of 2′-deoxy-3-isoadenosine (**16**α) exhibited a positive Cotton effect with a peak at 300 mμ, $[M]^{25}_{300}$ 2060 ± 200°, and a trough at 272 mμ, $[M]^{25}_{272}$ −6700 ± 700°. Measurement of the ORD of the β anomer could not be extended below 320 mμ owing to a prohibitively high ϵ/[M] ratio in the short-wavelength region. However, the ORD curve was plain positive from 589 to *ca.* 370 mμ, with a broad peak at 350 mμ, $[M]^{25}_{350}$ 123 ± 10°, and indication of an incipient negative Cotton effect in the lower wavelength region. The molecular amplitude of 88 observed for the α anomer at the peak lies within the range of 50–120° observed for the usual purine nucleosides.[41]

(41) T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, *Tetrahedron Letters*, 695 (1964).

The Conformation of Gramicidin S in Solution

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Abstract: The conformation of the homodetic cyclic decapeptide antibiotic, Gramicidin S, has been investigated. Measurements were made of the ultraviolet and infrared spectra, peptide hydrogen–deuterium exchange, optical rotatory dispersion, and the circular dichroism of the molecule in solution. The results indicate that most of the models that have been proposed so far do not explain the solution-state properties of the molecule. However, the structure proposed by Scheraga and co-workers, "a structure similar to the β type, with two intramolecular hydrogen bonds," and the mixed α,β structure proposed by Hodgkin and Oughton cannot be ruled out.

The conformation of the cyclic homodetic decapeptide, Gramicidin S, has received renewed attention due to the recent potential energy calculations of Liquori's group[1] and Scheraga's group.[2,3] On the basis of these calculations, Liquori and co-workers have suggested that Gramicidin S (GS, formula I) possesses

$$\left[\begin{matrix}\text{L-Pro-L-Val-L-Orn-L-Leu-D-Phe}\\ \text{D-Phe-L-Leu-L-Orn-L-Val-L-Pro}\end{matrix}\right]$$

I

two single turns of the right-handed α-helical fold, each one facing the other, imparting a twofold axis of symmetry to the molecule. Similar calculations by Scheraga and co-workers have, however, preferred a structure similar to the antiparallel intramolecular β structure for the molecule, with two hydrogen bonds.

Earlier to these two papers, several other workers had also proposed various models for GS. Abbott and Ambrose,[4] on the basis of infrared dichroic studies in the 4500–5000-cm^{-1} region, have suggested an α-ribbon type of conformation for GS. However, X-ray diffraction analysis of GS and possible models proposed by Hodgkin and Oughton[5] to explain the data argue against this suggestion on general grounds, such as the symmetry of the crystal. Thin film dialysis work of Craig and collaborators[6] also argues against this type of α or any other compact structure, and suggests a rather open structure for GS.

Another possible model is the intramolecular antiparallel β form with four hydrogen bonds. This has been favored by Hodgkin and Oughton to explain the X-ray analysis data best. This model has also been favored by Schwyzer.[7] In this structure, out of the possible eight peptide bonds, four are engaged in hydrogen bonding within the molecule, and because of primary structural restrictions, the β form is antiparallel. This model has a truly twofold axis of symmetry. The objections to this model are the infrared work of Abbott and Ambrose, and also the preliminary deuterium exchange studies of Jaffee and Craig,[8] that suggest the easy removal of the peptide hydrogens. It is noteworthy here that the calculations of Scheraga's group predict a structure similar to the β form with only two intramolecular hydrogen bonds. This might make the molecular conformation more flexible.

It is to be pointed out that in both of the above models, the side chains are not rigidly fixed in space, even though Abbott and Ambrose talk about a parallel stacking of the side chains in the crystal.

The third possible model was proposed again by Hodgkin and Oughton, and this model was a little more involved. This is the mixed α,β structure, where the valine to ornithine is an α-type move, ornithine to leucine a β-type move, and leucine to phenylalanine is an α move. This model packs like an α-helix, has a true twofold axis, four intramolecular hydrogen bonds, and is strain-free.

