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# Three New Cholestane Bisdesmosides from *Nolina recurvata* Stems and Their Inhibitory Activity on cAMP Phosphodiesterase and Na$^+$/K$^+$ ATPase

Yoko TAKAASHI,[a] Yoshihiro MIMAKI,*,[a] Aiko KAMEYAMA,[a] Minpei KURODA,[a]
Yutaka SASHIDA,*,[a] Tamotsu NIKAIDO,[b] Kazuo KOIKE,[b] and Taichi OHMOTO[b]

*School of Pharmacy, Tokyo University of Pharmacy and Life Science (formerly, Tokyo College of Pharmacy),[a] 1432-1, Horinouchi, Hachioji, Tokyo 192-03, Japan and School of Pharmaceutical Sciences, Toho University,[b] 2-2-1, Miyama, Funabashi, Chiba 274, Japan.* Received January 20, 1995; accepted March 15, 1995

**Fresh stems of *Nolina recurvata* were found to contain three new cholestane bisdesmosides. Their structures were determined on the basis of spectroscopic data and acid-catalyzed hydrolysis to be (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1-*O*-β-D-glucopyranoside 16-*O*-α-L-rhamnopyranoside (1), (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1,16-di-*O*-β-D-glucopyranoside (2) and (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside} 16-*O*-α-L-rhamnopyranoside (3), respectively. The prominent downfield shift of the aglycone 19-Me of 3, compared with that of 1 and 2, is likely caused by the interaction between the 19-Me and 6‴-Me of the rhamnose attached to C-2 of the inner glucose, evidence for which was provided through molecular mechanics and molecular dynamics calculation studies and by the nuclear Overhauser effect (NOE) correlation spectrum. The isolated compounds and their derivatives were evaluated for their inhibitory activity on cAMP phosphodiesterase and Na$^+$/K$^+$ ATPase.**

**Key words** *Nolina recurvata*; Agavaceae; cholestane bisdesmoside; conformational analysis; cAMP phosphodiesterase inhibition; Na$^+$/K$^+$ ATPase inhibition

In our continuous studies of the bioactive compounds contained in plants of the family Liliaceae, we have isolated a number of steroidal glycosides, some of which, *e.g.* those from *Smilax sieboldii*,[1] *Ornithogalum saundersiae*,[2] *Lilium hansonii*,[3] *L. ragale*,[4] *L. henryi*,[4] *Agapanthus inapertus*[5] and *Allium giganteum*,[6] exhibited potent inhibitory activity on cAMP phosphodiesterase. Our next target was of plants of the family Agavaceae because the occurrence of steroidal compounds in several Agavaceae plants, especially those belonging to the representative genera *Agave* and *Yucca*, is well documented,[7] and some of them are potentially of commercial importance as sources of steroidal hormones. We have now undertaken a chemical investigation of *Nolina recurvata* stems, an Agavaceae plant indigenous to Mexico with a stem swelling in the shape of sake bottle in the proximal part, and from it we isolated three new cholestane bisdesmosides (**1**—**3**). This paper refers to the structural elucidation of the new compounds based on spectroscopic data and acid-catalyzed hydrolysis, and also to the conformation of the saccharide part of **3** revealed through the molecular mechanics and molecular dynamics calculation studies and by the nuclear Overhauser effect (NOE) correlation spectrum. The inhibitory activity exhibited by the isolated compounds and their derivatives on cAMP phosphodiesterase and Na$^+$/K$^+$ ATPase is also described.

The concentrated 1-butanol-soluble phase of the methanolic extract of *N. recurvata* stems was repeatedly chromatographed on Diaion HP-20, silica-gel, Sephadex LH-20 and on octadecylsilanized (ODS) silica-gel to give compounds **1**—**3**.

Compound **1** was obtained in a yield of 0.028% (fresh weight) as a white amorphous powder, $[\alpha]_D$ −26.7° (methanol) with a molecular formula, $C_{39}H_{66}O_{13}$, deduced from elemental analysis combined with the data of the negative-ion FAB-MS exhibiting an $[M]^-$ peak at *m/z* 742. A broad IR absorption near 3420 cm$^{-1}$ was attributable to hydroxyl groups. The $^1$H-NMR spectrum showed signals due to two tertiary methyl protons at δ 1.32 and 0.98, three secondary methyl protons at δ 1.18 (d, $J=6.9$ Hz) and 0.86 (3H × 2, d, $J=6.5$ Hz), one olefinic proton at δ 5.58 (br d, $J=5.2$ Hz) and two anomeric protons of the sugar moieties at δ 5.27 (br s) and 4.99 (d, $J=7.7$ Hz). The $^{13}$C-NMR spectrum (Table 1) showed 39 resonance lines supporting the molecular formula deduced from the MS and elemental analysis; 27 of them could be due to the aglycone part and 12 to two monosaccharides. One of the monosaccharides was suggested to be a deoxyhexose by a distinct methyl proton doublet at δ 1.69 (5.2 Hz).

Acid hydrolysis of **1** with 1 N hydrochloric acid in dioxane–$H_2O$ (1 : 1) yielded an aglycone (**1a**), as well as L-rhamnose and D-glucose. The $^1$H-NMR spectrum of **1a** exhibited five methyl signals typical of steroids appearing at δ 1.39 and 1.27 (each s), 1.20 (d, $J=7.0$ Hz) and 0.90 (3H × 2, d, $J=6.5$ Hz), and four exchangeable protons at δ 6.33, 6.24, 6.02 and 5.88 as distorted singlet or doublet signals, which were coupled to the methine signals at δ 4.16, 3.98, 3.84 and 4.79, respectively, indicating the presence of four secondary hydroxyl groups. These $^1$H-NMR data and a detailed inspection of the $^{13}$C-NMR confirmed that **1a** was identical to the aglycone of schubertoside D, *i.e.* (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol, isolated by us from *Allium schubertii*.[8] The absolute configuration of C-22 was ascertained by converting the aglycone to 3β,22-bis-(*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) derivatives, followed by application of the advanced Mosher's method.[9] The steric hindrance around the hydroxyl groups of C-1 and C-16 was considered to prevent the bulky MTPA group from

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* To whom correspondence should be addressed.

