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Structure Elucidation of Sch 20561, a Cyclic Dehydropeptide Lactone — a Major Component of W-10 Antifungal Antibiotic

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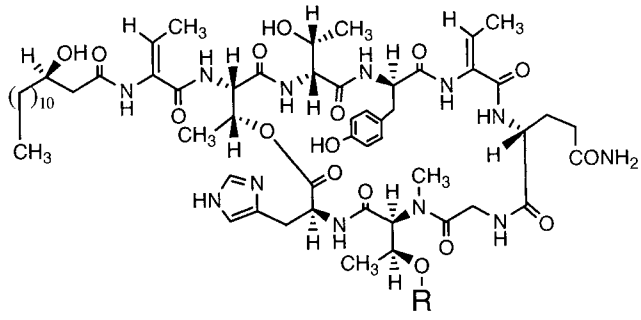
Antibiotic W-10 is a fermentation complex produced by the bacterium *Aeromonas* sp. W-10. The cyclic dehydropeptide lactones Sch 20562 (**1**) and Sch 20561 (**2**) are the major components of this fermentation complex and are of biological interest in view of their unique structural features and potent antifungal activity. The chemical degradation studies that were utilized in the assignment of structure **2** for Sch 20561 are described here. The structure determination of **2** made use of the ozonolytic cleavage of the dehydropeptide units to form fragments that were sequenced by mass spectrometry. The cyclic dehydropeptide lactone Sch 20561 (**2**) was found to be the aglycone of Sch 20562 (**1**) and these two natural products were correlated by a chemical transformation involving the deglucosidation of **1** to form **2**.

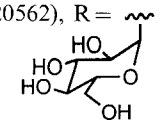
Sch 20561 and Sch 20562 are the major components of antibiotic W-10 complex, a fermentation product that is produced by a novel Gram-negative bacterium designated as *Aeromonas* sp. W-10 NRRL B-11053. The compounds are of biological interest in view of their potent antifungal activity against yeasts and dermatophytes.[1] In the preceding paper, we have described the chemical degradation studies that led to the assignment of the structure and stereochemistry for Sch 20562 as the α-glucosidic cyclic dehydrononapeptide lactone **1**.[2] We report here the chemical degradation studies used in the elucidation of the structure and stereochemistry for Sch 20561 as the cyclic dehydropeptide lactone **2** which is the aglycone of the glucoside **1** (Figure 1).

Results and Discussion

The physical data and preliminary hydrolytic experiments (Scheme 1), indicated that Sch 20561 (**2**) was a macrocyclic dehydropeptide lactone closely related to Sch 20562 (**1**).[2] The common features in the two compounds were evident in their aminoacid composition, a lactonic structure that undergoes a facile methanolysis to form a methyl ester **3**, an *N*-terminal D-β-hydroxymyristic acid (Hma) residue, and two α-aminocrotonic acid (Aca) units **5**. The presence of Hma was established by the identification of **4** in the ozonolysis of **3**; the presence of Aca units was established from the ^{1}H NMR signals at δ 1.80, 5.82 and from the high ε value of the

Fig. 1.



1 (Sch 20562), R = 

2 (Sch 20561), R = H

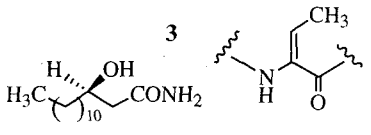
Scheme 1.

Molecular formula: $C_{57}H_{86}O_{16}N_{12}$
MW: 1195
p*K*a: 6.1
Ninhydrin: negative

2 $\xrightarrow{6\,N\,HCl}$ His (1), Thr (2), Glu (1), Gly (1), Tyr (1), NH_3 (3)

$\lambda_{max}^{MeOH/OH^-}$ 240 mμ (ε 25300), 292 mμ (ε 2600)
IR: 1750 cm^{-1} ^1H NMR: δ 1.80 (2 × CH_3)
^{13}C NMR: no δ 70~93 resonances

2 $\xrightarrow{MeOH/ET_3N}$ Methyl ester

3 $\xrightarrow{O_3}$ 

4 (Hma) **5**

Abbreviations: Aca = α-aminocrotonic acid; Asp = aspartic acid; Asn = asparagine; Gln = glutamine; Glu = glutamic acid; Gly = glycine; His = histidine; Hma = β-hydroxymyristic acid; Thr = threonine; Tyr = tyrosine.