The last model has been proposed by Warner[9] on the basis of molecular model studies. This is simply a model of two fused hexagons to form a naphthalene-type conformation for GS, with each peptide oxygen forming a corner of the hexagon. It has been claimed that this model maximizes the hydrophobic interactions among the side chains, a point that has not been given consideration in the previous models. No mention has been made of intramolecular hydrogen bonds. There is a hole in the center of each of the hexagons

(1) A. Liquori, P. De Santis, A. L. Kovacs, and L. Mazzarella, *Nature*, 211, 1039 (1966).
(2) G. Vanderkooi, S. J. Leach, G. Némethy, and H. A. Scheraga, *Biochemistry*, 5, 2991 (1966).
(3) H. A. Scheraga, R. A. Scott, G. Vanderkooi, S. J. Leach, K. D. Gibson, T. Ooi, and G. Némethy in "International Symposium on the Conformation of Biopolymers," University of Madras, Madras, India, Jan 18–21, 1967, Proceedings, in press.
(4) N. B. Abbott and E. J. Ambrose, *Proc. Roy. Soc.* (London), A219, 17 (1953).
(5) D. C. Hodgkin and B. M. Oughton, *Biochem. J.*, 65, 752 (1957).
(6) L. C. Craig, E. J. Harfenist, and A. L. Paladini, *Biochemistry*, 3, 764 (1964).
(7) R. Schwyzer, *Chimia* (Aarau), 12, 53 (1958); *Record Chem. Progr.* (Kresge-Hooker Sci. Lib.), 20, 147 (1959).
(8) L. C. Craig, *Science*, 144, 1093 (1964).
(9) D. T. Warner, *Nature*, 190, 120 (1961).

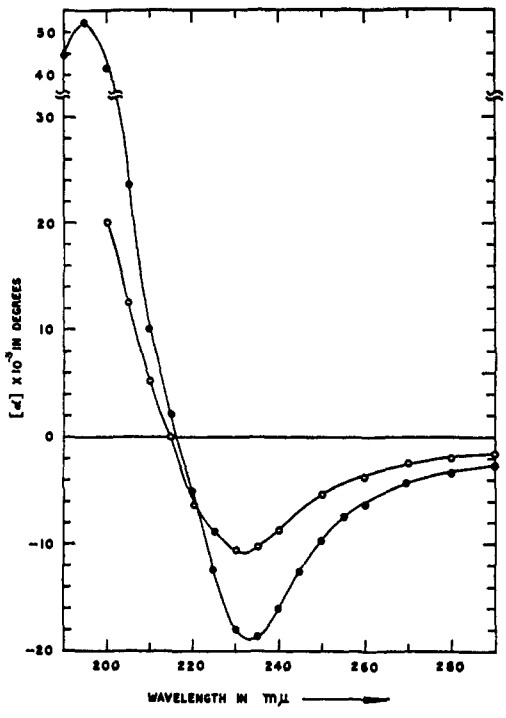

Figure 1. Optical rotatory dispersion of: -●-, Gramicidin S dihydrochloride dihydrate in ethanol, concentration 0.76 mg/ml; -○-, disarcosyl Gramicidin S dihydrochloride dihydrate in ethanol, concentration 1.56 mg/ml. All measurements were made at room temperature.

of this compact structure, through which the ornithyl side chains can enter the other side of the molecule. One side of the molecule is hydrophobic and the other hydrophilic. The two phenylalanyl groups are held together closely maximizing their hydrophobic contact. Rothen's surface studies on GS, quoted in Craig's article,[8] seem to support the contention that one side of the molecule is hydrophobic and the other side polar. However, there are serious objections to the Warner model. As mentioned earlier, the thin film dialysis work of Craig favors a rather open structure for GS. A more serious difficulty with this model is that this requires all the peptide bonds to be in the *cis* conformation, an energetically expensive situation. An all *cis* peptide model for GS no doubt confers a very compact structure and maximizes the nonpolar contacts, but whether the energy gained by hydrophobic bonding will more than overcome the energy expenditure involved in making all the peptide bonds *cis* is debatable. The results of the present paper show that a great number of the peptide bonds in GS are in fact in the *trans* conformation.

