Fiskin, A. M., Peterson, G., & Brady, F. O. (1977) *Ultramicroscopy 2*, 389.
Galdes, A., Vašák, M., Hill, H. A. O., & Kägi, J. H. R. (1978a) *FEBS Lett. 92*, 17.
Galdes, A., Hill, H. A. O., Bremner, I., & Young, B. W. (1978b) *Biochem. Biophys. Res. Commun. 85*, 217.
Glickson, J. O., Dadok, J., & Marshall, G. R. (1974) *Biochemistry 13*, 11.
Hvidt, A., & Nielsen, S. O. (1966) *Adv. Protein Chem. 21*, 287.
Kägi, J. H. R., & Vallee, B. L. (1960) *J. Biol. Chem. 235*, 3460.
Kägi, J. H. R., & Vallee, B. L. (1961) *J. Biol. Chem. 236*, 2435.
Kägi, J. H. R., Himmelhoch, S. R., Whanger, P. D., Bethune, J. L., & Vallee, B. L. (1974) *J. Biol. Chem. 249*, 3537.
Kissling, M. M., & Kägi, J. H. R. (1977) *FEBS Lett. 82*, 247.
Kojima, Y., & Kägi, J. H. R. (1978) *Trends Biochem. Sci. 3*, 90.
Kojima, Y., Berger, C., Vallee, B. L., & Kägi, J. H. R. (1976) *Prod. Natl. Acad. Sci. U.S.A. 73*, 3413.
Kopple, K. O., Ohniski, M., & Go, A. (1969) *J. Am. Chem. Soc. 91*, 4264.
Kopple, K. O., Go, A., Logan, R. H., & Savrda, J. (1972) *J. Am. Chem. Soc. 94*, 973.
Lerch, K. (1979) in *Metallothionein* (Kägi, J. H. R., & Nordberg, M., Eds.) Birkhäuser, Basel (in press).
Margoshes, M., & Vallee, B. L. (1957) *J. Am. Chem. Soc. 79*, 4813.
McDonald, C. C., & Phillips, W. D. (1969) *J. Am. Chem. Soc. 91*, 1513.
Molday, R. S., Englander, S. W., & Kallen, R. G. (1972) *Biochemistry 11*, 150.
Richards, F. M. (1963) *Annu. Rev. Biochem. 32*, 269.
Roberts, G. C. K., & Jardetzky, O. (1970) *Adv. Protein Chem. 24*, 447.
Rupp, H., Voelter, W., & Weser, U. (1974) *FEBS Lett. 40*, 176.
Sadler, P. J., Bakka, A., & Begnon, P. J. (1978) *FEBS Lett. 94*, 315.
Thiers, R. (1957) *Methods Biochem. Anal. 5*, 273.
Urry, D. W., Ohniski, M., & Walter, R. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 111.
Von Dreele, P. H., Brewster, A. I., Scheraga, H. A., Ferger, M. F., & Du Vigneaud, V. (1971) *Proc. Natl. Acad. Sci. U.S.A. 68*, 1028.
Von Dreele, P. H., Rae, I. O., & Scheraga, H. A. (1978) *Biochemistry 17*, 956.
Wagner, G., Wüthrich, K., & Tschesche, H. (1978) *Eur. J. Biochem. 86*, 67.
Wyssbrod, H. R., & Gibbons, W. A. (1973) *Surv. Prog. Chem. 6*, 209.

