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Natural Abundance ^{15}N Nuclear Magnetic Resonance Spectroscopic Evidence for the Structural Relationship between the Peptide Antibiotics Thiostrepton and Siomycin-A

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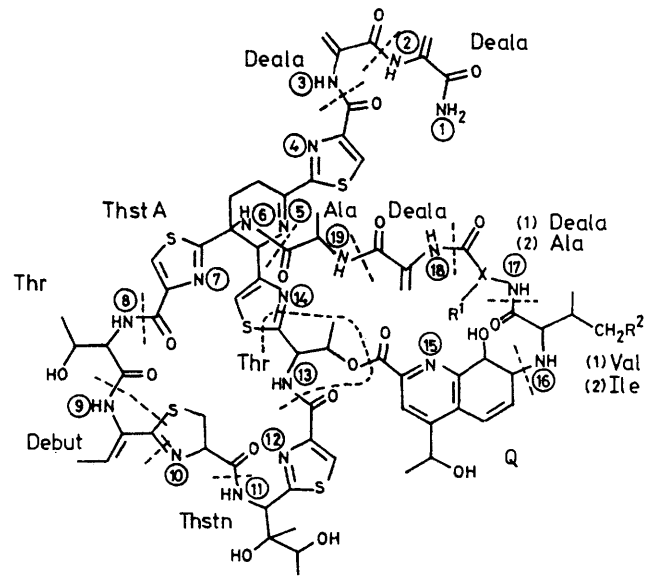
Summary Nitrogen-15 n.m.r. spectral comparison between the antibiotics thiostrepton and siomycin-A has permitted further evidence for the structure of the latter to be obtained.

As a result of chemical degradations[1] and a ^{13}C n.m.r. study[2] the tentative structure (**1**) has been proposed for the peptide antibiotic siomycin-A isolated from *Streptomyces sioyaensis*.[3] We now provide further evidence for the complete structure of siomycin-A by comparing its ^{15}N n.m.r. spectrum with that of the closely related antibiotic thiostrepton (**2**) of known constitution.[2,4] The structural difference between the two antibiotics was previously suggested to be the substitution of an Ile-Ala dipeptide unit of (**2**) by a Val-Deala residue in (**1**).[1,2]

Nitrogen-15 n.m.r. spectroscopy is a promising tool for studies of complex molecules of biological interest[5–9] since the chemical shift range of nitrogen covers about 800 p.p.m.[10] With the availability of n.m.r. spectrometers operating at higher fields for ^{15}N, the severe sensitivity problems associated with this nucleus can be overcome[9] even for compounds of the size investigated here.



(**1**) X = C, R^1 = CH_2, R^2 = H
(**2**) X = CH, R^1 = R^2 = Me

Amino-acid sequence information in peptides from ^{15}N n.m.r. spectra seems to depend on solvent.[5,11,12] Of the 19 nitrogen atoms present in both (**1**) and (**2**), 16 have almost identical chemical shifts. As a result of the structural difference in the nature of the dipeptide units, the 2 nitrogen atoms involved in the dipeptide units and the following Deala-nitrogen are expected to exhibit different chemical shifts because of the effect of the neighbouring residues.[11,12] On the basis of well established α-, β-, γ-, δ-, and ε-effects[13,14] the Table contains tentative signal assignments for the amine and for all the amide nitrogens. As a consequence of the loss of a deshielding δ-effect the Val-nitrogen of (**1**) appears 5·1 p.p.m. upfield relative to the Ile-nitrogen signal of (**2**). From the observed chemical shifts Deala-nitrogen resonances are close to those of Ala-nitrogens. The dehydrobutyrin (Debut)-nitrogen resonates a little upfield because of one shielding γ-effect, the Thr-nitrogens further upfield because of two such effects, and the thiostreptine (Thstn)-nitrogen at the highest field because of three.

Signal assignment of the two backbone Deala-nitrogens of (**1**) is not unambiguous. It has been established that a given amino-acid nitrogen involved in a peptide resonates at the lowest possible field when the contributions from the preceding residue across a carbonyl group amount to two δ-effects and no shielding ε-effect.[11] Since this is the case for (**1**) the assignment of the lower-field shift to the Val-following Deala unit is preferred. It is not clear at the moment why the Ala-preceding Deala-nitrogen signal of (**1**) is shifted by 4·7 p.p.m. upfield relative to that of (**2**) as a result of the sequence effect.

