The 3_{10} Helical Conformation of the Amino Terminal Decapeptide of Suzukacillin. 270 MHz ^{1}H NMR Evidence for Eight Intramolecular Hydrogen Bonds

M. Iqbal and P. Balaram*

Contribution from the Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India. *Received November 18, 1980*

Abstract: The amino terminal suzukacillin decapeptide fragment, Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe, the two pentapeptides Boc-Aib-Pro-Val-Aib-Val-OMe and Boc-Ala-Aib-Ala-Aib-Aib-OMe, and the tripeptide Boc-Ala-Aib-Aib-OMe have been studied by 270-MHz ^{1}H NMR spectroscopy. By use of solvent dependence of chemical shifts in a $CDCl_3$–$(CD_3)_2SO$ system and temperature dependence of amide NH chemical shifts in $(CD_3)_2SO$, the intramolecularly hydrogen bonded NH groups in these peptides have been identified. The tripeptide possesses one hydrogen bond, both pentapeptides show evidence for three intramolecular hydrogen bonds, and the decapeptide has eight NH groups participating in hydrogen bonding. An Ala(1)-Aib(2) β turn is proposed for the tripeptide. Both pentapeptides favor 3_{10} helical conformations composed of three consecutive β turns. The decapeptide adopts a 3_{10} helical conformation with some flexibility at the Val(5)-Ala(6) segment. The proposed conformations are consistent with the known stereochemical preferences of Aib residues.

Suzukacillin,[1] a 24 residue microbial peptide related to alamethicin,[2,3] has been shown to alter cation permeability of model membranes and forms transmembrane channels, whose conductance is dependent on the membrane potential.[4] Like alamethicin, suzukacillin contains a high proportion of α-aminoisobutyric acid (Aib). Considerable attention has been focused on the conformations of alamethicin and its fragments, with a view toward delineating the structural requirements for channel formation.[5-9] The two polypeptides differ in their chain length, with suzukacillin being longer. The tetrapeptide fragment Val-Aib-Val-Ala (residues 3–6) in suzukacillin (Figure 1) is absent in alamethicin. NMR,[5,9] IR,[7,8] CD,[9] and crystallographic[6,10-12] studies of alamethicin fragments and model Aib containing peptides have established the propensity of Aib rich sequences to adopt 3_{10} helical conformations. In particular, the amino terminal hexapeptide of alamethicin has been shown to fold in 3_{10} helical fashion, a feature initiated by the Aib-Pro-Aib sequence.[7-9] It was therefore of interest to examine the effect of the two Val residues on the conformation of the amino terminal segment of suzukacillin, since Val residues show a low preference for helical conformations.[13] The 270-MHz ^{1}H NMR studies reported in this paper establish a 3_{10} helical conformation, stabilized by eight intramolecular hydrogen bonds, for the amino terminal decapeptide fragment Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe (**1**) in $CDCl_3$ and $(CD_3)_2SO$ solutions. The results are compared with studies on the two pentapeptide fragments Boc-Aib-Pro-Val-Aib-Val-OMe (**2**) and Boc-Ala-Aib-Ala-Aib-Aib-OMe (**3**) to establish the effect of chain length on peptide folding.

Experimental Section

The peptides Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe (**1**), Boc-Aib-Pro-Val-Aib-Val-OMe (**2**), Boc-Ala-Aib-Ala-Aib-Aib-OMe (**3**), and Boc-Ala-Aib-Aib-OMe (**4**) (Boc = *tert*-butoxycarbonyl) were synthesized by solution phase procedures.[5] Couplings were mediated by dicyclohexylcarbodiimide (DCC) and DCC/1-hydroxybenzotriazole. The decapeptide **1** was prepared by coupling the acid derived from **2** with the amine derived from **3**. Boc groups were removed with use of 98% formic acid while methyl esters were saponifieid in 2 N NaOH/methanol. Details of synthetic procedures will be described elsewhere. All peptides were homogeneous by TLC on silica gel and yielded 270-MHz ^{1}H NMR spectra fully consistent with their structures. The pentapeptides **2** and **3** also yield single crystals suitable for X-ray diffraction, from methanol–methyl acetate.

^{1}H NMR spectra (270 MHz) were recorded on a Bruker WH-270 FT NMR spectrometer at the Bangalore NMR Facility. The ^{2}H resonance of $CDCl_3$ and $(CD_3)_2SO$ served as the internal field-frequency lock. All chemical shifts are expressed as δ (ppm) downfield from internal tetramethylsilane. Spectra were recorded at concentrations of 10 mg/mL.

