# Spectroscopic Studies on the Conformation of Gramicidin A'. Evidence for a New Helical Conformation*

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ABSTRACT: Absorption, circular dichroism, and nuclear magnetic resonance studies show the conformation of gramicidin A' (GA'), a commercial preparation (Nutritional Biochemicals) of gramicidin, and hydrogenated gramicidin A' to be highly solvent dependent. Infrared data in dimethyl sulfoxide indicate no change of backbone conformation on hydrogenation of gramicidin A'. In trifluoroethanol it is proposed, on the basis of a marked hypochromism and a circular dichroism with positive bands at 224 and 212 nm and a negative band at 194 nm, that in this solvent hydrogenated gramicidin A' occurs in a left-handed helical conformation. Nuclear magnetic resonance and optical rotatory dispersion data provide evidence for similar conformations in trifluoroethanol and in dimethyl sulfoxide. Since the αCH–NH coupling constants of gramicidin A' in dimethyl sulfoxide are much larger than expected for the α-helical conformation, this set of spectroscopic data is taken as evidence for a new helical conformation and is consistent with the recently proposed $\pi_{L,D}$ helices.

Gramicidin A is a linear pentadecapeptide with the following sequence: HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2CH_2OH$ (Sarges and Witkop, 1964, 1965; Ishii and Witkop, 1963; Hinman *et al.*, 1950). It is of particular interest due to its capacity to selectively transport cations across a lipid bilayer by the formation of transmembrane channels (Hladky and Haydon, 1970; Krasne *et al.*, 1971). Concentration studies on equilibrium conductance indicate that two molecules of GA'[1] are required per channel (Goodall, 1970; Tosteson *et al.*, 1968). It has recently been proposed that the gramicidin A transmembrane channel is formed by head-to-head attachment of two left-handed $\pi_{L,D}$-type helices (Urry, 1971; Urry *et al.*, 1971) and that the mechanism is one in which the ion moves along the core of the helix by an ion-induced relaxation of the channel's helical conformation. The present communication reports absorption, optical rotation, and proton magnetic resonance (pmr) data which bear on the conformation of gramicidin A and demonstrate a marked solvent dependence which is important to X-ray crystallographers and others interested in the conformation of the transmembrane channel.

Previously pmr studies of GA' in $Me_2SO$-$d_6$ indicated that this molecule exists in an ordered non-α-helical conformation with tryptophan indole N*H* hydrogens exposed to the solvent (Glickson *et al.*, 1972). The present study shows that in trifluoroethanol, in which the conformation of GA' is similar to its conformation in $Me_2SO$-$d_6$, GA' exists as a left-handed helix which may be one of the $\pi_{L,D}$ helices recently described (Urry, 1971; Urry *et al.*, 1971). Determination of the relative stability of ordered as opposed to disordered structures of GA' is also of considerable importance. Toward this end pmr spectra of GA' have been measured in strongly denaturing media, trifluoroacetic acid and 6 M guanidine deuteriochloride. Surprisingly, evidence for retention of some ordered structure was obtained in these solvents.

## Experimental Section

Gramicidin was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. Nuclear magnetic resonance analysis showed the commerical gramicidin to contain 72% gramicidin A, 9% gramicidin B, and 19% gramicidin C (Glickson *et al.*, 1972). These three congeners differ by the amino acid in position 11, which is L-Trp. L-Phe, and L-Tyr for GA, GB, and GC, respectively. Amino acid analysis of the commercial product was consistent with the nmr analysis, and both nuclear magnetic resonance and amino acid analysis verified the hydrogenation which was carried out by the method of Ruttenberg *et al.* (1966).

Optical data were obtained on a Cary 60 with 6001 Circular Dichrolism Accessory, on a Cary 14, and on a Beckman IR-11 with an IR-12 interchange. Temperature of the sample was monitored with the YSI Model 42SC telethermometer equipped with a hypodermic probe.

Pmr spectra were measured on a Varian Associates HR-220 spectrometer equipped with an SS-100 computer which was employed in averaging of spectra for signal-to-noise enhancement. Temperature measurements in the pmr studies, which were obtained by sample replacement with ethylene glycol, were accurate to ±2°.

Trimethyl phosphate-$d_9$ (TMP-$d_9$) and dimethyl sulfoxide-$d_6$ ($Me_2SO$-$d_6$, 99.5% D) were purchased from Diaprep Corp., Atlanta, Ga. Methanol-$d_4$ (99% D), *p*-dioxane-$d_8$, 2,2,2-trifluoroethanol-$d_3$, trifluoroacetic acid-*d*, and deuterium oxide (99.7% D) were supplied by Merck, Sharp and Dohme (Montreal, Can.). Spectroquality nondeuterated solvents were obtained from Matheson Coleman & Bell, Cincinnati, Ohio, and from Burdick and Jackson, Muskegon, Mich.

## Results and Discussion

Optical Spectra. The ultraviolet absorption data on hydrogenated gramicidin A' in several solvents are given in Figure 1. In distilled water, trimethyl phosphate, and trifluoro-

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[1] Abbreviations used are: GA, GB, GC, and GD, gramicidins A, B, C, and D, respectively; TMP-$d_9$, trimethyl phosphate-$d_9$; TS, tyrocidin S.

