

# 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  $\mu\text{g/ml}$ . 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  $\text{IC}_{50}$  of 1–4 on cAMP phosphodiesterase were 0.7, 1.2, 1.1 and 2.0 ( $\times 10^{-5} \text{ M}$ ), respectively, which are more potent than that of papaverine ( $\text{IC}_{50}$   $3.0 \times 10^{-5} \text{ M}$ ).

**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,<sup>1)</sup> 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.<sup>2)</sup> 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*.<sup>3)</sup> 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  $\mu\text{g/ml}$ . The methanolic extract was partitioned between 1-butanol and  $\text{H}_2\text{O}$ . 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,  $[\alpha]_D -62.0^\circ$  (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,  $\text{C}_{45}\text{H}_{74}\text{O}_{19}$ , was determined by elemental analysis, negative-ion FAB-MS ( $m/z$  917  $[\text{M}-\text{H}]^-$ ) and  $^{13}\text{C}$ -NMR spectrum. The IR spectrum featured a strong absorption at  $3390 \text{ cm}^{-1}$  due to hydroxyl groups, and characteristic absorptions at 975, 915, 895 and  $860 \text{ cm}^{-1}$ , with the absorption at  $895 \text{ cm}^{-1}$  being of greater intensity than that at  $915 \text{ cm}^{-1}$ , implying the presence of a (25*R*)-spiroacetal moiety in the molecule.<sup>4)</sup> The  $^1\text{H}$ -NMR spectrum of 1 showed signals for two tertiary methyl groups at  $\delta$  1.18 and 0.87 (each s), three secondary methyl groups at  $\delta$  1.72 (d,  $J=6.2 \text{ Hz}$ ), 1.12 (d,  $J=7.0 \text{ Hz}$ ) and 0.69 (d,  $J=5.4 \text{ Hz}$ ), and three anomeric protons at  $\delta$  6.33 (br s), 4.97 (d,  $J=7.8 \text{ Hz}$ ) and 4.85 (d,  $J=7.2 \text{ Hz}$ ). The signal at  $\delta$  1.72 was due to the methyl group of 6-deoxyhexopyranose.<sup>2a)</sup> The  $^{13}\text{C}$ -NMR spectrum showed

