=12.2, 4.6 Hz, H-6′a), 4.56 (1H, q-like, J=7.7 Hz, H-16), 4.49 (1H, dd, J=11.1, 6.4 Hz, H-6‴a), 4.42 (1H, dd, J=11.1, 7.0 Hz, H-6‴b), 4.36 (1H, dd, J=9.8, 9.8 Hz, H-3′), 4.30 (1H, br dd, J=7.0, 6.4 Hz, H-5‴), 4.23 (1H, dd, J=12.2, 2.0 Hz, H-6′b), 4.04 (1H, dd, J=9.8, 7.9 Hz, H-2′), 3.87 (1H, ddd, J=9.8, 4.6, 2.0 Hz, H-5′), 3.59 (1H, dd, J=10.5, 2.5 Hz, H-26a), 3.50 (1H, dd, J=10.5, 10.5 Hz, H-26b), 2.28, 2.22, 2.21, 2.20, 2.19, 2.10, 2.01, 1.99, 1.97 and 1.96 (each 3H, s, Ac × 10), 1.54 (3H, d, J=6.2 Hz, H-6″), 1.36 (3H, s, H-19), 1.14 (3H, d, J=6.9 Hz, H-21), 0.90 (3H, s, H-18), 0.70 (3H, d, J=5.4 Hz, H-27).

Partial Hydrolysis of 1 A solution of **1** (60 mg) in 0.2 N HCl (dioxane–H_2O, 1 : 1, 6 ml) was heated at 100 °C for 30 min. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU column and purified by silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) to give a partial hydrolysate (**1d**) (19 mg) and L-rhamnose. Compound **1d**: a white amorphous powder, $[\alpha]_D^{23}$ −68.0° (c=0.10, MeOH). Negative-ion FAB-MS *m/z*: 771 [M−H]$^-$: IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2920 (CH), 1440, 1370, 1255, 1235, 1200, 1165, 1070, 1040, 975, 935, 915, 895, 860. ^{1}H-NMR (pyridine-d_5) δ: 5.22 (1H, d, J=7.8 Hz, H-1‴), 4.92 (1H, d, J=7.7 Hz, H-1′), 3.57 (1H, dd, J=10.4, 2.8 Hz, H-26a), 3.49 (1H, dd, J=10.4, 10.4 Hz, H-26b), 1.12 (3H, d, J=6.9 Hz, H-21), 0.98 (3H, s, H-19), 0.87 (3H, s, H-18), 0.69 (3H, d, J=4.8 Hz, H-27). L-Rhamnose: TLC Rf 0.75 ($CHCl_3$–MeOH–H_2O, 20 : 10 : 1).

Compound 2 A white amorphous powder, $[\alpha]_D^{23}$ −72.0° (c=0.10, MeOH). *Anal.* Calcd for $C_{45}H_{72}O_{19}\cdot 2H_2O$: C, 56.71; H, 8.04. Found: C, 56.74; H, 7.96. Negative-ion FAB-MS *m/z*: 915 [M−H]$^-$, 770 [M−rhamnosyl]$^-$, 754 [M−galactosyl]$^-$. IR ν_{max}^{KBr} cm: 3410 (OH), 2940 (CH), 1445, 1375, 1240, 1040, 975, 915, 895, 860, 780. ^{1}H-NMR (pyridine-d_5) δ: 6.30 (1H, br s, H-1″), 5.08 (1H, br, H-7), 4.97 (1H, d, J=7.7 Hz, H-1‴), 4.85 (1H, d, J=7.8 Hz, H-1′), 3.56 (1H, dd, J=10.5, 2.6 Hz, H-26a), 3.46 (1H, dd, J=10.5, 10.5 Hz, H-26b), 1.70 (3H, d, J=

