N-SUBSTITUTED DERIVATIVES OF EM 49 STRUCTURE-ACTIVITY RELATIONSHIPS

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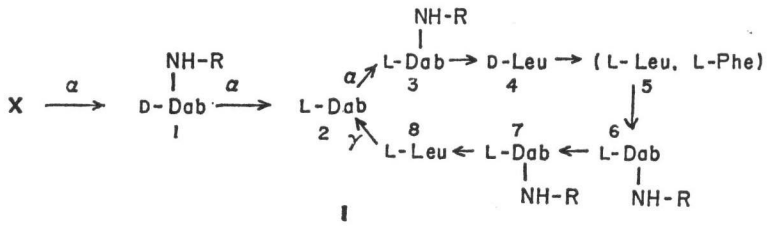
EM49 is a mixture of closely related peptide antibiotics with a broad spectrum of antimicrobial activity[1,2]. It was isolated from cultures of a strain of *Bacillus circulans* and its structure has been recently determined[3]. According to a recent nomenclature proposal[4], it belongs to a class of antibiotics named octapeptins. Like all octapeptins, EM49 is constituted of a cyclic heptapeptide with a side chain attached to the α-amino group of one of the 2,4-diaminobutyric acid residues. The side chain includes an additional diaminobutyric acid residue acylated with a C-10 or C-11 β-hydroxy fatty acid. Structural studies[3] of EM49 revealed 2.5 residues of L- and D-leucine and 0.5 of L-phenylalanine in addition to 5 residues of L- and D- 2,4-diaminobutyric acid. The total structure of EM49 is represented by **I**, R = H.



X=residues of 3-hydroxy-8-methylnonanoic acid
3-hydroxydecanoic acid
3-hydroxy-8-methyldecanoic acid

In addition to a broad spectrum of antibacterial activity, EM49 exhibits substantial antifungal and antitrichomonal activities *in vitro*. It is also active against systemic infections in mice caused by *Streptococcus pyogenes* and *Escherichia coli*[2]. Locally induced bacterial and candidal infections in mice and rats, can be successfully treated with EM49[5].

Like many other similar antibiotics, EM49 has a rather unfavorable therapeutic ratio (2~4). To explore the possibility of improving this ratio we undertook the synthesis of a number of derivatives.

The purpose of this communication is to report the synthesis and antibacterial activity of a series of EM49 derivatives in which the δ-amino groups of the 2,4-diaminobutyric acid residues have been substituted by acylation or alkylation. Other types of modification of the EM49 structure have also been explored with the same purpose and these studies will be the subject of future reports.

Chemistry

Three groups of N-substituted EM49 derivatives were synthesized:

a) aminoacyl derivatives (**I**, R=R′–NH(CH)$_n$CO–, with R″ on the CH)

b) hydroxyethyl derivatives (**I**, R=R‴–CH(OH)–CH$_2$–)

c) diguanide derivatives (**I**, R=H$_2$N–C(=NH)–NH–C(=NH)–)

For the synthesis of aminoacyl derivatives, protected amino acids were used in the form of their active esters and the reaction was carried out at pH 7.5~8.0 in dimethylformamide. The reaction was usually allowed to proceed overnight. The protecting groups were easily removed with either trifluoroacetic acid (for the *t*-butyloxycarbonyl group), hydrogen bromide solution in acetic acid or by hydrogenolysis (for the benzyloxycarbonyl group). The trifluoroacetate or hydrobromide salts thus obtained were transformed into hydrochlorides using an anion-exchange resin in the chloride form.

For the synthesis of the hydroxyethyl derivatives[6], EM49 was allowed to react with an alkyl or aryl epoxide. The temperature and the time of the reaction varied. Styrene oxide reacted under neutral conditions at room temperature with exclusion of oxygen, giving **30** (Table 1). Propylene oxide required basic medium (pH~8.0) and a low temperature to give **31**, and epoxyalkylaryl ethers required an elevated temperature to react with EM49, giving **32** and **33**.

