Chloropeptins, New Anti-HIV Antibiotics Inhibiting gp120-CD4 Binding from *Streptomyces* sp.

II. Structure Elucidation of Chloropeptin I

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The structure of chloropeptin I, a gp120-CD4 binding inhibitor having a potent anti-HIV activity, was elucidated by ^{1}H and ^{13}C NMR experiments and chemical degradation. It is a peptide antibiotic consisting of six aryl amino acids residues and an α-oxo aryl acid some of which have chlorine atoms.

Chloropeptins I (**1**) and II (**2**) are peptide antibiotics isolated from a culture broth of *Streptomyces* sp. WK-3419 as potent inhibitors against gp120-CD4 binding[1,2]. They inhibit strongly both the cytopathic effect assayed in HIV-1-infected MT-4 cells and the syncytium formation in co-cultured HIV-1-infected and uninfected MOLT-4 cells[2]. **1** is a new compound although **2** was identified with complestatin[3] which has been reported to inhibit the hemolysis of erythrocytes sensitized by the complement system[4]. In this paper, we wish to report the structure of **1**, elucidated *via* combination of NMR analysis and chemical degradation.

Results and Discussion

1 was obtained as a yellow-brown powder: mp >300°C; $[\alpha]_D^{26}$, −18.7° (c=0.16, DMSO); UV λ_{max}^{MeOH} nm (ε), 214 (64,600), 239 (sh), 285 (sh), 291 (14,600) and 304 (sh). **1** was positive to EHRLICH's reagent, but negative to ninhydrin and Sakaguchi reagents. The molecular formula was determined to be $C_{61}H_{45}N_7O_{15}Cl_6$ by HRFAB-MS (m/z 1325.1093 (M^+), calcd for 1325.1105). The IR spectrum revealed an amide carbonyl absorption at 1640 cm^{-1}. In the ^{13}C and ^{1}H NMR spectra (Table 1), the signals of six amide carbonyl carbons, five amide protons and one *N*-methyl group were observed. These data indicate the presence of six amide bonds originated from six amino acids. In the ^{1}H NMR spectrum, six methine proton signals and two methylene proton signals were observed. Additionally, in the aromatic region, eighteen proton signals and a couple of high-field shifted *m*-coupled signals at δ 5.70 and 5.99 (J=2.0 Hz) were observed. These results suggest that **1** is a peptide antibiotic containing aryl amino acid residues. The signal patterns of the aromatic region show the presence of seven aromatic ring residues as follows. Each three singlet signals at δ 7.28 (2H), δ 7.36 (2H) and δ 7.82 (2H) indicate the presence of 1,3,4,5-tetra-substituted symmetrical benzene rings. A couple of *m*-coupled signals are originated from a 1,3,4,5-tetra-substituted asymmetrical benzene ring. A pair of doublet signals at δ 6.74 (2H, J=8.5 Hz) and δ 7.08 (2H, J=8.5 Hz) and four double-doublet signals at δ 7.19, δ 6.79, δ 7.14 and δ 7.82 (1H, J=8.0, 2.0 Hz, respectively) revealed the presence of two *p*-substituted benzene ring systems. The seventh aromatic ring residue was deduced to be a 7-substituted indole ring system from the following results: **1** was positive to EHRLICH's reagent; the remaining three aryl proton signals at δ 7.22 (J=8.0 Hz), δ 6.90 (J=8.0 Hz) and δ 7.08 (J=8.0 Hz) indicated the presence of 1,2,3-trisubstituted benzene ring; and a singlet signal at δ 7.64 (1H) and a signal of exchangeable proton at δ 10.57 which were assigned at C-2 and NH group, respectively, were observed.