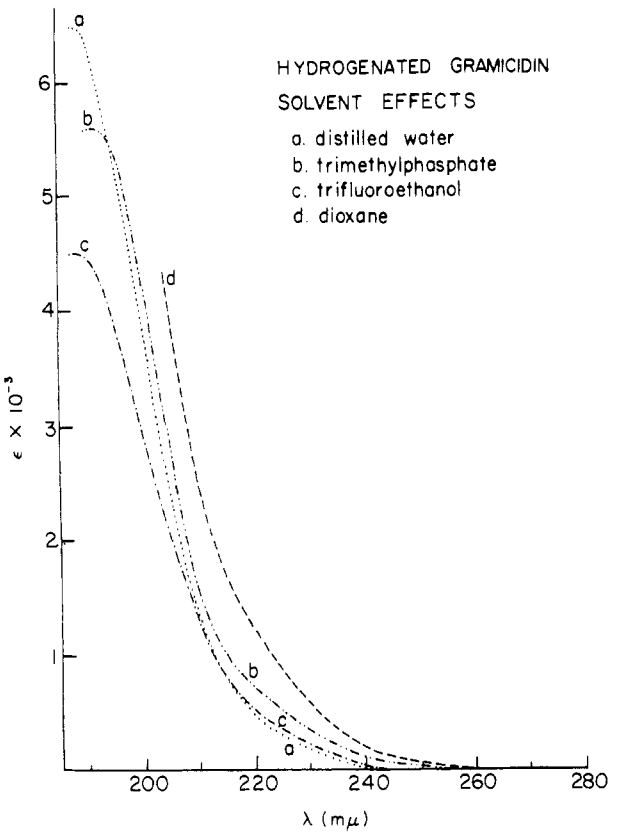


FIGURE 1: Solvent effects on the absorption spectrum of hydrogenated gramicidin A'. The marked hypochromism in trifluoroethanol (TFE) indicates helix formation.

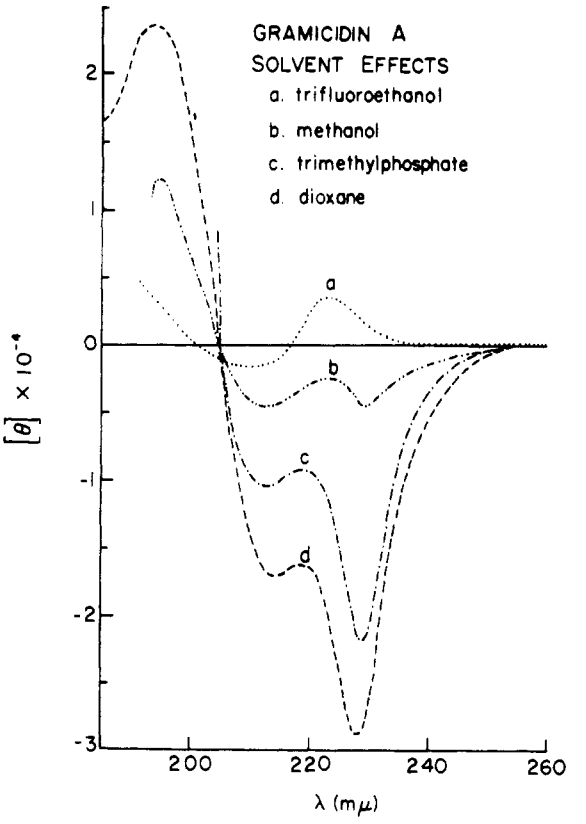


FIGURE 2: Solvent effects on the circular dichroism patterns of gramicidin A'.

ethanol, the mean residue molar extinction coefficients for the major band are $6.5 \times 10^3$, $5.6 \times 10^3$, and $4.5 \times 10^3$, respectively. While the peak cannot be reached in dioxane due to high solvent absorption, it appears that this absorption will be substantially greater than in trifluoroethanol. Representative values of mean residue molar extinction coefficients for polypeptides in $\alpha$-helical, disordered, and $\beta$-structure conformations are $4 \times 10^3$, $7 \times 10^3$, and $8 \times 10^3$, respectively. In general, the 190-nm peptide band in intramolecularly hydrogen-bonded helical structures can be expected to be hypochromic, whereas it is hyperchromic for more extended and intra- or intermolecularly hydrogen-bonded $\beta$ structures. Accordingly, absorption data on hydrogenated gramicidin A' are indicative of helix. Gramicidin A' also exhibits hypochromism in trifluoroethanol. The mean residue extinction coefficients at 193 nm are 11,200 in trimethyl phosphate, 10,600 in methanol, and 8300 in trifluoroethanol. Hypochromism is still observed in the presence of 27% aromatic residues.