Infrared Studies on the Conformation of Synthetic Alamethicin Fragments and Model Peptides Containing α-Aminoisobutyric Acid†

Ch. Pulla Rao, R. Nagaraj, C. N. R. Rao, and P. Balaram*

ABSTRACT: Infrared studies of synthetic alamethicin fragments and model peptides containing α-aminoisobutyric acid (Aib) have been carried out in solution. Tripeptides and larger fragments exhibit a strong tendency to form β turns, stabilized by 4 → 1 10-atom hydrogen bonds. Dipeptides show less well-defined structures, though C_5 and C_7 conformations are detectable. Conformational restrictions imposed by Aib residues result in these peptides populating a limited range of states. Integrated intensities of the hydrogen-bonded N-H stretching band can be used to quantitate the number of intramolecular hydrogen bonds. Predictions made from infrared data are in excellent agreement with nuclear magnetic resonance and X-ray diffraction studies. Assignments of the urethane and tertiary amide carbonyl groups in the free state have been made in model peptides. Shifts to lower frequency on hydrogen bonding are observed for the carbonyl groups. The 1–6 segment of alamethicin is shown to adopt a 3_{10} helical structure stabilized by four intramolecular hydrogen bonds. The fragments Boc-Leu-Aib-Pro-Val-Aib-OMe (12–16) and Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe (11–16) possess structures involving 4 → 1 and 5 → 1 hydrogen bonds. Supporting evidence for these structures is obtained from proton nuclear magnetic resonance studies.

The presence of α-aminoisobutyric acid (Aib)[1] in the polypeptide ionophore alamethicin (Mueller & Rudin, 1968; Martin & Williams, 1976; Pandey et al., 1977a) and the more recent demonstration of its occurrence in the closely related antibiotics suzukacillin (Jung et al., 1976), emerimicins (Pandey et al., 1977b), antiamoebins (Pandey et al., 1977c), and trichotoxin A-40 (Irmscher et al., 1978) have stimulated interest in the conformations of peptides containing Aib residues. The presence of Aib introduces considerable stereochemical constraints on the conformations of acyclic peptides. The propensity of Aib residues to initiate β turns and to generate 3_{10} helical structures has been demonstrated in solution by NMR (Nagaraj et al., 1979) and by X-ray diffraction in the solid state (Shamala et al., 1977, 1978; Prasad

† From the Solid State and Structural Chemistry Unit (Contribution No. 46) and the Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India. *Received June 5, 1979.* This work has been supported by the University Grants Commission. R.N. was the recipient of a fellowship from the Department of Atomic Energy, Government of India.

* Address correspondence to this author at the Molecular Biophysics Unit.

[1] Abbreviations used: Aib, α-aminoisobutyric acid; DCC, *N,N*-dicyclohexylcarbodiimide; Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; –OMe, methyl ester; –OBz, benzyl ester.

0006-2960/80/0419-0425$01.00/0

et al., 1979; Smith et al., 1977). Theoretical studies have also led to the conclusion that Aib residues are energetically limited to a narrow region of conformational space, in the left- and right-handed helical regions (Marshall & Bosshard, 1972; Burgess & Leach, 1973). The structures deduced for Aib-containing peptides in solution by NMR have also been observed in the solid state, suggesting that the stereochemical constraints introduced by Aib residues significantly reduce the problems due to the presence of multiple conformations of similar energies (Nagaraj et al., 1979). Aib-containing sequences are therefore attractive models for developing and refining both experimental and theoretical approaches to peptide conformational analysis. An earlier report from this laboratory described the application of IR spectroscopy as a probe for the development of secondary structure in the amino-terminal segment of alamethicin (Rao et al., 1979). In this paper we describe detailed IR studies of model Aib-containing peptides and synthetic alamethicin fragments, with a view toward exploring the application of IR methods as a tool for the study of the conformation of complex peptides.

Materials and Methods

Peptides were synthesized by solution-phase methods using dicyclohexylcarbodiimide-mediated couplings. Specific procedures for the synthesis of Z-Aib-Pro-OMe (**7**), Z-Aib-Pro-Aib-OMe (**9**), Z-Aib-Pro-Aib-Ala-OMe (**10**), and Z-Aib-Pro-NHMe (**8**) have been described earlier (Nagaraj et al., 1979; Prasad et al., 1979). Larger fragments were built-up by using the DCC–1-hydroxybenzotriazole method (Konig & Geiger, 1970). All compounds were chromatographically homogeneous on silica gel and yielded 100- and 270-MHz ^{1}H NMR spectra fully consistent with their structures. Full details of the synthetic procedures will be published elsewhere.

