A Comparison of the Solution and Crystal Conformations for the Alkali Metal Ion Complex of Antamanide†

Dinshaw J. Patel* and Alan E. Tonelli

ABSTRACT: Two cis X-Pro peptide bonds have been suggested for the alkali metal complex of antamanide in solution from an analysis of ^1H and ^{13}C nmr data (D. J. Patel, *Biochemistry 12*, 667 (1973); A. E. Tonelli, *Biochemistry 12*, 689 (1973)). The X-ray data on the structure of antamanide–Li in the crystal have located these cis peptide bonds at Pro_2–Pro_3 and Pro_7–Pro_8 (I. L. Karle *et al.*, *Proc. Nat. Acad. Sci. U. S. 70*, 1836 (1973)). The conformation 2,7-*cis* complex, reported as one of two possible candidates for the structure of antamanide–Na in solution, and the conformation of antamanide–Li in the crystal exhibit similar backbone (φ,ψ,ω) rotation angles. Both conformations predict the same amino acids participating in two type 1 ← 4 intramolecular hydrogen bonds necessary for stabilizing the backbone conformation and possess the same two cis peptide bonds to facilitate cyclization of this all-L decapeptide. Deviations of $<60°$ in the ψ rotation angles for four of the ten residues are observed between the two conformations. For these residues, the ψ rotation angles in the crystal are in high energy regions of the Ramachandran (φ,ψ) plot. The solution and crystal structures exhibit similar arrangements of the eight carbonyl groups in the vicinity of the metal ion binding site, but it is the crystallographic analysis that permits a definite identification of the ion binding site to be made. The four carbonyl oxygens at positions 1, 3, 6, and 8 and one solvent molecule coordinate the metal ion in a square pyramidal arrangement in the crystal. Hence, both the overall molecular shape and the arrangement of carbonyl groups in the metal ion binding site are remarkably similar in the solution and crystal structures.

The conformation of antamanide–Na has been intensively investigated by solution spectral techniques coupled with conformational calculations (Ivanov *et al.*, 1971; Faulstich *et al.*, 1972; Patel, 1973a; Tonelli, 1973). A subsequent X-ray analysis of antamanide–Li in the crystalline state (Karle *et al.*, 1973) permits a comparison between data observed in solution with that observed in the crystal.

Results

Cis peptide bonds were observed at Pro_2–Pro_3 and Pro_7–Pro_8 for the structure of antamanide–Li in the crystal (Karle *et al.*, 1973). Since the X-ray data are isostructural for the lithium antamanide and sodium [Phe^4,Val^6] antamanide, the backbone conformation of the crystalline peptide appears to be independent of the metal ion.

Ivanov *et al.* (1971) and Faulstich *et al.* (1972) considered only trans peptide bonds in their attempts to elucidate the structure of antamanide–Na in solution. Since the crystallographic data are only consistent with two cis peptide bonds, these structures will not be considered further.

A collaborative effort involving nuclear magnetic resonance (nmr) solution investigations (Patel, 1973a) and conformational calculations (Tonelli, 1973) predicted two cis peptide bonds for the conformation of antamanide–Na in solution. Two possibilities dependent on the location of the cis peptide bonds were considered: 1,6-*cis* complex, Val_1–Pro_2; Phe_6–Pro_7; 2,7-*cis* complex, Pro_2–Pro_3; Pro_7–Pro_8. The experimental data were interpreted to be more consistent with conformation 1,6-*cis* complex (Patel, 1973a; Tonelli, 1973). Since cis peptide bonds are observed in the X-ray structure at Pro_2–Pro_3 and Pro_7–Pro_8 (Karle *et al.*, 1973), 1,6-*cis* complex is a less likely candidate for the structure of antamanide–Na in solution.

