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Strevertenes, Antifungal Pentaene Macrolides Produced by *Streptoverticillcum* LL-30F848

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Abstract: This report describes new polyenes isolated from fermentations with *Streptoverticillium* sp. LL-30F848. Structure elucidation using a variety of spectroscopic techniques, including extensive NMR studies, revealed that these pentaene macrolides lacked the otherwise common hemiketal-tetrahydropyran and aminoglycoside moieties, but still carried a carboxylic acid group. The unambiguous assignment of NMR signals attributed to the olefinic region of the pentaenes was possible for the first time, and the relative stereochemistry of the macrolide was established according to ROESY correlations. Strevertene A is the principal pentaene of the antibiotic polyene complex produced.
Keywords: Natural products; polyenes; pentaenes, biologically active compounds; antifungal antibiotics

In our research program to discover new ergosterol biosynthesis inhibitors,[1] culture LL-30F848, taxonomically classified as a *Streptoverticillium* sp. was found to produce strongly inhibitory components, which at elevated concentrations also prevented growth of the test organism, a mutant of *Saccharomyces cerevisiae*. Bioactivity guided isolation and purification yielded a complex of polyenes responsible for the inhibition of ergosterol biosynthesis and strong antifungal activity. The UV chromophores of the constituents suggested that they were a series of related pentaenes. Comparison of molecular weights and spectroscopic properties with those of pentaenes reported in the literature, indicated that the compounds were new. The best characterized pentaenes such as the filipins,[2,3] elizabethin,[4] chainin[5] and fungichromin[6] belong to a group referred to as methylpentaenes, where one end of pentaene chain is substituted with a methyl group, and are readily recognizable by typical UV_{max} at 355, 338 and 322 nm. Regular pentaenes, such as the eurocidins[7] (see Figure 6) have UV_{max} at 350, 332 and 318 nm, whereas the UV spectra of oxopentaenes[8] reveal no fine structure (compare insert of Figure 1), but exhibit broad absorptions between 300 to 400 nm with maxima around 360 nm. Considering that a wide variety of actinomycetes have been reported to produce polyenes,[3] relatively few have been isolated from *Streptoverticillii*. As the present pentaene macrolides are produced by a *Streptoverticillium* sp., they were named strevertenes.

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RESULTS AND DISCUSSION

Isolation. Since biological assays had indicated that the polyenes were largely associated with cell material, the strevertenes were recovered from tank fermentations only by extraction of the pelleted cell cake while the supernatant was discarded. Slurrying the harvested pellet with methanol, filtration, and concentration of the filtrate yielded an extract from which the acidic polyenes were separated by adsorption chromatography on HP-20® or Amberchrom® CG 161dm resins. All absorbed material was recovered from the resin by successive elutions with 30%, 60%, and 100% methanol. The 60% methanol fraction containing the acidic strevertenes was further purified by preparative RP-HPLC yielding individual components of the complex (Compare Figure 1).

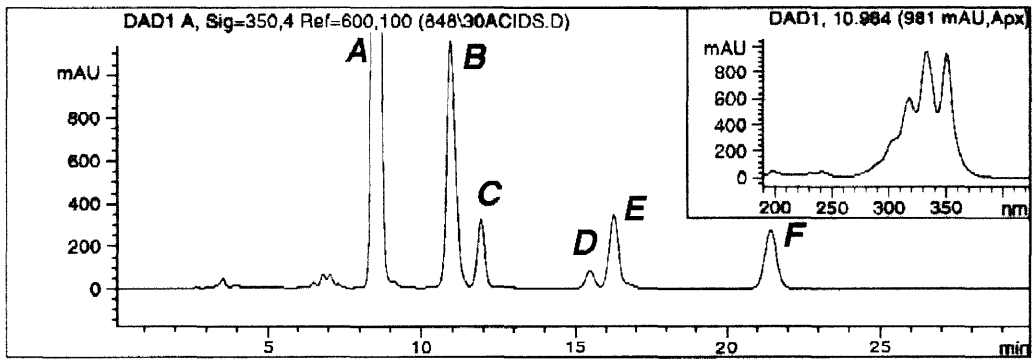

Figure 1. Chromatogram of the acidic strevertene complex using a C_{18} column (YMC ODS-A, 0.46 x 25 cm) and isocratic elution with 65% MeOH

Structure elucidation. The structure of strevertene A (1) was readily determined by spectroscopic analysis. A molecular formula of $C_{31}H_{48}O_{10}$ was calculated from HRFAB mass spectrometry data {$[M+Na]^+$ cal: 603.3145, found: 603.3131}. The number of carbon atoms was also evident from 31 resolved ^{13}C-NMR signals recorded on 65 mM DMSO-d_6 solutions (Table 2). In addition to the expected 10 signals for the olefinic carbon chain, the chemical shifts of the remaining ^{13}C signals indicated the presence of two carboxyl, five methylene, ten methine (of which seven were oxygenated), and three methyl groups. ^{1}H-NMR data (Table 1), in combination with COSY, DEPT, HMQC, HETCOR and D_2O exchange experiments, placed 42 of the 48 hydrogen atoms on carbons, leaving 6 bonded to oxygen atoms.

Connectivities were deduced from COSY and HMBC experiments, in conjunction with HMQC and/or HETCOR data, as shown in Figure 2 Although initially acquired on MeOH-d_4 solutions, only the NMR data of 1 recorded in DMSO allowed an unambiguous assignment. Here, the OH derived proton signals were sharp and provided valuable HMBC correlations that aided in the establishment of the correct sequence of methylene groups and hydroxymethine functions.