6.2 Hz, H-6″), 1.13 (3H, s, H-19), 1.12 (3H, d, J=6.9 Hz, H-21), 0.77 (3H, s, H-18), 0.70 (3H, d, J=5.3 Hz, H-27).

Acid Hydrolysis of 2 Compound **2** (100 mg) was treated with 1 N HCl (dioxane–H_2O, 1 : 1, 6 ml) at 100 °C for 3 h. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU column and was chromatographed on silica-gel with $CHCl_3$–MeOH (12 : 1) to give an aglycon (**2a**) (19 mg). Compound **2a**: a white amorphous powder, $[\alpha]_D^{23}$ −52.0° (c=0.10, MeOH). CI-MS m/z (%): 447 $[M+H]^+$ (42), 429 (60), 410 (100), 393 (33), 353 (8), 314 (15), 303 (13), 285 (17), 281 (16), 267 (16), 139 (53), 115 (31). EI-MS m/z (%): 428.2914 $[(M-H_2O)^+$, Calcd for $C_{27}H_{40}O_4$: 428.2927] (35), 395 (21), 314 (10), 299 (11), 281 (16), 239 (8), 159 (14), 139 (100), 105 (27). IR ν_{max}^{KBr} cm^{-1}: 3405 (OH), 2930 (CH), 1445, 1375, 1255, 1235, 1170, 1150, 1090, 1070, 1050, 1005, 975, 920, 895, 860, 840. ^{1}H-NMR (pyridine-d_5) δ: 6.22 (1H, br d, J=4.1 Hz, OH), 6.09 (1H, br d, J=4.1 Hz, OH), 5.20 (1H, s, OH), 5.15 (1H, br, H-7), 4.68 (1H, m, H-3), 4.56 (1H, q-like, J=7.1 Hz, H-16), 4.20 (1H, m, H-2), 3.56 (1H, dd, J=10.5, 3.4 Hz, H-26a), 3.46 (1H, dd, J=10.5, 10.5 Hz, H-26b), 1.12 (3H, d, J=6.8 Hz, H-21), 1.09 (3H, s, H-19), 0.79 (3H, s, H-18), 0.69 (3H, d, J=5.5 Hz, H-27). ^{1}H-NMR (pyridine-d_5–methanol-d_4, 10 : 1) δ: 5.13 (1H, br, H-7), 4.54 (1H, ddd, J=11.5, 9.1, 5.3 Hz, H-3), 4.53 (1H, q-like, J=6.6 Hz, H-16), 4.10 (1H, ddd, J=11.6, 9.1, 5.0 Hz, H-2), 3.54 (1H, dd, J=10.5, 3.0 Hz, H-26a), 3.44 (1H, dd, J=10.5, 10.5 Hz, H-26b), 2.34 (1H, dd, J=13.4, 5.3 Hz, H-4 equatorial), 2.27 (1H, br d, J=16.6 Hz, H-6a), 2.20 (1H, dd, J=11.9, 11.6 Hz, H-1 axial), 2.10 (1H, dd, J=11.9, 5.0 Hz, H-1 equatorial), 2.09 (1H, dd, J=13.4, 11.5, H-4 axial), 2.07 (1H, br dd, J=16.6, 5.7 Hz, H-6b), 1.12 (3H, d, J=6.8 Hz, H-21), 1.07 (3H, s, H-19), 0.78 (3H, s, H-18), 0.71 (3H, d, J=5.5 Hz, H-27).

Compound 3 A white amorphous powder, $[\alpha]_D^{23}$ −26.0° (c=0.10, MeOH). *Anal.* Calcd for $C_{45}H_{70}O_{19}\cdot 5/2H_2O$: C, 56.30; H, 7.87. Found: C, 56.18; H, 7.54. Negative-ion FAB-MS m/z: 913 $[M-H]^-$, 768 $[M-$rhamnosyl$]^-$, 752 $[M-$galactosyl$]^-$. UV λ_{max}^{MeOH} nm (log ε): 237 sh (4.07), 243 (4.11), 251 sh (3.93). IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2950 (CH), 1450, 1375, 1240, 1045, 980, 915, 900, 865, 815. ^{1}H-NMR (pyridine-d_5) δ: 6.36 (1H, br s, H-1″), 5.74 (1H, br d, J=5.6 Hz, H-11), 5.24 (1H, br d, J=5.0 Hz, H-7), 5.00 (1H, d, J=7.8 Hz, H-1‴), 4.89 (1H, d, J=7.6 Hz, H-1′), 4.70 (1H, ddd, J=11.3, 9.0, 5.4 Hz, H-3), 4.39 (1H, ddd, J=11.4, 9.0, 5.2 Hz, H-2), 3.57 (1H, dd, J=10.5, 2.6 Hz, H-26a), 3.47 (1H, dd, J=10.5, 10.5 Hz, H-26b), 2.67 (1H, dd, J=13.4, 5.4 Hz, H-4 equatorial), 2.52 (1H, dd, J=11.9, 11.4 Hz, H-1 axial), 2.44 (1H, dd, J=11.9, 5.2 Hz, H-1 equatorial), 2.43 (1H, br d, J=16.7 Hz, H-6a), 2.28 (1H, dd, J=13.4, 11.3, H-4 axial), 2.26 (1H, br dd, J=16.7, 5.0 Hz, H-6b), 2.07 (1H, dd, J=17.0, 5.6 Hz, H-12a), 1.99 (1H, br d, J=17.0 Hz, H-12b), 1.72 (1H, d, J=6.2 Hz, H-6″), 1.28 (3H, s, H-19), 1.09 (3H, d, J=6.9 Hz, H-21), 0.78 (3H, s, H-18), 0.70 (3H, d, J=5.3 Hz, H-27).

