

New Diterpene Glycosides of the Fungus *Acremonium striatisporum* Isolated from a Sea Cucumber

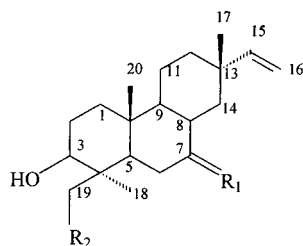
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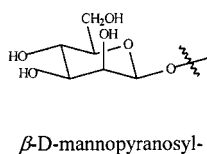
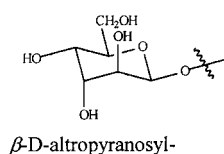
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Three new diterpene glycosides, virescensides O (**1**), P (**2**), and Q (**3**), have been isolated from a marine strain of *Acremonium striatisporum* KMM 4401 associated with the holothurian *Eupentacta fraudatrix*. Their structures were determined on the basis of HRMALDIMS and NMR data as β -D-altropyranosido-19-isopimara-8(14),15-diene-7 α ,3 β -diol (**1**), β -D-altropyranosido-19-7-oxoisopimara-8(9),15-diene-3 β -ol (**2**), and β -D-mannopyranosido-19-isopimara-7,15-diene-3 β -ol (**3**). The cytotoxic activity of the virescensides was examined.

In our search for secondary metabolites from marine fungi with cytotoxicity and/or novel chemical structures, we have previously isolated two new diterpene altrosides, virescensides M and N, from a marine strain of *Acremonium striatisporum* originally separated from the holothurian *Eupentacta fraudatrix*.¹ Further investigation for metabolites of this fungal strain has now led to the isolation of three new cytotoxic glycosides, virescensides O, P, and Q (**1**, **2**, and **3**). We report herein the isolation and structures of compounds **1–3** and their cytotoxic activity.



	R ₁	R ₂	
1	H, α -OH	β -D-altropyranosyl-	$\Delta^{8,14}$
2	O	β -D-altropyranosyl-	$\Delta^{8,9}$
2a	O	OH	$\Delta^{8,9}$
3	H	β -D-mannopyranosyl-	$\Delta^{7,8}$
3a	H, H	OH	$\Delta^{8,9}$



Results and Discussion

The fungus was cultured for 21 days on rice medium specially modified by us.¹ The CHCl_3 –MeOH (2:1, v/v) extract of the culture of *A. striatisporum* was fractionated by Si gel column chromatography followed by reversed-

phase and normal-phase HPLC to yield individual glycosides **1–3**. The structures of new compounds **1–3** were established by the interpretation of spectral data (NMR and HRMALDIMS), as well as by comparison of their spectra with those of related compounds.

The molecular formula of virescenside O (**1**) was determined as $\text{C}_{26}\text{H}_{42}\text{O}_8$ on the basis of HRMALDIMS and ^{13}C NMR spectra. A close inspection of the ^1H and ^{13}C NMR spectral data (Table 1) of **1** by DEPT and ^1H – ^{13}C 2D NMR shift-correlated measurement (HMQC) revealed the presence of three quaternary methyls (δ 26.1, C-17; 23.9, C-18; 15.5, C-20); seven methylenes (δ 37.8, C-1; 28.8, C-2; 31.1, C-6; 18.8, C-11; 34.4, C-12; 72.3, C-19; 63.5, C-6'), including two oxygen-bearing ones; seven oxygenated methines (δ 79.3, C-3; 72.6, C-7; 101.5, C-1'; 71.7, C-2'; 71.9, C-3'; 67.0, C-4'; 77.1, C-5'), including one methine linked to an anomeric carbon; two tertiary (δ 47.8, C-5; 45.9, C-9) and three C–C-bonded saturated quaternary carbons (δ 42.8, C-4; 38.6, C-10; 37.5, C-13), and one monosubstituted (148.9, 110.0) and one trisubstituted (140.6, 132.3) double bond.

