A STUDY OF THE SECONDARY STRUCTURE OF ILAMYCIN B_1 BY 300 MHz PROTON MAGNETIC RESONANCE*

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1. Introduction

Recent PMR and X-ray studies have shown that a common conformational feature of several cyclic polypeptide antibiotics is the β-turn associated with antiparallel pleated sheet structures [1–17]. In PMR parameters, the β-turn is characterized by an amide proton resonance that is shifted to high field because of the magnetic anisotropy of the vicinal peptide moiety and has a low temperature coefficient due to intramolecular hydrogen bonding. Signals with a small α-CHNH coupling constant reflecting the vicinal dihedral angle at the corner are also observed.

The cyclic peptides studied so far have an even number of residues, including a glycine or a D-amino acid which is energetically favored to form the corner of a β-turn [16–20]. The purpose of this study is to see if a cyclic peptide with an odd number of residues and with only L-amino acids can have an antiparallel β-type conformation.

The cyclic peptide studied is ilamycin B_1, a cyclic heptapeptide consisting of only L-amino acid residues (L-*N*-methyl leucine, L-tryptophan derivative, L-2-amino-trans-4-hexenoic acid, L-leucine, L-*N*-methyl leucine, L-alanine and L-3-nitrotyrosine). It is produced by *Streptomyces islandicus* and is an analogue of ilamycin, an antibiotic inhibiting the growth of mycobacteria [21–23].

2. Experimental

Material: Ilamycin B_1 was obtained from *Streptomyces islandicus n. sp.* and purified by a previously published procedure [23]. 0.06 M DMSO-d_6 solution was used for measurement.

NMR equipment: Varian Associates' 220 MHz and 300 MHz spectrometers with superconducting magnets, variable temperature, and tracked field sweep decoupling devices were used. The decoupling was done with a Wavetek VCO driven from the recorder sweep pot through a variable resistor network.

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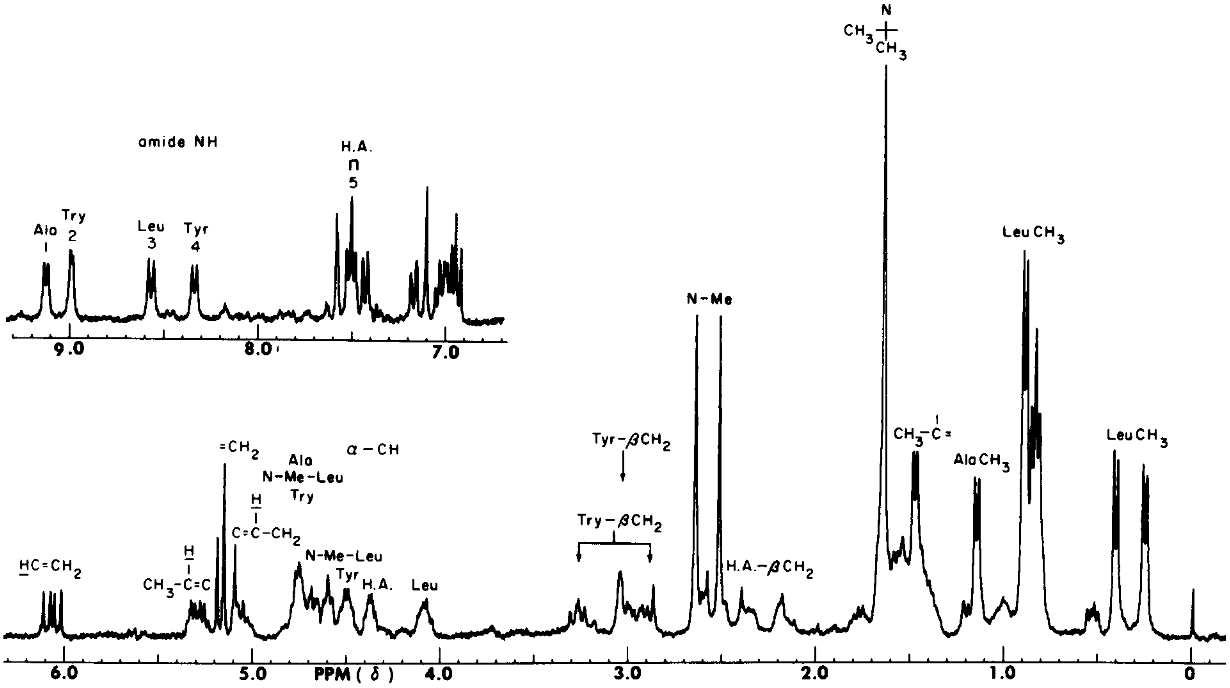


Fig. 1. 300 MHz Spectrum of ilamycin B_1 in DMSO-d_6.

3. Results

The spectrum of ilamycin B_1 at 300 MHz in DMSO-d_6 is shown in fig. 1. Five amide proton resonances are observed in the region 7.50–9.13 ppm downfield from internal TMS. One of these is shifted to extremely high field and superimposed on the tryptophan and 3-nitrotyrosine ring proton resonances. The assignments indicated in the spectrum are based on results of the following decoupling experiments: The respective amide proton resonances were linked with the corresponding α-CH protons (the α-CHNH coupling constants are 6, 3, 9 and 7.5 Hz from lower to higher field respectively for the four lower field resonances). Then all amide protons were exchanged by deuterium to eliminate α-CHNH splitting. Decoupling between α-CH and 2-CH protons was used to identify specific residues. Each β-CH can be assigned to a specific residue using the reference value of each amino acid and/or peptide derivative. The α-CH resonances of alanine, the tryptophan derivative, 3-nitrotyrosine, and 2-amino-trans-4-hexenoic acid were identified by this procedure. The remaining α-CH resonances of leucine and the two *N*-methyl leucines were assigned by the change in signal shape upon amide proton deuteration. The other assignments are indicated on the spectrum. Note that one of the three isopropyl methyl protons of leucine residues is shifted to very high field at 0.43 and 0.27 ppm below TMS, whereas the other two appear around 0.9 ppm.