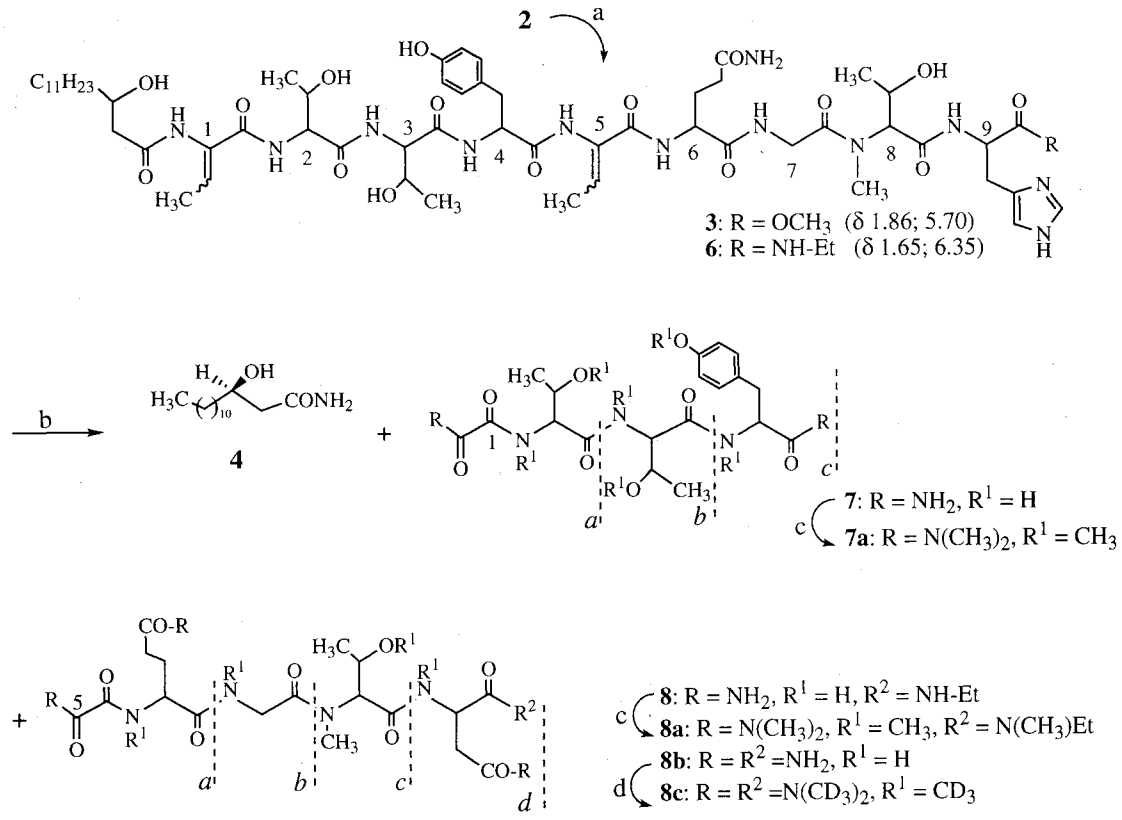
absorbance of the UV peak at 240 mμ.[2] The major difference between the two compounds was evident in the ^{13}C NMR spectrum of **2** which indicated the absence of a glucose unit in the molecule. The approach used for the structure elucidation of **2** paralleled that described for **1** and is based on the ozonolytic methodology that was developed for the selective cleavage of dehydropeptides.[2]

The preliminary ozonolysis of the acyclic methyl ester **3** also provided at the onset of this study, the known tripeptide **7**,[2] and the tetrapeptide **8b**. Perdeuteriomethylation was utilized to sequence **8b** so as to differentiate the methyl group of *N*-Me-Thr in **8c**, from the methyl groups introduced by the alkylation step. The cyclic lactone Sch 20561 (**2**) was aminolysed with ethylamine to afford the acyclic dehydropeptide amide **6** wherein the ethylamide functionality was introduced as a tag for the lactonic carbonyl in **2** (Scheme 2). This aminolysis was found to proceed with a concomitant isomerization of the dehydropeptide units as seen in the ^1H NMR olefinic resonances of **6**; minor side-products resulting from conjugate addition of $EtNH_2$ to the dehydropeptide units were also identified, but were not characterized. The acyclic ethylamide **6** was utilized in the chemical degradations aimed at obtaining the full amino acid sequence of **2**. Thus, ozonolysis of **6** followed by reductive work-up with Me_2S and ammonolysis of the intermediate oxalimides afforded the fragments D-Hma-NH_2 (**4**), and the tripeptide NH_2-oxalyl-L-Thr-D-*allo*-Thr-D-Tyr-NH_2 (**7**) that were characterized previously,[2] and the tetrapeptide fragment **8**. The sequence of fragment **7** was confirmed from the EI mass spectrum fragmentation pattern of its permethylation[3] product **7a**.

Amino acid analysis of **8** showed the composition Asp, Glu, Gly. The Asp detected in the amino acid composition was a new residue that was not originally present in **2** or **6**, and it is generated[2] by the ozonolysis of the *C*-terminal His unit in **6**. Additionally, the ^1H NMR spectrum of **8** showed the presence of an *N*-methyl resonance at δ 2.95 that was ascribed to an *N*-Me-Thr unit. The tetrapeptide **8** contained an *N*-terminal oxalamide group and a *C*-terminal Asn-NH-Et residue. Permethylation of **8** afforded **8a** that had the fragment ions *a*~*d* in the EI mass spectrum in agreement with the amino acid sequence NH_2-oxalyl-Gln-Gly-*N*-Me-Thr-Asn-NH-Et for the tetrapeptide **8**.[4] As expected for the ozonolytic cleavage of a dehydropeptide,[2] the *N*-terminal oxalamide group of **7** and the nitrogen of the amide group in **4** are derived from one of the Aca units linking these two fragments, and the same functionalities in **8** and **7** are derived from the other Aca units linking these latter two fragments. The full sequence for the acyclic dehydrononapeptide could then be reconstructed by replacing the said functionalities with two Aca units to link the three fragments **4**, **7**, **8**, and also by replacing the Asp unit with a His unit; on this basis, structure **6** was assigned to the full sequence in the acyclic dehydrononapeptide precursor of these ozonolytic fragments, which was also the aminolysis product of the lactone **2**.

