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THE HELICAL CONFORMATIONS OF 14- AND 16-RESIDUE FRAGMENTS OF SUZUKACILLIN, A MEMBRANE CHANNEL-FORMING POLYPEPTIDE

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The suzukacillin fragments, Boc-Ala-Aib-Aib-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (14), Boc-Ala-Aib-Ala-Aib-Aib-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (16G) and the completely apolar 16-residue peptide in which the glutamine residue has been replaced by alanine (16A) have been studied by 270 MHz ^{1}H-HMR, in C^2HCl_3 and $(C^2H_3)_2SO$ solution. Intramolecularly hydrogen-bonded NH groups have been identified by temperature and solvent dependence of chemical shifts. Peptides 14 and 16A adopt folded 3_{10} helical conformations stabilized by 11 and 13 hydrogen bonds, respectively. In peptide 16G there are 12 intramolecular hydrogen bonds, with the glycine NH being solvent-exposed, in contrast to 14 and 16A

The 3_{10} helix [1] is a structural feature found over short segments in protein crystal structures [2]. Incipient 3_{10} helical segments may serve as nucleation sites for peptide folding [3]. The only poly-peptide for which a 3_{10} helical structure has been proposed is poly-α-aminoisobutyric acid [4]. Recent structural studies have established a tendency for α-aminoisobutyric acid (Aib)-containing peptides to form Type III β-turn structures [5–7], which on repetition generate a 3_{10} helix. Recent interest in the conformation of α-aminoisobutyric acid-containing peptides, stems from the extensive occurrence of this residue in alamethicin [8], suzukacillin [9] (Fig. 1) and related membrane channel-forming peptides [10]. Studies in this laboratory on synthetic alamethicin [5,11] and suzukacillin fragments [12–14] suggest that these hydrophobic polypeptides adopt highly folded rodlike 3_{10} helical structures. These proposals have been based on NMR and X-ray analyses of short segments, together with infrared and CD studies of longer peptides. Since helical peptide conformations are stabilized by intramolecular hydrogen bonds, ^{1}H-NMR techniques, which allow delineation of hydrogen-bonded NH groups, have proved most useful in the analysis of these systems. However, the application of ^{1}H-HMR requires the observation of well-resolved amide NH resonances in oligopeptides, a condition that becomes difficult to satisfy in the study of moderately large peptides, at readily accessible magnetic field strengths. In our studies so far, we have provided a detailed analysis of the amide NH resonances of the 1–10 [12] and 11–21 [13,14] fragments of suzukacillin. In the present report we examine the larger 14- (Boc-Ala-Aib-Aib-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe, **14**) and 16- (Boc-Als-Aib-Ala-Aib-Aib-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe, **16G**) residue central fragments of suzukacillin and the related 16-residue peptide (Boc-Ala-Aib-Ala-Aib-Aib-Ala-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe, **16A**) and present

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Abbreviation: Aib, α-aminoisobutyric acid.

evidence for 3_{10} helical folding in these sequences. The results support a structure incorporating five turns of a 3_{10} helix stabilized by 12 intramolecular hydrogen bonds, for the 6–21 fragment of suzukacillin.

Experimental section

All peptides were synthesized by solution phase procedures as described for alamethicin I [15]. Peptides were characterized by 270 MHz ^{1}H-NMR spectra and found to be homogeneous by TLC on silica gel. ^{1}H-NMR studies were carried out on a Bruker WH-270 FT-NMR spectrometer at the Bangalore NMR Facility as reported earlier [11,12]. Spectral widths of 3012 Hz were used and the data after Fourier transformation were stored in 8K memory locations, yielding a digital resolution of 0.367 Hz/point. All chemical shifts are expressed as δ(ppm) downfield from tetramethylsilane. Solvent titration and variable temperature measurements were performed at a peptide concentration of 10 mg/ml as described earlier [12,13].

(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

Fig. 1. Sequences of suzukacillin (a) and alamethicin (b).

Results

Assignment of resonances

The NH resonances in the 270 MHz ^{1}H-NMR spectra of the peptides **14**, **16A** and **16G** are shown in Figs. 2–4, under varying conditions. A remarkable feature of the spectra is that there is very little overlap of the NH resonances. With the help of spectra in solvent mixtures of different composition, recorded as a function of temperature, all the individual NH resonances can be identified in the three peptides. In the case of peptide **14**, distinct resonances are observed for eight NH groups in

TABLE I

CHEMICAL SHIFTS OF NH GROUPS IN PEPTIDES

Values in parentheses are $J_{HNC^{\alpha}H}$ values in Hz, wherever measured. Starred resonances correspond to glutamine sidechain carboxamide NH protons.

