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

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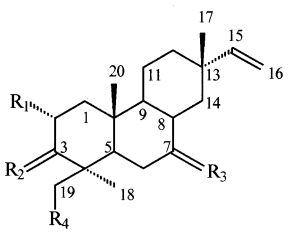
Four new diterpene glycosides, virescenosides R (**1**), S (**2**), T (**3**), and U (**4**), have been isolated from a marine strain of *Acremonium striatisporum* KMM 4401 associated with the holothurian *Eupentacta fraudatrix*. Their structures have been elucidated on the basis of HRFABMS, 1D and 2D NMR (^{1}H, ^{13}C, DEPT, COSY-45, COSY-RCT, HSQC, HMBC, and NOESY spectra), and the results of acidic hydrolysis as 19-*O*-{*β*-D-glucopyranosyl(1→6)-*β*-D-altropyranosyl}-isopimara-7,15-diene-2α,3*β*-diol (**1**), 19-*O*-*β*-D-altropyranosyl-3-oxo-isopimara-8(14),15-diene-7α-ol (**2**), 19-*O*-*β*-D-altropyranosyl-3,7-dioxo-isopimara-8,-15-diene (**3**), and 19-*O*-*β*-D-altropyranosyl-3,7-dioxo-isopimara-8(14),15-diene (**4**). The cytotoxic activity of the virescenosides was examined.

In our search for secondary metabolites from marine fungi with cytotoxity and/or novel chemical structures, we have previously isolated five new diterpene altrosides, virescenosides M−Q, from a marine strain of *Acremonium striatisporum* originally separated from the holothurian *Eupentacta fraudatrix*.[1,2] Further investigation for metabolites of this fungal strain has now led to the isolation of four new cytotoxic glycosides, virescenosides R−U (**1**−**4**). We report herein the isolation and structures of compounds **1**−**4** and their cytotoxic activity.

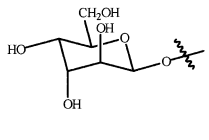
The fungus was cultured for 21 days on specially modified rice medium.[1] The $CHCl_3$−MeOH (2:1, v/v) extract of the culture of *A. striatisporum* was fractionated by Si gel column chromatography followed by reversed-phase and normal-phase HPLC to yield individual glycosides **1**−**4**. The structures of new compounds **1**−**4** were established by the interpretation of spectral data (NMR and HRFABMS), as well as by comparison of their spectra with those of related compounds.

The FAB mass spectrum of virescenoside R (**1**) exhibited a quasimolecular ion peak at *m*/*z* 643 $[M - H]^-$ in the negative ion mode and at *m*/*z* 645 $[M + H]^+$ and 667 $[M + Na]^+$ in the positive ion mode. The molecular formula of **1** was determined as $C_{32}H_{52}O_{13}$ on the basis of a high-resolution FABMS (positive ions) peak at *m*/*z* 667.3312 ($C_{32}H_{52}O_{13}Na$ requires *m*/*z* 667.3306) and was in accordance with ^{13}C NMR data. Initial examination of the 1-D proton and one-bond ^{1}H−^{13}C correlation NMR data suggested the presence of two sugars (anomeric signals at δ^1H 5.46 → δ^{13}C 99.2 and δ^1H 5.41 → 100.2). This was supported by fragment ions in the positive ion mass spectrum which were consistent with the loss of a C6-terminal sugar (*m*/*z* 483 $[M + H - sugar]^+$) and with the loss of both C6-sugars (*m*/*z* 321 $[M + H - 2\ sugars]^+$).

