The Conformation of the Sodium Complex of a Biologically Active Analog of Antamanide in the Crystalline State†

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ABSTRACT: Biologically active synthetic analogs of antamanide, that have been prepared so far, have all shown the ability to form a complex with sodium ions. The conformation of the sodium complex of [Phe^4-Val^6]antamanide (II) was established by crystal structure analysis of the bromide salt using X-ray diffraction. The complex crystallizes in space group $P2_12_12_1$ with cell dimensions $a = 12.880 \pm 0.005$, $b = 38.860 \pm 0.015$, and $c = 13.955 \pm 0.006$ Å. Eight peptide linkages are in the trans conformation while the Pro_2–Pro_3 and Pro_7–Pro_8 peptide bonds are cis. The outline of the ring resembles that of a saddle with an upper and a lower cavity. Carbonyl oxygen atoms of Val_1, Pro_3, Val_6, and Pro_7 separate the two cavities. The pentacoordinated Na^+ resides in the upper cavity with four Na–O ligands to O(1), O(3), O(6), and O(8) and the fifth ligand to the O atom of ethanol, the solvent from which the crystal was grown. The phenyl rings of Phe_4, Phe_6, Phe_9, and Phe_{10} are folded toward the polypeptide ring so as to make a more globular molecule. The complex has a charged interior and a lipophilic exterior. The NH···Br^- bonds are the only hydrogen bonds formed. Several other NH groups are shielded and do not participate in hydrogen bonding. The sodium [Phe^4-Val^6]-antamanide·C_2H_5OH complex is essentially isostructural (but not isomorphous) with lithium antamanide·CH_3CN.

The cyclic decapeptide antamanide (I) counteracts the toxicity of phallotoxins by preventing their accumulation in liver cells (see *e.g.*, Wieland (1972) and references therein). A necessary condition for biological activity appears to be the formation of a Na^+ complex. Antamanide selectively complexes with Na^+ rather than K^+. Numerous analogs of antamanide have been synthesized by the solid phase method and examined for their antitoxic activity. With one exception, all the analogs that form stable Na^+ complexes are biologically active (Wieland, 1972). The Na^+ complex of the symmetric analog (II), [Phe^4-Val^6]antamanide, was chosen for a conformational study in the solid state by single crystal X-ray diffraction analysis. The results will be compared to the conformation found in the crystal of the lithium antamanide·CH_3-CN complex (Karle *et al.*, 1973; Karle, 1974).

(all-L-) Pro(8)-Phe(9)-Phe(10)-Val(1)-Pro(2) / Pro(7)-Phe(6)-Phe(5)-Ala(4)-Pro(3) — I; Pro(8)-Phe(9)-Phe(10)-Val(1)-Pro(2) / Pro(7)-Val(6)-Phe(5)-Phe(4)-Pro(3) — II (all-L-)

Experimental Section

Crystals of the sodium complex of [Phe^4-Val^6]antamanide were grown in an ethanol solution of NaBr and the symmetric antamanide. They were provided by Drs. Tr. Wieland, W. Eurgermeister, and H. Faulstich of the Max Planck Institut für Medizinische Forschung, Heidelberg. In order to prevent deterioration upon drying, a single crystal was enclosed in a thin-walled capillary with a drop of mother liquor for the X-ray diffraction experiment. During the measurement of the intensities of the reflections, three reflections from three different zones were monitored. The values of the monitors decreased slowly at first, and then more rapidly, indicating a deterioration of the crystal. Since each monitor lost intensity at the same rate, it was possible to rescale the intensity data to the values that would have been measured in the absence of deterioration. A visual examination of the crystal indicated a surface deterioration. Several other crystals sealed in capillaries at the same time as the one used for the data collection, but not exposed to X-rays, showed similar deterioration; hence the conclusion can probably be drawn that the crystal was not affected by the X-rays but rather by lack of contact with sufficient mother liquor.

Intensity data were collected with the θ–2θ scan technique on a four-circle automatic diffractometer using a scan of $2.0° + 2\theta(\alpha_2) - 2\theta(\alpha_1)$ and a scan speed of 2°/min. Lorentz and polarization corrections were made and normalized structure factors, $|E_h|$, were obtained with the aid of a K curve. The cell parameters and other pertinent data are listed in Table I.

The coordinates of the Br^- ion, one of 91 atoms in the asymmetric unit, were readily determined from the Harker sections of the Patterson function computed with $(|E_h|^2 - 1)$ as coefficients. For the initial E map, phases obtained from the Br^- ion alone which satisfied the criteria that $|E_h| > 1.5$ and $|F_h|_c > k|F_h|_o$, where k is a fraction determined by the relative amount of known structure (Karle, 1968), were used as input into the tangent formula (Karle and Hauptman, 1956) to compute phases for the remaining $|E_h| > 1.1$. The first E map revealed 24 atoms which were used to calculate an improved set of phases. Those phases obeying the criteria mentioned above were used as input into the tangent formula. The *modus operandi* was to extend the known phase set without any refinement of the phases. Refinement of the phases, when a relatively heavy atom is present, results in convergence to the heavy atom phases and the loss of the effect of the lighter atoms. The second E map contained all the atoms in the complex except the atoms in the side groups of Phe^5 and Val^6. The remaining atoms were subsequently located in difference maps.

Full-matrix least-squares refinement, with weighting based on counting statistics, was performed with 4533 data with $|F_o| > 5.0$. Four passes were required for one cycle of refine-

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TABLE I: Crystallographic Data for the Br^- Salt of the Sodium [Phe[4]-Val[6]]Antamanide·C_2H_5OH Complex.