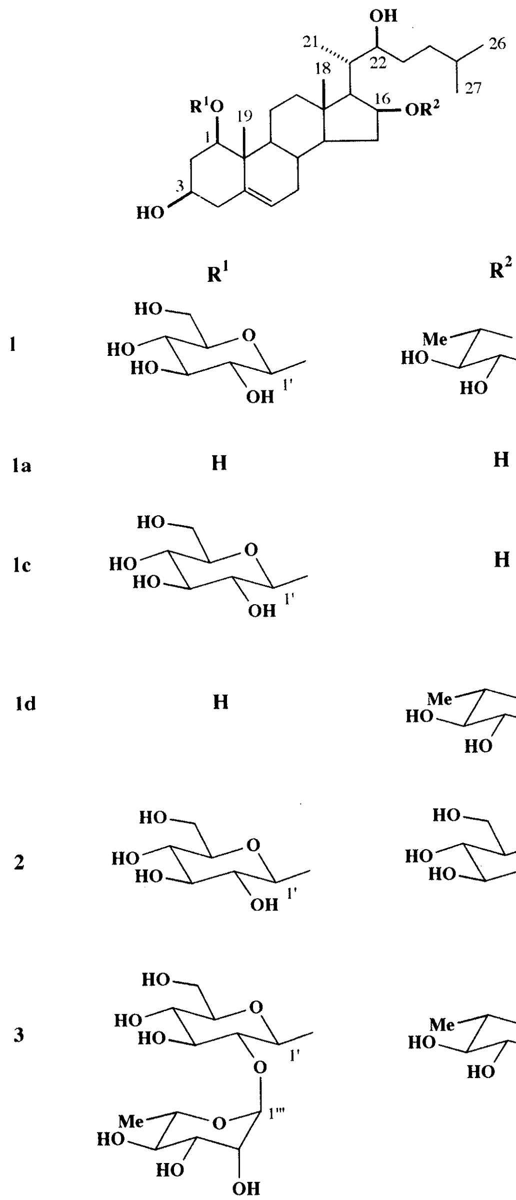


Chart 1

being introduced into the hydroxyl groups.

In the $^{13}$C-NMR spectrum of **1**, assignments of the saccharide part composed of D-glucose and L-rhamnose were performed by comparing the signals with those of authentic methyl glycosides[10]; the 6 resonance lines, $\delta$ 104.9, 72.6, 72.7, 74.0, 70.9 and 18.4 were assigned to an $\alpha$-L-rhamnopyranosyl unit and the 6 other lines, $\delta$ 101.3, 75.4, 78.7, 72.5, 78.1 and 63.7 to a $\beta$-D-glucopyranosyl unit, indicating that each monosaccharide was directly attached to the aglycone without being substituted by another monosaccharide. The $\beta$-configuration of D-glucose was reinforced by the large $J$ values of the anomeric proton (7.7 Hz). The $\alpha$-configuration of the L-rhamnose was readily confirmed by their $^{13}$C shifts; remarkable differences in the $^{13}$C shifts at C-3 and C-5 were recognized between $\alpha$-, and $\beta$-L-rhamnopyranosides.[10]

Table 1. $^{13}$C-NMR Spectral Data for Compounds **1**, **1a**, **1c**, **1d**, **2** and **3**[a)]

| C | **1** | **1a** | **1c** | **1d** | **2** | **3** |
|---|---|---|---|---|---|---|
| 1 | 82.9 | 78.2 | 82.9 | 78.2 | 82.8 | 83.6 |
| 2 | 37.6 | 44.0 | 37.6 | 44.0 | 37.6 | 37.7 |
| 3 | 68.0 | 68.2 | 68.0 | 68.2 | 68.0 | 68.1 |
| 4 | 43.7 | 43.6 | 43.8 | 43.6 | 43.8 | 43.9 |
| 5 | 139.7 | 140.4 | 139.6 | 140.4 | 139.6 | 139.7 |
| 6 | 124.7 | 124.5 | 124.8 | 124.4 | 124.8 | 124.8 |
| 7 | 31.9 | 32.3 | 32.0 | 32.1 | 31.8 | 31.9 |
| 8 | 33.2 | 33.3 | 33.3 | 33.2 | 33.2 | 33.3 |
| 9 | 50.3 | 51.5 | 50.4 | 51.4 | 50.3 | 50.5 |
| 10 | 42.8 | 43.5 | 42.8 | 43.5 | 42.8 | 42.8 |
| 11 | 23.9 | 24.2 | 23.8 | 24.2 | 23.8 | 24.1 |
| 12 | 40.5 | 41.2 | 41.0 | 40.8 | 40.6 | 40.7 |
| 13 | 42.0 | 42.4 | 42.4 | 42.0 | 42.2 | 42.1 |
| 14 | 55.1 | 55.3 | 55.1 | 55.2 | 55.3 | 55.2 |
| 15 | 35.8 | 37.5 | 37.4 | 35.9 | 37.3 | 35.9 |
| 16 | 82.2 | 75.4 | 75.4 | 82.1 | 82.7 | 82.2 |
| 17 | 58.0 | 58.4 | 58.3 | 58.1 | 58.2 | 58.1 |
| 18 | 11.9 | 15.3 | 15.3 | 11.9 | 13.8 | 11.9 |
| 19 | 14.8 | 13.9 | 14.8 | 13.9 | 14.8 | 15.0 |
| 20 | 36.1 | 36.2 | 36.1 | 36.1 | 36.0 | 36.1 |
| 21 | 13.5 | 13.7 | 13.8 | 13.4 | 12.6 | 13.6 |
| 22 | 73.2 | 71.5 | 71.5 | 73.2 | 73.2 | 73.2 |
| 23 | 34.4 | 32.0 | 32.0 | 34.4 | 33.8 | 34.5 |
| 24 | 36.8 | 36.8 | 36.8 | 36.8 | 36.7 | 36.8 |
| 25 | 28.7 | 28.5 | 28.4 | 28.7 | 28.9 | 28.7 |
| 26 | 22.8 | 22.8 | 22.8 | 22.8 | 23.0 | 22.8 |
| 27 | 22.9 | 23.0 | 23.0 | 22.9 | 23.1 | 22.9 |
| 1′ | 101.3 |  | 101.3 |  | 101.3 | 99.8 |
| 2′ | 75.4 |  | 75.4 |  | 75.4 | 79.9 |
| 3′ | 78.7 |  | 78.6 |  | 78.7 | 77.0 |
| 4′ | 72.5 |  | 72.4 |  | 72.5 | 72.6 |
| 5′ | 78.1 |  | 78.1 |  | 78.1 | 77.8 |
| 6′ | 63.7 |  | 63.6 |  | 63.7 | 63.7 |
| 1″ | 104.9 |  |  | 105.0 | 106.9 | 104.9 |
| 2″ | 72.6 |  |  | 72.5 | 75.7 | 72.6 |
| 3″ | 72.7 |  |  | 72.7 | 78.8 | 72.7 |
| 4″ | 74.0 |  |  | 74.0 | 71.8 | 74.0 |
| 5″ | 70.9 |  |  | 71.0 | 78.1 | 70.9 |
| 6″ | 18.4 |  |  | 18.4 | 63.0 | 18.4 |
| 1‴ |  |  |  |  |  | 101.6 |
| 2‴ |  |  |  |  |  | 72.8 |
| 3‴ |  |  |  |  |  | 72.9 |
| 4‴ |  |  |  |  |  | 74.3 |
| 5‴ |  |  |  |  |  | 69.4 |
| 6‴ |  |  |  |  |  | 19.1 |