Results

Assignments of Resonances. The NH resonances of the pentapeptides Boc-Aib-Pro-Val-Aib-Val-OMe (**2**) and Boc-Ala-Aib-Ala-Aib-Aib-OMe (**3**) in $CDCl_3$ and $(CD_3)_2SO$ are shown in Figure 2. The high field NH singlet at δ 5.03 in **2** is unambiguously assigned to the Aib(1) NH group, since urethane hydrogens appear at considerably higher field than amide hydrogens, in $CDCl_3$.[5,14,15] The assignment of the Aib(4) NH to the δ 7.27 singlet is unequivocal. The two doublets at δ 7.365 and 7.337 in $CDCl_3$ are due to the two Val NH groups. However, a specific assignment cannot be made at the present. The assignments in $(CD_3)_2SO$ are based on solvent titration experiments, in which spectra were recorded in $CDCl_3$–$(CD_3)_2SO$ mixtures of varying composition. Similarly in pentapeptide **3**, the doublet at δ 5.58 in $CDCl_3$ is assigned to Ala(1) NH and the doublet at δ 7.58 to Ala(3). The three Aib NH singlets cannot be unambiguously assigned to specific residues. However, a distinction may be made after delineation of hydrogen bonded NH groups, on the basis of conformational arguments, as discussed later. The $(CD_3)_2SO$ assignments were made as in the case of **2**. The chemical shifts of the various NH groups in **2** and **3** in the two solvents are summarized in Table I. Data for the tripeptide fragment Boc-Ala-Aib-Aib-OMe are also included (Figure 2).

The low-field regions of the 270-MHz ^{1}H NMR spectrum of the decapeptide Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe (**1**) in $CDCl_3$ and $(CD_3)_2SO$ are shown in Figure 3. In both solvents nine distinct sets of NH resonances are observed:

(1) Jung, G.; Konig, W. A.; Liebfritz, D.; Ooka, T.; Janko, K.; Boheim, G. *Biochim. Biophys. Acta* **1976**, *433*, 164-181.
(2) Mueller, P.; Rudin, D. O. *Nature (London)* **1968**, *217*, 713-719.
(3) Pandey, R. C.; Carter Cook, J., Jr.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 8469-8483.
(4) Boheim, G.; Janko, K.; Liebfritz, D.; Ooka, T.; Konig, W. A.; Jung, G. *Biochim. Biophys. Acta* **1976**, *433*, 182-199.
(5) Nagaraj, R.; Shamala, N.; Balaram, P. *J. Am. Chem. Soc.* **1979**, *101*, 16-20.
(6) Prasad, B. V. V.; Shamala, N.; Nagaraj, R.; Chandrasekaran, R. C.; Balaram, P. *Biopolymers* **1979**, *18*, 1635-1646.
(7) Rao, Ch. P.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *Biochemistry* **1980**, *19*, 425-431.
(8) Rao, Ch. P.; Nagaraj, R.; Rao, C. N. R.; Balaram, P. *FEBS Lett.* **1979**, *100*, 244-248.
(9) Nagaraj, R.; Balaram, P. *Biochemistry* **1981**, *20*, 2828-2835.
(10) Prasad, B. V. V.; Shamala, N.; Nagaraj, R.; Balaram, P. *Acta Crystallogr., Sect. B* **1980**, *36*, 107-110.
(11) Shamala, N.; Nagaraj, R.; Balaram, P. *J. Chem. Soc., Chem Commun.* **1978**, 996-997.
(12) Smith, G. D.; Duax, W. L.; Czerwinski, E. W.; Kendrick, N. E.; Marshall, G. R.; Mathews, F. S. In "Peptides: Proceedings of the Fifth American Peptide Symposiumσqt, Goodman, M.; Meienhofer, J., Eds.; Wiley: New York, 1977; pp 277-279.
(13) Chou, P. Y.; Fasman, G. D. *J. Mol. Biol.* **1977**, *115*, 135-175.
(14) Bystrov, V. F.; Portnova, S. L.; Tsetlin, V. I.; Ivanov, V. T.; Ovchinnikov, Yu. A. *Tetrahedron* **1965**, *25*, 493-515.
(15) Pysh, E. S.; Toniolo, C. *J. Am. Chem. Soc.* **1977**, *99*, 6211-6219.

