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Structure of Cypemycin, a New Peptide Antibiotic

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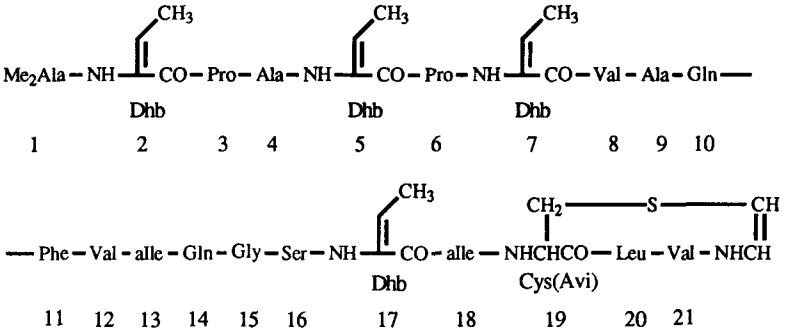
Abstract: The structure of cypemycin, a new peptide antibiotic, was determined by means of FAB-MS, NMR, and amino acid analysis. The data have revealed cypemycin as being a structurally unique peptide antibiotic that contains a sulfide bridge at its C-terminus as well as four α,β-unsaturated amino acids.

Recently, we reported the isolation of cypemycin from the fermentation broth of *Streptomyces* sp. OH-4156[1] and characterization of its biological properties. The antibiotic showed antimicrobial activity against *Micrococcus luteus* (MIC = 0.2 μg/ml) together with cytotoxicity against P388 leukemia cells *in vitro*. In this communication, we disclose the structure of cypemycin (**1**), which is a unique peptide antibiotic possessing a sulfide bridge at its C-terminus and four α,β-unsaturated amino acids.



AlaMe2; N-dimethylalanine
Dhb; (E)-2,3-didehydro-2-aminobutylic acid
Cys(Avi); [(Z)-2-aminovinyl]cysteine

Fig. 1 The structure of cypemycin (**1**).

The physico-chemical properties of cypemycin (**1**) were already reported in a previous paper.[1] Antibiotic **1** was negative to ninhydrin, but showed strong absorption at 1660 cm^{-1} and 1530 cm^{-1} in IR spectrum, suggesting the antibiotic to be a peptide. The nominal mass of the molecular weight was determined to be 2094 by FAB-MS, in which the quasi-molecular ion peaks $[M+H]^+$, $[M+Na]^+$ and $[M-H]^-$ appeared at m/z 2095, 2117 and 2093, respectively. The amino acid analysis of the hydrolyzate of **1** (6N HCl, 130°C, 3 hr) gave the amino acid composition of **1** as Ser, 2Glx, 2Pro, Gly, 2Ala, Cys, 3Val, 2alle, Leu, and Phe (Table 1). However, the sum of the molecular weights of those amino acid residues was only about 1600, which was

much lower than that determined by FAB-MS. The shortage suggested the presence of some unusual amino acids in the molecule.

Table 1 Amino acid analysis of the hydrolyzate of cypemycin (**1**).

	Molar ratio			Molar ratio	
Residue	found	calcd.	Residue	found	calcd.
Ser	1.1	1	Cys	0.7	1
Glx	2.1	2	Val	2.5	3
Pro	1.9	2	alle	1.6	2
Gly	1.3	1	Leu	1.1	1
Ala	2.0	2	Phe	0.9	1
			Total	15.2	16

