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Sch 486058: A Novel Cyclic peptide of Actinomycete Origin

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Abstract The structure of a novel cyclic peptide (**1**) produced by *Actinomycete* sp. has been assigned on the basis of extensive NMR and mass spectral data.

Keywords fermentation, *Actinomycete* sp., cyclic peptide, NMR, MS, Sch 486058

During the course of search for biologically active metabolites from microbial sources, we have isolated a cyclic peptide (Fermentation #89-02408) produced by *Actinomycete* sp. (Schering Collection, SCC 2186). The isolation and purification was carried out according to scheme shown in Fig. 1. In this paper, we describe the structure elucidation of this novel cyclic peptide.

Sch 486058 (**1**, major fraction) is a cyclic peptide (Rydon (+) and ninhydrin (−) with a MW of 1530 by SIMS; *m/z* 1531 $(M+H)^+$ and 1529 $(M-H)^-$; UV λ_{max} 220 and 280 nm.

^{13}C NMR spectrum of **1** in CD_3OD/CD_3OH (APT, DEPT) indicated the presence of seven methyls (10.4, 12.3, 16.3, 16.6, 18.7, 20.2, 20.2), twelve methylenes (25.4, 26.4, 27.8, 30.2×2, 30.4×2, 30.8, 34.6, 36.0, 36.9, 42.7), three methines (31.0, 34.5, 37.7), three CH_2N (43.3, 44.0, 44.9), one CH_2O (64.1), twelve CHO/CHN (50.5, 53.7, 54.0, 54.9, 56.4, 56.5, 59.6, 60.1, 60.2, 65.5×2, 69.0), eleven =CH (116.6×2, 117.5, 129.5×2, 130.1×2, 131.5×2, 136.9, 143.0), five quaternary carbons (128.6, 140.6, 157.6, 159.0, one *C overlapped), and sixteen CON/COOH (170.8, 171.5, 171.6, 171.8, 171.9, 172.3, 172.6, 172.7, 173.0×2, 173.4, 173.6, 174.7, 175.1, 178.2, 180.7). The above data gave a composition of $C_{69}H_{102}N_{20}O_{20}$ (MW=1530.75) with 29 degrees of unsaturation which included four rings. HRMS for $(M+Na)^+$ ion [$C_{69}H_{102}N_{20}O_{20}Na$; calc. 1553.7477 and obsd. 1553.7448] confirmed the composition.

Amino acid analysis indicated the presence of one mole each of aspartic acid, glutamine, serine, histidine, threonine, arginine, tyrosine, valine, D-phenylalanine, three moles of glycine, and about 1/2 mole of D,L-isoleucine. CIMS of the acid hydrolysate mixture confirmed the above amino acids. The ions *m/z* $(M+1)^+$ and $(M+NH_4)^+$ were Gly (76 and 93), Val (118 and 135), Ile (132), Ser (106 and 123), Phe (166 and 183), Tyr (182 and 199), Gln (147), Asp (90 and 107 after loss of CO_2), Thr (120 and 137), and Arg (175, weak). CIMS studies also indicated the presence of an additional, present in both aqueous and organic layers, component with *m/z* 245 $(M+1)^+$ and 227 $(M+1-H_2O)^+$. HRMS confirmed the chemical composition of *m/z* 245 as $C_{12}H_{25}N_2O_3$ (obsd: 245.1839). CAMIKES studies of *m/z* 245 $(M+1)^+$ indicated *m/z* 229, 227,199, 188, 184, 171, 170, 154, 142, 141, 132, 86 and 68 in the spectrum.

The lack of reasonable amounts of Ile in the acid hydrolysis mixture with concurrent presence of an additional product with the above chemical composition suggested that the side chain is a dipeptide consisting of two Ile units with a free COOH group.

Peptide sequencing through enhanced Akabori reaction

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has been well established [1∼3] and its application to **1** (Table 1) indicated the presence of one arginine residue which was converted to ornithine followed by conversion of an amide function (Asn or Gln) to hydrazide. The appearance of *m/z* 1389, 1404, and 1277 confirmed the presence of two Ile residues outside the cyclic peptide moiety.