*a*) Spectra were measured in pyridine-$d_5$.

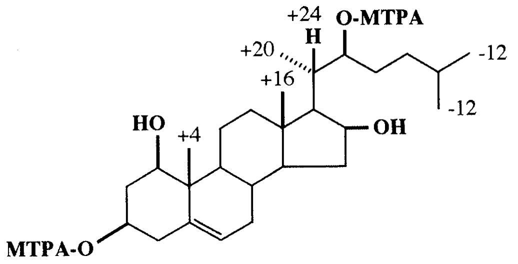


Fig. 1. Chemical Shift Differences between **1a** ($S$)-MTPA Ester (**1a″**) and **1a** ($R$)-MTPA Ester (**1a′**)

$\Delta\delta$ (Hz) $= \delta_{(S)\text{-MTPA}} - \delta_{(R)\text{-MTPA}}$.

On comparison of the $^{13}$C signals of the aglycone moiety of **1** with those of **1a**, glycosylation-induced downfield shifts were observed at C-1 and C-16 in **1**, accounting for the rhamnose and glucose linkages to the C-1 and C-16 hydroxy positions, respectively, or C-16 and C-1. The $^1$H-NMR spectrum of the nonaacetate derivative (**1b**) of **1**, which was prepared by treatment with acetic anhydride in pyridine, offered further support for the above findings. The 1-H and 16-H methine protons of **1b** appeared at $\delta$

3.62 (dd, $J=11.8$, 4.1 Hz) and 4.62 (ddd, $J=7.8$, 7.8, 4.7 Hz), whereas 3-H and 22-H shifted extremely downfield, by +0.9 ppm (δ 4.76, m) and +1.42 ppm (δ 5.43 br dd, $J=9.9$, 3.2 Hz), respectively, by *O*-acetylation compared with those of **1**. Mild hydrolysis of **1** with 0.2 N hydrochloric acid gave two partial hydrolysates (**1c**, **1d**). The $^1$H- and $^{13}$C-NMR spectra of **1c** and **1d** showed the presence of a β-D-glucopyranosyl unit in **1c** and an α-L-rhamnopyranosyl unit in **1d**. Downfield shifts by *O*-glycosylation were observed at C-1 in **1c** and at C-16 in **1d** in comparison of the $^{13}$C signals with those of **1a**. The structures of **1c** and **1d** were assigned as the 1-*O*-β-D-glucopyranoside and 16-*O*-α-L-rhamnopyranoside of **1a**, respectively. Thus, the complete structure of **1** was characterized as (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1-*O*-β-D-glucopyranoside 16-*O*-α-L-rhamnopyranoside.

The spectral properties of **2** ($C_{39}H_{66}O_{14}$) were essentially identical to those of **1**. The $^1$H- and $^{13}$C-NMR spectra exhibited signals due to two β-D-glucopyranosyl residues. Acid hydrolysis of **2** gave **1a** and D-glucose. The linkage positions of the two glucose moieties to the aglycone C-1 and C-16 were confirmed by detecting the downfield shifts by *O*-glycosylation at C-1 and C-16 and comparing the $^{13}$C-NMR of **2** with that of **1a**. The structure of **2** was shown to be (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1,16-di-*O*-β-D-glucopyranoside.