The ethylamino group in **6** identified the terminal His-9 as the residue forming the lactonic carbonyl in **2**. The hydroxyl group of the residue involved in this lactone functionality was then identified as outlined in Scheme 3. The free hydroxyl groups in compound **2** were protected as the tetrahydropyranyloxy derivatives and the resulting per-THP-**2** was ozonized followed by reductive work-up and methanolysis. The methanolysis step cleaved the intermediate oxalimide formed in the ozonolysis of the Aca units and also liberated the hydroxyl group involved in the lactone bond of **2**, to afford the fragments **4a** and **9** that were characterized previously.[2] The sequence in the tripeptide **9** was

Scheme 2.



Reagents: (a) $EtNH_2$/DMF, (b) i. O_3/MeOH/−78°C ii. Me_2S iii. NH_3-MeOH iv. chromatography, (c) DMSO/NaH/CH_3I, (d) DMSO/NaH/CD_3I

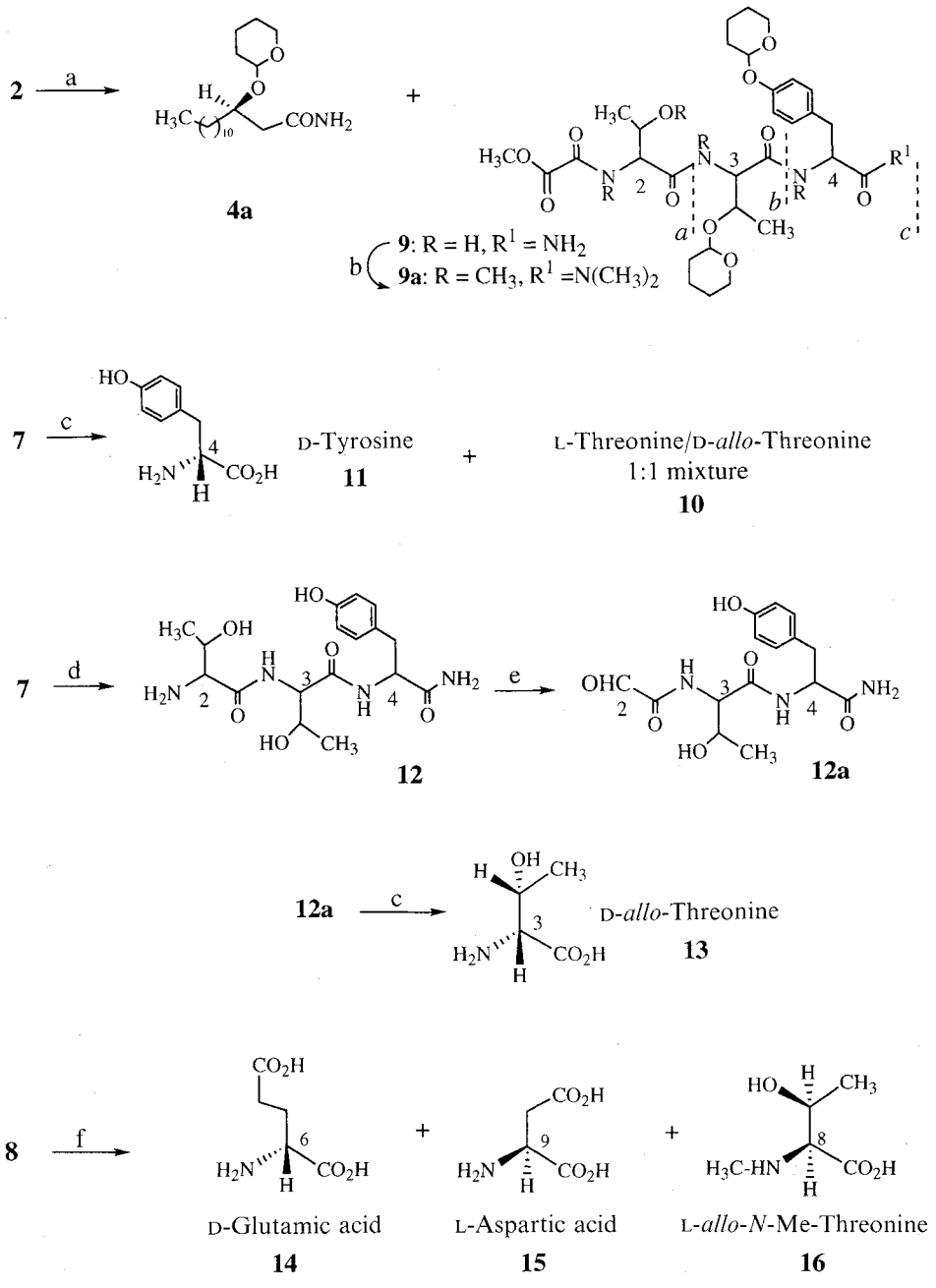
confirmed from the EIMS fragmentation pattern of its permethylated product **9a**. The formation of **4a**, in conjunction with the fragment ion *a* at *m/e* 216 in the MS of **9a**, and the identification of the terminal ethylamide in **6**, established that the lactone bond in **2** involved the hydroxyl group of the threonine-2 unit and the carboxyl group of the terminal histidine-9.

The information generated to this point, elucidated the sequence and cyclic lactonic structure **2** for Sch 20561 wherein the stereochemistry of only the *N*-terminal Hma residue was defined.