The circular dichroism spectra of gramicidin A' in various solvents are given in Figure 2. The circular dichroism (CD) pattern is seen to be highly variable with different solvents. The variability may be due to changes in backbone conformation or due to changes in tryptophan contributions or both. Absorptions of tryptophan occur in the uv spectra near 223 and 193 nm. The CD spectra after hydrogenation continue to show variation with solvent in a manner qualitatively similar to that of GA', that is, the ellipticity in the 220-nm wavelength range is most negative in dioxane and most positive in trifluoroethanol, with that of other solvents being in between in correct order. This is consistent with the backbone conformation being the same after hydrogenation with large contributions due to the aromatic transitions in the nonhydrogenated antibiotic. Infrared studies in $Me_2SO$ on GA' and hydrogenated GA' exhibited, in both cases, bands at 1665 (amide I) and 1535 $cm^{-1}$ (amide II). This provides evidence that in $Me_2SO$ hydrogenation does not alter backbone conformation.

Using the mixed solvents of dioxane and trifluoroethanol, the transition from one CD pattern to the other occurs between 40 and 80% trifluoroethanol (see Figure 3). This is consistent with the formation of two separate conformations. The CD spectrum in trifluoroethanol is of particular interest (see Figure 4) with positive extrema at 224 and 212 nm and a negative extremum at 194 nm. This general spectrum in trifluoroethanol is characteristic of left-handed helices but is red shifted from the $\alpha$-helix positions of 222, 208, and 190–192 nm in the same solvent (see, for example, Quadrifoglio and Urry, 1968, and Holzwarth and Doty, 1965).

In addition the temperature effect provides evidence for an essentially completely ordered state near 7°. Even so, the magnitude of the ellipticities is about one-third that of $\alpha$-helical polypeptides.

Optical rotatory dispersion of GA' and hydrogenated GA' is of interest in correlating infrared (ir) and nuclear magnetic resonance (nmr) data obtainable in $Me_2SO$ with the CD studies in trifluoroethanol. The optical rotatory dispersion (ORD) of GA' in $Me_2SO$ is most similar to that in trifluoroethanol and indicates the presence of a positive band just below 240 nm. Similarly, the ORD of hydrogenated GA' in $Me_2SO$ shows the long-wavelength bands to be positive as in trifluoroethanol; therefore, the ORD shows the conformation in $Me_2SO$ to be similar to that in trifluoroethanol. Also,

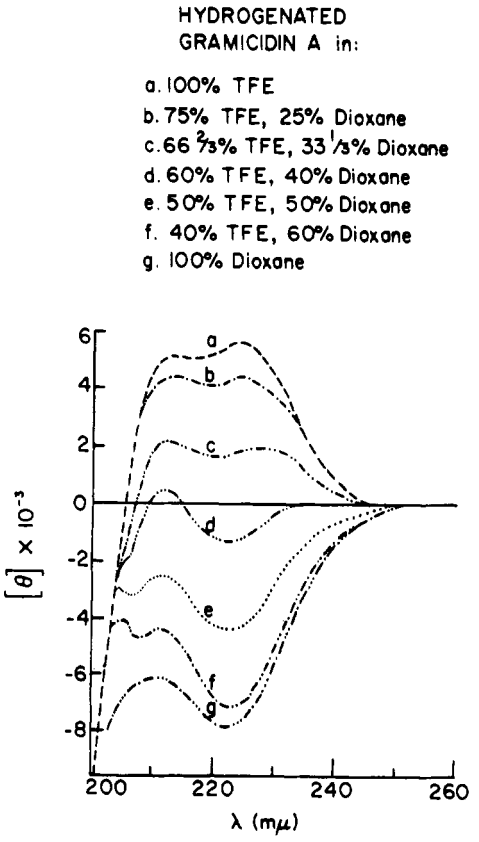


FIGURE 3: Circular dichroism of a solvent elicited conformational change for hydrogenated gramicidin A'. The transition, occurring between 40 and 80% TFE, demonstrates a change in backbone conformation.

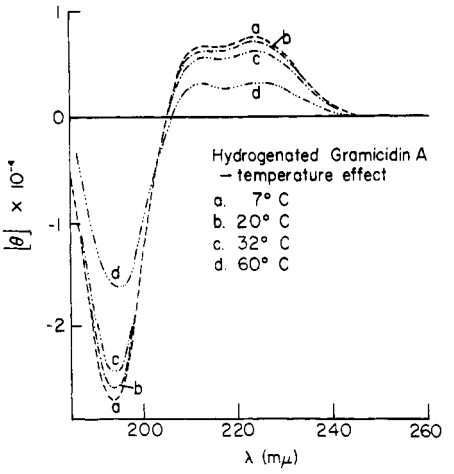


FIGURE 4: Temperature effect on the CD pattern of hydrogenated gramicidin A' in TFE indicating a stable conformation being reached at 7°. The CD pattern is characteristic of left-handed helices but distinguishable from that of the $\alpha$ helix.

as will be shown below, solvent studies on GA' using pmr (Glickson *et al.*, 1972) with sensitive ring-current-shifted protons show the conformations in $Me_2SO$ and trifluoroethanol to be very similar. The several $\alpha$CH–NH coupling constants that could be resolved for gramicidin A' are greater than 7 Hz (Glickson *et al.*, 1972), whereas a value of no more than 6 Hz is expected for $\alpha$ helices (Ramachandran *et al.*, 1971) of L and D residues with some values expected to be as low as 3 Hz.

