CONFORMATIONAL STATES OF ANTAMANIDE AND ITS ANALOGS IN SOLUTIONS

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Macrocyclic compounds capable of complexing alkali-metal ions and thereby inducing the cationic permeability of various membrane systems (so-called ionophors) have recently been widely used in biochemical investigations (see [1] and the references which it gives). A characteristic feature of the majority of the compounds of this type studied (valinomycin, the enniatins, the nactins, etc.) is the high K/Na selectivity of complex formation and of induced ionic flows, amounting to 10,000:1 in valinomycin [1, 2, 6]. Since the possibility of inducing the transport of sodium ions through biological membranes is of considerable interest, a task which has now become urgent is the search for membrane-active compounds with sodium selectivity. In this connection, attention is attracted by the cyclic decapeptide antamanide (I) (Fig. 1), which complexes sodium ions in organic solvents considerably more effectively than potassium ions (stability constants of the complexes in ethanol 2500 and 200 liters/mole, respectively) [3].

In order to study the reasons for the sodium specificity of the complex formation of antamanide and to find membrane-active complexes based on it, we have investigated the conformation of antamanide and its Na complex with the aid of a wide variety of physicochemical methods. A spatial structure of the Na complex which satisfied all known experimental results has been given in a previous paper [4]. The structure found served as a basis for an analysis of the relationship between structure and complex-forming properties in the antamanide series [5].

The present paper gives the results of a study of the conformational states of free antamanide in various solvents (for a preliminary communication, see [5]). Together with antamanide we studied perhydroantamanide (II), in which the phenyl chromophoric groups have been replaced by cyclohexyl groups [7] and [Val^6, Ala^9]antamanide (III) [8], the symmetrical primary structure of which substantially facilitates the interpretation of spectral results.*

Spectral Characteristics

A. Circular Dichroism (CD) Curves. On considering the CD curves of antamanide (Fig. 2), it can be seen that on passing from a nonpolar medium [heptane–dioxane (5:2)] to more polar media, particularly those containing hydroxy groups, the intensities and positions of the Cotton effects change, and in some cases (curve 6 and, apparently, 5) the sign undergoes reversal and the number of Cotton effects changes. Such considerable changes in the CD curves cannot be explained by changes in the electronic structures of the amide and phenyl chromophoric groups on solvation; in our opinion, they are connected with conformational rearrangements of the antamanide with the change in the solvent. The CD curves of the analog (III)

* Perhydroantamanide (II) is characterized by a somewhat higher solubility in organic solvents than (I) and (III), which leads to the appearance of membrane activity [5, 7]. [Val^6, Ala^9]antamanide (III) possesses a substantially higher tendency to complex formation with Na^+ and K^+ than antamanide (stability constants of the complexes in ethanol 25,000 and 1000 liters/mole, respectively) [5, 8].

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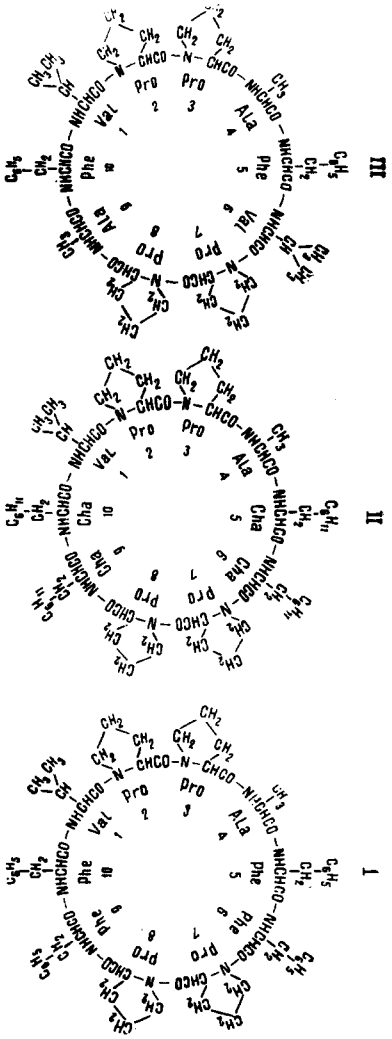

Fig. 1. Structure of antamanide (I), perhydroantamanide (II), and [Val^6, Ala^9]antamanide (III).

(Fig. 3) are extremely close to the corresponding curves of (I), which shows the similarity of the predominating conformations of the two cyclodecapeptides. In addition, a comparison of curves 1, 3, and 5 (see Figs. 2 and 3) leads to the conclusion that in antamanide the transition from "nonpolar" forms to "polar" forms takes place at lower concentrations of ethanol than in (III). The observed differences in the CD curves (for example, the decreased intensity of the Cotton effect in (III) in the 270-250 nm region) are frequently connected also with a lower number of aromatic chromophoric groups.

Previously, on considering the ORD curves of antamanide, we came to the conclusion that several forms participate in the conformational equilibrium [4]. In view of the existence of an isosbestic point, it was assumed that two main forms take part in this equilibrium. However, on the CD curves of compounds (I-III) the nonmonotonic nature of the change in the curves in the short-wave region (below 212 nm) with an increase in the concentration of ethanol in heptane can be seen (curves 1, 3, and 5 in Figs. 2-4), which shows the existence of a larger number of conformers. The most clearly shown feature can be found on the CD curves of perhydroantamanide (II), which has a considerably simpler form than that of compounds (I) and (III) because of the absence of phenyl chromophoric groups and the chiral-optical effects connected with them. For example, the passage from a mixture of heptane and dioxane (5:2) to a mixture of heptane and ethanol (9:1) is accompanied by a change in the sign of the CD curve in the 200-212-nm region, and with a further increase in the polarity of the medium (passage to 96% ethanol or a 1:1 mixture of ethanol in water) the sign of the CD curves returns to what it was before (negative) (Fig. 4). Nevertheless, if information for nonpolar media (curves 1 in Figs. 2-4) is disregarded, the CD curves of compounds (I-III), like the ORD curves of antamanide (I), have isosbestic points at 206-215 nm.