The methyl group at C-2 (δ_C 13.9, δ_H 1.04 ppm) showed a two-bond correlation to C-2 at 46.4 ppm and two three-bond HMBC's to the carboxyl group at 173.8 ppm and the hydroxymethine carbon at 71.6 ppm. A COSY correlation between a hydroxyl proton (δ_H 4.94 ppm, ^3J = 3.4 Hz) ascertained the link to the methine proton at 3.63 ppm. This hydroxyl proton (3-OH) displayed important HMBC's to the methine carbon at 46.4 ppm and the methylene carbon at 41.3 ppm, corroborating its location vicinal to the methyl group. Similar observations were made for the 5-hydroxyl proton (δ_H 5.22 ppm, ^3J = ~ 1 Hz) and the 7-hydroxyl proton (δ_H 4.71 ppm, ^3J = 3.3 Hz). The HMBC's from the sharp 5.22 ppm signal (5-OH) to the methylene carbons at 41.3 and 43.8 ppm demonstrated that this unit was the central hydroxymethine in the 1,3-polyol chain followed by the unit bearing the proton at 4.71 ppm which was correlated to the methylene carbons at 43.8 ppm and. 38.5 ppm. The proton signals of this methylene group (δ_C 38.5 ppm) resonated at 1.09 ppm and were linked, as indicated by COSY and HMBC, to two methylene groups (δ_C 22.6; δ_H 0.88, 1.71 ppm; and δ_C 37.1; δ_H 0.89, 1.21 ppm), the latter of which connected to a hydroxymethine group distinguished by the well resolved carbon resonance at 68.2 ppm. From this point, the sequence could be extended to a methylene group (δ_C 40.9, δ_H 1.44, 1.53 ppm) which in turn was bonded to a well distinguished hydroxymethine unit (δ_C 67.2, δ_H 3.38 ppm). The linkage between the latter hydroxymethine and the adjacent methine group (position 14, δ_C 55.9, δ_H 2.50 ppm) was somewhat difficult to establish, as virtually no coupling between the proton signals at 2.50 and 3.38 ppm occurred, but HMBC tied these groups together unambiguously.

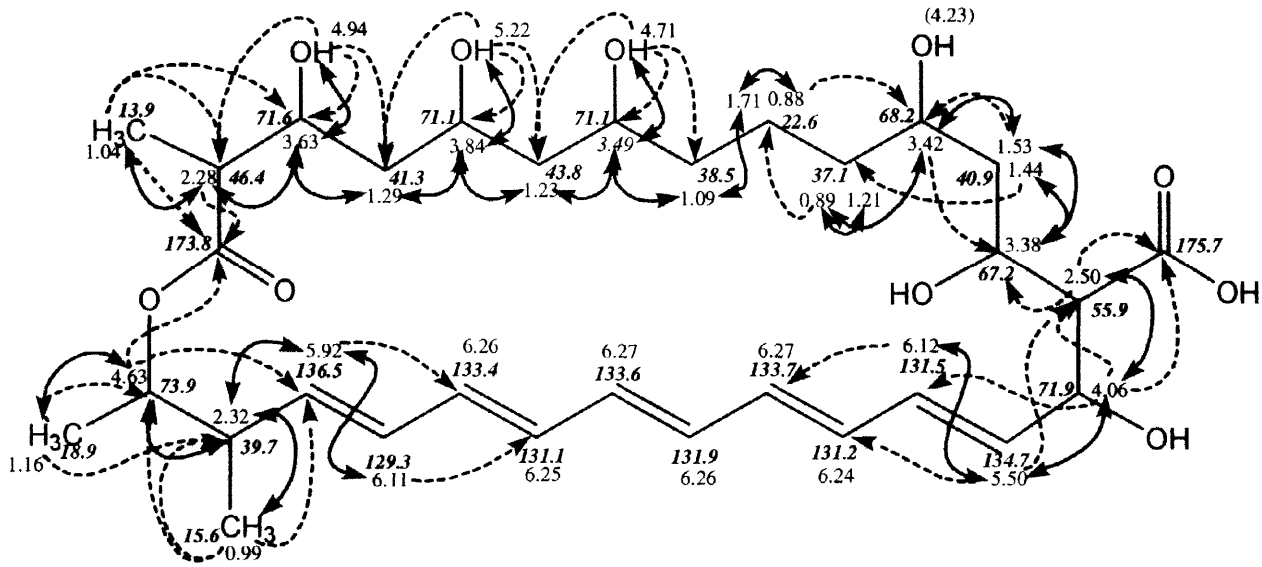

Figure 2. Connectivities in strevertene A as established by HMBC (⟶) and COSY (⟷)

The sharp triplet at 4.06 ppm coupled with proton signals at 2.50 and 5.50 ppm and thus formed the bridge between the carboxyl group bearing methine and the olefinic region of the molecule. As the conjugation and length of a polyene chromophore is indicated from its characteristic UV spectrum, the UV

curve of **1** (see insert in Figure 1) defined its chromophore as a series of five E-conjugated double bonds (unsubstituted pentaene), that formed the backbone of this molecule. The sequential assignment of NMR signals to all 10 C-H units was possible despite extensive signal overlap in the proton spectrum. HETCOR data served as a basis for this assignment, because here the olefinic carbon region was completely resolved and allowed an unambiguous pairing of carbon and hydrogen atoms. The well separated proton signals at both ends of the pentaene region then facilitated the unambiguous assignment of the inner carbon atoms via long range couplings. For example, H-16 (δ_H 5.50 ppm) could be linked to H-17 (δ_H 6.12 ppm{δ_C 131.5 ppm}) by DQF-COSY and to C-18 (δ_C 131.2 ppm) via a strong three-bond HMBC, then C-19 (δ_C 133.7 ppm) could be placed next due to a strong HMBC from H-17. HSQC-TOCSY and HMQC-TOCSY experiments confirmed these assignments and also reached to C-20 (δ_C 131.9 ppm). Similarly, beginning with H-25 (δ_H 5.92 ppm -{C-25, δ_C 136.5 ppm}), the resonances for C-24, C-23, C-22 and C-21 were also assigned. Thus, all olefinic NMR signals were assigned, an accomplishment not previously reported for pentaene macrolides. Continuing the sequential assignments from C-25 to methyl-substituted C-26 (δ_C 39.7 ppm) to C-27 (δ_C 73.9 ppm) was straightforward by using COSY and HMBC. Finally, the closure of the macrocyclic lactone ring was indicated by a three-bond HMBC from H-27 (δ_H 4.63 ppm) to C-1 (δ_C 173.8 ppm). This completed the structure elucidation of strevertene A (**1**), illustrated in Figure 2 above.