The correlations observed in the ^1H – ^1H COSY and HMQC spectra of **1** and double resonance experiments indicated the presence of the following isolated spin systems: $-\text{CH}_2-\text{CH}_2-\text{CHOH}-$ (C-1–C-3), $>\text{CH}-\text{CH}_2-\text{CHOH}-$ (C-5–C-7), $-\text{CH}=\text{CH}_2$ (C-15–C-16), $-\text{CH}_2-\text{O}-$ (C-19). Furthermore, an additional spin system involved one anomeric proton, four oxymethine ones, and protons of a hydroxymethyl group (C-1'–C-6'). The ^1H – ^1H COSY spectrum of **1** contained a cross-peak attributed to long-range coupling between the olefinic proton at δ 5.57 (H-14) and a carbonyl proton at δ 4.39 (H-7). In addition, H-14 was allylically coupled to a signal at δ 2.40 (H-9), which was further correlated with two signals at δ 1.60 (H-11 α) and 1.45 (H-11 β). This information together with the data obtained from the ^1H – ^{13}C HMBC spectrum (Table 1) indicated that **1** was a diterpene monoside with a tricyclic aglycon structure.

A direct comparison of ^1H and ^{13}C NMR spectra of **1** with those of the glycosides M and N¹, and virescensides A–C, obtained earlier from the terrestrial fungus *Acremonium luzulae*^{2–5} suggests that virescenside O has the structure of an isopimaradienic altroside. The ^{13}C NMR spectrum of **1** contained signals for a monosubstituted double bond at δ 148.9 (d) and 110.0 (t). The proton signals of a typical

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Table 1. ^1H and ^{13}C NMR Data of Virescenoside O (**1**) in $\text{C}_5\text{D}_5\text{N}$ (J , Hz)

atom	δ_{C}	δ_{H}	$^1\text{H}-^1\text{H}$ COSY	HMBC (C)	NOESY (H)
1	37.8 t	1 α : 1.23 m 1 β : 1.73 m	1 β , 2 α,β 1 α , 2 α,β		3, 9 11 β , 20
2	28.8 t	2 α : 2.02 m 2 β : 1.95 m	1 α,β , 2 β , 3 1 α,β , 2 α , 3		19a, 20 1 α , 5, 18
3	79.3 d	3.67 dd (4.8, 11.0)	2 α,β		
4	42.8 s				
5	47.8 d	2.16 dd (2.3, 12.8)	6 α,β		3, 9, 18
6	31.1 t	6 α : 2.23dt (2.3, 13.0) 6 β : 1.91 td (3.4, 12.8)	5, 6 β , 7 5, 6 α , 7		18 19b, 20
7	72.6 d	4.39 t (3.4)	6 α,β , 14		14
8	140.6 s				
9	45.9 d	2.40 m	11 α,β		1 α , 5
10	38.6 s				
11	18.8 t	11 α : 1.60 m 11 β : 1.45 m	9, 11 β , 12 α,β 9, 11 α , 12 α,β		
12	34.4 t	1.45 m	11 α,β		
13	37.5 s				
14	132.3 d	5.57 d (1.9)	7, 9		7, 17
15	148.9 d	5.81 dd (10.7, 17.5)	16a,b		17
16	110.0 t	16a: 4.94 dd (1.5, 10.7) 16b: 5.03 dd (1.5, 17.5)	15, 16b 15, 16a	13	17
17	26.1 q	1.09 s		12, 13, 14, 15	14, 15, 16b
18	23.9 q	1.57 s		3, 4, 5, 19	3, 5, 6 α , 19a,b
19	72.3 t	19a: 4.24 d (10.0) 19b: 4.49 d (10.0)	19b		1 1 , 2 β , 18, 20 6 β , 18, 20
20	15.5 q	0.88 s		1, 5, 9, 10	1 β , 2 β , 11 β , 19a,b
1 1	101.5 d	5.54 d (1.7)	2 1		19a,b
2 1	71.7 d	4.63 dd (1.7, 4.9)	1 1 , 3 1		
3 1	71.9 d	4.76 dd (3.3, 4.9)	2 1 , 4 1		
4 1	67.0 d	4.82 dd (3.3, 8.1)	3 1 , 5 1		
5 1	77.1 d	4.57 m	4 1 , 6 1 a,b		
6 1	63.5 t	6 1 a: 4.40 dd (5.3, 11.4) 6 1 b: 4.51 dd (3.7, 11.4)	5 1 , 6 1 b 5 1 , 6 1 a		4 1