In spite of the renewed interest in the conformation of GS, there have not been too many experimental results pertaining to the solution-state conformation of this molecule. Infrared spectra in the solid state have been reported by Abbott and Ambrose, and by Schwyzer. Craig and collaborators have reported on the thin film dialysis and a partial Cotton effect curve of GS in aqueous solution.[10] In this paper, several experimental results will be presented and the merits of the various proposed conformational models will be discussed.

(10) M. A. Ruttenberg, T. P. King, and L. C. Craig, *J. Am. Chem. Soc.*, 87, 4196 (1965).

Experimental Section

Gramicidin S dihydrochloride dihydrate and disarcosyl Gramicidin S dihydrochloride dihydrate [cyclo(Sar-L-Val-L-Orn-L-Leu-D-Phe-Sar-L-Val-L-Orn-L-Leu-D-Phe)·2HCl·2H_2O] were both obtained as gifts from Professor N. Izumiya, Laboratory for Biochemistry, Faculty of Science, Kyushu University, Fukuoka, Japan. All measurements were made on these hydrochlorides in solution.

Optical rotatory dispersion (ORD) measurements were made using a Cary Model 60 spectropolarimeter. Cells of path lengths ranging from 1.0 to 0.01 cm were used and the runs were made at ambient temperature. These measurements were done at the University of Minnesota, Minneapolis, Minn. Circular dichroism (CD) measurements were made using a Jasco-Durrum spectropolarimeter (ORD-CD-UV-5) at the University of California Cardiovascular Research Center. Grateful thanks are due Professor Jen Tsi Yang for these measurements.

Ultraviolet spectral measurements were made using a Cary 14 spectrophotometer at ambient temperature. A pair of matched 1-cm quartz cells were used, and the instrument was purged with dry pure nitrogen.

Infrared spectra were recorded using a Perkin-Elmer Model 521 spectrophotometer. Spectra were obtained in potassium bromide and in Nujol mull. Solution spectra in deuterium oxide were obtained using Irtran II cells.

Deuterium oxide was 99.8% pure and was obtained from the Atomic Energy Commission, Trombay, India.

Molecular model studies were made using Dreiding models and the Fisher–Hirschfelder–Taylor space-filling models.

Results

The ultraviolet spectrum of GS in water solution reveals no peptide $\pi-\pi^*$ band maximum until at least as low as 188 mμ. The residue molar extinction coefficient at 190 mμ is close to 9000. There is just a trace of a shoulder in the $n-\pi^*$ absorption region, *i.e.*, at about 210 mμ. In general, the absorption spectral features reflect more the characteristics of cyclic peptides (large extinction coefficients, low-lying bands) than the conventional conformations such as the α-helix, β form, etc.

The optical rotatory dispersion (ORD) of GS in ethanol (*c* 0.76 mg/ml) is given in Figure 1. A trough is encountered at 232 mμ with a trough specific rotation of −19,000°. The crossover point occurs at about 215 mμ, and then a peak at 195 mμ with a peak height of 52,000°. The ORD in aqueous solution (*c* 0.2 mg/ml) is reported in a note by Ruttenberg, King, and Craig.[10] The dispersion curves in the two solvents are very similar, except the trough in ethanol is slightly enhanced and blue shifted. This could be because of either solvent shift or aggregation, but we believe that, if this effect is real at all, it is due primarily to a solvent shift (see Discussion).

Even though the dispersion profile looks in the first glance akin to that of a right-handed α-helix, closer inspection of the peak positioning, the crossover point, and the magnitudes of the rotations suggests that the right-handed helix is not necessarily present. No anomalous dispersion was observed in the phenylalanyl absorption region (260 mμ) in these experiments. Also to be noted is the fact that the ORD of GS is very similar in the trough, crossover, and peak positioning to those of two other analogs of the same family, *i.e.*, Tyrocidin B, the ORD in aqueous solution of which has been reported by Ruttenberg, *et al.*, and disarcosyl GS, the ORD of which is reported in Figure 1 along with GS. This suggests that the conformations of all these three molecules are very similar.