The structures of the minor strevertene components (B, C, E and F) were readily elucidated by applying the same NMR techniques as described above for strevertene A (**1**). In principle, the structures can be deduced from the observed ^{1}H- and ^{13}C-NMR chemical shifts by comparison to those of **1** (see Tables 1 and 2). It is noteworthy that the chemical shifts within the polyol and pentaene chains were very consistent for these compounds, but changes in the substitution patterns (e.g. the 2-ethyl group in B, or 27-isopropyl group in E) generally invoked small changes in the chemical shifts for H-14/C-14 and 14a on the opposite side of the molecule.

The electrospray (LC/MS - positive ion detection) mass spectra of strevertene A, B, C, D, E and F revealed a molecular ion in each case, and six consecutive losses of 18 mass units reflecting the progressive elimination of six hydroxyl groups. Further, a weak but significant fragment ion was observed at M/Z 257 for A and B, at M/Z 271 for C and D, and at M/Z 285 for E and F, consistent with their proposed structures, as shown in Figure 3. The negative electrospray mass spectra contained the $[M]^-$ ions of strevertene A, B, C, D, E and F as the principal signal and one additional minor signal which fortuitously was also diagnostic for the C-2 substitution pattern. A M/Z 365 ion was observed for A, C, and E, whereas a signal at M/Z 379 was revealed for B, D, and F.

strevertene	R_1	R_2
A	$-CH_3$	$-CH_3$
B	$-CH_2CH_3$	$-CH_3$
C	$-CH_3$	$-CH_2CH_3$
D	$-CH_2CH_3$	$-CH_2CH_3$
E	$-CH_3$	$-CH(CH_3)_2$
F	$-CH_2CH_3$	$-CH(CH_3)_2$

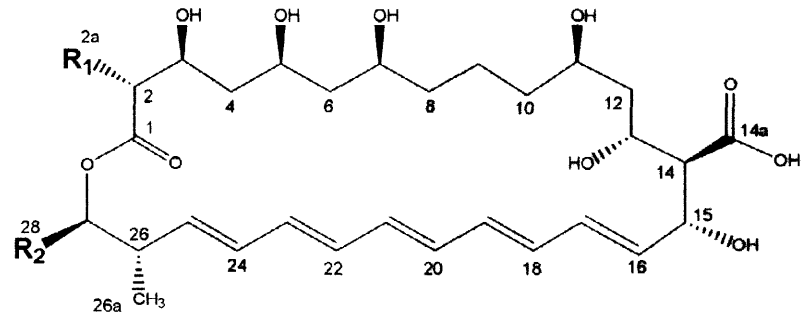

Figure 3. Structures of acidic strevertenes produced by *Streptoverticillium* LL-30F848

Relative configuration. With the determination of the pentaenes' planar structure complete, only the stereochemistry of the strevertenes remained to be elucidated. While their absolute stereochemistry cannot be determined by NMR techniques alone, the relative spatial arrangement of hydrogen atoms can be inferred from NOE measurements using NOESY or ROESY. Since all polyene macrolides have a rigid "paper clip-like" structure (as seen from X-ray studies[9]), ROESY measurements were applied, as demonstrated previously,[10] to assign the relative stereochemistry around the macrocyclic ring. The observed ROESY data, reflecting spatial orientation of protons and the distances between them, were entered in the CHARM computer modeling program as constraints for dynamics simulations and calculations of energy minima considering a series of configurations. The result then permitted a presentation of the relative stereochemistry as illustrated for strevertene E in Figure 4.

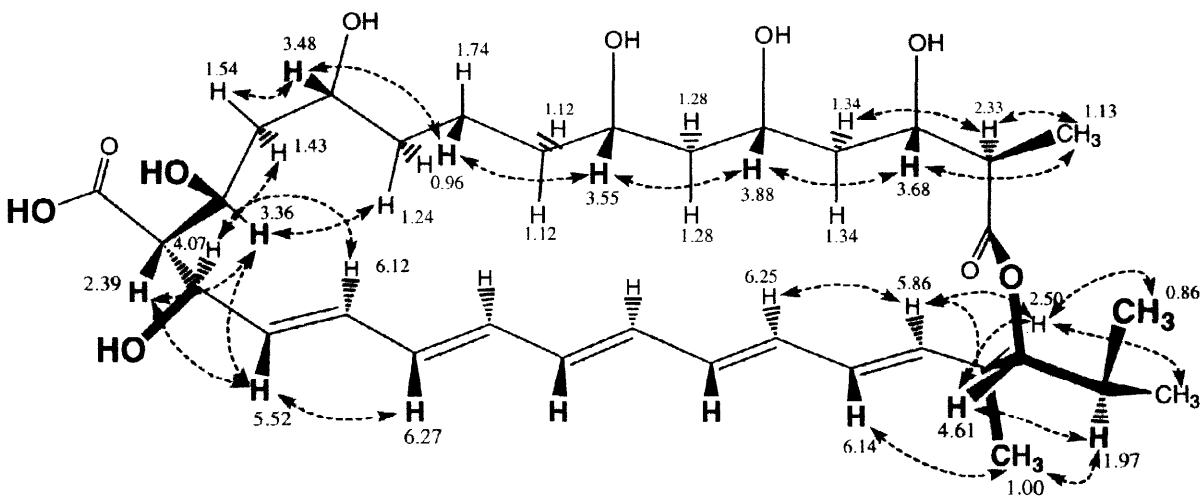

Figure 4. Key ROESY correlations reflect the relative stereochemistry of the pentaene macrolide

