Conformational Studies of Polypeptide Antibiotics. Proton Magnetic Resonance of Stendomycin†

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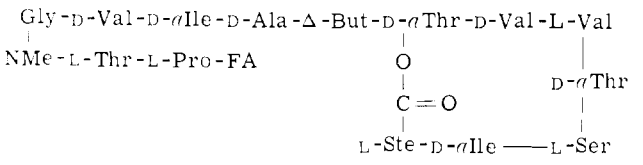
ABSTRACT: The proton magnetic resonance spectrum of the polypeptide antibiotic stendomycin is analyzed in trifluoroethanol. The techniques for differentiating between solvent-exposed and solvent-shielded peptide protons (deuteron–proton-exchange rates, chemical shift temperature dependence, and trifluoroethanol solvent mixtures) are discussed as they relate to stendomycin. Taken together, these three methods point to a structure having all the peptide protons shielded to varying degrees from the solvent. Conformational details are discussed. These involve folding of the lactone ring using β and β-like turns, and a left-handed α-helical segment for the series of D-amino acids in the linear segment.

Stendomycin, an antifungal tetradecapeptide antibiotic, was isolated by Thomson and Hughes (1963) from *Streptomyces endus*. Bodansky *et al.* (1969) determined the primary structure of the major component (I). FA is a fatty acid, Δ-But



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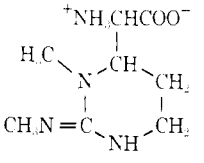

II

is dehydrobutyrene and L-Ste is stendomycidine (II) (Bodansky *et al.*, 1969; Marconi and Bodansky, 1970).

Optical rotatory dispersion studies by Bodansky and Bodansky (1968) led them to conclude that stendomycin has a preferred conformation in solution. Urry and Ruiter (1970) conducted circular dichroism (CD) and infrared (ir) experiments on stendomycin. The shape of the CD pattern of stendomycin in trifluoroethanol was almost identical to that of poly(D-glutamic acid), but exhibited only half the magnitude. The ir results were also consistent with substantial α-helical structure.

It is the purpose of this work to present the results of proton magnetic resonance (pmr) studies of stendomycin. The techniques for distinguishing between solvent-exposed and solvent-shielded peptide protons (Kopple *et al.*, 1969; Ohnishi and Urry, 1969; Urry and Ohnishi, 1970; Pitner and Urry, 1972) will be examined as they apply to stendomycin. More detailed descriptions of solution conformations consistent with the experimental data will be given.

Experimental Section

Spectra were recorded using a Varian Associates HR-220 spectrometer. Sample concentrations were 10% (w/v). Tetramethylsilane was used as an internal reference. Probe temperature was determined by measuring the chemical shift difference between resonances of ethylene glycol or methanol. Decoupling experiments were performed with a field tracking decoupling device fabricated in this laboratory; one multiplet may be irradiated continuously as the remainder of the spectrum is scanned.

Stendomycin was a gift from Eli Lilly and Co. and was used without further purification.

Results

Spectral Assignments. The pmr spectra of stendomycin in trifluoroethanol-d_3 and CD_3OD are presented in Figure 1. The upper spectrum in each pair was recorded within 15 min after sample preparation. The lower in each pair was obtained using samples dissolved in a CD_3OD–D_2O mixture and evaporated overnight at 50° under vacuum; this procedure is necessary to exchange all of the peptide protons. The peptide proton regions of the spectra are shown in Figure 2. The peaks are numbered in order of increasing field as they appear in trifluoroethanol. The peak positions were observed in trifluoroethanol–CH_3OH mixtures at several solvent concentration ratios so that the numbers below each peak identify resonances corresponding to identical peptide protons in the two solvents.

Assignment of multiplets to specific protons or groups of protons in the stendomycin molecule is complicated by several factors. Many amino acids appear more than once in the fourteen amino acid sequence; there are three valines, three threonines (two *allo*-threonines and one *N*-methylthreonine), and two *allo*-isoleucines. Nine of the fourteen amino acids have only one proton in the β position resulting in a large number of similar splitting patterns for the α-proton resonances. Even though there is one dominant member of the stendomycin family (Bodansky *et al.*, 1969), there are members in which *allo*-isoleucine is substituted by valine or leucine; these minor components may be expected to complicate the pmr spectrum.