14			**16A**			**16G**	
NH	δ		NH	δ		NH	δ
	C^2HCl_3	$(C^2H_3)_2SO$		C^2HCl_3	$(C^2H_3)_2SO$	$((C^2H_3)_2SO)$	
S_1	7.21	8.49	S_1	7.00	8.41	S_1	8.35
S_{2*}	7.92	8.19	T_2	7.98	8.15	T_2	8.21
T_3	8.10	7.98	S_3	7.93	7.86	S_{3*}	8.11
S_4	7.79	7.91	D_4	7.80(6.2)	7.83	S_4	7.97
S_5	7.79	7.80	S_5	7.81	7.82	D_5	7.96
D_6	7.89	7.80	D_6	7.78(7.4)	7.77	S_6	7.94
S_7	7.69	7.74	S_7	7.79	7.77	S_7	7.80
D_8	7.89	7.74	S_8	7.60	7.75	D_8	7.77
S_{9*}	7.65	7.74	D_9	7.72(4.4)	7.72	D_9	7.77
D_{10}	7.80	7.67	S_{10}	7.47	7.70	D_{10}	7.77
S_{11}	7.41	7.67	D_{11}	7.67(4.4)	7.60(8.1)	S_{11}	7.70
D_{12}	7.81	7.58	D_{12}	7.45(5.2)	7.35(7.2)	S_{12*}	7.70
S_{13}	7.30	7.37	S_{13}	7.30	7.35	S_{13}	7.58
S_{15}	7.97	7.05	D_{14}	5.91	7.20(4.4)	D_{14}	7.58(4.0)
S_{15}	7.09	7.13	S_{15}	7.07	7.03	S_{15}	7.37
—	—		—	—	—	D_{16}	7.17(5.1)
—	—	—	—	—	—	S_{17}	7.05

can be assigned to the valine, glutamine and two leucine residues. Of the nine singlets, seven arise from α-aminoisobutyric acid residues, which lack a hydrogen at C^{α}, while two are due to the glutamine sidechain carboxamide group. The latter, S_2 and S_9, were identified on the basis of their broadening at elevated temperatures, as compared to other singlets, in $(C^2H_3)_2SO$. This behaviour of glutamine sidechain protons has been noted earlier [13, 14, 16]. The chemical shifts of the various resonances are summarized in Table I.

In peptide **16A**, resonances corresponding to all the 15 NH groups can be identified as described for **14**. Representative spectra are shown in Fig. 2. There are eight singlets (S_n) due to Aib-NH groups, six doublets (D_n) due to the valine, two leucine and three alanine residues and one triplet (T_n) assigned to the lone glycine residue. Of these, the urethane NH of Ala(1), D_{14}, can be readily assigned due to its highfield position in C^2HCl_3. As in **14**, this resonance is broadened at most solvent compositions, but a doublet is detectable at concentrations between 14.5 and 20% $(C^2H_3)_2SO$ (Fig. 3d). With the exception of D_{14} and T_2, the other resonances cannot be assigned to specific residues. Assignments of the Ala(1)-NH (D_{16}) and Gly-NH (T_2) were made in similar fashion in peptide **16G** (Fig. 4). The glutamine carboxamide sidechain protons (S_3, S_{12}) were assigned to singlets, which broadened with increasing temperature in $(C^2H_3)_2SO$, as in the case of **14**. The chemical shifts of the various NH resonances in peptides **14**,