Thus, if it is assumed that in nonpolar media there is one preferred conformation of compounds (I-III), the nature of the change of their CD curves with a change in the solvent is in harmony with three main forms participating in the conformational equilibrium. We have called them A, B, and C. Curves 1 in Figs. 2-4 correspond to form A, which exists in nonpolar media; the addition of small amounts (10-20%) of ethanol (curves 2) displaces the equilibrium in the direction of form B, which, with a further increase in the ethanol and then in the water content, gradually changes into form C (curves 4 and 5). The hypothesis put forward was confirmed in the course of a further investigation of compounds (I-III) by IR and NMR spectroscopy.

B. IR and NMR Spectra. As can be seen from Fig. 5, the IR spectra of compounds (I-III) in dilute solutions of $CHCl_3$ are extremely similar. The absence in the amide A region of bands at 3400-3500 cm^{-1} shows the participation of all six NH groups in intramolecular hydrogen bonds (intra-HBs). The characteristics of the NMR spectra of compounds (I-III) are shown in Figs. 6-11 and in Tables 1 and 2. In an analysis of the spectra of chloroform solutions (Figs. 6-8), no additional signals that could show a trans $\rightleftarrows$ cis isomerization of the tertiary amide groups were found. In the spectrum of antamanide ($CDCl_3$, 30°C, Fig. 6) in the region of the signals from the amide NH groups that is most important for conformational investigations (6-9 ppm), only three signals out of six were seen; the others are concealed beneath the signals of the protons of the phenyl groups and of the solvent ($CHCl_3$ as an impurity in the $CDCl_3$). On passing to perhydroantamanide (II), this region becomes substantially simpler in view of the absence of aromatic protons (see Fig. 7). In this case it is possible to see all six NH signals which, as in the sodium complex of antamanide [4], are grouped by the values of the chemical shifts and their temperature dependences and the spin–spin coupling constants of the protons in the NH–CH fragments into three pairs (see Table 2), thereby showing the conformational similarity of the "diametrically" opposite amino acid residues (see Fig. 1). The assignment of the signals of Cha^6 and Cha^9 shown in Table 1 was made on this basis. In agreement with the pri-

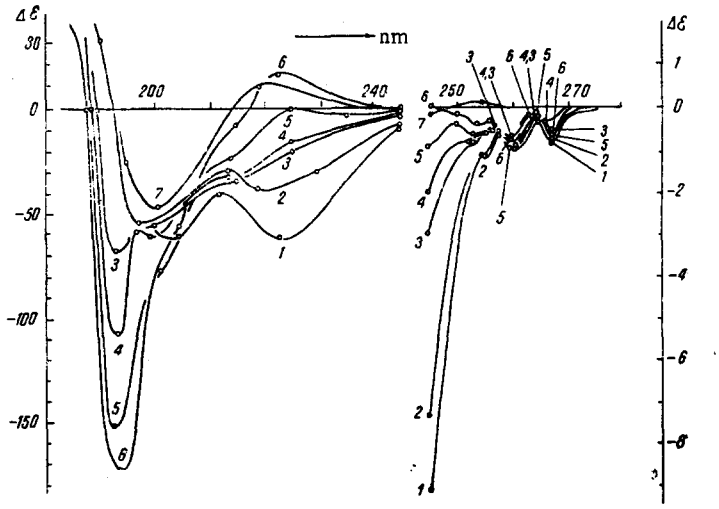

Fig. 2. CD curves of antamanide (I) in: 1) heptane–dioxane (5:2); 2) acetonitrile; 3) heptane–ethanol (9:1); 4) trifluoroethanol; 5) ethanol; 6) water–ethanol (3:1); 7) ethanol+NaCl.

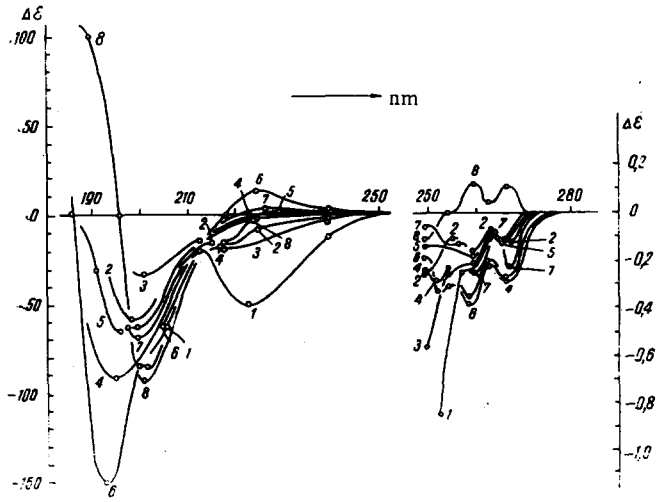

Fig. 3. CD curves of [Val^6, Ala^9]antamanide (III): 1) heptane–dioxane (1:1); 2) acetonitrile; 3) heptane–ethanol (4:1); 4) trifluoroethanol; 5) ethanol; 6) water–ethanol (2:1); 7) ethanol+KCl; 8) ethanol+NaCl.

mary structure, there is a second-order axis of rotation in [Val^6, Ala^9]antamanide (III), as follows from its NMR spectrum, which changes only slightly on cooling to −35°C and contains the signals from five pairs of structurally equivalent amino acid residues (see Fig. 8). On the whole, by comparing the values of δ, $\Delta\delta/\Delta T$, and $^3J_{NH-CH}$ for chloroform solutions (see Table 2) it may be concluded that the conformational characteristics of compounds (I-III) in this solvent are monotypical, which agrees completely with the features of the circular dichroism and IR spectra given above.