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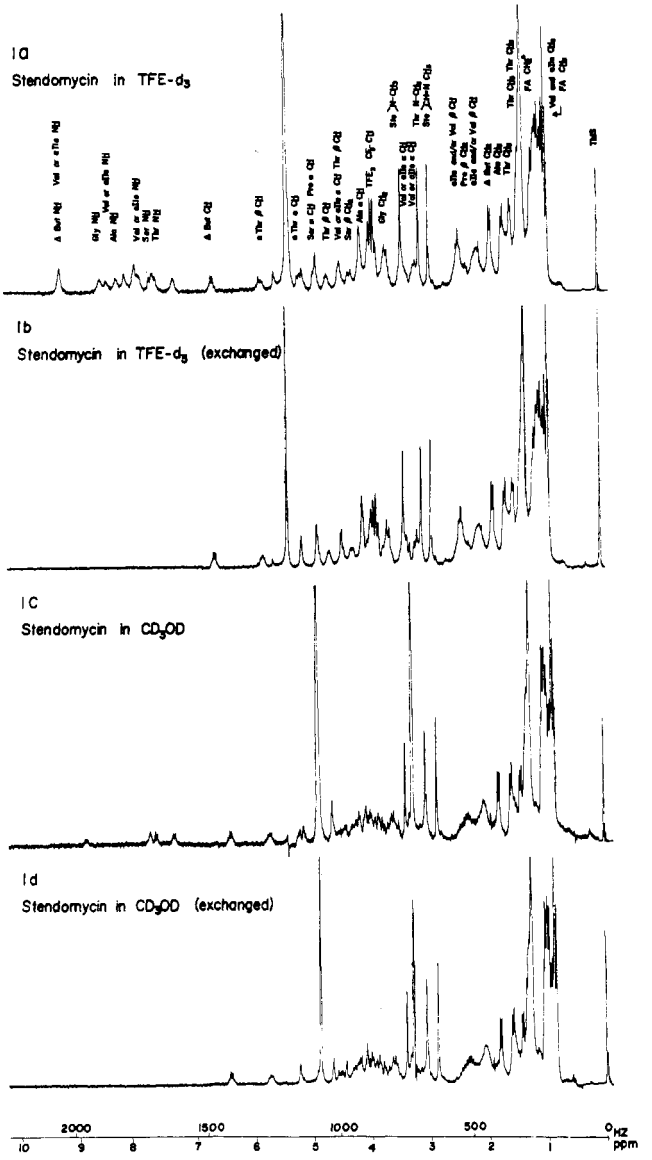

FIGURE 1: 220-MHz pmr spectra of stendomycin in (a) deuteriotrifluoroethanol, (b) deuteriotrifluoroethanol with peptide protons exchanged, (c) deuteriomethanol, and (d) deuteriomethanol with peptide protons exchanged. Spectra were recorded at 15°.

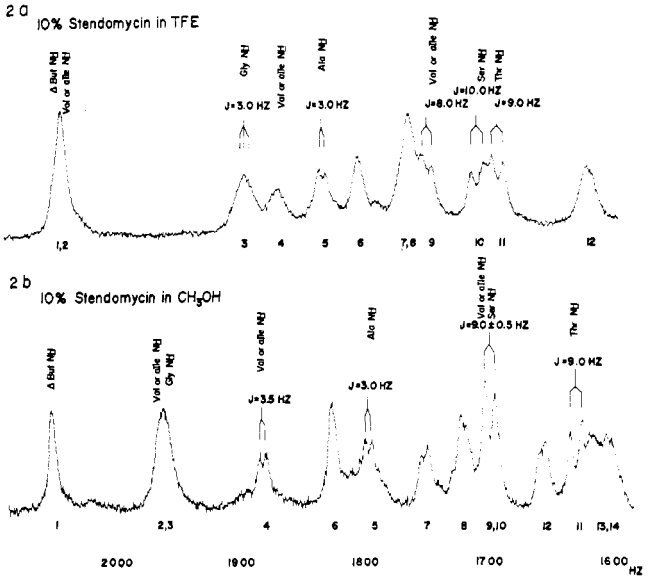

FIGURE 2: 220-MHz pmr spectra of the peptide proton resonances in (a) trifluoroethanol and (b) methanol. Spectra were recorded at 20°.