Infrared (IR) spectra were recorded in $CHCl_3$ and CCl_4 solutions by using peptide concentrations of $\sim 5 \times 10^{-3}$ and 1×10^{-4} M, respectively. The solvents were dried and distilled before use. All spectra were obtained on a Perkin-Elmer 580 spectrometer. For $CHCl_3$ solutions a variable path length cell with NaCl windows was used and path lengths employed were 3.4 and 2.2 mm. Spectra in CCl_4 were recorded by using a 10-cm path length, fused silica cell. Integral band intensities were calculated as described by Ramsay (1952). ^{1}H NMR studies were carried out on a Bruker WH-270 FT-NMR spectrometer or on a Varian HA-100 spectrometer as described earlier (Nagaraj et al., 1979).

Results and Discussion

Model Peptides. Figure 1 shows the NH stretching region (ν_{NH}) of the IR spectra of Z-Aib-Aib-OMe (**1**) and Z-Aib-Ala-OMe (**2**) in dilute solutions of $CHCl_3$ and CCl_4. At the concentrations used in these ($\sim 5 \times 10^{-3}$ M in $CHCl_3$; 10^{-4} M in CCl_4), intermolecular aggregation is negligible. This is borne out by the absence of any significant change in the ν_{NH} bands as a function of concentration in these ranges. The intense band at ~ 3430 cm^{-1} in $CHCl_3$ corresponds to ν_{NH} free while the broader band at ~ 3400 cm^{-1} reflects the presence of intramolecular hydrogen-bonded species (ν_{NH} bonded). In the less polar solvent CCl_4, **1** shows two free NH bands at 3440 and 3416 cm^{-1} and hydrogen-bonded absorption at 3393 and 3330 cm^{-1}. **2** also shows the presence of a broad band at 3322 cm^{-1} in CCl_4. The presence of ν_{NH} bands between 3400 and 3330 cm^{-1} in model compounds like acetylamino acid methylamides has been attributed to the existence of structures stabilized by five-atom (C_5) and seven-atom (C_7) hydrogen-bonded rings (Figure 2) (Tsuboi et al., 1958; Avignon et al., 1969). In both **1** and **2** the almost extended C_5 structures

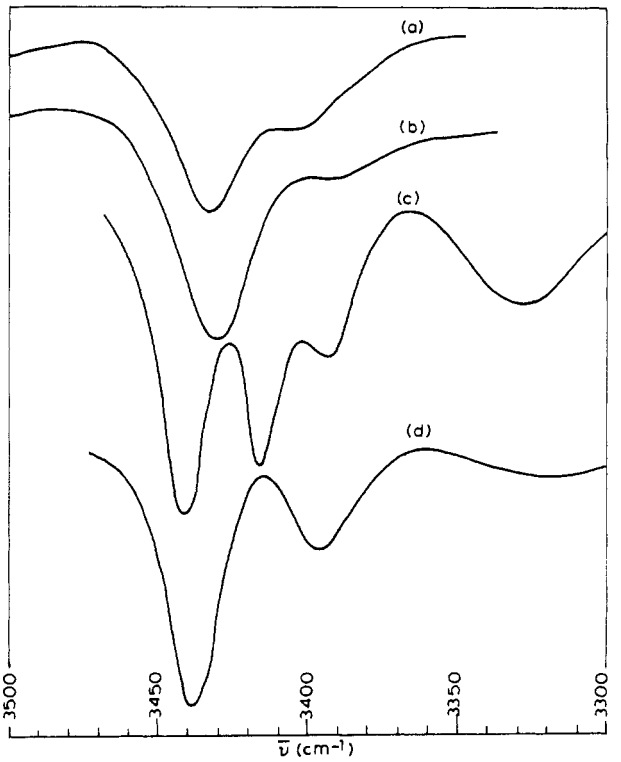

FIGURE 1: ν_{NH} bands of dipeptides. (a) Z-Aib-Aib-OMe (**1**) in $CHCl_3$; (b) Z-Aib-Ala-OMe (**2**) in $CHCl_3$; (c) **1** in CCl_4; (d) **2** in CCl_4.

