Four Novel Withanolide-Type Steroids from the Leaves of *Solanum cilistum*

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Four novel withanolide-type steroids named cilistols p, pm, p1 and u (1—4, respectively), were isolated from the leaves of *Solanum cilistum*. The respective structures were characterized by spectroscopic means as follows: cilistol p (1) was (22*R*,24*R*,25*R*,26*S*)-1-oxo-22,26-epoxy-3α,5α-cycloergostane-6β,17α, 24,25,26-pentaol 26-*O*-β-D-glucopyranoside, cilistol pm (2) corresponded to the 6-*O*-methyl ether derivative of 1; cilistol p1 (3) was represented as the 24-*O*-methyl ether of 1, and cilistol u (4) was shown to be the epoxide between C-24 and -25, presumably bearing cilistols p, pm and p1 by ring-opening.

Key words *Solanum cilistum*; Solanaceae; withanolide-type steroid; 3,5-*cyclo*-steroid; cilistol

In our search for bioactive steroidal glycosides with potent anti-cancer[1] and anti-herpes simplex virus type-1 (HSV-1)[2] activities among the *Solanum*-genera plants, we have found the occurence of withanolide-type steroids,[3—5] which are characteristic constituents, being quite different from the so-far obtained spirostanol, furostanol, spirosolane and solanidane glycosides,[6] from the leaves of *Solanum cilistum*. We reported twelve withanolide-type steroids, named cilistols a, b, d, q, f, g, v, t, i, j, y and w.[3—5] In a continuing study on the steroidal constituent in the leaves, we have isolated four additional novel withanolide-type steroids. Herein, we describe their structural characterization.

The leaves (560 g) of this plant was extracted with hot MeOH repeatedly and was concentrated under reduced pressure to give an extract (68.0 g), which was defatted with hexane. The insoluble layer was chromatographed on Diaion HP-20P by eluting with H_2O, 30, 50, 70 and 90% aq. MeOH, successively. The respective fractions were subsequently subjected to a variety of column chromatographies such as silica gel ($CHCl_3$: MeOH=20 : 1→10 : 1, $CHCl_3$: MeOH : H_2O= 9 : 1 : 0.1→8 : 2 : 0.2), Chromatorex ODS (60% MeOH→90% MeOH→MeOH), and Sephadex LH-20 (MeOH) to afford four whithanolide-type steroids, named cilistols p (**1**, 14.8 mg), pm (**2**, 102.8 mg), p1 (**3**, 35.2 mg) and u (**4**, 25.5 mg).

Cilistol p (**1**) was obtained as an amorphous powder, $[\alpha]_D$ −77.8° (MeOH). A *quasi*-molecular ion peak at *m/z* 655 $[M+H]^+$ in the positive FAB-MS and elemental analysis of **1** indicated its molecular formula to be $C_{34}H_{54}O_{12}$. At first, signals (in pyridine-d_5) due to two steroidal angular methyl groups (each 3H, s, at δ 0.86, 1.47) at C-18 and -19, respectively, one secondary methyl group (3H, d, J=6.7 Hz at δ 1.38) at C-21, H-22 (δ 5.10, br d, J=12.2 Hz), one β-D-glucopyranosyl moiety (H-1, d, J=7.9 Hz at δ 5.35; H-2, t-like, J=8.3 Hz at δ 4.00; H-3, t-like, J=8.3 Hz; H-4, t-like, J=8.3 Hz at δ 4.25; H-5, m, at δ 3.94; H-6, dd, J=4.9, 12.2 Hz at δ 4.39; H′-6, br d, J=12.2 Hz at δ 4.49) and H-26 (an acetal proton, s, at δ 5.59) on the ^{1}H-NMR spectrum could be easily assigned by comparing with those co-existing withanolide-type steroids so far obtained.[3—5] Furthermore, the ^{1}H–^{1}H shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC, Fig. 1) techniques led to the assignments of the signals due to two methylene-protons (each 1H, t-like, J=4.9 Hz, at δ 0.08, 0.89) being characteristic in respect to appearance in very high field, two tertiary methyl groups (each 3H, s, at δ 1.79, 2.06) adjacent to the oxygen function, a carbonyl group, and an oxygen-bearing methine-proton (br s, at δ 3.46) to be H_2-4, H_3-27; H_3-28 and H-6. The signals due to a methine group at C-3 (δ_H 1.27, m, and δ_C 15.7), a methylene group at C-4 (δ_H 0.08, 0.89, δ_C 18.0) and a quaternary carbon at C-5 (δ_C 52.9) were correlated to each other between H-3 and H_2-4 in the ^{1}H–^{1}H COSY and between C-5 and H_3-19 in the HMBC, establishing the presence of a 3,5-*cyclo* structure. Thus, the plain structure of **1** was represented as shown in Fig. 1. The nuclear Overhauser enhancement spectroscopy (NOESY, Fig. 2) was observed between H-4 (δ 0.89) and H-3 (δ 1.29), H-4 (δ 0.89) and H-6 (δ 3.46), H_2-4 (δ 0.08, 0.89) and H_3-19 (δ 1.47). Since the H-6 signal appeared as a broad singlet at δ 3.46, the orientation of the hydroxyl group at C-6 was concluded as β-side, taking into consideration the correlation between the coupling constant and the vicinal angle on the stereo model. Furthermore, concerning the relative configurations at C-22, -24, -25 and -26, based upon the result of the NOESY between H-22 and H-23, H-22 and H_3-28, respectively, and H-26 and H_3-27 as illustrated in Fig. 2 and X-ray analysis of cilistol a,[3,4] they were concluded to be 22*R*,24*R*, 25*R*,26*S*. Consequently, the structure of **1** was (22*R*,24*R*,25*R*, 26*S*)-1-oxo-22,26-epoxy-3α,5α-cycloergostane-6β,17α, 24,25,26-pentaol 26-*O*-β-D-glucopyranoside.