As can be seen from Figure 2, the peptide region of the pmr spectrum contains many resonances having small α-CH–NH couplings; this makes corresponding decoupling experiments difficult. One simplifying feature is that there are no aromatic amino acids whose resonances often overlap with peptide proton resonances complicating assignments. The assignment of multiplets to resonances is indicated in Figure 1. In approaching the problem of assignments, the publication by McDonald and Phillips (1969) locating resonance positions of amino acids in random-coil polypeptides was very helpful. Identification of resonances was achieved in the majority of cases by performing decoupling experiments not only in trifluoroethanol-d_3 and CD_3OD, but also in various mixtures of the two solvents. Overlapping peaks, impossible to identify in one solvent alone, could be separated by adding various amounts of the other solvent. This procedure was especially helpful for α-proton resonances.

The extremely low-field quartet (1462 Hz) is the β-CH of Δ-But; its position is characteristic for such olefinic protons. It is coupled to the Δ-But methyl resonance at 407 Hz. The low field very sharp NH singlet at 2045 Hz (1) is that of Δ-But; it exhibits no coupling and its low-field position is consistant with the Δ-But olefinic system. The sharp singlet structure is obscured by an overlapping doublet in trifluoroethanol, but is clearly evident in CH_3OH (Figure 2).

The multiplet at 1279 Hz corresponds to the β-CH of the D-*a*Thr involved in the lactone bond; it is identical in structure in exchanged and unexchanged samples, and is no longer located at low field when the lactone bond is broken (Bodansky *et al.*, 1969). Decoupling indicates that the resonance at 1230 Hz is the corresponding α-CH, and the doublet at 324 Hz is the corresponding methyl group. The α-CH is coupled to the NH doublet at 1686 Hz (11).

The group of resonances at 1075 Hz contains two triplets, one with a small coupling constant and one having a large coupling. This is more clearly seen in the exchanged sample in CD_3OD; the two triplets come at 1032 and 1000 Hz. The only α-CH resonances of stendomycin which can exhibit this structure are those of L-Ser and L-Pro. The smaller coupling constant triplet is that of L-Ser. This triplet is not coupled to any resonances in the region of the spectrum in which the β-CH_2 resonances of Pro usually appear (McDonald and Phillips, 1969). It is coupled to the two multiplets at 937 and 948 Hz which are therefore assigned as β-CH_2 resonances of L-Ser. The triplet of L-Ser is also coupled to the NH at 1705 Hz (10).

The group of resonances at 1075 Hz also contains the proline triplet; it is coupled to β-CH_2 resonances at 440 and 465 Hz.

The β-CH multiplets of the two other Thr residues are located at 1028 and 980 Hz; they decouple from methyl CH_3 resonances at 305 and 295 Hz which are obscured by the large peak of the fatty acid methylene protons.

There is a Val or *a*Ile α-CH multiplet centered around 985 Hz; it decouples from a β-CH resonance at 475 Hz. Since the Val and the Ile β-CH resonances occur very close together in the random-coil spectra (McDonald and Phillips, 1969), the identity of this α-CH resonance is ambiguous. It is coupled to the NH multiplet at 1741 Hz (9).