For further study, we chose [Val^6, Ala^9]antamanide (III), since it had simpler NMR spectra than compounds (I) and (II). Like the CD curves, the NMR spectra of compound (III) changed markedly on the addition of hydroxyl-containing solvents. For example, in the presence of 3 vol.% of CH_3OH the $NH^{1,6}$ and $NH^{4,9}$ signals shifted upfield by 0.34 and 0.05 ppm, respectively, and the signals from $NH^{5,10}$ underwent a paramagnetic shift by 0.16 ppm (see Fig. 9 and Table 2). A further increase in the concentration of CH_3OH led to a change in the direction of the shifts of the NH signals. The position of the extremum at ~4 vol. % of CH_3OH agrees with the CD results. The $^3J_{NH-CH}$ constants changed simultaneously with the chemical shifts; such behavior of the NH signals on passing from a nonpolar to a polar medium has also been observed and been studied in detail for the cyclodecadepsipeptide valinomycin (see Fig. 4 [11] and Fig. 9). It

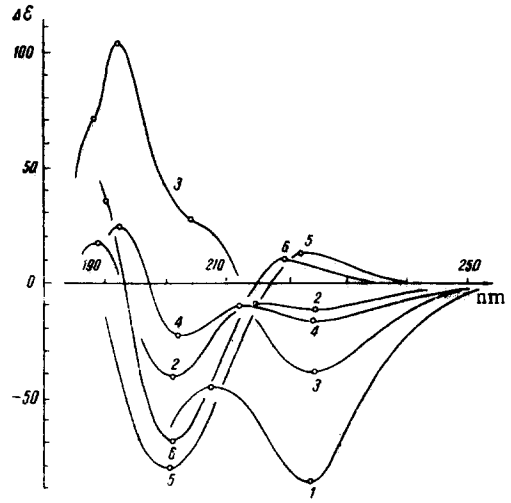

Fig. 4. CD curves of perhydroantamanide (II): 1) heptane–dioxane (5 : 2); 2) acetonitrile; 3) heptane–ethanol (9 : 1); 4) ethanol; 5) water–ethanol (1 : 1); 6) ethanol + NaCl.

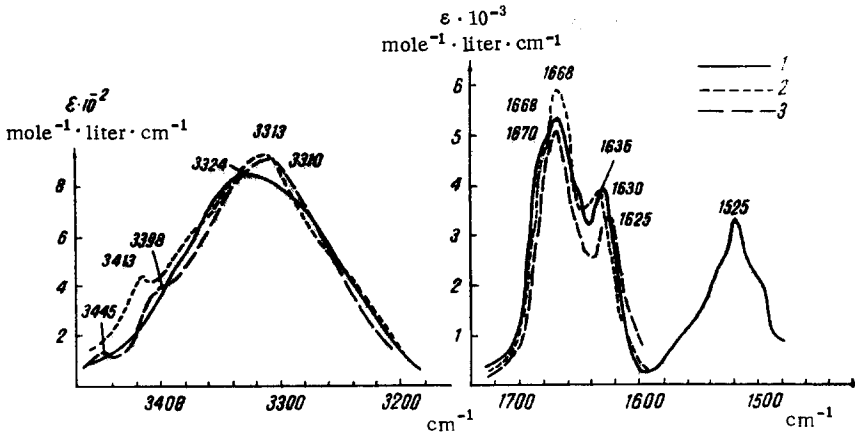

Fig. 5. IR spectra of antamanide (I) and its analogs (II) and (III): 1) antamanide (I); 2) perhydroantamanide (II); 3) [Val^6, Ala^9]antamanide (III).

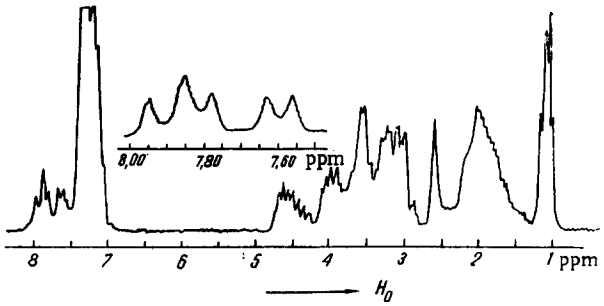

Fig. 6. NMR spectrum of antamanide (I) in chloroform.

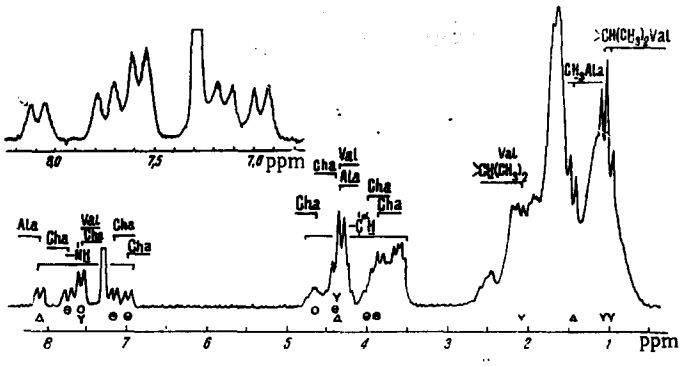

Fig. 7. NMR spectrum of perhydroantamanide (II) in chloroform at 50°C. The symbols denote the bound protons. The assignment of the signals is shown above the spectrum.