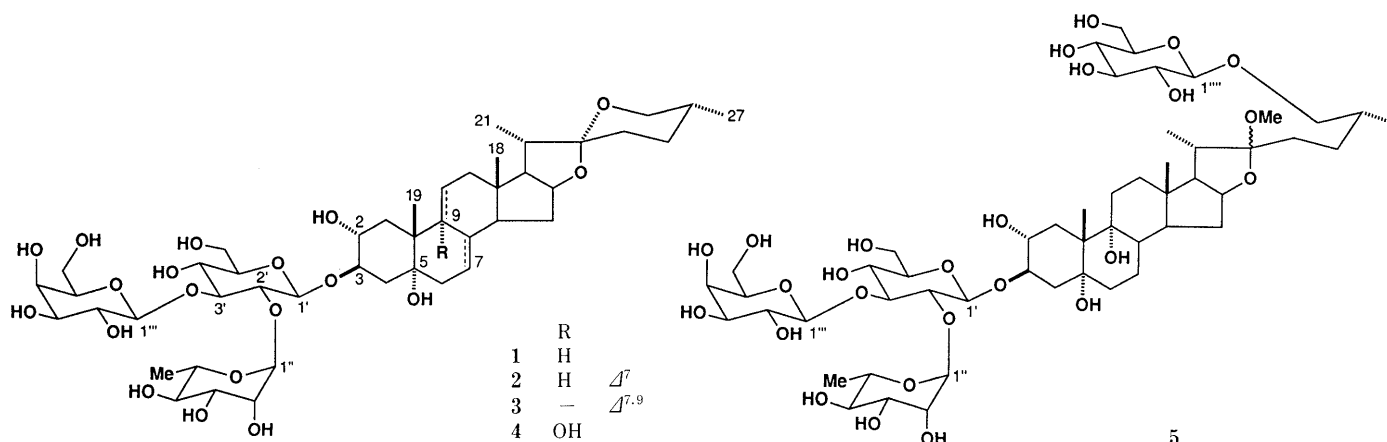


Chart 1

four signals in the field lower than 100 ppm; the signals at  $\delta$  105.1, 102.2 and 100.8 were due to the anomeric carbons, and the signal at  $\delta$  109.2 was assignable to the C-22 carbon of the spirostan skeleton.<sup>5)</sup> 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<sub>2</sub>O (1:1) at 80 °C for 1.5 h gave a steroidal sapogenin (C<sub>27</sub>H<sub>44</sub>O<sub>5</sub>) (**1a**) together with D-glucose, D-galactose and L-rhamnose. The <sup>13</sup>C-NMR spectrum of **1a** showed seven signals between 60–110 ppm; the signals at  $\delta$  109.2 (C), 81.3 (CH), 66.9 (CH<sub>2</sub>), 63.1 (CH) were readily assigned to the C-22, C-16, C-26 and C-17 carbons of the spirostan skeleton,<sup>5)</sup> and the signals at  $\delta$  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 <sup>1</sup>H-NMR signals at  $\delta$  6.12 (br), 6.02 (br) and 5.25 (brs) in pyridine-*d*<sub>5</sub>, which disappeared upon the addition of methanol-*d*<sub>4</sub>, accompanied by the signals at  $\delta$  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 <sup>1</sup>H-NMR spectrum of **1a** verified the presence of a -CH<sub>2</sub>-CH(OH)-CH(OH)-CH<sub>2</sub>- 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-2 $\alpha$ ,3 $\beta$ -diol (yuccagenin) on the basis of spectroscopic evidence.<sup>6)</sup> These spectral data indicated a

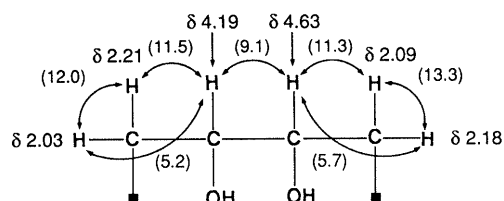


Fig. 1. Partial Structure of **1a** Shown by the <sup>1</sup>H-NMR Spectrum in Pyridine-*d*<sub>5</sub>-Methanol-*d*<sub>4</sub> (10:1)

*J* values (Hz) are given in parentheses.

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 ( $J_{H2-H1axial} = 11.5$  Hz,  $J_{H2-H3} = 9.1$  Hz and  $J_{H3-H4axial} = 11.3$  Hz in pyridine-*d*<sub>5</sub>-methanol-*d*<sub>4</sub>, 10:1) that the C-2 and C-3 hydroxyl groups have  $\alpha$ -equatorial and  $\beta$ -equatorial configurations, respectively. Comparison of the <sup>13</sup>C-NMR spectrum of **1a** with that of (25*R*)-5 $\alpha$ -spirostane-2 $\alpha$ ,3 $\beta$ -diol (gitogenin)<sup>5,7)</sup> revealed the presence of a 5 $\alpha$ -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*)-5 $\alpha$ -spirostane-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ -triol.

Assignments of the <sup>13</sup>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,<sup>5,8)</sup> which indicated the existence of a terminal  $\alpha$ -L-rhamnopyranosyl unit ( $\delta$  102.2, 72.4, 72.8, 74.1, 69.6 and 18.6), a terminal  $\beta$ -D-galactopyranosyl unit ( $\delta$  105.1, 72.4, 75.2, 70.1, 77.4 and 62.1) and a 2,3-disubstituted  $\beta$ -D-glucopyranosyl unit ( $\delta$  100.8, 77.2, 89.3, 69.6, 77.8 and 62.2) in **1**. The <sup>1</sup>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  $\delta$  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 <sup>13</sup>C-NMR spectrum of which showed the presence of a terminal  $\beta$ -D-galactopyranosyl unit ( $\delta$  106.4, 73.0, 75.1, 70.2, 77.4 and 62.1) and a 3-substituted  $\beta$ -D-glucopyranosyl unit ( $\delta$  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*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*-[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranose. The saccharide group was concluded to be linked to the C-3 hydroxy position of the aglycon because in the