The projection shown in Figure 4 corresponds to the absolute configuration of filipin III, which is currently the only pentaene macrolide with established stereochemistry.[11,12] One important aspect of the

assignment was the observed strong spatial interaction of the three hydroxymethine protons at positions 3 (δ_H 3.68 ppm), 5 (δ_H 3.88 ppm) and 7 (δ_H 3.55 ppm) which placed them on the same face of the molecule. This positioning also requires 'syn' configuration of all three hydroxyl groups which, independently, is also suggested from the small coupling constants (<4Hz) of their proton signals.[12] The correlations could also be followed through the entire pentaene region since the olefinic hydrogen atoms have to be placed in a strictly alternating mode. This 'syn' configuration is revealed in the ROESY couplings between H-15 (δ_H 4.07 ppm) and H-17 (δ_H 6.12 ppm) and between H-23 (δ_H 6.25 ppm) and H-25 (δ_H 5.86 ppm). The additional strong spatial coupling of H-25 to H-26 (δ_H 2.50 ppm) indicated their 'cis' configuration which is consistent with the observed ROESY cross peak between H-24 (δ_H 6.14 ppm) and H-26a (δ_H 1.00 ppm). Significantly, ROESY correlations across the ring between H-25 (δ_H 5.86 ppm) and H-2 (δ_H 2.33 ppm) as well as H-4 (δ_H 1.34 ppm) were noted with an intensity slightly stronger than the H-25 to H-28 coupling. Further ROESY correlations exist across the ring between several protons (δ_H 6.27 ppm to 1.12 and 1.28 ppm). Outside the ring, a relatively weak cross-peak between the methyl groups 2a on one side and 28a, 29 on the other, was also noticed. Altogether, the relative stereochemistry of the strevertenes can be presented as shown in Figure 4. Further work is currently being conducted to also resolve the absolute stereochemistry of these compounds.

Characteristics of isolated components. To unambiguously establish the presence of a free carboxyl group, the methyl ester of strevertene A(**1**) was synthesized by reacting with diazomethane in MeOH. The reaction was followed by HPLC using a RAININ Microsorb-MV C_{18} column (5μ, 100Å, 4.6 x 150 mm) eluted with 65% MeOH/ 35% 0.1M NH_4OAc at pH5 and a flow of 1mL/min. Under these conditions, **1** eluted at 7.2 min. whereas the retention time of the methyl ester **1a** was 12.8 min. The NMR data for **1a** were expectedly similar to those of **1** with small changes around position 14 indicative of the location of the methyl ester. The presence of a methoxyl group was inferred by new signals in the ^{1}H- and ^{13}C-NMR spectrum at 3.57 ppm and 50.9 ppm respectively, which showed a three-bond HMBC to the ^{13}C-resonance at 172.6 ppm. Also, all of the hydroxyl proton signals of **1a** were detected in the ^{1}H-NMR spectrum away from the residual water peak and assigned their respective positions through COSY and HMBC (see Table 1).

In an attempt to produce additional methyl ester (**1a**) by exposing **1** to acidic methanol (1% H_2SO_4 in MeOH) at room temperature, we found that a new compound was generated. After 30 min, HPLC analysis revealed that a more hydrophobic compound was produced, also extractable into ethyl acetate, but its retention time was different from that of the methyl ester. NMR analysis of the product suggested that it did not contain a methyl ester. A close inspection of the NMR data indicated convincingly that the carboxyl

Table 1. Assigned ^{1}H-NMR shift values of selected strevertenes dissolved in DMSO-d_6

H	A (1)	B	E	1a	1b
2	2.28 *(qd, 7.1, 7.3)*	2.17 *(m)*	2.33 *(qd, 6.9)*	2.28 *(qd, 7.1, 7.3)*	2.24 *(qd, 7.0)*
3	3.63 *(m)*	3.64 *(m)*	3.68 *(m)*	3.64 *(m)*	3.90 *(m)*
4	1.29 *(m)* 2H	1.31*(m)* 2H	1.34 *(m)* 2H	1.30 *(m)* 2H	1.39 *(m)* 2H
5	3.84 *(m)*	3.86 *(m)*	3.88 *(m)*	3.84 *(m)*	3.71 *(m)*
6	1.23 *(m)* 2H	1.29 *(m)* 2H	1.28 *(m)* 2H	1.24 *(m)* 2H	1.30 *(m)* 2H
7	3.49 *(m)*	3.54 *(m)*	3.55 *(m)*	3.51 *(m)*	3.43 *(m)*
8	1.09 *(m)* 2H	1.09 *(m)* 2H	1.12 *(m)* 2H	1.09 *(m)* 2H	1.14 *(m)* 2H
9	1.71 *(m)*, 0.88 *(m)*	1.76 *(m)*, 0.93 *(m)*	1.74 *(m)*, 0.96 *(m)*	1.71 *(m)*, 0.93 *(m)*	1.47 *(m)*, 0.93 *(m)*
10	0.89 *(m)*, 1.21 *(m)*	0.93 *(m)*, 1.22 *(m)*	0.96 *(m)*, 1.24 *(m)*	0.92 *(m)*, 1.21 *(m)*	1.34 *(m)*, 1.48 *(m)*
11	3.42 *(m)*	3.45 *(m)*	3.48 *(m)*	3.42 *(m)*	4.59 *(m)*
12	1.44 *(m)*, 1.53 *(m)*	1.42 *(m)*, 1.57 *(m)*	1.43 *(m)*, 1.54 *(m)*	1.49 *(m)*	1.70 *(m)*, 1.82 *(m)*
13	3.38 *(m)*	3.36 *(m)*	3.36 *(m)*	3.38 *(m)*	4.30 *(m)*
14	2.50 *(dd, 9.5)*	2.47 *(dd, 9.5)*	2.39 *(dd)*	2.62 *(dd, 10.2, 4.2)*	2.76 *(d)*
15	4.06 *(t, 9.5)*	4.06 *(t, 9.5)*	4.07 *(t, 9.5)*	4.09 *(dd, 10.2, 9.0)*	4.56 *(dd, 9.5, 9.0)*
16	5.50 *(dd, 15.3, 9.5)*	5.50 *(dd, 15.3, 9.5)*	5.52 *(dd, 15.3, 9.0)*	5.50 *(dd, 14.8, 9.0)*	5.55 *(dd, 15.3, 9.0)*
17	6.12 *(dd, 15.3)*	6.12 *(dd, 15.3)*	6.12 *(dd, 15.3)*	6.15 *(dd, 14.8)*	5.94 *(dd, 15.3)*
18	6.24	6.26	6.27	6.24	6.23 *(dd)*
19-22	6.26 - 6.27	6.26 - 6.27	6.26 - 6.27	6.26 - 6.27	6.26 - 6.27
23	6.25	6.25	6.25	6.26	6.25 *(dd)*
24	6.11 *(dd, 15.6)*	6.13 *(dd, 15.6)*	6.14 *(dd, 15.6)*	6.11 *(dd, 15.4, 8.6)*	6.08 *(dd, 15.6)*
25	5.92 *(dd, 15.6, 6.6)*	5.96 *(dd, 15.6, 6.6)*	5.86 *(dd, 15.6, 6.6)*	5.93 *(dd, 15.4, 6.4)*	5.89 *(dd, 15.6, 6.6)*
26	2.32 *(qdd, 9.0, 6.9, 6.6)*	2.36 *(qdd, 9.0, 6.9, 6.6)*	2.50 *(qdd, 9.2, 6.8, 6.6)*	2.34 *(qdd, 9.0, 6.9, 6.6)*	2.34 *(qdd, 9.0, 6.9, 6.6)*
27	4.63 *(dd, 9.0, 6.3)*	4.66 *(dd, 9.0, 6.3)*	4.61 *(dd, 9.2, 3.0)*	4.64 *(dd, 9.0, 6.3)*	4.76 *(dd, 9.0, 6.3)*
28	1.16 *(d, 6.3)*	1.19 *(d, 6.3)*	1.97 *(qd, 6.9, 3.0)*	1.17 *(d, 6.3)*	1.13 *(d, 6.3)*
29	-	-	0.82 *(d, 6.9)*	-	-
2a	1.04 *(d, 7.1)*	1.46 *(m)*, 1.73 *(m)*	1.13 *(d, 6.9)*	1.05 *(d, 7.1)*	0.93 *(d, 6.9)*
26a	0.99 *(d, 6.9)*	1.00 *(d, 6.9)*	1.00 *(d, 6.8)*	1.00 *(d, 6.9)*	0.93 *(d, 6.9)*
28a	-	-	0.86 *(d, 6.9)*	-	-
2b	-	0.80 *(t, 7.3)*	-	-	-
3a (OH)	4.96 *(d, 3.4)*	5.06 *(d, 3.4)*	4.96 *(d)*	4.95 *(d, 3.1)*	n.d.
5a (OH)	5.22 *(d, ~ 1)*	5.29*(s)*	5.23 *(s)*	5.22 *(d, ~ 1)*	n.d.
7a (OH)	4.71 *(d, 3.3)*	4.77 *(d, 3.2)*	4.73 *(d)*	4.71 *(d)*	n.d.
11a (OH)	4.23 *(s, br)*	4.21 *(d)*	n.d.	4.21 *(d, 4.7)*	n.d.
13a (OH)	n.d.	n.d.	n.d.	4.73 *(d)*	n.d.
15a (OH)	n.d.	n.d.	n.d.	5.12 *(d, 4.8)*	n.d.
14c (OCH_3)				3.57 *(s)*	