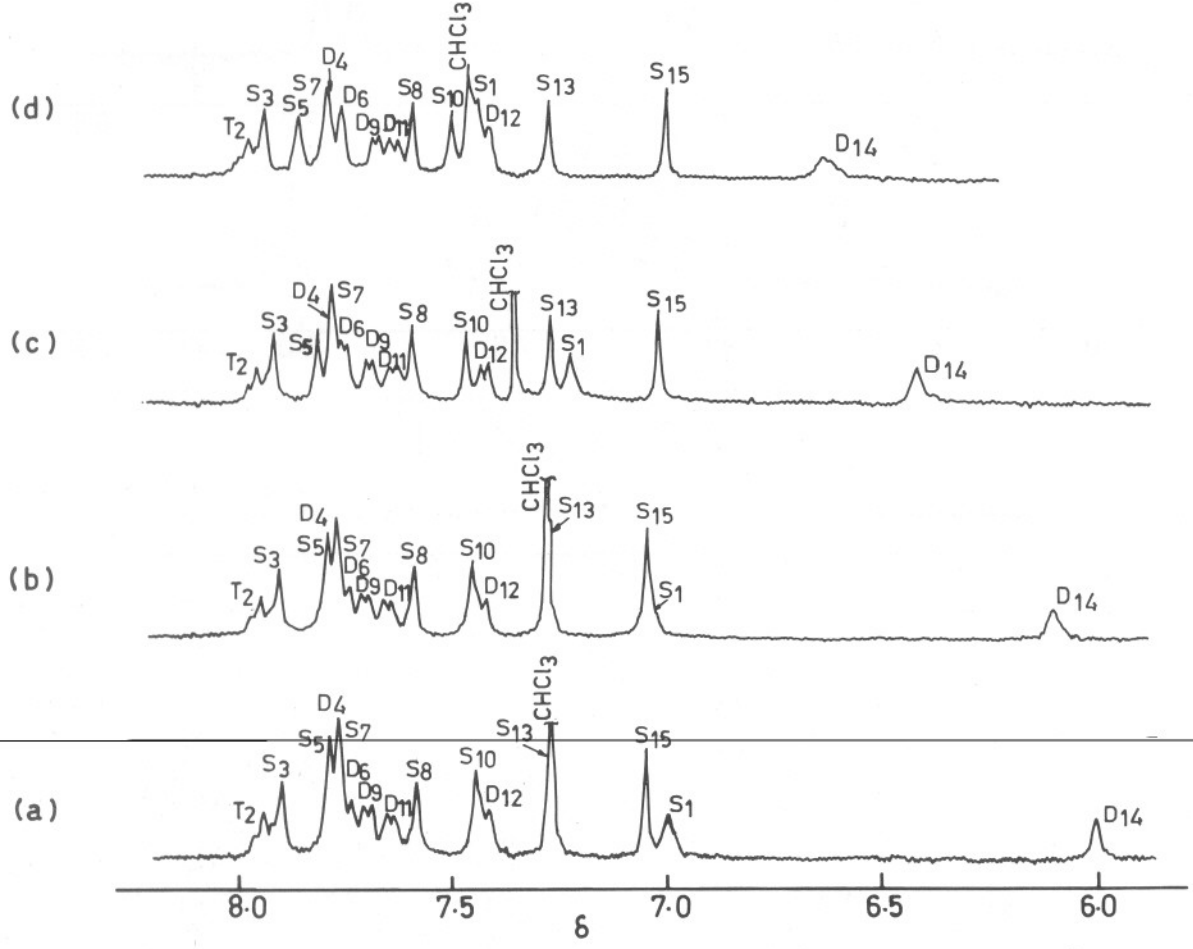

Fig. 3. Partial 270 MHz ^1H NMR spectra of peptide **16A** (NH region) in $C^2HCl_3/(C^2H_3)_2SO$ mixtures. (a) 1% $(C^2H_3)_2SO$; (b) 2% $(C^2H_3)_2SO$; (c) 6.5% $(C^2H_3)_2SO$; (d) 14.5% $(C^2H_3)_2SO$. Peptide concentration 0.0066 M.

can be assigned to the valine, glutamine and two leucine residues. Of the nine singlets, seven arise from α-aminoisobutyric acid residues, which lack a hydrogen at C^{α}, while two are due to the glutamine sidechain carboxamide group. The latter, S_2 and S_9, were identified on the basis of their broadening at elevated temperatures, as compared to other singlets, in $(C^2H_3)_2SO$. This behaviour of glutamine sidechain protons has been noted earlier [13, 14, 16]. The chemical shifts of the various resonances are summarized in Table I.