Cilistol pm (**2**) was obtained as an amorphous powder with $[\alpha]_D$ −65.2° (MeOH). A *quasi*-molecular ion peak at *m/z* 669 $[M+H]^+$ in the positive FAB-MS and elemental analysis of **2** indicated its molecular formula to be $C_{35}H_{56}O_{12}$. By comparing the ^{1}H- and ^{13}C-NMR spectra (in pyridine-d_5) of **2** with those of **1**, **2** was shown to be almost identical with **1**. Its molecule weight was higher by 14 mass units than that of **1**, suggesting **2** to be a methyl derivative of **1**. Appearance of the methoxyl signal at δ 3.30 (3H, s) in the ^{1}H-NMR spectrum supported this assumption. The HMBC observation between methyl protons at δ_H 3.30 and C-6 at δ_C 81.6 led to the conclusion that the methyl group links to the hydroxyl at C-

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Table 1. ^{13}C-NMR Data for Cilistols p (**1**), pm (**2**), p1 (**3**), and u (**4**) in Pyridine-d_5

Position	1	2	3	4
C- 1	217.6	216.9	217.7	217.6
- 2	39.9	39.5	38.8	39.8
- 3	15.7	14.4	15.7	15.6
- 4	18.0	18.5	18.0	18.0
- 5	52.9	53.1	52.9	52.9
- 6	72.2	81.6	72.2	72.2
- 7	38.8	35.1	38.6	38.8
- 8	30.0	30.3	30.0	29.9
- 9	47.6	47.5	47.6	47.5
-10	36.0	33.0	36.0	35.9
-11	22.6	22.5	22.6	22.6
-12	32.8	32.7	32.8	32.6
-13	49.0	49.7	49.0	48.8
-14	50.3	50.1	50.3	50.2
-15	24.4	24.4	24.4	24.3
-16	38.1	38.0	38.0	37.9
-17	85.3	85.3	85.2	85.0
-18	15.1	15.1	15.2	15.1
-19	15.3	13.9	15.3	15.2
-20	43.8	43.8	43.7	43.7
-21	10.5	10.4	10.3	10.2
-22	70.8	70.8	70.3	68.0
-23	41.0	41.0	39.9	33.9
-24	72.9	73.0	77.3	61.4
-25	74.9	74.9	75.2	61.6
-26	102.0	102.1	102.0	100.2
-27	22.6	22.7	22.4	17.4
-28	25.4	25.4	19.0	18.7
6-OMe		56.6		
24-OMe			50.1	
Glc				
C-1	99.7	99.7	99.5	95.8
-2	74.8	74.8	74.8	74.6
-3	78.8	78.8	78.8	78.5
-4	71.4	71.4	71.3	71.6
-5	79.1	79.1	79.0	78.6
-6	62.3	62.3	62.3	62.8