Table I summarizes the backbone (φ,ψ,ω) rotation angles published for the 2,7-*cis* complex in solution from model building and conformational calculations (Patel, 1973a; Tonelli, 1973) and compares them with those observed in the antamanide–Li crystal structure (Karle *et al.*, 1973). The angles are defined according to the 1970 IUPAC–IUB convention (Kendrew *et al.*, 1970). The same data are plotted on Ramachandran φ,ψ maps in Figure 1. Photographs of CPK models of the crystal and solution structures are presented in Figure 2.

Discussion

(i) φ,ψ,ω *Rotation Angles.* The φ,ψ,ω rotation angles (Table I), φ,ψ Ramachandran plots (Figure 1), and CPK models (Figure 2) of the 2,7-*cis* complex conformation for antamanide–Na in solution, as derived from model building (Patel, 1973a) and conformational calculations (Tonelli, 1973), and the conformation of antamanide–Li in the crystal (Karle *et al.*, 1973) permit a detailed comparison between the two structures. Table I indicates that of the 30 rotation angles necessary to define the backbone conformation of the alkali ion complex of antamanide, the crystal and solution structures exhibit 26 rotation angles which are within $\pm 20°$ of each other. Only the ψ angles of Ala_4 and Phe_5 (and of Phe_9 and Phe_{10} by symmetry) differ ($\sim 60°$) for the solution and crystal conformations. Since the deviations from 0° of ψ_{Ala_4} and ψ_{Phe_9} compensate (symmetric about $\psi = 0°$) the deviations of ψ_{Phe_5} and $\psi_{Phe_{10}}$ (see Figure 1), the solution and crystalline structures are remarkably similar (see Figure 2). This is in contrast to a recent statement that the conformation found in the crystalline state is different from any of the conformations proposed for the sodium antamanide complex in solution on the basis of nmr data (Karle *et al.*, 1973).

† From the Bell Laboratories, Murray Hill, New Jersey 07974. *Received August 20, 1973.*

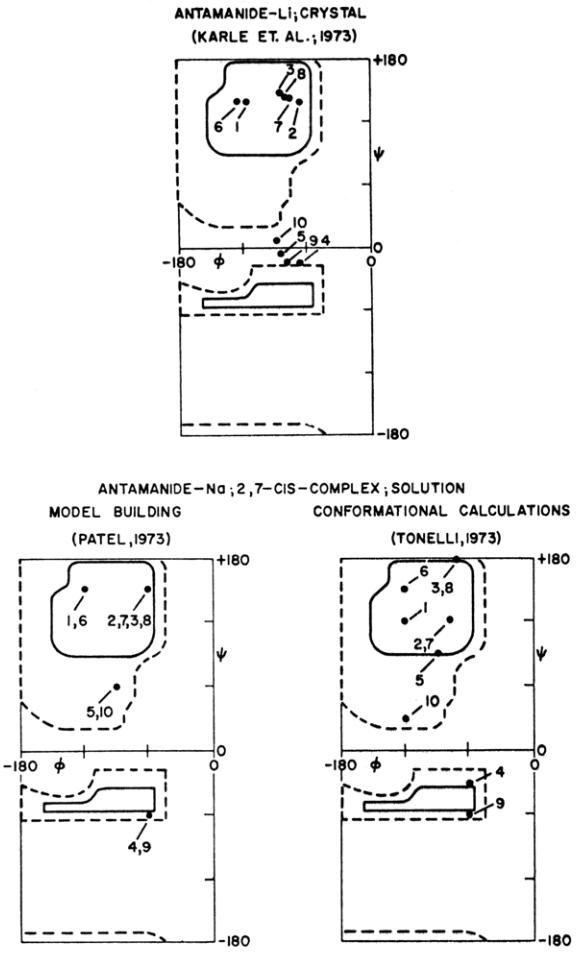


FIGURE 1: A comparison of the Ramachandran (φ,ψ) plots (Ramachandran and Sasiskharan 1968) for antamanide–Li in the crystal with those for the 2,7-*cis* complex conformation in solution as derived from model building and conformational calculations.