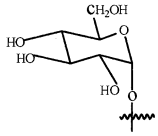
The aglycon moiety of glycoside **1** was found by extensive NMR spectroscopy (^{1}H, ^{13}C, DEPT, COSY-45, COSY-RCT, HSQC, HBMC, and NOESY spectra) (Table 1) to be the same as that of virescenoside A (**5**), isolated earlier from the terrestrial fungus *Acremonium luzulae*.[3−6] Acid hydrolysis of virescenoside R gave **1a**, which was identical to virescenol A by NMR and optical rotation. The sugar part of the hydrolysate was treated first with (+)-2-octanol in the presence of trifluoroacetic acid and then with pyridine−acetic anhydride. Analysis of the resulting acetylated (+)-2-octyl glycosides by capillary GC led to the identification of D-altrose and D-glucose.[7] The identification of each sugar as well as their sequence, interglycosidic linkage, and configuration of glycosidic bonds in **1** were determined by 1D and 2D NMR including HBMC and NOESY (Table 1) and various ^{1}H−^{1}H COSY experiments. A long-range ^{1}H−^{13}C correlation (δ^1H 5.46 → δ^{13}C 70.0) revealed a linkage



	R_1	R_2	R_3	R_4	
1	OH	H, *β*-OH	H	*α*-D-glucopyranosyl(1 → 6)-*β*-D-altropyranosyl-	$\Delta^{7,8}$
1a	OH	H, *β*-OH	H	OH	$\Delta^{7,8}$
2	H	O	H, α-OH	*β*-D-altropyranosyl-	$\Delta^{8,14}$
3	H	O	O	*β*-D-altropyranosyl-	$\Delta^{8,9}$
4	H	O	O	*β*-D-altropyranosyl-	$\Delta^{8,14}$
5	OH	H, *β*-OH	H	*β*-D-altropyranosyl	$\Delta^{7,8}$
6	H	H, *β*-OH	H, α-OH	*β*-D-altropyranosyl	$\Delta^{8,14}$
7	H	H, *β*-OH	O	*β*-D-altropyranosyl	$\Delta^{8,9}$



β-D-altropyranosyl-



α-D-glucopyranosyl-

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Table 1. ^{1}H and ^{13}C NMR Data of Virescenoside R (**1**) in C_5D_5N (*J*, Hz)

atom	δ_C	δ_H	HMBC	NOESY
1	46.5 CH_2	α: 1.35 m		3
		β: 2.28 dd (4.1, 12.7)	3, 5, 10, 20	20
2	67.8 CH	4.48 m		19a,b, 20
3	83.7 CH	3.48 d (9.5)	1, 2, 4, 18, 19	1α, 5, 18
4	43.6 C			
5	51.2 CH	1.40 m		3, 9
6	24.4 CH_2	α: 2.00 m		18
		β: 2.22 m		20
7	122.2 CH	5.38 m		14*β*
8	135.1 C			
9	52.4 CH	1.68 m		5, 12α,14α
10	36.1 C			
11	20.5 CH_2	α: 1.47 m		
		β: 1.28 m		17
12	36.2 CH_2	α: 1.27 m		
		β: 1.37 m		17
13	36.9 C			
14	46.1 CH_2	α: 2.03 brd (14.5)		
		β: 1.94 brd (14.5)	7, 8, 9, 12, 13	7, 17
15	150.5 CH	5.85 dd (10.8, 17.5)	12, 13, 14, 16, 17	12α, 14α, 17
16	109.4 CH_2	a: 4.95 dd (1.5, 10.8)	13, 15	
		b: 5.00 dd (1.5, 17.5)	13, 15	12α, 14*β*, 17
17	21.4 CH_3	0.88 s	12, 13, 14, 15	11*β*, 12*β*, 14*β*, 15, 16b
18	25.7 CH_3	1.29 s		3, 6α, 19a,b
19	70.0 CH_2	a: 3.85 d (10.0)		6*β*, 18, 20, 1-Alt
		b: 4.44 d (10.0)	3, 4, 5, 18, 1-Alt	6*β*, 18, 20, 1-Alt
20	15.7 CH_3	1.05 s	1, 5, 9, 10	1*β*, 2, 6*β*, 19a,b
		Alt (1→C-19)		
1	99.2 CH	5.46 d (1.2)	19	19a,b, 5-Alt
2	71.1 CH	4.53 dd (1.2, 4.4)	3,4-Alt	
3	72.1 CH	4.70 dd (3.3, 4.4)	1,2,4,5-Alt	
4	65.1 CH	4.86 dd (3.3, 9.4)	5-Alt	
5	74.2 CH	4.49 m		
6	67.6 CH_2	a: 4.09 dd (4.1, 10.5)		1-Glc
		b: 4.63 dd (3.2, 10.5)		1-Glc
		Glc (1→6Alt)		
1	100.2 CH	5.41 d (3.7)	5,6-Alt	6a,b-Alt
2	73.4 CH	4.10 dd (3.7, 9.5)	3-Glc	
3	74.8 CH	4.70 t (9.5)	1,2,4-Glc	
4	71.2 CH	4.22 t (9.7)		
5	74.3 CH	4.47 m		
6	62.5 CH_2	a: 4.35 dd (5.6, 12.2)		
		b: 4.46 dd (2.6, 12.2)		