6. Consequently, the structure of **2** could be represented as the 6-*O*-methyl ether of **1**.

Cilistol p1 (**3**) was isolated as an amorphous powder with $[\alpha]_D$ −125.0° (MeOH). A *quasi*-molecular ion peak at *m*/*z* 669 $[M+H]^+$ in the positive FAB-MS and elemental analysis of **3** indicated its molecular formula to be $C_{35}H_{56}O_{12}$, which is identical with that of **2**. The ^{1}H-NMR spectrum (in pyridine-d_5) resembled those of **1** and **2**; however, a methoxyl signal newly appeared at δ 3.50 and the signals due to H_3-27 and H_3-28 shifted toward higher field by 0.13 and 0.17 ppm, respectively, by comparing with those of **1**. The ^{13}C-NMR signal at δ 77.3 assignable to C-24 was shifted by +4.40 ppm in comparison with that of **1**. Accordingly, it was apparent that the methyl group was attached to the hydroxyl group at C-24. The structure of **3** could be represented as the 24-*O*-methyl ether derivative of **1**.

Cilistol u (**4**) was obtained as an amorphous powder, $[\alpha]_D$ −102.0° (MeOH). A *quasi*-molecular ion peak at *m*/*z* 637 $[M+H]^+$ in the positive FAB-MS and elemental analysis of **4** indicated its molecular formula to be $C_{34}H_{52}O_{11}$. The whole pattern of the ^{1}H-NMR spectrum (in pyridine-d_5) resembled that of **1**; however, the signals due to H_3-27 and H_3-28 appeared at δ 1.43 and 1.26, being higher field by 0.36 and 0.80 ppm, respectively. Therefore, **4** was regarded as an

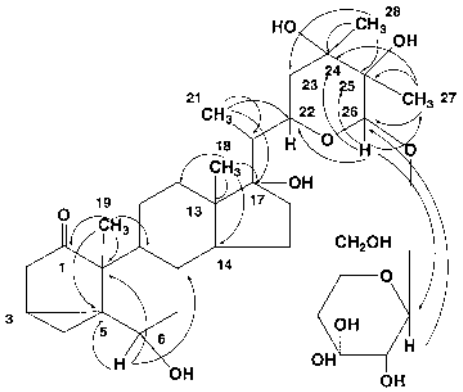


Fig. 1. Significant Correlations Observed in the HMBC Spectrum of **1**

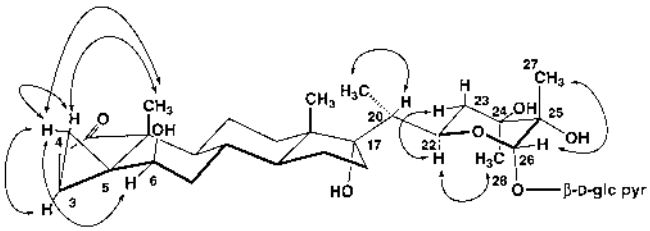
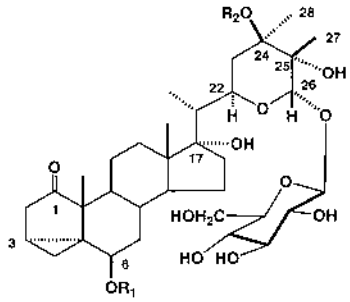


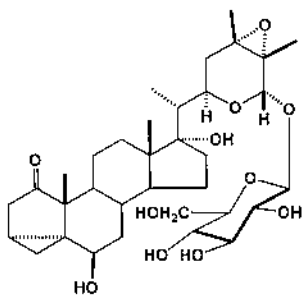
Fig. 2. Significant Correlations Observed in the NOESY Spectrum of **1**



Cilistol p (1) R_1=R_2=H

Cilistol pm (2) R_1=Me, R_2=H

Cilistol p1 (3) R_1=H, R_2=Me



Cilistol u (4)

Fig. 3. The Structures of Cilistols **1**—**4**

epoxy compound at C-24 and -25 corresponding to **1**. Consequently, the structure of **4** was (22*R*,24*S*,25*R*,26*S*)-1-oxo-22,26;24,25-diepoxy-3α,5α-cycloergostane-6β,17α,26-triol 26-*O*-β-D-glucopyranoside.