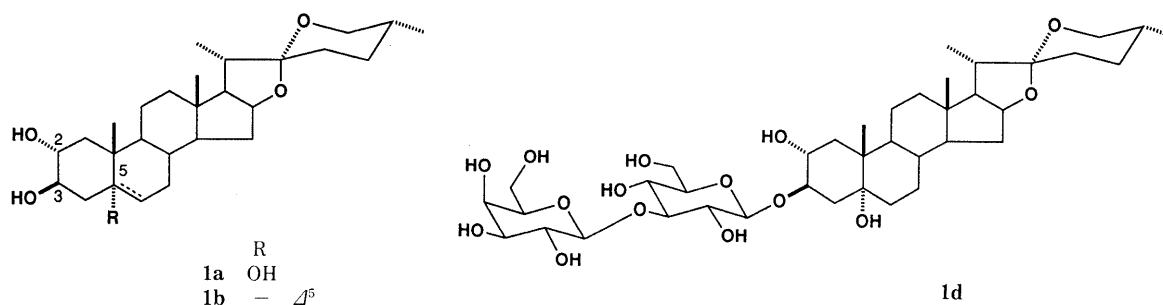


Chart 2

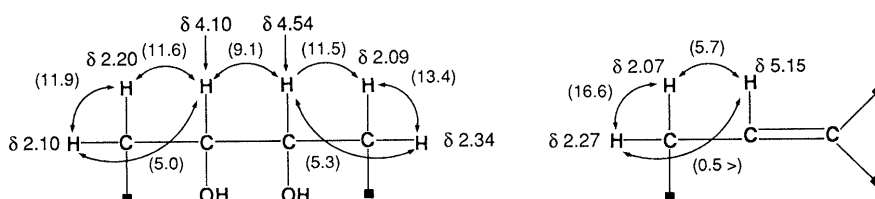


Fig. 2. Partial Structure of **2a** Shown by the <sup>1</sup>H-NMR Spectrum in Pyridine-*d*<sub>5</sub>-Methanol-*d*<sub>4</sub>

$^{13}\text{C}$ -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*)-5 $\alpha$ -spirostane-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ -triol 3-*O*-{*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*-[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -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** ( $\text{C}_{45}\text{H}_{72}\text{O}_{19}$ ) with 1 N hydrochloric acid yielded an aglycon ( $\text{C}_{27}\text{H}_{42}\text{O}_5$ ) (**2a**). The  $^1\text{H}$ -NMR spectrum of **2a** displayed signals for two tertiary methyl groups at  $\delta$  1.09 and 0.79 (each s), and two secondary methyl groups at  $\delta$  1.12 (d,  $J=6.8$  Hz) and 0.69 (d,  $J=5.5$  Hz), similarly to **1a**. The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of **2a** showed the existence of a trisubstituted olefinic group in **2a** [ $^1\text{H}$ -NMR:  $\delta$  5.15 (1H, br);  $^{13}\text{C}$ -NMR:  $\delta$  139.3 (C) and 116.0 (CH)]. Furthermore, double resonance experiments in the  $^1\text{H}$ -NMR spectrum of **2a** verified that a  $-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}(\text{OH})-\text{CH}_2-$  group and a  $-\text{CH}_2-\text{CH}=\text{C}-$  group are present in **2a** (Fig. 2), indicating **2a** to be a 7,8-dehydro ( $\Delta^7$ ) or 9,11-dihydro ( $\Delta^9$ ) derivative of **1a**; the latter was readily ruled out by the  $^{13}\text{C}$ -NMR data. In the  $^{13}\text{C}$ -NMR spectrum of **2a**, the signals at  $\delta$  40.5 (C), 41.1 ( $\text{CH}_2$ ) and 21.8 ( $\text{CH}_2$ ) 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  $\delta$  55.1 (CH), 43.7 (CH) and 37.3 ( $\text{CH}_2$ ) 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 established as (25*R*)-5 $\alpha$ -spirost-7-ene-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ -triol, and the structure of **2** as (25*R*)-5 $\alpha$ -spirost-7-ene-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ -

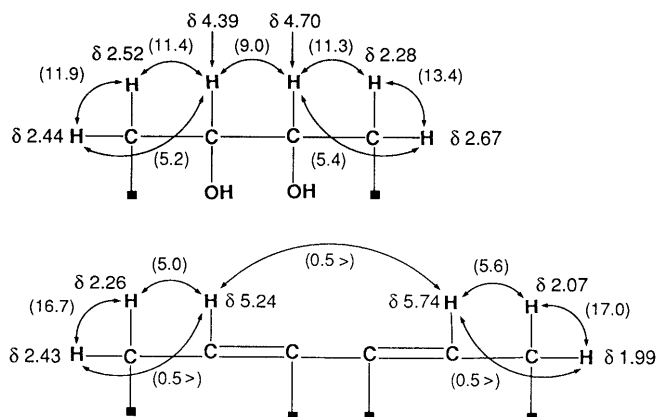


Fig. 3. Partial Structure of **3** Shown by the  $^1\text{H}$ -NMR Spectrum in Pyridine- $d_5$

triol 3-*O*-{*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*-[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranoside}, named agapanthussaponin B.