*Pmr Spectra.* Proton magnetic resonance (pmr) spectra of GA' in dioxane, TMP-$d_9$, methanol-$d_4$, and trifluoroethanol-$d_3$ appear in Figure 5. Insets show the spectra in undeuterated methanol and trifluoroethanol in the low-field region where N*H* and aromatic C*H* resonances occur. In $Me_2SO$-$d_6$, the solvent in which the best-resolved pmr spectra of GA' were obtained, extensive assignment of resonances to specific hydrogens was accomplished by means of deuterium exchange of labile hydrogens, model compound studies, spin decoupling, and hydrogenation of tryptophan indole rings (Glickson *et al.*, 1972). Such a systematic assignment of resonances was not attempted in the solvents employed in the current study; however, rough assignment was accomplished by comparison to the spectrum of GA' in $Me_2SO$-$d_6$.

Thus, the tryptophan indole N*H* resonance was readily identified by its characteristic low-field position at 2342 Hz in TMP-$d_9$, at 2256 Hz in $CH_3OH$, and at 1982 Hz in trifluoroethanol (Glickson *et al.*, 1969, 1971, 1972). In dioxane-$d_8$ the indole N*H* resonance overlaps with peptide N*H* peaks. The high-field shift of indole N*H* resonances in dioxane-$d_8$ could result from disruption or weakening of hydrogen bonds involving the indole N*H* protons. This is suggested by studies of the effect of hydrogen bonding on the chemical shift of the indole N*H* resonance of skatole (3-methylindole) (J. D. Glickson and W. D. Phillips, unpublished data). In $Me_2SO$-$d_6$, a strong hydrogen-bonding solvent, the indole N*H* resonance occurred at 2358 Hz, whereas in *n*-tetradecane the same resonance was observed at 1615 Hz ($3 \times 10^{-2}$ M skatole, 23° in both solvents). The absence of temperature dependence of the indole N*H* resonance in the latter solvent was consistent with the total absence of hydrogen bonding. The high-field shift of about 750 Hz associated with disruption of indole N*H* hydrogen bonds of skatole suggests that tryptophan indole N*H* resonances may be similarly sensitive to hydrogen bonding. In trifluoroethanol we expect most of the indole N*H* hydrogens of GA' to be hydrogen bonded either to the solvent or as a result of intermolecular aggregation. Consequently, another mechanism is probably responsible for the high-field shift of the indole N*H* resonance in this solvent (see below). The formyl C*H* absorption is observed at 1815 Hz in dioxane-$d_8$, 1825 Hz in TMP-$d_9$, 1804 Hz in methanol, and 1781 Hz in trifluoroethanol. Whereas this resonance is a singlet in trifluoroethanol and $Me_2SO$-$d_6$ (Glickson *et al.*, 1972), close inspection of the formyl C*H* resonance in methanol-$d_4$ (Figure 5) reveals that it consists of four distinct components at 1906, 1822, 1800, and 1735 Hz. The relative intensities of these resonances in the order of increasing field position are 12.4, 40.4, 29.2, and 18.0%, and their total intensity corresponds to 0.9 ± 0.1 proton (using the aromatic C*H* resonances as internal standards). The effect of methanol on the formyl C*H* resonances of GA' is both reproducible and reversible. Thus, after evaporating the methanol, the usual spectrum of GA' in $Me_2SO$-$d_6$ is obtained, a single formyl C*H* peak of undiminished intensity. The number of formyl C*H* resonances of GA' could result from chemically distinct components whose formyl C*H* resonances can be resolved in methanol but not in $Me_2SO$ or trifluoroethanol and/or from conformationally distinct components whose rate of interconversion is slow relative to their chemical shift differences in methanol. Replacement of valine-1 by isoleucine, as reported by Gross and Witkop (1965) for GA, GB, and GC could perturb the resonance of the nearby formyl C*H* proton; however, the relative