Molecular formula	$C_{66}H_{82}N_{10}O_{10}(NaBr)(C_2H_5OH)$
Mol wt	1324.4
Color	Colorless
Habit	Stout prism
Size (mm)	0.45 × 0.45 × 0.90
Space group	$P2_12_12_1$
a (Å)	12.880 ± 0.005
b (Å)	38.860 ± 0.015
c (Å)	13.955 ± 0.006
Volume (cm^3)	6984.7
Z	4
Density (calcd) (g/cm^3)	1.259
Radiation	Cu Kα
Wavelength (Å)	1.54178
No. of independent reflections	5258

ment with anisotropic thermal factors, refining 26 atoms in each pass with some overlap of atoms from the previous pass. As in the lithium antamanide·CH_3CN complex (Karle *et al.*, 1973), the Na^+ complex of [Phe[4]-Val[6]]antamanide also has a solvent molecule, C_2H_5OH, involved in a ligand to the metal ion. However, difference maps indicated two positions for the O atom of the C_2H_5OH. Using either position led to unacceptably high thermal values; hence, the two different sites, labeled EO1 and XO1 (Table II), were weighted at $^2/_3$ and $^1/_3$, respectively, for the last pass of refinement. The refinement was terminated after the fifth pass of anisotropic least-squares refinement, equivalent to more than one cycle, for reasons of economy. At this point the agreement factor, R, is 12.0% (see paragraph at end of paper regarding Supplementary Material). No hydrogen atoms were included in the structure factor calculations or in the refinement. Fractional coordinates and thermal factors for the 91 atoms are listed in Table II, bond lengths and angles for the peptide chain are shown in Table III, while Table IV contains the conformational angles.

Results

The Complex. The complex sodium [Phe[4]-Val[6]]antamanide·C_2H_5OH is essentially isostructural with lithium antamanide·CH_3CN except in the areas where the side groups differ. The stereodiagrams in Figure 1 show three views of the Na^+ complex. Each view is at right angles to the other two views. In the decapeptide ring there are eight trans peptide linkages while the Pro_2–Pro_3 and Pro_7–Pro_8 linkages are in the cis

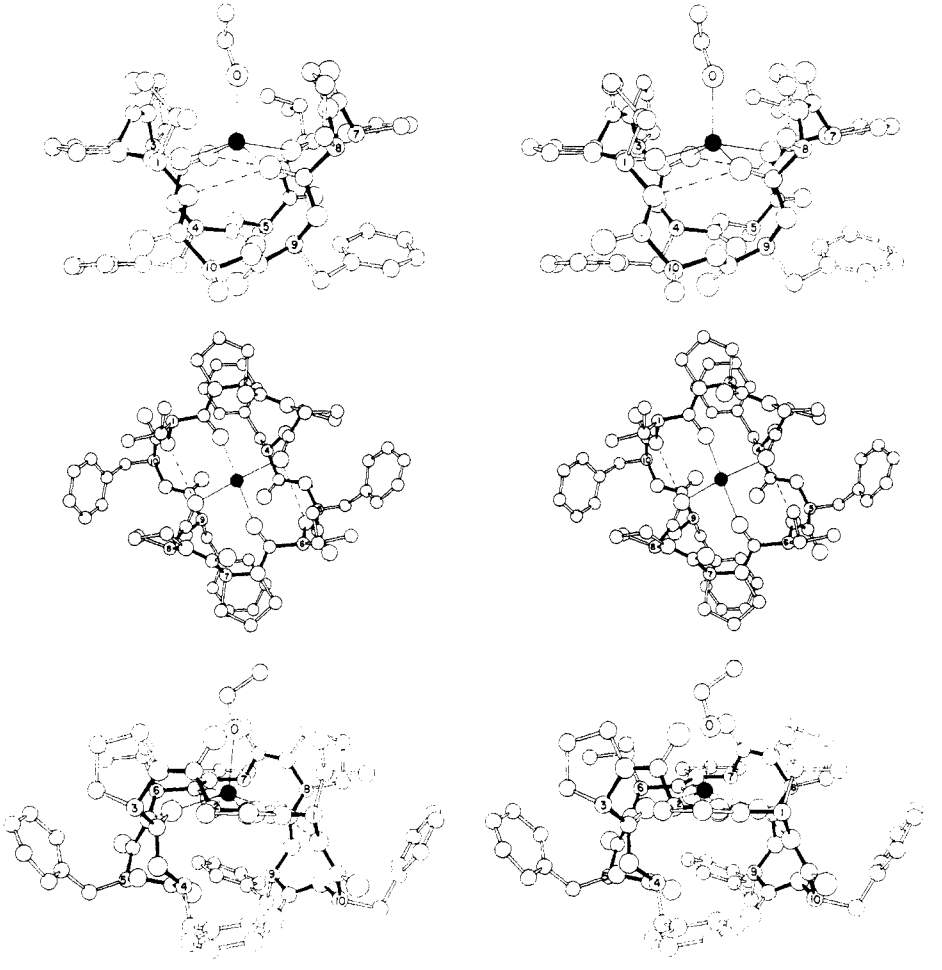
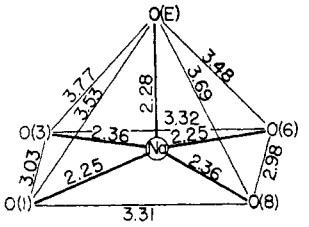


FIGURE 1: Stereodiagrams of the sodium [Phe[4]-Val[6]]antamanide·C_2H_5OH complex. The Na^+ is represented by the black atom and the 30-membered polypeptide ring is outlined by the black bonds. The C^α atom of each peptide unit is numbered. Light solid lines represent the Na–O ligands while the broken lines represent intramolecular NH···O approaches which are somewhat too long to be considered hydrogen bonds. Each view is at 90° to the other two views: (upper) the phenyl groups on Phe_5 and Phe_{10} have been omitted for clarity; (middle) the C_2H_5OH moiety has been omitted; (lower) the complete complex. The diagrams were drawn from the crystallographic coordinates by a computer program (Johnson, 1965).