Stereochemistry of the Amino Acid Units

The absolute stereochemistry of the amino acid residues in **2** was determined by the isolation and characterization of the individual amino acids formed in the hydrolysis of fragments **7** and **8** (Scheme 3). Hydrolysis of **7** afforded, as found previously,[2] the amino acid unit-4 characterized as D-tyrosine, and a mixture **10** comprising of Thr derived from the Thr-2 and Thr-3 units. The [α] value, together with the integration for two methyl doublets at δ 1.24, 1.32, established that **10** was a 1 : 1 mixture of D-*allo*-threonine and L-threonine. A minor chemical modification of **7** was used to allow the selective isolation of the Thr-3 unit from this tripeptide. Thus, the *N*-terminal oxalamide of fragment **7** was hydrolyzed under mild acidic conditions to afford **12** and the Thr-2 was destroyed by oxidizing the vicinal amino alcohol with sodium periodate followed by hydrolysis of the resulting *N*-glyoxylamide intermediate **12a** to afford the Thr-3 unit which was characterized as D-*allo*-threonine **13**. Based on the identification of Thr-3 as **13** and the identification of **10** as being comprised of a 1 : 1 mixture of D-*allo*-threonine and L-threonine, Thr-2 unit was assigned the L-threonine configuration. Hydrolysis of

Scheme 3.



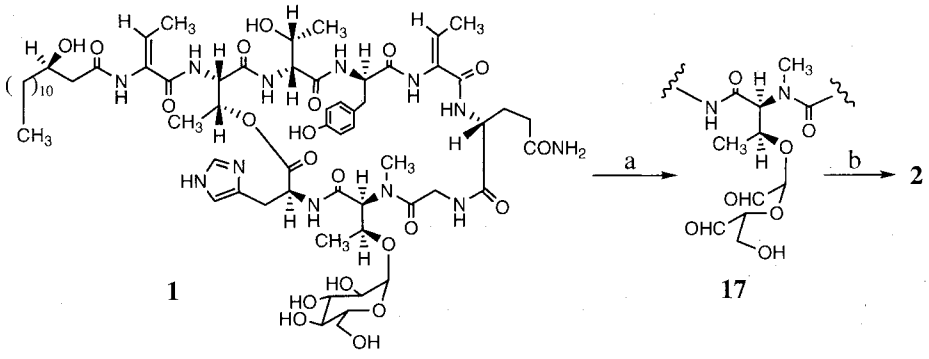
Reagents: (a) i. DHP/*p*-TSA ii. O_3/MeOH iii. Me_2S iv. Et_3N/MeOH, (b) NaH/DMSO/CH_3I, (c) 6N HCl/110°C, (d) i. 1N HCl/70°C, (e) $NaIO_4$/THF/H_2O, (f) i. 6N HCl/110°C 24 hours ii. silica-gel chromatography iii. $CuCO_3$/separation iv. H_2S.

fragment **8** afforded the amino acids derived from Gln-6, Asn-9, and Thr-8 residues and these were characterized as D-glutamic acid **14**, L-aspartic acid **15**, and *N*-methyl-L-*allo*-threonine[4] **16**.

Stereochemistry of the Dehydro Units

The ^1H NMR chemical shifts in DMSO-d_6 for the vinylic proton quartets of *N*-Ac-Aca-OCH_3 are characteristic for the *E*- and *Z*-isomers and correlated well

Scheme 4.



Reagents: (a) $NaIO_4$/MeOH - H_2O, (b) i. Zn dust/MeOH/AcOH ii. chromatography.

for the assignment of the *E* stereochemistry for the Aca units in Sch 20562 (**1**).[2] The vinylic proton quartets at δ 5.80, 5.84 for the two Aca units in Sch 20561 (**2**) also, were found to be in close agreement with the chemical shift for this proton in *N*-Acetyl-*E*-Aca-OCH_3 at δ 5.90; the corresponding signal for a *Z* configuration would be expected to be at a lower field as in *N*-Acetyl-*Z*-Aca-OCH_3 (δ 6.48)[2] which in turn correlates well with the chemical shift observed in the aminolysis product **6** (δ 6.35). On this basis, we have assigned the *E*-configuration for the two Aca units in **2**.

The above chemical degradation studies enabled us to assign the structure and stereochemistry **2** for Sch 20561. The compound was thus established to be the aglycone of Sch 20562 (**1**),[2] the major component of the W-10 antibiotic complex.

Deglucosidation of Sch 20562 (**1**) to Sch 20561 (**2**)

In view of the similarities in the structures of the two major components **1** and **2** of the W-10 antibiotic complex, it was considered of interest to correlate the two compounds through a chemical transformation. Such a correlation involving the deglucosidation of **1** to **2** was preferred since the reverse process would require the selective protection of several hydroxyl groups in the aglycone molecule. Additionally, we had developed methodology using mild reaction conditions for the deglucosidation of the *N*-methyl-L-*allo*-threonine unit in an ozonolysis fragment of **1**.[2] That methodology was applied to the intact glucoside **1**, as shown in Scheme 4. Thus, sodium periodate cleavage of the vicinal glycols of Sch 20562 (**1**) followed by zinc dust reduction of the intermediate glyoxal acetal **17** afforded the deglucosidated product **2**. The TLC mobility and antifungal activity of **2** derived from **1** was compared with the natural product by running a TLC (silica gel) of titred amounts of the two samples followed by overlaying (filter paper barrier) of the developed TLC plate on a nutrient agar plate, preinnoculated with *Saccharomyces cerevisiae*, and incubation at 37°C. The resulting zones of inhibition of growth of the organism, and the TLC mobilities of the two samples established that the deglucosidation product derived from **1** was identical with the natural product **2**.