(ii) *Type 1 ← 4 Bends.* The conformation called 2,7-*cis* complex is stabilized by type 1 ← 4 intramolecular hydrogen bonds between

$$Pro_8\ C{=}O \cdots H{-}N\ Val_1$$

$$Pro_3\ C{=}O \cdots H{-}N\ Phe_6$$

(Patel, 1973a; Tonelli, 1973). The same hydrogen bonds were observed in the crystal of antamanide–Li (Karle *et al.*, 1973) with an oxygen–nitrogen distance of 3.00–3.05 Å, a value slightly larger than those observed for most O···H–N hydrogen bonds (2.70–2.95 Å).

The rotation angles at positions 2 and 3 for the type 1 ← 4 bend derived by model building (Patel, 1973a), conformational calculations (Tonelli, 1973), and X-ray analysis (Karle *et al.*, 1973) in the alkali metal ion complex of antamanide are presented in Table II. These may be compared with the type I and type III bends proposed by Venkatachalam (1968) from conformational calculations and evaluated in a literature search of the bends in the available protein crystallographic data (Crawford *et al.*, 1973; Lewis *et al.*, 1973).

	φ_2,ψ_2	φ_3,ψ_3
type I	−60, −30	−90,0
type III	−60, −30	−60, −30

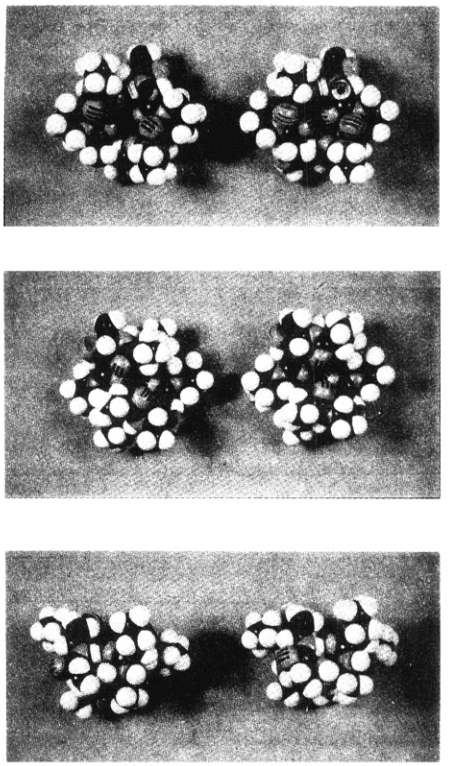


FIGURE 2: Three views of a comparison of the structure of the alkali metal ion complex of antamanide in the crystal (left) and the conformation 2,7-*cis* complex (right). The top and middle plates are two perspectives of the cavity and the bottom plate indicates the type 1 ← 4 bend and the orientation of the peptide protons. The side chains of Val_1 and the aromatic groups of Phe_5, Phe_6, Phe_9, and Phe_{10} have been left out to present a clearer view of the model.

Ramachandran *et al.* (1972) have undertaken further calculations and have refined the (φ,ψ) values originally calculated by Venkatachalam (1968); these are: type I, ϕ_2,ψ_2 (−50, −40); ϕ_3,ψ_3 (−120,60) for L-amino acids at positions 2 and 3. It may therefore be stated that the rotation angles determined in the crystal for residues 2 and 3 in the type 1 ← 4 bend are similar to those originally predicted by Venkatachalam (1968), while the rotation angles determined for these residues in the solution conformation 2,7-*cis* complex are similar to the modified values of Ramachandran *et al.* (1972).