The compounds **1**—**4** with the 3,5-*cyclo* structure obtained

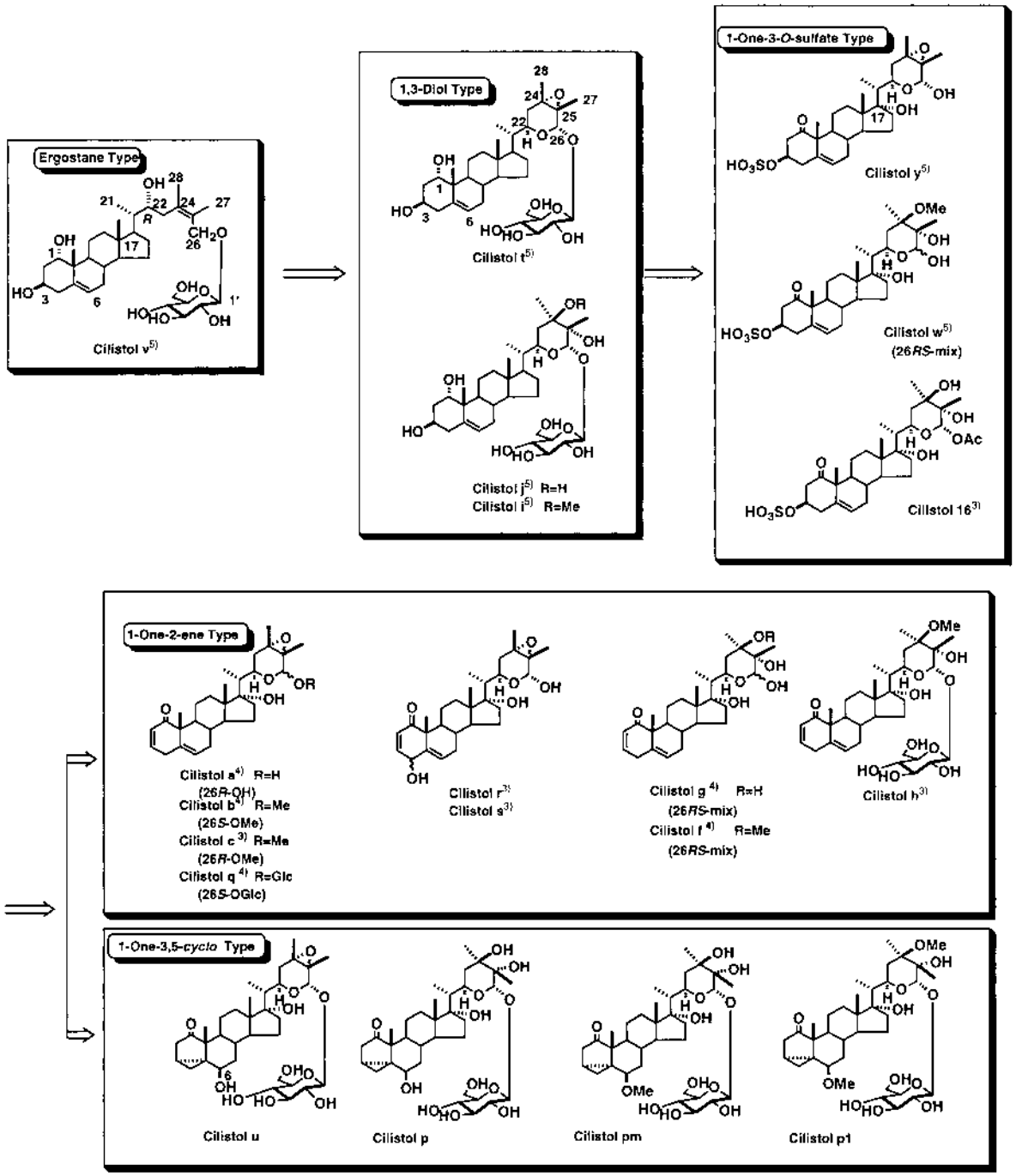


Fig. 4. Hypothetical Biogenetic Pathway

at this time were suspected to be produced secondarily from 3-*O*-sulfate type such as cilistols y and w[5] during extraction and separation; however, reinvestigation by extraction using a mixture solvent of acetone and water at room temperature substantiated the occurrence of **1**—**4**; therefore, they are regarded as genuine constituents.

In so far as **1**—**4** have the acetal group at C-26 and the 3,5-*cyclo*-propane ring, they are distinguishable from other withanolides obtained from solanaceous plants.

Taking into account the withanolide type-steroids so far obtained from the title plant, a biogenetic hypothetical scheme would be proposed as shown in Fig. 4.

