Approximate Treatment of the Conformational Characteristics of the Cyclic Deca-L-peptide Antamanide and Its Sodium Complex in Solution†

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ABSTRACT: Several conformations for the cyclic deca-L-peptide antamanide and its Na^+ complex, which are consistent with the conformation-sensitive information obtained in recent proton magnetic resonance and ^{13}C nuclear magnetic resonance investigations, are generated and presented. Restriction of each residue to those conformations corresponding to a low intramolecular energy and possessing a dihedral angle between N–H and C^α–H^α, which is consistent with the observed vicinal spin–spin coupling for the residue in question, permits a reduction in the myriad of possible cyclic conformations to a manageable number. Generated conformations with cis peptide bonds between Val_1-Pro_2 and Phe_6-Pro_7 are presented as the most probable candidates for the solution conformations of antamanide and its Na^+ complex. The dipole moments of the generated cyclic conformations are calculated and are found to vary widely among the possible structures. Hence, measurements of the dipole moments of antamanide and its Na^+ complex in strong and weak hydrogen-bond acceptor solvents are suggested as a means of selecting the correct solution conformations from the several proposed.

Earlier we (Tonelli *et al.*, 1971) generated and proposed a low-energy solution conformation for antamanide in $CDCl_3$ which was consistent with the then available nuclear magnetic resonance (nmr) and circular dichroism (CD) data. This conformation is characterized by all-trans peptide bonds and solvent-exposed amide protons. However, as outlined by Patel (1973a) more recently available infrared (ir), CD, and nmr data (Ivanov *et al.*, 1971; Patel, 1973a,b) suggest the existence of an antamanide solution conformer with all six of its amide protons participating in intramolecular hydrogen bonds in nonaqueous weak hydrogen-bond acceptor solvents. In addition, recent investigations of proline-containing cyclic and linear peptides (Torchia *et al.*, 1972a,b; Deber *et al.*, 1971, 1972; Torchia, 1972) have revealed the solvent-dependent presence of cis as well as trans X-Pro peptide bonds, where X may be proline.

In light of the above studies and because the nmr data for the Na^+ complex of antamanide (Ivanov *et al.*, 1971; Patel, 1973b) are now also available, it is believed that a reinvestigation, or more accurately, an expanded investigation of the conformational characteristics of antamanide and its Na^+ complex is warranted. Consequently, cyclic conformations of antamanide and its Na^+ complex, which are consistent according to a "Karplus-like" relation with the measured vicinal coupling constants between the amide and α protons, are computer generated. Only cyclic structures with individual low-energy residue conformations are considered, and both X-Pro imide bonds, Val_1-Pro_2 and Phe_6-Pro_7, are allowed to adopt the cis as well as the trans conformation. In addition, the dipole moment of each low-energy conformation is estimated in an attempt to find an additional physical property through which competing structures may be differentiated.

Details of Calculations

The methods used to generate low-energy cyclic peptide conformations which are consistent with nmr data are detailed elsewhere (Tonelli, 1971a, 1972; Tonelli and Brewster, 1972; Bovey *et al.*, 1972). Specific treatment of the deca-L-peptide antamanide is presented in our previous study (Tonelli *et al.*, 1971). In brief, each residue in the deca-L-peptide is restricted to those conformations φ (rotation about the N–C^α bond) consistent with the measured amide to α-proton coupling constants J_{NC^α} according to either of the "Karplus-like" relations

$$J_{NC^\alpha} = \begin{bmatrix} 8.5 \cos^2 \varphi' \ (0° \leq \varphi' \leq 90°) \\ 9.5 \cos^2 \varphi' \ (90° \leq \varphi' \leq 180°) \end{bmatrix}$$

(Karplus, 1959, 1963; Barfield and Karplus, 1969) or

$$J_{NC^\alpha} = 8.9 \cos^2 \varphi' - 0.9 \cos \varphi' + 0.9 \sin^2 \varphi'$$

(Bystrov *et al.*, 1969), where φ' is the dihedral angle between N–H and C^α–H^α and is directly related to the N–C^α skeletal bond rotation angle φ.