The circular dichroism (CD) of GS is illustrated in Figure 2. The measurements were made in aqueous

solution of concentration 0.198 mg/ml. The noise level below 195 mμ cast doubts on the reliability of the results below this wavelength, and hence the curve is presented only down to 199 mμ. However, it can be observed qualitatively that the CD spectrum remains until as low as 195 mμ, and perhaps even lower. The curve is broad, asymmetric, and has a minimum centered around 213 mμ ($[\theta] = -23{,}500°$). No shoulders were observed in the CD curve at 222 or 206 mμ, the values typical of the α-helix, suggesting the absence of any appreciably detectable amounts of the helical fold. No CD bands were noticed under these experimental conditions in the phenylalanyl absorption region. This suggests that there is no rigid orientation of the side chains at least in solution. The CD curve in the first glance looks similar to that of the antiparallel intramolecular β form,[11] but a closer look at the important details such as the magnitudes, crossover point, peaks, etc., rules out the possibility that the molecule exists entirely in the β form.

The infrared spectrum of GS reveals distinct amide I and amide II absorption bands at 1637 and 1530 cm^{-1}, respectively. Spectra in Nujol mull and in KBr pellets did not differ in peak positions. The ratio of the optical densities of the amide II to amide I was about 0.30. The presence of the amide II in the 1530-cm^{-1} region is in itself indication of the presence of *trans* peptide conformation. If one assumes that the peptide groups in helical poly α-L-glutamic acid are all in the *trans* conformation, then the ratio of the amide II to amide I optical densities of 0.4 encountered there[12] can be taken to be the calibration value for the *trans* peptide conformation. With this "calibration," we find that out of the eight secondary amides in GS at least six are in the *trans* conformation. Further, since there are two prolyl residues in the molecule, one can surmise that all the secondary amide groups in GS are possibly in the *trans* peptide conformation.

The values of the amide I and amide II frequencies reported here agree well with the reported values by Schwyzer,[13] also the relative intensities, but not with the values reported by Abbott and Ambrose[4] (1646 and 1538 cm^{-1}). Abbott and Ambrose concluded from this and from the dichroism studies at 4500–5000 cm^{-1} that GS has an α-fold, while the present results seem to contradict this.

Studies were also conducted on the time dependence of the NH to ND exchange of the peptide hydrogens when GS was dissolved in heavy water. The infrared method of Blout and co-workers[12] was followed, which measures the time-dependent decay of the amide II peak at 1530 cm^{-1} upon deuteration. GS was dissolved in deuterium oxide and let stand for various times. After each given time, the solution was rapidly evaporated to dryness under vacuum, and the infrared spectrum in the 1800–1400-cm^{-1} region in Nujol mull was recorded. (The infrared spectra in heavy water solution itself were tried using Irtran II cell windows, but the interference fringes produced by the cell material caused difficulties in the observations.) It was found that the amide II band at 1530 cm^{-1} almost completely disappeared in 15 min indicating that all the amide hydrogens in the molecule were exchanged for deuterium in this period. The amide I peak at 1637 cm^{-1} did not show any variation upon deuteration. These results suggest a rather open structure for GS in solutions.

(11) See for example, E. Iizuka and J. T. Yang, *Proc. Natl. Acad. Sci. U. S.*, **55**, 1175 (1966).
(12) E. R. Blout, C. de Loze, and A. Asadourian, *J. Am. Chem. Soc.*, **83**, 1895 (1961).
(13) R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **40**, 624 (1957).

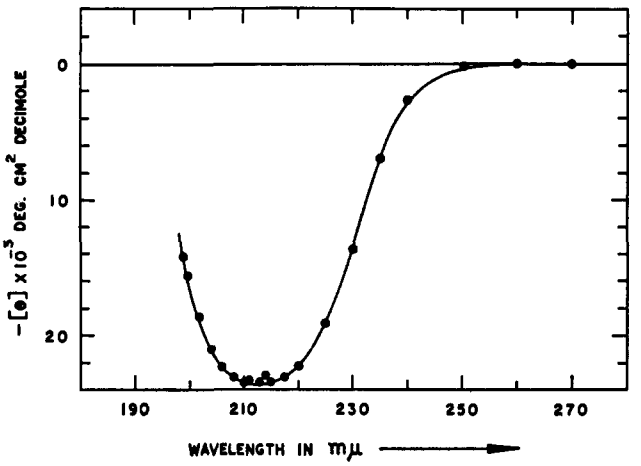

Figure 2. Circular dichroism of Gramicidin S dihydrochloride dihydrate in water at room temperature; concentration 0.198 mg/ml.