0002-7863/81/1503-5548$01.25/0

Table I. NMR Parameters of Peptide NH Groups in Suzukacillin Fragments 2, 3, and 4

peptide	residues	δ_{NH} $CDCl_3$	δ_{NH} $(CD_3)_2SO$	$d\delta/dT$ $[(CD_3)_2SO]$, ppm/°C	$J_{HNC^{\alpha}H}$, Hz[b] $CDCl_3$	$J_{HNC^{\alpha}H}$, Hz[b] $(CD_3)_2SO$
Boc-Aib-Pro-Val-Aib-Val-OMe (2)	Aib-(1)	5.033	7.646	0.005		
	Val-(3)[a]	7.365	7.505	0.002	8.5	8.1
	Aib-(4)	7.274	7.537	0.002		
	Val-(5)[a]	7.337	7.178	0.001	9.5	8.5
Boc-Ala-Aib-Ala-Aib-Aib-OMe (3)	Ala-(1)	5.581	7.175	0.006	2.7	5.1
	Aib-(2)[a]	6.759	8.301	0.005		
	Ala-(3)	7.581	7.742	0.003	5.1	4.8
	Aib-(4)[a]	7.310	7.591	0.002		
	Aib-(5)[a]	7.202	7.290	0.001		
Boc-Ala-Aib-Aib-OMe (4)	Ala-(1)	5.003	7.112	0.0066	4.0	5.9
	Aib-(2)[a]	6.540	7.883	0.0046		
	Aib-(3)	7.175	7.391	0.0013		

[a] Assignment of these resonances is not unequivocal, but is supported by the conformational arguments discussed in the text. [b] Errors in J values are ~±0.5 Hz.

(a) - Ac-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol

(b) - Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol

Figure 1. Sequencs of suzukacillin (a) and alamethicin I (b).

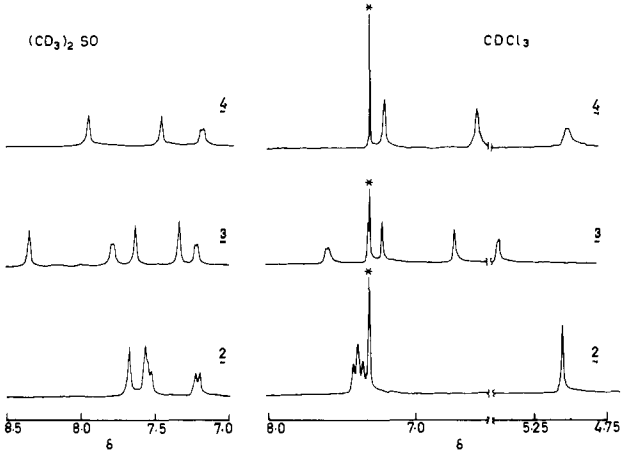

Figure 2. NH proton resonances (270 MHz) of Boc-Aib-Pro-Val-Aib-Val-OMe (**2**), Boc-Ala-Aib-Ala-Aib-Aib-OMe (**3**), and Boc-Ala-Aib-Aib-OMe (**4**) in $CDCl_3$ and $(CD_3)_2SO$.

five singlets due to the Aib residues and four doublets due to the two Ala and two Val residues. Of these the Aib(1) NH can be unequivocally assigned by virture of its high-field position in $CDCl_3$. The corresponding assignments of these NH resonances (denoted as singlets S_1, S_2, S_5, S_7, and S_8 and doublets D_3, D_4, D_6, D_9) in $(CD_3)_2SO$ are based on solvent titration studies. Double irradiation experiments allowed the assignment of D_3 and D_4 to Ala residues while D_6 and D_9 were assigned to the Val residues. Assignment of the amide NH groups to specific residues is not possible at present. However, the studies described below establish that all eight amide NH groups are involved in intramolecular hydrogen bonding, allowing deductions about molecular conformation, even in the absence of specific assignments.

Determination of Hydrogen Bonded NH Groups. The involvement of peptide NH groups in intramolecular hydrogen bonding may be established with the use of various criteria like rates of hydrogen-deuterium exchange,[16] solvent dependence of chemical shifts,[17] and temperature dependence of chemical shifts in hydrogen bonding solvents (e.g., $(CD_3)_2SO$).[18] The temperature dependence of NH chemical shifts in $(CD_3)_2SO$ in the pentapeptides **2** and **3** is shown in Figure 4. The solvent dependence of chemical shifts in $CDCl_3$–$(CD_3)_2SO$ mixtures is shown in Figure 5. The temperature coefficients ($d\delta/dT$) are summarized in Table I. In **2** both Val NH protons and the Aib(2) NH show very small changes in chemical shift on going from a poor hydrogen bonding solvent like $CDCl_3$ to a strongly hydrogen bond accepting solvent like $(CD_3)_2SO$. Further, these three NH groups also yield low $d\delta/dT$ values (≲0.002 ppm/°C). This behavior is characteristic of solvent shielded, intramolecularly hydrogen bonded NH groups. In contrast the Aib(1) NH shows a large solvent shift (2.61 ppm) and also has a high $d\delta/dT$ value (0.005 ppm/°C), suggesting that this group is exposed to solvent.