NMR Assignments for Sch 20561 (**1**)

Most of the proton and carbon chemical shifts of Sch 20561 (**2**) were assigned and the data is summarized in Table 1. The assignments are based upon 2D (^1H-^1H), COSY, HMBC, HMQC, and HMQC-TOCSY (15 msec). HMBC allowed most of the amino acid connections.

Conclusion

In summary, we describe here our studies on the structure elucidation of the antifungal antibiotic Sch 20561 (**2**). Ozonolysis of the dehydropeptide units was utilized as an effective method to accomplish the selective

Table 1. ^{13}C and ^{1}H NMR chemical shifts of Sch 20561 (**2**).

Unit	Atom No.	^{13}C δ	^{1}H δ (mult.)
D-Hma	C_1	170.3	
	C_2	43.6	2.16, 2.25
	C_3	67.3	3.80 (bm)
	C_4	36.9	1.33 (bs)
	C_{5-13}	29.0	1.22 (bs)
	C_{14}	13.9	0.85 (t)
E-Aca-1	NH		9.40
	C_1	164.2	
	C_2	130.3	
	C_3	121.1	5.81 (q)
	C_4	13.3	1.79 (d)
L-Thr-2	NH		7.58 (d)
	C_1	168.4	
	C_2	55.1	4.68
	C_3	71.1	5.20 (m)
	C_4	15.8	1.19 (d)
D-allo-Thr-3	C_1	170.0	
	C_2	58.8	3.80
	C_3	67.0	4.28
	C_4	20.4	1.01 (d)
D-Tyr-4	NH		8.25
	C_1	170.2	
	C_2	54.5	4.50
	C_3	36.2	2.75, 2.90
	$C_{1'}$	127.5	
	$C_{2'}$	130.0	6.94 (d)
	$C_{3'}$	114.8	6.60 (d)
	$C_{4'}$	155.6	
E-Aca-5	NH		9.40
	C_1	164.2	
	C_2	131.1	
	C_3	120.5	5.70 (q)
	C_4	13.1	1.81 (d)
D-Gln-6	NH		8.25
	C_1	171.2	
	C_2	52.4	4.33
	C_3	26.7	1.80, 2.05
	C_4	31.5	2.15
	C_5	174.0	
Gly-7	NH		7.90 (t)
	C_1	169.4	
	C_2	40.8	3.90, 4.02
N-Me-L-*allo*-Thr-8	$N\text{-}CH_3$	31.5	2.80
	C_1	168.4	
	C_2	62.6	4.63
	C_3	64.6	4.14 (m)
	C_4	19.7	1.03 (d)
L-His-9	NH		8.02
	C_1	170.2	
	C_2	52.3	4.52
	C_3	28.6	2.96
	$C_{2'}$	134.8	7.59
	$C_{4'}$	114.8	6.58
	$C_{5'}$	131.4	

^{1}H Chemical shifts in (DMSO-d_6 400 MHz), ^{13}C data in (DMSO-d_6 100.6 MHz).

cleavage of the peptide into three fragments which were sequenced by mass spectrometry. The stereochemistry of the amino acid units was assigned by isolation of free amino acids from the hydrolysates of selected fragments. The stereochemistry of the α-aminocrotonic acid units was assigned by NMR spectroscopy. The NMR resonances of **2** were assigned using two dimensional NMR techniques. A high content of D-amino acids and the presence of the *E*-Aca residues are some of the unique features in this class of dehydropeptides. Microbial products which are cyclic peptides containing Aca residues are not common and our literature search showed only two other natural products *viz.* the antifungal antibiotics stendomycin, and the herbicolins that have been reported previously.[5,6] The structures of **1** and **2** are closely related to those of herbicolins A and B[6] and this may indicate that the producing organisms for these antifungal cyclic dehydropeptides may be related taxonomically.

Experimental

General Procedures

(a) Amino acid analyses were performed on total hydrolysates (6 N HCl/110°C/18 hours) of the peptides (5 mg) and are expressed as relative ratios. (b) Permethylations were performed by methodology described previously[3] as follows: A solution of the peptide (10~20 mg) in DMSO (0.2 ml) was added with stirring

at rt to a solution of methylsulfinyl carbanion (1~2 ml), freshly prepared from DMSO (1~2 ml) and sodium hydride (20~40 mg) at 80°C. After 5 minutes, MeI or CD_3I (0.1 ml) was added and the reaction was worked up after 1 hour by diluting with ice/water, acidification to pH 4 with dilute acid and extratction with CH_2Cl_2. The major product from the permethylation was isolated by TLC on silica-gel.

Sch 20561 (**2**)

The fermentation and isolation of **2** from antibiotic W-10 complex has been described previously.[1] The compound was obtained as a white crystalline solid from MeOH: mp 160~166°C; $[\alpha]_D$ −88° (5% aq. pyridine, $c=0.9$); IR (nujol) 1750 cm^{-1}; λ_{max} (MeOH/OH^-) 240 mμ (ε 25,300), 292 mμ (ε 2,600); MS (FAB) *m/z* 1195 (MH^+); Amino acid analysis His (1), Thr (2), Glu (1), Gly (1), Tyr (1), NH_3 (3).