Discussion

The ultraviolet spectrum of GS does not show any maximum until as far down as 188 mμ. The value of the residue molar extinction coefficient at 190 mμ is about 9000, a value much higher than that of the α-helix (4100), the antiparallel β form (about 7500 for poly-L-lysine), or the randomly coiled form (6900).[14] The absorption maximum and extinction coefficients of GS seem, thus, to disfavor the presence of the α-helix, the β form, or the random coil or any combination of these conformations.

The ORD and CD properties of GS are the most interesting in terms of conformational studies. While the values of b_0 (−720 in water[10] with λ_0 212 mμ) and the Cotton effect trough at 233 mμ of −16,500° seem to suggest a complete right-handed α-helix in the molecule, one should view these results with some caution. It has been recently shown by Goodman and others[15] that poly N-methyl-(L-)alanine which does not form an α-helix shows an ORD curve surprisingly similar to that of the right-handed α-helix. Balasubramanian and Wetlaufer have reported[16] the ORD of cyclic peptides such as diketopiperazines and cyclic tetra-L-alanine, and these show similarities in the ORD and other spectroscopic properties with those of the righthanded α-helix. They have also suggested that any rigid peptide system that allows the proper orientation of the perturbing vicinal groups and also the proper geometric disposition of identical peptide chromophores would be prototypic to the helix, *i.e.*, will show closely related spectral properties. Thus it

(14) K. Rosenheck and P. Doty, *Proc. Natl. Acad. Sci. U. S.*, **47**, 1775 (1961).
(15) M. Goodman and M. Freed, *J. Am. Chem. Soc.*, **89**, 1264 (1967); J. E. Mark and M. Goodman, *ibid.*, **89**, 1267 (1967).
(16) D. Balasubramanian and D. B. Wetlaufer in "International Symposium on the Conformation of Biopolymers," University of Madras, Madras, India, Jan 18–21, 1967, Proceedings, in press. See also B. J. Litman and J. A. Schellman, *J. Phys. Chem.*, **69**, 978 (1965).

is entirely feasible that conformations other than the α-helix could give rise to the ORD pattern as is observed in GS.

In fact there is more evidence that the ORD of GS does not reflect the α-helical fold or the antiparallel β form. The crossover point in GS occurs at about 215 mμ, which is quite different from that encountered in either the complete helix or the complete β form. The structure cannot be a mixture of the α and the β forms, or mixtures of the random coil with either of these conformations, since the experimental peak of the Cotton effect is observed at 195 mμ with a peak rotation of 52,000°. The mixed α and β conformation, or in fact any conformation with mixtures of any of the above structures, would be expected to have different crossover points, extrema, and rotation values. Thus the ORD curve seems to owe its origin to a conformation other than the α-helix, the antiparallel β form, the disordered chain, or mixtures of these forms.

The rotatory dispersion of GS is formally very similar to those of Tyrocidine B[10] and cyclodeca-(Sar-L-Val-L-Orn-L-Leu-D-Phe)$_2$, *i.e.*, disarcosyl GS. The dispersion of the latter is shown in Figure 1 with that of the GS. They differ in their extremum rotations, but the positions of the troughs and crossovers are almost identical, suggesting that all these three molecules have very similar molecular conformations. The structure in the latter two compounds may not be as fully formed as in GS, judging from the magnitudes of the rotations in these cases and the slight blue shift of the crossover point at least in the case of disarcosyl GS.

The origin of the Cotton effect at about 215 mμ is suspected to be the n–π^* peptide transition, although the contributions from the secondary aromatic absorption around 220 mμ cannot be totally disregarded. Under these experimental conditions, however, no anomalous dispersion in the 260-mμ aromatic absorption region was detected. Also to be noticed is the slight blue shift and enhancement of the trough depth (by 2500°) of GS in ethanol compared to the water solution. This could be because of either aggregation of the GS molecules in ethanol in this concentration (0.76 mg/ml) or a slight solvent shift associated with the n–π^* transition. In the light of the suggestion by Ruttenberg, King, and Craig[10] that the aggregation of GS is predominantly hydrophobic in nature, one expects less aggregation in ethanol compared to in water. Thus this behavior in ethanol is presumably due to the solvent dependency of the peptide n–π^* band. The anomalous dispersion in the 240–190-mμ region actually suggests two Cotton effects, and the lower wavelength positive Cotton effect is almost definitely due to the peptide π–π^* band.