From the COSY, HMQC and HMBC data, the structures of six amino acid residues (A to F) and a 2-oxoaryl acid residue (G) were determined to be as shown in Fig. 1. Chemical shifts of the quaternary carbons, C-3 and 5 of C, E and G residues (δ 122.1, 121.9 and 122.8) indicated that they attached to chlorine

Table 1. ^{13}C and ^{1}H NMR spectral data of chloropeptin I (**1**) in DMSO-d_6.

	^{13}C		^{1}H	(Hz)
A (4-hydroxyphenylglycine)				
C=O	171.5	s		
Cα	55.9	d	5.04 1H d	6.5
NH			8.41 1H d	6.5
1	127.8	s		
2,6	128.4	d	7.08 2H d	8.5
3,5	115.4	d	6.74 2H d	8.5
4	157.3	s		
B (*N*-methyltyrosine)				
C=O	168.6	s		
Cα	61.5	d	5.06 1H d	
Cβ	35.1	t	3.02 2H m	
N-CH_3	31.2	q	2.99 3H s	
1'	134.1	s		
2'	130.3	d	7.19 1H dd	8.0, 2.0
3'	123.0	d	7.14 1H dd	8.0, 2.0
4'	156.2	s		
5'	121.5	d	6.79 1H dd	8.0, 2.0
6'	131.6	d	7.82 1H dd	8.0, 2.0
C (3,5-dichloro-4-hydroxyphenylglycine)				
C=O	169.3	s		
Cα	51.7	d	5.16 1H d	6.0
NH			8.79 1H d	6.0
1'	131.3	s		
2',6'	127.2	d	7.36 2H s	
3',5'	122.1	s		
4'	148.8	s		
D (4-dihydroxyphenylglycine)				
C=O	168.1	s		
Cα	55.0	d	5.61 1H d	8.5
NH			8.25 1H d	8.5
1'	126.4	s		
2'	112.2	d	5.70 1H d	2.0
3'	150.6	s		
4'	141.8	s		
5'	126.2	s		
6'	125.9	d	5.99 1H d	2.0
E (3,5-dichloro-4-hydroxyphenylglycine)				
C=O	169.0	s		
Cα	53.5	d	5.41 1H d	8.5
NH			8.19 1H d	
1'	132.2	s		8.5
2',6'	126.7	d	7.28 2H s	
3',5'	121.9	s		
4'	148.2	s		
F (tryptophan)				
C=O	169.3	s		
Cα	54.9	d	5.08 1H m	
Cβ	26.7	t	3.12 2H m	
NH			8.90 1H d	6.0
1'			10.57 1H bs	
2'	126.0	d	7.64 1H d	2.0
3'	107.0	s		
3'a	129.1	s		
4'	116.7	d	7.22 1H d	8.0
5'	118.7	d	6.90 1H t	8.0
6'	120.8	d	7.08 1H d	8.0
7'	125.6	s		
7'a	135.6	s		
G (2-(3,5-dichloro-4-hydroxyphenyl)-2-oxoacetic acid)				
C=O	164.5	s		
Cα	185.4	s		
1'	127.2	s		
2',6'	130.4	d	7.82 2H s	
3',5'	122.8	s		
4'	157.8	s		
phenol			9.41 1H s	
			9.42 1H bs	
			9.95 1H bs	
			10.06 1H bs	

Fig. 1. Partial structures of chloropeptin I (**1**).