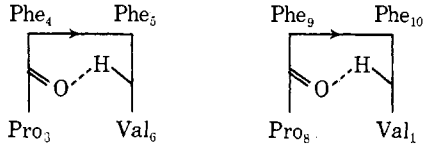
ANGLES	DEG.
O(1)NaO(3)	82
O(3)NaO(6)	92
O(6)NaO(8)	80
O(8)NaO(1)	91
O(E)NaO(1)	103
O(E)NaO(3)	109
O(E)NaO(6)	100
O(E)NaO(8)	105

FIGURE 2: The coordination polyhedron for the Na^+ showing lengths and angles for the Na–O ligands.

conformation. The 30-membered ring is folded into a shape resembling a saddle forming an upper cavity and a lower cavity with the carbonyl oxygen atoms from residues 1, 3, 6, and 8 separating the two cavities. The upper cavity is bounded by four prolyl and two valyl groups, see, *e.g.*, the upper view in Figure 1, while the lower cavity is surrounded by the side groups of four phenylalanyl residues, see, *e.g.*, the lower view in Figure 1.

The Na^+ resides in the upper cavity and participates in five Na–O ligands: four ligands to O(1), O(3), O(6), and O(8) in the [Phe^4-Val^6]antamanide and one to the O atom of the C_2H_5OH. The coordination polyhedron is approximately a square pyramid as shown in Figure 2. The average Na–O distance is 2.30 Å as compared to 2.44 Å for the more usual octahedrally coordinated Na^+. As in the lithium antamanide·CH_3CN complex, the solvent molecule, which in this case is C_2H_5OH instead of CH_3CN, becomes an integral part of the complex by participating in a ligand with the pentacoordinated metal ion. The solvent molecule acts as a plug in the cup-shaped upper cavity and the nonpolar end of the C_2H_5OH completes the hydrophobic outer surface around the upper cavity.

In the Li^+ complex, the average Li–O distance is 2.12 Å, whereas in the Na^+ complex the average Na–O distance is 2.30 Å. Thus the upper cavity must expand in order to accommodate the Na^+ ion. The greatest expansion, 0.29 Å, takes place between O(1)···O(8) and O(3)···O(6). As a consequence, the two moieties near the bottom of the molecule come closer together; *e.g.*, in the Li^+ complex, the O(4)···O(9) distance is 3.76 Å, whereas in the Na^+ complex, the O(4)···O(9) separation is only 3.41 Å. Furthermore, there are two intramolecular hydrogen bonds in the Li^+ complex, O(3)···HN(6) at 3.05 Å and O(8)···HN(1) at 3.00 Å. The equivalent separations in the Na^+ complex, *i.e.*



have increased to 3.29 and 3.18 Å, respectively, as a consequence of introducing a larger metallic ion, and can no longer be considered as hydrogen bonds.

Antamanide and its biologically active analogs selectively complex with Na^+ rather than K^+ (Ivanov *et al.*, 1971; Wieland, 1972). The K^+ ion is considerably larger than Na^+. If the antamanide moiety retains the same conformation in forming a K^+ complex, then it seems unlikely that the upper part of the molecule can expand sufficiently to accommodate K–O ligands which usually are greater than 2.7 Å. One may speculate that a K^+ ion could be placed somewhat farther above the plane of the four O atoms, O(1), O(3), O(6), and O(8), involved in ligand formation (as shown in the upper and lower stereodiagrams of Figures 1) in order to accommodate

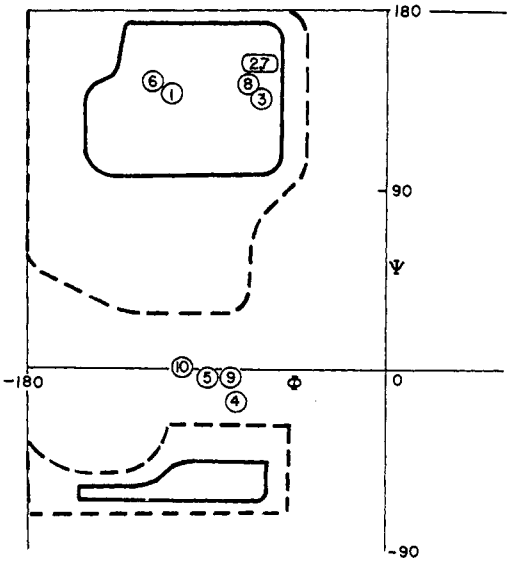


FIGURE 3: Conformational map. The numbers refer to the ten peptide residues in the sodium [Phe^4-Val^6]antanamide·C_2H_5OH complex. Dashed lines enclose low energy regions while the solid lines enclose the lowest energy regions as defined by Ramachandran and Sasisekharan (1968).

the longer M^+–O distances, and, in addition, that two solvent molecules might make ligands to the K^+ ion so that the coordination number is at least six. Or, alternatively, the K^+ may occupy the interior of the lower cavity, where six O atoms could be available for ligand formation; specifically, O(4) and O(9) in addition to atoms O(1), O(3), O(6), and O(8) which separate the upper cavity from the lower cavity. Crystals of a potassium antamanide complex suitable for an X-ray diffraction analysis are not yet available.

