Six New Withanolide-Type Steroids from the Leaves of *Solanum cilistum*

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Six new withanolide-type steroids, designated cilistols v, t, i, j, y and w (1—6, respectively), were obtained from the leaves of *Solanum cilistum*. Their respective structures were characterized by spectroscopic means as follows: Cilistol v (1) was (22*R*,24*Z*)-1α,3β,22,26-tetrahydroxyergost-5,24-diene 26-*O*-β-D-glucopyranoside, which is regarded as the precursor of other withanolide-type steroids included in this title plant. Cilistol t (2) was (22*R*,24*S*,25*R*,26*S*)-24,25;22,26-diepoxy-1α,3β,26-trihydroxyergost-5-ene 26-*O*-β-D-glucopyranoside, and cilistols j (3) and i (4) corresponded to the substances probably formed by the cleavage of the epoxy ring at C-24 and 25 of 2. Cilistol y (5) was 3-*O*-sulphonyl (22*R*,24*S*,25*R*,26*R*)-1-oxo-24,25; 22,26-diepoxy-3β,17α,26-trihydroxyergost-5-ene 26-*O*-β-D-glucopyranoside, and cilistol w (6) corresponded to the substance obtained by the fission of the epoxy ring at C-24 and 25 of 5. The occurrence of these withanolide type steroids from *Solanum* genera is rare and worthy of note.

Key words *Solanum cilistum*; Solanaceae; withanolide-type steroid; cilistol

Our studies on the constituents of *Solanum*-genera plants have resulted in the isolation of numerous steroidal glycosides: spirostane, furostane, solasodane and solanidane glycosides, some of which were found to exhibit anti-cancer[1] and anti-herpes simplex virus (HSV-1)[2] activities. As a further extension of these studies, we began to investigate the constituents of *Solanum cilistum*, isolated six withanolide-type steroids which were different from the steroidal glycosides obtained to date from other *Solanum* plants and reported these in a preceding paper.[3] In a continuing study on constituents of the leaves of the title plant, we have isolated additional six withanolide-type steroids. Herein we describe their structural characterization.

A methanolic extract of 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 then treated with hexane. The insoluble layer was chromatographed on Diaion HP-20P by successively eluting with H_2O, 30, 50, 70 and 90% aq. MeOH. The respective fractions were subsequently subjected to a variety of column chromatographies such as Chromatorex ODS ($H_2O \rightarrow$MeOH, gradiently), Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$–MeOH=20 : 1→10 : 1, $CHCl_3$–MeOH–H_2O=9 : 2 : 0.1→8 : 2 : 0.2) to afford six whithanolide-type steroids, called cilistols v (**1**, 28.6 mg), t (**2**, 102.8 mg), i (**3**, 35.2 mg), j (**4**, 57.4 mg), y (**5**, 23.5 mg) and w (**6**, 344.5 mg).

Cilistol v (**1**) was obtained as an amorphous powder, $[\alpha]_D$ −23.4° (MeOH). A *quasi*-molecular ion peak at *m*/*z* 609 $[M+H]^+$ in the positive FAB-MS and elemental analysis of **1** indicated its molecular formula to be $C_{34}H_{56}O_9$. The ^{1}H-NMR spectrum (in pyridine-d_5) displayed signals for two tertiary methyl groups (δ 0.90, 1.14), two tertiary methyl groups (δ 1.82, 1.90) adjacent to the double bond, a secondary methyl group (δ 1.19, d, J=6.7 Hz), three oxygenated methine protons [δ 4.06 (br d, J=12.8 Hz), 4.15 (br s), and 4.73 (overlapped)], one set of oxygenated methylene protons [δ 4.63, 4.73 (each 1H, ABq, J=11.0 Hz)] and one olefinic proton (δ 5.64, m) together with one β-glucopyranosyl moiety [anomeric proton: δ 4.97 (d, J=7.9 Hz)]. The ^{13}C-NMR spectrum (Table 1, in pyridine-d_5) showed 34 signals composed of five methyl groups (δ 12.0, 13.0, 17.8, 19.5, 19.9), one hydroxymethyl (δ 70.1), three oxygenated methine carbons (δ 66.1, 71.0, 72.7), and two double bonds (δ 123.5, 127.8, 132.9, 140.2) including one β-glucosyl moiety. The ^{1}H–^{1}H shift correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear

Table 1. ^{13}C-NMR Data for Cilistols v (**1**), t (**2**), i (**3**), j (**4**), y (**5**) and w (**6**) in Pyridine-d_5

	1	**2**	**3**	**4**	**5**	**6**
C- 1	72.7	72.7	72.7	72.7	210.6	210.5
-2	40.3	40.3	40.3	40.2	46.0	45.9
-3	66.1	66.1	66.1	66.1	74.0	74.3
-4	43.0	43.3	43.3	43.2	37.5	37.7
-5	140.2	140.2	140.2	140.1	135.8	135.9
-6	123.5	123.5	123.5	123.5	126.0	126.2
-7	32.3	32.3	32.4	32.3	31.6	31.7
-8	41.7	41.8	41.8	41.7	32.3	32.4
-9	41.7	41.8	41.8	41.7	42.8	42.9
-10	42.1	42.8	42.1	42.1	52.9	52.9
-11	24.9	24.7	24.8	24.8	39.3	39.2
-12	39.9	39.9	39.9	39.9	32.6	32.7
-13	42.9	42.1	42.8	42.8	48.3	48.4
-14	56.6	56.7	56.7	56.6	50.6	50.7
-15	27.9	27.5	27.5	27.5	22.8	22.9
-16	20.7	20.7	20.7	20.7	24.0	24.1
-17	53.6	52.9	53.7	53.7	84.9	85.0 85.2
-18	12.0	11.9	12.0	12.0	15.0	15.2
-19	19.9	19.9	19.9	19.9	18.9	18.8
-20	43.3	39.3	39.3	39.3	43.9	43.8 44.2
-21	13.0	13.3	13.3	13.4	10.1	10.3 10.7
-22	71.0	67.0	69.3	69.8	67.0	74.3 74.4
-23	35.1	30.2	35.1	37.0	33.9	41.1
-24	127.8	61.2	77.3	73.0	63.0	73.2 74.3
-25	132.9	61.8	75.5	75.2	63.4	75.3 77.3
-26	70.1	95.5	101.7	101.7	92.7	98.3 99.1
-27	17.8	17.1	22.3	22.5	17.3	16.2 22.7
-28	19.5	18.7	18.8	19.9	18.8	23.7 25.5
24-OMe			50.4			49.7
Glc						
C-1	103.5	99.9	99.1	99.1		
-2	75.3	74.6	74.1	74.8		
-3	78.5	78.5	78.8	78.7		
-4	71.7	71.7	71.4	71.4		
-5	78.1	78.5	79.0	79.0		
-6	62.8	62.8	62.3	62.3		

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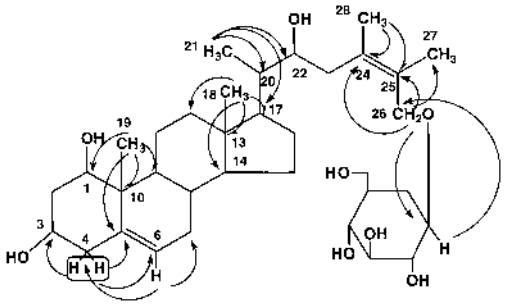
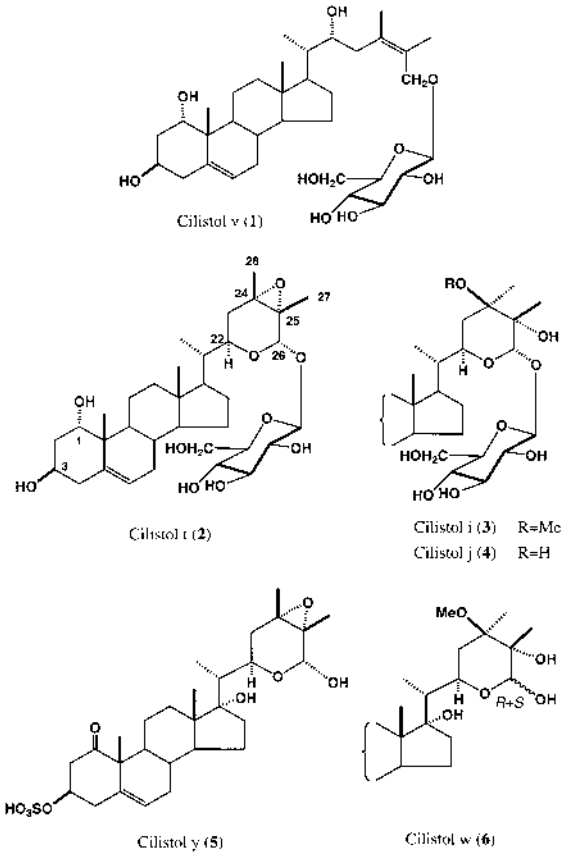