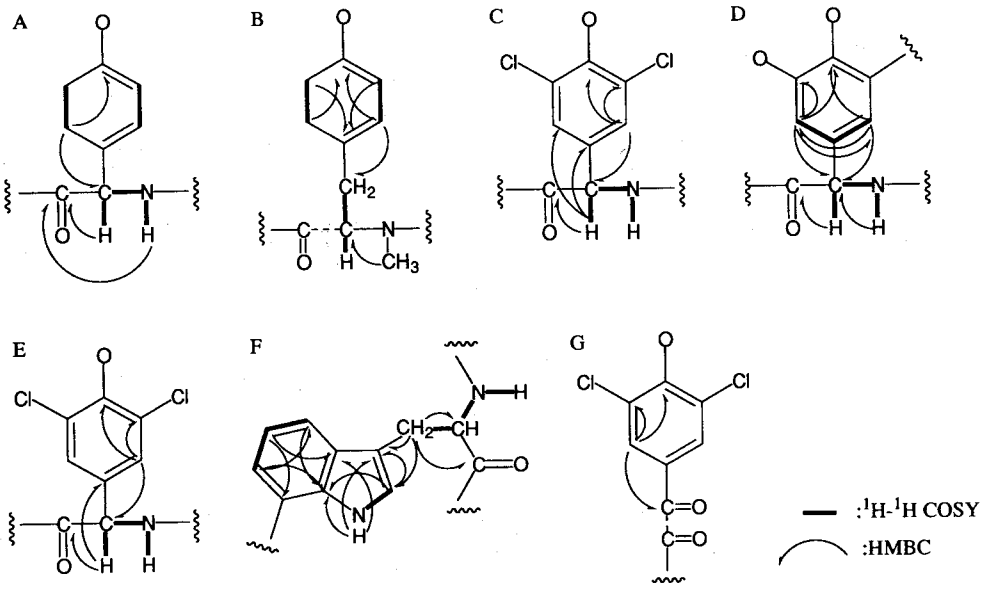


Fig. 2. HMBC correlation and NOEs of chloropeptin I (**1**).

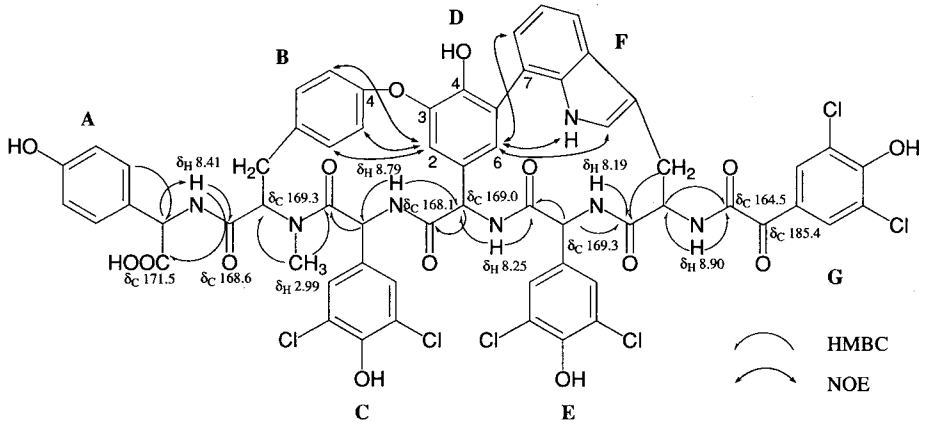
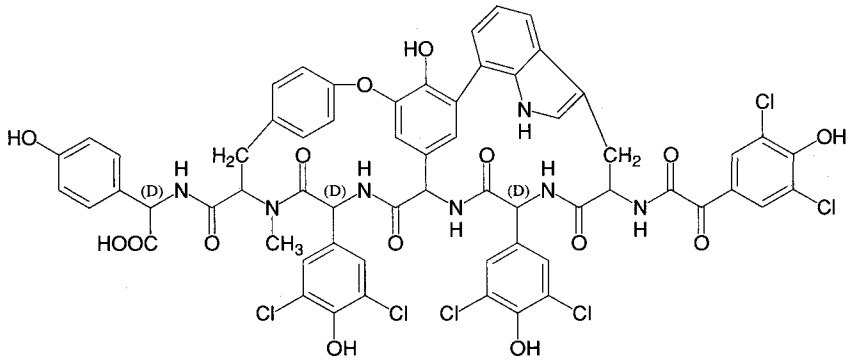


Fig. 3. The structure of chloropeptin I (**1**).



atoms.