Among the bond angles for the peptide residues, several values differ considerably from the averages. These values are circled in Table III. They occur in the Pro_3 and Pro_8 residues which are adjacent to the cis peptide bonds. Similar deviations from average angular values for the same angles also occur in the lithium antamanide complex.

The conformational angles for the sodium [Phe^4-Val^6]-antamanide·C_2H_5OH and lithium antamanide·CH_3CN are compared in Table IV. In the decapeptide ring, the ϕ_i and ψ_i values are quite similar for the two complexes. The largest difference for comparable ϕ_i is only 13° while the largest difference for comparable ψ_i is 11°. In the eight side groups that can be compared, the χ_i values are also quite similar for the Li^+ and Na^+ complexes except for Pro_8 and Pro_7 where the differences are of the order of 30°. The conformation of the pyrrolidine ring in the prolyl residue has been found to be quite flexible in various compounds (see Karle (1972) and references therein) and therefore consistent with the differences in χ_i found for the two complexes. An inspection of the thermal factors for the pyrrolidine rings (Table II) shows that they are much larger for the C^γ atom in each prolyl ring, *i.e.*, atoms C(22), C(32), C(72), and C(82), than for the other atoms in the ring, thus indicating some disorder in the pyrrolidine rings.

The ϕ, ψ conformational map for the Na^+ complex is shown in Figure 3. The values for six of the residues fall within a low energy region as defined by Ramachandran and Sasisekharan (1968). Four residues, Phe_4, Phe_5, Phe_9, and Phe_{10}, however, fall in the higher energy region near $\psi = 0°$. The ϕ, ψ map for the Li^+ complex is, of course, almost identical to Figure 3.

The sequence of the peptide groups in [Phe^4-Val^6]antamanide (II) has twofold rotation symmetry. No advantage is taken

TABLE II: Fractional Coordinates[a] and Thermal Factors.[b]

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Br^-	0.5452	0.3404	0.8508	7.59	8.82	4.57	−1.75	0.50	−0.41
Na^+	0.6903	0.3931	0.3490	3.50	4.40	4.19	0.30	−0.28	0.20
N1	0.8167	0.3005	0.2820	3.44	3.26	4.89	−0.24	−0.16	−0.39
C1	0.8996	0.3125	0.3433	5.59	5.97	3.63	−1.04	−0.37	−1.69
C′1	0.8506	0.3298	0.4324	1.59	4.11	4.86	0.60	0.09	1.09
O1	0.7769	0.3498	0.4195	2.93	4.77	4.36	0.47	−0.69	−0.15
N2	0.8898	0.3270	0.5191	3.40	6.20	2.92	−0.33	0.03	0.09
C2	0.8426	0.3452	0.6004	4.02	4.78	4.93	0.59	−0.00	−0.09
C′2	0.8579	0.3836	0.5938	2.93	5.43	3.65	−0.28	−0.78	−0.78
O2	0.9361	0.3960	0.5546	1.96	7.61	7.76	−1.50	1.00	−1.08
N3	0.7857	0.4046	0.6329	3.29	5.95	4.09	0.06	0.47	−0.54
C3	0.6800	0.3933	0.6681	2.81	5.18	4.64	−0.48	−0.86	−0.96
C′3	0.6186	0.3825	0.5811	3.01	3.64	4.13	−0.26	−0.24	−0.31
O3	0.6165	0.3981	0.5030	3.26	4.19	4.52	0.34	0.01	1.15
N4	0.5613	0.3536	0.5950	1.10	4.55	4.38	0.99	−0.56	0.88
C4	0.5188	0.3349	0.5127	2.12	3.91	4.64	0.80	−0.05	0.33
C′4	0.4237	0.3538	0.4691	4.76	5.00	3.31	−1.38	−0.27	−0.81
O4	0.3930	0.3456	0.3892	6.54	6.05	4.46	1.73	−0.71	−0.57
N5	0.3757	0.3781	0.5217	1.80	5.03	5.03	0.13	0.08	−0.51
C5	0.2789	0.3951	0.4842	2.69	7.18	5.16	0.75	−0.48	−0.89
C′5	0.3092	0.4281	0.4269	5.65	6.07	4.90	1.34	−0.86	−0.39
O5	0.2378	0.4462	0.3953	3.62	9.58	8.69	1.67	−0.99	2.16
N6	0.4116	0.4357	0.4126	3.41	6.00	5.60	0.08	−0.06	0.34
C6	0.4401	0.4631	0.3454	2.99	4.76	6.84	−0.66	−0.18	−0.38
C′6	0.5030	0.4449	0.2646	3.99	3.64	4.38	−0.54	−0.61	0.20
O6	0.5547	0.4200	0.2802	3.54	4.04	5.30	1.19	0.54	1.48
N7	0.4935	0.4568	0.1761	4.67	5.29	5.18	1.03	−1.01	1.28
C7	0.5489	0.4386	0.0979	6.14	5.80	4.56	0.79	−0.30	0.30
C′7	0.6668	0.4441	0.1077	5.74	4.00	5.52	−0.62	0.79	0.11
O7	0.7012	0.4704	0.1454	7.68	4.35	8.95	−2.88	0.08	−0.84
N8	0.7262	0.4178	0.0758	3.61	5.86	5.55	−1.27	−1.24	−0.21
C8	0.6890	0.3848	0.0390	2.82	4.78	4.61	−1.27	0.70	1.04
C′8	0.6452	0.3641	0.1226	1.96	3.81	3.39	0.42	0.29	−0.43
O8	0.6865	0.3637	0.2010	3.52	4.22	3.66	−0.55	−1.03	−0.60
N9	0.5670	0.3443	0.0978	2.43	3.59	2.96	−0.39	0.03	−0.12
C9	0.5075	0.3261	0.1741	3.00	3.56	3.62	−0.80	−0.18	0.48
C′9	0.5588	0.2944	0.2127	3.94	4.25	3.13	0.04	−0.20	0.41
O9	0.5214	0.2793	0.2817	3.47	5.61	6.06	1.28	1.15	1.23
N10	0.6475	0.2850	0.1710	2.34	4.09	3.67	0.05	−0.79	−0.01
C10	0.7072	0.2547	0.2091	2.08	3.81	4.20	−0.55	−0.42	−0.17
C′10	0.8013	0.2673	0.2701	4.15	4.60	2.50	2.17	1.57	1.03
O10	0.8559	0.2439	0.3025	3.43	5.18	5.34	0.47	0.29	0.30
C11	0.9630	0.3411	0.2869	3.64	9.33	4.71	−2.27	0.14	−1.06
C12	1.0549	0.3546	0.3495	4.58	8.60	5.95	−0.05	1.05	−0.12
C13	1.0082	0.3244	0.1954	5.20	8.92	6.89	−0.97	−0.32	−1.36
C21	0.9008	0.3312	0.6905	7.66	8.44	3.61	2.26	−1.30	0.92
C22	0.9793	0.3093	0.6559	7.59	10.55	5.80	3.95	−0.76	1.73
C23	0.9760	0.3028	0.5471	2.55	5.96	6.48	1.74	−1.91	1.23
C31	0.6350	0.4269	0.7122	9.59	4.36	6.55	−1.54	0.53	−3.26
C32	0.7240	0.4529	0.7176	15.35	2.65	14.93	−2.33	2.28	−3.27
C33	0.8034	0.4419	0.6396	3.89	5.39	8.04	−1.65	1.31	−1.69
C41	0.4889	0.2986	0.5387	3.03	4.39	6.67	−0.11	0.03	0.84
C42	0.5866	0.2770	0.5671	2.29	3.58	4.91	−0.66	−0.97	0.15
C43	0.6478	0.2649	0.4985	2.45	6.57	4.23	0.85	−0.23	0.30
C44	0.7370	0.2441	0.5227	6.06	7.10	4.42	0.69	0.65	0.24
C45	0.7528	0.2363	0.6178	3.47	5.72	5.20	−0.12	−0.72	0.53
C46	0.6861	0.2489	0.6884	3.63	6.62	4.92	0.21	0.45	1.55
C47	0.6046	0.2698	0.6609	4.64	5.56	4.20	−0.59	0.11	0.51
C51	0.2030	0.4033	0.5661	3.26	9.92	5.32	0.61	−0.59	−1.36
C52	0.2469	0.4250	0.6509	3.89	9.25	6.65	2.21	1.84	−1.20
C53	0.2941	0.4099	0.7258	7.64	15.17	10.53	2.91	1.24	−2.57