Compound **3**, $C_{45}H_{76}O_{17}$, was a more polar constituent than **1** and **2**. The presence of three sugars in **3** was apparent from the three anomeric proton signals at δ 6.48 (br s), 5.26 (br s) and 4.91 (d, $J=7.8$ Hz). Three anomeric carbon signals were also observed at δ 104.9, 101.6 and 99.8. On comparison of the $^{13}$C-NMR spectrum of **3** with that of **1**, a set of additional signals, corresponding to a terminal α-L-rhamnopyranosyl unit appeared in **3**, and the chemical shifts of the signals due to the glucose moiety attached to C-1 of the aglycone were significantly different, while all other signals were almost in line with those of **1**. It was observed that the $^{13}$C signal of C-2 of the glucose was displaced downfield at δ 79.9 (+4.5 ppm), accompanied by upfield shifts of C-1 (−1.5 ppm) and C-3 (−1.7 ppm), as compared with those of **1**, indicating that the C-2 position of the glucose was the glycosylated position to which the additional L-rhamnose was linked. Partial hydrolysis of **3** with 0.2 N hydrochloric acid furnished **1** and L-rhamnose. Consequently, the structure of **3** was formulated as (22*S*)-cholest-5-ene-1β,3β,16β,22-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside} 16-*O*-α-L-rhamnopyranoside.

Table 2. $^1$H-NMR Chemical Shift Assignments of **3**$^{a)}$

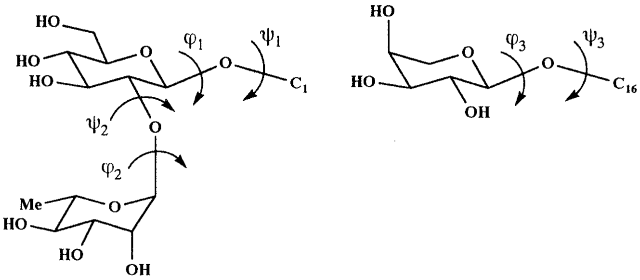
| Position | $^1$H | *J* (Hz) |
|---|---|---|
| 1 | 3.90 dd | 12.2, 4.0 |
| 2eq | 2.67 br d | 12.2, 4.0 |
| 2ax | 2.33 ddd | 12.2, 12.2, 12.2 |
| 3 | 3.76 br ddd | 12.2, 12.2, 3.4 |
| 4eq | 2.56 dd | 12.2, 3.4 |
| 4ax | 2.69 dd | 12.2, 12.2 |
| 5 | — | |
| 6 | 5.60 br d | 5.8 |
| 7eq | 1.93 br dd | 13.8, 4.7 |
| 7ax | 1.50 | |
| 8 | 1.52 | |
| 9 | 1.54 | |
| 10 | — | |
| 11eq | 2.94 ddd | 11.3, 3.1, 3.1 |
| 11ax | 1.74 | |
| 12eq | 2.11 br d | 12.2 |
| 12ax | 1.49 | |
| 13 | — | |
| 14 | 0.95 m | |
| 15α | 2.28 ddd | 13.1, 7.6, 7.6 |
| 15β | 1.61 ddd | 13.1, 13.1, 4.0 |
| 16 | 4.36 ddd | 7.6, 7.6, 4.0 |
| 17 | 1.97 dd | 11.0, 7.6 |
| 18 | 1.05 s | |
| 19 | 1.49 s | |
| 20 | 2.21 br dq | 11.0, 6.7 |
| 21 | 1.19 d | 6.7 |
| 22 | 3.98 br dd | 8.9, 3.7 |
| 23 | 1.81 br ddd | 13.1, 13.1, 3.7 |
| | 1.52 | |
| 24 | 1.72 m | |
| | 1.42 m | |
| 25 | 1.57 | |
| 26 | 0.88 d | 6.7 |
| 27 | 0.88 d | 6.7 |
| 1′ | 4.88 d | 7.6 |
| 2′ | 4.23 dd | 8.9, 7.6 |
| 3′ | 4.16 dd | 8.9, 8.9 |
| 4′ | 3.92 dd | 8.9, 8.9 |
| 5′ | 3.81 ddd | 8.9, 6.4, 2.7 |
| 6′ | 4.44 dd | 11.9, 2.7 |
| | 4.20 dd | 11.9, 6.4 |
| 1″ | 5.23 br s | |
| 2″ | 4.43 br d | 3.1 |
| 3″ | 4.38 dd | 8.9, 3.1 |
| 4″ | 4.23 | |
| 5″ | 4.25 | |
| 6″ | 1.67 d | 5.8 |
| 1‴ | 6.40 br s | |
| 2‴ | 4.68 br d | 3.4 |
| 3‴ | 4.56 dd | 9.2, 3.4 |
| 4‴ | 4.27 dd | 9.2, 9.2 |
| 5‴ | 4.85 dq | 9.2, 6.1 |
| 6‴ | 1.78 d | 6.1 |

*a)* Spectrum was measured in pyridine-$d_5$–methanol-$d_4$ (10 : 1).

The complete assignment of the $^1$H-NMR of **3** was performed by interpretation of the $^1$H–$^1$H correlation spectroscopy (COSY) combined with the phase-sensitive NOE correlation spectroscopy (PHNOESY) spectra recorded in a mixed solvent of pyridine-$d_5$–methanol-$d_4$ (10 : 1) to eliminate the exchangeable protons and minimize signal overlap (Table 2). On comparison of the 19-Me signal of the aglycone part of **3** with those of **1** and **2**, it was found that the 19-Me of **3** shifted prominently downfield, by 0.19 ppm, from that of **1**, and by 0.26 ppm from that of **2**. Furthermore, the 6‴-Me of the rhamnose attached to the C-2 of the inner glucose resonated at a relatively lower field (δ 1.80) compared with that of the usual terminal rhamnoses of oligosaccharides (about δ 1.70).[11] These observations seemed to arise from the interaction between the 19-Me and the 6‴-Me. In order to obtain evidence for this estimation, molecular mechanics and molecular dynamics calculation studies were performed on **3**. The starting geometries were generated by the Metropolis Monte Carlo search method[12] and submitted to energy minimization using the Discover-cff91 force field program.[13] The local minima thus found were taken as starting structures for molecular dynamics calculations *in vacuo* at 300 K. The calculation results are shown in Table 3, in which the glycosidic torsion angles are expressed as $\varphi$ (H1–C1–O1–$C_x$) and $\psi$ (C1–O1–$C_x$–$H_x$). The torsion angles calculated were consistent with the NOE correaltions between the anomeric protons and each proton of another substituted saccharide or the aglycone. In the preferred conformation of **3**, as illustrated in Fig. 2, the 19-Me of the aglycone was revealed to be in close proximity to 6‴-Me of the rhamnose at a distance of 3 Å, which was supported by an NOE correlation between them. In conclusion, the steric proximity of the methyl groups resulted in reduced electron density around the protons of each methyl group, which caused downfield shifts of the methyl protons.

