CARBON-13 NMR STUDIES OF EM 49 AND RELATED OCTAPEPTINS

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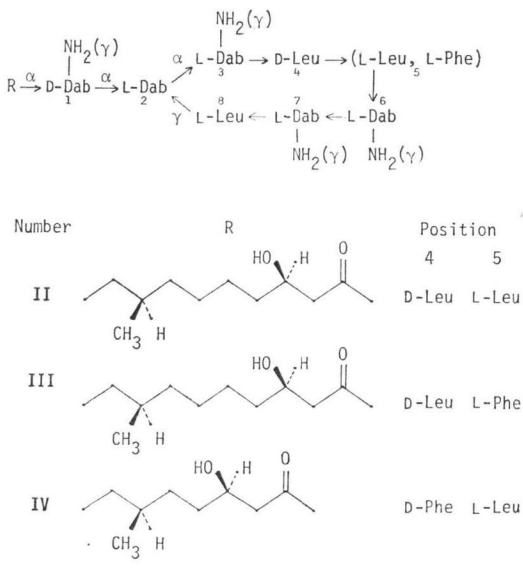
EM 49 is a mixture of closely related peptide antibiotics isolated from cultures of a strain of *Bacillus circulans*[1~3]. Its structure and similarities with polymyxins have been discussed[4]. The peptide antibiotics have been classified as octapeptins[5] and we report here the comparison of their cmr data including carbon-13 spin-lattice relaxation behavior of EM 49 in solution.

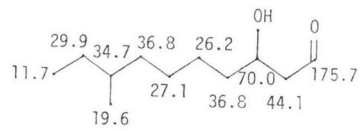
Proton-decoupled carbon-13 NMR spectra of EM 49 (**I**) (R=complex mixture, Fig. 1) revealed the presence of eight quartets at δ (ppm) 11.6, 14.3, 19.6, 21.5, 21.9, 22.1, 22.9 and 23.2, sixteen triplets at 26.1, 27.2, 28.7, 29.3(2), 29.8, 30.3, 31.4, 36.4, 36.7, 37.3(2), 37.4(2), 39.8, 40.3 and 44.2, eighteen doublets at 24.9, 25.3(2), 34.6, 51.7, 52.3, 52.7, 53.0(2), 53.5, 53.9, 55.2, 69.9, 128.0, 129.5(2) and 129.9(2) and twelve singlets at 137.5, 172.6(2), 173.4, 173.8(3), 174.1, 174.4, 174.7, 175.4, and 175.6 ppm. The relative intensities of some carbons were less than one, consistent with nature of the complex.

Fig. 1.



The power of ^{13}C NMR spectrometry was immediately apparent when under the conditions of long pulse delay (3~5 times the longest T_1) the quantities of Phe component, determined by comparing with the intensity of CHOH carbon at δ 69.9, were found to be in agreement with the amino acid analysis, *e.g.*, **I** (0.5 Phe) and **IV** (1.0 Phe).

To simplify the assignments of ^{13}C NMR data EM 49 was chromatographically separated into two major components; octapeptins A_1 (**II**) and B_1 (**III**). Noise decoupled spectra are shown in Fig. 2 and the data are presented in Table 1. The analysis of the data indicated the same acyl moiety for II and III, and by the process of elimination the following assignments were made.



The absence of Phe carbons and the presence of three CH_2 (39.6~40.2 ppm) and six CH_3 (21.4~23.3 ppm) carbons were consistent with the presence of three Leu molecules in **II**. The appearance of δ 14.3 resonance in **I** indicated minor components such as octapeptins A_3 and B_3 containing a linear acyl function. However, limited quantities prevented detailed analysis of cmr data.

Octapeptin C (**IV**) has been assigned a different structure containing D-Phe instead of D-Leu at position 4 (Fig. 1)[6]. The comparison of cmr data of **III** and **IV** indicated that the above substitution resulted in an upfield shift (δ c*=137.5 (**III**)→136.7 (**IV**)) of the quarternary carbon of the Phe ring, whereas, the α-CH carbon of Phe shifted downfield (δ CH=55.1 (**III**)→56.2 (**IV**)). These differences in the chemical shifts are not unexpected considering the nature of the amide bond in a chiral environment. The acyl function, which has two carbons less, was assigned the following chemical shifts.

The chemical shifts of CHOH and CH_3 carbons of the acyl functions in **II**, **III** and **IV** suggested that the stereochemistry was identical for both the fatty acids.