In the pentapeptide **3** the Ala(3) NH and two Aib NH groups appear to be hydrogen bonded. This is evident from their insensitivity to the nature of the solvent and their low $d\delta/dT$ values. The Ala(1) NH and one of the Aib NH groups are exposed to solvent and exhibit large solvent shifts and temperature dependences. The tripeptide fragment Boc-Ala-Aib-Aib-OMe was also studied for comparison and the results are summarized in Table I. Here, one Aib NH shows evidence of being involved in intramolecular hydrogen bonding, while the Ala NH and second Aib NH are free.

The results of solvent titration and variable temperature experiments for the decapeptide **1**, carried out at a concentration of 10 mg/mL, are shown in Figure 6. It is clearly seen that Aib(1) NH shows a large downfield shift (2.049 ppm) in $(CD_3)_2SO$, relative to $CDCl_3$, and also has a relatively high $d\delta/dT$ value in $(CD_3)_2SO$ (0.0044 ppm/°C). These parameters are characteristic of a solvent-exposed proton. Of the other eight NH resonances, seven show extremely small $d\delta/dT$ values and are insensitive to changes in solvent. One Aib NH (S_2) shows a small solvent shift (0.297 ppm) on going from $CDCl_3$ to $(CD_3)_2SO$ but has a rather high $d\delta/dT$ value in $(CD_3)_2SO$ (0.004 ppm/°C). These results imply that the decapeptide **1** possesses seven strong intramolecular hydrogen bonds, while an eighth NH group also shows some evidence for participating in such an interaction. $d\delta/dT$ values in $(CD_3)_2SO$ were also measured at a lower peptide concentration of 1 mg/mL (Table II). There is no significant concentration dependence of $d\delta/dT$ values, implying that intermolecular shielding of NH groups is not likely, at these concentrations. The results suggest that peptide aggregation may not be an important feature in $(CD_3)_2SO$. Measurements up to 50 mg/mL of peptide did not show large changes in $d\delta/dT$ values. Temperature coefficients in $CDCl_3$ follow a pattern similar to that in $(CD_3)_2SO$ (Table II). However, a detailed interpretation of $d\delta/dT$ values in non-hydrogen bonding solvents like $CDCl_3$ should be approached with

(16) Stern, A.; Gibbons, W. A.; Craig, L. C. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 734–741.

(17) Pitner, T. P.; Urry, D. W. *J. Am. Chem. Soc.* **1972**, *94*, 1399–1400.

(18) Kopple, K. D.; Ohnishi, M.; Go, A. *J. Am. Chem. Soc.* **1969**, *91*, 4264–4272.

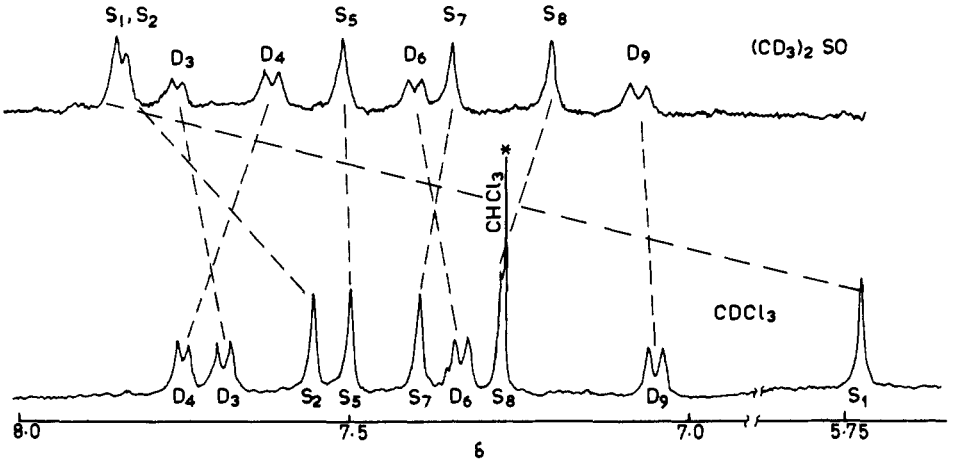

Figure 3. NH proton resonances of Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe (**1**) in $CDCl_3$ and $(CD_3)_2SO$.