coupling constants {Hz}, n.d. = not determined

Table 2. Observed ^{13}C-NMR shift values of selected strevertenes dissolved in DMSO-d_6

assignment	mult (DEPT)	A(1)	B	C	E	1a	1b
1	s	173.8	173.2	174.2	174.3	173.7	175.2
2	d	46.4	54.4	46.3	46.2	46.4	46.4
3	d	71.6	71.0	71.3	71.34	71.5	72.5
4	t	41.3	41.5	41.4	41.5	41.3	42.4
5	d	71.1	71.4	71.2	71.27	71.0	72.3
6	t	43.8	43.7	43.8	43.9	43.8	44.5
7	d	71.1	71.3	71.1	71.18	71.0	72.2
8	t	38.5	38.5	38.5	38.6	38.5	38.5
9	t	22.6	22.8	22.6	22.5	22.6	20.3
10	t	37.1	37.1	37.1	37.2	37.0	35.1
11	d	68.2	68.2	68.2	68.1	68.0	76.9
12	t	40.9	40.5	40.8	40.7	40.2	32.6
13	d	67.2	67.1	67.2	67.2	66.9	61.8
14	d	55.9	57.8	56.2	56.8	58.4	56.5
15	d	71.9	71.8	71.8	71.7	71.4	72.8
16	d	134.7	134.4	134.7	134.7	134.0	134.1
17	d	131.5	131.8	131.7	131.7	132.1	131.48
18	d	131.2	131.2	131.2	131.2	130.9	132.4
19	d	133.7	133.8	133.6	133.5	133.9	134.3
20	d	131.9	131.84	131.9	131.9	131.7	133.2
21	d	133.6	133.7	133.6	133.4	133.6	134.3
22	d	131.1	131.0	131.0	131.0	131.1	132.6
23	d	133.4	133.5	133.4	133.3	133.4	134.3
24	d	129.3	129.2	129.4	129.5	129.3	131.5
25	d	136.5	136.7	136.6	136.6	136.6	136.1
26	d	39.7	39.4	37.9	35.8	39.7	41.0
27	d	73.9	74.4	78.0	80.2	73.8	74.2
28	q	18.9	19.1	(t) 25.3	(d) 28.7	18.9	19.7
29	q			9.3	19.8		
2a	q	13.9	(t) 22.4	14.3	14.5	13.9	20.3
2b	q		11.4				
14a	s	175.7	174.2	175.4	175.3	172.6	172.7
26a	q	15.6	15.6	15.7	15.4	15.7	17.1
28a	q				15.1		
14c (OMe)						50.9	

group had undergone preferential reaction with one of the endogenous hydroxyl groups to form a lactone instead of the methyl ester. Both the ^{1}H- and ^{13}C-NMR signals of the product had considerably shifted by comparison to those of starting compound 1. Especially indicative of the structural changes were the shifts for two of the hydroxyl group-bearing carbons from 68.2 and 67.2 ppm to 76.9 and 61.8 ppm, respectively. The carbon signal at 76.9 ppm linked to the proton at 4.59 ppm by HMQC was assigned to position 11

due to the coupling with the proton at 1.82 ppm and ROESY correlations to the protons at 1.34, 1.47 and 2.76 ppm. The latter also revealed strong spatial couplings to H-15 (δ_H 4.56 ppm) and H-13 (δ_H 4.30 ppm) Significantly, strong spatial couplings were also observed between H_a-12 (δ_H 1.82 ppm), H-13 (δ_H 4.30 ppm) and H-16 (δ_H 5.55 ppm) denoting the close distance between them. Chemical shifts as well as observed couplings are evidence for the δ-lactone structure. Our models of the macrolide only allowed for the 11-hydroxyl group to undergo lactonization with the 14-carboxyl group since both were spatially close and on the same face of the molecule. However, formation of lactone **1b** is still somewhat surprising as the rigidity of molecule **1** does not readily allow a contact of these groups unless the 12-methylene group swings out of the plane and facilitates the ring closure.