ABX system of a vinyl group at δ 5.81 (1H, dd, 10.7, 17.5, Hz), 5.03 (1H, dd, 1.5, 17.5, Hz), and 4.94 (1H, dd, 1.5, 10.7, Hz) in the spectrum of **1** indicated the C-15, C-16 position of this double bond.⁶⁻⁹ Furthermore, the position of the C-17 methyl group (δ 1.09, s) and of the exo-vinyl group at C-13 were argued by $^1\text{H}-^{13}\text{C}$ HMBC and NOE measurements (Table 1). The stereochemistry at C-13 in **1** was assigned to be the same as sandaracopimaradienic derivatives on the basis of the similarity of C-15–C-17 chemical shifts for these compounds.¹⁰⁻¹³

Localization of the trisubstituted double bond (δ 140.6, s, 132.3, d) at the C-8, C-14 position was evident from the $^1\text{H}-^1\text{H}$ COSY and HBMC correlations. A direct comparison of ^{13}C NMR shifts of **1** with the values published for 7 α -hydroxysandaracopimar-8(14),15-dienic derivatives¹³⁻¹⁵ confirmed this deduction. The small coupling constants of the H-7 signal at δ 4.39 (1H, t, 3.4) indicated that **1** contained an allylic secondary alcohol function with an axial configuration. Furthermore, in comparison with the spectra of sandaracopimaric acid^{10,12} the shift of the C-9 signal from δ 50.7 to 45.9 may be explained by the γ -effect of an axial hydroxyl at C-7. The COSY and HMBC data allowed the assignment of the signal at δ 79.3 (C-3) to a secondary hydroxyl-bearing carbon, adjacent to a quaternary sp^3 C carbon. The relative stereochemistry of the proton at C-3 was defined on the basis of the $^1\text{H}-^1\text{H}$ coupling constants (J = 4.8, 11.0 Hz) observed between H-3 and H-2 α,β and assigned as axial.

The ^1H NMR spectrum of **1** showed two signals corresponding to an AB system coupling at δ 4.24 and 4.49 (each 1H, each d, 10.0 Hz), which were consistent with the presence of CH_2O - group linked to a quaternary sp^3 C carbon. The position and stereochemistry of the methyl (1.57, s) and hydroxymethyl (72.3, t) groups at C-4 and methyl group (0.88, s) at C-10 were established on the basis of NOEs and HBMC data. Furthermore, NOE correlations

between H-2 β and H₃-20 as well as between H-3 α and H-5 α indicated a trans ring fusion between rings A and B. The NOESY spectrum exhibited the cross-peaks H-1 α /H-9 α and H-5 α /H-9 α , indicating these protons to be on the same side of the molecule. NOEs were also observed between H-3 α and H₃-18 and between H-7 β and H-14. All these data are consistent with a $\Delta^{8(14),15}$ -sandaracopimaradienic skeleton with an equatorial hydroxyl group at C-3 and an axial hydroxymethyl and hydroxyl groups at C-4 and C-7 for the aglycon part of **1**. The strong NOEs from H-1 1 to H-19a indicated that the sugar moiety was linked at C-19.

A comparison of the ^{13}C NMR spectrum of **1** with the data published for α - and β -D-altropyranoses as well as a good coincidence of carbon signals due to the glycosidic moiety with those of virescenosides A, M, and N together with magnitudes of $^1\text{H}-^1\text{H}$ spin coupling constants in ^1H NMR spectra of **1**^{1,5,16-18} elucidated the presence of a β -D-altropyranoside unit of C1 form in **1**. Acid hydrolysis of virescenoside O gave D-altropyranose and 1,6-anhydro- β -D-altropyranose, which were identified by NMR spectra and optical rotation. On the basis of all the data above, the structure of virescenoside O was established as β -D-altropyranosido-19-sandaracopimara-8(14),15-diene-3 β ,7 α -diol.