The CD spectrum of GS reinforces the view that the optical properties indicate a conformation different from the complete right-handed helix, the complete β form, a complete random chain, or mixtures of these. The absence of any band or shoulder at either 222 mμ or 206 mμ rules out the α-helix. While the CD band encountered at 213 mμ is reminiscent of the antiparallel β form, there is no crossover at about 206 mμ or a positive CD band centered at 195 mμ, both characteristic of the antiparallel β form.[11] This rules out the possibility that GS exists as a 100% β form. The experimental curve could be compatible with a partial β structure. But the absence of even a trend toward a minimum at 192 mμ, in the CD, and more definitively, the absence of a negative Cotton effect in the ORD curve characteristic of the disordered chain, rules out a partially disordered chain. It can then be definitively said that the models proposed by Abbott and Ambrose, Liquori and co-workers, or Schwyzer do not satisfy the observed ORD and CD properties of GS in solution.

The observation of the amide I band at 1637 and the amide II band at 1530 cm^{-1} in GS and the fact that the ratio of the optical densities of the amide II to the amide I band is close to 0.3 indicate that almost all the secondary amide groups in GS are in the *trans* conformation. Since Hodgkin and Oughton have indicated that at least four of the peptides have to be *cis* in order to fold the molecule in the α fold, this is another point in disfavor of the Abbott–Ambrose and the Liquori models. Again, the Warner model is also to be questioned on this basis, since this requires all the peptides to be in the *cis* form. The present data, which are in agreement with Schwyzer's values, are more in favor of a structure akin to the β form. However, no shoulder or a weak band in the 1685-cm^{-1} range, characteristic of the antiparallel β form, was observed. One could still argue then in favor of a partial β form. It should be pointed out, however, that cyclic peptides of various lengths show, in general, infrared spectra very similar to those of the conventional polypeptide structures, even though molecular models prohibit such conformations in these compounds. For example, cyclic tetra-L-alanine shows infrared frequencies at 1680 (m), 1650 (s), and 1540 (m) cm^{-1}, the latter two reminiscent of the helix, although this molecule does not form a helix.[16] Similarly, cyclic (Gly-L-Leu-Gly-L-Leu-Gly) displays the amide I and the amide II bands at 1651 and 1537 cm^{-1} again akin to the α-helix.[17] Thus, one has to be a little cautious in interpreting the conformation of cyclic peptides by comparison with data on the conventional linear polypeptide structures. The case of a cyclic decapeptide such as GS however is slightly different in that the loop is large enough to accommodate, in principle, the helical or the intramolecular β structures. The present infrared data of GS are then compatible with a conformation similar to the β form, or a rather flexible cyclic structure. In any case the Abbott–Ambrose, Liquori, Schwyzer, and Warner models seem inadequate to explain the infrared data.

Hydrogen–deuterium exchange studies are a little more helpful. It is known that when the amide hydrogens are involved in hydrogen bonding to form the α-helix, the exchange of these for deuterium in heavy water solution is very slow, often of the order of several days. But when the polypeptide is in the disordered conformation, deuterium exchange is complete within 10 min.[12] The deuterium exchange experiments on GS reported above reveal that essentially all the amide II peak was lost within 15 min in D_2O. Furthermore the amide I peak showed no variation upon deuteration, whereas in the β form, a shift from 1638 to 1610 cm^{-1} has been noted under similar conditions.[18] This argues against any type of a compact conformation

(17) G. W. Kenner, P. J. Thomson, and J. M. Turner, *J. Chem. Soc.*, 4149 (1958).