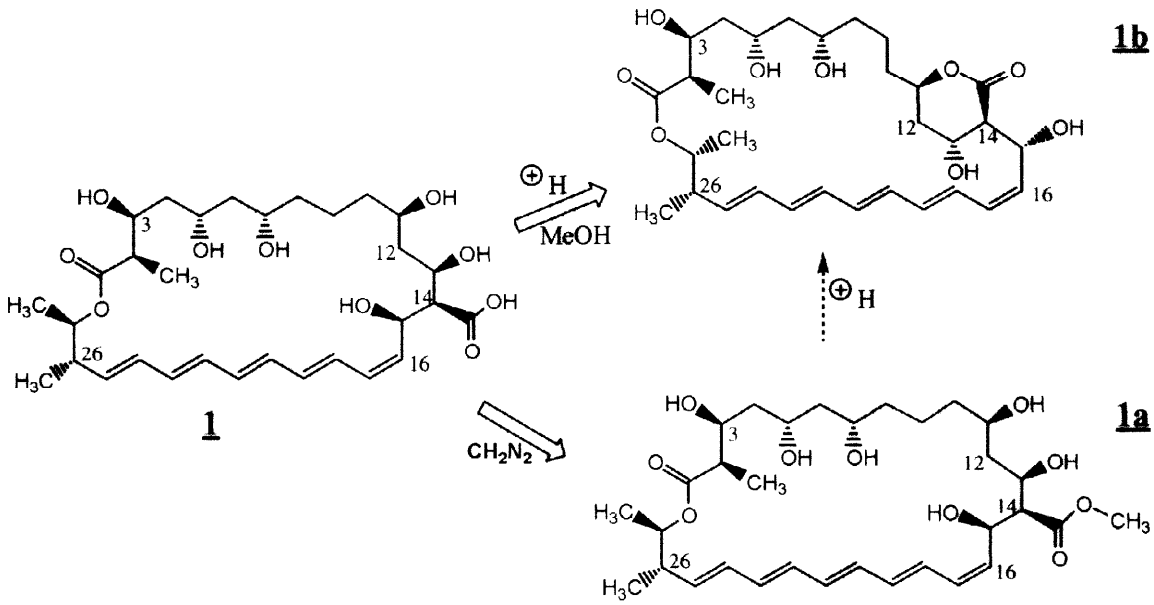

Figure 5. Reactions of strevertene A involving the carboxyl group

Additional evidence for the formation of the lactone was derived from treating **1** with acid (HCl) in a mixture of acetonitrile/water which afforded the same product **1b**, although more by-products were generated under these conditions. In solution, this compound (**1b**) also seems to degrade faster than **1**, perhaps due to the additional strain on the molecule.

Biological Activities. The major compound, strevertene A, as well as the complex, were determined to inhibit the growth of phytopathogenic fungi such as *Venturia inaequalis, Pyrenophora teres, Botrytis cinerea, Uromyces fabae, Cercospora beticola, Plasmopara viticola, Puccinia recondita f.sp. tritici* and *Erysiphe graminis f.sp. tritici* with MIC's between 5-25 μg/mL. The strevertenes are also active on a variety of yeasts.

DISCUSSION

Over the years, a relatively large number of polyenes have been reported as metabolic products of actinomycetes[3] as these compounds are easily recognized by their characteristic UV spectra. Remarkably however, the structures of only a few are actually known. Aside from the oxopolyenes,[8] defined structures of some two dozen, naturally occurring polyenes have been reported. Among them are the clinically important drugs amphotericin B and nystatin A. This class of compounds is known for its antifungal activity and has also been indicated to inhibit ergosterol biosynthesis.[13] A side by side comparison revealed that ergosterol biosynthesis inhibition by filipin III, the strevertenes, or pimaricin was about equal, but they were a magnitude more potent than nystatin, in contrast to a previous report.[14] Although the strevertenes represent a new family of pentaenes which do inhibit ergosterol biosynthesis, the well-known physicochemical liabilities of this class precluded further development.

The recently patented acidic pentaene macrolides Mer-K1093[15] and MA-2664[16] (also produced by a *Streptoverticillium* sp.) represent regioisomeres of the strevertenes. The neutral polyenes AB023a and b[17] are closely related, since they bear a methyl instead of a carboxyl group. Rectilavendomycin[18] may also belong to this group, although its structure was not established entirely.

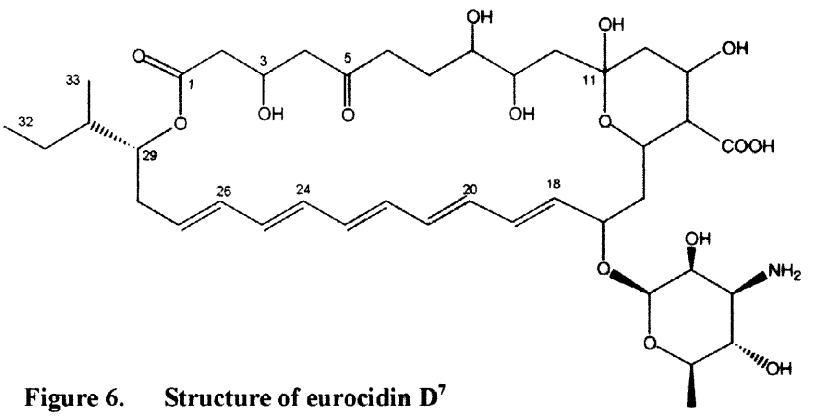

Figure 6. Structure of eurocidin D[7]

Other recently reported polyenes produced by *Streptoverticillii* include the methylpentaene fungichromin[19] and the eurocidins[7] (Figure 6) which are considered regular pentaene macrolides. A mutated *Streptoverticillium eurocidium* has also been reported to produce the regular tetraene macrolide YS-822A.[20]