TABLE II (*Continued*)

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C54	0.3465	0.4326	0.8019	9.32	15.78	6.06	2.93	−0.24	−1.27
C55	0.3419	0.4677	0.7895	14.55	9.93	12.03	1.71	1.47	3.33
C56	0.3103	0.4804	0.7104	10.96	10.19	9.08	1.35	2.28	−1.86
C57	0.2594	0.4589	0.6287	10.22	10.05	10.52	2.20	4.09	−2.19
C61	0.5174	0.4868	0.4134	10.98	5.47	12.56	−4.41	5.97	−5.13
C62	0.5842	0.5100	0.3537	8.93	14.31	13.04	1.60	2.67	−3.00
C63	0.4392	0.5095	0.4635	15.04	12.03	18.42	−2.16	5.19	−8.62
C71	0.5131	0.4590	0.0061	10.99	10.57	2.91	2.70	−1.38	2.64
C72	0.4339	0.4844	0.0382	15.42	14.35	3.76	4.65	−2.94	1.75
C73	0.4234	0.4865	0.1431	7.82	5.19	10.90	1.72	−3.09	2.28
C81	0.7889	0.3673	−0.0029	5.14	8.40	6.53	0.15	1.74	−0.77
C82	0.8671	0.3964	−0.0105	3.61	11.89	13.83	−1.90	1.42	−0.33
C83	0.8443	0.4216	0.0694	3.64	7.94	8.91	−2.57	0.62	−0.07
C91	0.3930	0.3163	0.1374	3.31	5.10	6.87	−1.32	−1.31	1.25
C92	0.3323	0.3482	0.1154	1.47	5.79	4.86	−0.06	0.07	−0.02
C93	0.3079	0.3725	0.1857	3.81	6.55	5.78	0.68	0.07	−0.10
C94	0.2450	0.4036	0.1631	3.26	5.72	9.37	0.16	−0.09	0.74
C95	0.2033	0.4067	0.0695	8.23	8.50	9.07	1.57	−3.25	1.05
C96	0.2311	0.3814	0.0026	8.39	10.37	6.85	1.29	−3.19	2.65
C97	0.2946	0.3529	0.0222	3.68	10.48	6.48	1.14	−1.45	1.59
C101	0.7414	0.2307	0.1308	3.98	5.24	5.14	−0.69	1.24	−0.30
C102	0.8037	0.2484	0.0556	3.51	8.17	4.67	2.78	−0.42	−1.33
C103	0.7672	0.2651	−0.0275	7.72	6.72	6.24	−0.39	−1.68	−1.28
C104	0.8269	0.2825	−0.0943	8.22	7.80	4.33	0.79	1.12	−0.01
C105	0.9352	0.2853	−0.0826	6.88	10.84	6.06	0.72	4.33	−0.43
C106	0.9763	0.2696	−0.0008	6.49	11.83	6.10	2.94	0.84	0.31
C107	0.9193	0.2524	0.0644	7.47	9.18	5.17	1.64	1.43	0.14
EO1	0.8152	0.4341	0.3321	7.01	10.96	11.04	−2.75	2.11	−2.05
XO1	0.8504	0.4255	0.3819	7.59	7.59	6.22	−3.14	0.26	1.86
EC11	0.8843	0.4547	0.3538	9.88	15.64	10.46	−7.05	0.03	−1.20
EC12	0.9833	0.4571	0.3030	6.46	19.54	12.62	−3.44	−2.38	3.00
WO	0.3291	0.3336	0.7245	15.29	16.18	9.34	1.90	1.58	1.89