Fig. 1. Significant Correlations Observed in the HMBC Spectrum of **1**



multiple bond correlation (HMBC) techniques, the latter of which is illustrated in Fig. 1, allowed the unambiguous assignments that the hydroxyl group attached to C-1, -3 and -26, the double bonds existed at C-5 and -24 and the glucosyl linkage bound to the hydroxyl at C-26 along with the assignments of all carbon signals and part of proton signals. Thus, the plain structure for **1** is represented as shown in Fig. 1. The stereo configurations of the hydroxyl groups at C-1 and -3 were suggested to be α and β, respectively, since the proton signals at C-1 and 3 appeared as a broad singlet ($W_{1/2}$= 5.3 Hz) and multiplet ($W_{1/2}$=21.3 Hz), respectively. The hydroxyl configuration at C-22 and the geometry at the double bond at C-24 were deduced as *R* and *Z*, respectively, reasoning that **1** would be turned into a coexistent withanolide-type steroid, such as cilistol t (**2**) as mentioned below and observation of the nuclear Overhauser effect spectroscopy (NOESY) between methyl groups at C-27 and C-28. Consequently, the structure of **1** was established as a new compound: (22*R*,24*Z*)-1α,3β,22,26-tetrahydroxyergost-5,24-diene 26-*O*-

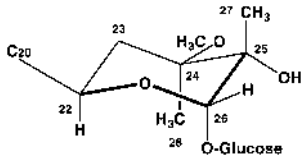


Fig. 2. Configurations of the Substituents at the Lactol Moiety

β-D-glucopyranoside.

Cilistol t (**2**) was obtained as an amorphous powder showing $[\alpha]_D$ −54.4° (MeOH). A *quasi*-molecular peak due to $[M+H]^+$ at *m/z* 623 in the positive FAB-MS and elemental analysis gave the molecular formula $C_{34}H_{54}O_{10}$ for **2**. The ^{1}H-NMR spectrum showed signals due to four tertiary methyl groups at δ 0.64, 1.10, 1.39, 1.42, one secondary methyl group at δ 1.09 (d, *J*=6.7 Hz), and three oxygenated methine protons at δ 4.13 (br s), 4.45 (br d, *J*=11.0 Hz), 4.72 (m); the latter three were assigned as H-1β, H-3α and H-22, respectively, by comparison with those of **1**, along with signals due to β-D-glucopyranosyl moiety. Moreover, a characteristic singlet signal at δ 5.65 was ascribable to the acetal proton of H-26 by comparison with that of cilistol a, (22*R*,24*S*,25*R*,26*R*)-1-oxo-22,26;24,25-diepoxy-17α,26-dihydroxy-ergost-2,5-diene (**7**), described in the preceding paper.[3] Among a total of 34 signals in the ^{13}C-NMR spectrum (Table 1), significant ones at δ 61.2, 61.8 (two quaternary carbons adjacent to methyl group and epoxide), 66.1, 67.0, 72.7 (three oxygenated methine carbons), 95.5 (an acetal carbon), 99.9 (anomeric carbon), and 123.5 and 140.2 (two olefinic carbons) could be assigned to C-24, -25, -3, -22, -1, -26, glucopyranosyl C-1, and C-6 and -5, respectively, by comparison with those of **1** and **7**. The analysis of ^{1}H–^{1}H COSY, HMQC, and HMBC spectra has led to the unambiguous assignments of the above and other signals. Concerning configurations at C-22, 24, 25 and -26 were conceived as identical with those of **7**, whose structure was obtained by X-ray analysis. Accordingly, the structure of **2** was determined to be (22*R*,24*S*,25*R*,26*S*)-24,25;22,26-diepoxy-1α,3δ,26-trihydroxyergost-5-ene 26-*O*-β-D-glucopyranoside.