between H1-Alt and C-19 of the aglycon. This interpretation was confirmed by a strong NOESY cross-peak between H-19a,b and H1-Alt. Similarly, a long-range correlation (δ^1H 5.41 → δ^{13}C 67.6) and the downfield chemical shift of C6-Alt (67.6), and the NOESY cross-peak between H1-Glc and H6a,b-Alt, assigned the linkage between these two sugar units.

A comparison of the ^{13}C NMR spectrum of **1** with published data for α- and *β*-D-altropyranoses and α-methyl-D-altropyranoside together with magnitudes of ^{1}H−^{1}H and C1-Alt/H1-Alt (162.6 Hz) spin-coupling constants in the NMR spectra of **1** elucidated the presence of a *β*-D-altropyranoside unit of C1 form in **1**.[8−10] Similarly, the terminal sugar was determined as α-D-glucopyranose of C1 form based on ^{13}C chemical shifts and ^{1}H−^{1}H and C1-Glc/H1-Glc (167.5 Hz) spin-coupling constants. Thus, the structure of virescenoside R (**1**) was determined as 19-*O*-{α-D-Glcp(1→6)-*β*-D-Altp}-isopimara-7,15-diene-2α,3*β*-diol.

The ^{13}C and ^{1}H NMR spectra of the sugar moieties of virescenosides S, T, and U showed a close similarity of all proton and carbon chemical shifts and proton multiplicities (Table 2). The acid hydrolysis of the sum of these virescenosides gave D-altrose as one of the sugars which was identified by GLC of the corresponding acetylated (+)-2-octyl glycoside, using authentic samples prepared from D- and L-altrose. Proton NMR coupling constant values for the sugar portion of virescenosides S, T, and U and NOESY correlations (Table 2 and the Experimental Section) elucidated the presence of a *β*-D-altropyranoside unit of C1 form in these glycosides.

In HRFABMS virescenoside S (**2**) gave a quasimolecular ion at *m/z* 503.2707 $[M + Na]^+$. These data, coupled with ^{13}C NMR spectral data (DEPT), established the molecular formula of **2** as $C_{26}H_{40}O_8$. The ^{13}C NMR spectra of the aglycon part of **2** (Table 2) showed signals characteristic of a saturated ketone group, one secondary alcohol function, and two double bonds at 213.6 (C), 71.8 (CH), 148.3 (CH), 139.6 (C), 132.6 (CH), and 110.6 (CH_2).