EXPERIMENTAL

General. A Hewlett-Packard 1100 or 1090 M LC system with diode array detection (monitored at 350 nm) employing a variety of columns was used for the analysis of fractions or to check the purity of isolated components. (Columns: RAININ Microsorb C_{18}, YMC ODS-A (C_{18}) reverse phase column (4.6x250 mm), eluted isocratically with 0.2M TFA/MeOH/water in a ratio of 10:65:25. In some instances a system of 65% MeOH and 35% 0.1M NH_4OAc buffer at pH 5 was used instead. (monitored at 300 nm).
Preparative HPLC separations were accomplished on a MODCol™ C18 column (100 Å Kromasil C18, 10m, 2.54 x 25 cm) using an isocratic system of 60% MeOH/water with the effluent being monitored at 350 nm by a variable wavelength detector (LDC). HP-20 or Amberchrom CG 161m chromatography was performed on self-packed open columns (21 x 300 mm) by step gradient elution using the indicated solvent mixtures. Fractions from all columns were generally collected by hand and pooled according to peaks observed on HPLC analysis.
NMR spectra were obtained on Bruker AMX 300, 400 or 500 MHz NMR instruments employing standard pulse sequences. Chemical shifts of ^1H and ^{13}C NMR signals were determined in ppm relative to the solvent signals of residual DMSO at δ_H 2.49 ppm and δ_C 39.50 ppm.

UV spectra were recorded using a Hewlett-Packard Model 8450A spectrometer, or obtained "on the fly" during HPLC analysis on HP 1100 or 1090M instruments equipped with a diode array detector.
IR spectra were obtained with a Nicolet 20AXB FT-IR spectrometer.
FAB mass spectra were recorded using a VG-ZAB SE high performance mass spectrometer and a VG 11-250 data system. LC-ESI mass spectra were obtained on a Finnigan LCQ mass spectrometer.

Isolation and purification of strevertenes A - F from large scale fermentations

Whole mash (567 L.) was mixed with 7 L (1.25%) toluene for 20 min and subsequently filtered using a Millipore Ceraflo® (Ceramic Microfilter) system. The collected retentate was washed with 200 L of water before it was reslurried in 200 L MeOH for 1/2 hour. The broth filtrate and wash were discarded. Renewed filtration of the reslurried mix on the Ceraflo® system provided 200 L methanolic permeate. The solid retentate was discarded.

The permeate was concentrated to ca. 60 L volume and diluted to 25% MeOH with distilled water. On diluting the solution became turbid and the colloid solution was again filtrated using the Ceraflo® system. The obtained, emulsion-like retentate (~6 L) was slurried with 4 kg washed HP-20 resin for 1 hour and the resin was then collected into an open glass column II (12 x 150 cm). Its clear effluent and the 10 L of 30% MeOH wash ware added to the previously filtered permeate. The filtered permeate was passed onto a 60 L HP-20 column I followed by a wash with 180 L of 30% MeOH. Effluent and wash were subsequently discarded, as polyenes were not present.

Both HP-20 column were eluted with 60% MeOH to obtain the acid strevertenes followed by a 100% MeOH wash to obtain neutral polyenes and other material. Column II was eluted with 10 L of 60% MeOH (IIA) followed by a washing with 100% MeOH (IIB) until the resin appeared white. Column I was eluted with 300 L of 60% MeOH [IA] and other material was then desorbed from the resin with 120 L of 100% MeOH [IB].

To concentrate, the 60% MeOH eluates (IA + IIA) were pooled and diluted with the same amount of water and loaded onto a 30 L HP-20 column. The absorbed polyenes were then stripped from the column with 60 L MeOH (100%) [Fraction IIIA] and concentrated to ca 5 L.

The concentrate of fraction IIIA diluted to 25% MeOH with DI water and loaded onto a Kiloprep® (Millipore 12-L cartridge with 55-105 μm Bondapak C_{18}) column. The column was eluted with an aqueous methanol step gradient of 30, 60 and 100% MeOH. Fractions were taken at regular intervals and cuts containing similar ratios of the same compounds were pooled and concentrated until an emulsion formed.

Fractions 1-7 were discarded as they contained mostly impurities. The major polyene, essentially pure strevertene A (ca. 3.9g), was found in fractions 7 and 8. Fractions 12-17 (780 mg) containing only strevertene A and B were pooled and concentrated to 4L in water as were fractions 18-24 and fractions 25-28. Each of the above pools was passed over an Amberchrome XAD column (10 x 5 cm) loaded in water and eluted with 100% MeOH. Reverse phase preparative chromatography of the last pooled fractions (25-28) afforded samples of the minor strevertenes B (105 mg), E (36 mg) and F (27 mg).

Preparation of strevertene A -methylester (1a) Strevertene A (**1**) [70 mg] was dissolved in 15 mL methanol and diazomethane, previously prepared as an ethereal solution, was then added in portions to generate the ester **1a**. The reaction was followed by HPLC analysis showing that the peak at 8.4 min. was turning over to a new peak at 9.4 min. When the reaction was ca. 85% complete, the bulk of the solvent was evaporated under reduced pressure. The remaining solution (ca. 3 mL) was poured into 30 mL of EtOAc and

extracted twice with 10 mL water. Ester **1a** remained in the EtOAc phase (93% pure by HPLC), whereas unreacted **1** was found almost quantitatively in the aqueous phase. The crude ester **1a** [53.6 mg] was used for spectroscopy without further chromatographic purification.

Preparation of strevertene A -lactone (1b) Dry strevertene A (**1**) [107 mg] was dissolved in 5 mL methanol and then added to a 10 mL methanol solution containing 1.5% H_2SO_4. The reaction mixture was then let stand for approximately 30 min. when HPLC analysis revealed that the peak for **1** at 8.4 min had disappeared and a major peak at 12.0 min was produced instead. The chromophore of this peak was unchanged. The reaction mixture was concentrated to approximately 10 mL after 280 mg solid Na_2CO_3 had been added. This was then poured into ethyl acetate (20 mL) and the solution was transferred into an extraction funnel. Shaking with 25 mL of a 0.1 M $NaHCO_3$ solution and two water washes provided a neutral reacting ethyl acetate phase. The solvent was evaporated, the residue was then dissolved and dried from MeOH twice and finally re-dissolved in 40 mL t-BuOH, frozen and lyophilized. The dry product [24.2 mg] was found to be the lactone **1b**.