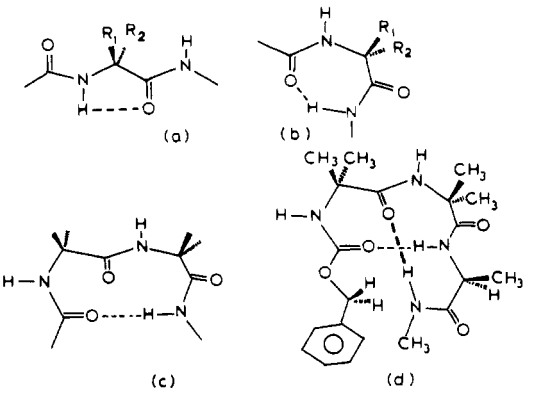

FIGURE 2: Schematic representation of intramolecular hydrogen-bonded structures. (a) C_5; (b) C_7; (c) C_{10} or β turn; (d) incipient 3_{10} helical conformation of Z-Aib-Aib-Ala-NHMe (**6**).

appear to be present in $CHCl_3$, while the folded C_7 conformation becomes more populated in CCl_4. The presence of a C_7 conformation for Ac-Aib-NHMe in CCl_4 has been previously noted (Aubry et al., 1978).

In order to study the effect of lengthening the peptide chain, the compounds Z-Ala-Aib-Ala-OMe (**3**), Z-Aib-Aib-Ala-OMe (**4**), Z-Aib-Aib-Aib-OMe (**5**), and Z-Aib-Aib-Ala-NHMe (**6**) were examined, and their ν_{NH} bands in dilute $CHCl_3$ solution are shown in Figure 3. While **3** exists predominantly in non-hydrogen-bonded conformations (ν_{NH} 3430 cm^{-1}) with a weak band at 3367 cm^{-1} indicating a small population of C_7 structures, **4**, **5**, and **6** clearly show strong ν_{NH}-bonded peaks at 3366, 3369, and 3356 cm^{-1}, respectively. Earlier NMR and X-ray diffraction studies have established the tendency of Aib residues to adopt ϕ and ψ values that favor the formation of type III β turns or 3_{10} helical structures (Nagaraj et al., 1979; Shamala et al., 1977, 1978; Prasad et al., 1979). The band at ~ 3360 cm^{-1} is therefore assigned to the β-turn conformations (C_{10}) stabilized by intramolecular 4 → 1 hydrogen bonds (Figure 2c). Tripeptides **3** and **4** differ only by a single

Table I: Infrared Data (ν_{NH} and ν_{CO}) for Model Aib-Containing Di- and Tripeptides

compd	ν_{NH} (cm^{-1})[c]	no. of H bonds	ν_{CO} (cm^{-1})
Z-Aib-Aib-OMe (1)[a]	3433, 3400		1740, 1724, 1675
Z-Aib-Aib-OMe (1)[b]	3440, 3416, 3396, 3330		
Z-Aib-Ala-OMe (2)[a]	3430, 3395		1740, 1724, 1675
Z-Aib-Ala-OMe (2)[b]	3438, 3396, 3322		
Z-Ala-Aib-Ala-OMe (3)[a]	3430, 3367		1738, 1714, 1701, 1671
Z-Aib-Aib-Ala-OMe (4)[a]	3428 (1.178), 3366 (1.44)	1	1740, 1717, 1685, 1670
Z-Aib-Aib-Aib-OMe (5)[a]	3428 (1.070), 3369 (1.38)	1	1738, 1718, 1686, 1668
Z-Aib-Aib-Ala-NHMe (6)[a]	3425 (1.023), 3356 (2.82)	2	1715, 1655–1685

[a] Solvent was $CHCl_3$. [b] Solvent was CCl_4. [c] Values in parentheses are integrated peak intensities. Units are mol^{-1} L cm^{-2} ($\times 10^{-4}$).