Cilistol i (**3**) was isolated as an amorphous powder with $[\alpha]_D$ −56.5° (MeOH). The elemental analysis and a *quasi*-molecular ion peak at *m/z* 655 $[M+H]^+$ in the positive FAB-MS provided the molecular formula of $C_{35}H_{58}O_{11}$. A study comparing the ^{1}H-NMR spectra of **3** with those of **2** revealed that signals due to H_3-27 and -28 shifted toward the lower field at δ 1.63 and 1.92, and a new methoxyl signal occurred in **3**. Thus, it was presumed that **3** might be biogenetically formed and involve the cleavage of an epoxy ring at C-24 and -25 from **2**. The facts: 1) that the difference NOE experiment showed the correlations between H-26 and H_3-27, H_3-27 and OCH_3, OCH_3 and H_3-28, and H-22 and H_3-28 and 2) that the fission of the epoxy ring at C-24 and -25 involved an *S*N2 reaction, and 3) that the methoxyl group probably originated from MeOH and was assumed to be introduced from the anticoplanar side to leave an oxonium ion at C-25 allowed determination of the stereo configurations of the substituents at the lactol ring moiety as illustrated in Fig. 2. Thus, the structure of **3** was determined to be (22*R*,24*R*,25*R*,26*S*)-22,26-epoxy-24-*O*-methyl-1α,3β,24,25,26-pentahydroxyergost-5-ene 26-*O*-β-D-glucopyranoside.

Cilistol j (**4**) was obtained as an amorphous powder with

$[\alpha]_D$ −63.7° (MeOH). From evidence of the elemental analysis and a *quasi*-molecular ion peak at *m/z* 641 $[M+H]^+$ in the positive FAB-MS, the molecular formula was given to be $C_{34}H_{56}O_{11}$. The ^{1}H-NMR spectra of **4** and **3** were almost identical except for the disappearance of a methoxyl group at δ 3.60 in **4**. A carbon signal at δ 73.0 ascribable to C-24 shifted by +4.3 ppm in comparison with that of **3**. Therefore the structure of **4** was characterized as a desmethyl compound of **3**.

Cilistol y (**5**) was obtained as an amorphous powder with $[\alpha]_D$ +8.6° (MeOH). The positive FAB-MS showed a *quasi*-molecular ion peak at *m/z* 578 $[M+Na+H]^+$, its molecular formula was derived as $C_{28}H_{42}O_9S$ by combination with the elemental analysis. The presence of a sulfate group and its location were supported by the elemental analysis and a distinguishing lower shift to δ 5.13 of H-3 signal (m, overlapped with water signal) in the ^{1}H-NMR spectrum. Moreover, the occurrence of carbonyl group at C-1 was verified by the HMBC correlation between C-19 methyl protons and C-1 carbonyl carbon. Other ^{1}H- and ^{13}C-signals were assigned by comparison with those of **7**. Consequently, the structure of **5** was established as (22*R*,24*S*,25*R*,26*R*)-1-oxo-22,26;24,25-diepoxy-3β,17α,26-trihydroxyergost-5-ene 3-*O*-sulfate.

Cilistol w (**6**) was obtained as an amorphous powder with $[\alpha]_D$ +12.6° (MeOH). From the evidence of a *quasi*-molecular ion peak at *m/z* 610 $[M+Na+H]^+$ in the positive FAB-MS and the elemental analysis, its molecular formula was determined to be $C_{29}H_{46}O_{10}S$. The ^{1}H-NMR spectrum showed several splitting signals as pair sets originated from the respective H_3-18 (δ 0.77, 0.79), H_3-19 (δ 1.25, 1.26), H_3-21 (δ 1.36, 1.37, each d, J=6.7 Hz), H_3-27 (δ 1.87, 2.01), H_3-28 (δ 1.92, 2.13), OCH_3 (δ 3.61, 3.62), H-22 (δ 4.31, 4.95, each br d, J=12.2 Hz) and H-26 (δ 5.48, 5.60, each br s), as well as cilistols f ang g reported earlier.[3] It has become apparent that in the glycol system by opening the epoxy ring at C-24 and -25 just like **6**, cilistols f and g,[3] both compounds with *R* and *S* configurations at the C-26 hemiacetal center were present in an inseparable equilibrium state. A comparative study on the ^{1}H- and ^{13}C-NMR spectra with those of **5** with the help of the ^{1}H–^{1}H COSY, HMQC and HMBC techniques has resulted in the conclusion that the structure of **6** could be represented as (22*R*,24*R*,25*R*,26*Φ*)-1-oxo-22,26-epoxy-24-*O*-methyl-3β,17α,24,25,26- pentahydroxyergost-5-ene 3-*O*-sulfate.