The general features of the ^{1}H and ^{13}C NMR spectra (Table 2 and the Experimental Section) of the aglycon part of **2** closely resembled those of virescenoside O (**6**) with the exception of proton and carbon signals belonging to the A ring.[2] The correlation observed in the COSY-45, COSY-RCT, and HSQC spectra of **2** indicated the presence of the following isolated spin systems: - -CH_2- -CH_2- - (C-1- -C-2) and - -CH_2−O- - (C-19). In the HBMC experiment the methyl singlet at δ 1.41 (H_3-18) showed long-range correlations with C-3 (213.6), C-4 (52.5), C-5 (49.7), and C-19 (73.1). These data and correlations observed in the COSY-45 and NOESY spectra indicated the position and stereochemistry of the methyl and hydroxymethyl groups at C-4 and the position of the carbonyl group at C-3. The strong NOEs from H1-Alt to H-19a,b and the downfield chemical

Table 2. ^{1}H and ^{13}C NMR Data of Virescenosides S (**2**), T (**3**), and U (**4**) in C_5D_5N (*J*, Hz)

	2		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.3 CH_2	1α: 1.50 m 1*β*: 1.92 m	34.9 CH_2	α: 1.55 m *β*: 1.92 ddd (2.9, 5.7, 13.0)	37.6 CH_2	α: 1.43 m *β*: 1.92 m
2	36.4 CH_2	2α: 2.39 m 2*β*: 2.94 td (5.1, 14.4)	35.9 CH_2	α: 2.42 ddd (3.0, 4.5, 11.4) *β*: 2.95 td (5.6, 11.4)	36.2 CH_2	α: 2.38 dt (4.6, 13.4) *β*: 2.97 td (5.3, 13.6)
3	213.6 C		211.6 C		212.7 C	
4	52.5 C		52.1 C		52.1 C	
5	49.7 CH	2.53 dd (2.7, 13.0)	50.9 CH	2.23 dd (3.7, 14.3)	52.0 CH	2.00 dd (7.8, 11.1)
6	31.3 CH_2	α: 1.97 dt (2.7, 13.3) *β*: 1.75 td (3.2, 13.0, 13.3)	36.2 CH_2	α: 2.68 dd (3.7, 17.4) *β*: 2.89 dd (14.3, 17.4)	37.9 CH_2	α,*β*: 2.70 m
7	71.8 CH	4.39 brt	197.6 C		197.6 C	
8	139.6 C		128.9 C		134.5 C	
9	45.2 CH	2.40 m	163.1 C		50.0 CH	1.94 m
10	38.3 C		39.2 C		35.7 C	
11	18.9 CH_2	α: 1.54 m *β*: 1.45 m	23.2 CH_2	α,*β*: 2.07 m	19.1 CH_2	α: 1.36 m *β*: 1.53 m
12	34.1 CH_2	α,*β*: 1.42 m	33.4 CH_2	α: 1.50 m *β*: 1.21 m	33.9 CH_2	α,*β*: 1.46 m, 1.56 m
13	37.5 C		34.3 C		38.7 C	
14	132.6 CH	5.61 d (1.9)	33.7 CH_2	α: 2.55 brd (17.7) *β*: 2.14 brd (17.7)	143.9 CH	6.96 dd (1.2, 2.9)
15	148.3 CH	5.76 dd (10.6, 17.4)	145.6 CH	5.73 dd (10.8, 17.4)	146.6 CH	5.85 dd (10.5, 17.5)
16	110.6 CH_2	a: 4.92 dd (1.5, 10.6) b: 4.99 dd (1.5, 17.4)	111.5 CH_2	a: 4.92 dd (1.5, 17.4) b: 4.97 dd (1.5, 10.8)	111.7 CH_2	a: 5.01 dd (1.2, 10.5) b: 5.07 dd (1.2, 17.4)
17	25.7 CH_3	1.09 s	27.5 CH_3	0.96 s	25.6 CH_3	1.05 s
18	21.4 CH_3	1.41 s	20.7 CH_3	1.24 s	20.8 CH_3	1.28 s
19	73.1 CH_2	a: 4.17 d (10.0) b: 4.25 d (10.0)	72.3 CH_2	a: 4.05 d (10.0) b: 4.33 d (10.0)	72.8 CH_2	a: 4.07 d (9.8) b: 4.27 d (9.8)
20	14.8 CH_3	1.08 s	17.7 CH_3	1.37 s	14.1 CH_3	1.10 s
		Alt (1→C-19)				
1	100.4 CH	5.48 d (1.3)	100.4 CH	5.45 d (1.3)	100.4 CH	5.43 d (1.4)
2	72.0 CH	4.51 dd (1.2, 3.6)	71.9 CH	4.51 dd (1.3, 3.3)	72.0 CH	4.49 dd (1.4, 3.7)
3	72.6 CH	4.74 dd (2.7, 3.6)	72.6 CH	4.74 t (3.3)	72.6 CH	4.74 m
4	66.3 CH	4.76 dd (3.3, 7.5)	66.3 CH	4.78 dd (3.3, 10.8)	66.2 CH	4.78 dd (3.1, 7.8)
5	76.6 CH	4.55 m	76.7 CH	4.55 m	76.6 CH	4.53 m
6	63.3 CH_2	a: 4.38 dd (6.7, 12.2) b: 4.56 m	63.3 CH_2	a: 4.37 dd (6.3, 12.3) b: 4.52 m	63.3 CH_2	a: 4.34 dd (6.3, 12.3) b: 4.52 m