Physico-chemical Properties:

4,6,8,12,14,16-hexahydroxy-15-carboxyl-3,27,28-trimethyloxacylooctacosa-17,19,21,23,25-pentaen-2-one) or **strevertene A**. $C_{31}H_{48}O_{10}$ = 580.71
$[\alpha]$ ^{25}D= -37 ± 2(c = 0.6 %); UV (MeOH) λ_{max}[nm] (ε): 233 (4,950), 240 (4,970), 303 (20,000), 317 (36,800), 332 (64,300), 350 (62,400); IR (KBr) 3409(br), 2976, 1725, 1632, 1586, 1452, 1403, 1380, 1325, 1194, 1109, 1008; ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); MS(pES) = M/Z 581 (100%), 563 (90%), 545 (15%), 527 (20%), 509 (25%), 491 (8%), 491 (4%), 473 (1%), 257 (2%); MS(nES) $[M-H]^-$ = M/Z 579; HRFABMS $[M+Na]^+$=M/Z 603.3131, calcd. for $C_{31}H_{48}O_{10}Na$ = M/Z 603.3145, (Δ = - 1.4 mmu)

4,6,8,12,14,16-hexahydroxy-3-ethyl-15-carboxyl-27,28-dimethyloxacyclooctacosa-17,19,21,23,25-pentaen-2-one or **strevertene B**: $C_{32}H_{50}O_{10}$ = 594.73
UV (MeOH) λ_{max}[nm] (ε): 233 (4,950), 240 (4,970), 303 (20,000), 317 (36,800), 332 (64,300), 350 (62,400); ^{1}H-NMR (see Table 1); ^{13}C NMR (see Table 2); MS(pES) = M/Z 595 (100%), 577 (85%), 559 (13%), 541 (15%), 523 (25%), 405 (8%), 505 (3%), 257 (2%); MS(nES) $[M-H]^-$ = M/Z 593

4,6,8,12,14,16-hexahydroxy-15-carboxyl-3,27-dimethyl-28-ethyloxacyclooctacosa-17,19,21,23,25-pentaen-2-one or **strevertene C**: $C_{32}H_{50}O_{10}$ = 594.73
UV (MeOH) λ_{max}[nm] (ε): 233 (4,950), 240 (4,970), 303 (20,000), 317(36,800), 332 (64,300), 350 (62,400); ^{13}C-NMR (see Table 2), MS(pES) = M/Z 595 (100%), 577 (55%), 559 (10%), 541 (15%), 523 (18%), 405 (8%), 505 (1%), 257 (2%); MS(nES) $[M-H]^-$ = M/Z 593;

4,6,8,12,14,16-hexahydroxy-15-carboxyl-27-methyl-3,28-diethyloxacyclooctacosa-17,19,21,23,25-pentaen-2-one or **strevertene D**: $C_{33}H_{52}O_{10}$ = 608.76
UV (MeOH) λ_{max}[nm] (ε): 233 (4,950), 240 (4,970), 303 (20,000), 317(36,800), 332 (64,300), 350 (62,400); MS(pES) = M/Z 609 (100%), 591 (45%), 573 (8%), 555 (10%), 537 (10%), 519 (1%), 271 (0.2%); MS(nES) $[M-H]^-$ = M/Z 607;

4,6,8,12,14,16-hexahydroxy-15-carboxyl-3,27-dimethyl-28-isopropyloxacyclooctacosa-17,19,21,23,25-pentaen-2-one or **strevertene E**: $C_{33}H_{52}O_{10}$ = 608.76
UV (MeOH) λ_{max}[nm] (ε): 233 (4,950), 240 (4,970), 303 (20,000), 317(36,800), 332 (64,300), 350 (62,400); ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); MS(pES) = M/Z 609 (100%), 591 (70%), 573 (10%), 555 (8%), 537 (15%), 519 (1%), 285 (1%); MS(nESpray) $[M-H]^-$ = M/Z 607;

4,6,8,12,14,16-hexahydroxy-3-ethyl-15-carboxyl-27-methyl-28-isopropyloxacyclooctacosa-17,19,21,23,25-pentaen-2-one or **strevertene F**: $C_{34}H_{54}O_{10}$ = 622.79
UV (MeOH) λ_{max}[nm] (ε): 233 (4,950), 240 (4,970), 303 (20,000), 317(36,800), 332 (64,300), 350 (62,400); MS(pES) = M/Z 623 (100%), 605 (65%), 587 (10%), 569 (12%), 551 (15%), 533 (2%), 285 (1%); MS(nES) $[M-H]^-$ = M/Z 621;

*4,6,8,12,14,16-hexahydroxy-15-metboxycarbonyl-3,27,28-trimethyloxacyclooctacosa-17,19,21,23,25-pentaen-2-one (**1a**)* or **strevertene A - methylester**: $C_{32}H_{50}O_{10}$ = 594.73
UV (MeOH) λ_{max}[nm] (ε): 233 (4,950), 240 (4,970), 303 (20,000), 317(36,800), 332 (64,300), 350 (62,400); ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); MS(pES) = M/Z 595 (100%), 577 (90%), 559 (15%), 541 (25%), 523 (35%), 505 (10%), 491 (2%), 487 (1%)

*1,17,19,21,26-pentahydroxy-12,13,16-trimethyl-14,28-dioxabicylo[24.2.2]triaconta-2,4,6,8,10-pentaen-15,29-dione (**1b**)* or **strevertene A - lactone**: $C_{31}H_{46}O_9$ = 562.69
UV (MeOH) λ_{max}[nm] (ε): 234 (4,980), 242 (5,000), 303 (20,000), 318(36,800), 333 (64,300), 352 (62,400); ^{1}H NMR (see Table 1); ^{13}C NMR (see Table 2); MS(pES) = M/Z 563 (60%), 545 (100%), 527 (20%), 509 (10%), 491 (4%), 473 (2%)

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