TABLE 1. Chemical Shifts (ppm) of the NMR Signals of the CH Groups of Compounds (I-III) in $CDCl_3$

Fragment	Residue	Antamanide [28], 53°C	Perhydroantamanide, 50°C	[Val^6, Ala^9]Antamanide, 30°C
$C(CH_3)_2$	Val*	1,01(7Hz), 1,08(7Hz)	1,00 (7,0 Hz) 1,07 (7,0Hz)	0,94 (6,8 Hz) 0,87 (6,8Hz)
CH_3	Ala*	1,16 (7 Hz)	1,46 (7,4 Hz)	1,42 (7,3Hz)
CH_2(β, γ)	Pro	1,78 1,83 2,12	1,5—2,6	1,5—2,6
C^β H	Val	2,05	2,06	2,13
C^α H	Val	4,49	4,37	4,38
	Ala	4,35	4,37	4,51
	Pro	3,85 3,95 4,09	3,5—4,1	3,0—4,1
	Phe/Cha†	4,11 4,64 4,67	3,90 3,83 4,65 4,40	3,82
C_6H_5/C_6H_{11}	Phe/Cha	7,1—7,4	1,3—2,0	7,1—7,3

* The spin—spin coupling constants are given in parentheses.
† The values of the chemical shifts of the $C^\alpha H$ groups are shown in order of increasing chemical shifts of the neighboring NH protons corresponding to them (see Table 2).

may be assumed that the changes observed in the spectra of compounds (I-III) are, as in the case of valinomycin, connected with a conformational rearrangement of the molecule and consequent cleavage of the intra-HBs.

To determine the participation of the NH groups in intra-HBs, the temperature gradients of the chemical shifts of the NH groups and the rates of deuterium exchange, NH → ND, were measured (see Table 2). The results obtained permit a differentiation of the intra-HBs in $CDCl_3$ according to their strength. In perhydroantamanide (II) the strongest bonds are formed by the NH groups of the valine residue and one of the cyclohexylalanine residues ($\Delta\delta/\Delta T = 1 \cdot 10^{-3}$ ppm/deg). NH groups of the other two Cha residues with $\Delta\delta/\Delta T = 5.7$ and 4.2 ppm/deg give less strong intra-HBs. We propose the following explanation of the negative temperature gradients observed for compounds (II) and (III) in $CDCl_3$ (see Table 2). In the energetically favorable conformational state of the molecule of the cyclopeptide, the NH groups to which the negative gradients correspond participate in weak intra-HBs. These intra-HBs are due, in the first place, to the fact that in the predominating conformation, stabilized in this case by nonvalent interactions of the atoms, the corresponding NH and C=O groups are located close to one another. With a rise in the temperature, this conformation becomes less rigid, and the weak intra-HBs break. As a result, the corresponding NH groups acquire the capacity for entering into intermolecular association on interacting with the carbonyl groups of other molecules of the same compound. Since the intermolecular bonds must be stronger than the weak intra-HBs, a downfield shift of the signal with a rise in the temperature (negative temperature gradient) takes place. With a further rise in the temperature it is to be expected that the NH shift will reach an extremum and then the signal will shift upfield because of the rupture of the intermolecular H bonds. Apparently, under the conditions of our experiments the temperature corresponding to the extremum was not reached. Thus, we assume that the NH^4 and NH^9 groups of compounds (II) and (III) form the weakest intra-HBs of the six present in form A. Their cleavage is apparently connected with the presence in the IR spectra of weak bands of free NH groups appearing in the form of inflections on the high-frequency side of the main amide A bands (see Fig. 5). The greatest intensity of these bands (at 3413 cm^{-1}) is observed in per-

TABLE 2. Parameters of the NMR Signals of the NH Groups of Compounds (I-III)

Parameter of the spectrum	Antamanide [28], $CDCl_3$	Perhydroantamanide, $CDCl_3$	[Val^6, Ala^9]Antamanide		
			$CDCl_3$	3% CH_3OH in $CDCl_3$ or 2% CD_3OD in $CDCl_3$	CH_3OH or CD_3OD
	Val^1	Val^1 Cha^6	Val^1, Val^6	Val^1, Val^6	Val^1, Val^6
δ, ppm	7,66	7,58 7,58	7,75	7,40	7,90
$^3J_{NN-CH}$, Hz	7,1	6,8 6,8	6,6	7,0	6,0
$\Delta\delta/T\ 10^{-3}$, ppm/deg	—	1,0 1,0	2,0	0	7,2
$\tau_{1/2}$	—	— —	—	~5 days	<5 min
	Ala^4	Ala^4 Cha^9	Ala^4, Ala^9	Ala^4, Ala^9	Ala^4, Ala^9
δ, ppm	8,01	8,08 7,76	7,60	7,57	7,86
$^3J_{NH-CH}$, Hz	8,5	8,0 8,5	8,5	8,6	7,8
$\Delta\delta/T\ 10^{-3}$, ppm/deg	—	—3,3 —6,0	—2,0	—2,3	4,7
$\tau_{1/2}$	—	— —	—	2 h	<5 min
	Phe^5; Phe^6; Phe^9; Phe^{10}	Cha^5; Cha^{10}	Phe^5, Phe^{10}	Phe^5, Phe^{10}	Phe^5, Phe^{10}
δ, ppm	7,05; 7,45; 7,88	6,97; 7,16	7,24	7,40	7,43
$^3J_{NH-CH}$, Hz	7,0 8,0 6,0	7,0 7,3	7	7,1	8,0
$\Delta\delta/T\ 10^{-3}$, ppm/deg	—	4,25; 5,7	—	4,4	11,2
$\tau_{1/2}$	—	— —	—	~3 days	30 min