shift of C-19 (*δ* 73.1) indicated that the sugar moiety was linked at C-19. On the basis of the above data, the structure of virescenoside S was established as 19-*O*-*β*-D-altropyranosyl-3-oxo-isopimara-8(14),15-diene-7α-ol (**2**). This agrees with the molecular mass difference of 2 mass units between virescenoside O and **2**.

The molecular formula of virescenoside T (**3**) was determined as $C_{26}H_{38}O_8$ by pseudomolecular ions at *m*/*z* 479.2672 $[M + H]^+$ and 501.2476 $[M + Na]^+$ in HRFABMS (positive mode) and ^{13}C NMR analyses. The aglycon part of **3** was found by extensive NMR (^{1}H and ^{13}C NMR, COSY-45, COSY-RCT, HBMC, and NOESY) and UV spectroscopy (Table 2 and Experimental Section) to closely resemble that of virescenoside P (**7**) with the exception of proton and carbon signals belonging to the A ring.[2] The ^{13}C NMR spectrum of **3** showed a peak at *δ* 211.6, consistent with a saturated ketone. Correlations observed in the COSY-45 and HSQC spectra of **3** indicated the presence of an isolated spin system corresponding to the sequence $-CH_2-CH_2-$ -(C-1- -C-2). The long-range correlation of the methyl proton signal at *δ* 1.24 (H_3-18) with the carbon signal at *δ* 211.6 and the downfield chemical shift of C-4 (*δ* 52.1) placed a ketone group at C-3 of ring A. The position and stereochemistry of the methyl (1.24, s) and hydroxymethyl (72.3, CH_2) groups at C-4 were established on the basis of NOEs and HBMC data. The attachment of the sugar moiety to C-19 was established by the correlation of H1-Alt and H-19a,b in the NOESY spectrum and the downfield chemical shift of C-19 (*δ* 72.3). The above data showed that the structure of **3** is 19-*O*-*β*-D-altropyranosyl-3,7-dioxo-isopimara-8,15-diene.