A further reduction in the number of residue conformations considered in the computer search for cyclic structures is achieved by requiring each residue to adopt a conformation of low intramolecular energy. Conformational energy maps (Brant *et al.*, 1967; Schimmel and Flory, 1967, 1968) calculated for trans peptide bond dipeptide fragments are used in the selection of low-energy residue conformations.[1]

Adoption of standard polypeptide geometry (Brant and Flory, 1965; Ramachandran and Venkatachalam, 1968), in-

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[1] The conformational energy maps of the residues in the immediate vicinity of a cis peptide bond have not been published. Since the Val_1-Pro_2 and Phe_6-Pro_7 peptide bonds are allowed to adopt the cis as well as the trans conformation, it is not possible to estimate the intramolecular conformational energy of those generated cyclic structures containing at least one cis peptide bond. Instead, it is assumed that the allowed or low-energy conformations of residues neighboring a cis peptide bond are the same as those of the trans peptide bond conformers. This assumption receives partial justification from approximate potential energy estimates made for cis and trans peptide bond oligomers of proline (Tonelli, 1970) and alanine (Tonelli, 1971b).

cluding planar peptide bonds, permits a computer test for ring closure using the transformation of virtual bond vectors method (Brant and Flory, 1965). A space filling molecular model is constructed for each cyclic conformation generated and is observed for interactions, such as steric overlaps and intramolecular hydrogen bonds, longer in range than those considered in the calculation of the dipeptide conformational energy maps. Stabilization attributable to intramolecular hydrogen bonding is estimated following the method of Brant (1968). Since there are four imide bonds in antamanide (between Val_1-Pro_2, Pro_2-Pro_3, Phe_6-Pro_7, and Pro_7-Pro_8), there are 16 distinct conformers in all which differ in their cis peptide bond content and distribution. However, we assume that only the peptide bonds between Val_1-Pro_2 and Phe_6-Pro_7 can adopt the cis conformation on the basis of previous studies of the conformational characteristics of cis and trans peptide bond containing proline oligomers and polymers (Deber *et al.*, 1970; Tonelli, 1970; Torchia and Bovey, 1971), which show that in the residue sequence -X-Pro-Pro- it is the X-Pro peptide bond that is most likely to be cis.

The dipole moment of each low-energy cyclic conformation generated was calculated following the matrix method of Flory (Flory and Schimmel, 1967; Brant *et al.*, 1969). Adjacent α-carbon atoms may be connected by ficticious bonds termed virtual bonds (Brant and Flory, 1965). Providing the peptide bond is held in a fixed conformation the distance between adjacent α-carbon atoms or the length of the virtual bond remains constant, independent of rotations about the N–C^α and C^α–CO bonds. Calculation of the dipole moment of a polypeptide involves expression of each of the peptide group dipole moments in a reference frame along its virtual bond. Adoption of the group dipole moment used by Brant and Flory (1965) leads to

$$m = \begin{bmatrix} -0.63 \\ 3.65 \\ 0.0 \end{bmatrix} \text{D}$$

for peptide bonds, where *m* is the peptide group dipole moment expressed in the usual coordinate system (Brant and Flory, 1965) along a virtual bond. Based on the calculations of Yan *et al.* (1970), it is assumed that the partial charge distribution about the peptide bond is the same for both trans and cis conformations.

In the calculation of the dipole moment of the *trans*-M conformation (Ivanov *et al.*, 1971), two of whose peptide bonds are *ca.* 30° from the planar, trans conformation, the peptide group dipole moment expressed along the virtual bond spanning the nonplanar peptide bond is

$$m = \begin{bmatrix} -0.42 \\ 3.45 \\ 0.68 \end{bmatrix} \text{D}$$

When transforming (Brant and Flory, 1965) the peptide group dipole moments of the two nonplanar peptide groups to a common reference frame, which is necessary in the vector summation of group dipole moments (Flory and Schimmel, 1967; Brant *et al.*, 1969), it was assumed that the rotation angle about these bent or nonplanar peptide bonds was 0° (trans) instead of 30°.

Calculated Results

Three overall classes of conformations were generated for antamanide and its Na^+ complex: classes I and II antamanide conformations characterized by six and no intramolecularly hydrogen-bonded amide protons, respectively, and class complex Na^+ antamanide conformations characterized by two strong intramolecular hydrogen bonds as discussed by Patel (1973b). The proposed conformations of antamanide and its Na^+ complex are presented in Tables I and II together with their calculated dipole moments. Conformation *trans*-II (Tonelli *et al.*, 1971) was generated previously and is not discussed further. We were unable to generate a low-energy all-trans peptide bond conformation (*trans*-I) for antamanide with each of the amide protons participating in an intramolecular hydrogen bond. The lowest energy all-trans peptide bond conformation found for the Na^+–antamanide complex (*trans*-complex) is very similar to the structure generated by Patel (1973b) in his model-binding studies.[2] A cavity lined with the carbonyl groups of the Val_1, Pro_2, Phe_6, and Pro_7 residues in a tetrahedral arrangement would appear to allow the *trans*-complex conformer to bind metal ions.