Acid hydrolysis of **3** ( $\text{C}_{45}\text{H}_{70}\text{O}_{19}$ ) gave several unidentified artifactual sapogenols; no genuine aglycon could be obtained. The UV and  $^{13}\text{C}$ -NMR spectra of **3** indicated that **3** has a conjugated tetrasubstituted olefinic group [UV:  $\lambda_{\text{max}}$  237 shoulder (sh) ( $\log \epsilon$  4.07), 243 ( $\log \epsilon$  4.11) and 251 sh ( $\log \epsilon$  3.93);  $^{13}\text{C}$ -NMR:  $\delta$  142.6 (C), 135.6 (C), 121.2 (CH) and 117.5 (CH)]. Detailed analysis of the  $^1\text{H}$ - $^1\text{H}$  correlation spectroscopy ( $^1\text{H}$ - $^1\text{H}$  COSY) spectrum of **3** revealed two structural fragments composed of **3**,  $-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}(\text{OH})-\text{CH}_2-$  and  $-\text{CH}_2-\text{CH}=\text{C}-\text{C}=\text{CH}-\text{CH}_2-$  (Fig. 3), leading to the structure of the aglycon moiety of **3** as (25*R*)-5 $\alpha$ -spirosta-7,9-diene-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ -triol. The UV spectrum of **3** agreed well with that of a reported steroidal compound with a  $\Delta^{7,9}$  system [ $\lambda_{\text{max}}$  236 ( $\log \epsilon$  4.11), 243 ( $\log \epsilon$  4.16) and 251 ( $\log \epsilon$  4.00)].<sup>9</sup> Thus, the structure of **3** was established as (25*R*)-5 $\alpha$ -spirosta-7,9-diene-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ -triol 3-*O*-{*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*-[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranoside}, named agapanthussaponin C.

Compound **4**, composed of the molecular formula  $\text{C}_{45}\text{H}_{74}\text{O}_{20}$ , which was confirmed by elemental analysis, negative-ion FAB-MS and  $^{13}\text{C}$ -NMR spectrum, has one more oxygen atom than does **1**. The  $^{13}\text{C}$ -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 resonant at  $\delta$  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  $\delta$  76.7 showed  $^2J_{\text{C-H}}$  and  $^3J_{\text{C-H}}$  correlations with the H-4 equatorial proton signal at  $\delta$  2.35 (dd,  $J=13.6$ , 5.9 Hz), the H-1 equatorial at  $\delta$  2.09 (dd,  $J=12.1$ , 5.3 Hz) and the H-19 methyl at  $\delta$  1.23 (s), resulting in the assignment of the signal to C-5. Another quaternary carbon signal at  $\delta$  77.4 showed correlations with an H-12 equatorial at  $\delta$  1.48 (1H, brd,  $J=12.8$  Hz) and an H-19 methyl (Fig. 4). Furthermore, in the  $^{13}\text{C}$ -NMR spectrum of **4**, the signals at  $\delta$  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  $^1\text{H}$ - $^1\text{H}$  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 9 $\alpha$ -hydroxyl group was evident, and the structure of **4** was established as (25*R*)-5 $\alpha$ -spirostane-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -tetrol 3-*O*-{*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*-[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranoside}, named agapanthussaponin D.

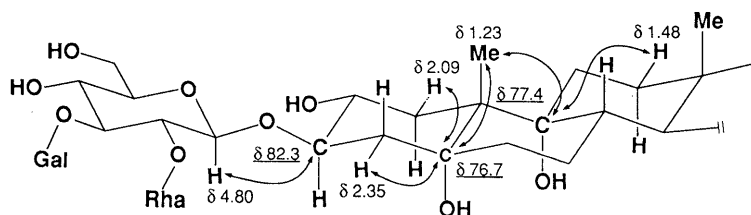


Fig. 4.  $^1\text{H}$ - $^{13}\text{C}$  Long-Range Correlations of **4** in Pyridine- $d_5$ -Methanol- $d_4$  (10:1)

Underlined figures indicate  $^{13}\text{C}$ -NMR chemical shifts.