The molecular formula of virescenoside U (**4**) was determined as $C_{26}H_{38}O_8$ by pseudomolecular ions at *m*/*z* 479.2625 $[M + H]^+$ and 501.2474 $[M + Na]^+$ in the HRFABMS (positive mode) and ^{13}C NMR analyses. A close inspection of the ^{1}H and ^{13}C NMR spectral data (Table 2) of the aglycon part of virescenoside T by DEPT and HSQC revealed the presence of three quaternary methyls (*δ* 25.6, C-17; 20.8, C-18; 14.1, C-20); six methylenes (*δ* 37.6, C-1; 36.2, C-2; 37.9, C-6; 19.1, C-11; 33.9, C-12; 72.8, C-19), including one oxygen-bearing methylene; two tertiary (*δ* 52.0, C-5; 50.0, C-9) and three saturated quaternary carbons (*δ* 52.1, C-4; 35.7, C-10; 38.7, C-13); one carbonyl group (*δ* 212.7, C-3); and a monosubstituted double bond (146.6, C-15; 111.7, C-16). The remaining functionality, corresponding to the carbon signals at *δ* 197.6 (C), 143.9 (CH), and 134.5 (C), suggested the presence of the trisubstituted enone chromophore. The UV spectrum showed a λ_{max} at 247 nm (log ϵ 3.4), consistent with the enone system in structure **4**.

The correlations observed in the COSY-45, COSY-RCT, and HSQC spectra of the aglycon part of **4** indicated the presence of the following isolated spin systems: - -CH_2- CH_2- (C-1- -C-2), >$CH-CH_2-$ (C-5−C-6), - -$CH=CH_2-$ (C-15−C-16), - -CH_2-O- (C-19). Furthermore, the COSY-45 spectrum of **4** contained a cross-peak attributed to allylic coupling between the olefinic proton at *δ* 6.96 (H-14) and a signal at 1.94 (H-9), which was in turn coupled with two signals at *δ* 1.36 (H-11α) and 1.53 (H-11*β*). On the basis of this information together with the data obtained from the

HBMC spectrum (Experimental Section) a diterpene altroside with a tricyclic aglycon structure was indicated for **4**.

A direct comparison of ^{1}H and ^{13}C NMR spectra of **4** with those of the glycosides M−Q,[1,2] and virescenosides A−C, obtained earlier from the terrestrial fungus *Acremonium luzulae*[6,11−13] suggests that virescenoside U has the structure of an isopimaradienic altroside. The proton signals of a typical ABX system of a vinyl group at δ 5.85 (1H, dd, 10.5, 17.5 Hz), 5.07 (1H,dd, 1.2, 17.4 Hz), and 5.01 (1H, dd, 1.2, 10.5 Hz) indicated a monosubstituted double bond located at the C-15, C-16 position of this double bond.[4,14−16] Furthermore, the positions of the C-17 methyl group (δ 1.05, s) and of the exo-vinyl group at C-13 were confirmed by HBMC and NOE measurements (Experimental Section). The long-range correlation of the methyl proton signal at δ 1.05 (H_3-17) with the carbon signal at 143.9 (C-14) and the downfield chemical shift of H-14 (δ 6.96) indicated the 8(14)-en-7-one position for the trisubstituted enone chromophore in **4**. The stereochemistry at C-13 in **4** was assigned to be the same as sandaracopimaradienic derivatives on the basis of the similarity of the C-15−C-17 chemical shifts for these compounds.[17−20]

The NMR spectrum of **4** showed two signals corresponding to an AB system coupling at δ 4.07 and 4.27 (each 1H, d, 9.8 Hz), which was consistent with the presence of a CH_2O- - group linked to a quaternary sp^3 carbon. The position and stereochemistry of the methyl (1.28, s) and hydroxymethyl (72.8, CH_2) groups at C-4 and methyl group (1.10, s) at C-10 were established as for compound **3**. The location of the carbonyl group at C-3 was evident from the COSY-45 spectra and downfield chemical shift of C-4 (δ 52.1). The NOE correlation between H-2β and H_3-20 and H-19a as well as between H_3-18 and H-5 indicated a trans ring fusion between rings A and B. All of these data are consistent with a $\Delta^{8(14),15}$-isopimaradienic skeleton with a carbonyl function at C-3, an axial hydroxymethyl group at C-4, and a 7-keto group conjugated with a C-8/C-14 double bond in **4**. The strong NOEs from H1-Alt to H-19a,b and the downfield chemical shift of C-19 (δ 72.8) indicated that the sugar moiety was linked at C-19. On the basis of these data, the structure of virescenoside U was established as 19-*O*-β-D-altropyranosyl-3,7-dioxo-isopimara-8(14),15-diene.