The D-Ala α-CH multiplet is centered at 905 Hz; its quartet

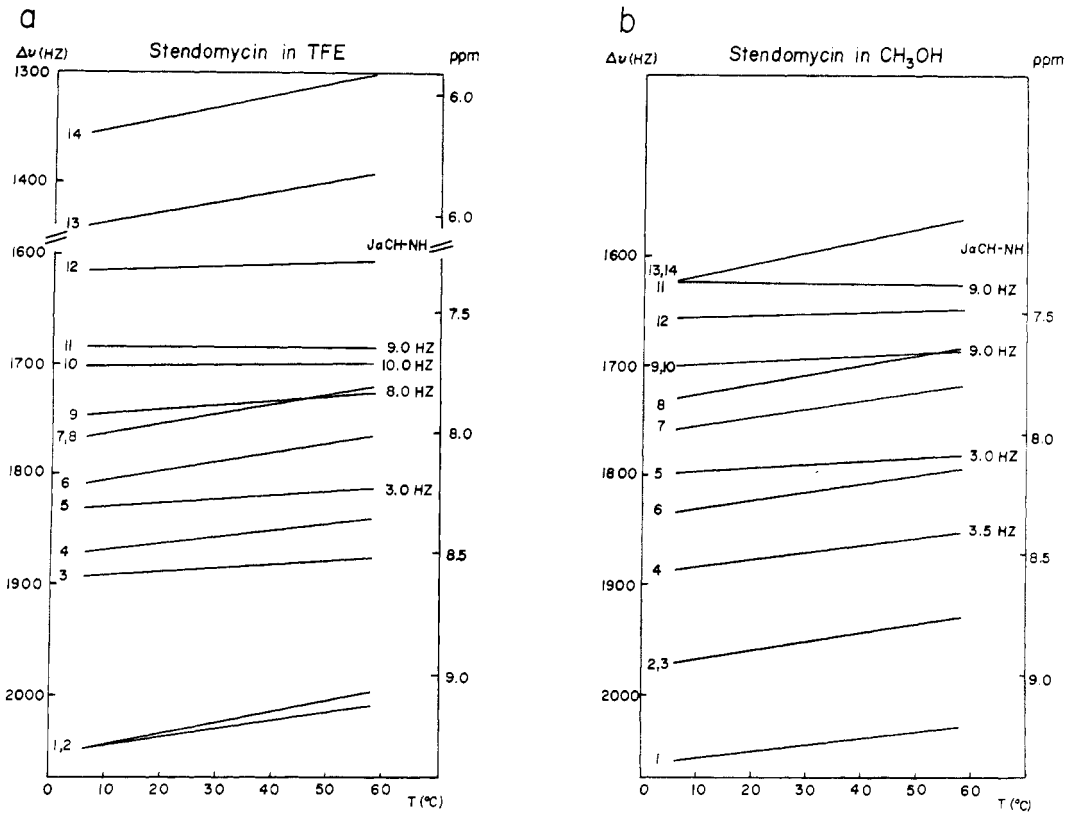

FIGURE 3: Chemical shift temperature dependence of the peptide proton resonances in (a) trifluoroethanol and (b) methanol.

structure is more clearly evident in mixtures of trifluoroethanol-d_3 and CD_3OD. It decouples from the CH_3 doublet at 362 Hz and from the NH resonance at 1830 Hz (5).

Two of the peptide NH multiplets appear to be triplets in trifluoroethanol-d_3, the ones at 1863 and 1890 Hz. Again, solvent mixtures help by revealing the triplet-like structure of the multiplet at 1863 Hz in trifluoroethanol is, in fact, due to the superposition of two multiplets, a large doublet and a smaller multiplet probably resulting from one of the minor members of the stendomycin family. So the triplet at 1890 Hz (3) is due to Gly, since it is the only amino acid whose NH resonance can have triplet structure. This triplet decouples from the α-CH_2 resonance located at 800 Hz.

The three sharp singlets at 746, 678, and 640 Hz are N-CH_3 resonances. The singlet farthest upfield shows doublet structure in trifluoroethanol and corresponds to the L-Ste methyl group attached to the nitrogen not in the six-membered ring; its doublet structure is due to protonation of the adjacent nitrogen. This NH resonance occurs at 1342 Hz in trifluoroethanol, but exchanges much too rapidly to be observed in trifluoroethanol-d_3. The other L-Ste CH_3 resonance, the one corresponding to the methyl group attached to a nitrogen in the L-Ste ring, is identified by analogy with a spectrum for Ste published by Marconi and Bodansky (1970). The remaining *N*-methyl resonance (678 Hz) is due to the N-CH_3 of *N*-Me-L-Thr.

There are two α-CH resonances at 725 and 694 Hz coupled, respectively, to NH resonances 1860 (4) and 2035 Hz (2). Both of these appear to be either Val or *a*Ile α-CH resonances because they couple to β-CH resonances at 510 and 495 Hz, respectively.

The large peak at 285 Hz is the fatty acid methylene protons. The high field group of multiplets are those due to the *a*Ile Val, and fatty acid methyl protons. The resonance of the NH proton attached to the nitrogen in the L-Ste ring comes at 1425 Hz; its exchange is too rapid to be observed in trifluoroethanol-d_3.

Temperature Dependence. Figure 3 gives the temperature dependence of the peptide proton resonances (1–12) and the proton resonances of the stendomycidine ring (13, 14) in trifluoroethanol and CH_3OH. The resonances are numbered in order of increasing field as they appear in trifluoroethanol. Resonances 13 and 14 begin to collapse in CH_3OH as temperature is increased, so that by 58° they are very broad (>50 Hz); in trifluoroethanol these resonances merely undergo upfield shifts with increasing temperature exhibiting no broadening.