TABLE I.  $^{13}\text{C}$ -NMR Spectral Data for Compounds **1**, **1a**, Gitogenin, **1b**, **1d**, **2**, **2a** and **3–5**<sup>a)</sup>

| C     | <b>1</b> | <b>1a</b>          | Gitogenin          | <b>1b</b>          | <b>1d</b> | <b>2</b>           | <b>2a</b>          | <b>3</b> | <b>4</b> | <b>5</b>           |
|-------|----------|--------------------|--------------------|--------------------|-----------|--------------------|--------------------|----------|----------|--------------------|
| 1     | 40.0     | 40.7               | 46.5               | 46.6               | 39.9      | 39.9 <sup>b)</sup> | 39.8               | 39.2     | 35.1     | 35.0               |
| 2     | 71.0     | 73.3 <sup>b)</sup> | 73.1               | 72.7               | 71.1      | 70.5               | 73.1               | 70.8     | 70.8     | 70.9               |
| 3     | 82.9     | 73.7 <sup>b)</sup> | 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 <sup>b)</sup> | 43.4               | 38.6     | 40.6     | 40.7               |
| 5     | 73.6     | 73.9               | 45.3               | 141.3              | 73.6      | 73.1               | 73.3 <sup>c)</sup> | 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 <sup>b)</sup> | 32.3 <sup>b)</sup> | 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 <sup>b)</sup> | 32.2 <sup>b)</sup> | 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 <sup>b)</sup> |
| 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 <sup>b)</sup> |
| 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 <sup>c)</sup> |
| 4'''' |          |                    |                    |                    |           |                    |                    |          |          | 71.9               |
| 5'''' |          |                    |                    |                    |           |                    |                    |          |          | 78.6 <sup>c)</sup> |
| 6'''' |          |                    |                    |                    |           |                    |                    |          |          | 63.0               |

a) Spectra were measured in pyridine-*d*<sub>5</sub>. b, c) Assignments may be interchangeable in each column.

Compound **5** (C<sub>52</sub>H<sub>88</sub>O<sub>26</sub>) presented a positive Ehrlich's test,<sup>10)</sup> and the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra indicated **5** to be a 22-methoxyfurostanol saponin.<sup>5)</sup> Enzymatic hydrolysis of **5** with  $\beta$ -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*- $\beta$ -D-glucopyranosyl-(25*R*)-5 $\alpha$ -furostane-2 $\alpha$ ,3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ ,22 $\xi$ ,26-hexol 3-*O*-{*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-*O*-[ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 3)]- $\beta$ -D-glucopyranoside}.