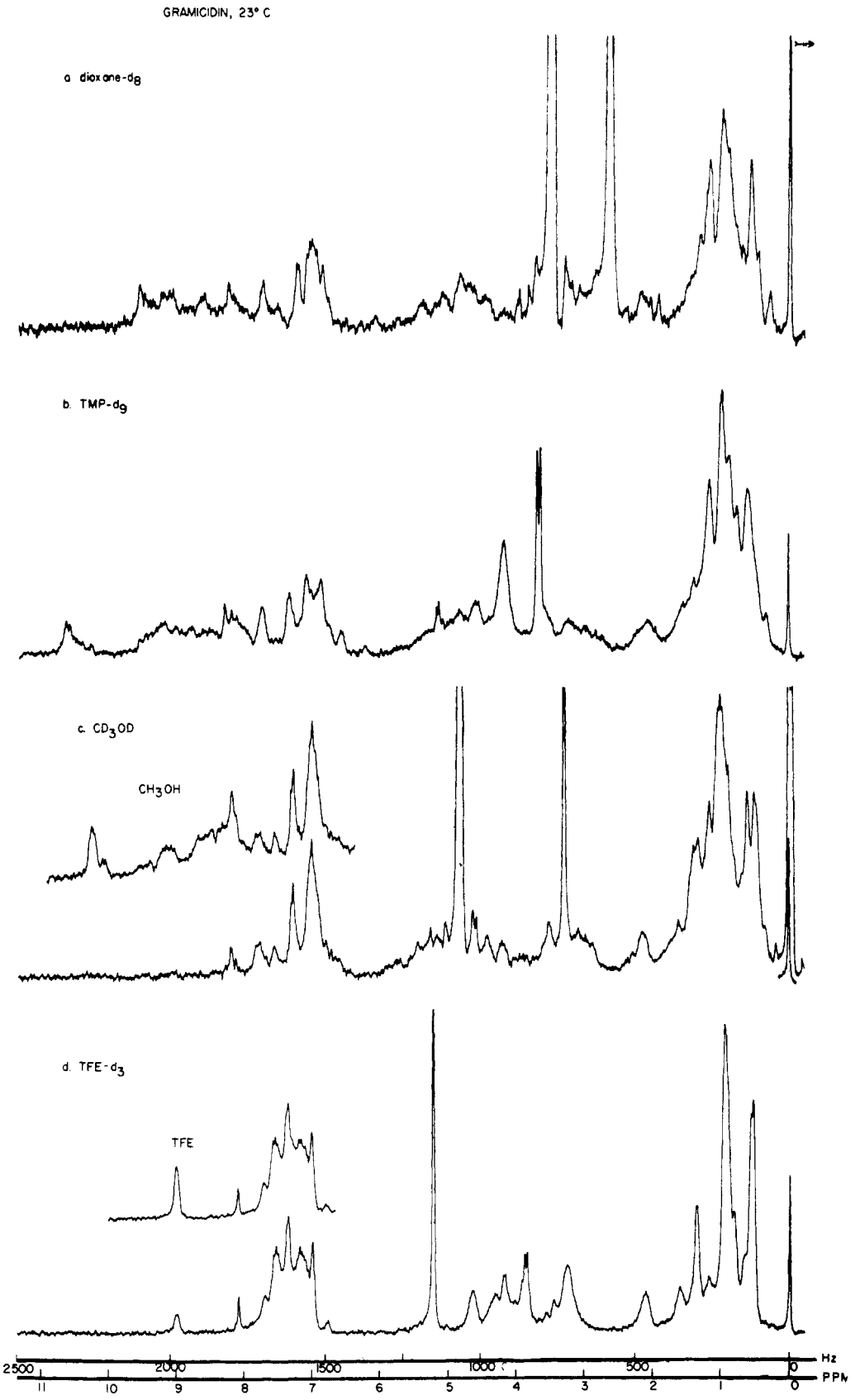


FIGURE 5: Pmr spectra of GA′ at 23° in (a) dioxane-$d_8$, (b) TMP-$d_4$, (c) $CD_3OD$, and (d) in 2,2,2-TFE-$d_3$. Concentrations are 10% (w/v) except in dioxane in which a saturated solution (~3.6%) was employed. Low-field insets to spectra c and d were measured in undeuterated methanol and 2,2,2-TFE, respectively.

intensities of the formyl resonances in methanol do not agree with the report of these authors that the isoleucine congener constitutes about 0–20% of GA′. On this basis the latter explanation is unlikely.

Peptide N*H* and aromatic C*H* resonances occur between 1490 and 2200 Hz in dioxane-$d_8$, TMP-$d_9$, and methanol, but in trifluoroethanol the peptide N*H* resonances are shifted to high field where they overlap with the aromatic C*H* protons between 1490 and 1720 Hz. A high-field shift was also observed for the tryptophan indole N*H* resonance. That such a high-field shift of N*H* resonances need not result from a conformational change in GA′ is suggested by spectral studies of gramicidin S, now referred to as tyrocidin S (TS). CD studies on solvent and temperature effects indicate that TS has a very stable conformation and that it has the same conformation in methanol as in trifluoroethanol (Quadrifoglio and Urry, 1968; D. W. Urry, unpublished data), but the peptide N*H* resonances of this antibiotic shift from 1690–1960 Hz (Stern *et al.*, 1968) in methanol to 1590–1715 Hz in trifluoroethanol.

Residual protons in the deuterated solvents contribute intense resonances to the spectra in Figure 5: dioxane-$d_8$, 770 Hz ($H_2O$) and 580 Hz ($C_4HD_7O_2$); TMP-$d_9$, 920 Hz ($H_2O$) and 810 Hz ($C_3HD_8O_4$); methanol-$d_4$, 1060 Hz (OH) and 725 Hz ($CHD_2OD$); trifluoroethanol, 1170 Hz (O*H*) and 855 Hz ($CF_3CHDOD$). The $\alpha$C*H* absorptions range between 800 and 1250 Hz, whereas the 600- to 800-Hz region contains resonances originating from ethanolamine and tryptophan methylene protons. Resonances near 400 Hz probably originate from the valine $\beta$C*H* hydrogens. The leucine $\beta$ and $\gamma$C*H* peaks are obscured by more intense methyl absorptions.