Diguanides of EM49 were prepared easily by a reaction of EM49 with dicyanodiamide in an alcoholic solution at the elevated temperature.[7,8]

Table 1. A comparison of the antimicrobial spectra of EM49 and its derivatives

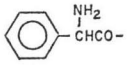
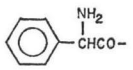
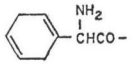
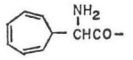
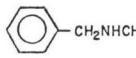
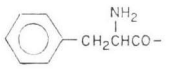
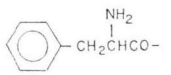
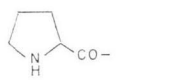
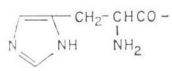
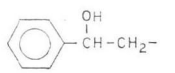
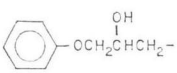
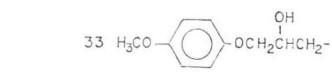
No.	R[a]	Minimal inhibitory concentration (μg/ml)						LD_{50} mg/kg (mice, s.c.)	ED_{50}[b]
		Staphylococcus aureus 209P	*Streptococcus pyogenes* C203	*Escherichia coli* SC8294	*Pseudomonas aeruginosa* SC8329	*Candida albicans* SC5314	*Trichomonas vaginalis* SC8560		
1	H_2NCH_2CO-	≥100	9.4	3.9	6.3	50	62.5	140	
2	 (D,L)	10.9	1.6	≥12.5	>100	≥100	12.5	189	
3	 (D)	≥50	1.4	>9.4	>100	>100	25	252	
4		10.9	1.2	>12.5	>100	≥100	25	189	
5		3.9	0.7	50	>50	>50	9.4	>600	
6		6.3	2.4	≥50	≥50	≥50	>25	>400	>400
7	$CH_3CH(NH_2)CO-$	75	9.4	21.3	50	>75	>75	162	
8	$H_2NCH_2CH_2CO-$	62.5	9.4	12.5	25	≥100	≥100	56	
9	$CH_3CH_2CH(NH_2)CO-$ (D)	75	4.7	15.6	>50	>100	25	120	
10	$(CH_3)_2CHCH(NH_2)CO-$	37.5	3.1	18.7	>100	>100	25	135	
11	$(CH_3)_2CHCH_2CH(NH_2)CO-$	3.1	0.8	9.4	75	31.2	25	449	23.3
12	$(CH_3)_2CHCH_2CH(NHCH_3)CO-$	18.7	1.6	18.7	>100	≥100	25	68	
13	$CH_3CH_2CH(CH_3)CH(NH_2)CO-$	6.3	0.8	12.5	≥100	37.5	43.7	253	
14	$CH_3CH_2CH_2CH_2CH(NH_2)CO-$	3.9	1.4	9.4	10.9	≥50	25	>600	
15	$H_2NCH_2CH_2CH_2CH_2CH_2CO-$	>100	6.3	37.5	>100	>100	>100	42	
16	$CH_3CH_2CH_2CH_2CH_2CH_2CH(NH_2)CO-$	12.5	4.7	>50	75	≥50	≥25	>600	
17	$H_2NCH_2CH_2CH(NH_2)CO-$	>50		>50	6.3	>50	25		
18	$H_2NCH_2CH_2CH_2CH_2CH(NH_2)CO-$	12.5	0.6	12.5	12.5	>100	25	94	
19	$CH_3CH(OH)CH(NH_2)CO-$	3.1	1.0	12.5	>100	>100	50	>727	
20	$H_2NCH_2CH_2CH(OH)CO-$	>50		25	>50	>50	>50		

Table 1. (continued)