In HRMALDIMS virescenoside P (**2**) gave a quasimolecular ion at m/z 503.2630 [M + Na]. These data, coupled with ^{13}C NMR spectral data (DEPT), established the molecular formula of **2** as $\text{C}_{26}\text{H}_{40}\text{O}_8$. The general features of the UV and ^1H and ^{13}C NMR spectra of **2** (Table 2 and the Experimental Section) closely resembled those of virescenoside M¹ with the exception of proton and carbon signals belonging to the ring A.

Correlations observed in the $^1\text{H}-^1\text{H}$ COSY and HMQC spectra and double resonance experiments on **2** indicated the presence of an isolated spin system corresponding to the sequence $-\text{CH}_2-\text{CH}_2-\text{CHOH}-$ (C-1–C-3). Thus, this

Table 2. ^1H and ^{13}C NMR Data of Virescenosides P (**2**) and Q (**3**) in $\text{C}_5\text{D}_5\text{N}$ (J , Hz)

atom	2		3	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
1	34.6 t	1 α : 1.23 m 1 β : 1.73 m	38.3 t	1 α : 1.15 m 1 β : 1.78 m
2	28.1 t	2 α : 1.91 m 2 β : 2.02 m	28.6 t	2 α : 1.88 m 2 β : 2.03 m
3	77.5 d	3.47 dd (4.5, 11.4)	79.0 d	3.57 dd (4.0, 11.9)
4	42.7 s		42.6 s	
5	50.0 d	1.75 dd (3.3, 14.5)	51.3 d	1.28 m
6	37.1 t	6 α : 2.89 dd (3.3, 18.0) 6 β : 3.24 dd (14.5, 18.0)	24.3 t	6 α : 2.04 m 6 β : 2.30 m
7	199.5 s		122.0 d	5.33 m
8	128.4 s		135.4 s	
9	164.2 s		52.1 d	1.60 m
10	39.6 s		35.3 s	
11	23.0 t	11 α : 2.07 m 11 β : 2.07 m	20.4 t	11 α : 1.47 m 11 β : 1.30 m
12	33.9 t	12 α : 1.50 m 12 β : 1.21 m	36.2 t	12 α : 1.32 m 12 β : 1.45 m
13	34.4 s		36.9 s	
14	33.7 t	14 α : 2.56 brd (17.6) 14 β : 2.12 brd (17.6)	46.1 t	14 α : 2.02 brd (15.0) 14 β : 1.95 brd (15.0)
15	145.9 d	5.74 dd (10.8, 17.4)	150.4 d	5.88 dd (10.7, 17.5)
16	111.4 t	16a: 4.90 dd (1.5, 17.4) 16b: 4.97 dd (1.5, 10.7)	109.4 t	16a: 4.96 dd (1.5, 10.7) 16b: 5.03 dd (1.5, 17.5)
17	27.7 q	0.94s	21.4 q	0.90 s
18	23.0 q	1.35 s	24.1 q	1.42 s
19	71.9 t	19a: 4.16 d (10.4) 19b: 4.70 d (10.4)	71.3 t	19a: 4.22 d (10.2) 19b: 4.54 d (10.2)
20	17.5 q	1.21 s	15.6 q	0.95 s
1 1	101.2 d	5.45 d (1.4)	102.5 d	4.90 d (0.9)
2 1	71.9 d	4.56 dd (1.4, 4.4)	71.7 d	4.54 dd (0.9, 3.3)
3 1	72.5 d	4.74 dd (3.2, 4.4)	75.6 d	4.13 dd (3.3, 9.2)
4 1	66.7 d	4.78 dd (3.2, 8.5)	68.9 d	4.58 t (9.2)
5 1	76.5 d	4.49 m	78.8 d	3.88 ddd (2.6, 5.4, 9.2.)
6 1	63.5 t	6 1 a: 4.36 dd (6.0, 12.0) 6 1 b: 4.47 dd (3.5, 12.0)	62.8 t	6 1 a: 4.38 dd (5.4, 11.5) 6 1 b: 4.55 dd (2.6, 11.5)