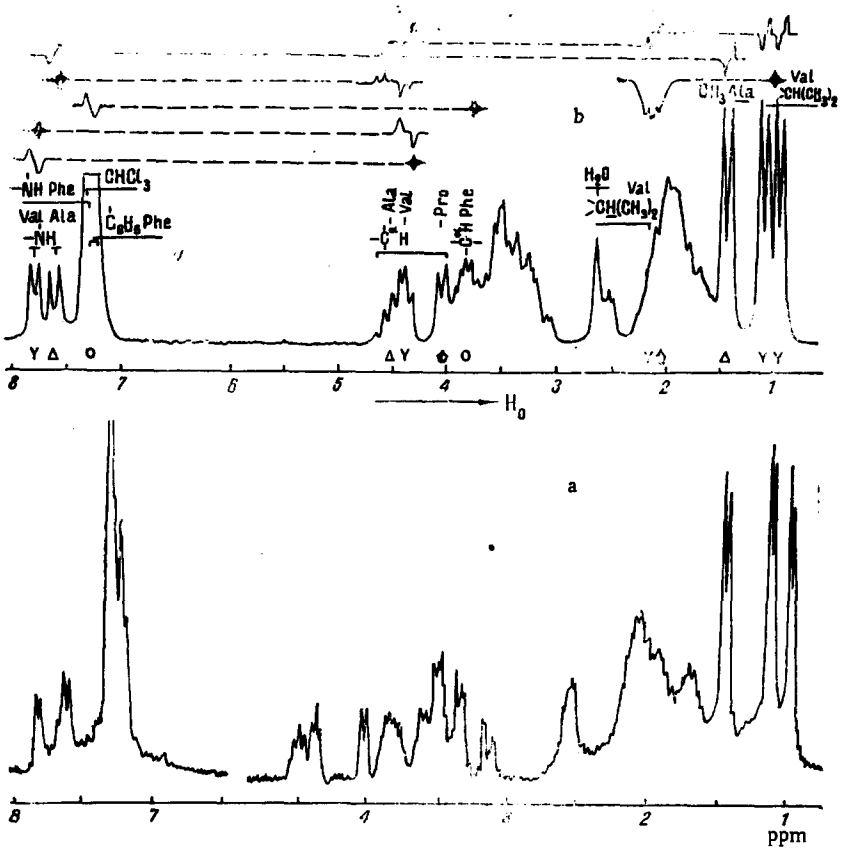

Fig. 8. NMR spectra of [Val^6, Ala^9]antamanide (III) in chloroform: a) 200-MHz spectrum at 20°C; b) 100-MHz spectrum at 30°C (the signals obtained by the INDOR method, by means of which the assignment of the signal shown by the symbols was performed, are shown above the spectrum).

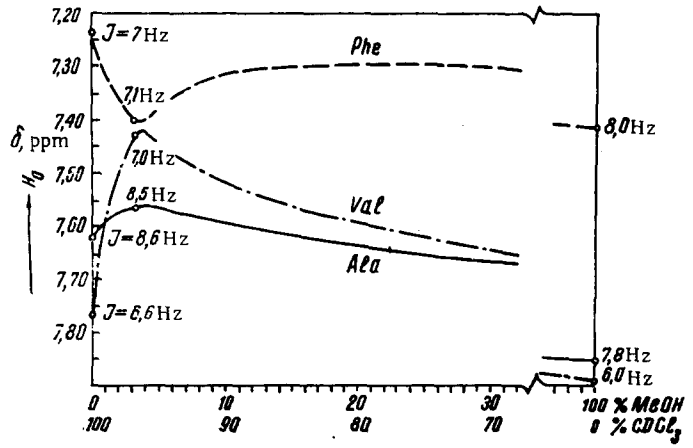

Fig. 9. Dependence of the chemical shifts of the NH protons and the $^3J_{NH-CH}$ constants in the spectrum of [Val^6, Ala^9]-antamanide (III) on the composition of the $CDCl_3-CH_3OH$ mixtures.

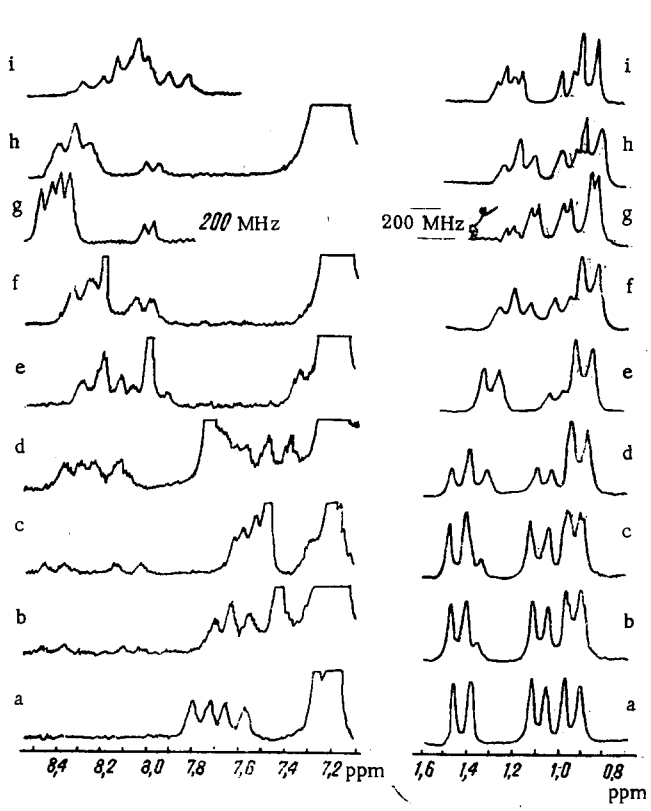

Fig. 10. Changes in the NMR spectrum of [Val^6, Ala^9]-antamanide (III) on the addition of $(CD_3)_2SO$ to $CDCl_3$ at 30°C: a) $CDCl_3$; b) 5.7% of $(CD_3)_2SO$; c) 9.1% of $(CD_3)_2SO$; d) 23.3% of $(CD_3)_2SO$; e) 48% of $(CD_3)_2SO$; f) 77% of $(CD_3)_2SO$; g) $(CD_3)_2SO$ at 20°C, 200 MHz; h) $(CD_3)_2SO$; i) $(CD_3)_2SO$ at 68°C. The left-hand spectra show the region of the NH protons and the right-hand spectra that of the CH protons.

hydroantamanide, which agrees with the higher absolute values of the negative temperature gradients of the chemical shifts than for compound (III).