The two alanine methyls absorb at 264 Hz in $Me_2SO$-$d_6$ (Glickson *et al.*, 1972). Consequently, the distinct resonances at 256 Hz (dioxane-$d_8$), 257 Hz (TMP-$d_9$), 257 or 300 Hz (methanol-$d_4$), and 304 Hz (trifluoroethanol-$d_3$) probably originate from alanine methyl resonances.

The leucine and valine methyl absorptions occur in two groupings: some are clustered near 210 Hz, the absorption frequency associated with solvated leucine and valine methyl protons, whereas others are shifted to high field at frequencies associated with leucine and valine methyl protons that are located near the faces of tryptophan rings (Glickson *et al.*, 1972). In $Me_2SO$-$d_6$ three valines and one leucine contribute to the "solvated" methyl absorption at 182 Hz, whereas one valine and three leucines have methyl resonances ring current shifted to 127 Hz. Similar high-field ring-current shifts in pmr spectra of proteins have been assigned to leucine, valine, isoleucine, and methionine methyl protons in the proximity of aromatic side chains of tryptophans, phenylalanines, and histidines of proteins (McDonald and Phillips, 1967, 1969). Because of their strong dependence on the relative orientations of methyl protons and aromatic rings (Johnson and Bovey, 1958), the ring-current-shifted methyl resonances often reflect very subtle conformational changes of peptides and proteins (McDonald *et al.*, 1971). The ring-current-shifted methyl resonances in the spectra of GA′ support the conclusion drawn from optical spectra that in dioxane, TMP, methanol, trifluoroethanol, and $Me_2SO$ this antibiotic exists in ordered conformations.

Comparison of the pmr spectra of GA′ in the various solvents (Figure 5), particularly in the conformationally sensitive region of methyl absorption, reveals that the spectra gradually change with the solvent in the order dioxane, TMP, methanol, and trifluoroethanol. The N*H* absorptions are not included in this comparison because they may be influenced by various effects other than the conformation of the molecule, *e.g.*, effects of solvent on hydrogen bonding of exposed N*H* protons. The spectrum in dioxane is most similar to that in TMP, but is distinctly different from the spectra in trifluoroethanol and $Me_2SO$ (Glickson *et al.*, 1972), which are strikingly similar.

The same order of solvent dependence was also noted in CD spectra of normal and hydrogenated GA′ (see Figures 2 and 3). From the latter results, it has been concluded that the backbone conformation of GA′ is changing in these solvents. These conformational changes contribute, at least partially, to the changes observed in the pmr spectra; however, other effects may also be operative. Thus, Sarges and Witkop (1965) noted that in dioxane and in some other solvents GA′ existed as a dimer. Since they studied 1% solutions, whereas pmr measurements here cited involve 10% solutions, more extensive aggregation may occur in the pmr experiments. Indeed, significant line broadening is noted in dioxane and TMP solutions (Figure 5) which probably reflects increased dipolar relaxation rates of the slower tumbling GA′ aggregates. Some ring-current shifts may result from intermolecular interactions which bring methyl groups of one molecule into the ring current field of an indole ring of a neighboring molecule. Since no concentration dependence was noted in these pmr spectra between 5 and 10% (w/v), intermolecular interactions could involve rather stable aggregates.

The reversibility of the spectral changes with solvent was determined by lyophilizing the solvent and redissolving the sample in $Me_2SO$-$d_6$. In all cases the normal spectrum in $Me_2SO$-$d_6$ was obtained.

Overlap of resonances of GA′ in trifluoroethanol solution (Figure 5) prevents measurement of peptide $\alpha$C*H*–N*H* coupling constants which yield estimates of backbone dihedral angles and help distinguish between various left-handed helices that may be consistent with the optical data. However, in $Me_2SO$ solution, in which the conformation of this antibiotic is similar to its conformation in trifluoroethanol, all the coupling constants that were measured were greater than 8.0 Hz, except one which was 7.0 Hz (Glickson *et al.*, 1972). A value of no more than 6 Hz is expected for $\alpha$ helices (Ramachandran *et al.*, 1971) of L and D residues with some values expected to be as low as 3 Hz. Consequently, a left-handed $\alpha$-helical conformation appears unlikely, however the $\pi_{L,D}$ helices are left-handed helices with anticipated peptide $\alpha$C*H*–N*H* coupling constants between 7.0 and 9.5 Hz. In $Me_2SO$ and trifluoroethanol solution GA′ may therefore assume one of these conformations.

*Denaturation Studies.* Optical and pmr spectra indicate that in dioxane, TMP, methanol, trifluoroethanol, and $Me_2SO$, GA′ assumes an ordered orientation. Using high-field ring-current-shifted methyl resonances as criteria for ordered structure, we have examined pmr spectra of GA′ in trifluoroacetic acid and 6 M guanidine deuteriochloride solutions, two of the most strongly denaturing media of proteins, in an effort to convert GA′ to the disordered state.