Acid Hydrolysis of 3 Compound **3** (50 mg) was subjected to acid hydrolysis identically to **2** to give several artifactural sapogenols. The structures remain to be determined.

Compound 4 A white amorphous powder, $[\alpha]_D^{23}$ −50.0° (c=0.10, MeOH). *Anal.* Calcd for $C_{45}H_{74}O_{20}\cdot 3/2H_2O$: C, 56.18; H, 8.07. Found: C, 56.24; H, 7.96. Negative-ion FAB-MS m/z: 933 $[M-H]^-$, 915 $[M-H_2O]^-$, 788 $[M-$rhamnosyl$]^-$, 772 $[M-$galactosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2930 (CH), 1445, 1375, 1240, 1175, 1065, 1045, 980, 960, 915, 895, 865. ^{1}H-NMR (pyridine-d_5–methanol-d_4) δ: 6.24 (1H, br s, H-1″), 4.93 (1H, d, J=7.8 Hz, H-1‴), 4.80 (1H, d, J=7.3 Hz, H-1′), 4.71 (1H, ddd, J=11.4, 9.1, 5.9 Hz, H-3), 4.59 (1H, q-like, J=7.4 Hz, H-16), 4.35 (1H, ddd, J=12.1, 9.1, 5.3 Hz, H-2), 3.54 (1H, br d, J=10.5, H-26a), 3.47 (1H, dd, J=10.5, 10.5 Hz, H-26b), 2.78 (1H, dd, J=12.1, 12.1 Hz, H-1 axial), 2.35 (1H, dd, J=13.6, 5.9 Hz, H-4 equatorial), 2.24 (1H, dd, J=13.6, 11.4, H-4 axial), 2.09 (1H, dd, J=12.1, 5.3 Hz, H-1 equatorial), 1.96 (1H, ddd, J=13.0, 13.0, 5.9 Hz, H-8), 1.82 (1H, ddd, J=12.8, 12.8, 3.6 Hz, H-12 axial), 1.68 (1H, d, J=6.2 Hz, H-6″), 1.48 (1H, br d, J=12.8 Hz, H-12 equatorial), 1.23 (3H, s, H-19), 1.09 (3H, d, J=6.9 Hz, H-21), 0.92 (3H, s, H-18), 0.68 (3H, d, J=5.3 Hz, H-27).

Compound 5 A white amorphous powder, $[\alpha]_D^{23}$ −54.0° (c=0.10, MeOH). *Anal.* Calcd for $C_{52}H_{88}O_{26}\cdot H_2O$: C, 54.44; H, 7.91. Found: C, 54.21; H, 7.66. Negative-ion FAB-MS m/z: 1127 $[M-H]^-$, 965 $[M-$glucosyl$]^-$. IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 2910 (CH), 1440, 1365, 1295, 1250, 1035, 905, 885, 800. ^{1}H-NMR (pyridine-d_5) δ: 6.32 (1H, br s, H-1″), 4.98 (overlapping with H_2O signal, H-1‴), 4.86 (1H × 2, d, J=7.7 Hz, H-1′, H-1⁗), 3.26 (3H, s, OMe), 1.72 (1H, d, J=6.2 Hz, H-6″), 1.26 (3H, s, H-19), 1.17 (3H, d, J=6.9 Hz, H-21), 1.01 (3H, d, J=6.6 Hz, H-27), 0.93 (3H, s, H-18).

Enzymatic Hydrolysis of 5 Compound **5** (25 mg) was dissolved in an AcOH/AcONa buffer (pH 5) with β-glucosidase (25 mg), and the mixture was incubated at room temperature overnight. The crude products were chromatographed on silica-gel with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) to yield **4** (12 mg) and D-glucose. D-Glucose: TLC Rf 0.39 (n-BuOH–Me_2CO–H_2O, 4 : 5 : 1).

Assay of cAMP Phosphodiesterase Activity The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously.[13] 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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