The IC<sub>50</sub> of the isolated saponins and their derivatives

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

| Compounds  | IC <sub>50</sub> ( $\times 10^{-5}$ M) |
|------------|----------------------------------------|
| <b>1</b>   | 0.7                                    |
| <b>1a</b>  | —                                      |
| <b>1d</b>  | 44.7                                   |
| <b>2</b>   | 1.2                                    |
| <b>2a</b>  | —                                      |
| <b>3</b>   | 1.1                                    |
| <b>4</b>   | 2.0                                    |
| <b>5</b>   | —                                      |
| 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,<sup>11</sup> 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  $\delta$ -values referring to tetramethylsilane (TMS) as the internal standard. Assignments of the <sup>13</sup>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 octadecylsilylanized (ODS) silica-gel (Nacalai Tesque) were used for column chromatographies. TLC was carried out on precoated Kieselgel 60 F<sub>254</sub> (0.25 mm thick, Merck) and RP-18 F<sub>254</sub> S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H<sub>2</sub>SO<sub>4</sub> 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.  $\times$  250 mm or 4.6 mm i.d.  $\times$  250 mm, ODS, 5  $\mu$ m) or a TSK-gel Silica-60 column (Tosoh, 4.6 mm i.d.  $\times$  250 mm, silica-gel, 5  $\mu$ m). 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 [<sup>3</sup>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<sub>2</sub>O and extracted with *n*-BuOH. The *n*-BuOH-soluble phase was chromatographed on silica-gel with CH<sub>2</sub>Cl<sub>2</sub>–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<sub>2</sub>O 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<sub>3</sub>–MeOH–H<sub>2</sub>O (30:10:1; 20:10:1; 7:4:1) and ODS silica-gel with MeOH–H<sub>2</sub>O (4:1; 1:1) to give **1** (1.29 g), a mixture of **2** and **4**, as was shown by the <sup>1</sup>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<sub>2</sub>O, and the solution was left standing overnight. The reaction mixture was poured into ice-water and extracted with CHCl<sub>3</sub>. The extract was washed with H<sub>2</sub>O and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The acetate was chromatographed on silica-gel with hexane–Me<sub>2</sub>CO (3:2) and hexane–CHCl<sub>3</sub>–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<sub>3</sub>–MeOH–H<sub>2</sub>O (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<sub>2</sub>O, 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<sub>45</sub>H<sub>74</sub>O<sub>19</sub>·3/2H<sub>2</sub>O: C, 57.13; H, 8.20. Found: C, 57.01; H, 8.07. Negative-ion FAB-MS *m/z*: 917 [M–H]<sup>–</sup>, 772 [M–rhamnosyl]<sup>–</sup>, 756 [M–galactosyl]<sup>–</sup>. IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>–1</sup>: 3390 (OH), 2930 (CH), 1445, 1375, 1240, 1205, 1040, 975, 940, 915, 895, 860, 830, 810, 780. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 6.33 (1H, brs, 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<sub>2</sub>O, 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<sub>3</sub>–MeOH (12:1), and then with CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O (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.<sup>6</sup> Compound **1a**: a white amorphous powder,  $[\alpha]_D^{23}$  –54.0° (*c*=0.10, MeOH). EI-MS *m/z* (%): 448.3190 [M<sup>+</sup>, Calcd for C<sub>27</sub>H<sub>44</sub>O<sub>5</sub>: 488.3189] (4), 428 (2), 375 (11), 316 (22), 301 (12), 287 (13), 139 (100), 115 (19). IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>–1</sup>: 3405 (OH), 2925 (CH), 1445, 1375, 1255, 1240, 1205, 1175, 1095, 1045, 1000, 975, 955, 935, 915, 895, 860, 795. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 6.12 (1H, br, OH), 6.02 (1H, br, OH), 5.25 (1H, brs, 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). <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>–methanol-*d*<sub>4</sub>, 10:1)  $\delta$ : 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<sub>2</sub>O (1 ml) and treated with (–)- $\alpha$ -methylbenzylamine (5 mg) and Na[BH<sub>3</sub>CN] (8 mg) in EtOH (1 ml) at 40 °C for 4 h, followed by acetylation with Ac<sub>2</sub>O (0.3 ml) in pyridine (0.3 ml). The reaction mixture was passed through a Sep-Pak C<sub>18</sub> cartridge (Waters), initially with H<sub>2</sub>O–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- $\alpha$ -methylbenzylamino]-l-deoxyalditol acetate derivatives of the monosaccharides, which was then analyzed by HPLC.<sup>12</sup> Derivatives of D-glucose, D-galactose and L-rhamnose were detected.

**Acetylation of 1** Compound **1** (52 mg) was acetylated with Ac<sub>2</sub>O in pyridine and the crude acetate was purified by silica-gel column chromatography with hexane–CHCl<sub>3</sub>–EtOAc (1:1:2) to yield the corresponding decaacetate (**1c**) (36.5 mg) as a white amorphous powder. Compound **1c**: IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>–1</sup>: 3510 (OH), 2940 (CH), 1740 (C=O), 1435, 1365, 1220, 1170, 1125, 1040, 975, 945, 910, 890, 860, 830, 795. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 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, brs, 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  $\times$  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<sub>2</sub>O, 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<sub>3</sub>–MeOH–H<sub>2</sub>O (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]<sup>–</sup>. IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>–1</sup>: 3380 (OH), 2920 (CH), 1440, 1370, 1255, 1235, 1200, 1165, 1070, 1040, 975, 935, 915, 895, 860. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 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 *R*<sub>f</sub> 0.75 (CHCl<sub>3</sub>–MeOH–H<sub>2</sub>O, 20:10:1).