Based on ^2J and ^3J long range correlations from HMBC, the amide connectivities of each residues were established as shown in Fig. 2. Biaryl ether bond between C-4 of Tyr (B) and C-3 of 3,4,5-trisubstituted phenylglycine (D) was confirmed by the findings that the chemical shifts of these carbon atoms were down-field shifted at δ 156.2 and δ 150.6, respectively, and that NOEs were observed between 2-H of D and 3-H, 5-H and 6-H of B, respectively (Fig. 2). The second cyclic system was deduced to be the connection between C-5 of D and C-7 of indole residue, because the strong NOEs were observed between 6-H of D and 1-H, 2-H and 6-H of indole residue. Thus, the structure of **1** was elucidated as shown in Fig. 3.

Configurations of amino acid residues were investigated based on chemical degradation. Acid hydrolysis (6N HCl, 120°C, 20 hours) of **1** gave 4-hydroxyphenylglycine (**3**) and 3,5-dichloro-4-hydroxyphenylglycine (**4**). The absolute configuration of **3** was determined to be D, corresponding to Rf value of a standard sample in chiral TLC. **4** was also determined to be D-configuration from its optical rotation ($[\alpha]_D = -65°$, $c = 0.1$, in H_2O)[3].

As described above, the structure of **1** was elucidated on the basis of NMR and chemical degradation method. **1** has six aryl amino acids and an α-keto-aryl acid residues. (D)-4-hydroxyphenylglycine and (D)-3,5,-dichloro-4-hydroxyphenylglycine were determined *via* acid hydrolysis, while the configurations of the other three amino acids, *N*-methyltyrosine, 3,4-dihydroxyphenylglycine and tryptophan remain to be clarified. If the residues of 3,5-dichloro-4-hydroxyphenylglycine, 3,5-substituted-4-hydroxyphenylglycine and 2-(3,5,-dichloro-4-hydroxyphenyl)-2-oxo-acetic acid would be biosynthesized *via* 4-hydroxyohenylglycine, all of the 3,5-substituted-4-hydroxyphenylglycine residues should have D configuration. Another compound (**2**) isolated from *Streptomyces* sp. WK-3419 was identified with complestatin. The substituted position at Trp of **1** differs from that of **2**. The mechanism of biosynthesis of **1** and **2** in *Streptomyces* sp. WK-3419 is of interest. The configurations of *N*-mehtyltyrosine, 4-hydroxyphenyl-

glycine and tryptophan residues are going to discuss in a separate paper[6].

Experimental

1 and **2** were obtained from a culture broth of *Streptomyces* sp. WK-3419 as described previously[1]. FAB-MS spectrum was obtained with a JEOL model JMS-AX505 HA spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Varian XL-400 spectrometer. Optical rotation was measured with a Jasco DIP-370 polarimeter.

Acid Hydrolysis of **1**

A solution of **1** (65 mg) in 6 N HCl (3 ml) was heated at 120°C for 20 hours in a vacuumed sealed tube, and then concentrated to dryness. The residue was dissolved in water and then purified by HPLC (Shiseido Capcell Pak C18 SG, 20 mm i.d. × 250 mm, mobile phase; 0.05% trifluoroacetic acid) to give **3** (1.5 mg, HRFAB-MS: *m/z* 168.0669 $(M+H)^+$, calcd. 168.0661 for $C_8H_{10}O_3N$) and **4** (4.0 mg, HRFAB-MS: *m/z* 235.9882 $(M+H)^+$, calcd. 235.9881 for $C_8H_8O_3NCl_2$). **3** was identified as D-4-hydroxyphenylglycine by comparison with authentic samples of D and L isomers: Rf values of D- and L- isomers of 4-hydroxyphenylglycine in chiral TLC [Chiral HPTLC plate; CHIR, Merck Art. 14101, mobile phase; acetonitrile - methanol - water (4 : 1 : 1)] were 0.63 and 0.75, respectively.

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