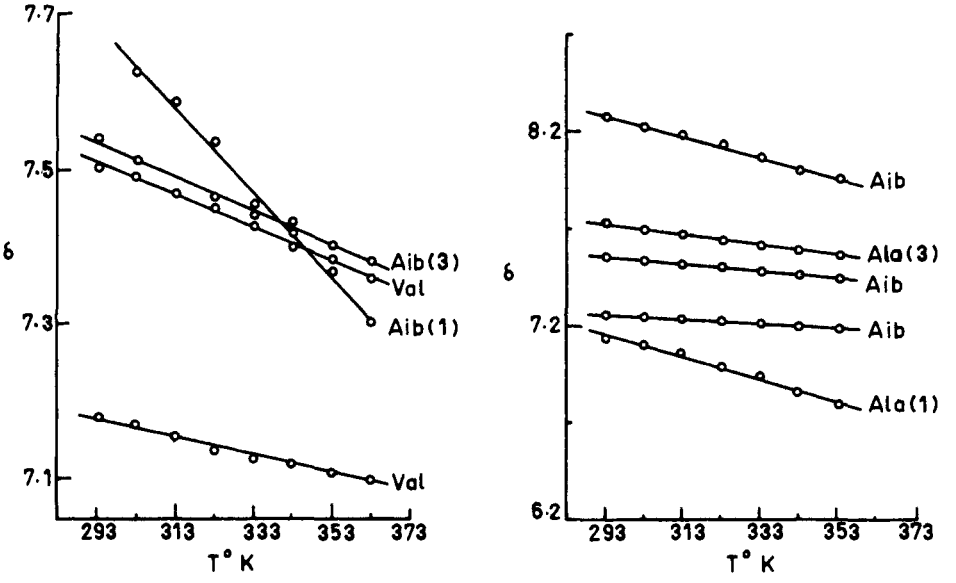

Figure 4. Temperature dependence of NH chemical shifts in $(CD_3)_2SO$: (left) **2** and (right) **3**.

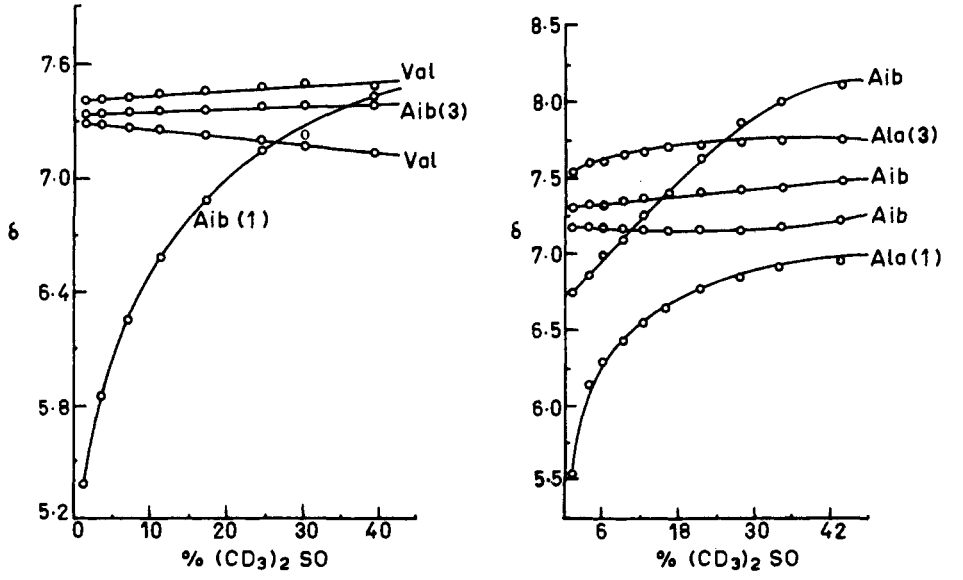

Figure 5. Solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures: (left) **2** and (right) **3**.

caution and we will elaborate on these results elsewhere.

Discussion

In the case of stereochemically constrained Aib containing peptides, only a restricted range of conformational states are accessible in solution.[5-9] It therefore becomes possible to relate the observed NMR parameters of NH groups to preferred molecular conformations, without the attendant problems of dynamic averaging, which frequently obscure NMR studies of acyclic peptides. Earlier studies of model Aib peptides have established

