High Field ^{15}N Nuclear Magnetic Resonances Spectroscopy of Peptides. Assignments in Viomycin Sulphate

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Summary All 13 resonances from the natural abundance 36·48 MHz ^{15}N n.m.r. spectrum of viomycin have been observed and assignments made, largely on the basis of selective low power ^{1}H decoupling experiments in com-

bination with off-resonance effects, which confirm the structure of the guanidinium group at pH 2·8.

THE antibiotic peptide viomycin (I), a member of the tuberactinomycin group, has been extensively studied by chemical, *X*-ray, and spectroscopic methods.[1] An important feature of the structure in both crystal and solution is the presence of the transannular hydrogen bond between the proton at N-9 and the carbonyl oxygen at C-21.

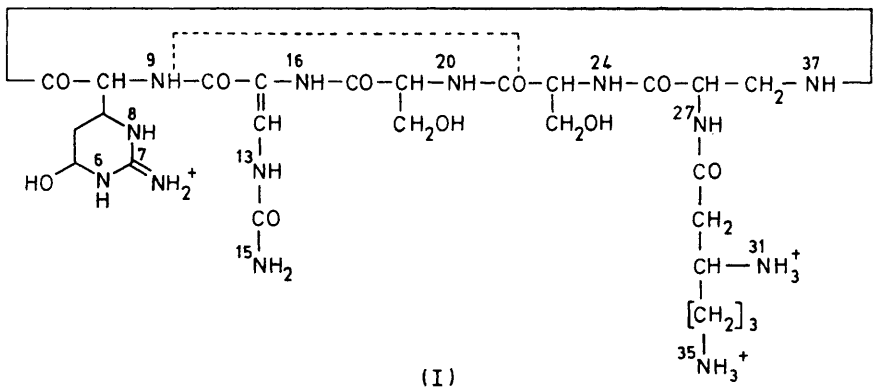
Published data[2] on ^{15}N n.m.r. spectra of peptides are so far restricted to quite simple molecules, and the complex interplay of sequence, H-bonding, and medium effects upon ^{15}N chemical shifts makes assignment by predictive methods unreliable. Indeed by considering chemical shifts alone only a partial assignment has been made[3a] for the relatively simple ^{15}N n.m.r. spectrum of the peptide gramicidin S.

The chemical shifts from the 36·48 MHz natural abundance ^{15}N n.m.r. spectrum of (I), together with those from the high frequency (low field) region of its ^{1}H n.m.r. spectrum, are shown in the Table. We have confirmed the assignments in the ^{1}H n.m.r. spectrum, quoted by Wakamiya and Shiba,[1] with the additional observation of broad $-NH_3^+$ resonances at δ 8·09 and 8·33. In spite of the wide range of chemical environments for the 13 nitrogen atoms, consideration of ^{15}N chemical shifts alone allowed unambiguous assignment of only the two lowest frequency ^{15}N resonances (see Table) on the basis of ^{15}N chemical shift substituent parameters.[2,4] The ^{15}N assignments shown in the Table were obtained by the technique of specific low power (*ca.* 1350 Hz) irradiation of the proton resonances. Two complementary effects are observed in the ^{15}N n.m.r. spectrum. First, the ^{15}N resonance of the nitrogen atom whose directly bonded proton is irradiated appears as a sharp singlet, and second, other resonances display reduced ^{15}N–^{1}H one-bond coupling.[5] All ^{15}N resonances are inverted by the negative ^{15}N–(^{1}H) nuclear Overhauser effect (n.O.e.). The maximum observable n.O.e. in the limit of predominant ^{15}N–^{1}H dipole–dipole relaxation is a function of both field strength and correlation time (τ_c) for reorientation of the N–H vectors.[3] For ^{15}N n.m.r. observation at 36·48 MHz we calculate, in the manner described previously,[3a] that a value for τ_c of *ca.* $1{\cdot}3 \times 10^{-9}$ s will give a ^{15}N signal nulled by the n.O.e. Our observation of inverted ^{15}N resonances then means that $\tau_c < 1{\cdot}3 \times 10^{-9}$ s. Extrapolating to zero, this reduced coupling as a function of the irradiation frequency provides confirmation of the frequency of the bonded proton.† The sensitivity of the method is demonstrated by the assignments of N-6 and N-37 where the proton resonances are separated by only 0·12 p.p.m. However, the small chemical shift separations for H-7 and H-15 and H-13 and H-20 (*ca.* 0 and 0·04 p.p.m., respectively) result in uncertainties in ^{15}N assignments within these two groups of resonances. Since the ^{15}N assignments for the two amino-groups had been previously established assignments for the protons of these groups were obtained from the decoupling experiments.