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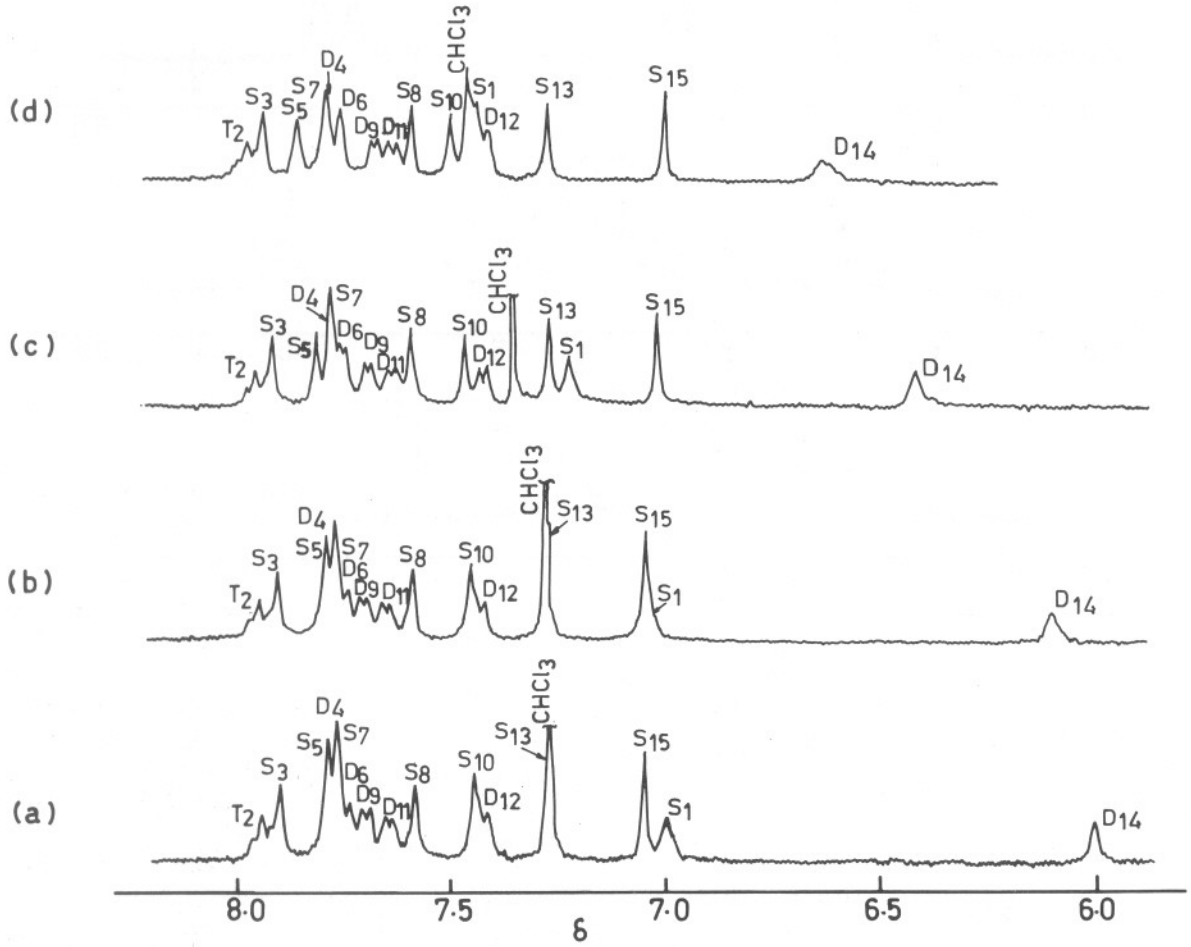

Fig. 3. Partial 270 MHz ^1H NMR spectra of peptide **16A** (NH region) in $C^2HCl_3/(C^2H_3)_2SO$ mixtures. (a) 1% $(C^2H_3)_2SO$; (b) 2% $(C^2H_3)_2SO$; (c) 6.5% $(C^2H_3)_2SO$; (d) 14.5% $(C^2H_3)_2SO$. Peptide concentration 0.0066 M.