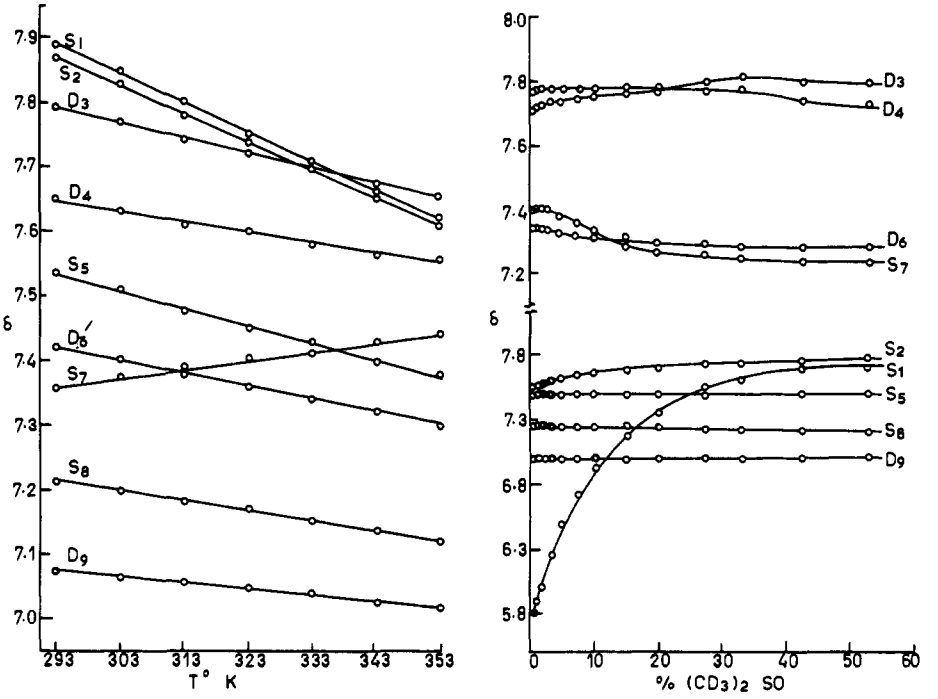

Figure 6. Left: Temperature dependence of NH chemical shifts for **1** in $(CD_3)_2SO$. Right: Solvent dependence of NH chemical shifts for **1** in $CDCl_3$–$(CD_3)_2SO$ mixtures.

Table II. NMR Parameters of Peptide NH Groups in Suzukacillin Decapeptide Fragment 1

residues[a]		δ_{NH}		$d\delta/dT$,[b] ppm/°C		$J_{HNC^{\alpha}H}$,[c] Hz	
		$CDCl_3$	$(CD_3)_2SO$	$CDCl_3$	$(CD_3)_2SO$	$CDCl_3$	$(CD_3)_2SO$
Aib(1)	S_1	5.742	7.891	0.0063	0.0044 (0.0052)		
Aib	S_2	7.575	7.872	0.0033	0.0040 (0.0040)		
Ala	D_3	7.714	7.795	0.0026	0.0022 (0.0026)	6.0	4.7
Ala	D_4	7.779	7.649	0.0012	0.0016 (0.0015)	4.8	7.0
Aib	S_5	7.515	7.536	0.0029	0.0026 (0.0029)		
Val	D_6	7.344	7.421	0.0018	0.0020 (0.0017)	5.5	5.2
Aib	S_7	7.409	7.367	0.0011	−0.0010 (0.0016)		
Aib	S_8	7.284	7.213	0.0017	0.0015 (0.0014)		
Val	D_9	7.041	7.077	0.0010	0.0010 (0.0008)	5.9	5.8

[a] The assignment of S_1 to Aib(1) NH is unambiguous. Other assignments are not to specific residues. Val and Ala NH groups have been assigned by using spin decoupling. [b] $d\delta/dT$ values in both solvents were measured at a peptide concentration of 10 mg/mL. Values in parentheses for $(CD_3)_2SO$ correspond to experiments done at a concentration of 1 mg/mL. [c] Errors in J values are ca. ±0.5 Hz.

that these residues show a very strong preference for 3_{10} helical conformations ($\phi \sim \pm 60°$, $\psi \sim \pm 30°$).[5-12] Type III β turns,[19] stabilized by 4→1 hydrogen bonds, have been established by X-ray crystallography in the peptides benzoxycarbonyl-Aib-Pro-NHMe,[6] benzoxycarbonyl-Aib-Aib-Ala-OMe,[10] and benzoxycarbonyl-Aib-Pro-Aib-Ala-OMe.[5] These conformations have been shown to persist in solution by NMR and IR studies.[7,8] The tendency of the type III β turns to propogate as 3_{10} helical segments has been demonstrated in the crystal structure of tosyl-$(Aib)_5$-OMe[11] and in solution of the amino terminal alamethicin hexapeptide, benzoxycarbonyl-Aib-Pro-Aib-Ala-Aib-Ala-OMe.[9] These studies of Aib peptides have also established the strong tendency of Aib-X and X-Aib sequences to form β-turn conformations (type I or type III),[19] even where X is a Pro residue. The structural preferences of Aib residues, together with the NMR results outlined earlier, permit us to postulate conformations for the suzukacillin fragments, which are compatible with the spectral data. The NMR studies lead to the following conclusions:

1. The tripeptide **4** favors a solution conformation in which one Aib NH is intramolecularly hydrogen bonded.

2. In the pentapeptide **2** a structure possessing three hydrogen bonds involving the Val(3), Aib(4), and Val(5) NH groups is preferred.