The temperature dependence of amide proton chemical shifts (fig. 2) shows that the two upfield resonances are less temperature dependent, whereas the remaining three have the same dependence as the amide proton of *N*-methyl acetamide. This indicates that the amide protons of the 2-amino-trans-4-hexenoic acid and 3-nitrotyrosine residues are hydrogen bonded and form β-turns. Consistent with this interpretation is the hydrogen-deuterium exchange of the amide protons at room temperature in 10% D_2O-DMSO. The order for exchange is $1 > 2 \gg 3 > 5 > 4$ (numbers are indicated on the spectrum): proton 1 exchanges within 2 hr, 2 takes 3 hr, 3 takes several days, and 4 and 5 take much

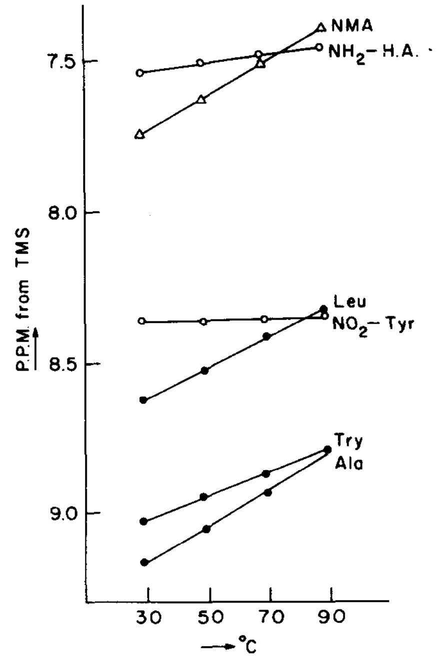
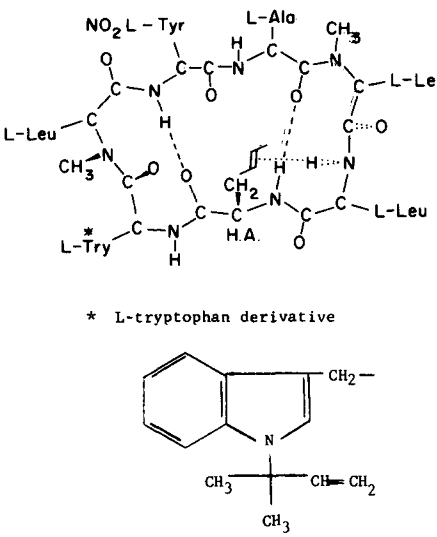


Fig. 2. Temperature dependence of amide proton resonances for ilamycin B_1 in DMSO-d_6. NMA: *N*-methyl acetamide. NH_2-H.A.: 2-NH_2-trans-hexenoic acid.

longer. The slow exchange of 4 and 5 is indicative of the rigid secondary structure, i.e., the hydrogen bonds, of the molecule.

4. Discussion

A proposed secondary structure of ilamycin B_1 is shown in fig. 3. All amide configurations are *trans*. There are β-turns with hydrogen bonding between the amide proton of 3-nitrotyrosine and the carbonyl of 2-amino-trans-4-hexenoic acid and between the amide proton of the hexenoic acid and the carbonyl of alanine. One corner of the turn is occupied by the tryptophan derivative and an *N*-Me-leucine; another *N*-Me-leucine and leucine are positioned at the other corner. The high field shift of one of the leucyl isopropyl methyl resonances can be explained from this structure in terms of the ring current effect of the indole ring of tryptophan or the nitrophenol ring (or both) depending on the orientation of the side chains. The coupling constant between α-CH and β-CH could not be observed because of overlapping of other signals, hence no information is available on the orientation of these residues.



* L-tryptophan derivative

Fig. 3. A proposed secondary structure of ilamycin B_1.

The configurations of amide carbonyls other than those involved in hydrogen bonding may be inferred from steric considerations. The simple steric argument that the bulky side chain of the tryptophan derivative at the beginning of the turn may prefer to be placed in an equatorial-like position suggests that the carbonyl between them points upward. Moreover, this configuration suggests that the amide proton [2] with the small coupling constant belongs to the tryptophan derivative residue due to the following simple steric argument. When a bulky side chain is placed at the α-carbon at the beginning of the turn, empirically the coupling constant of the NH–CH of the corner residue is small since the approximately equatorial position of the group requires the dihedral angle of NH–CH to approach 90°. A similar feature in the amide signal region was observed previously in the phenylalanine residue of gramicidin-S [7, 13] and the isoleucine of oxytocin derivatives [15]. The same sort of argument may be made for the other turn. But in this case, if the carbonyl at the amide linkage between *N*-Me-leucine and leucine is pointed down, not only is the amide hydrogen near the double bond region of the hexenoic acid, but the leucine side chain between them is located in an approximately equatorial position making the interaction sterically feasible. Such an interaction could cause the high field shift of this amide proton compared to the other nonhydrogen bonded protons [1, 2] and slower rate of H–D exchange. The amide linkage between alanine and 3-nitrotyrosine which is not involved in either a β-turn or a hydrogen bond cannot be predicted by such a preliminary observation. One function of these residues may be to ease the steric crowding.

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