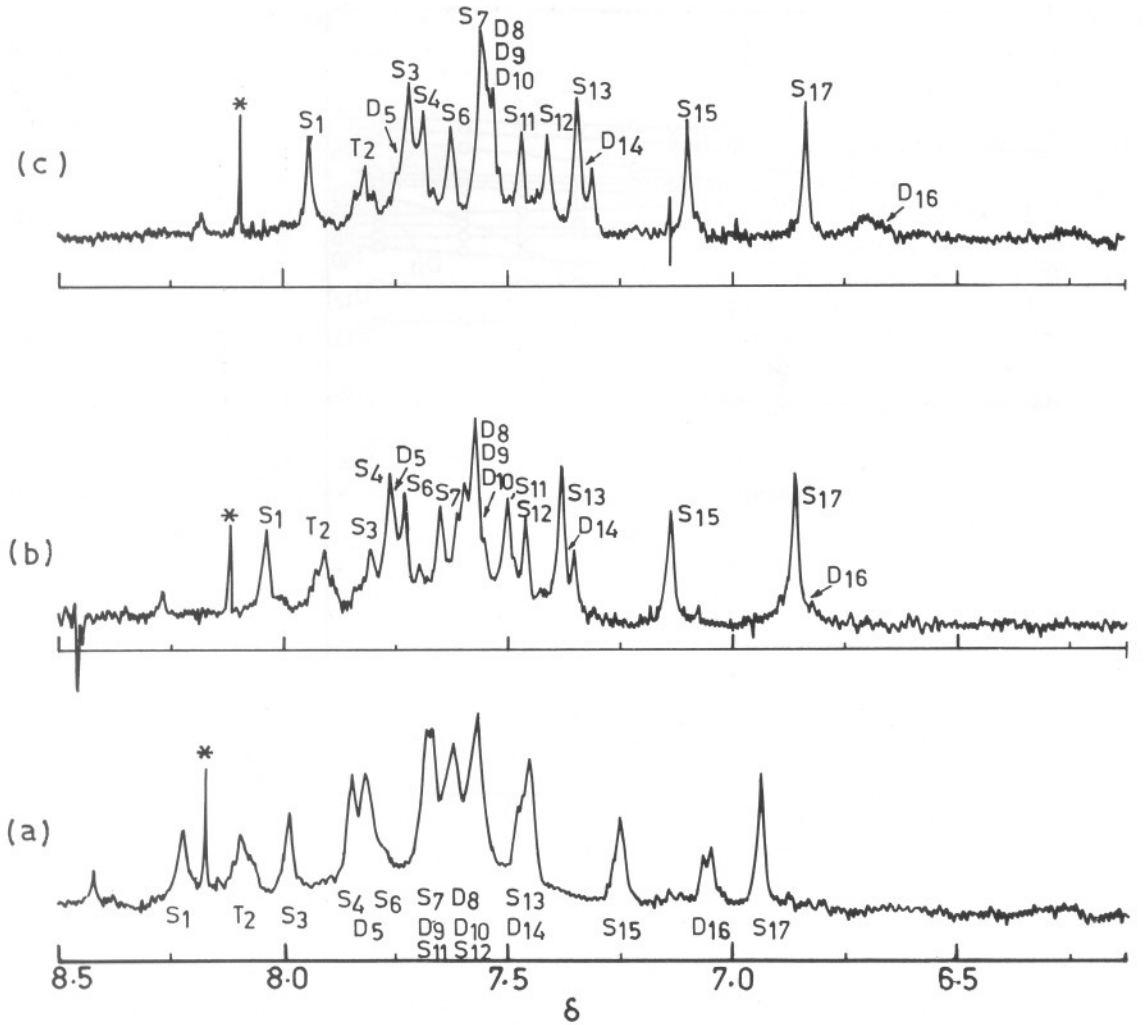

Fig. 4. Partial 270 MHz ^{1}H-NMR spectra (NH region) of peptide **16G** in $(C^2H_3)_2SO$ at different temperatures. (a) 295 K; (b) 325 K; (c) 345 K. Peptide concentration 0.0064 M. Starred peak corresponds to a solvent impurity.

16A and **16G** are summarized in Table I. While unambiguous assignment to specific residues is not possible with the present data, the interpretation of results aimed at establishing hydrogen-bonded NH groups remains independent of detailed assignments.

Delineation of hydrogen-bonded NH groups

The involvement of NH groups in intramolecular hydrogen bonds was established using solvent dependence of chemical shifts in C^2HCl_3/$(C^2H_3)_2SO$ mixtures and temperature dependence of chemical shifts in $(C^2H_3)_2SO$ and C^2HCl_3 [11–13]. Fig. 5 shows the results of solvent titration experiments for peptides **14** and **16A**. The temperature dependences of NH chemical shifts in C^2HCl_3 and $(C^2H_3)_2SO$ were established for **14** and **16A**, while data for **16G** were obtained in $(C^2H_3)_2SO$. The temperature coefficient ($d\delta/dT$) values in peptides **14**, **16A** and **16G** are summarized in Table II.

In peptide **14** only two resonances S_1 and D_{14} show large downfield shifts with increasing concentration of the strongly hydrogen-bonding solvent, $(C^2H_3)_2SO$, indicating their exposure to the solvent. The chemical shifts of the other 13