[a] The first 40 atoms after Br^- and Na^+ are the backbone atoms $N_iC_i^{\alpha}C_i'O_i$ of the ten peptide groups, the following 46 atoms are those in the side groups where $C_i^{\beta} = Ci1$, $C_i^{\gamma} = Ci2$, etc., and the last five entries represent the ethanol molecule with a disordered O atom (EO1 and XO1) and a possible water molecule (WO). [b] The thermal parameters are expressed in the form $T = \exp[-1/4(B_{11}h^2a^{*2} + B_{22}k^2b^{*2} + B_{33}l^2c^{*2} + 2B_{12}hka^*b^* + 2B_{13}hla^*c^* + 2B_{23}klb^*c^*)]$ where the B_{ij} values are in Å^2 units.

TABLE III: Bond Lengths (Å) and Angles (deg).

Angles	i = 1	2	3	4	5	6	7	8	9	10	Av i = 1–10	Av for Polypeptides[a]
$C_{i-1}'N_iC_i^{\alpha}$	120.1	120.9	125.1	120.1	120.0	119.4	118.3	126.4	119.0	119.8	120.9	122
$N_iC_i^{\alpha}C_i'$	108.3	112.0	107.0	111.5	109.4	105.2	110.1	108.3	114.9	110.2	109.6	111
$C_i^{\alpha}C_i'N_{i+1}$	123.1	120.2	113.6	118.8	119.6	117.9	115.1	113.0	116.4	119.5	117.7	116
$C_i^{\alpha}C_i'O_i$	117.9	118.9	125.5	119.2	117.5	121.7	120.5	122.2	120.2	114.4	119.8	120.5
$N_{i+1}C_i'O_i$	118.6	120.2	120.9	122.0	122.9	120.3	124.3	124.3	123.4	126.1	122.3	123.5
$C_i'C_i^{\alpha}C_i^{\beta}$	107.8	109.4	110.9	109.7	111.7	110.9	106.9	111.0	108.9	111.3		
$N_iC_i^{\alpha}C_i^{\beta}$	108.2	104.8	102.7	111.8	111.0	100.9	102.6	104.0	110.9	112.2		
Bonds	Val	Pro	Pro	Phe	Phe	Val	Pro	Pro	Phe	Phe		
N_i–C_i^{α}	1.446	1.468	1.513	1.465	1.505	1.467	1.483	1.460	1.491	1.504	1.480	1.455
C_i^{α}–C_i^{β}	1.586	1.562	1.555	1.507	1.537	1.656	1.576	1.570	1.607	1.502	1.556	
C_i^{α}–C_i'	1.547	1.509	1.507	1.552	1.561	1.559	1.540	1.525	1.499	1.559	1.536	1.51
C_i'–O_i	1.238	1.242	1.247	1.226	1.238	1.195	1.234	1.217	1.226	1.234	1.230	1.24
C_i'–N_{i+1}	1.316	1.352	1.359	1.346	1.366	1.325	1.352	1.313	1.332	1.319	1.338	1.325

[a] See, *e.g.*, Marsh and Donohue (1967).

TABLE IV: Conformational Angles[a] in Alkali Ion–Antamanide Complexes.

(a) Sodium [Phe⁴-Val⁶]Antamanide·C_2H_5OH

	Val	Pro	Pro	Phe	Phe	Val	Pro	Pro	Phe	Phe
i	1	2	3	4	5	6	7	8	9	10
ϕ_i	−112	−69	−68	−75	−91	−117	−70	−71	−79	−102
ψ_i	142	150	139	−17	5	146	149	147	−4	1
ω_i	177	−11	−165	−176	170	−177	−3	−172	176	176
χ_i^1	−61, −179	5	−11	−65	54	−86, 161	−5	−16	−63	56
χ_i^2				−78, 104	75, −88				−63, 120	88, −89

(b) Lithium Antamanide·CH_3CN

	Val	Pro	Pro	Ala	Phe	Phe	Pro	Pro	Phe	Phe
i	1	2	3	4	5	6	7	8	9	10
ϕ_i	−115	−65	−81	−69	−84	−123	−74	−69	−79	−90
ψ_i	138	139	148	−13	−6	140	145	145	−13	8
ω_i	179	−4	−174	175	−178	−172	−3	−175	172	173
χ_i^1	−68, 170	12	22		66	−176	25	−21	−60	59
χ_i^2					84, −95	−87, 94			−56, 125	88, −93

[a] The convention followed is that proposed by the IUPAC-IUB Commission on Biochemical Nomenclature (1970).

of this molecular symmetry in the crystal system. Space group $P2_12_12_1$ contains three twofold screw axes, but no twofold rotation axis. The appearance of the complex in Figure 1 and the similarity of the conformational angles for the pairs of residues i and $i + 5$ show that the complex does indeed contain a near twofold rotation axis. The major exception to twofold symmetry in the complex is the C_2H_5OH moiety. If the solvent in which the complex was formed had been acetone or acetonitrile, both of which accommodate a twofold axis, the complex may have had a true crystallographic twofold axis.