(18) P. Doty, K. Imahori, and E. Klemperer, *Proc. Natl. Acad. Sci. U. S.*, **44**, 424 (1958).

for GS, be it the helix or the completely hydrogen-bonded intramolecular β form. These results on GS are in favor of a conformation that is rather flexible, with perhaps weak, if any, hydrogen bonds. Craig[8] has also arrived at a similar conclusion based on preliminary exchange studies.

The Hodgkin–Oughton Mixed α,β Model and the Scheraga Model. The Liquori, Abbott–Ambrose, Schwyzer, and Warner models are inadequate to explain all the solution properties of GS. However, the mixed α,β model and the Scheraga model need to be studied more closely. The former model is obtained by folding the molecule roughly in a horseshoe shape, with all the peptides in the *trans* form, the Val–Orn move of the α-type move (*i.e.*, the NH bonds pointing in the same way, perpendicular to the plane of the ring), the Orn–Leu in a β-type move (NH bonds pointing in opposite ways), and the Leu–Phe in an α move. The two D-Phe side chains come close to each other with a possible hydrophobic interaction in solution. There is a hole in the center of this model, similar to the Warner model. Although four hydrogen bonds have been allowed to form, inspection of the Drieding models reveals that two of these are nonlinear, and thus weak. In aqueous solution, even if the hydrogen bonds are only of marginal stability, the side-chain hydrophobic interactions might serve to maintain the mixed α,β model. The "bend" of the horseshoe that carries the ornithines is the hydrophilic part and the open ends the hydrophobic part of the molecule. The mixed α,β model is not a mixture of partial α and partial β forms. While the spectral properties of this model are unknown at present, the present data need not be considered inconsistent with this structure. Unpublished surface studies of Rothen, quoted by Craig,[8] reveal that one side of the molecule is hydrophobic and the other polar, a fact that is again not inconsistent with this model. The mixed α,β structure is also "open" to a greater degree than the Warner *cis*-peptide model, and thus could explain the dialysis results.[6,19] It is also noteworthy that when L-Phe is substituted for D-Phe in GS, the possible hydrophobic contact is lost and the model becomes more open and less rigid.

The Scheraga model is a structure "somewhat similar to the β-pleated sheet," but has only two intramolecular hydrogen bonds. Substitution of L-Phe for the D isomer would result in a major alteration of the structure. The Scheraga model is reported to agree quite well with the X-ray data. Since most of the dihedral angles lie in the larger of the allowed regions of the dipeptide maps, it is conceivable that even when the hydrogen bonds are of only marginal stability, an essentially similar structure would be maintained in solution. Since Scheraga and co-workers claim that this model is to be regarded as an initial one obtained by the variational method, and also that this model is only somewhat similar to the β sheet form, one cannot disregard the validity of this structure for Gramicidin S.

In summary, the infrared, ultraviolet, hydrogen exchange, ORD, and CD measurements in solution, and also the dialysis and surface studies on GS, suggest that most of the proposed models are not totally satisfactory. These data do not, however, rule out a conformation somewhat similar to the β form, or a rather flexible Warner-type structure with *trans* peptides. In this connection then, the mixed α,β structure of Hodgkin and Oughton and the Scheraga model cannot be unequivocally disregarded, since among all the proposed structures for GS, these two are consistent with several more properties of GS in solution than the rest.[20]

Acknowledgments. The author wishes to express his grateful appreciation to Professor N. Izumiya of the Laboratory of Biochemistry, Faculty of Science, Kyushu University, Fukuoka, Japan, for the gifts of samples of Gramicidin S and disarcosyl Gramicidin S. Thanks are also due Dr. Jen Tsi Yang, of the University of California Cardiovascular Research Center, San Francisco, Calif., for the CD measurements.

(19) M. A. Ruttenberg, T. P. King, and L. C. Craig, *Biochemistry*, **5**, 2857 (1966).

(20) NOTE ADDED IN PROOF. Recently a suggestion has been made (see *e.g.*, E. Schroder and K. Lubke, Ed., "The Peptides," Vol. I, Academic Press Inc., New York, N. Y., 1965, p. 271) to classify cyclic peptides as homodetic and heterodetic. Peptides of the former class contain peptide bonds exclusively; in heterodetic cyclic peptides ring closure is brought about *via* one or several other functional groups.