Increasing the temperature of such a complex molecule as stendomycin, causes a change in the vibrational states of the molecule. The molecule is very compact, so that this repopulation can change the average magnetic environment experienced by the peptide protons. It is this effect that one attempts to eliminate by conducting temperature-dependence experiments in two solvents in which relative chemical shift temperature dependence has been shown to be different for solvent-exposed and solvent-shielded peptide protons (Pitner and Urry, 1972). For gramicidin S the ratios of the temperature coefficients of solvent exposed to the coefficients of intramolecularly hydrogen-bonded peptide protons are 1.6 and 3.3 in trifluoroethanol and methanol, respectively. So that in two solvents in which the conformation has been shown to be the same (Pitner and Urry, 1972), this method may offer a less ambiguous delineation between types of peptide protons.

The temperature dependence plots are remarkably similar in both solvents, the behavior of each resonance closely paralleling that of the corresponding resonance in the other

solvent. There is no clear classification of two types of peptide protons as there is with gramicidin S. In both solvents, resonances 10, 11, and 12 show small temperature coefficients, whereas, the remainder shift upfield with temperature. It is important to note that very little change is observed in the α-CH–NH coupling constants with temperature.

Trifluoroethanol–CH_3OH Solvent Mixtures. The chemical shifts of the peptide NH and L-Ste NH resonances in solvent mixtures of trifluoroethanol and CH_3OH are presented in Figure 4. None of the peptide NH resonances exhibit large upfield shifts with increasing mole per cent trifluoroethanol as did the solvent-exposed peptide protons of gramicidin S (Pitner and Urry, 1972). The two NH resonances of the L-Ste ring do, shifting upfield about 1 ppm, approximately the same shift as the solvent-exposed peptide protons of gramicidin S. There are, however, five resonances, 2, 5, 8, 9, and 11, which shift definitely downfield as did the intramolecularly hydrogen-bonded protons of gramicidin S. The α-CH–NH coupling constants which could be followed throughout the range of solvent mixtures show very little change.

Exchange Studies. The spectrum of stendomycin in trifluoroethanol-d_3 is shown in Figure 1. Within 15 min after mixing, little noticeable exchange has taken place between the solvent deuterons and peptide protons. The NH resonances of the L-Ste ring do not, however, show up in the deuterated solvent, since they exchange immediately. After 2 hr at 20° the NH protons corresponding to resonances 1, 3, and 5 have almost completely exchanged. The remainder of the NH protons exchange very slowly. After 2 hr at 60° protons 6, 7, and 12 have exchanged, and after 6 hr at 60° protons 4 and 10 have exchanged. The remaining protons, 2, 8, 9, and 11 exchange very slowly; only 2 exhibits a very slight decrease in intensity after 4 and 10 have completely exchanged. In CD_3OD exchange is more rapid; only resonances 2, 8, 9, and 11 remain after 15 min. These are the same protons which exchange very slowly in trifluoroethanol-d_3.

Discussion

Methods of Delineating Peptide Protons. The three methods of delineating peptide protons according to whether they are exposed to the solvent or not (chemical shift temperature dependence, deuterium–proton-exchange rates, and trifluoroethanol–solvent mixture titrations) have been shown to be quite effective when dealing with smaller polypeptides (Kopple *et al.*, 1969; Ohnishi and Urry, 1969; Urry and Ohnishi, 1970; Pitner and Urry, 1972). Agreement between the three methods with gramicidin S is very good. With stendomycin, an opportunity presents itself to compare these three methods and discuss possible problems which may be encountered when dealing with larger molecules.

As will be discussed below, experimental evidence points to a compact folded conformation, for the lactone ring, and suggests a right-handed helical segment for the series of D-amino acids in the tail. With such a folded conformation, great care must be exercised in interpreting temperature-dependence results; slight conformational changes which alter relative spatial orientations of peptide protons and nearby peptide moieties can cause significant chemical shift changes due to the magnetic anisotropy of the peptide moiety. Also, slight conformational changes near the olefinic group of Δ-But can cause chemical shift changes to proximate peptide protons. Even in the case of smaller polypeptides, it is important to remember that raising the temperature can effect a conformational change. However, in the case of