D-Hma-*E*-Aca-L-Thr-D-*allo*-Thr-D-Tyr-*E*-Aca-D-Gln-Gly-*N*-Me-L-*allo*-Thr-L-His-OCH_3 (**3**)

A suspension of **1** (1.0 g) in MeOH (70 ml) was treated with Et_3N (3 ml) and the resulting solution was stored at rt for 24 hours. The crude product obtained by evaporation of the solvent was purified by chromatography on silica gel (30 g). The major product was eluted with CH_2Cl_2 - MeOH - NH_4OH - H_2O (85 : 15 : 1 : 1), dissolved in MeOH and the solution was diluted with Et_2O to afford **3** as a white powder (0.75 g): mp 130~133°C; $[\alpha]_D$ −25° (*c* 0.4, MeOH); IR (nujol) cm^{-1}; UV λ_{max} (MeOH/OH^-) 240 mμ (ε; ^1H NMR (DMSO-d_6, 100 MHz) δ 0.89 (t, 3H, $J=7$ Hz), 1.08 (m, 9H), 1.86 (d, 6H, $J=7$ Hz), 2.78 (s, 3H), 3.64 (s, 3H), 5.70 (m, 2H), 6.65 (d, 2H, $J=8$ Hz), 7.06 (d, 2H, $J=8$ Hz), 7.59 (s, 1H), 9.20 (bm, 1H), 9.54 9s, 1H). *Anal* Calcd for $C_{58}H_{90}O_{17}N_{12}\cdot H_2O$: C, 55.94; H, 7.45; N, 13.50. Found: C, 55.65; H, 7.45; N, 13.40.

Ozonolysis of **3**: Ozone was bubbled into a solution of **3** (0.65 g) in MeOH (15 ml) at −78°C until a blue color developed; excess O_3 was removed with a stream of N_2 followed by the addition of Me_2S (2 ml). The solution was stirred at rt until a starch-iodide test was negative, and then cooled in an ice bath and saturated with NH_3. The solution was stored for 24 hours and was then evaporated under reduced pressure. The residue was chromatographed on silica gel (30 g). The least polar spot eluted with CH_2Cl_2 - MeOH - NH_4OH - H_2O (60 : 30 : 3 : 2) was crystallized from MeOH-Et_2O to afford D-β-hydroxymyristamide (**4**) as colorles crystals, identical (TLC, NMR, rotation) with an authentic sample[2] of **4**: (0.12 g) mp 110~111°C; $[\alpha]_D$ −4.9° (*c* 0.4, DMF). *Anal* Calcd for $C_{14}H_{29}O_2N\cdot 0.8CH_3OH$: C, 66.08; H, 12.06; N, 5.21. Found: C, 65.91; H, 12.42; N, 5.56.

The next compound eluted with the same solvent was crystallized from Me_2CO/H_2O to afford NH_2-oxalyl-L-Thr-D-*allo*-Thr-D-Tyr-NH_2 (**7**) as colorless crystals, identical (TLC, NMR, rotation) with an authentic sample[2] of **7**: (0.2 g) mp 245~248°C; $[\alpha]_D$ +8.7° (*c* 0.35, DMF). *Anal* Calcd for $C_{19}H_{27}O_8N_5$: C, 50.32; H, 6.00; N, 15.44. Found: C, 50.06; H, 5.98; N, 15.18.

The column was washed out with 10% NH_4OH-MeOH and the polar product was rechromatographed on silica gel (5 g). Elution with CH_2Cl_2 - MeOH - NH_4OH - H_2O (60 : 30 : 3 : 2) afforded the most polar product which was isolated as a white amorphous powder from MeOH-Et_2O characterized as NH_2-oxalyl-D-Gln-Gly-*N*-Me-L-*allo*-Thr-L-Asn-NH_2 (**8b**): (0.15 g) mp 135~139°C; $[\alpha]_D$ −14.2° (*c* 0.35, DMF), −26.7° (*c* 0.36, H_2O), −15.9° (*c* 0.4, MeOH); *Anal* Calcd for $C_{18}H_{30}O_9N_8$: C, 43.03; H, 6.02; N, 22.30. Found: C, 43.22; H, 6.05; N, 21.78. Amino acid analysis Glu (1), Gly 1), Asp (1), NH_3 (4).

Perdeuteriomethylation of **8b** afforded **8c**: EIMS *m/e* 285 (*a*), 359 (*b*), 491 (*c*), 706 (M^+).

D-Hma-*E*-Aca-L-Thr-D-*allo*-Thr-D-Tyr-*E*-Aca-D-Gln-Gly-*N*-Me-L-*allo*-Thr-L-His-NHEt (**6**)

A solution of **2** (1.3 g) in DMF (15 ml) and ethylamine (4 ml) was stored at rt for 7 days and was then evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (40 g). Elution with CH_2Cl_2 - MeOH - NH_4OH - H_2O (90 : 10 : 1 : 1) afforded **6** as an amorphous white powder from MeOH-Et_2O: (0.93 g) mp 144~148°C; $[\alpha]_D$ −15.3° (*c* 0.5, MeOH), −4.7° (*c* 0.47, DMF); ^1H NMR (DMSO-d_6, 300 MHz) δ 0.86 (t, 3H, $J=7$ Hz), 1.02 (m, 12H); 1.65, 1.79 (d, 6H, $J=7$ Hz), 2.84 (s, 3H), 5.65, 6.35 (m, 2H). *Anal* Calcd for $C_{59}H_{93}O_{16}N_{13}\cdot 2H_2O$: C, 55.52; H, 7.66; N, 14.26. Found: C, 55.59; H, 7.69; N, 13.94.