Several low-energy conformations with one or two cis peptide bonds at Val_1-Pro_2 and/or Phe_6-Pro_7 were generated. However, none of the generated cyclic conformers possessing a single cis peptide bond was consistent with the nmr data for antamanide in weak hydrogen-bond acceptor solvents (all N–H's intramolecularly hydrogen bonded), although several (see Patel, 1973b) did exhibit a carbonyl oxygen cavity for the binding of metal ions.

On the other hand, when both the Val_1-Pro_2 and Phe_6-Pro_7 peptide bonds were fixed in the cis conformation low-energy cyclic structures consistent with the nmr data for antamanide and its Na^- complex were found. The lowest energy structures found (see Table I) for antamanide with all amide protons participating in intramolecular hydrogen bonds and with all amide protons exposed to solvent are designated 1,6-*cis*-I and -II, respectively, according to Patel (1973a,b). Actually, many low-energy cyclic structures with cis Val_1-Pro_2 and Phe_6-Pro_7 peptide bonds, in addition to the conformer 1,6-*cis*-II presented in Table I, were found. Confomer 1,6-*cis*-II is simply a representative example of the apparently flexible class of antamanide conformers with amide protons exposed to nonaqueous strong hydrogen-bond acceptor solvents. Following Patel (1973b), the generated conformation of the Na^+–antamanide complex containing cis Val_1-Pro_2 and Phe_6-Pro_7 peptide bonds is called the 1,6-*cis*-complex.

In addition to the conformations generated in this study, those structures with cis Pro_2-Pro_3 and Pro_7-Pro_8 peptide bonds (2,7-cis conformers) obtained by Patel (1973a,b) through molecular model building studies and the conformations *trans*-M and *trans*-M-complex proposed by Ivanov *et al.* (1971) and Faulstich *et al.* (1972) for antamanide and its Na^+ complex, respectively, are also listed in Tables I and II for purposes of comparison and discussion (see below).

Discussion of Results

The three classes of proposed antamanide and Na^+–antamanide conformations (trans, 1,6-cis, and 2,7-cis) are distin-

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[2] The values of the rotation angles for the various antamanide and Na^+-antamanide complex conformers given by Patel (1973a,b) and presented here in Tables I and II are identical. This coincidence is a result of searching for the lowest energy conformation generated here by computer which most closely approximated the conformer obtained from Patel's molecular model-building studies. Such a procedure was followed, because it is a bit difficult to read off rotation angles from molecular models and we wanted to find the lowest energy (intramolecular) cyclic conformer which still retains all of the gross features (J_{NC^α}, hydrogen bonds, etc.) of the molecular model derived structure.

TABLE I: Proposed Solution Conformations of Antamanide.

Conformer	Rotation Angle[a]	Val$_1$,Phe$_6$	Pro$_2$,Pro$_7$	Pro$_3$,Pro$_8$	Ala$_4$,Phe$_9$	Phe$_5$,Phe$_{10}$	Calcd Dipole Moment (D)
1,6-*cis*-I	φ	60,30	100–120	100–120	30	30,90	10.3–11.3
	ψ	300,330	310–330	270	120	120	
	ω	180	0	0	0	0	
2,7-*cis*-I	φ	60	120	120	60	60	7.1
	ψ	300	330	150	300	300	
	ω	0	180	0	0	0	
trans-M[b]	φ	240	120	120	90	240	2.8–8.7
	ψ	270	120–150	120–150	150–180	90	
	ω	~30	0	0	0	0	
1,6-*cis*-II	φ	30	120	120	60	30	12.9
	ψ	300	330	120	300	360	
	ω	180	0	0	0	0	
2,7-*cis*-II	φ	60	100	120	60	90	0.9–7.6
	ψ	270–330	300	300	270–330	330	
	ω	0	180	0	0	0	
trans-II	φ	90	102	122	90	90	16.8
	ψ	300,270	310	125	120	330	
	ω	0	0	0	0	0	

[a] All rotation angles are expressed according to the convention of Edsall *et al.* (1966). [b] Proposed by Ivanov *et al.* (1971) and Faulstich *et al.* (1972).