(19) Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425–1436.

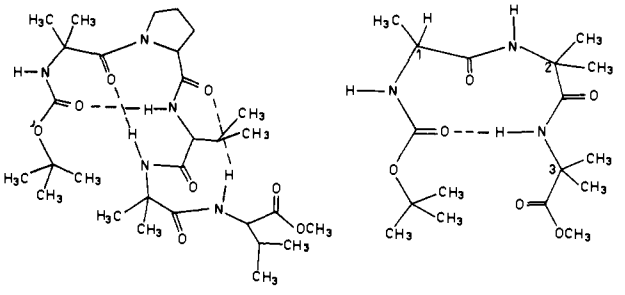

Figure 7. Proposed hydrogen bonded conformations for **2** (left) and **4** (right).

3. The pentapeptide **3** adopts a solution conformation in which the Ala(3) NH and two Aib NH groups are hydrogen bonded.

4. The decapeptide **1** assumes a highly folded conformation stabilized by seven strong intramolecualr hydrogen bonds, while one Aib NH groups is weakly bonded. The only fully solvent exposed NH in this pepetide is the Aib(1) NH group.

A conformation for the tripeptide **4** compatible with the NMR data and the known structural preferences of Aib residues is illustrated in Figure 7. The proposed structure consists of an Ala(1)-Aib(2) β turn, stabilized by a 4→1 hydrogen bond between the urethane CO and the NH of Aib(3). The Aib(2) and Ala(1) NH groups are solvent exposed. For the pentapeptide **2**, a 3_{10}

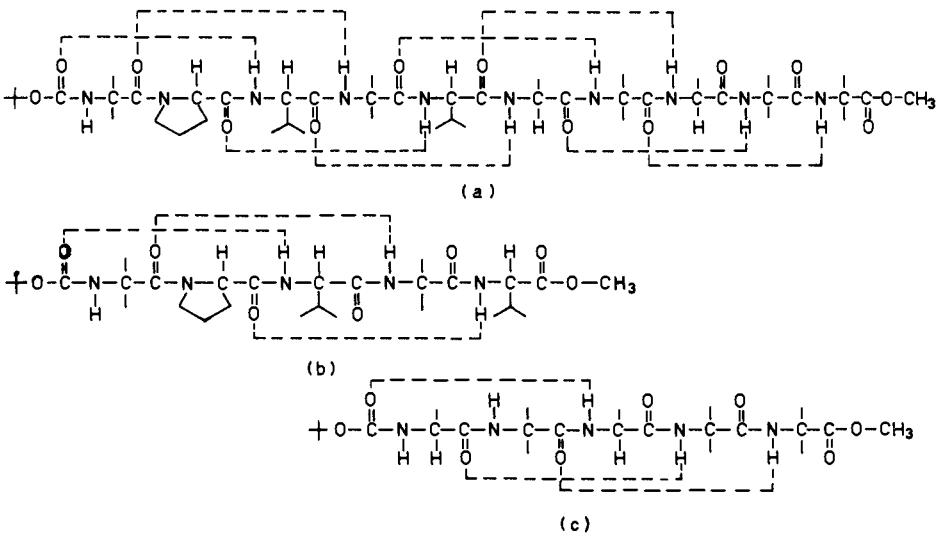

Figure 8. Schematic hydrogen bonding schemes for peptides: (a) **1**, (b) **2**, and (c) **3**.

helical structure composed of three consecutive type III β turns is suggested by the NMR data. The β turns have Aib(1)-Pro(2), Pro(2)-Val(3), and Val(3)-Aib(4) as the corner residues. The Val(3), Aib(4), and Val(5) NH groups participate in 4→1 hydrogen bonds with the urethane, Aib(1), and Pro(2) CO groups, respectively (Figure 7). For the pentapeptide **3** 3_{10} helical structure is again compatible with experimental results. The Ala(3), Aib(4), and Aib(5) NH groups form three intramolecular 4→1 hydrogen bonds, which stabilize three consecutive type III β turns, with Ala(1)-Aib(2), Aib(2)-Ala(3), and Ala(3)-Aib(4) as the corner residues. A schematic representation of the hydrogen bonding pattern is shown in Figure 8c.