Ozonolysis of **6**: Ozone was bubbled into a solution of **6** (1.0 g) in MeOH (50 ml) at −78°C until a blue color developed; excess O_3 was removed with a stream of N_2 followed by the addition of Me_2S (2 ml). The solution was stirred at rt until a starch-iodide test was negative, and then cooled in an ice bath and saturated with NH_3. The solution was stored for 24 hours and was then evaporated under reduced pressure. The residue was chromatographed on silica gel (30 g). The least polar spot eluted with CH_2Cl_2 - MeOH - NH_4OH - H_2O (60 : 30 : 3 : 2) was crystallized from EtOAc to afford D-β-hydroxy-

myristamide (**4**) as colorless crystals (0.19 g) identical (TLC, mp, NMR, rotation) with an authentic sample[2] of **4**.

The next product eluted was crystallized from Me_2CO/H_2O to afford NH_2-oxalyl-L-Thr-D-*allo*-Thr-D-Tyr-NH_2 (**7**) as colorless crystals (0.15 g), identical (TLC, mp, NMR, rotation) with an authentic sample[2] of **7**.

The product from the most polar fractions (0.35 g) was rechromatographed on silica gel (10 g). Elution with CH_2Cl_2 - MeOH - NH_4OH - H_2O (60 : 30 : 3 : 2) afforded the polar product which was isolated as a white amorphous powder from MeOH-Et_2O and characterized as NH_2-oxalyl-D-Gln-Gly-*N*-Me-L-*allo*-Thr-L-Asn-NH-Et **8**: (0.15 g) mp 120~124°C; $[\alpha]_D$ −26.5° (*c* 0.43, MeOH), −13.1° (*c* 0.4, DMF); ^{1}H NMR (DMSO-d_6, 100 MHz) δ 0.98 (t, 3H, J=7 Hz), 1.05 (d, 3H, J=7 Hz), 2.95 (s, 3H), 3.18 (q, 2H). *Anal* Calcd for $C_{20}H_{24}O_9N_8 \cdot 0.8H_2O$: C, 44.08; H, 6.58; N, 20.56. Found: C, 44.14; H, 6.45; N, 20.26.

Permethylation of **8** afforded **8a**: EIMS *m/e* 270 (*a*), 341 (*b*), 470 (*c*), 684 (M^+).

MeO-Oxalyl-L-Thr-[*O*-tetrahydropyranyl-D-*allo*-Thr]-[*O*-tetrahydropyranyl D-Tyr]-NH_2 (**9**)

A solution of **2** (0.5 g) in DMF (3 ml) was diluted with CH_2Cl_2 (10 ml) and dihydropyran (3 ml) and treated with *p*-TSA (0.05 g). The resulting gel on stirring at rt for 20 minutes turned into a clear solution and after an additional hour was treated with KOAc (0.2 g) and stirred with H_2O and CH_2Cl_2 (50 ml). The organic layer was then washed with brine, dried over Na_2SO_4, concentrated to a low volume and diluted with Et_2O. The resulting solid per-THP **2** (IR in nujol: 5.7 μ) was dissolved in MeOH (40 ml) and ozonized at −78°C. Excess O_3 was removed with a N_2 stream followed stirring with Me_2S (1 ml) until a negative starch-iodide test was obtained. Et_3N (1 ml) was added and the solution was stored at rt for 24 hours and was then evaporated to dryness. The crude product was chromatographed on silica-gel (20 g). Elution with 2% MeOH-CH_2Cl_2 afforded D-3-tetrahydropyranyloxy-myristamide **4b** (0.17 g), identical (TLC, mp, NMR, rotation) with an authentic sample[2] of **4b**. Further elution with the same solvent afforded **9** (0.09 g), identical (TLC, mp, NMR, rotation) with an authentic sample[2] of **9**.

Permethylation of **9** afforded **9a**: EIMS *m/e* 216 (*a*), 415 (*b*), 720 (M^+).

D-Tyrosine from Amino Acid Unit #4

A solution of **7** (0.25 g) in 6 N HCl (10 ml) was heated at 110°C for 17 hours and was then evaporated under reduced pressure. The residue was dissolved in H_2O (4 ml), NH_4OH was added to pH 8, the solution was stirred with Darco (0.1 g) and filtered thrugh a 0.45 μm filter. The clear filtrate was concentrated and the suspension was heated NH_4OH was added to redissolve the solid until a clear solution was obtained. AcOH was added to pH 5~6 and the crystals obtained on cooling the solution in an ice bath for 6 hours were filtered and dried to afford D-tyrosine **11** (45 mg): mp 278~284°C; $[\alpha]_D$ +7° (*c* 0.45, 5 N HCl); identical (NMR, TLC) with an authentic sample of the L-isomer. The filtrate was evaporated to dryness and the residue was chromatographed on silica gel (10 g) eluting with CH_2Cl_2 - MeOH - NH_4OH - H_2O (60 : 45 : 3 : 2). The fractions homogeneous in Thr were evaporated to afford a crystalline 1 : 1 mixture **10** of D-*allo*-Thr and L-Thr (40 mg): $[\alpha]_D$ −25° (*c* 0.33, 5 N HCl); ^{1}H NMR (D_2O, 60 MHz) δ 1.23 (d, 3H, J=7 Hz), 1.34 (d, 3H, J=7 Hz), 3.58 (d, 1H, J=5 Hz), 3.84 (d, 1H, J=4 Hz).