TABLE. ^{15}N and ^{1}H chemical shifts[a] and assignments for the NH groups of viomycin.[b]

Group	^{15}N δ/p.p.m.	^{1}H δ
N-6	77·9 (d)	8·73
N-7	62·7 (t)[c]	7·04
N-8	63·0 (d)	7·99
N-9	85·3 (d)	8·17
N-13	95·2 (d)[d]	9·86
N-15	51·4 (t)[c]	7·04
N-16	88·4 (d)	9·42
N-20	96·2 (d)[d]	9·90
N-24	102·1 (d)	9·19
N-27	102·0 (d)	9·04
N-31	22·1 (br s)	8·33
N-35	12·3 (br s)	8·09
N-37	92·1 (d)	8·61

[a] ^{15}N Chemical shifts are in p.p.m. to high frequency of the $^{15}NH_4^+$ resonance from 5M $^{15}NH_4{}^{15}NO_3$ in 2N HNO_3, measured in the pulse-Fourier transform mode at 36·48 MHz (Buker WH 360) with ^{1}H noise decoupling; multiplicities are in parentheses. ^{15}N Spectra for double resonance assignments were accumulations of 20,000 transients in *ca.* 4·5 h. ^{1}H Chemical shifts are in p.p.m. to high frequency of hexamethyldisiloxane for spectra at 360 MHz, measured using the ^{1}H decoupling coil of the ^{15}N probehead for transmission and detection. [b] 0·3M Solution in 90% H_2O–10% D_2O, pH adjusted to *ca.* 2·8, sample in 10 mm o.d. tube. [c] Assignments may be interchanged. [d] Assignments may be interchanged.



(I)

† This method has also been applied to obtain a full assignment of proton-bearing carbon resonances in the 50·3 MHz ^{13}C n.m.r. spectrum of (I).

as broad singlets because of exchange of the bonded protons with solvent water at an intermediate rate at this pH (2·8). The work of Blomberg *et al.*[6] on the ^{15}N n.m.r. spectra of the amide group of glutamine and the guanidinium group of arginine indicates that more extreme pH's (<2 or >5) would be required for collapse of the multiplet structure of the other 11 ^{15}N resonances of (I). That the ^{15}N resonances of the guanidinium group are two doublets and a triplet confirms[1] the structure of this grouping as $-NH-C(=NH_2^+)-NH-$. Of the 6 peptide (–NH–CO–) linkages in the molecule, both the proton and nitrogen resonances of the viomycidine residue (H-9 and N-9) are at the lowest frequency. We speculate that this may be due to the involvement of these two atoms in the intramolecular hydrogen bond.

^{15}N N.m.r. spectra of (I), measured at 9·12 (Bruker HFX-13) and 18·24 MHz (Bruker WH-180), showed 11 and 12 resolved resonances, respectively. This demonstrates the advantage of the observation at higher frequency (36·48 MHz) in resolving all 13 resonances. We have made an approximate comparison between the sensitivities of 18·24 (25 mm o.d. tube) and 36·48 MHz (10 mm o.d. tube) ^{15}N n.m.r. spectral observations for (I) which showed that spectra of similar quality could be obtained at the lower frequency with the larger quantity of sample in *ca.* one tenth of the time required for the higher frequency observations. However, the larger tube requires approximately 10 times more sample.

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