(iii) *Metal Ion–Carbonyl Interactions.* The ^{13}C spectra of the Na complex of Val_6,Ala_9–antamanide (Bystrov *et al.*, 1972) and that of antamanide (Patel, 1973a) have been reported recently. Two carbonyl resonances shift downfield by 2.3 ppm while two others shift downfield by 1.5 ppm on complexation (Bystrov *et al.*, 1972), and all four are assigned to the carbonyls complexing the metal ion (Bystrov *et al.*, 1972; Patel, 1973a). Since these shifts reflect conformational changes on complexation as well as coordination to the metal ion, it is not clear whether the four carbonyls in the complex are oriented with the same angle and distance to the metal ion. In the crystal structure, the average Li···O distance is 2.11 Å for the complexing oxygens of residues 1, 3, 6, and 8, with the Li ion 0.4 Å above a plane made by these oxygen atoms (Karle *et al.*, 1973).

For 2,7-*cis* complex, the carbonyl oxygens of Val_1, Phe_6, Ala_4, and Phe_9 possess the geometry for complexing the metal ion. In addition, the carbonyls of Pro_2, Pro_7, Pro_3, and Pro_8 may form part of the cavity (Patel, 1973a). The carbonyls of

TABLE I: A Comparison of the φ,ψ,ω Rotation Angles (Convention of Kendrew *et al.*, 1970) for the 2,7-*cis* Complex Conformation in Solution as Derived from Model Building and Conformational Calculations with Those Determined for Antamanide–Li in the Crystal.

	Val_1	Pro_2	Pro_3	Ala_4	Phe_5	Phe_6	Pro_7	Pro_8	Phe_9	Phe_{10}
					Antamanide–Li, Crystal (Karle *et al.*, 1973)					
φ	−115	−65	−83	−67	−84	−123	−74	−69	−78	−88
ψ	138	139	147	*−14*	*−6*	139	144	144	*−15*	*7*
ω	178	−3	−173	176	−178	−171	−3	−176	172	173
					Antamide–Na; 2,7-*cis* Complex; Solution (Patel, 1973a; Tonelli, 1973) Model Building (Patel, 1973a)					
φ	−120	−60	−60	−60	−90	−120	−60	−60	−60	−90
ψ	150	150	150	*−60*	*60*	150	150	150	*−60*	*60*
ω	180	0	−180	180	−180	−180	0	−180	180	180
					Conformational Calculations (Tonelli, 1973)					
φ	−120	−78	−70	−60	−90	−120	−78	−70	−60	−120
ψ	120	120	180	*−30*	*90*	150	120	180	*−60*	*30*
ω	180	0	−180	180	−180	−180	0	−180	180	180

Val_1, Pro_3, Phe_6, and Pro_8 coordinate the Li ion in the crystal (Karle *et al.*, 1973), with the carbonyl groups of Ala_4, Phe_9, Pro_2, and Pro_7 in the vicinity of the ion binding site. Thus, the solution and crystal structures predict that the same carbonyl groups are in the vicinity of the metal ion binding site and with similar orientations (see Figure 2), but it is the crystallographic analysis that permits a definite identification of those four carbonyls that coordinate the metal ion in the crystal.

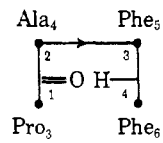
A most interesting result deduced from the crystal structure is the presence of one solvent molecule coordinated to the alkali metal ion resulting in a square pyrimidal geometry of ligands attached to it (Karle *et al.*, 1973). There is no information on this point available from the solution investigations, and it is not clear whether a square pyrimidal geometry exists for the complex in solution. In fact, when the 2,7-*cis* complex was built from CPK models an attempt was made to generate a cavity with tetrahedral coordination of the metal ion to four carbonyl ligands, but no consideration was given to one solvent molecule as an additional ligand.