Taking into account the withanolide-type steroids so far obtained from the title plant, the following hypothesis for a sequential biogenetic route can be suggested: the precursor of withanolide-type steroid; cilistol v (**1**)→1,3-diol type; cilistols t (**2**), i (**3**) and j (**4**)→1-one-3-*O*-sulfate type; cilistols y (**5**) and w (**6**)→1-one-2-ene type: cilistols a, b, d, q, f and g.[3]

The occurrence of the withanolide-type steroids from the *Solanum* genera is very rare, this report is probably the second to the earlier our paper[3] and it is worthy of note.

Experimental

General Experimental 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-DX 303HF. NMR spectra were measured in pyridine-d_5 on a JEOL α-500 spectrometer (500 MHz) and chemical shifts were referred 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 from Dr. Masaharu Matsui (National Research Institute of Vegetables, Ornamental Plants and Tea) and cultivated at the Botanical Garden of Kumamoto University.

Extraction and Isolation A methanolic extract of 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 treated with hexane. The insoluble layer was chromatographed on Diaion HP-20P by eluting successively with H_2O, 30, 50, 70 and 90% aq. MeOH. The respective fractions eluted with 50, 70, and 90% MeOH were subsequently subjected to a variety of column chromatographies such as silica gel, Chromatorex ODS, and Sephadex LH-20 to afford six whithanolide-type steroids, termed cilistols v (**1**, 28.6 mg), t (**2**, 102.8 mg), i (**3**, 35.2 mg), j (**4**, 57.4 mg), y (**5**, 23.5 mg), and w (**6**, 344.5 mg).

Cilistol v (**1**): An amorphous powder, $[\alpha]_D^{23}$ −23.4° (c=0.23, MeOH). Pos. FAB-MS (*m/z*): 609 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.70 (3H, s, H_3-18), 1.14 (3H, s, H_3-19), 1.19 (3H, d, J=6.7 Hz, H_3-21), 1.82 (3H, s, H_3-28), 1.90 (3H, s, H_3-27), 3.99 (1H, m, glc H-5), 4.03 (1H, dd, J=7.9, 8.6 Hz, glc H-2), 4.06 (1H, br d, J=12.8 Hz, H-22), 4.15 (1H, br s, $W_{1/2}$=5.3 Hz, H-1), 4.22 (1H, t-like, J=8.6 Hz, glc H-3), 4.27 (1H, t-like, J=8.6 Hz, glc H-4), 4.41 (1H, dd, J=4.8, 9.4 Hz, glc H-6), 4.54 (1H, d, J=9.4 Hz, glc H′-6), 4.63, 4.73 (each 1H, ABq, J=11.0 Hz, H_2-26), 4.73 (1H, overlapped, $W_{1/2}$=21.3 Hz, H-3), 4.97 (1H, d, J=7.9 Hz, glc H-1), 5.64 (br s, H-6). ^{13}C-NMR: see Table 1. *Anal*. Calcd for $C_{34}H_{56}O_9$: C, 67.07; H, 9.27. Found: C, 66.84; H, 9.22.

Cilistol t (**2**): An amorphous powder, $[\alpha]_D^{23}$ −54.4° (c=0.62, MeOH). Pos. FAB-MS *m/z* 623 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.64 (3H, s, H_3-18), 1.07 (3H, d, J=6.7 Hz, H_3-21), 1.10 (3H, s, H_3-19), 1.39 (3H, s, H_3-28), 1.43 (3H, s, H_3-27), 3.93 (1H, m, glc H-5), 4.07 (1H, t-like, J=8.3 Hz, glc H-2), 4.15 (1H, br s, H-1), 4.23 (1H, t-like, J=7.5 Hz, glc H-3), 4.25 (1H, t-like, J=7.5 Hz, glc H-4), 4.40 (1H, dd, J=4.9, 11.6 Hz, glc H-6), 4.45 (1H, br d, J=11.0 Hz, H-22), 4.54 (1H, dd, J=2.2, 11.0 Hz, glc H′-6), 4.72 (1H, m, H-3), 5.36 (d, J=7.9 Hz, glc H-1), 5.62 (1H, m, H-6), 5.65 (1H, s, H-26). ^{13}C-NMR: see Table 1. *Anal*. Calcd for $C_{34}H_{54}O_{10}$: C, 65.55; H, 8.74. Found: C, 65.43; H, 8.69.