Similar analysis of cmr data of polymyxin B

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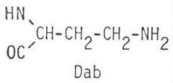
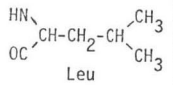
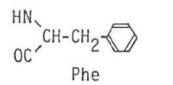
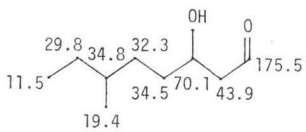
Table 1. ^{13}C-NMR chemical shifts for octapeptins

Amino acid	Literature[a,b]	II[c]	III	IV
 Dab	171.6	173.9,173.7(2),172.7(2)	174.0(2),173.5,172.7,172.6	173.7,173.6(2),172.7,172.3
	51.4	53.7, 52.7, 52.3, 51.7	53.8, 52.8, 52.2, 51.6	53.9, 52.8, 52.4, 51.6
	28.4	31.3, 30.2, 29.4(2), 28.8	31.4, 30.2, 29.6, 29.2, 28.7	31.5, 30.3, 29.5(2), 28.8
	40.0	37.6(2), 37.4(3)	37.4(5)	37.6(2), 37.5(2), 37.1(1)
 Leu	180.8	175.4, 174.7, 174.4	174.7, 174.5	175.5, 174.6
	54.8	53.7(1), 53.2(2)	53.0(2)	53.1(2)
	41.7	40.3, 40.1, 39.8	40.2, 39.6	40.2, 39.8
	25.5	25.3(3)	25.3, 24.9	25.3, 24.6
	23.3	23.3, 23.0, 22.8	23.0, 22.8	23.1, 22.9
	21.7	22.1, 21.9, 21.4	22.2, 21.9	21.8, 21.5
 Phe	178.8		174.0	173.9
	57.0		55.1	56.7
	38.6		36.4	36.4
	138.8(c*)		137.5(c*)	136.7(c*)
	129.4(2)		130.0(2)	129.9(2)
	130.1(2)		129.5(2)	129.7(2)
	127.6(1)		127.9(1)	128.1(1)

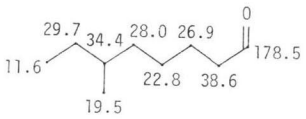
[a] Dab 2HCl; Leu and Phe in presence of $NaHCO_3$.

[b] These values are comparable with those reported in literature, *e.g.*, DESLAURIERS, R.; A. C. M. PAIVA, K. SCHAUMBURG & IAN C. P. SMITH, Biochemistry 14: 878, 1975

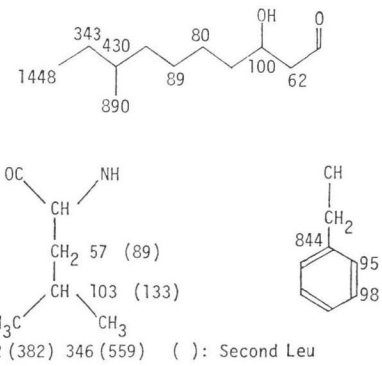
[c] The assignments of amide carbons are tentative.



in D_2O (unpublished data) as well as of a double pulse experiment, $(180°\text{–}t_1\text{–}90°\text{–}T)_n$ where t_1= 0.60 second, resulted in defining the acyl moiety as:



Finally the carbon-13 relaxation behavior of **I** was studied to understand the molecular motion of a medium ring peptide in D_2O (Fig. 3). Relaxation time (T_1, m sec) data obtained for a limited number of carbons resulted in values which are comparable to the relaxation data of nonapeptide hormones oxytocin and lysine-vasopressin[7], *e.g.*, T_1 (CO)$\simeq$1100 and T_1 (CH)$\simeq$ 55 m sec, indicating a similar degree of rigidity. However the fatty acid component exhibited a much greater conformational freedom than the cyclic ring component.



Experimental

Carbon magnetic resonance (cmr) spectra were recorded on a Varian XL–100–15 spectrometer operating at 25.16 MHz, equipped with FOURIER Transfrom accessories from Nicolet Technology Corporation. The spectrometer was internally locked to the deuterium frequency (15.4 MHz) of the solvent. Solutions of the compounds in

Fig. 2.