**Compound 2** A white amorphous powder,  $[\alpha]_D^{23}$  –72.0° (*c*=0.10, MeOH). *Anal.* Calcd for C<sub>45</sub>H<sub>74</sub>O<sub>19</sub>·2H<sub>2</sub>O: C, 56.71; H, 8.04. Found: C, 56.74; H, 7.96. Negative-ion FAB-MS *m/z*: 915 [M–H]<sup>–</sup>, 770 [M–rhamnosyl]<sup>–</sup>, 754 [M–galactosyl]<sup>–</sup>. IR  $\nu_{\max}^{\text{KBr}}$  cm<sup>–1</sup>: 3410 (OH), 2940 (CH), 1445, 1375, 1240, 1040, 975, 915, 895, 860, 780. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 6.30 (1H, brs, 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<sub>2</sub>O, 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<sub>3</sub>-MeOH (12:1) to give an aglycon (**2a**) (19 mg). Compound **2a**: a white amorphous powder,  $[\alpha]_D^{23} -52.0^\circ$  ( $c=0.10$ , MeOH). CI-MS  $m/z$  (%): 447 [ $M+H$ ]<sup>+</sup> (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<sub>27</sub>H<sub>40</sub>O<sub>4</sub>: 428.2927] (35), 395 (21), 314 (10), 299 (11), 281 (16), 239 (8), 159 (14), 139 (100), 105 (27). IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3405 (OH), 2930 (CH), 1445, 1375, 1255, 1235, 1170, 1150, 1090, 1070, 1050, 1005, 975, 920, 895, 860, 840. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 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). <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>-methanol-*d*<sub>4</sub>, 10:1)  $\delta$ : 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,  $[\alpha]_D^{23} -26.0^\circ$  ( $c=0.10$ , MeOH). Anal. Calcd for C<sub>45</sub>H<sub>70</sub>O<sub>19</sub>·5/2H<sub>2</sub>O: C, 56.30; H, 7.87. Found: C, 56.18; H, 7.54. Negative-ion FAB-MS  $m/z$ : 913 [ $M-H$ ]<sup>-</sup>, 768 [ $M$ -rhamnosyl]<sup>-</sup>, 752 [ $M$ -galactosyl]<sup>-</sup>. UV  $\lambda_{max}^{MeOH}$  nm (log  $\epsilon$ ): 237 sh (4.07), 243 (4.11), 251 sh (3.93). IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3410 (OH), 2950 (CH), 1450, 1375, 1240, 1045, 980, 915, 900, 865, 815. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 6.36 (1H, brs, 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 artifactual sapogenols. The structures remain to be determined.

**Compound 4** A white amorphous powder,  $[\alpha]_D^{23} -50.0^\circ$  ( $c=0.10$ , MeOH). Anal. Calcd for C<sub>45</sub>H<sub>74</sub>O<sub>20</sub>·3/2H<sub>2</sub>O: C, 56.18; H, 8.07. Found: C, 56.24; H, 7.96. Negative-ion FAB-MS  $m/z$ : 933 [ $M-H$ ]<sup>-</sup>, 915 [ $M-H_2O$ ]<sup>-</sup>, 788 [ $M$ -rhamnosyl]<sup>-</sup>, 772 [ $M$ -galactosyl]<sup>-</sup>. IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3400 (OH), 2930 (CH), 1445, 1375, 1240, 1175, 1065, 1045, 980, 960, 915, 895, 865. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>-methanol-*d*<sub>4</sub>)  $\delta$ : 6.24 (1H, brs, 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,  $[\alpha]_D^{23} -54.0^\circ$  ( $c=0.10$ , MeOH). Anal. Calcd for C<sub>52</sub>H<sub>88</sub>O<sub>26</sub>·H<sub>2</sub>O: C, 54.44; H, 7.91. Found: C, 54.21; H, 7.66. Negative-ion FAB-MS  $m/z$ : 1127 [ $M-H$ ]<sup>-</sup>, 965 [ $M$ -glucosyl]<sup>-</sup>. IR  $\nu_{max}^{KBr}$  cm<sup>-1</sup>: 3380 (OH), 2910 (CH), 1440, 1365, 1295, 1250, 1035, 905, 885, 800. <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>)  $\delta$ : 6.32 (1H, brs, H-1''), 4.98 (overlapping with H<sub>2</sub>O 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  $\beta$ -glucosidase (25 mg), and the mixture was incubated at room temperature overnight. The crude products were chromatographed on silica-gel with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O (20:10:1) to yield **4** (12 mg) and D-glucose. D-Glucose: TLC  $R_f$  0.39 (*n*-BuOH-Me<sub>2</sub>CO-H<sub>2</sub>O, 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.<sup>13)</sup> 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 [<sup>3</sup>H]adenosine as the only labelled compound to be counted.

**Acknowledgement** We thank Dr. Y. Shida, Mrs. C. Sakuma and Mrs. Y. Katoh of the Central Analytical Center of our college for measuring the mass and 2D NMR spectra.

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