Virescenosides R, S, T, and U exhibited cytotoxic action against tumor cells of Ehrlich carcinoma ($IC_{50} = 25-60\ \mu M$) in vitro. These glycosides showed a weak cytotoxic effect on developing eggs of the sea urchin *Strongylocentrotus intermedius* ($IC_{50} = 100-150\ \mu M$).

Experimental Section

General Experimental Procedure. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. UV spectra were recorded on a Specord UV−vis spectrometer in MeOH. ^{1}H and ^{13}C NMR spectra were recorded in both $CDCl_3$ and C_5D_5N on a Bruker DPX-300 spectrometer operating at 300 and 75.4 MHz, respectively, using TMS as an internal standard. FABMS spectra were measured in a glycerol matrix on a AMD-604S mass spectrometer. GLC analyses were performed on a Agilent 6850 Series GC system equipped with a HP-5MS column and a temperature program of 100 to 250 °C at 5 °C min^{-1}. Helium was used as the carrier gas. Preparative HPLC was carried out on a Beckman apparatus equipped with an RIDK-22 refractometric detector, using Diasphere-110-C18 (5 μm, 4 × 250 mm) and Zorbax SIL (5 μm, 4 × 150 mm) columns.

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 extracted three times 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 40% MeOH in $CHCl_3$ (total volume 3 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 Diasphere-110-C18 column eluting with a step gradient from 45% to 75% MeOH in H_2O and then by normal-phase HPLC on a Zorbax SIL column with EtOAC−$(CH_3)_2$CO−EtOH (100:20:15) to give **1** (15 mg) and EtOAC−$(CH_3)_2$CO (70:30) to yield **2** (4.5 mg), **3** (5 mg), and **4** (3.0 mg).

19-*O*-{α-D-Glcp(1→6)-*β*-D-Altp}-isopimara-7,15-diene-2α,3*β*-diol (1): colorless amorphous solid; $[\alpha]^{20}_D$ +12° (*c* 0.6, MeOH); ^{1}H and ^{13}C NMR spectra (C_5D_5N), see Table 1; HRFABMS (positive ion) *m*/*z* 667.3312 $[M + Na]^+$ (calcd for $C_{32}H_{52}O_{13}Na$, 667.3306).

19-*O*-*β*-D-Altropyranosyl-3-oxo-isopimara-8(14),15-diene-7α-ol (2): colorless amorphous solid; $[\alpha]^{20}_D$ −42.5° (*c* 0.4, MeOH); ^{1}H and ^{13}C NMR spectra (C_5D_5N), see Table 2; HBMC correlation (H/C) H-16b/C-13; Me-17/C-12, C-13, C-14, C-15; Me-18/C-3, C-4, C-5, C-19; M-20/C-1, C-5, C-9, C-10; H-1^1/C-2^1; NOESY correlation (H/H) 1α/9, 1*β*/11α,20, 2*β*/20, 5/1α,9,18, 6α/18, 6*β*/19b,20, 7/14, 9/1α,5, 9/1α, 11α/1*β*, 11*β*/20, 14/7,17, 15/17, 16b/17, 17/14,15,16b, 18/5,6α,19b, 19a/1^1,20, 19b/6*β*,18, 20/1*β*,2*β*,6*β*,11*β*,19a, 1^1/19a,b,5^1; HRFABMS (positive ion) *m*/*z* 503.2617 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_8Na$, 503.2621).