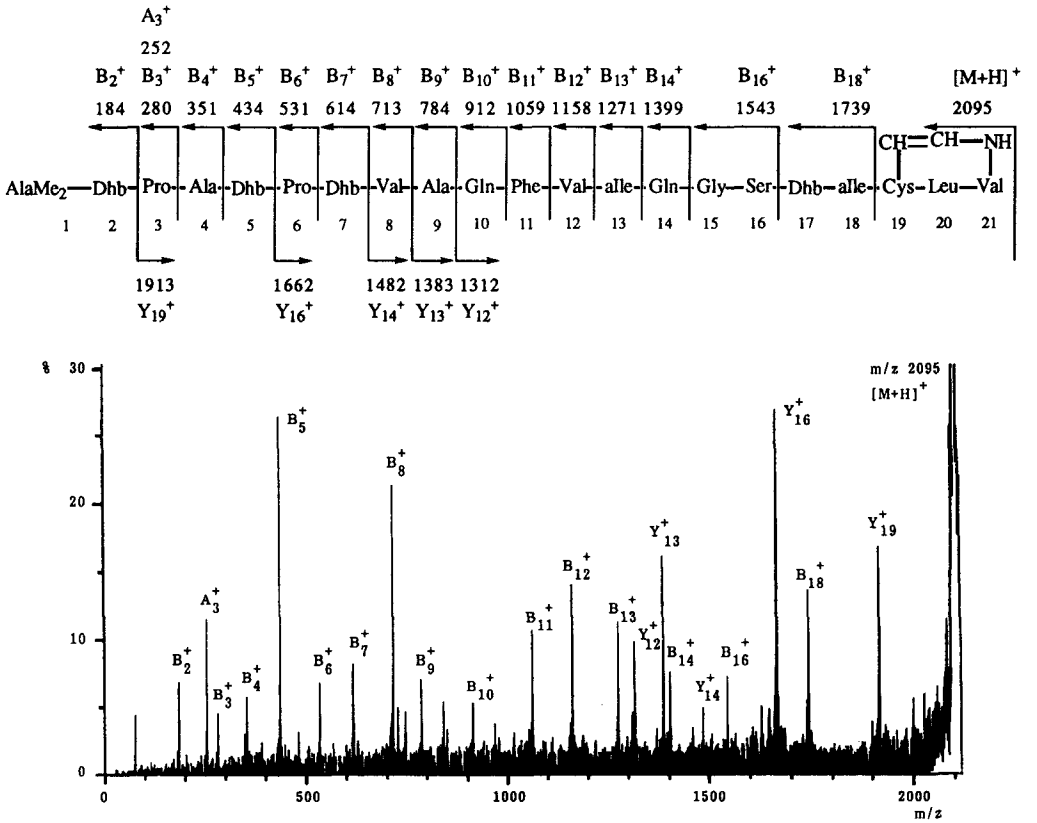


Fig. 2 FAB-B/E linked scan spectrum of cypemycin (**1**). (matrix; Magic Bullet)

The ^{1}H-NMR spectrum of **1** showed four coupled methin-methyl systems, an AMX spin system, and an N-dimethyl signal together with the signals due to the residues determined by amino acid analysis. Further analysis by COSY, HOHAHA, and ROESY (τ_m=100ms) experiments enabled us to assign those signals due to

the unusual amino acids. Chemical shifts of the four methin-methyl systems together with NOE between the methin and singlet amide signal in each system revealed the presence of four 2,3-didehydro-2-aminobutyric acids (Dhb). The NOEs also proved the configurations of all double bonds to be E. The AMX spin system was assigned to be an 2-aminovinyl (Avi) group, and the NOE between the Cys-β and Avi-1' showed the connection of this unit to the sulfur atom of Cys. The vicinal coupling constant of the double bond (J=11Hz) revealed Z-configuration for the double bond of the Avi moiety. The remaining N-dimethyl signal correlated in the ROESY spectrum to the α-proton of an Ala residue, which lacked the amide proton signal. Accordingly, the N-dimethyl group was assigned to an $AlaMe_2$ residue, which was placed at the N-terminal position of **1**. The presence of the N-dimethyl group at the N-terminus of **1** explains the negative reaction to ninhydrin. These substructures along with the normal amino acid residues gave a molecular formula of $C_{99}H_{154}N_{24}O_{24}S$, which is consistent with the molecular weight determined by FAB-MS. Further analysis including HMQC and HMBC experiments allowed the assignment of all ^{1}H and ^{13}C-signals to elucidate the structures of those unusual amino acids as well as residues observed in amino acid analysis (Table 2).

The blocked N-terminus and the presence of four α,β-unsaturated amino acids hampered the sequential analysis by degradation study. Thus, the sequential analysis of **1** was performed by means of fragmentation in FAB-MS and NMR method. The FAB-B/E linked scan (m/z 2095 $[M+H]^+$ as a parent ion) spectrum with collision-induced degradation (CID)[2] showed remarkable fragment ion peaks due to a series of acylium ions starting from the N-terminus (Fig. 2), then the sequence from $AlaMe_2$-1 to Gln-14 was ambiguously determined. On the other hand, the NMR sequential analysis was performed based on NOE correlations and ^{1}H-^{13}C long-range couplings. The interresidual NOE as shown in Fig. 3 proved the sequence from $AlaMe_2$-1 to Pro-3, from Ala-4 to Cys-19, and from the C-terminal Val-21 to Cys(Avi)-19 through a sulfide bridge. Furthermore, ^{1}H-^{13}C long-range couplings complemented the sequence from Pro-3 to Ala-4, from Pro-6 to alle-18, and the position of the Cys(Avi) bridge. Although only Leu-20 did not afford clear interresidual NOE or ^{1}H-^{13}C long-range coupling due to the signal broadening, Leu-20 should be placed between Cys-19 and Val-21 to satisfy the above FAB-MS and NMR data.