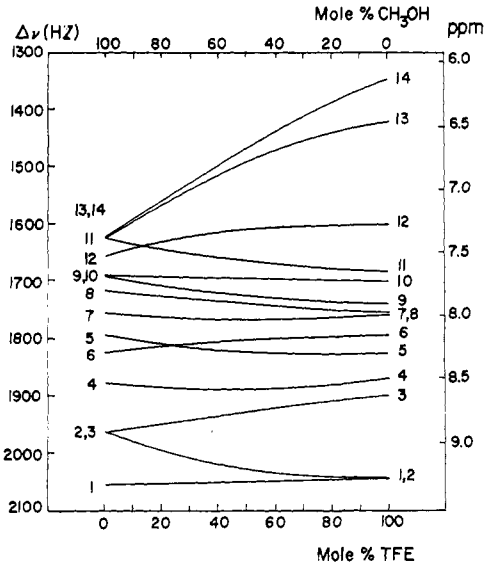

FIGURE 4: Peptide proton resonance positions in mixtures of trifluoroethanol and methanol.

stendomycin, there is little change in α-CH–NH coupling constants with temperature; this indicates that the conformation about the corresponding α-CH–NH bond does not change very much. Caution must be exercised here also; a relatively small amount of stendomycin with the tail in a random-coil conformation in rapid equilibrium with the ordered conformation would change the coupling constant by an experimentally unobservable amount. CD results (Urry and Ruiter, 1970) are consistent with a partial unfolding of a helical segment with temperature in trifluoroethanol.

Similar considerations apply to solvent mixture titrations. As mentioned above, little observable change is detected in the α-CH–NH coupling constants as the concentration ratio of trifluoroethanol to CH_3OH is changed. There is, however, a decrease in intensity of the CD pattern of about 20%, but no change in pattern shape in going from pure trifluoroethanol to pure CH_3OH. This result is very similar to the result obtained by raising the temperature of a solution of stendomycin in trifluoroethanol (Urry and Ruiter, 1970). When compared with results for gramicidin S (Pitner and Urry, 1972), Figure 4 suggests that the peptide protons of stendomycin are all shielded from the solvents, none exhibit large upfield shifts. The two NH protons of the stendomycidine ring do shift upfield; this indicates these protons are exposed to the solvent. One must be careful, however, when comparing the guanidine-like NH protons of the stendomycidine ring with the peptide protons because of the large difference in acidity.

Exchange results indicate that in both trifluoroethanol-d_3 and CD_3OD, peptide protons 2, 8, 9, and 11 are shielded extremely well from the solvent, possibly through the formation of very well protected hydrogen bonds. Protons 4 and 10 are not quite as well shielded followed by 6, 7, and 12. The most exposed peptide protons are 1, 3, and 5.

When comparing the three methods, it is always important to bear in mind that the exchange results are observations of kinetic processes, and the temperature dependence and trifluoroethanol titration results are observations concerned with an average property of states in equilibria. The most slowly exchanging protons, 2, 8, 9, and 11, all shift downfield as the trifluoroethanol concentration is increased (Figure 4). These two results are consistent with behavior observed for

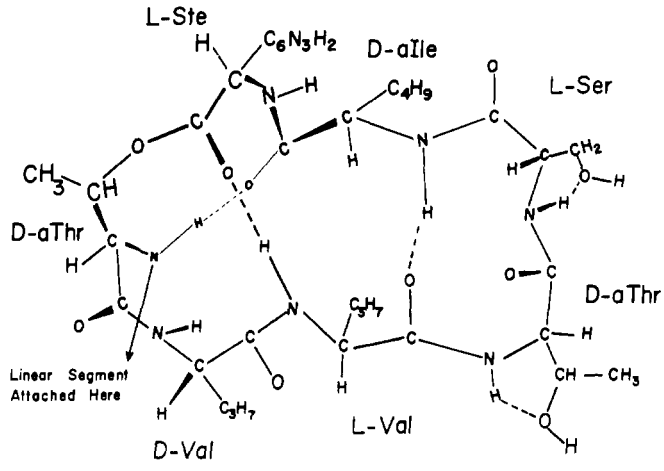

FIGURE 5: One possible conformation for the lactone ring of stendomycin. The ring has been partially unfolded to reveal more clearly the β-turn aspects. It is included to facilitate reading of the discussion.

the internally hydrogen-bonded protons of gramicidin S. One proton, 5, is inconsistent in that it shifts downfield and exchanges very rapidly. Protons 9 and 11 have low-temperature coefficients in agreement with exchange results, but 2 and 8 have definitely positive temperature coefficients in conflict with their slow exchange. Protons 4 and 10, which have faster exchange rates, show almost no net change in going from CH_3OH to trifluoroethanol; proton 4 has a fairly large temperature coefficient, and 10 has a small coefficient. Protons 6 and 12, which exchange faster, shift slightly upfield in the trifluoroethanol titration; 7 exhibits a dip with no net chemical shift change. Protons 6 and 7 have definitely positive temperature coefficients, but that of 12 is small. Of the most rapidly exchanging, 1 and 3 shift upfield as trifluoroethanol is added, and 5 shifts downfield; all have positive temperature coefficients. The stendomycidine NH protons 13 and 14 exchange very rapidly and shift upfield more than 1 ppm as trifluoroethanol is added. They also have temperature coefficients higher than the peptide protons.