metabolite has the same structure as virescenoside M except that it possesses one less hydroxyl group. The magnitudes of the vicinal coupling constants (4.5, 11.4 Hz) between H-3 (δ 3.47) and H-2 α,β (δ 2.02, 1.91) revealed an equatorial configuration of the alcohol function at C-3.

The relative stereochemistry of **2** was defined by analysis of NMR chemical shifts and coupling constant values and by NOESY correlations (Table 2 and the Experimental Section). The CD spectrum of **2a** obtained upon acid hydrolysis of **2** showed the characteristic Cotton effects at 324 (positive), 258 (negative), and 210 (positive) nm, which were in good agreement with those for methyl 7-oxo-13-epi-pimar-8,15-dien-18-oate.⁹ These data led us to the conclusion that the aglycon **2a** belonged to the normal 5 α -pimarane series, and the structure of virescenoside P was established as β -D-altropyranosido-19-7-oxoisopimar-8,15-diene-3 β -ol.

The molecular formula of virescenoside Q (**3**) was established as $\text{C}_{26}\text{H}_{42}\text{O}_7$ on the basis of HRMALDIMS and ^{13}C NMR spectra. The carbon signals of the aglycon part of **3** (Table 2) were very similar to those of virescenoside B¹⁹ except for the chemical shifts of C-3, C-18, and C-19, and accordingly it is proposed that **3** has the same aglycon structure as virescenoside B.

Acid hydrolysis of virescenoside Q gave **3a**, which was identical to isovirescenoside B by NMR spectra and optical rotation. Besides **3a**, acid hydrolysis of **3** gave D-mannose, which was identified by optical rotation and by GLCMS as the corresponding aldononitrile peracetate. These data and the magnitudes of H-1 1 –H-6 1 and C-1 1 –H-1 1 (158 Hz) spin-coupling constants in the NMR spectra of **3**^{16–18} and NOE data (see Experimental Section) elucidated the pres-

ence of a β -D-mannopyranoside unit in **3**. On the basis of all the above data, the structure of virescenoside Q was established as β -D-mannopyranosido-19-isopimar-7,15-diene-3 β -ol.

It was shown that virescenosides O, P, and Q exhibited cytotoxic action against tumor cells of Ehrlich carcinoma (IC_{50} = 20–100 μM) in vitro. Virescenoside P showed cytotoxic effects on developing eggs of the sea urchin *Strongylocentrotus intermedius* (MIC_{50} = 5.0 μM).

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded in both CDCl_3 and pyridine on a Bruker DPX-300 spectrometer operating at 300 and 75.4 MHz with TMS as internal standard. HRMALDIMS analyses were carried out with a Bruker Biflex time-of-flight mass spectrometer equipped with a UV-nitrogen laser (337 nm). CD spectra were obtained with a JASCO model J-500. UV spectra were recorded on a Specord UV-vis spectrometer in MeOH. GLCMS analyses were done on a Hewlett-Packard HP6890 GG system, with an HP-5MS capillary column (30.0 m \times 250 μm \times 0.25 μm) at 210 $^\circ\text{C}$. Helium was used as the carrier gas, and the ionizing voltage was 70 eV. Optical rotations were measured by a Perkin-Elmer 141 polarimeter.

Cultivation of *A. striatisporum*. The cultivation of the fungus was performed as previously reported.¹

Extraction and Isolation. At the end of the incubation period, the mycelium and medium were homogenized and thrice extracted then with a mixture of CHCl_3 –MeOH (2:1, v/v, ca. 2 L). After evaporation of the solvent, the residual material (4 g) was passed over normal-phase silica, which was eluted first with CHCl_3 (500 mL) followed by a step gradient from 5% to 20% MeOH in CHCl_3 (total volume 2 L). Fractions of 10 mL were collected and combined by TLC examination.

