Tetrahedron Letters No. 3, pp 185 - 188, 1976. Pergamon Press. Printed in Great Britain.

CARBON-13 NMR STUDIES OF PEPTIDE ANTIBIOTICS, THIOSTREPTON AND SIOMYCIN A: THE STRUCTURE RELATIONSHIP

Kazuo Tori,* Katsuya Tokura, Kei Okabe, Mitsuo Ebata and Hideo Otsuka

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 Japan

and

Gabor Lukacs

Institut de Chimie des Substances Naturelles, C.N.R.S., 91190-Gif-sur-Yvette, France

(Received in Japan 13 November 1975; received in UK for publication 8 December 1975)

The structure of thiostrepton (1), a peptide antibiotic isolated from Streptomyces azureus,[1] has been investigated by chemical degradation for a long time.[2] Many degradation products have hitherto been revealed, e.g., thiostreptonic acid, thiostreptoic acid, thiostreptine, many other thiazole-4-carboxylic acid derivatives, several quinaldic acid derivatives, and several amino acids: two Ala's, Ile, Thr, Cys, butyrine (But) including two moles of pyruvic acid from dehydroalanines (Deala).[2] Thereafter, a tentative structure of 1 has been proposed by an X-ray crystallographic analysis[4] as shown in FIG. 1 (except for the Deala residue in a parenthesis). However, it is not a final one because the presence of one or two additional alanine precursors were suggested in the molecule;[3] it has been postulated[4] that the residue(s), if they occur, should be an extension of the long side chain.

Siomycin A (2),[5] another peptide antibiotic isolated from Streptomyces sioyaensis,[5a] having a structure quite similar to that of 1,[6] has also been studied by degradative methods;[6] 2 has a Val residue instead of Ile and lacks one Ala residue as compared with 1.[6] A tentative structure of 2 has been proposed as a result.[6b]

We wish to report here ^{13}C NMR spectroscopic studies of these two antibiotics to elucidate their structure relationship.

The natural-abundance ^{1}H-noise-decoupled 15-MHz ^{13}C FT NMR spectra of 1 and 2 in DMSO-d_6 and $CDCl_3$-CD_3OD (a 8 : 2 mixture) at ambient temperatures exhibited very broad CH_2- and CH-signals for an

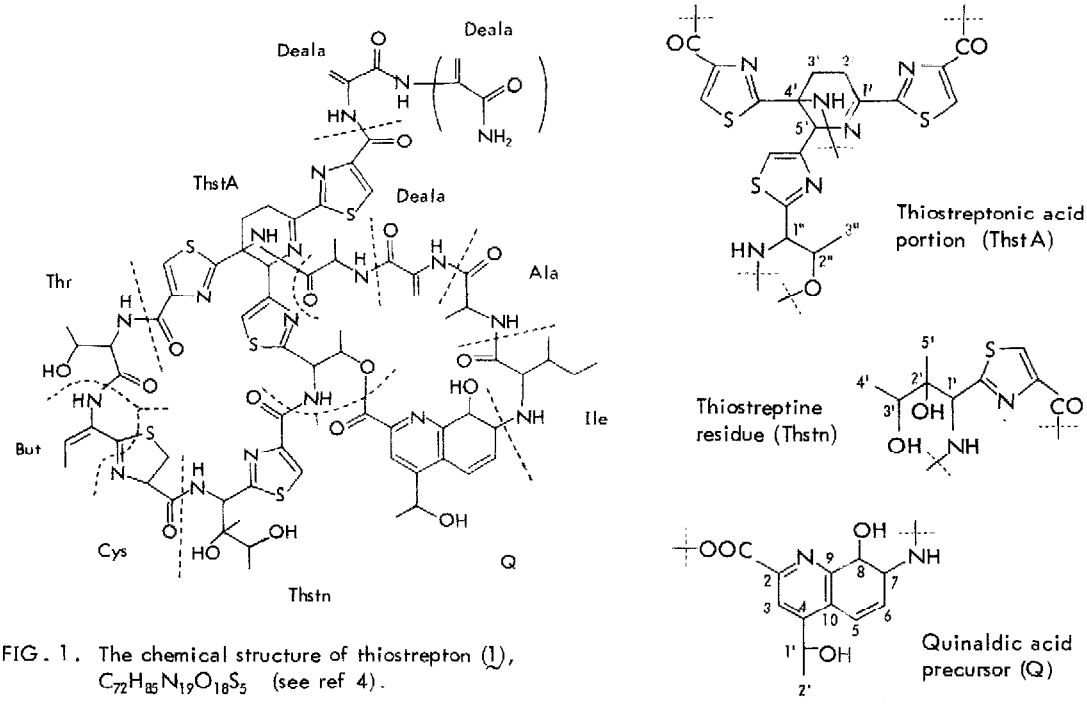

FIG. 1. The chemical structure of thiostrepton (1), $C_{72}H_{85}N_{19}O_{18}S_5$ (see ref 4).