Experimental

General Procedures Optical rotations were determined on a JASCO DIP-1000 polarimeter (l=0.5). FAB-MS were obtained in a glycerol matrix in the positive ion mode using a JEOL JMS-DX300 and JMS-DX303HF. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer (500 MHz) and chemical shifts were referenced to tetramethylsilane (TMS). Column chromatography was carried out on silica gel 60 (230—400 mesh, Merck), Sephadex LH-20 (25—100 nm, Pharmacia Fine Chemicals), MCI gel CHP-20P (75—150 μm, Mitsubishi Kasei), Chromatorex ODS (30—50 μm, Fuji Silysia Chemical Ltd.), and TLC was performed on precoated silica gel 60F_{254} (0.2 mm, Merck).

Plant Material Seeds of the title plant were provided by Dr. Masaharu Matsui (National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, Ano, Mie, Japan) and cultivated at the Botanical Garden of Kumamoto University.

Extraction and Isolation The leaves (560 g) of this plant was extracted with hot MeOH repeatedly and evaporated under reduced pressure to give an extract (68.0 g), which was defatted with hexane. The insoluble layer was chromatographed on Diaion HP-20P by eluting with H_2O, 30, 50, 70 and 90% aq. MeOH, successively. The respective fractions were subsequently subjected to a variety of column chromatographies such as silica gel ($CHCl_3$: MeOH = 20 : 1→10 : 1, $CHCl_3$: MeOH : H_2O = 9 : 2 : 0.1), Chromatorex ODS (60% MeOH→80% MeOH→90% MeOH→MeOH), and Sephadex LH-20 (MeOH) to afford four whithanolide-type steroids, named cilistols p (**1**, 14.8 mg), pm (**2**, 102.8 mg), p1 (**3**, 35.2 mg) and u (**4**, 25.5 mg).

Cilistol p (**1**): Amorphous powder, $[\alpha]_D^{23}$ −77.8° (c=0.23, MeOH). Pos. FAB-MS (m/z): 655 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.07 (1H, t-like, J=4.9 Hz, H-4), 0.86 (3H, s, H_3-18), 0.89 (1H, t-like, J=4.9 Hz, H′-4), 1.27 (1H, m, H-3), 1.38 (3H, d, J=6.7 Hz, H_3-21), 1.47 (3H, s, H_3-19), 1.79 (3H, s, H_3-27), 2.06 (3H, s, H_3-28), 2.10 (1H, d, J=18.0 Hz, H-2), 2.34 (1H, t-like, J=12.8 Hz, H-23), 2.47 (1H, m, H-20), 2.67 (1H, t-like, J=12.8 Hz, H′-23), 2.84 (1H, dd, J=4.0, 18.0 Hz, H′-2), 3.46 (1H, br s, H-6), 3.99 (1H, m, glc H-5), 4.00 (1H, t-like, J=8.3 Hz, glc H-2), 4.22 (1H, t-like, J=8.3 Hz, glc H-3), 4.25 (1H, t-like, J=8.3 Hz, glc H-4), 4.39 (1H, dd, J=4.9, 12.0 Hz, glc H-6), 4.49 (1H, br d, J=12.0 Hz, glc H′-6), 5.10 (1H, br d, J=12.2 Hz, H-22), 5.36 (1H, d, J=8.3 Hz, glc H-1), 5.59 (1H, s, H-26). ^{13}C-NMR: see Table 1. *Anal.* Calcd for $C_{34}H_{54}O_{12}$: C, 62.36; H, 8.31. Found: C, 62.23; H, 8.33.