The pmr spectra of GA′ in trifluoroacetic acid and trifluoroacetic acid-*d* (Figure 6) and in 6 M guanidine deuteriochloride (in 31.2% $D_2O$–$Me_2SO$-$d_6$ at 83°) contain distinct high field shifted methyl resonances, which indicate that some ordered structure is retained (see also Figure 7). Instability of tryptophan in acid hinders spectral measurements in trifluoroacetic acid, but the extent of decomposition (as judged by the appearance of red color in the solution)

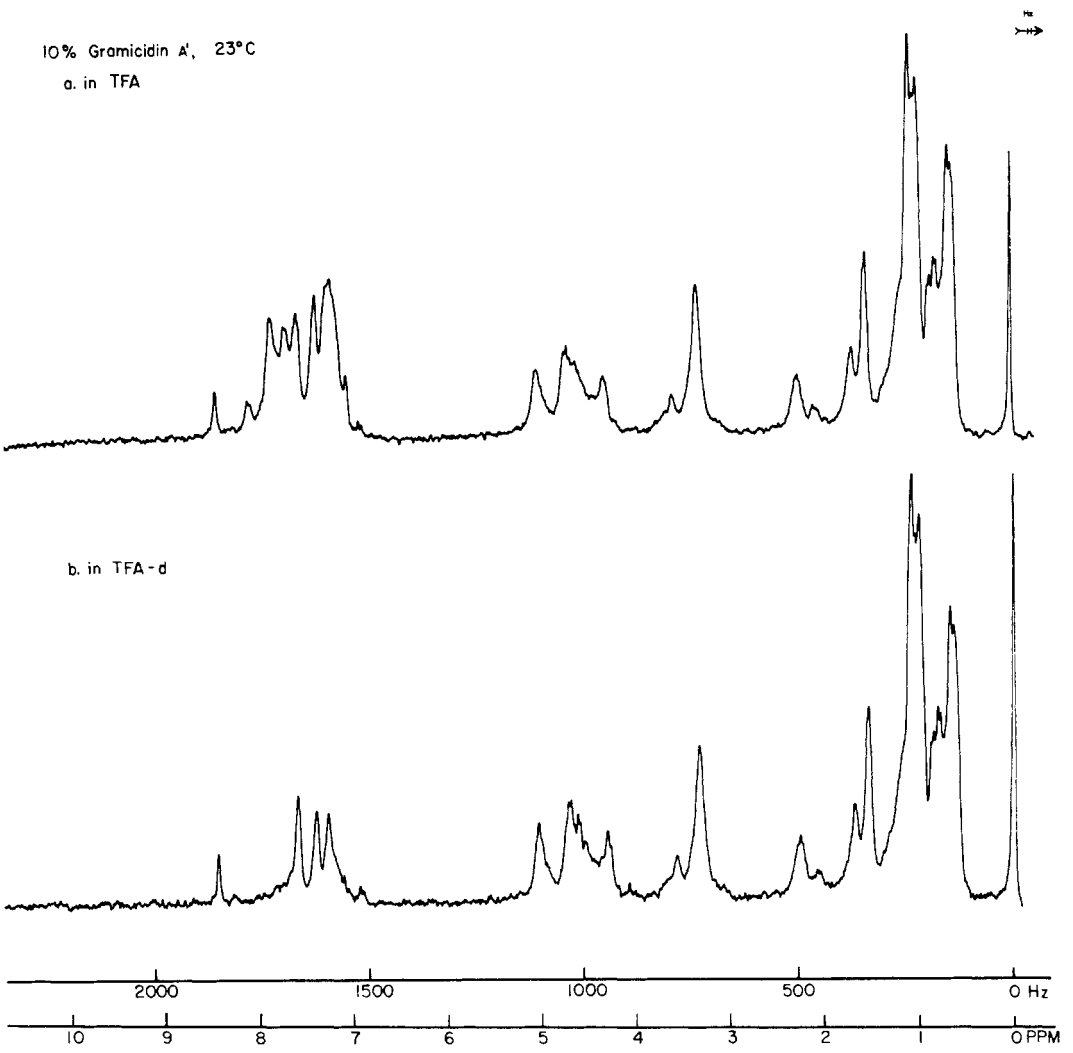


FIGURE 6: Pmr spectra of 10% GA′ (w/v) at 23° in (a) TFA and (b) TFA-*d*.