All of the peptide proton delineation methods point toward a structure having limited solvent exposure of the peptide protons. The temperature dependence is very similar in both trifluoroethanol and CH_3OH. There are no protons which shift upfield markedly as the mole per cent trifluoroethanol is increased in trifluoroethanol–CH_3OH mixtures. Exchange is very slow for all the protons in trifluoroethanol and for four in CH_3OH. These results are in agreement with conclusions of Bodansky and Bodansky (1968), who found no reaction with the modified Rydon reagent suggesting all peptide protons were "inside" the molecule.

It is unfortunate for comparison purposes that some of the peptide protons could not be solvent exposed; even so, a more complete picture of the solution conformation of stendomycin can be obtained than could have been possible had only one method been available. Exchange results alone would not have pointed clearly to the conformation having shieded peptide protons, and the two equilibrium methods could not in any way have predicted exchange rates. The methods, taken together, indicate that exchange takes place when peptide protons are exposed to the solvent as the molecule unfolds for a very short period of time.

Solution Conformation. In proposing detailed conformations, caution must be exercised. There may be, and often is, more than one conformation which agrees with experimental data. Commonly, one is dealing with an ensemble of conformations which may be in rapid equilibrium, so that the resulting pmr spectrum is a time average of these conformations. Bearing these considerations in mind, conformations will now be presented which are consistent with experimental data and steric limitations. Space-filling models were used to construct the conformations.

The primary structure of stendomycin (Bodansky *et al.*, 1969) contains a lactone ring of seven amino acids, the ester linkage formed between the carboxyl group of L-Ste and the hydroxyl group of D-*a*Thr. This ring may be folded, using β and β-like turns (Venkatachalam, 1968; Urry and Ohnishi, 1970). One of these has the L-Ser and D-*a*Thr at the corners of the turn with the end peptide moiety carboxyl and nitrogen in the most sterically favored above plane–below plane conformation (Urry and Ohnishi, 1970). The peptide proton of D-*a*Ile is hydrogen bonded to the carboxyl oxygen of L-Val. This allows the peptide protons of L-Ser and D-*a*Thr to be hydrogen bonded to their respective hydroxyl oxygens. This β turn is consistent with the following experimental observations. The L-Ser α-CH–NH coupling is 10 Hz as would be expected from the trans orientation (Ramachandran *et al.*, 1971) of the two protons, dictated by the most stable geometric conformation (Urry and Ohnishi, 1970). The exchange rate of the L-Ser NH is slow, but not slow enough to be in a β-turn hydrogen bond. There is one peptide NH resonance (8) not identified, having a small coupling constant, very slow exchange rate, and relatively high-field position (Urry and Ohnishi, 1970) in the peptide region of the spectrum. These three characteristics would all be consistent with the D-*a*Ile peptide NH forming the β turn.

Another β-type turn has the β-carbon of the D-*a*Thr which is involved in the ester linkage, at one corner and L-Ste at the other corner. The β-carbon of D-*a*Thr has a configuration analogous to that of a D-amino acid; therefore, the β turn has at its corners one L-amino acid and one D residue. By analogy with the most sterically favored conformation of the peptide moiety in such a β turn, the carboxyl oxygen of the ester linkage is pointed in the correct direction. The turn is formed by a hydrogen bond between the D-*a*Thr peptide nitrogen and D-*a*Ile carboxyl oxygen. This conformation is supported by the following observations. The peptide proton of D-*a*Thr (number 11) is very slowly exchanging and is located at relatively high field as would be expected from its involvement in β-turn formation. The α-CH–NH coupling of D-*a*Thr is large (9 Hz) consistent with the trans orientation of the two protons dictated by the β turn.