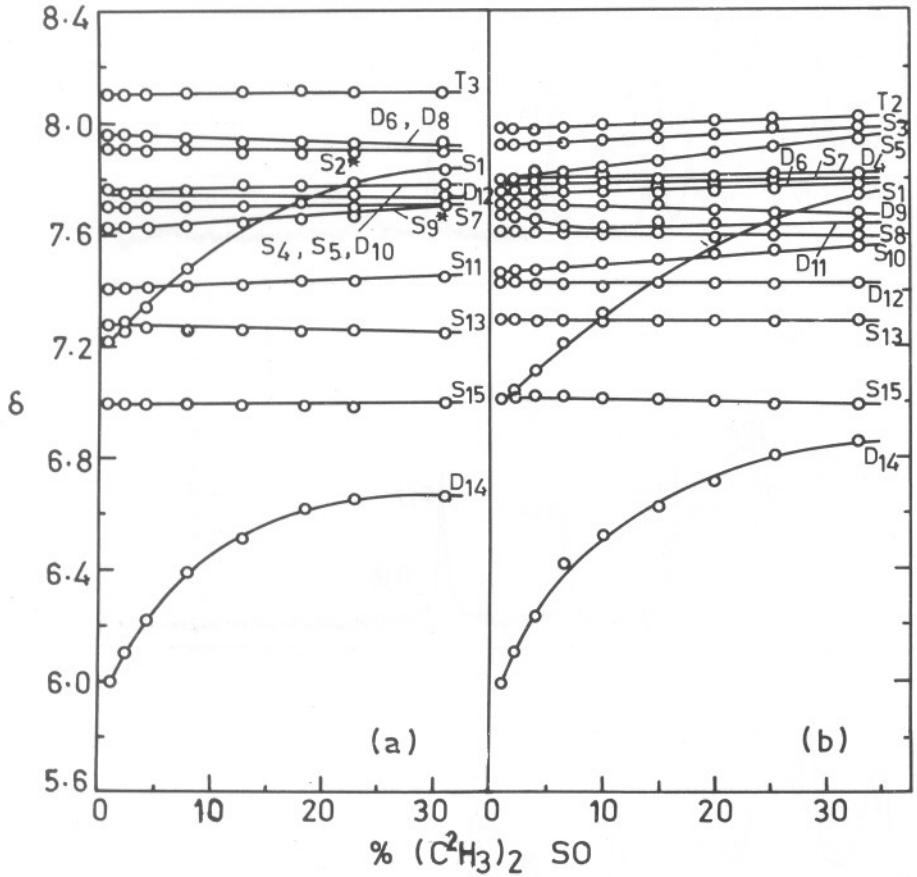

Fig. 5. Solvent dependence of NH chemical shifts in $C^2HCl_3/(C^2H_3)_2SO$ mixtures. (a) **14**; (b) **16A**.

NH resonances (11 backbone and two sidechain) are insensitive to changes in solvent composition. Of the 15 NH groups, S_1, S_2 (glutamine sidechain) and D_{14} (Ala(1)) show $d\delta/dT$ values greater than $4 \cdot 10^{-3}$ ppm/K in $(C^2H_3)_2SO$, characteristic of solvent-exposed NH resonances. S_9 (glutamine sidechain) also has a relatively high $d\delta/dT$ value of $3.6 \cdot 10^{-3}$ ppm/K. The remaining 11 NH resonances have small $d\delta/dT$ values in $(C^2H_3)_2SO$, typical of solvent-shielded or intramolecularly hydrogen-bonded NH protons [17]. The temperature coefficients together with the solvent dependence of chemical shifts suggest that 11 backbone NH groups are shielded from solvent and involved in hydrogen-bonding in **14**. The sidechain NH protons show high $d\delta/dT$ values, but are relatively insensitive to solvent composition. While $d\delta/dT$ values in strongly hydrogen-bonding solvents such as $(C^2H_3)_2SO$ have been widely used in delineating intramolecularly hydrogen-bonded NH groups in peptides, studies in relatively non-polar solvents such as C^2HCl_3 have found less use. Recently it has been suggested that intramolecularly hydrogen-bonded and free NH groups should yield low $d\delta/dT$ values in C^2HCl_3, whereas intermolecularly hydrogen-bonded groups my have high values [18]. Alternatively, high $d\delta/dT$ values in C^2HCl_3 may reflect structural transitions. The data in Table II clearly show that resonances known to be solvent-exposed in $(C^2H_3)_2SO$, also have high $d\delta/dT$ values in C^2HCl_3. The absence of discontinuities in the solvent titration curves makes the possibility of a solvent-induced transition unlikely. The C^2HCl_3 data indicate that the free NH groups probably participate in intermolecular hydrogen-bonding at the concentrations

TABLE II

TEMPERATURE COEFFICIENTS OF NH GROUPS IN PEPTIDES

Starred resonances correspond to glutamine sidechain carboxamide NH protons.