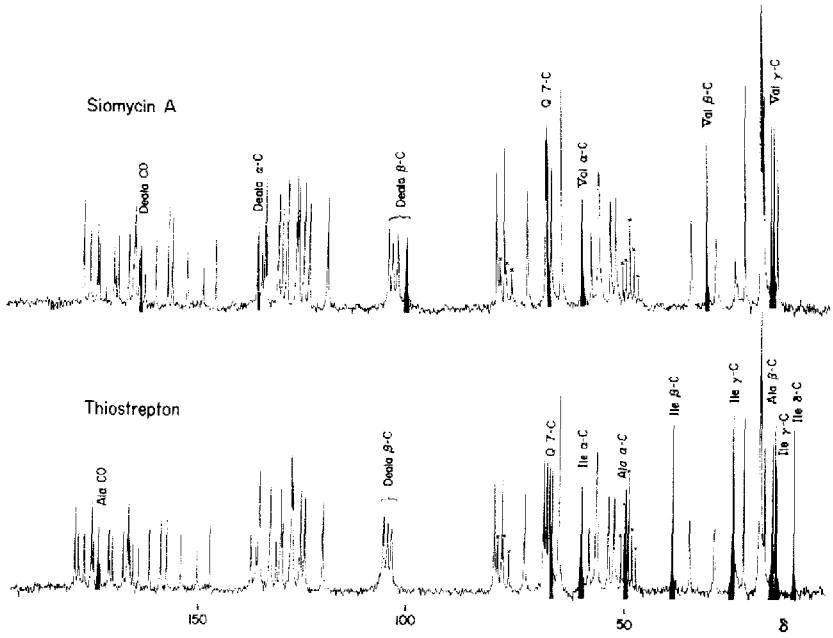

FIG. 2. The ^1H-noise-decoupled ^{13}C FT NMR spectra of thiostreptone and siomycin A at 25 MHz in $CDCl_3$–CD_3OD (8 : 2) at 70° (taken with a JEOL FX-100 spectrometer, 10-mm tubes; 120 mg cm^{-3}; pulse flipping angle, 90°; number of transients, 5,000); x, solvent peaks.

TABLE. ^{13}C Chemical Shifts[a] and Tentative Assignments

	Thiostrepton (1)	Siomycin A (2)
C-$\underline{C}H_3$	11.6(Ile δ); 15.4, 15.9(Ile γ, Ala β); 16.5(Ala β); 23.0(Q 2'); 18.8, 19.0, 19.2×3	15.4(Ala β); 16.5, 17.1(Val γ×2); 23.0(Q 2'); 19.0×2, 19.4×3
C(S)-$\underline{C}H_2$	25.0(ThstA 2'); 25.3(Ile γ); 29.7(ThstA 3'); 35.2(Cys β)[c]; 39.2(Ile β)	25.1(ThstA 2'); 29.6(ThstA 3'); 35.2(Cys β)[c]; 31.8(Val β)
C-$\underline{C}H$		
N(O)-$\underline{C}H$	49.9(Ala α); 52.4, 53.7(Ala α, ThstA 1"); 56.2, 56.4(Thr α, Thstn 1'); 59.6(Ile α); 64.9(ThstA 5'); 66.2(Q 7)[b]; 67.1(Thr β); 79.7(Cys α)[c]; 64.9, 68.4, 68.6, 72.6 (Q 8, 1', Thstn 3', ThstA 2")	52.4, 53.7(Ala α, ThstA 1"); 56.2, 56.4 (Thr α, Thstn 1'); 59.9(Val α); 64.8(ThstA 5'); 67.1(Thr β); 68.0(Q 7);[b] 79.7(Cys α)[c]; 64.8, 68.4, 68.8, 72.5 (Q8, 1', Thstn 3', ThstA 2")
N(O)-$\underline{C}$	58.2(ThstA 4'); 77.7(Thstn 2')	58.0(ThstA 4'); 77.7(Thstn 2')
C=$\underline{C}H_2$	103.0, 103.9, 104.8(Deala β)	100.5, 102.7, 104.0, 104.9(Deala β)
C=$\underline{C}H$	118.8(But β); 122.9, 123.6, 125.4, 125.8 127.8, 130.6, 133.0	118.8(But β); 123.0, 124.1, 125.4, 125.8 128.0, 130.6, 132.7
C=$\underline{C}$	127.8, 129.2(Q 4, 10)	128.0, 129.1(Q 4, 10)
C=$\underline{C}$-N	133.0, 133.9, 135.0(Deala α); 144.2(But α); 147.0, 150.6×2, 153.7, 155.3, 157.7, 160.0; 169.5(Ala CO); 161.5, 162.0, 162.4×2, 162.7, 163.5, 166.1, 166.6, 167.3, 169.0, 170.5×2, 170.7, 172.1, 173.7, 174.2	132.7, 133.8, 134.7×2(Deala α); 144.2(But α); 147.0, 150.6×2, 153.9, 155.1, 157.7, 160.0; 161.1(Deala CO); 161.5, 162.1, 162.4×2, 162.7, 163.7, 166.0, 166.6, 167.3, 168.9, 170.5×2, 170.7, 172.1, 173.7, 173.9
N=$\underline{C}$		
$\underline{C}O$		
N=$\underline{C}$-S		