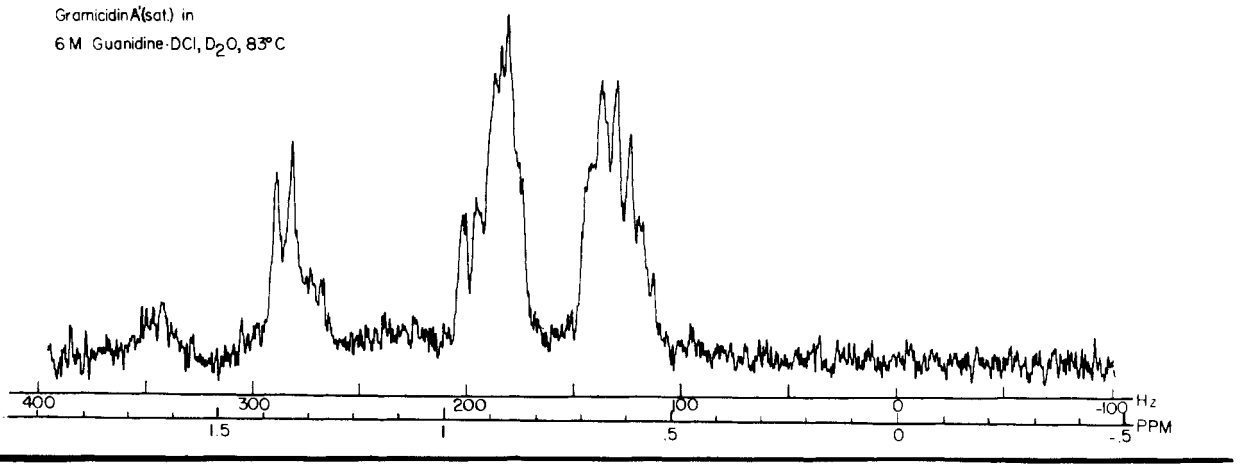


FIGURE 7: The high-field region of the pmr spectrum of GA′ (saturated) in 6 M guanidine·DCl in 31.2% $D_2O$–$Me_2SO$-$d_6$ (v/v) at 83° (25 multiscan averaged spectra). The most prominent resonances originate from methyl hydrogens of alanine (280 Hz), valine and leucine in solvated environments (180 Hz), and valine and leucine in close proximity to indole rings (130 Hz).

was minimized by rapid spectral measurements. Figure 5b shows that partial deuterium exchange of tryptophan C*H* hydrogens occurs in trifluoroacetic acid-*d* (Bak *et al.*, 1967). In trifluoroacetic acid, like in trifluoroethanol, the peptide N*H* resonances experience a strong high-field shift which makes them overlap with tryptophan C*H* absorptions.

No other polypeptide has, to our knowledge, been reported to contain order under such extreme denaturing conditions.

This retention of order may be associated with the highly hydrophobic side chains, and perhaps also with intermolecular interactions which stabilize folded conformations. They may also be due to the alternating L and D residues in the primary sequence of GA′ giving rise to intramolecular interactions between side chains, even in an extended conformation.

## Conclusions

Optical and magnetic resonance measurements indicate that the conformation of GA′ is strongly solvent dependent. In trifluoroethanol, and presumably also in $Me_2SO$, the antibiotic exists as a left-handed helix, the data for which are consistent with the family of $\pi_{L,D}$ helices proposed for the transmembrane channel structure of GA′ (Urry, 1971; Urry *et al.*, 1971). The mobility of the conformation of GA′, indicated by the ease with which solvent changes alter the backbone structure, might engender local ion-induced conformational relaxations, which are believed to occur in the transmembrane channel (Urry, 1971; Urry *et al.*, 1971). This motility is also consistent with conformational energy calculations which indicate that the $\pi_{L,D}$ helix lies in a broad energy minimum in which a number of local, energetically similar conformational energy minima exist. Transition between these local minima could be induced by solvent changes and interactions with cations.

Reviewing the arguments for a new helical conformation, the absorption data (demonstrating a hypochromism in trifluoroethanol argue for helix formation in this solvent, and the CD data in trifluoroethanol indicate a left-handed helix. The CD pattern, however, is readily differentiable from that characteristic of $\alpha$ helices, and, in particular, the pmr data (which, with the ORD, indicate similar conformations in trifluoroethanol and $Me_2SO$) provide data on the $\phi$ dihedral angle which is not possible with either $\alpha$ helices or $3_{10}$ helices. More specifically, a left-handed helix of GA′ containing as it does alternating L and D residues with one glycine in the position of a D residue, would have $\alpha$CH–NH dihedral angles of 0° for the L residues and 120° for the D residues. Based on the empirical correlations of dihedral angle and coupling constant (Ramachandran *et al.*, 1971), this would require that of the 14 possible doublets eight peptide N*H* coupling constants would be near 6 Hz and 6 would be around 3 Hz. Whenever resolved and verified by decoupling experiments, the peptide N*H* resonances of ordered GA′ in $Me_2SO$ exhibit coupling constants of greater than 7 Hz. This means that the left-handed helical conformation of GA′ has $\alpha$CH–NH dihedral angles near 180°, which corresponds to a $\phi$ dihedral angle of near 120° (using the nomenclature of IUPAC–IUB, 1970). These results are what are to be expected of $\pi_{L,D}$ helices.

## Acknowledgment

The authors thank B. Starcher for providing the amino acid analyses for gramicidin A′ and hydrogenated gramicidin A′, and the Mental Health Board of Alabama for financial support.

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