The isolated compounds and their derivatives were assayed for inhibitory activity on cAMP phosphodiesterase and $Na^+/K^+$ ATPase to find new positive inotropic agents. The cAMP phosphodiesterase inhibition test also provided a useful tool for the primary screening of biologically active compounds present in natural sources.[14] The $Na^+/K^+$ ATPase inhibition has recently been suggested to be related to the redifferentiation of human leukemia cells.[15] The cholestane bisdesmosides (**1**—**3**) and the aglycone (**1a**) exhibited medium inhibitory activity on cAMP phosphodiesterase, while the monodesmosides (**1c** and **1d**) showed none at all (Table 4). As for the inhibitory activity on $Na^+/K^+$ ATPase, only the aglycone (**1a**) was a weak inhibitor, showing 29% inhibition at a sample concentration of 100 $\mu$M. Cholesterol was examined and showed no activity at all. The aglycone (**1a**) is a cholesterol derivative with three hydroxyl groups at C-1, -16 and -22, and the hydroxyl groups introduced into cholesterol were considered to contribute to the inducement of the weak activity of **1a**.

Table 3. Torsion Angles of the Saccharide Part of **3**



| $\varphi$ | Torsion angles | $\psi$ | Torsion angles |
|---|---|---|---|
| 1 | 43.9° | 1 | 19.8° |
| 2 | 51.8° | 2 | 23.8° |
| 3 | 28.7° | 3 | −47.2° |

**Experimental**

Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 spectrophotometer, UV on a JASCO V-520 UV/VIS spectrophotometer, and MS on a VG AutoSpec E or Hitachi M-80 instrument. Elemental analysis was carried out using a Perkin-Elmer 240B elemental analyzer. 1D NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for $^1$H-NMR) and 2D NMR on a JEOL A-500 spectrometer (500 MHz for $^1$H-NMR). Chemical shifts are given as $\delta$-values with reference to tetramethylsilane (TMS), the internal standard. Silica-gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-Kasei) and 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 using a Tosoh HPLC system (Tosoh: pump, CCPM; controller, CCP controller PX-8010; detector UV-8000) equipped with a TSK-gel Silica-60 column (Tosoh, 4.6 mm i.d. × 250 mm, silica-gel, 5 $\mu$m) and a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 $\mu$m). The liquid scintillation counter used was an Aloka LSC-903 instrument. Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase, cyclic AMP and ouabain sensitive dog kidney $Na^+/K^+$ ATPase were obtained from Sigma, and [$^3$H]cAMP was from the Radiochemical Center. All other chemicals used were of biochemical-reagent grade.

**Isolation** Commercially available fresh stems of *N. recurvata* (2.5 kg)

Table 4. Inhibitory Activity on cAMP Phosphodiesterase of the Isolated Compounds and Their Derivatives

| Compounds | $IC_{50}$ ($\times 10^{-5}$ M) |
|---|---|
| **1** | 35.3 |
| **1a** | 34.2 |
| **1c** | 500< |
| **1d** | 500< |
| **2** | 37.1 |
| **3** | 24.0 |
| Papaverine | 3.0 |

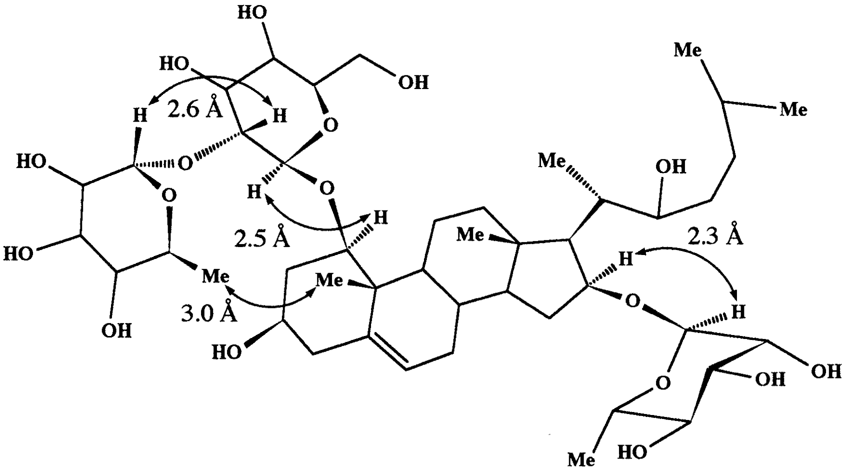


Fig. 2. Calculated Preferred Conformations of **3**

Arrows indicate the NOE correlations.

were cut into pieces and extracted with MeOH under reflux. The extract was concentrated almost to dryness under reduced pressure and the crude residue, after dilution with $H_2O$, was extracted with *n*-BuOH. The *n*-BuOH-soluble phase was fractionated on a silica-gel column, eluted with a gradient mixture of $CH_2Cl_2$–MeOH (9 : 1; 4 : 1; 2 : 1), and finally with MeOH to give four fractions (I—IV). Fraction I was chromatographed on silica-gel, eluted with $CHCl_3$–MeOH (5 : 1) and $CHCl_3$–MeOH–$H_2O$ (20 : 10 : 1), Sephadex LH-20 with MeOH, and on ODS silica-gel with MeOH–$H_2O$ (7 : 3) to give compound **1** (690 mg; 0.028%) and **2** (129 mg; 0.005%). Fraction III contained a considerable amount of saccharides, the removal of which was performed by column chromatography on Diaion HP-20 with an increasing amount of MeOH in $H_2O$. The fractions, eluted with 80% MeOH and 100% MeOH, were combined and chromatographed on silica-gel, eluted with $CHCl_3$–MeOH–$H_2O$ (30 : 10 : 1; 20 : 10 : 1), Sephadex LH-20 with MeOH, and on ODS silica-gel with MeOH–$H_2O$ (7 : 3; 3 : 2) to give compound **3** (25.7 mg; 0.001%).