Fractions containing the desired compounds were further purified by reversed-phase HPLC on a Silasorb-ODS column (10 μ m, 9.6 \times 200 mm, 220 nm) eluting with a step gradient from 52% to 75% MeOH in H₂O and then by normal-phase HPLC on a Zorbax SIL column (5 μ m, 4.6 \times 150 mm) using EtOAc–(CH₃)₂CO (70:30) as eluent to yield **1** (6 mg), **2** (4.5 mg), and **3** (4.2 mg).

β -D-Altropyranosido-19-sandaracopimara-8(14),15-diene-3 β ,7 α -diol (1): colorless amorphous solid; $[\alpha]_D^{20}$ –44° (c 0.5, MeOH); ¹H and ¹³C NMR spectra (C₅D₅N), see Table 1; HRMALDIMS *m/z* 505.2790 (calcd for C₂₆H₄₂O₈Na, 505.2772).

β -D-Altropyranosido-19-7-oxoisopimara-8,15-diene-3 β -ol (2): colorless amorphous solid; $[\alpha]_D^{20}$ +31° (c 0.2, MeOH); UV (MeOH) λ_{\max} (log ϵ) 248 (3.7) nm; ¹H and ¹³C NMR spectra (C₅D₅N), see Table 2; HMBC correlation (H/C) H-16a,b/C-13, C-15; Me-17/C-12, C-13, C-14, C-15; Me-18/C-3, C-4, C-5, C-19; Me-20/C-1, C-5, C-9, C-10; NOESY correlations (H/H) 1 α /3,5, 1 β /11 β , 2 β /19b,20, 3/5,18, 5/18, 6 β /19b,20, 11 α /15, 12 α /15, 14 α /16b,17, 14 β /17, 15/17, 16b/17, 18/19a, 19a,b/20,1¹; HRMALDIMS *m/z* 503.2630 (calcd for C₂₆H₄₀O₈Na, 503.2616).

β -D-Mannopyranosido-19-isopimara-7,15-diene-3 β -ol (3): colorless amorphous solid; $[\alpha]_D^{20}$ –20° (c 0.45, MeOH); ¹H and ¹³C NMR spectra (C₅D₅N), see Table 2; HMBC correlation (H/C) H-15/C-13, C-17; H-16a,b/C-13; Me-17/C-12, C-13, C-14, C-15; Me-18/C-3, C-4, C-5, C-19; Me-20/C-1, C-5, C-9, C-10; H-1¹/C-2¹; NOESY correlation (H/H) 1 α /3,9, 1 β /11 α , 20, 2 β /19a,b,20, 3/5,18, 5/9, 6 α /18, 6 β /19b,20, 7/14 β , 9/12 α , 11 β /17, 20, 12 α /14 α ,15, 14 α /15,16b, 14 β /17, 15/17, 16b/17, 18/19a,b, 19a,b/20,1¹, 19b/6 β , 1¹/3¹,5¹, 3¹/5¹; HRMALDIMS *m/z* 489.2809 (calcd for C₂₆H₄₂O₇Na, 489.2823).

Acidic Hydrolysis of Virescenoside P (2). A solution of compound **2** (5 mg) in 0.1 M TFA (1 mL) was heated in a stoppered reaction vial for 30 min. The water layer was extracted with CHCl₃. The residue obtained after evaporation of the extract was chromatographed on a Si gel column (0.8 \times 6 cm), eluting first with hexane and finally with a solvent system of hexane–ethyl acetate (60:40), to yield 1.8 mg of **2a**.