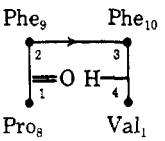
(iv) *Conformational Energies.* Although all 20 φ and ω rotation angles are very similar, there are some differences in the ψ rotation angles that need further consideration (Table I, Figure 1). The ψ angles for residues Ala_4, Phe_9, Phe_5, and Phe_{10} are located within $\pm 15°$ of $\psi = 0°$ for the crystalline structure of antamanide–Li, which represents an unfavorable region of the Ramachandran plot as derived from semiempirical functions by various groups (for a review, see Ramachandran and Sasisekharan (1968)). The structure of the 2,7-*cis* complex took this feature of the conformational map into account and our values for the ψ angles of residues 4, 9, 5, and 10 are located symmetrically about $\psi = 0°$, but in the low energy regions of the Ramachandran plot. Crystal packing forces probably compensate for the unfavorable energy of these ψ values in the crystal (see Tonelli and Brewster, 1972). Alternately, the presence of a metal ion provides a favorable energy of complexation which compensates for the unfavorable energy of their ψ values in the crystal. There are small energy barriers, if any, between the crystal structure and 2,7-*cis* complex.

It is instructive to refer to the X-ray analysis by Zalkin *et al.* (1966) on ferrichrome A in the crystal. The φ,ψ coordinates for the six amino acids that make up the cyclic hexapeptide have been reported. The rotation angles of three of the residues are given as (ϕ,ψ): Orn-3 (−145,−160); Orn-1 (−104,5); Gly (82,−2). While the rotation angles of the remaining three amino acids are well within the low energy contours of the Ramachandran plot, the φ,ψ values of Orn_3 are just at the outermost low energy contour, and the φ,ψ rotation angles of Ser_2 and Gly are outside the low energy regions of the Ramachandran plot.

The crystal structure of the K complex of the depsipeptide enniatin B has been reported by Dobler *et al.* (1969). Rama-

TABLE II





	φ,ψ		φ,ψ	
	Ala_4	Phe_5	Phe_9	Phe_{10}
Model building	−60,−60	−90,60	−60,−60	−90,60
Conformational calculations	−60,−30	−90,90	−60,−60	−120,30
X-Ray analysis	−67,−14	−84,−6	−78,−15	−88,7

chandran plots for N-alkylated amino acids and ester residues have been calculated by Ovchinnikov *et al.* (1971), Maigret and Pullman (1973), Ramachandran *et al.* (1972), and A. E. Tonelli (unpublished results). Though the rotation angles φ,ψ for the ester groups $(+60,-120)$ are in low energy regions of the φ,ψ plot for D ester residues, those of the N-alkylated peptide groups $(-60,+120)$ are outside the φ,ψ plot for L-N-alkylated amino acids.

Thus, it appears that in the crystal structures of ferrichrome A-Fe (Zalkin *et al.*, 1966), enniatin B-K (Dobler *et al.*, 1969), and antamanide–Li (Karle *et al.*, 1973) the rotation angles of a few residues are $<30°$ outside the low energy contours of the φ,ψ maps derived from conformational calculations owing most probably to favorable crystalline packing forces and/or the energy of complexation.

The calculated dipole moment of the 2,7-*cis* complex backbone was reported to be 9.2 D (Tonelli, 1973) and may be compared to the value of 9.4 D calculated for the crystalline Li complex structure. The symmetric deviations from $\psi = 0°$ in Ala_4, Phe_5, Phe_9, and Phe_{10} (see Figure 1) for the 2,7-*cis* complex conformation produce the near coincidence of calculated dipole moments for the structure in the crystal and solution. Conformational energies (sum of independent residue energies) of 12 and 11 kcal/mol are estimated for the 2,7-*cis* complex conformations derived from model building (Patel, 1973a) and conformational calculations (Tonelli, 1973), respectively, compared to a higher intramolecular conformational energy for the structure of the complex in the crystal (Karle *et al.*, 1973). At this time, it is not possible to estimate the favorable interaction between the positively charged ion and the carbonyl oxygens on complexation and hence this was not taken into consideration in calculating the conformational energies.