The chemical shifts assignments based upon the analysis of COSY, HMQC, and HMQC-TOCSY spectra are presented in Table 2. A small number of cyclic peptides have been subject of comparable studies [4∼7]. The AA sequence was established on the basis of NOESY data. Strong $H\alpha_i \rightarrow NH_{i+1}$, $H\beta_i \rightarrow NH_{i+1}$, and some $NH_i \rightarrow NH_{i+1}$ interactions were observed and the most important ones are shown in Fig. 3. It was possible to assign Gln instead of Asn on the basis of NOE data. Most difficult part was sorting out the connection of Asp in the AA sequence. HMBC data resulted in significant improvements in the AA sequence, *e.g.* the complete assignment of the dipeptide side chain and established connectivity between CH_2 protons of Gly_1 with α-CO of Asp at δ 174.7, and between CH_2 protons of Asp with α- and γ-CO of Asp at δ 174.7 and 173.0 (Fig. 3).

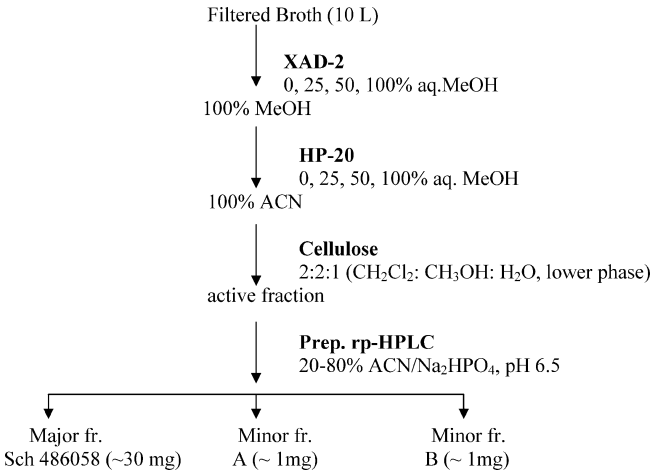

Fig. 1 Isolation of Sch 486058.

The original activity was based on agar diffusion assay using 6 mm paper disc. The MIC for the major component (**1**) was $>128\ \mu$g/ml against most fungi, however, we could not get end points on both yeast and fungi. The MIC for the minor, structurally related components (A and B) were $0.25\ \mu$g/ml and $<0.0625\ \mu$g/ml against *Candida*, respectively, and were inactive against *Aspergillus*. Due to lack of material and desired potency, the project was terminated.

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Table 1

m/z	Structure
1489 ($(M+1)^+-42$)	Arg→Ornithine ($-CH_2NHC=NH(NH_2)\rightarrow -CH_2NH_2$)
1504 ($(M+1)^+-42+15$)	Gln→hydrazide ($-CH_2CONH_2\rightarrow -CH_2CONHNH_2$)
1389 ($(M+1)^+-42-131+31$)	Arg→Ornithine (loss of one Ile+$NHNH_2$)
1404 (1389+15)	(loss of one Ile+$NHNH_2$+Gln→hydrazide)
1277 (1489−131−113+31)	(loss of two Ile+$NHNH_2$)

m/e	structure
245 $(M+1)^+$	$C_{12}H_{25}N_2O_3$
229 $(M+1)^+$ - 16	loss of NH_2
227 $(M+1)^+$ - 18	loss of H_2O (diketopiperazine $C_{12}H_{23}N_2O_2$)
86 $(M+1)^+$ - 159	loss of 2xC_4H_9 and COOH ($C_3H_6N_2O$)

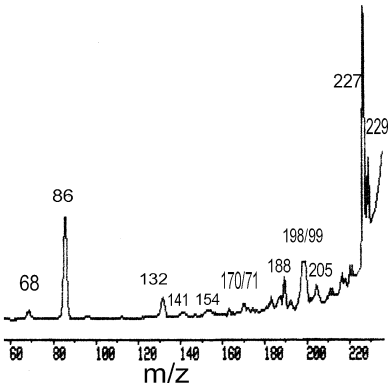

Fig. 2 The CAD MIKES of side chain, *m/z* 245 $(M+1)^+$ and the major ions.