It is natural that it is just the NH^4 and NH^9 groups that are solvated primarily by methanol in $CDCl_3$–CD_3OD (98:2). This follows from the comparatively high rate of the deuterium exchange in compound (III) (see Table 2). The preferential nature of the cleavage of the $NH^4\cdots O=C$ and the $NH^9\cdots O=C$ intra-HBs also depends on the participation in their formation of the carbonyl groups of the proline residues (see Figs. 12 and 13), which effectively interact with alcohols [32]. So far as concerns solutions of the analog (III) in methanol, judging from the values of $\tau_{1/2}$ and of $\Delta\delta/\Delta T$, they lack intra-HBs. The somewhat retarded rate of deuterium exchange of the $NH^{5,10}$ groups is apparently due to the nonequivalence of the steric environments of the $NH^{5,10}$ and $NH^{1,6}$ groups, since the extremely high value of $\Delta\delta/\Delta T$ of $11.2 \cdot 10^3$ ppm/deg excludes the possibility of the existence of intra-HBs.

Thus, in spite of certain difficulties arising in the interpretation of the spectral results, on their basis it is possible to conclude that the conformational transition A → B → C observed with the gradual addition of methanol or ethanol to solutions of compounds (I-III) in nonpolar organic solvents is accompanied by the rupture of the system of intra-HBs: first the bonds with the participation of the $NH^{4,9}$ groups are cleaved with the formation of form B, and then form C, which does not contain intra-HBs, predominates. It must be mentioned that we have observed a similar type of conformational equilibrium previously for the cyclododecadepsipeptide valinomycin [11]. Furthermore, compounds (I-III) differ sharply in their conformational mobility from another cyclodecapeptide – gramicidin S – which scarcely changes its spatial structure and the intra-HB system of the main peptide chain when the solvent is varied [12].

Interesting information has been obtained in a study of the NMR spectra of [Val^6, Ala^9]antamanide (III) in media containing dimethyl sulfoxide. The addition of this solvent to a solution of (III) in chloroform or methanol is accompanied by the appearance of new signals that can be found in all the regions of the spec-

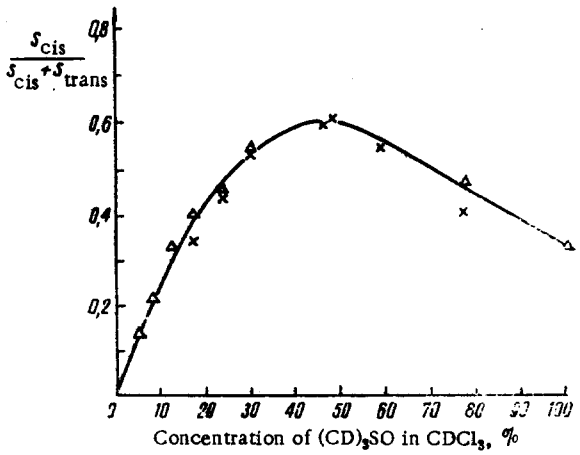

Fig. 11. Dependence of the amount of cis form of [Val^6, Ala^9]antamanide (III) on the composition of $CDCl_3-(CD_3)_2SO$ mixtures; × – Val, Δ – Ala.

trum. As can be seen from Fig. 10, with a gradual increase in the concentration of $(CD_3)_2SO$ in $CDCl_3$ to a ratio of 1 : 1 two new signals appear in the weak-field region (8.0-8.5 ppm) at first hardly perceptible and then with ever-increasing intensity; these have been assigned by means of the INDOR method to the Ala^{1,6} and Phe^{4,9} groups.* In the 0.8-1.5-ppm region a symbatic increase in the intensity of the additional signals corresponding to the C-methyl groups of the alanine and valine residues is found. In our opinion, the phenomenon observed is connected with the cis–trans isomerism of the tertiary amide groups formed with the participation of the proline residues.†

The coalescence of the signals from the valine C-methyl groups in $(CD_3)_2SO$ solution is found at ~ 130°C, which shows a considerable height of the energy barrier of the mutual conversion of the cis and trans forms of [Val^6, Ala^9]antamanide (III), exceeding the height of the barriers found in linear proline derivatives (characteristic for these are coalescence temperatures of ~ 80°C [16]). It will be shown below that in form A of [Val^6, Ala^9]antamanide (and, consequently, in the B and C forms) all the amide bonds possess the trans configuration. Consequently, the conformers appearing on the addition of $(CD_3)_2SO$ have the cis configuration of one or more amide bonds. From the ratio of the areas of the C-methyl signals and from the 200-MHz spectra and those of the amide protons we have determined the dependence of the molar fraction of the cis form on the concentration of dimethyl sulfoxide. On using for this purpose the signals either of the valine or of the alanine groups, practically identical results were obtained (see Fig. 11), from which it follows that the amount of cis form may reach 60%.

Spatial Structure of Antamanide in Nonpolar Solvents

The structure of form A has been established by the successive consideration of all the possible conformations of the cyclopeptides (I-III) with a second-order axis of symmetry and six intra-HBs. Of these, those conformations were selected in which the orientation of the protons in the NH–CH fragments agreed with the $^3J_{NH-CH}$ constants given in Table 2 (i.e., Φ^1 and $\Phi^6 \sim 150°$, ~ -90 or $60 \pm 30°$, Φ^4 and $\Phi^9 - 120 \pm 20°$, Φ^5 and $\Phi^{10} \sim -150°$, ~ -90 or $60 \pm 25°$, taking into account the corresponding stereochemical relationships [13, 24]).‡ Here, the possibility of the realization both of the trans ($\omega \sim \pm 180°$) and the cis ($\omega \sim 0°$) configurations of the tertiary amide bonds was taken into account. The results of the analysis showed that the conditions mentioned are satisfied by only two types of structures with all the amide bonds in the trans configuration; their conformational parameters Φ and Ψ were determined by taking into account literature information on peptides containing proline residues [15, 19-22] and intra-HBs of various types [21, 23]. Below

* It is interesting to note that the $^3J_{NH-CH}$ constant found for the NH^{1,6} groups is 10.7 Hz (with a correction for the electronegativity of the substituents 11.7 Hz [13]), which is greater than any other value of $^3J_{NH-CH}$ observed hitherto.