Another β-type turn has the D-Val at one corner, and both the α- and β-carbons of D-*a*Thr at the other. The turn is formed by hydrogen bonding the peptide nitrogen of L-Val to the carboxyl oxygen of L-Ste. This β turn is different in that it contains the α- and β-carbons and the hydroxyl oxygen of the D-*a*Thr; it therefore, contains one more atom than the standard β turn. This turn is supported experimentally by a very slowly exchanging peptide proton with a large α-CH–NH coupling (8 Hz) at high field (9). The peptide proton cannot be identified definitely as Val. As discussed in the results section, it could be Ile. If it were Val, there would be consistency between experimental data and the proposed turn.

The skewing of the lactone ring which results from the above β and β-like turns places the NH of L-Ste very close to the carboxyl oxygen of D-*a*Thr. This hydrogen bond results in a β turn with L-Ser and D-*a*Ile at the corners. It is a turn, however, not having the end peptide carboxyl oxygen and nitrogen

in the most sterically favored conformation; the relative above and below plane positions are reversed. However, the skewing of the ring resulting from the formation of the other turns makes this hydrogen bond sterically feasible. The peptide proton of the D-Val in the ring is sterically shielded from the solvent very effectively by the bulky valyl side chain.

The tail of stendomycin contains a series of D-amino acids. It was suggested from CD results (Urry and Ruiter, 1970) that this sequence of D-amino acids could form a left-handed α helix, there being a hydrogen bond between the D-Ala peptide nitrogen and the *N*-Me-L-Thr carboxyl oxygen, one between the D-*a*Ile nitrogen and L-Pro oxygen, and one between the D-Val nitrogen and fatty acid oxygen. This is supported by an α-CH–NH coupling constant of about 3.0 Hz for D-Ala which is consistent with an α helix (Ramachandran *et al.*, 1971).

The folding of the lactone ring and the α-helical segment shields ten protons from the solvent. The other two peptide protons of Δ-But and Gly can be involved in hydrogen bonds by folding the tail over the ring resulting in a compact overall structure. The NH of Δ-But is in an ideal position to hydrogen band with the carboxyl oxygen of the adjacent D-*a*Thr. The tail can then be brought over the ring allowing the Gly NH to hydrogen bond with one of the carboxyl oxygens across the ring; the oxygen of L-Ser is ideally located for this. These hydrogens would be exposed to the solvent if the tail were moved away from the ring due to intramolecular motion; this idea is consistent with their rapid exchange rates (1 and 3).

Another possible conformation is obtained by reversing the above–below plane orientation of the carboxyl oxygen of the ester linkage, relative to the β turn having L-Ste and the β-carbon of D-*a*Thr at the corners. This reversal does not result in unfavorable steric interactions, due to the skewing of the remainder of the ring. It, in fact, places the β-methyl of the lactone D-*a*Thr in a more favorable steric location. This conformation does not allow the glycine to form a hydrogen bond as easily by folding the tail over the ring. Additional conformations may be considered by varying the position of the β turns within the ring moiety. In general, there is the perspective that the ring moiety contains β turns with the linear segment in a left-handed α-helical conformation. The peptide C–O moieties of the helical segment hydrogen bond to peptide N–H moieties of the ring component.

Although the pmr spectra are extremely sharp, the possibility of dimer or other small polymer formation cannot be ruled out. If limited association did occur, this could account for some of the shielding of peptide protons from the solvent.

An interesting feature of the stendomycin lactone ring is the alternating sequence of D- and L-amino acids. This alternation has been shown to be of primary steric importance in the formation of certain types of ion-transport channels (Urry, 1971; Urry *et al.*, 1971). Examination of space-filling models of stendomycin reveals that one turn of a β helix (Urry, 1972a,b) can be formed by a hydrogen bond between the lactone D-*a*Thr carboxyl oxygen and the peptide nitrogen of D-*a*Ile. The side chains of alternating D- and L-amino acids point outward from the helical axis, as does the tail. This suggests a possible mechanism to explain the ion-transport properties (Goodall and Urry, 1972)[1] of stendomycin. Molecules could stack ring on top of ring held together by hydrogen bonds between peptide nitrogens and carboxyl oxygens of adjacent molecules; the hydrophobic fatty acid tail would reach into the lipophilic membrane. This is an interesting example of the recently proposed "Concept of Cyclic Conformations with Linear Conformational Correlates" (Urry 1972a,b). The channel formed as described above would be similar to the β^6-(π_{L,D^6}) helix of gramicidin A (Urry *et al.*, 1971; Urry, 1972a).

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