Table 2 ^{1}H and ^{13}C NMR Data for Sch 486058 in CD_3OH/CD_3OD[a]

AA		^{13}C[b]	^{1}H (mult, *J* in Hz)[b]	AA		^{13}C[b]	^{1}H (mult, *J* in Hz)[b]
Gly 1	CO	171.8		Tyr	CO	175.1	
	α	43.4	4.50, 3.38		α	56.4	4.23 m
	NH		8.4		β	35.9	3.05, 3.20
					γ-C_1	128.9	
Phe	CO	172.3			C_2, C_6	131.5	7.18 (d, 7.0)
	α	59.8	3.86 m		C_3, C_5	116.6	6.70 (d, 7.0)
	β	34.6	3.30, 3.46		C_4	157.6	
	γ-C_1	140.6			NH		7.53 (bd)
	C_2, C_6	130.1	7.26				
	C_3, C_5	129.5	7.26	Thr	CO	171.9	
	C_4	127.5	7.14		α	60.1	4.96
	NH		8.28 (d, 6.0)		β	69.2	4.18
					γ	18.6	1.17 (d, 6.5)
Gly 3	CO	171.6			NH		7.94 (d)
	α	44.3	4.16, 2.90 (bd)				
	NH		NA	Gly 2	CO	171.5	
					α	45.0	3.85, 3.90
Gln	CO	172.7			NH		7.63
	α	53.7	4.48				
	β	30.4	1.71, 1.80	Val	CO	170.8	
	γ	34.6	1.90, 2.21		α	60.1	
	$\underline{CO}NH_2$	178.2			β	31.0	4.32
	$CO\underline{NH_2}$		6.32, 7.72		γ,γ'	20.2, 20.1	1.99
	NH		8.22 (d)		NH		1.05 (d)
							7.8
His	CO	173.4		Asp	CO	174.7	
	α	50.6			α	54.9	
	β	36.9	4.86		β	30.8	4.40 (b)
	γ-C_1	NA	3.21, 2.40		γ	173.0	2.86 (br), 2.96 (br)
	C_2	136.9			NH		
	C_4	143.0	8.09				8.87 (d, 5.0)
	NH		7.60 (br)	Ile 1	CO	180.7	
			8.32			α	65.5
Ser	CO	173.0			β	37.8	3.75
	α	56.5			γ-CH_3	16.6	1.97
	β	64.1	4.62 m		γ-CH_2	25.4	0.89 (d)
	NH		3.71, 3.77		δ-CH_3	12.3	0.85, 1.22
	OH		8.37 (d)		NH		0.70 (t)
			5.25				NA
Arg	CO	173.6		Ile 2	CO	172.6	
	α	54.0			α	65.5	
	β	30.2	4.60		β	34.6	3.44
	γ	26.3	1.75, 1.38		γ-CH_3	16.3	2.59
	δ	42.7	1.38, 1.70		γ-CH_2	27.1	1.00 (d)
	ε	159.3	3.17		δ-CH_3	10.5	1.17, 1.50
	NH				NH		0.87 (t)
	NH		8.3				8.80
			8.70 (d)				

[a] Instruments: Varian XL-400, GE-400. [b] The chemical shifts are in ppm with reference to internal TMS and coupling constants are in hertz.

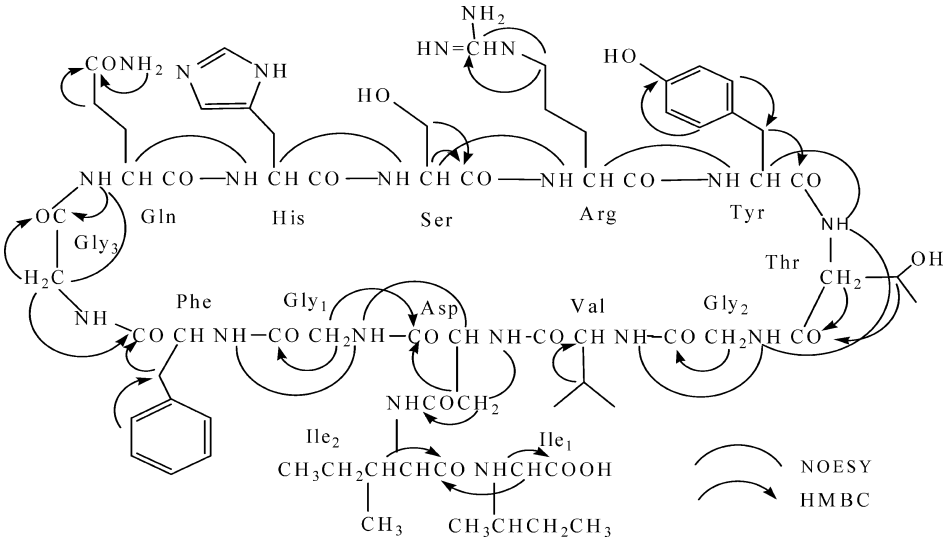

Fig. 3 Compound **1** with NOESY and HMBC interactions.

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