[a] ^{13}C NMR spectra were mainly determined with a Varian NV-14 FT NMR spectrometer at 15.087 MHz at 90° using a 8:2 mixture of $CDCl_3$-CD_3OD as a solvent in 8-mm tubes; concentrations were about 400 mg cm^{-3}; errors of δ_C (from internal TMS) are within 0.2; FT measurement conditions were as follows: spectral width, 3170 Hz; pulse flipping angle, ca. 30°; acquisition time, 0.6 sec; number of data points, 3706; number of transients, 300,000-360,000.

[b] This assignment is solely due to the proximity of the N$\underline{C}$H to Ile or Val.

[c] The thiazoline-ring carbon signals were assigned according to a recent study of althiomycin.[9]

accurate count of their carbon numbers to be made. The resonance broadening is probably due to the large globular molecular shape of these substances. However, at elevated temperatures (>70°), they become sharper in $CDCl_3$-CD_3OD and the carbon numbers were found to be 72 for 1 and 71 for 2. FIGURE 2 shows the 25-MHz ^{13}C NMR spectra of 1 and 2.

The ^{13}C signal assignments were, though not fully accomplished, carried out tentatively by ^1H noise[7] and single-frequency off-resonance[8] decoupling techniques and using known chemical-shift rules,[8] chemical shifts of the amino acids reported[8] and those observed for thiostreptine and methyl 4-(α-hydroxyethyl)-8-methoxyquinaldate (see the TABLE). It is clearly demonstrated, as seen from the TABLE, that the spectra

of 1 and 2 are quite similar to each other except for the signals of the Ile and one of the Ala residues and a signal (probably due to 7-NCH in Q) at δ_C 66.2 in 1, and those of the Val and one of the Deala residues and a signal at δ_C 68.0 in 2 (see the filled signals in FIG. 2).

The number of alanine precursors in 1 has frequently been discussed.[2-4] The ^{13}C spectra suggest that three and four Deala residues are present in 1 and 2, respectively. Our results from amino-acid analyses of 1, 2, and their $NaBH_4$-reduction[3] products were in agreement with the above spectral results. Signals due to $CH_2=C<$ of Deala and $-CH=C<$ of But almost disappeared from the ^{13}C spectra of the reduction products, each of which was a mixture consisting of several components.

As a result, it is concluded that thiostrepton has the structure 1 shown in FIG. 1 (including Deala in the parenthesis) if Hodgkin and coworkers' structure and suggestive comment proposed[4] are accepted, and that the structure of siomycin A is different from that of thiostrepton only in one portion: Val-Deala residue of the former[6] corresponds to Ile-Ala residue of the latter.[2]

Acknowledgements. We thank Prof. M. Bodanszky and the Squibb Institute for an authentic sample of thiostrepton used for a preliminary spectral study, ATCC for a strain of S. azureus, and JEOL Co. for the 25-MHz ^{13}C FT NMR spectra.

REFERENCES

(1) (a) J. Vandeputte and J. D. Dutcher, Antibiot. Ann. 560 (1955-1956); (b) M. Bodanszky, J. D. Dutcher and N. J. Williams, J. Antibiot. A16, 76 (1963), and references therein.
(2) (a) M. Bodanszky, J. Fried, J. T. Sheehan, N. J. Williams, J. Alicino, A. I. Cohen, B. T. Keeler and C. A. Birkhimer, J. Amer. Chem. Soc. 86, 2478 (1964); (b) M. Bodanszky, J. A. Scozzie and I. Muramatsu, Ibid. 91, 4934 (1969); and references therein.
(3) M. Bodanszky, J. A. Scozzie and I. Muramatsu, J. Antibiot. 23, 9 (1970).
(4) B. F. Anderson, D. C. Hodgkin and M. A. Viswamitra, Nature 225, 233 (1970).
(5) (a) H. Nishimura, S. Okamoto, M. Mayama, H. Otsuka, K. Nakajima, K. Tawara, M. Shimohira and N. Shimaoka, J. Antibiot. A14, 255 (1961); (b) M. Ebata, K. Miyazaki and H. Otsuka, Ibid. 22, 364 (1969).
(6) (a) M. Ebata, K. Miyazaki and H. Otsuka, Ibid. 22, 423 (1969); (b) Idem., Ibid. 22, 434 (1969).
(7) E. Wenkert, A. O. Clouse, D. W. Cochran and D. Doddrell, J. Amer. Chem. Soc. 91, 6879 (1969).
(8) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, New York (1972); J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York (1972).
(9) B. W. Bycroft and R. Pinchin, J.C.S. Chem. Comm. 121 (1975).