**Compound 1** An amorphous solid, $[\alpha]_D^{26}$ −26.7° ($c$=0.32, MeOH). *Anal.* Calcd for $C_{39}H_{66}O_{13}\cdot H_2O$: C, 61.56; H, 9.01. Found: C, 61.56; H, 8.79. Negative-ion FAB-MS $m/z$: 742 $[M]^-$, 579 $[M-\text{glucosyl}]^-$; IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3420 (OH), 2940 (CH), 1460, 1375, 1255, 1060, 1045, 975, 900, 825, 800, 695; $^1$H-NMR (pyridine-$d_5$) $\delta$: 5.58 (1H, br d, $J$=5.2 Hz, 6-H), 5.27 (1H, br s, 1″-H), 4.99 (1H, d, $J$=7.6 Hz, 1′-H), 4.54 (1H, dd, $J$=11.2, 2.5 Hz, 6′a-H), 4.49 (1H, br d, $J$=3.1 Hz, 2″-H), 4.44 (1H, dd, $J$=8.5, 3.1 Hz, 3″-H), 4.37 (1H, ddd, $J$=7.3, 7.3, 4.1 Hz, 16-H), 4.35—4.28 (3H, overlapping, 6′b-H, 4″-H and 5″-H), 4.24 (1H, dd, $J$=8.9, 8.9 Hz, 3′-H), 4.12 (1H, dd, $J$=8.9, 8.9 Hz, 4′-H), 4.05 (1H, dd, $J$=8.9, 7.6 Hz, 2′-H), 4.01 (1H, br d, $J$=10.4 Hz, 22-H), 3.98 (1H, dd, $J$=11.3, 3.7 Hz, 1-H), 3.94 (1H, m, 5′-H), 3.86 (1H, m, 3-H), 1.69 (3H, d, $J$=5.2 Hz, 6″-Me), 1.32 (3H, s, 19-Me), 1.18 (3H, d, $J$=6.9 Hz, 21-Me), 0.98 (3H, s, 18-Me), 0.86 (3H × 2, d, $J$=6.5 Hz, 26-Me and 27-Me).

**Acid Hydrolysis of 1** A solution of **1** (75.3 mg) in 1 N HCl (dioxane–$H_2O$, 1 : 1, 5 ml) was refluxed for 1 h under an Ar atmosphere. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column, and it was then transferred to a silica-gel column, eluted with $CHCl_3$–MeOH (19 : 1) and then with MeOH to give an aglycone (**1a**) (12.2 mg) and a mixture of monosaccharides with several decomposed compounds (41.7 mg). Compound **1a**: an amorphous solid, $[\alpha]_D^{27}$ −60.0° ($c$=0.10, $CHCl_3$). CI-MS $m/z$ (%): 416 $[M-H_2O]^+$ (71), 399 $[M-H_2O\times2+H]^+$ (100), 381 $[M-H_2O\times3+H]^+$ (70), 363 $[M-H_2O\times4+H]^+$ (18), 318 (8), 299 (29), 287 (48), 271 (23); IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3400 (OH), 2950 (CH), 1460, 1380, 1055, 1025, 1005, 985, 825; $^1$H-NMR (pyridine-$d_5$) $\delta$: 6.33 (1H, br d, $J$=4.9 Hz, 22-OH), 6.24 (1H, br s, 3-OH), 6.02 (1H, br d, $J$=5.8 Hz, 1-OH), 5.88 (1H, d, $J$=3.4 Hz, 16-OH), 5.65 (1H, br d, $J$=5.4 Hz, 6-H), 4.79 (1H, m, 16-H), 4.16 (1H, m, 22-H), 3.98 (1H, m, 3-H), 3.84 (1H, ddd, $J$=11.9, 5.8, 5.8 Hz, 1-H), 1.39 (3H, s, 19-Me), 1.27 (3H, s, 18-Me), 1.20 (3H, d, $J$=7.0 Hz, 21-Me), 0.90 (3H × 2, d, $J$=6.5 Hz, 26-Me and 27-Me).

**Identification of Monosaccharides of 1** To a sugar mixture (2.3 mg) in $H_2O$ (1 ml), (−)-α-methylbenzylamine (8 mg) was added, followed by Na[$BH_3CN$] (8 mg) in EtOH (1 ml). The mixture was left standing for 3 h at 40 °C, and after evaporation of the solvent completely, it was acetylated with $Ac_2O$ (0.3 ml) in pyridine (0.3 ml). The reaction mixture was passed through a Sep-Pak $C_{18}$ cartridge and eluted with $H_2O$–MeCN (4 : 1; 1 : 1; 1 : 9, each 5 ml). The $H_2O$–MeCN (9 : 1) fraction was then passed through a Toyopak IC-SP M cartridge (Tosoh) and eluted with EtOH (10 ml) to give a mixture of the 1-[($S$)-$N$-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which was then analyzed by HPLC.[16] Derivatives of D-glucose and L-rhamnose were detected.