† The equilibrium of the trans and cis forms of linear derivatives of proline has been shown previously for a large number of examples [14-16]. Recently, the appearance of additional signals due to cis–trans isomerism has also been detected in cyclic hexapeptides containing proline residues [17].

‡ In the present paper we use the conformational nomenclature proposed in 1970 by the IUPAC commission [18].

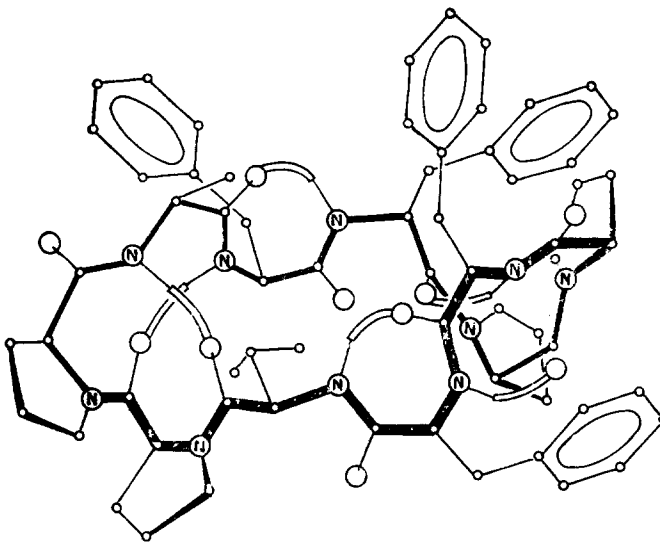

Fig. 12. Conformation of antamanide (I) in nonpolar media (side view).

we give the values of Φ and Ψ at which the distance between the ends of the peptide chain does not exceed 1 Å (possible deviations of the angles not greater than 10-15°).

1. Structure with intra-HBs at 1 → 9, 6 → 4, 10 → 7, 5 → 2, 9 → 6, 4 → 1.

	$Val^{1,6}$ and Phe^{6}	$Pro^{2,7}$	$Pro^{3,8}$	$Ala^{4,9}$ and Phe^{9}	$Phe^{5,10}$
Φ	−80	−60	−55	−100	60
Ψ	165	−40	−40	10	−70

2. Structure with intra-HBs at 1 → 9, 6 → 4, 10 → 5, 5 → 10, 9 → 6, 4 → 1.

	$Val^{1,6}$ and Phe^{6}	$Pro^{2,7}$	$Pro^{3,8}$	$Ala^{4,9}$ and Phe^{9}	$Phe^{5,10}$
Φ	20	−60	−60	−140	70
Ψ	150	−35	−35	− 30	−70.

The second structure is unlikely, since it corresponds to extemely high energies of closest-range interaction (for example, in residues 1 and 6 preceding the Pro^{2} and Pro^{7} residues [22]). Furthermore, the dipole moment calulated for it (2.4 D) is considerably lower than the experimental dipole moments [5.8 ± 0.3 D for (I) and 5.2 ± 0.3 D for (III)], while the dipole moment of the first structure (~4.5 D) agrees with the experimental value more satisfactorily.

From what has been said it follows that form A of cyclopeptides of the antamanide group corresponds to a structure of type 1. As can be seen from Fig. 12, it contains two intra-HBs of the 3 → 1 type and four intra-HBs of the 4 → 1 type; the formation of the latter fixed the proline residues in a conformation corresponding to a fragment of a 3_{10} helix. The coordinates Φ and Ψ of the amino acid residues in positions 1, 3, 4, 5, 6, 8, 9, and 10 correspond to the permitted regions on the corresponding maps; analogous conformations have been found previously by x-ray structural analysis and spectral methods in proteins [25] and various peptide systems [10, 11, 13, 24, 26]. Scheraga has shown that the conformations with Φ = 60° and Ψ = 40° which we have proposed for the $Pro^{2,7}$ residues preceding the $Pro^{3,8}$ residues are also found in the permitted conformational regions and can be realized in cyclic peptides [20] in spite of the fact that they possess a considerably higher energy than the conformations with Φ = 60°, Ψ = 120-180°, and some other authors consider them to be forbidden [15, 19].

As can be seen from Fig. 13, the conformations of the peptide chain I in form A and in the Na complex are extremely similar, and to a first approximation the transition of the conformation of the complex into conformation A can be represented as the result of the rotation of the planes of the secondary amide groups formed by Pro^{3} and Pro^{8}. In these circumstances, CO^{3} and CO^{8} are displaced from the central part of the molecule to its periphery, and the NH^{4} and NH^{9} groups are close to CO^{1} and CO^{6} and form intra-HBs with them; simultaneously, the CO^{1}, CO^{6}, CO^{5}, and CO^{10} groups are somewhat displaced from the center of the

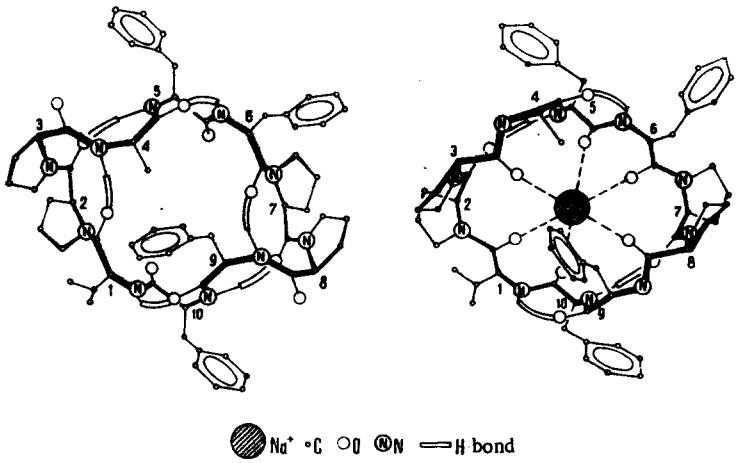

Fig. 13. Conformation of antamanide (I) in nonpolar media and of its Na^+ complex (view from above).

internal cavity. Thus, while in the complex six carbonyl groups are spatially close and are oriented within the molecule, ensuring effective ion–dipole interaction with the cation, in the free antamanide these groups are located at a considerably greater distance (6-12 Å), excluding any appreciable dipole–dipole repulsion whatever. In addition, form A is stabilized by two additional intra-HBs.