Crystallographic Packing. The manner in which the lithium antamanide·CH_3CN and the sodium [Phe⁴-Val⁶]antamanide·C_2H_5OH pack is quite different. The Li^+ complex forms a clathrate with large channels in which additional solvent molecules reside while there is no clathrate formation in the Na^+ complex. The packing is shown in Figure 4, where the view in one is perpendicular to that of the other. The only hydrogen bonding is to the Br^- ion with N(4)H···Br^- = 3.61 Å and N(9)H···Br^- = 3.46 Å. These hydrogen bonds tie the molecules into continuous chains parallel to the c axis (lower view in Figure 4). There is no hydrogen bonding between complexes since two of the NH groups, from residues 1 and 6, are in the interior of the molecule and the remaining two NH groups, from residues 5 and 10, are shielded by the phenyl moieties of the side groups and are not spatially available for hydrogen bonding.

There are nine O···C contacts between 3.25 and 3.70 Å. The oxygen atoms are from residues 2, 4, 5, 7, 9, and 10 and the carbon atoms principally from the side groups of Pro_3 and Phe_4. There are two C···C contacts less than 3.58 Å and 13 C···C between 3.58 and 3.80 Å, all involving carbon atoms from the side chains of residues Pro_2, Phe_4, Phe_5, Pro_7, Phe_9, and Phe_{10}.

Two peaks persisted in the difference maps which were considerably stronger than the peaks indicating hydrogen atoms. The stronger of the two peaks, labeled WO in Table II, is located 3.05 Å from O(10) and 3.36 Å from the Br^- ion. Thus, it is possible that the atom represents a water molecule cocrystallized in the cell which makes hydrogen bonds to O(10) and Br^-.

The other peak has already been mentioned in the Experimental Section. It appears to be an alternate position for the hydroxyl O atom in the ethanol. The two positions for the O atom are related by a rotation about the ethanol C–C bond. In approximately two-thirds of the complexes, the ethanol O atom forms a ligand to the Na^+ with Na–O = 2.28 Å, while in about one-third of the complexes, the ethanol O in the alternate position makes a weaker ligand to the Na^+ at 2.46 Å. The ethanol O in the alternate position may also participate in an OH···O hydrogen bond of length 2.89 Å to the carbonyl oxygen of Pro_2. Thermal factors for the side groups in Phe_5 and Val_6 are considerably larger than for all the other side groups indicating that there is positional disorder in this region. An inspection of the packing diagrams (Figure 4) shows that if ethanol escapes during the deterioration of the crystal, as described in the Experimental Section, a possible path through the crystal would be past Phe_5 and Val_6.

The lithium antamanide·CH_3CN crystal was much more stable while sealed in the capillary with its mother liquor than the sodium [Phe⁴-Val⁶]antamanide·C_2H_5OH. The difference could be accounted for in the electrophilic qualities of the solvents. The acetonitrile with its C≡N can exert a much stronger attraction for the metal ion than the C–OH in ethanol. Crystals grown from acetone probably have a M^+—O=C ligand of intermediate strength and stability.

Discussion

The conformation of a polypeptide in solution may not necessarily be the same as that determined for the crystalline state. For example, nuclear magnetic resonance (nmr) studies by Tonelli and Brewster (1972) indicate that the conformation found for *cyclo*(Gly-Gly-Gly-Gly-D-Ala-D-Ala) in the crystal-