***(R)*-, *(S)*-MTPA Derivatives of 1a** Compound **1a** (4.3 mg) was dissolved in dry $CH_2Cl_2$ (0.5 ml) and pyridine (0.3 ml), to which were added ($R$)-MTPA-Cl (20 mg) and 4-(dimethylamino)pyridine (3 mg), and the solution was allowed to stand at room temperature for 1 h. The reaction mixture was evaporated and, after dilution with $H_2O$, extracted with $Et_2O$ twice. The $Et_2O$ phase was dried over anhydrous $Na_2SO_4$ and chromatographed on silica-gel using hexane–$Me_2CO$ (12 : 1) and hexane–EtOAc (12 : 1) to yield the ($R$)-MTPA ester (**1a′**) (6.2 mg) of **1a**. Following this procedure, **1a** (4.3 mg) was converted to ($S$)-MTPA ester (**1a″**) (6.3 mg). $^1$H-NMR of **1a′** (chloroform-$d_1$) $\delta$: 7.61—7.36 (10H, aromatic protons), 5.62 (1H, br d, $J$=5.5 Hz, 6-H), 5.41 (1H, br dd, $J$=7.3, 7.3 Hz, 22-H), 4.87 (1H, m, 3-H), 4.33 (1H, ddd, $J$=7.6, 7.6, 4.5 Hz, 16-H), 3.57—3.50 (overlapping with OMe signals, 1-H), 3.55 and 3.50 (each 3H, s, OMe × 2), 2.15 (1H, m, 20-H), 1.02 (3H, s, 19-Me), 0.90 (3H, d, $J$=6.9 Hz, 21-Me), 0.87 (3H, d, $J$=6.4 Hz, 26-Me), 0.85 (3H, d, $J$=6.4 Hz, 27-Me), 0.84 (3H, s, 18-Me). $^1$H-NMR of **1a″** (chloroform-$d_1$) $\delta$: 7.60—7.38 (10H, aromatic protons), 5.63 (1H, br d, $J$=5.4 Hz, 6-H), 5.43 (1H, br dd, $J$=7.0, 7.0 Hz, 22-H), 4.86 (1H, m, 3-H), 4.40 (1H, m, 16-H), 3.58—3.50 (overlapping with OMe signals, 1-H), 3.55 and 3.52 (each 3H, s, OMe × 2), 2.21 (1H, m, 20-H), 1.03 (3H, s, 19-Me), 0.95 (3H, d, $J$=6.9 Hz, 21-Me), 0.88 (3H, s, 18-Me), 0.84 (3H, d, $J$=6.6 Hz, 26-Me), 0.82 (3H, d, $J$=6.7 Hz, 27-Me).

**Acetylation of 1** Compound **1** (30 mg) was acetylated with $Ac_2O$ in pyridine and the crude acetate was chromatographed on silica-gel using hexane–$Me_2CO$ (3 : 2) to give the corresponding nonaacetate (**1b**) (23.9 mg). IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 2955 (CH), 1755 (C=O), 1435, 1365, 1230, 1125, 1035, 975, 950, 925, 900, 835, 695; $^1$H-NMR (pyridine-$d_5$) $\delta$: 5.78 (1H, dd, $J$=9.8, 9.8 Hz, 3′-H), 5.74 (1H, dd, $J$=10.1, 3.2 Hz, 3″-H), 5.71 (1H, dd, $J$=3.2, 1.5 Hz, 2″-H), 5.63 (1H, dd, $J$=10.1, 9.6 Hz, 4″-H), 5.61 (1H, br d, $J$=6.1 Hz, 6-H), 5.54 (1H, dd, $J$=9.8, 9.8 Hz, 4′-H), 5.47 (1H, dd, $J$=9.8, 8.1 Hz, 2′-H), 5.43 (1H, br dd, $J$=9.9, 3.2 Hz, 22-H), 5.29 (1H, d, $J$=1.5 Hz, 1″-H), 4.97 (1H, d, $J$=8.1 Hz, 1′-H), 4.76 (1H, m, 3-H), 4.62 (1H, ddd, $J$=7.6, 7.6, 4.7 Hz, 16-H), 4.54 (1H, dd, $J$=12.1, 4.7 Hz, 6′a-H), 4.45 (1H, dd, $J$=12.1, 2.1 Hz, 6′b-H), 4.34 (1H, dd, $J$=9.6, 6.3 Hz, 5″-H), 4.12 (1H, ddd, $J$=9.8, 4.7, 2.1 Hz, 5′-H), 3.62 (1H, dd, $J$=11.8, 4.1 Hz, 1-H), 2.22, 2.17, 2.13, 2.11, 2.08, 2.05, 2.04, 2.03 and 1.99 (each 3H, s, Ac), 1.42 (3H, d, $J$=6.3 Hz, 6″-Me), 1.15 (3H, d, $J$=6.9 Hz, 21-Me), 1.12 (3H, s, 19-Me), 1.05 (3H, d, $J$=6.6 Hz, 26-Me or 27-Me), 1.00 (3H, d, $J$=6.5 Hz, 26-Me or 27-Me), 0.99 (3H, s, 18-Me).