In the decapeptide **1** the NMR results favor a highly folded helical conformation, in which seven NH groups (2 Ala, 2 Val, 3 Aib) are strongly hydrogen bonded. One Aib NH S_2 shows a relatively high $d\delta/dT$ value in $(CD_3)_2SO$ (0.004 ppm/°C). However, this resonance shows a small solvent shift in the $CDCl_3$–$(CD_3)_2SO$ system. It is likely that this NH is involved in a weaker intramolecular hydrogen bond, which is further destabilized in the polar solvent $(CD_3)_2SO$. $d\delta/dT$ values as high as 0.0043 ppm/°C have been taken as diagnostic of intramolecular hydrogen bonds.[20] An examination of the decapeptide primary structure shows that the strongly β-turn-favoring sequences like Aib-X, X-Aib, and Pro-X occur throughout the peptide, with the sole exception of the Val(5)-Ala(6) segment. We therefore propose that the Aib NH resonance S_2 be assigned to Aib(7) NH, since a distortion at the Val(5)-Ala(6) residues would weaken the 4→1 intramolecular hydrogen bond between the Aib(7) NH and the Aib(4) CO groups. The proposed hydrogen bonding schemes for **1** is shown in Figure 8a. The hydrogen bonding schemes in the two pentapeptide fragments **2** and **3** are illustrated for comparison in Figure 8. The eight intramolecular hydrogen bonds in **1** stabilize three turns of a 3_{10} helix.[21] The presence of L-amino acids in the sequence would favor right-handed chain folding (negative ϕ and ψ values), a feature supported by crystal structure determinations of model peptides.[5,6,12] The α-helical conformation has been excluded since a 5→1 hydrogen bonding scheme would not be compatible with the presence of eight intramolecular hydrogen bonds in **1**. Furthermore, Aib peptides have been repeatedly shown to favor 4→1 hydrogen bonded β-turn structures.[5-12] The proposed structure is so far the most highly folded conformation demonstrated for a small linear oligpeoptide in solution. It is also the longest 3_{10} helical segment, for which experimental support has been provided.

In the preceding discussion of peptide conformation, we have not used the information available in the vicinal coupling constant ($J_{HNC^{\alpha}H}$) for the Ala and Val residues. The J values for these residues in peptides **1**, **2**, **3**, and **4** in $CDCl_3$ and $(CD_3)_2SO$ are presented in Tables I and II. In each case the values obtained are consistent with the right-handed 3_{10} helical conformation ($\phi \sim -60°$). It may be noted that the Karplus–Bystrov[22] curves which relate J and ϕ are steep in the region −40° to −80°. As a consequence J values <8 Hz are reasonably consistent with ϕ values $\sim -60° \pm 20°$. We believe that for the peptides discussed in this paper, delineation of intramolecular hydrogen bonds taken together with the known stereochemical preferences of Aib residues afford a more reliable guide to backbone conformation. It is however noteworthy that the only J values which change appreciably between $CDCl_3$ and $(CD_3)_2SO$ in the smaller fragments are the Ala(1) NH groups in **3** and **4**. This presumably reflects distortion of the Ala(1)-Aib(2) β turn in $(CD_3)_2SO$. Two Ala doublets (D_3 and D_4) show significant changes in J on going from $CDCl_3$ to $(CD_3)_2SO$. Of these, D_4 changes by as much as 2.2 Hz, suggeting that it may be the Ala(6) NH, since conformational flexibility at the Val(5)-Ala(6) segment is possible in **1**.

The amino terminal decapeptide of suzukacillin thus favors a 3_{10} helical conformation. A similar structural preference has already been noted for smaller alamethicin fragments. It therefore appears that conformational constraints introduced by Aib residues override the tendency of Val residues to adopt nonhelical structures. The insertion of the Val-Aib-Val-Ala segment in suzukacillin does not affect the propensity of the chain to adopt a 3_{10} helical conformation. Since this is the major primary structural difference between alamethicin and suzukacillin, we conclude that the two antibiotics adopt similar three-dimensional structures, i.e., a largely rod-like 3_{10} helical conformation, with a flexible polar tail and some conformational flexibility at the Gly-Leu segment (residues 11–12 in alamethicin and 15–16 in suzukacillin).[9] The two polypeptides have been shown to possess similar membrane activity.[4] It is now almost certain that similarities in their structures dictate that they must function by a common mechanism, the details of which await elucidation.

Acknowledgment. Financial support from the Department of Science and Technology and the University Grants Commission is gratefully acknowledged. P.B. is the recipient of a Career Award from the UGC. M.I. thanks the UGC for the award of a Teacher-Fellowship.

(20) Khaled, M. A.; Urry, D. W.; Okamoto, K. *Biochem. Biophys. Res. Commun.* **1976**, *72*, 162–169.
(21) Donohue, J. *Proc. Natl. Acad. Sci. U.S.A.* **1953**, *39*, 470–478.
(22) Bystrov, V. F. *Prog. NMR Spectrosc.* **1976**, *10*, 41–81.