(v) *Peptide Bonds and the Rotation Angle* ω. In their investigation of peptides containing L- and D-amino acids, Ramachandran *et al.* (1972) made the interesting observation that a glycine or a D-amino acid facilitates cyclization of an otherwise all L polypeptide sequence containing trans peptide bonds. Antamanide which contains only L-amino acids and no glycine residues exhibits poor cyclization yields of the linear polypeptide (Wieland, 1968). It is therefore not surprising to find that both the solution (Patel, 1973a; Tonelli, 1973) and crystal (Karle *et al.*, 1973) conformations of the alkali ion complex of antamide contain two cis X-Pro peptide bonds. The crystallographic data provided the interesting observation that these cis peptide bonds are located between the Pro–Pro sequence rather than the X–Pro sequence (where X is an amino acid other than proline) in the structure of antamanide–Li in the crystal (Karle *et al.*, 1973). In the absence of specifically labeled antamanide derivatives it was not possible to reach this identification from the solution investigations (Patel, 1973a).

The X-ray data further indicate that the peptide bonds do not deviate more then $\pm 10°$ from the planar form consistent with the recent suggestions of Winkler and Dunitz (1971).

(vi) *Evaluation of Procedures Used to Derive Solution Conformation.* The application of nmr spectroscopy coupled with conformational calculations has resulted in the elucidation of the conformations of several peptides in solution (for a review, see Bovey *et al.* (1972)). Currently available crystal data on some of these peptides now permit an evaluation of the spectral and theoretical procedures used in deriving conformations in solution.

The rotation angle φ is related to the vicinal coupling constant $J_{H^NH^\alpha}$ according to the Karplus relationship (Barfield and Karplus, 1969; Bystrov *et al.*, 1969, 1973; Ramachandran *et al.*, 1971). The φ values thus evaluated from the experimental nmr coupling constant data for antamanide–Na in solution are in excellent agreement with those determined in the structure of antamanide–Li in the complex (see Table I).

The temperature coefficient of the chemical shift of exchangeable protons (Kopple *et al.*, 1969) and their susceptibility to chemical exchange in a deuterated medium (Stern *et al.*, 1968) provide measures of whether the peptide proton is hydrogen bonded or buried on the one hand or exposed to solvent on the other. Application of this approach to antamanide–Na in solution suggests that the peptide protons of Val_1 and Phe_6 participate in strong intramolecular hydrogen bonds. The peptide protons of Phe_5 and Phe_{10} are either solvent shielded or participate in weak intramolecular hydrogen bonds and the peptide protons of Ala_4 and Phe_9 are exposed to solvent (Patel, 1973a). For 2,7-*cis* complex and the crystal structure, the peptide protons of Val_1 and Phe_6 participate in $1 \leftarrow 4$ type hydrogen bonds, Ala_4 and Phe_9 are exposed to solvent, while Phe_5 and Phe_{10} are shielded from solvent by the phenyl rings.

These intramolecular hydrogen bonds were predicted to be of the $1 \leftarrow 4$ type, though this conclusion was not based on experimental evidence (Patel, 1973a). This assumption was verified in the crystallographic analysis of antamanide–Li in the crystal (Karle *et al.*, 1973). Some caution is necessary, however, since recent crystallographic investigations have shown hydrogen bonds of the $1 \leftarrow 5$ type for valinomycin in the crystal (Duax *et al.*, 1972).

The nmr technique can approach the question of whether the X–Pro peptide bond is cis or trans in solution. Thus, in proton nmr spectra, the proline H^α resonance is a doublet with $J_{H^\alpha H^\beta} = 8.0, 0$ Hz for the cis X–Pro peptide bond and a multiplet at higher field for the trans X–Pro peptide bond (Torchia, 1971). Further, distinct C^β and C^γ carbon proline chemical shifts are observed for cis X–Pro and trans X–Pro peptide bond geometries (Dorman *et al.*, 1973). On the basis of such experimental evidence, two cis X–Pro peptide bonds were proposed for the structure of antamanide–Na in solution (Patel, 1973a). The crystallographic data independently support this conclusion and validate the nmr approach to the investigation of the geometry of X–Pro peptide bonds in polypeptides.