14			**16A**			**16G**	
	$d\delta/dT$(ppm/K)$\times(10^3)$		NH	$d\delta/dT$(ppm/K)$\times 10^3$)		NH	$d\delta/dT$(ppm/K)$(\times 10^3)$
	C^2HCl_3	$(C^2H_3)_2SO$		C^2HCl_3	$(C^2H_3)_2SO$		$(C^2H_3)_2SO$
S_1	6.0	6.0	S_1	5.4	5.9	S_1	5.7
S_{2*}	4.6	5.1	T_2	3.7	3.8	T_2	5.2
T_3	2.3	1.6	S_3	2.2	1.2	S_{3*}	5.3
S_4	2.2	2.0	D_4	1.9	1.2	S_4	2.5
S_5	2.2	1.0	S_5	2.2	1.7	D_5	2.5
D_6	1.8	2.2	D_6	1.7	1.6	S_6	2.5
S_7	1.5	1.2	S_7	1.6	1.6	S_7	0.1
D_8	1.8	1.2	S_8	1.2	0.9	D_8	2.0
S_{9*}	4.2	3.6	D_9	0.8	2.2	D_9	2.0
D_{10}	3.2	1.8	S_{10}	2.0	2.2	D_{10}	2.0
S_{11}	1.6	2.9	D_{11}	3.2	2.8	S_{11}	3.0
D_{12}	3.2	2.9	D_{12}	1.8	0.5	S_{12}	3.2
S_{13}	1.2	3.2	S_{13}	1.4	2.9	S_{13}	2.1
D_{14}	11.7	6.5	D_{14}	8.4	6.6	D_{14}	2.4
S_{15}	1.0	2.0	S_{15}	0.7	1.9	S_{15}	3.0
—	—	—	—	—	—	D_{16}	7.0
—	—	—	—	—	—	S_{17}	2.0

used. Association of the peptide Boc-Gln-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe (11–21 fragment of suzukacillin) has been demonstrated to occur in C^2HCl_3 via NH groups, which are not involved in intramolecular hydrogen bonds [14].

In peptide **16A** only resonances S_1 and D_{14} (Ala(1)-NH) have high $d\delta/dT$ values in $(C^2H_3)_2SO$ ($> 4 \cdot 10^{-3}$ ppm/K) and show significant solvent-dependence of chemical shifts (Table II and Fig. 5). All the other 13 NH groups have $d\delta/dT$ values less than $4 \cdot 10^{-3}$ ppm/K in $(C^2H_3)_2SO$ and also show very little solvent-sensitivity of chemical shifts. This suggests that 13 NH groups in **16A** probably participate in intramolecular hydrogen bonds.

In peptide **16G**, of the 17 NH resonances (15 backbone and two sidechain) D_{16} (Ala(1)-NH), S_1 and one sidechain NH (S_3) have high $d\delta/dT$ values in $(C^2H_3)_2SO$, characteristic of free NH groups. 12 backbone NH groups and one sidechain NH (S_{12}) have low $d\delta/dT$ values. Interestingly the Gly-NH (T_2) has a much higher $d\delta/dT$ value in peptide **16G**, as compared to peptide **14** and **16A**.

Discussion

The results presented above favour the following conclusions:

(i) Peptide **14** adopts a folded conformation in solution, stabilized by eleven intramolecular hydrogen bonds. Together with the known stereochemical preferences of α-aminoisobutyric acid residues for Type III β-turn or incipient 3_{10} helical structures [10], the data suggest that **14** is folded into a long stretch of 3_{10} helix stabilized by eleven $4 \rightarrow 1$ hydrogen bonds.

(ii) Peptide **16A** also folds into a 3_{10} helix stabilized by 13 intramolecular hydrogen bonds.

(iii) Peptide **16G**, which corresponds to the 6–21 fragment of suzukacillin, is also folded into a helical conformation stabilized by 12 hydrogen bonds involving backbone NH groups. In contrast to peptide **16A**, the Gly-NH in **16G** does not appear to participate in an intramolecular hydrogen bond in $(C^2H_3)_2SO$ solution.

Earlier studies from this laboratory have provided evidence for the 3_{10} helical folding of the

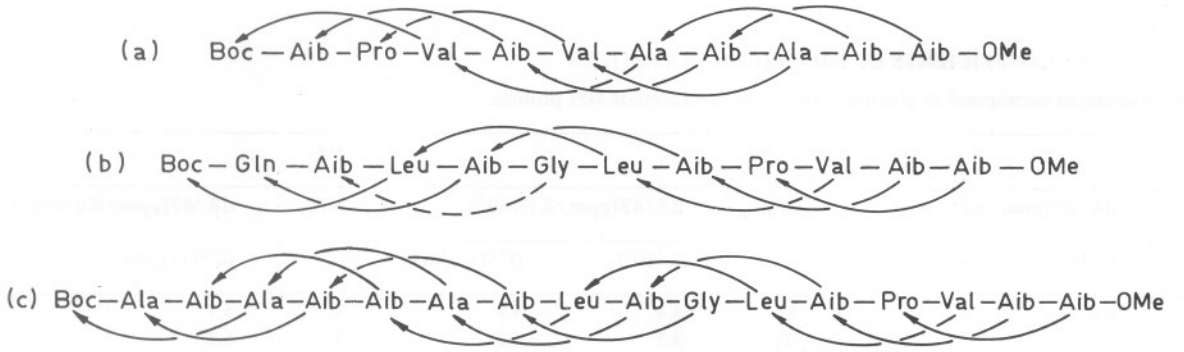

Fig. 6. Schematic representation of the hydrogen-bonding pattern in suzukacillin fragments and **16A**. (a) 1–10 [12]; (b) 11–21 [13]; (c) **16A**. Arrows represent NH to CO hydrogen bonds.