7-Oxoisopimara-8,15-diene-3 β ,19 β -diol (2a): colorless amorphous solid; $[\alpha]_D^{20}$ +44° (c 0.15, MeOH); UV (MeOH) λ_{\max} (log ϵ) 253 (4.04) nm; CD (3.10 \times 10^{–6} M, MeOH) $\Delta\epsilon_{324}$ +0.96, $\Delta\epsilon_{258}$ –0.15, and $\Delta\epsilon_{210}$ +1.64; ¹H NMR (300 MHz, J, CDCl₃) δ 3.49 (1H, dd, 4.8, 11.4, H-3), 1.73 (1H, dd, 3.5, 14.4, H-5), 2.57 (1H, dd, 3.5, 17.5, H-6 α), 2.33 (1H, dd, 14.4, 17.5, H-6 β), 2.36 (1H, brd, 17.7, H-14 α), 2.57 (1H, brd, 17.7, H-14 β), 5.67 (1H, dd, 10.8, 17.4, H-15), 4.83 (1H, dd, 1.4, 17.4, H-16a), 4.97 (1H, dd, 1.4, 10.8, H-16b), 3.43 (1H, d, 11.2, H-19a), 4.29 (1H, d, 11.2, H-19b), 1.02 (3H, s, H₃-17), 1.24 (3H, s, H₃-18), 1.06 (3H, s, H₃-20); ¹³C NMR (75.4 MHz, CDCl₃) δ 33.9 (t, C-1), 27.7 (t, C-2), 79.9 (d, C-3), 42.4 (s, C-4), 49.6 (s, C-5), 34.9 (t, C-6), 199.0 (s, C-7), 129.1 (s, C-8), 164.5 (s, C-9), 39.2 (s, C-10), 23.2 (t, C-11), 33.7 (t, C-12), 34.5 (s, C-13), 33.4 (t, C-14), 145.1 (d, C-15), 111.8 (t, C-16), 28.2 (q, C-17), 21.9 (q, C-18), 63.9 (t, C-19), 18.4 (q, C-20); EIMS *m/z* 318 [M]⁺ (6), 300 (3), 170 (10), 148 (100), 82 (99).

Acidic Hydrolysis of Virescenoside Q (3). A solution of compound **3** (5 mg) in 0.1 M TFA (1 mL) was heated in a boiling water bath for 1 h. The lipid part of the hydrolysate was purified as described above to yield 1.2 mg of **3a**. The residue obtained after evaporation of the water layer was purified on a Separon SGX NH₂ column (7 μ m, 3 \times 150 mm) eluting with 90% AcCN to yield 0.8 mg of D-mannose, $[\alpha]_D^{20}$ +14.1° (c 0.4, H₂O). Monosaccharide was treated with

NH₂OH·HCl (1 mg) and pyridine (0.5 mL) at 100 °C for 1 h. A solution obtained was heated with Ac₂O (0.5 mL) at 100 °C for 1 h and concentrated in vacuo to dryness. The aldononitrile peracetate was analyzed by means of GLC and GLCMS.

Isopimara-7,15-diene-3 β ,19-diol (3a): $[\alpha]_D^{20}$ +98° (c 0.16, CHCl₃); ¹H and ¹³C NMR spectra and optical rotation data obtained for **3a** were in agreement with published data^{7,19} for isovirescenol B.

Acidic Hydrolysis of Virescenoside O (1). Acidic hydrolysis of compound **1** (12 mg) was performed as described above for **2**. The residue obtained after evaporation of the water layer was purified on a Zorbax NH₂ column (5 μ m, 4.6 \times 150 mm) eluting with 90% AcCN to yield 1.7 mg of 1,6-anhydro- β -D-altropyranose (altrosan) and 1.2 mg of D-altrose, $[\alpha]_D^{20}$ +32.8° (c 0.6, H₂O). The ¹³C NMR spectrum obtained for the monosaccharide was in agreement with published data for D-altrose.²⁰ The acetylation of altrosan with Ac₂O and pyridine afforded the triacetate, $[\alpha]_D^{20}$ –166° (c 0.3, CHCl₃). Its ¹H NMR spectrum was in agreement with published data.²

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