In deriving solution conformations of peptides using model building and conformational calculations, extensive use is made of conformational maps to limit the number of structures consistent with the experimental data. Only those regions of conformational space within 5 kcal of the lowest energy regions are considered. This results in φ,ψ rotational angles for antamanide–Na in solution which are within the low energy contours of the Ramachandran plot (Figure 1). In contrast, the structure of antamanide–Li in the crystal exhibits four (φ,ψ) pairs which are in high-energy regions of conformational space (Figure 1). It should be stated that small changes in the ψ values of residues 4, 9, 5, and 10 for the structure in the crystal, as might occur on dissolution, would locate these residues in favorable areas of the conformational map with little change in the overall geometry of the complex and similar orientations of the carbonyls in the binding site. Under such conditions, the φ,ψ,ω rotation angles in the crystal and for 2,7-*cis* complex in solution would be the same.

(vii) *Comment on the Solvent-Dependent Conformations of Antamanide in Solution.* The X-ray data have located the two cis X–Pro peptide bonds at Pro_2–Pro_3 and Pro_7–Pro_8 in the conformation of antamanide–Li in the crystal (Karle *et al.*,

1973). At low Na ion concentration, a proton nmr study suggested the absence of cis–trans peptide bond isomerization for antamanide on complexation (Patel, 1973a). Thus, the solvent-dependent conformations of uncomplexed antamanide probably possess cis peptide bonds at Pro_2–Pro_3 and Pro_7–Pro_8.

The ^1H and ^{13}C nmr analysis of the solvent dependent conformations suggested cis X-Pro peptide bonds at either Val_1–Pro_2 and Phe_6–Pro_7 on the one hand or Pro_2–Pro_3 and Pro_7–Pro_8 on the other (Patel, 1973b). The former class of conformations designated 1,6-cis, which appeared to be more consistent with the experimental data, are now less likely, and consideration is given to the latter class, designated 2,7-*cis*.

The antamanide conformation in weak hydrogen bond acceptor, nonaqueous solvents (designated 2,7-*cis*-I) contains four strong and two weak intramolecular hydrogen-bonded protons and occurs in rapid equilibrium with a conformation with all peptide protons exposed to solvent (designated 2,7-*cis*-II) in strong hydrogen bond acceptor, nonaqueous media.

$$\text{2,7-}cis\text{-I} \rightleftarrows \text{2,7-}cis\text{-II}$$

These conformations have been defined in terms of φ,ψ,ω rotation angles (Patel, 1973b; Tonelli, 1973). The conformation 2,7-*cis*-I is rigid due to the presence of intramolecular hydrogen bonds and exhibits a backbone similar to the gramicidin S structure. The calculated dipole moment of 7.1 D (Tonelli, 1973) compares favorably with the experimental value of 5.2–5.8 D for antamanide in nonpolar solvents (Ovchinnikov *et al.*, 1972). Conformation 2,7-*cis*-II, which lacks intramolecular hydrogen bonds, exhibits a flexible structure, and the published rotation angles are representative of a single conformation from among a group of low-energy structures.

The conformation of antamanide in aqueous media has been suggested to be similar to the conformation of the metal ion complex of antamanide (Faulstich *et al.*, 1972; Patel, 1973a).

References

Barfield, M., and Karplus, M. (1969), *J. Amer. Chem. Soc. 91*, 1.

Bovey, F. A., Brewster, A. I., Patel, D. J., Tonelli, A. E., and Torchia, D. A. (1972), *Accounts Chem. Res. 5*, 193.

Bystrov, V. F., Ivanov, V. T., Kozmin, S. A., Mikhaleva, I. I., Khalilulina, K. Kh., Ovchinnikov, Yu. A., Fedin, E. I., and Petrovski, P. V. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 21*, 34.

Bystrov, V. F., Ivanov, V. T., Portnova, S. L., Balashova, T. A., and Ovchinnikov, Yu. A. (1973), *Tetrahedron 29*, 873.