Cilistol pm (**2**): Amorphous powder, $[\alpha]_D^{23}$ −65.2° (c=0.36, MeOH). Pos. FAB-MS (m/z): 669 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.10 (1H, t-like, J=6.1 Hz, H-4), 0.82 (3H, s, H_3-18), 0.89 (1H, t-like, J=4.9 Hz, H′-4), 1.12 (1H, m, H-3), 1.29 (3H, s, H_3-19), 1.37 (3H, d, J=7.3 Hz, H_3-21), 1.79 (3H, s, H_3-27), 2.04 (1H, d, J=17.1 Hz, H-2), 2.07 (3H, s, H_3-28), 2.35 (1H, t-like, J=12.8 Hz, H-23), 2.47 (1H, m, H-20), 2.67 (1H, t-like, J=12.8 Hz, H′-23), 2.70 (1H, br s, H-6), 2.82 (1H, dd, J=3.7, 17.1 Hz, H′-2), 3.30 (3H, s, OMe-6), 3.95 (1H, m, glc H-5), 4.01 (1H, t-like, J=8.6 Hz, glc H-2), 4.23 (1H, t-like, J=8.6 Hz, glc H-3), 4.26 (1H, t-like, J=8.6 Hz, glc H-4), 4.41 (1H, dd, J=4.9, 12.2 Hz, glc H-6), 4.50 (1H, br d, J=12.2 Hz, glc H′-6), 5.11 (1H, br d, J=12.2 Hz, H-22), 5.36 (1H, d, J=8.6 Hz, glc H-1), 5.60 (1H, s, H-26). ^{13}C-NMR: see Table 1. *Anal.* Calcd for $C_{35}H_{56}O_{12}$: C, 62.85; H, 8.44. Found: C, 62.73; H, 8.36.

Cilistol p1 (**3**): Amorphous powder, $[\alpha]_D^{23}$ −125.0° (c=0.26, MeOH). Pos. FAB-MS (m/z): 669 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.08 (1H, t-like, J=4.1 Hz, H-4), 0.86 (3H, s, H_3-18), 0.89 (1H, t-like, J=4.1 Hz, H′-4), 1.27 (1H, m, H-3), 1.36 (3H, d, J=6.7 Hz, H_3-21), 1.47 (3H, s, H_3-19), 1.64 (3H, s, H_3-27), 1.89 (3H, s, H_3-28), 2.04 (1H, t-like, J=13.3 Hz, H-23), 2.10 (1H, d, J=17.7 Hz, 2-H), 2.46 (1H, m, H-20), 2.60 (1H, t-like, J=13.3 Hz, H′-23), 2.82 (1H, dd, J=4.0, 17.7 Hz, H′-2), 3.46 (1H, br s, H-6), 3.50 (3H, s, OMe-24), 3.92 (1H, m, glc H-5), 3.98 (1H, t-like, J=8.5 Hz, glc H-2), 4.20 (1H, t-like, J=8.5 Hz, glc H-3), 4.23 (1H, t-like, J=8.5 Hz, glc H-4), 4.37 (1H, dd, J=4.9, 11.6 Hz, glc H-6), 4.48 (1H, br d, J=11.6 Hz, glc H′-6), 5.03 (1H, br d, J=12.2 Hz, H-22), 5.30 (1H, d, J=7.9 Hz, glc H-1), 5.46 (1H, s, H-26). ^{13}C-NMR: see Table 1. *Anal.* Calcd for $C_{35}H_{56}O_{12}$: C, 62.85; H, 8.44. Found: C, 62.97; H, 8.51.

Cilistol u (**4**): Amorphous powder, $[\alpha]_D^{23}$ −102.0° (c=0.15, MeOH). Pos. FAB-MS (m/z): 637 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.06 (1H, t-like, J=5.5 Hz, H-4), 0.85 (3H, s, H_3-18), 0.87 (1H, t-like, J=5.5 Hz, H′-4), 1.26 (3H, d, J=6.7 Hz, H_3-21), 1.26 (3H, s, H_3-28), 1.43 (3H, s, H_3-27), 1.44 (3H, s, H_3-19), 2.09 (1H, d, J=17.7 Hz, H-2), 2.19 (1H, t-like, J=11.0 Hz, H-23), 2.40 (1H, m, H-20), 2.57 (1H, br d, J=12.8 Hz, H′-23), 2.81 (1H, J=3.6, 14.0 Hz, H′-2), 3.43 (1H, br s, H-6), 3.92 (1H, m, glc H-5), 4.06 (1H, dd, J=8.6 Hz, glc H-2), 4.24 (1H, t-like, J=8.6 Hz, glc H-3), 4.24 (1H, t-like, J=8.6 Hz, glc H-4), 4.39 (1H, dd, J=5.5, 11.6 Hz, glc H-6), 4.52 (1H, br d, J=11.6 Hz, glc H′-6), 4.80 (1H, br d, J=11.6 Hz, H-22), 5.35 (1H, d, J=7.9 Hz, glc H-1), 5.63 (1H, s, H-26). ^{13}C-NMR: see Table 1. *Anal.* Calcd for $C_{34}H_{52}O_{11}$: C, 64.13; H, 8.23. Found: C, 64.22; H, 8.19.

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