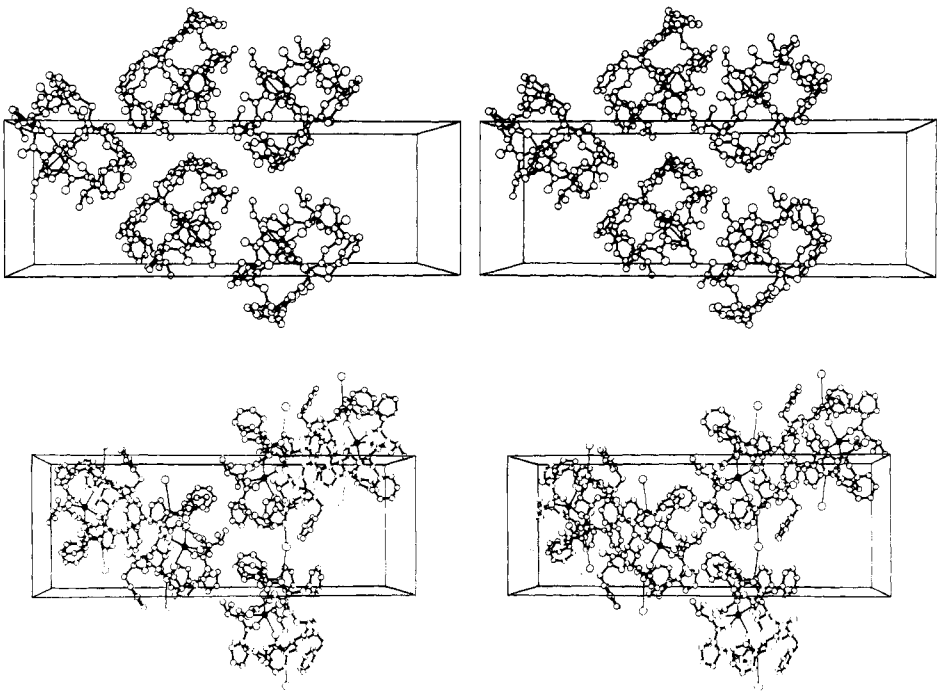


FIGURE 4: Stereodiagrams of the packing in the cell. Five neighboring complexes are drawn. (upper) The axial directions are $a \downarrow$, $b \rightarrow$, and c directed toward the viewer. (lower) This view is rotated by 90° from the upper view. The axial directions are $c \uparrow$, $b \rightarrow$, and a directed toward the viewer. The NH$\cdots$Br$^-$ hydrogen bonds, parallel to the c axis, are indicated by light lines.

line state (Karle *et al.*, 1970) is different than the conformations present in solution in dimethyl sulfoxide. Furthermore, the nature of the solvent may have a profound effect on the conformation.

Detailed spectroscopic studies on the solution conformation of antamanide and the Na^+ complex have been carried out by several laboratories (Ivanov *et al.*, 1971; Ovchinnikov *et al.*, 1972; Faulstich *et al.*, 1972; Patel, 1973; Tonelli, 1973). It has been established that uncomplexed antamanide exists in two distinct conformations, one in nonpolar solvents and the other in polar solvents, particularly when water is added. On the other hand, the conformation of the Na^+ complex in solution is independent of solvent and, in addition, the Na^+ complex conformation appears to be the same as that for the uncomplexed antamanide in the presence of water.

Recently Patel (1973) and Tonelli (1973) proposed cis peptide bonds for antamanide and sodium antamanide. Models were generated with individual low-energy residue conformations consistent with the results of proton magnetic resonance and ^{13}C nmr. Their most probable model for the decapeptide ring contained cis peptide bonds between Val_1–Pro_2 and Phe_6–Pro_7. Another model for the ring considered unlikely by Patel and Tonelli contained cis peptide bonds between Pro_2–Pro_3 and Pro_7–Pro_8, the cis linkages which have been found in the crystalline state. The comparison of conformational angles for the proposed solution model of the decapeptide ring containing Pro_2–Pro_3 and Pro_7–Pro_8 cis linkages and those resulting from the crystal structure analysis is shown in Figure 5. There is approximate correspondence between the two conformations for several residues. The differences occur chiefly for residues 4, 5, 9, and 10. In model building (Patel, 1973) and conformational calculations (Tonelli, 1973), the reasonable assumption was made that the ϕ, ψ values for each residue fall within a low energy region as designated in the Ramachandran plot. However, in the crystal, the ψ values found for Phe_4, Phe_5, Phe_9, and Phe_{10} are near 0°, an area of higher energy.

Crystal structure analyses have now been completed on two related, biologically active complexes: lithium antamanide·CH_3CN and sodium [Phe^4-Val^6]antamanide·C_2H_5OH. These complexes contain alkali ions of different radii, the polypeptides differ in the side groups for residues 4 and 6, and the ligated solvent molecules are different. Furthermore, the two complexes crystallize in different space groups, $P2_1$ and $P2_12_12_1$, and the environments in the crystal are quite different. The Li^+ complex crystal contains large channels in which additional solvent molecules reside, whereas in the Na^+ complex crystal, the molecules of the complex are relatively

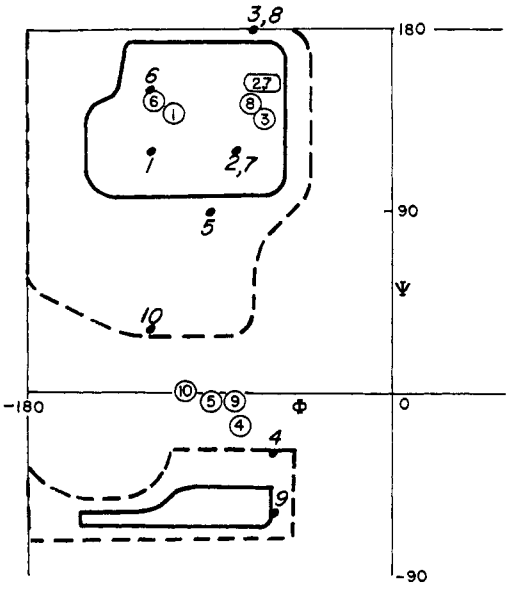


FIGURE 5: A comparison of the conformational angles for the sodium [Phe^4-Val^6]antamanide·C_2H_5OH complex in the crystalline state (○) and those proposed for the sodium antamanide solution model containing cis Pro_2-Pro_3 and Pro_7–Pro_8 peptide bonds (●) (Patel, 1973; Tonelli, 1973).

close packed. In spite of these differences, the two complexes are nearly isostructural. In other words, none of the factors which are different, either in the complex or in the environment, affect the conformation of the cyclic polypeptide. The constancy of the conformation in the solid state, along with the spectroscopic observations that the spectrum of the sodium antamanide complex is independent of the solvent, indicates that the conformation of the metal complex found in the solid state most probably represents the conformation in solution.

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Supplementary Material Available

A listing of structure factor amplitudes will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number Bio-74-2155.

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