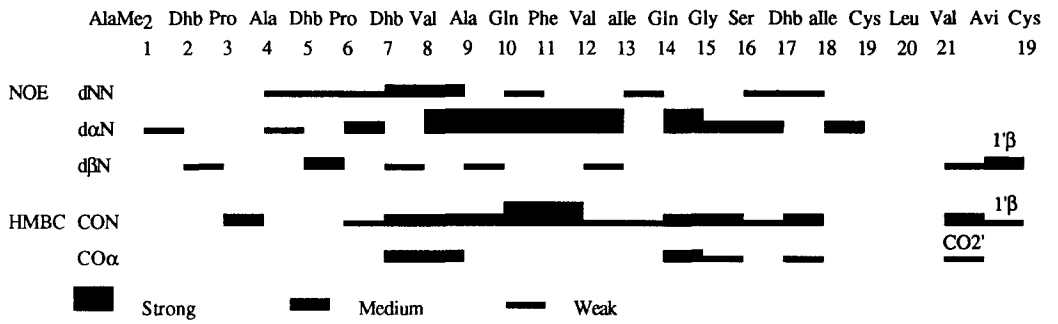


Fig. 3 Sequential NOE and HMBC correlations of cypemycin (**1**).

Consequently, the structure of cypemycin was determined as shown in Fig. 1. The presence of unusual building blocks including a sulfide bridge as well as four α,β-unsaturated amino acids revealed that, the antibiotic was structurally similar to a class of peptide antibiotics including nisin[3] and epidermin[4], which were proposed by Jung to be classified as lantibiotics.[5] However, these lantibiotics generally have many sulfide bridges in their molecule, in contrast, cypemycin possesses only one sulfide bridge at its C-terminus, and the major part of the molecule forms a single peptide chain. These characteristics of the structure suggest this antibiotic being a unique peptide related to lantibiotics.

Table 2. NMR data of cypemycin (1). (400MHz for ^{1}H, 100MHz for ^{13}C, in DMSO-d_6 at 30°C)

Residue		Chemical shifts (ppm) ^{1}H	Chemical shifts (ppm) ^{13}C
AlaMe$_2$	CO		171.90
-1	Me$_2$	2.26	41.10
	α	3.41	64.85
	β	1.14	11.51
Dhb-2	CON	9.71	166.65
	α		131.10
	β	5.70	121.23
	γ	1.71	12.02
Pro-3	CO		171.04
	α	4.28	60.68
	β	2.22,1.75	29.24
	γ	1.86,1.78	24.78
	δ	3.56,3.37	48.96
Ala-4	CON	8.00	172.04
	α	4.32	48.39
	β	1.32	17.03
Dhb-5	CON	8.95	165.90
	α		130.83
	β	5.74	122.27
	γ	1.66	12.02
Pro-6	CO		171.43
	α	4.28	61.02
	β	2.22,1.85	29.32
	γ	1.92,1.86	24.78
	δ	3.51	48.83
Dhb-7	CON	8.92	164.06
	α		129.78
	β	6.47	130.11
	γ	1.64	12.77
Val-8	CON	7.24	170.84
	α	4.14	58.61
	β	2.04	29.83
	γ	0.85	18.97
Val-8	δ	0.81	17.94
Ala-9	CON	7.86	171.90
	α	4.23	48.44
	β	1.20	17.35
Gln-10	CON	7.70	170.99
	α	4.12	52.48
	β	1.73,1.57	27.76
	γ	1.88	31.17
	δ,ε	7.03,6.65	173.51
Phe-11	CON	8.04	170.93
	α	4.54	54.22
	β	3.01,2.79	37.77
	1'		137.62
	2',6'	7.23	129.15
	3',5'	7.22	127.94
	4'	7.15	126.15
Val-12	CON	7.94	171.12
	α	4.15	58.13
	β	1.96	29.83
	γ	0.76	18.23
		0.78	19.11
alle-13	CON	7.79	171.32
	α	4.28	56.10
	β	1.82.	36.41
	γ	1.31,1.07	25.53
	δ	0.81	11.36
	ε	0.80	14.53
Gln-14	CON	7.95	171.52
	α	4.25	52.48
	β	1.90,1.80	27.67
	γ	2.13	31.33
	δ,ε	6.77,7.22	173.76
Gly-15	CON	8.09	169.36
Gly-15	α	3.79,3.88	42.26
Ser-16	CON	8.16	172.61
	α	4.20	56.10
	β	3.92,3.73	61.32
	OH	5.50	
Dhb-17	CON	9.65	165.05
	α		129.56
	β	6.57	130.83
	γ	1.68	12.86
alle-18	CON	7.47	170.62
	α	4.13	58.13
	β	1.89	35.25
	γ	1.36,1.18	25.30
	δ	0.84	11.26
	ε	0.87	14.72
Cys(Avi)	CON	7.65	169.42
-19	α	4.64	52.05
	β	3.08,2.85	37.04
	1'	5.47	99.84
	2'	7.11	132.09
	N-3'	8.70	
Leu-20	CON	7.46	172.38
	α	3.93	54.90
	β	1.63,1.48	39.38
	γ	1.63	24.20
	δ	0.93	22.27
		0.84	21.69
Val-21	CON	7.71	169.01
	α	3.92	59.57
	β	2.05	28.51
	γ	0.78	18.90
		0.89	19.47

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