Cilistol i (**3**): An amorphous powder, $[\alpha]_D^{23}$ −56.5° (c=0.46, MeOH). Pos. FAB-MS *m/z* 655 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.64 (3H, s, H_3-18), 1.11 (3H, s, H_3-19), 1.14 (3H, d, J=6.7 Hz, H_3-21), 1.63 (3H, s, H_3-27), 1.92 (3H, s, H_3-28), 3.60 (3H, s, OMe), 3.92 (1H, m, glc H-5), 3.96 (1H, t-like, J=8.6 Hz, glc H-2), 4.14 (1H, br s, H-1), 4.19 (1H, t-like, J=8.6 Hz, glc H-3), 4.25 (1H, t-like, J=8.6 Hz, glc H-4), 4.36 (1H, dd, J=5.2, 11.9 Hz, glc H-6), 4.48 (1H, dd, J=2.4, 11.9 Hz, glc H′-6), 4.74 (1H, m, H-3), 4.74 (1H, br d, J=11.0 Hz, H-22), 5.28 (d, J=7.9 Hz, glc H-1), 5.46 (1H, s, H-26), 5.62 (1H, m, H-6). ^{13}C-NMR: see Table 1. *Anal*. Calcd for $C_{35}H_{58}O_{11}$: C, 64.19; H, 8.93. Found: C, 63.92; H, 8.89.

Cilistol j (**4**): An amorphous powder, $[\alpha]_D^{23}$ −63.7° (c=0.19, MeOH). Pos. FAB-MS *m/z* 641 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.64 (3H, s, H_3-18), 1.11 (3H, s, H_3-19), 1.17 (3H, d, J=6.7 Hz, H_3-21), 1.78 (3H, s, H_3-27), 2.09 (3H, s, H_3-28), 3.96 (1H, m, glc H-5), 3.98 (1H, t-like, J=8.5 Hz, glc H-2), 4.14 (1H, br s, H-1), 4.20 (1H, t-like, J=8.6 Hz, glc H-3), 4.22 (1H, t-like, J=8.6 Hz, glc H-4), 4.39 (1H, dd, J=4.9, 12.2 Hz, glc H-6), 4.50 (1H, br d, J=12.2 Hz, glc H′-6), 4.75 (1H, m, H-3), 4.80 (1H, br d, J=11.0 Hz, H-22), 5.32 (1H, d, J=7.9 Hz, glc H-1), 5.60 (1H, s, H-26), 5.63 (1H, m, H-6). ^{13}C-NMR: see Table 1. *Anal*. Calcd for $C_{34}H_{56}O_{11}$: C, 63.72; H, 8.81. Found: C, 63.98; H, 8.78.

Cilistol y (**5**): An amorphous powder, $[\alpha]_D^{23}$ +8.6° (c=0.21, MeOH). Pos. FAB-MS *m/z* 578 $[M+Na+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.70 (3H, s, H_3-18), 1.22 (3H, s, H_3-19), 1.25 (3H, d, J=6.7 Hz, H_3-21), 1.33 (3H, s, H_3-28), 1.50 (3H, s, H_3-27), 4.70 (1H, br d, J=11.6 Hz, H-22), 5.13 (1H, overlapped, H-3), 5.43 (1H, m, H-6), 5.52 (1H, s, H-26). ^{13}C-NMR: see Table 1. *Anal*. Calcd for $C_{28}H_{42}O_9S$: C, 60.63; H, 7.63. Found: C, 60.88; H, 7.59.

Cilistol w (**6**): An amorphous powder, $[\alpha]_D^{23}$ +12.6° (c=0.71, MeOH). Pos. FAB-MS *m/z* 610 $[M+Na+H]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.77, 0.79 (each s, H_3-18), 1.25, 1.26 (each s, H_3-19), 1.36, 1.37 (each d, J=6.7 Hz, H_3-21), 1.86, 2.01 (each s, H_3-27), 1.94, 2.13 (each s, H_3-28), 3.61, 3.62 (each s, OMe), 4.31, 4.95 (each br d, J=11.6 Hz, H-22), 5.01 (1H, m, H-3), 5.48 (overlapped, H-6), 5.48, 5.60 (each s, H-26). ^{13}C-NMR: see Table 1. *Anal*. Calcd for $C_{29}H_{46}O_{10}S$: C, 59.37; H, 7.90. Found: C, 59.52; H, 7.84.

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