No.	R[a]	Minimal inhibitory concentration (μg/ml)						LD_{50} mg/kg (mice, s.c.)	ED_{50}[b]
		Staphylococcus aureus 209P	*Streptococcus pyogenes* C203	*Escherichia coli* SC8294	*Pseudomonas aeruginosa* SC8329	*Candida albicans* SC5314	*Trichomonas vaginalis* SC8560		
21		9.4	3.5	21.3	>100	50	25	435	
22		3.1	0.8	9.4	>100	25	21.9	>727	
23		2.0	0.3	7.8	>50	≥50	6.3	>600	470
24		75	9.4	37.5	>100	>100	≥100	189	
25		15.6	2.4	7.8	50	≥100	50	443	
26	H_2NNHCH_2CO-	4.7		2.4	3.9	21.8	>50	>300	>300
27	H_2NOCH_2CO-	3.9		2.4	3.1	21.8	≥50	284	73
28	 [c]	4.7	0.8	1.2	1.6	9.4	>50	190	>60
29		6.3	3.1	2.8	9.4	43.8	>50	190	>60
30		3.1		3.5	3.1	6.3	>12.5	>400	>400
31		37.5		2.4	9.4	50		169	>200
32		12.5		37.5	>100	>100			
33		6.3		9.4	25	75			
34	EM49	5.5	0.63	0.47	0.9	9.4	18.9	150	18.7

Blank space indicates that the compound was not tested.

[a] aminoacyl residues originating from naturally occurring amino acids are L-configuration unless otherwise marked.

[b] ED_{50} in mg/kg by subcutaneous route against mice infected systemically with *Escherichia coli* SC8294.

[c] only three δ-amino groups of EM49 were substituted.

Varying the amounts of dicyanodiamide, we were able to prepare tris and tetrakis derivatives of EM49 as indicated by the elemental analysis.

The homogeneity and purity of the derivatives were ascertained by paper and thin-layer chromatography, elemental analysis and amino acid analysis.

Biological Results

Minimal inhibitory concentrations (MIC) in

μg/ml for a group of bacteria, fungi, and protozoa are presented in Table 1. In addition, the ED_{50} values for several of the compounds against an experimental *Escherichia coli* (SC 8294) infection in mice are given. The details of the experimental biological procedure have already been described[2].

The majority of the derivatives obtained by acylation of EM49 showed fair to good activity against *Staphylococcus aureus* and *Streptococcus pyogenes*. Some of them (**5**, **6**, **11**, **14**, **19**, **22**, **23**, **26**, **27**) exhibited equal or better activity against the gram-positive organisms than EM49. Their antipseudomonal activity, except for **26** and **27**, was considerably lower than that of EM49. Among the derivatives with naturally occurring amino acids, the L-phenylalanyl derivative (**22**) showed good activity against gram-positive organisms, but the activity against gram-negative organisms was inferior to that of EM49. Antifungal activity of both compounds was comparable. It is noteworthy that the derivatives with the L- and D-isomers of phenylalanine (**22** and **23**, respectively) showed very similar antimicrobial activities and that the toxicity of both compounds was substantially lower than that of EM49. Good activity against gram-positive bacteria and low toxicity were obtained when the amino groups of EM49 were acylated with threonine residues (**19**). However, low activity against gram-negative organisms did not allow the selection of this derivative for further studies. Similar antimicrobial activity but slightly better antifungal activity was obtained with the derivative containing leucine residues (**11**). The derivatives that included in their structures residues of 2,4-diaminobutyric acid (**17**), proline (**24**), or 2-hydroxy-4-aminobutyric acid (**20**) were almost completely devoid of activity.

Alkylation of EM49 did not alter, in most cases, (**28**~**30**), the minimal inhibitory concentrations of EM49 against gram-positive and gram-negative bacteria. Compound **30**, in which there were two substituents in the short alkyl residue (hydroxyl and phenyl groups in the ethyl residue bound to the primary amino groups of EM49), exhibited completely comparable activities to those of EM49 against the tested bacteria. The toxicity of **30** was much lower than that of EM49, but was unable to modify the course (ED_{50} > 400 mg/kg) of a lethal infection in mice. The antifungal activity of the rest of these derivatives except for **28** and **30** was decreased, especially where the primary amino groups of EM49 (**32**, **33**) were hydroxyalkylated.

Acknowledgements

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