So far as concerns the B and C forms, to determine their conformational parameters a more extensive theoretical analysis of the molecule of the decapeptide or its fragments including the minimization of the potential energy with respect to several variables (analogous to the investigation performed for valinomycin [11] and the enniatins [27]) is necessary. We are performing this investigation at the present time.

Shortly after this study had been completed, an investigation of the conformational states of antamanide in solutions with the aid of CD curves, NMR spectra, and a theoretical conformational analysis was published [28]. The authors concerned came to the conclusion that in all the media considered (dioxane, chloroform, methanol, trimethyl phosphate, etc.) antamanide occupies a monotypical conformation characterized approximately by the following parameters:

	Val[1]	Phe[6]	Pro[2], Pro[7]	Pro[3], Pro[8]	Ala[4], Phe[9]	Phe[5], Phe[10]
Φ	90	90	102	122	90	90
Ψ	270	300	310	125	120	330
ω	0	0	0	0	0	0

Its distinguishing features are the absence of intra-HBs and the orientation of all the amide carbonyl groups on one side of the central plane of the ring; the dipole moment that we have calculated for the values of Φ and Ψ given is 16.6 D. Thus, the proposed structure seriously contradicts the results of IR spectroscopy and dipole-moment measurement reported in the present paper and therefore it cannot be considered as a preferred conformation of antamanide in nonpolar media. Nevertheless, it is not excluded that such a structure is possessed by form C which, as shown above, is the dominating form in solutions in CH_3OH or EtOH–H_2O (1:1). The $^3J_{NH-CH}$ constants measured in CH_3OH solutions (Table 2) agree with the coordinates $\Phi \approx 90°$ [28] for the amino acid residues in positions 1, 4, 5, 6, 9, and 10. In our view, one of the reasons for the erroneous conclusion of the American and German authors [28] must be considered the defective argumentation of the important hypothesis of the weak dependence of the spatial structure of antamanide on the composition of the medium. As has been shown above, the CD curves used for this purpose actually show the presence of a complex conformational equilibrium which is displaced when the solvent is changed. The measurement of the rate of deuterium exchange of the NH groups as the only method of detecting intra-HBs also appears unsatisfactory, since the kinetics of this reaction depend not only on the intra-HBs but on a large number of inadequately studied factors (in the first place, the steric screening of the NH groups and the features of the electronic structure of the different amide groups). Finally, we must mention the extreme simplicity of the theoretical conformational analysis, with the a priori assumption of improbable conformations in which the amino acid residues have the coordinates $\Phi = 210\text{-}270°$. This hypothesis contradicts both the calculated and the experimental results [11, 13, 24-26]. On the whole, the results of a comparison of this paper [28] and the present investigation convincingly show the necessity for using in the study of the conformational states of peptide systems the widest possible set of physicochemical and theoretical methods giving independent and mutually supplementary information.

Experimental

Before the physicochemical measurements, the cyclopeptides (I-III) were dried over P_2O_5 at 0.5 mm and 50°C for 16 h. The CD curves were measured on a Cary-60 spectropolarimeter with a Cary-6001 attachment for taking CD curves at concentrations of the solutions of $(0.1 \pm 1)^* \cdot 10^{-3}$ M and a temperature of 23-26°C; thickness of the cells 0.01-1 cm. The IR spectra were recorded on a UR-10 instrument with LiF and NaCl prisms. The cell thickness for measurements in the 3500-3200-cm^{-1} region was 20 mm and for 1750-1610 cm^{-1} it was 0.1 and 5 mm, the concentrations of the solutions being $\sim 7.8 \cdot 10^{-3} - 5.2 \cdot 10^{-4}$ M. The NMR spectra were recorded on JEOL 4H-100 and Varian HA-100 spectrometers with a working frequency of 100 MHz and stabilization of the resonance conditions on one sample, and also on a 200-MHz spectrometer (Branch of the Institute of Chemical Physics of the Academy of Sciences of the USSR). Tetramethylsilane was used as internal standard. The chemical shifts were determined with an accuracy of ± 0.005 ppm, the spin–spin coupling constants with an accuracy of ± 0.1 Hz, and the temperature with an accuracy of ± 2°C. The concentration of the solutions was 0.05 M. The assignment of the signals was made by the INDOR method (see Fig. 8) [29] or by double resonance. The dipole moments were measured on an instrument working by the beat method at a frequency of 1 MHz, and calculation was performed by Hedestrand's method [30]. The dipole moments for given coordinates Φ and Ψ were calculated by the successive summation of the vectors of the dipole moments of the amide groups; the magnitudes and directions of these moments have been determined previously [31]. The comparatively small contributions of the moments of the side hydrocarbon groups were not taken into account.

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Summary

1. A preferred conformation of antamanide in nonpolar solvents has been proposed with the aid of a wide group of physicochemical methods and theoretical analyses.

2. On the basis of spectroscopic results, the hypothesis has been put forward of the existence of two other forms of antamanide (B and C) which form on passing from nonpolar to polar media.

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