The ^{15}N signal of the primary amide in the side chain can be easily assigned by its chemical shift[8] without the help of the time-consuming ^{1}H-undecoupled spectrum. The lowest field signal of (**2**) in the amide region is attributed, in view of the β-effects, to that originating from its thiostreptonic acid (ThstA) portion. The thiazole, pyridine, and C=N nitrogens could not be unambiguously differentiated.[10]

TABLE. ^{15}N N.m.r. chemical shifts (p.p.m.) in $CDCl_3$–MeOH (8:2) solution, upfield from external $^{15}NO_3^-$ in H_2O and tentative signal assignments for the antibiotics siomycin-A (**1**) and thiostrepton (**2**).[a]

(**1**)	Nitrogen number	Assignment	(**2**)
55·2; 63·6; 64·1; 66·6	4; 5; 7; 10[b]	Azine-nitrogens	54·9; 63·5; 64·1; 66·1
72·0; 85·3; 87·5	12; 14; 15		72·2; 84·8; 87·4
250·1	17	Backbone Deala following Val	—
253·1	6	Thiostreptonic acid (ThstA) unit	253·2
253·8; 253·8	2; 3	Side-chain Deala	253·9; 253·9
—	18	Backbone Deala	254·8
—	17	Ala following Ile	255·3
256·8	19	Ala	256·8
259·2	9	Dehydrobutyrin (Debut)	259·3
259·5	18	Backbone Deala preceding Ala	—
262·9; 264·5	8; 13[b]	Thr	263·1; 264·7
265·6	11	Thiostreptine (Thstn) unit	265·4
280·4	1	Side-chain $CONH_2$	280·9
—	16	Ile	331·4
336·5	16	Val	—

[a] ^{15}N N.m.r. spectra of the antibiotics were recorded at 36·48 MHz and 25 °C with a Bruker WH-360 Fourier transform n.m.r. spectrometer using 1 g of substance in 4 ml of $CDCl_3$–MeOH (8:2) and 15 mm diameter sample tubes. Broad-band ^{1}H decoupling (2·7 W) was employed in the inverse-gated mode to provide a decoupled spectrum but without nuclear Overhauser enhancement. A spectral width of 14,000 Hz was acquired in 16K data points using a 50 μs (*ca.* 70° flip angle) pulse width. The acquisition time (with ^{1}H decoupling) of 0·57 s was preceded by a delay (without decoupling) of 3 s. A total of 15,500 scans were accumulated and transformed with an exponential line broadening of 0·6 Hz. [b] Specific assignments cannot be made.

On the basis of the highly sensitive ^{15}N n.m.r. chemical shift identities and differences between the spectrum of (**1**) and (**2**), the earlier proposal for the structure of siomycin-A as (**1**)[2,3] can be accepted with confidence. The ^{15}N signal assignment presented here will be of great help in future structural investigations of antibiotics of the thiostrepton family.

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[1] M. Ebata, K. Miyazaki, and H. Otsuka, *J. Antibiotics*, 1969, **22**, 423.
[2] K. Tori, K. Tokura, K. Okabe, M. Ebata, H. Otsuka, and G. Lukacs, *Tetrahedron Letters*, 1976, 185.
[3] M. Ebata, K. Miyazaki, and H. Otsuka, *J. Antibiotics*, 1969, **22**, 434.
[4] B. F. Anderson, D. C. Hodgkin, and M. A. Viswamitra, *Nature*, 1970, **225**, 233.
[5] G. E. Hawkes, E. W. Randall, and C. H. Bradley, *Nature*, 1975, 257, 767.
[6] D. Gust, R. B. Moon, and J. D. Roberts, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4696.
[7] D. E. Dorman, J. W. Paschal, and K. E. Merkel, *J. Amer. Chem. Soc.*, 1976, **98**, 6885.
[8] H. Depaire, J.-P. Thomas, A. Brun, W. E. Hull, A. Olesker, and G. Lukacs, *Tetrahedron Letters*, 1977, 1401.
[9] G. E. Hawkes, E. W. Randall, and W. E. Hull, *J.C.S. Chem. Comm.*, 1977, 546.
[10] M. Witanowski, L. Stefaniak, and H. Januszewski, 'Nitrogen NMR,' Plenum Press, 1973.
[11] T. B. Posner, V. Markowski, P. Loftus, and J. D. Roberts, *J.C.S. Chem. Comm.*, 1975, 769.
[12] D. Gattegno, G. E. Hawkes, and E. W. Randall, *J.C.S. Perkin II*, 1976, 1525.
[13] P. S. Pregosin, E. W. Randall, and A. I. White, *Chem. Comm.*, 1971, 1602.
[14] R. L. Lichter and J. D. Roberts, *J. Amer. Chem. Soc.*, 1972, **94**, 2495.