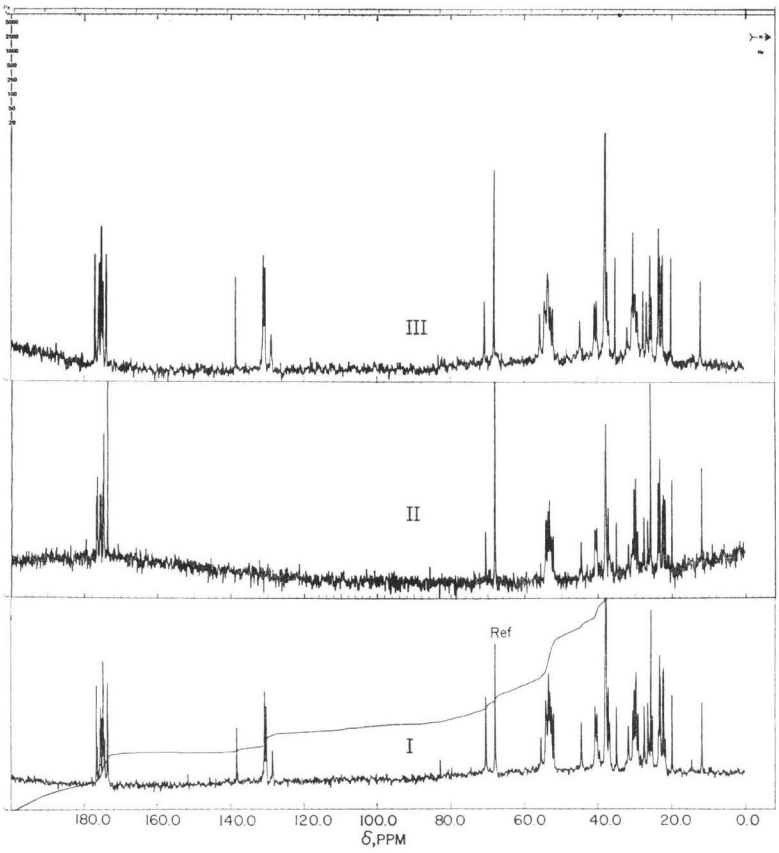
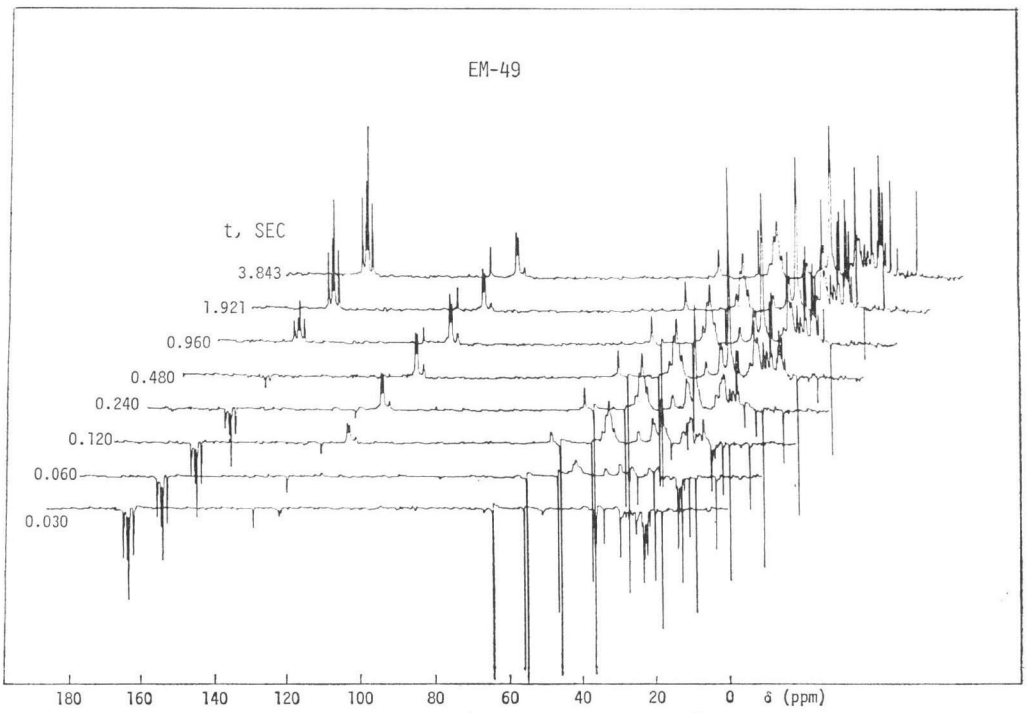

III
II
Ref
I
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
δ,PPM

Fig. 3.


EM-49
t, SEC
3.843
1.921
0.960
0.480
0.240
0.120
0.060
0.030
180
160
140
120
100
80
60
40
20
0
δ (ppm)

deuterium oxide containing dioxane as internal reference were used at 60°C. Typical spectra were obtained using sweep width, 3,000 Hz(QPD), pulse width, 9 μsec ($\simeq$40° tip angle), pulse interval, 5 or 10 sec and 8k data table. The chemical shifts (Table 1) were calculated with reference to δ dioxane=67.4 ppm from TMS.

Carbon-13 spin-lattice relaxation was carried out utilizing Varian FT-80A NMR spectrometer operating at 20 MHz and at ambient temperature. Concentration was approximately 400 mg/2 ml D_2O. Inversion-recovery pulse sequence, $(180°-t-90°-T)_n$ was used to calculate T_1 values utilizing Varian Software programs and a three-parameter non-linear least squares fit for the best values of T_1, M_0 and k to the following equation.

$$M_t=M_o\,(1+2\,ke^{-t/T_1})$$

The samples were available as chromatographically separated and purified materials.

Acknowledgements

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