**Partial Hydrolysis of 1** A solution of **1** (150 mg) in 0.2 N HCl (dioxane–$H_2O$, 1 : 1, 4 ml) was refluxed for 30 min under an Ar atmosphere. The reaction mixture was neutralized by passing it through an Amberlite IRA-93 ZU column, and was chromatographed on silica-gel eluted with $CHCl_3$–MeOH (6 : 1) to give partial hydrolysates, **1c** (18.1 mg) and **1d** (9.8 mg). Compound **1c**: an amorphous solid, $[\alpha]_D^{27}$ −11.4° ($c$=0.11, MeOH). Negative-ion FAB-MS $m/z$: 595 $[M-H]^-$; IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3400 (OH), 2940 (CH), 1460, 1380, 1260, 1155, 1065, 1030, 885, 825; $^1$H-NMR (pyridine-$d_5$) $\delta$: 5.60 (1H, br d, $J$=4.8 Hz, 6-H), 5.00 (1H, d, $J$=7.6 Hz, 1′-H), 1.29 (3H, s, 19-Me), 1.21 (3H, s, 18-Me), 1.15 (3H, d, $J$=6.8 Hz, 21-Me), 0.89 (3H × 2, d, $J$=6.5 Hz, 26-Me, 27-Me). Compound **1d**: an amorphous solid, $[\alpha]_D^{27}$ −29.1° ($c$=0.46, MeOH). Negative-ion FAB-MS $m/z$: 579 $[M-H]^-$; IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3430 (OH), 2920 (CH), 1450, 1375, 1250, 1115, 1045, 975, 900, 825, 800; $^1$H-NMR (pyridine-$d_5$) $\delta$: 5.64 (1H, br d, $J$=5.2 Hz, 6-H), 5.28 (1H, br s, 1″-H), 1.70 (3H, d, $J$=5.5 Hz, 6″-Me), 1.41 (3H, s, 19-Me), 1.21 (3H, s, 18-Me), 1.04 (3H, d, $J$=6.9 Hz, 21-Me), 0.86 (3H × 2, d, $J$=6.5 Hz, 26-Me and 27-Me).

**Compound 2** An amorphous solid, $[\alpha]_D^{26}$ −11.7° ($c$=0.29, MeOH). *Anal.* Calcd for $C_{39}H_{66}O_{14}\cdot3H_2O$: C, 57.62; H, 8.93. Found: C, 57.32; H, 8.40. Negative-ion FAB-MS $m/z$: 757 $[M-H]^-$; IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3400 (OH), 2940 (CH), 1455, 1370, 1255, 1155, 1065, 885, 825, 695; $^1$H-NMR (pyridine-$d_5$) $\delta$: 5.50 (1H, br d, $J$=5.2 Hz, 6-H), 4.97 (1H, d, $J$=7.6 Hz, 1′-H), 4.74 (1H, d, $J$=7.7 Hz, 1″-H), 1.25 (3H, s, 19-Me), 1.16 (3H, d, $J$=6.9 Hz, 21-Me), 1.03 (3H, s, 18-Me), 0.93 (3H × 2, d, $J$=5.9 Hz, 26-Me and 27-Me).

**Acid Hydrolysis of 2** A solution of **2** (10.1 mg) in 1 N HCl (dioxane–$H_2O$, 1 : 1, 2 ml) was treated identically to that of **1** to give **1a** (4 mg) and saccharide fraction (5.8 mg). D-Glucose in the saccharide fraction was identified by converting it into a 1-[($S$)-$N$-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative followed by HPLC analysis.

**Compound 3** An amorphous solid, $[\alpha]_D^{26}$ −45.4° ($c$=0.64, MeOH). *Anal.* Calcd for $C_{45}H_{76}O_{17}\cdot2H_2O$: C, 58.42; H, 8.72. Found: C, 58.60; H, 8.43. Negative-ion FAB-MS $m/z$: 887 $[M-H]^-$; IR $\nu_{\max}^{\mathrm{KBr}}$ cm$^{-1}$: 3400 (OH), 2940 (CH), 1450, 1375, 1120, 1040, 975, 905, 830, 805, 695; $^1$H-NMR (pyridine-$d_5$) $\delta$: 6.48 (1H, br s, 1‴-H), 5.58 (1H, br d, $J$=5.4 Hz, 6-H), 5.26 (1H, br s, 1″-H), 4.91 (1H, d, $J$=7.7 Hz, 1′-H), 1.80 (3H, d, $J$=6.2 Hz, 6‴-Me), 1.68 (3H, d, $J$=5.5 Hz, 6″-Me), 1.51 (3H, s, 19-Me), 1.19 (3H, d, $J$=6.9 Hz, 21-Me), 1.04 (3H, s, 18-Me), 0.86 (3H × 2, d, $J$=6.5 Hz, 26-Me and 27-Me).

**Partial Hydrolysis of 3** Compound **3** (10 mg) was subjected to partial hydrolysis, the same as **1**. The crude hydrolysate was chromatographed on silica-gel and eluted with $CHCl_3$–MeOH (5 : 1) to give **1** (1.2 mg) and L-rhamnose.

**Conformational Calculation** The starting geometries were generated by the Metropolis Monte Carlo search method[12] using the CONFSEARCH program,[17] and were submitted to energy minimization using the Discover-cff91 force field program.[13] The local minima thus found were taken as the starting structures for molecular dynamics calculations *in vacuo* at 300 K and at a time step of 1 fs. The equilibration time was 1 ps and the total simulation time was 1000 ps. Trajectory frames were saved every 0.01 ps. The trajections were then examined with the Analysis module of Insight II.[18] Calculations were performed on an IRIS Indigo Elan computer.

**Assay of cAMP Phosphodiesterase Activity** The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously.[14b,14c] The assay was 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, leaving [$^3$H]adenosine as the only labelled compound to be counted.

**Assay of $Na^+/K^+$ ATPase Activity** The $Na^+/K^+$ ATPase activity was assayed according to the reported method[19] with some modification. The reaction mixture composed of 50 mM Tris–HCl (pH 7.3, 37 °C), 3 mM ATP, 4 mM $Mg^{2+}$, 130 mM $Na^+$, 20 mM $K^+$, 0.02 units of $Na^+/K^+$ ATPase, with and without 0.1 mM test compound dissolved in dimethylsulfoxide (DMSO), was incubated for 15 min at 37 °C. The concentration of DMSO in the mixture was held at 5%. The reaction was terminated by the addition of 50% $CCl_3COOH$. The released inorganic phosphate was determined by a modification of the Fiske–Subbarow method.[20] To the test solution was added 0.5% sodium dodecyl sulfate, 0.1% 2,4-diaminophenol·2HCl in 1% $Na_2SO_3$ and 1% ammonium heptamolybdate in 1 M $H_2SO_4$. After being set aside 20 min, the absorbance at 660 nm was recorded.

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