TABLE II: Proposed Solution Conformation of the Na$^+$–Antamanide Complex.

Conformer	Rotation Angle	Val$_1$,Phe$_6$	Pro$_2$,Pro$_7$	Pro$_3$,Pro$_8$	Ala$_4$,Phe$_9$	Phe$_5$,Phe$_{10}$	Calcd Dipole Moment (D)
1,6-*cis*-Complex	φ	60	120	120	120	90	18.7
	ψ	300	330	120	120	120,150	
	ω	180	0	0	0	0	
2,7-*cis*-Complex	φ	60	102	110	120	90,60	9.2
	ψ	300,330	300	0	150,120	270,210	
	ω	0	180	0	0	0	
trans-Complex	φ	60	120	120	120	90	15.6
	ψ	300,330	330	125	120	270	
	ω	0	0	0	0	0	
trans-M-Complex[a]	φ	80	120	120	330	250	2.2
	ψ	20	130	270	150	110	
	ω	0	0	0	0	0	

[a] Proposed by Ivanov *et al.* (1971).

guishable primarily by the number (0 or 2) and placement (Val$_1$-Pro$_2$ and Phe$_6$-Pro$_7$ or Pro$_2$-Pro$_3$ and Pro$_7$-Pro$_8$) of their cis peptide bonds. It is clear from Patel's nmr solvent and Na$^+$ ion-dependent equilibrium exchange studies (Patel, 1973a,b) that the interconversion of conformers I (all N–H's intramolecularly hydrogen bonded) and II (all N–H's solvent exposed) and the complexation equilibria between I and the Na$^+$ complex do not involve isomerization of any peptide bonds. Thus, all three classes of conformations (I, II, and complex) must have an identical number and distribution of cis and trans peptide bonds.

On the basis of the C$^\alpha$ splitting patterns in the proton nmr spectra of antamanide in nonaqueous solvents and the chemical shifts of the C$^\beta$ and C$^\gamma$ resonances in the ^{13}C nmr spectra of antamanide and its Na$^+$ complex in CD$_3$CN, it appears, as Patel (1973a,b) has suggested, that two of the four imino acid residue peptide bonds (Val$_1$-Pro$_2$, Pro$_2$-Pro$_3$, Phe$_6$-Pro$_7$, and Pro$_7$-Pro$_8$) are cis and two are trans. This conclusion is further supported by the CD spectrum of perhydroantamanide in methanol (Faulstich *et al.*, 1972). We therefore conclude that antamanide dissolved in nonaqueous strong or weak hydrogen-bond accepting solvents and its Na$^+$ complex contains two cis peptide bonds. Of the proposed structures listed in Tables I and II, only those of the 1,6-cis and 2,7-cis families possess two cis peptide bonds.

As discussed by Patel (1973b), the 1,6-cis family of conformations appears preferable to the 2,7-cis conformers on the basis of the presence of a cluster of carbonyl groups in 1,6-*cis*-I, which may serve as a recognition site for the complexation of metal ions, and the consistency between the predicted and observed chemical-shift behavior of the amide protons on complexation and of the solvent dependency of the amide proton chemical shifts in the Na^+ complex. An additional factor favoring the 1,6-cis family of conformations, which was mentioned earlier in the discussion as a partial justification for neglecting the 2,7-cis conformers in the generation of antamanide structures, is the observation (Deber *et al.*, 1970; Tonelli, 1970; Torchia and Bovey, 1971) that the probability of finding the X-Pro peptide bond in the cis conformation in an X-Pro-Pro peptide sequence is considerably higher (a factor of two or more) than the probability for a cis Pro-Pro peptide bond. The preference of cis X-Pro peptide bonds over cis Pro-Pro peptide bonds can be traced (Tonelli, 1970) to the existence of a greater number of steric interferences between the pyrrolidine rings in an X-Pro-*cis*-Pro conformation as compared to those in X-*cis*-Pro-Pro conformers.

Finally, it is apparent from the calculated dipole moments presented in Tables I and II that, with the possible exception of class II conformers which are probably flexible with solvent-exposed amide protons, each of the three classes of conformations (I, II, and complex) may be distinguished between the two cis peptide bond families 1,6-cis and 2,7-cis by the large differences in their dipole moments. Such a differentiation is currently in progress utilizing a dielectric constant cell designed to measure the dipole moments of liquids in microquantities.

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