D-*allo*-Threonine from Amino Acid Unit #3

A solution of **7** (0.4 g) in 1 N HCL (10 ml) was heated at 65°C for 16 hours and was then evaporated under reduced pressure. The resulting ninhydrin positive **12** was dissolved in H_2O (10 ml), the pH of the solution was adjusted to neutrality with $NaHCO_3$ followed by the addition of $NaIO_4$ (0.318 g). After stirring for 3 hours, ethylene glycol (0.092 g) was added and after 0.5 hour the mixture was evaporated to dryness. The residue was suspended in MeOH and filtered. The filtrate was evaporated, dissolved in 6 N HCl (15 ml) and heated at 110°C for 16 hours. The hydrolysate was evaporated and chromatographed on silica gel (10 ml). Elution with CH_2Cl_2 - MeOH - NH_4OH - H_2O (60 : 30 : 3 : 2 v/v) yielded D-tyrosine followed by the more polar fraction which after recystallization from EtOH-HCl-aniline afforded D-*allo*-threonine **13** as colorless needles (45 mg): $[\alpha]_d$ −25° (5 N HCl, *c* 0.39); ^{1}H NMR (D_2O, 100 MHz) δ 1.24 (d, 3H, J=7 Hz), 3.80 (d, 1H, J=4 Hz), 4.25 (m, 1H); identical (NMR, TLC) with an authentic sample of D-*allo*-threonine.

D-Glutamic Acid, Glycine, *N*-Methyl-L-*allo*-threonine, and L-Aspartic Acid from Amino Acid Units #6, 7, 8 and 9

A solution of tetrapeptide **7** (0.6 g) in 6 N HCl (20 ml) was heated at 110°C for 18 hours and was then evaporated to dryness under reduced pressure. The product was chromatographed on silica gel (30 g). Elution with

CH_2Cl_2-MeOH-NH_4OH-H_2O (60:30:3:2 v/v) afforded 63 mg of *N*-methyl-L-*allo*-threonine **16** which was crystallized from MeOH as colorless needles: mp 226~230°C; $[\alpha]_D$ +15.3° (5 N HCl, *c* 0.55); identical (NMR, TLC, rotation) with an authentic sample of *N*-methyl-L-*allo*-threonine.[4] Subsequent fractions afforded glycine. The column was then eluted with 20% NH_4OH-MeOH (100 ml) and the eluate was evaporated to dryness. The resulting residue was dissolved in H_2O (10 ml), $CuCO_3$ (200 mg) was added, the suspension was heated on a steam-bath for 0.5 hour and filtered hot. The dark blue filtrate was stored in a refrigerator overnight and the insoluble ASP-copper salt was isolated by filtration. The filtrate containing the soluble GLU-copper salt was treated with excess H_2S and filtered. The clear filtrate was evaporated to dryness and the residue was recrystallized from H_2O-EtOH to afford D-glutamic acid **14** as colorless crystals (20 mg): mp 203~205°C; $[\alpha]_D$ −26.9° (5 N HCl, *c* 0.38), identical (TLC, NMR, rotation) with an authentic sample of D-glutamic acid. The insoluble ASP-copper salt was suspended in water (10 ml) and stirred with H_2S at rt. The resulting black suspension was then filtered through a celite pad and the clear filtrate was evaporated to dryness. The residue was recrystallized from H_2O-EtOH to afford L-aspartic acid **15** as colorless crystals (40 mg): $[\alpha]_D$ +22.8° (5 N HCl, *c* 0.4), identical (TLC, NMR, rotation) with an authentic sample of L-Aspartic acid.

Deglucosidation of Sch 20562 (**1**) to Sch 20561 (**2**)

A solution of $NaIO_4$ (0.32 g) in H_2O (10 ml) was added to a solution of **1** (1.0 g)[2] in MeOH (200 ml) and H_2O (10 ml). The mixture was stirred at 37°C for 16 hours and was then quenched with ethylene glycol (0.4 ml). After stirring for 15 minutes the solution was concentrated to qa 50 ml and was diluted with H_2O (25 ml). The resulting gelatinous product was filtered, washed with H_2O, dissolved in MeOH, evaporated and the residue was dried under high vaccum. The resulting crude glyoxal acetal **19** was dissolved in MeOH (100 ml) and AcOH (10 ml) and stirred at 60°C with Zn dust (4 g) for 48 hours. The mixture was then filtered, the filtrate was concentrated under reduced pressure, diluted with H_2O and the resulting gel was filtered and dried under high vaccum. The crude product (0.45 g) was chromatographed on silica gel (15 g) eluting with 20% MeOH-CH_2Cl_2. The fractions homogeneous in the less polar spot were combined (0.15 g) and cystallized from MeOH to afford **2**: (0.06 g) mp 158~164°C; $[\alpha]_D$ −84° (5% H_2O-pyridine, *c* 0.32), identical (TLC, NMR, bioautogram) with a comparison sample of **2**.

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