1–10 and 11–21 fragments of suzukacillin [12,13]. The present report establishes that these conformations are maintained over longer segments. Both the 8–21 suzukacillin fragment **14** and the apolar 16-residue peptide **16A** favour 3_{10} helical structures. In the latter, five complete turns of a 3_{10} helix are fully accommodated. The hydrogen-bonding schemes proposed for **16A**, together with those postulated for shorter fragments of suzukacillin are schematically illustrated in Fig. 6. In the 6–21 fragment **16G** there appears to be a loss of the hydrogen bond involving the Gly-NH, leading to a high $d\delta/dT$ value for this group in $(C^2H_3)_2SO$. Peptides **16A** and **16G** differ solely at residue 6. The presence of the carboxamide group in **16G** may conceivably affect the conformation either by sidechain backbone hydrogen-bonding [13] or by favouring intermolecular association. Aggregation of peptides stabilized by sidechain hydrogen-bonding groups could also lead to the observed differences between **16A** and **16G**. However, a detailed NMR study of the aggregation of the smaller 11–21 suzukacillin fragment revealed that peptide association is unimportant in $(C^2H_3)_2SO$ at the concentrations employed [14]. Further, helical α-aminoisobutyric acid peptides aggregate in apolar solvents such as C^2HCl_3, at concentrations used in NMR studies, without significant disruption of the conformation of individual molecules [14].

In the suzukacillin sequence, the proline residue at position 18 interrupts the chain of successive $4 \rightarrow 1$ hydrogen bonds, leaving the Gly-CO group free, in a 3_{10} helical structure. In both alamethicin and suzukacillin sequences there is the possibility of some conformational flexibility at the Gly-Leu-Aib-Pro segment [11]. The possibility of expansion of the 3_{10} helix into an α-helix in this region has already been considered from infrared studies of these fragments [6]. It may be noted that there are very small differences in ϕ, ψ values for the 3_{10} and α-helical conformations. These structures can be distinguished largely on the basis of their hydrogen-bonding patterns. Distorted conformations lying between these regular structures also explain the observed data. Transitions from the $4 \rightarrow 1$ hydrogen-bonded to $5 \rightarrow 1$ hydrogen-bonded structures in the central part of **16G** could lead to exposure of the Gly-NH. In longer sequences the additional hydrogen bond present in the 3_{10} helix would not lend very much additional stabilization as compared to the α-helix. It is quite likely that, while the 3_{10} helix is maintained over shorter segments, expansion to an α-helix must certainly be considered in the longer sequences. Resonance S_1, which is solvent-exposed in all three peptides, may be assigned to the Aib(2)-NH, which is not hydrogen-bonded in either 3_{10} or α-helical conformations.

The presence of L-amino acids in these sequences would favour chain-folding into right-handed helical conformations [19]. The $J_{HNC^{\alpha}H}$ values for all the NH doublets that can be measured are less than 8 Hz (Table I), which is compatible with $\phi \sim -40$ to $-70°$, required for right-handed 3_{10} or α-helical conformations [20]. The results of this study, together with earlier reports on small fragments, demonstrate that the apolar 1–21 segment of suzukacillin must adopt a largely 3_{10} or α-helical conformation. The close homology

of their sequences suggests that this conclusion is also valid for alamethicin.

These studies emphasize the utility of highfield NMR studies in the delineation of hydrogen-bonded NH groups, even in rather long acyclic peptide sequences, which adopt stereochemically rigid conformations. The availability of higher magnetic field strengths should permit detailed analysis of the complete sequences of membrane channel-forming polypeptides, by providing adequate spectral resolution. It is, however, clear that the α-aminoisobutyric acid containing channel-formers favour predominantly 3_{10} or α-helical conformations. Such helices cannot accommodate passage of cations through the helix interior, as proposed in the models for the gramicidin A transmembrane channel [21]. Membrane channels must arise by aggregation of apolar helices in the case of the α-aminoisobutyric acid containing polypeptides [10]. Structural investigations of such peptide aggragates should be valuable in developing detailed molecular mechanisms for the membrane activity of suzukacillin, alamethicin and related polypeptides.

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