19-O-*β*-D-Altropyranosyl-3,7-dioxo-isopimara-8,15-diene (3): colorless amorphous solid; $[\alpha]^{20}_D$ +23° (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (3.7) nm; ^{1}H and ^{13}C NMR spectra (C_5D_5N), see Table 2; HBMC correlation (H/C) H-15/C-13; 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-19b/C-3; H1-Altr/C2-Altr; NOESY correlation (H/H) 1α/5, 2*β*/19a,20, 5/1α,18, 6α/18, 6*β*/19a,b,20, 11*β*/20, 12α/17, 14α/16a,17, 14*β*/17, 15/12α,17, 16a/14α,17, 17/12α,14α,*β*,15,16a, 18/5,6α,19a,b, 19a/2*β*,18,20,1-Altr, 19b/6*β*,18,20,1-Altr, 20/1*β*,2*β*,6*β*,11*β*, 1-Altr/19a,b,5-Altr; HRFABMS (positive ions) *m*/*z* 479.2672 $[M + H]^+$ (calcd for $C_{26}H_{39}O_8$, 479.2645), 501.2476 $[M + Na]^+$.

19-*O*-*β*-D-Altropyranosyl-3,7-dioxo-isopimara-8(14),15-diene (4): colorless amorphous solid; $[\alpha]^{20}_D$ −30° (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ϵ) 247 (3.4) nm; ^{1}H and ^{13}C NMR spectra (C_5D_5N), see Table 2; HBMC correlation (H/C) Me-17/C-12, C-13, C-14, C-15; Me-18/C-4, C-5, C-19; Me-20/C-1, C-5, C-9, C-10; H-19b/C-4, C-5; NOESY correlation (H/H) 1*β*/20, 2*β*/19a,20, 5/18, 6α/18, 6*β*/19a,b,20, 14/17, 15/17, 16b/17, 17/11*β*,14,15, 18/5,6α,19a,b, 19a/2*β*,6*β*,18,20,1-Altr, 19b/6*β*,18,20,1-Altr, 20/1*β*,2*β*,6*β*,19a,b, 1-Altr/19a,b,5-Altr; HRFABMS (positive ions) *m*/*z* 479.2625 $[M + H]^+$ (calcd for $C_{26}H_{39}O_8$, 479.2645), 501.2474 $[M + Na]^+$.

Acidic Hydrolysis of Virescenoside R (1). A solution of compound **1** (9 mg) in 0.2 M TFA (1 mL) was heated in a stoppered reaction vial for 30 min. The water layer was extracted with $CHCl_3$. The residue obtained after evaporation of the extract was chromatographed on a Diasphere-110-C18 column (5 μm, 4 × 250 mm) eluting with 80% MeOH to yield 1.9 mg of **1a**. The residue obtained after evaporation of the water layer was purified on a Zorbax NH_2 column (5 μm, 4.6 × 15 mm) eluting with 90% AcCN to yield 1.0 mg of glucose and 0.8 mg of altrose. The monosaccharides were treated with (+)-2-octanol (0.2 mL) in the presence of trifluoroacetic acid (1 drop) in a stoppered reaction vial at 130 °C overnight.[7] The obtained mixtures were evaporated to dryness and acetylated with Ac_2O in pyridine. The acetylated (+)-2-octyl glycosides were analyzed by GLC using the corresponding authentic samples prepared from D- and L-glucose and d- and L-altrose.

Isopimara-7,15-diene-2α,3*β*,19-triol (1a): $[\alpha]^{20}_D$ −42° (*c* 0.2, $CHCl_3$); ^{1}H and ^{13}C NMR spectra and optical rotation data obtained for **1a** were in agreement with published data[3−5] for virescenol A.

Acidic Hydrolysis of Virescenosides S−U (2−4). A solution of a mixture of compounds **2**, **3**, and **4** (each 1 mg) in 0.1 M TFA (1 mL) was heated in a stoppered reaction vial for 45 min. The residue obtained after evaporation of the water layer was treated as described above for **1**. The absolute configuration of the monosaccharide was determined by GLC of the acetylated (+)-2-octyl glycoside using the corresponding authentic samples prepared from D- and L-altrose.

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