Bystrov, V. F., Portnova, S. L., Tsetlin, V. I., Ivanov, V. T., and Ovchinnikov, Yu. A. (1969), *Tetrahedron 25*, 493.

Crawford, J. I., Lipscomb, W. N., and Schellman, C. G. (1973), *Proc. Nat. Acad. Sci. U. S. 70*, 538.

Dobler, M., Dunitz, J. D., and Krajewski, J. (1969), *J. Mol. Biol. 42*, 603.

Dorman, D. E., Torchia, D. A., and Bovey, F. A. (1973), *Macromolecules 6*, 80.

Duax, W. L., Hauptmann, H., Weeks, C. M., and Norton, D. A. (1972), *Science 176*, 911.

Faulstich, H., Burgermeister, W., and Wieland, Th. (1972), *Biochem. Biophys. Res. Commun. 47*, 975.

Ivanov, V. T., Miroshnikov, A. I., Abdullaev, N. D., Senyavina, L. B., Arkhipova, S. F., Uvarova, N. V., Khalululina, K. Kh., Bystrov, V. F., and Ovchinnikov, Yu. A. (1971), *Biochem. Biophys. Res. Commun. 42*, 654.

Karle, I. L., Karle, J., Wieland Th., Burgermeister, W., Faulstich, H., and Witkop, B. (1973), *Proc. Nat. Acad. Sci. U. S. 70*, 1836.

Kendrew, J. C., Klyne, W., Lifson, S., Miyazawa, T., Nemethy, G., Phillips, D. C., Ramachandran, G. N., and Scheraga, H. A. (1970), *Biochemistry 9*, 3471.

Kopple, K. D., Ohnishi, M., and Go, A. (1969), *J. Amer. Chem. Soc. 91*, 4264.

Lewis, P. N., Momany, F. A., and Scheraga, H. A. (1973), *Biochim. Biophsica Acta 303*, 211.

Maigret, B., and Pullman, B. (1973), *Biochem. Biophys. Res. Commun. 50*, 908.

Ovchinnikov, Yu. A., Ivanov, V. T., Bystrov, V. F., and Miroshnikov, A. I. (1972), Chemistry and Biology of Peptides, Meienhofer, J., Ed., Ann Arbor, Mich., Ann Arbor Science Publishers.

Ovchinnikov, Yu. A., Ivanov, V. T., Evstratov, A. V., and Laine, I. A. (1971), Peptides 1969, Amsterdam, North-Holland Publishing Co., p 266.

Patel, D. J. (1973a), *Biochemistry 12*, 677.

Patel, D. J. (1973b), *Biochemistry 12*, 667.

Ramachandran, G. N., Chandrasekaran, R., and Kopple, K. D. (1971), *Biopolymers 10*, 2113.

Ramachandran, G. N., Chandrasekaran, R., and Kopple, K. D. (1972), *Progr. Peptide Res. 2*, 195.

Ramachandran, G. N., and Sasisekharan, V. (1968), *Advan. Protein Chem. 23*, 283.

Stern, A., Gibbons, W., and Craig, L. C. (1968), *Proc. Nat. Acad. Sci. U. S. 61*, 735.

Tonelli, A. E. (1973), *Biochemistry 12*, 689.

Tonelli, A. E., and Brewster, A. I. (1972), *J. Amer. Chem. Soc. 94*, 2851.

Torchia, D. A. (1971), *Macromolecules 4*, 440.

Venkatachalam, C. M. (1968), *Biopolymers 6*, 1425.

Wieland, Th. (1968), *Angew. Chem., Int. Ed. Engl. 7*, 204.

Winkler, F. W., and Dunitz, J. D. (1971), *J. Mol. Biol. 59*, 169.

Zalkin, A., Fornester, J. D., and Templeton, D. H. (1966), *J. Amer. Chem. Soc. 88*, 1810.