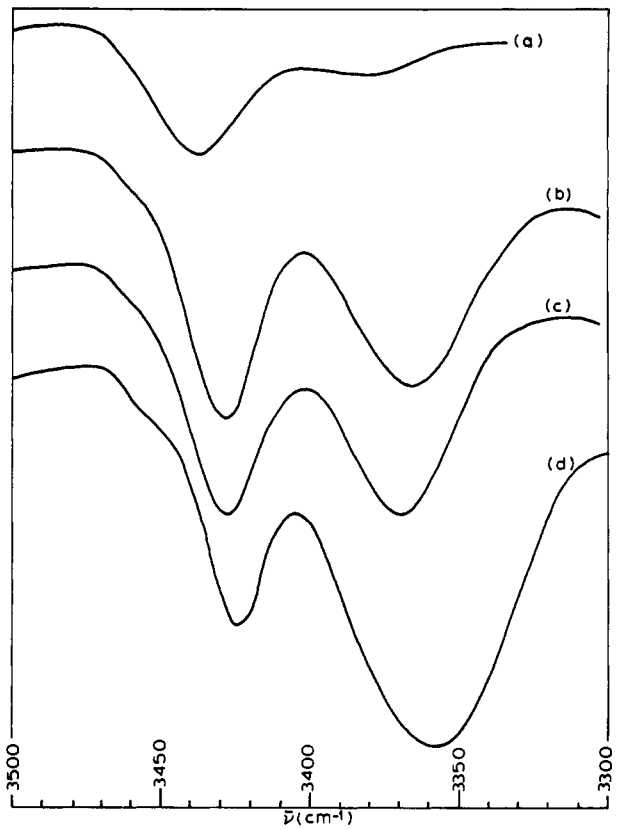

FIGURE 3: ν_{NH} bands of tripeptides in $CHCl_3$. (a) Z-Ala-Aib-Ala-OMe (3); (b) Z-Aib-Aib-Ala-OMe (4); (c) Z-Aib-Aib-Aib-OMe (5); (d) Z-Aib-Aib-Ala-NHMe (6).

substitution of a hydrogen at C(α) by a methyl group in the first residue. However, there is a dramatic difference in their IR spectra with **4** showing the presence of β-turn conformers in contrast to **3**. These results clearly emphasize the role of Aib residues in initiating β-turn formation. In an earlier study of the amino-terminal fragments of alamethicin, it was established that specific sequences of Aib-containing peptides tend to adopt β-turn structures in solution whenever they are stabilized by hydrogen-bond formation (Rao et al., 1979). A comparison of the spectra of these peptides suggests that the peptides capable of forming 4 → 1 hydrogen bonds do not show any increase in ν_{NH}-bonded intensity relative to ν_{NH} free on going from $CHCl_3$ to CCl_4 solution. Due to the limited conformational flexibility of Aib residues, the molecules tend to populate only a restricted range of structures. As a consequence, correlations between ν_{NH}-bonded integrated intensities become possible within a given peptide series. This permits the use of IR spectroscopy in quantitating the number of 10-atom hydrogen bonds in Aib-containing peptides in solution. The assumption made is that the peptides exclusively populate the hydrogen-bonded β-turn structures if available. The validity of this has been established by the excellent correlation between the IR predictions and NMR and X-ray results (Rao et al., 1979). Peptides **4** and **5** can form only a single β-turn structure. The presence of a type III β turn stabilized by a hydrogen bond between the CO group of the benzyloxycarbonyl function and the Ala(3) NH in **4** has been observed in the crystalline state by X-ray diffraction (B. V. V. Prasad, unpublished experiments). However, in **6**, conversion of the terminal carboxylic acid to a methylamide group allows the formation of two consecutive β turns, with -Aib-Aib- and -Aib-Ala- at the corners, stabilized by two 4 → 1 intramolecular hydrogen bonds. Figure 3 shows a marked increase in the ν_{NH}-bonded intensity in **6** as compared to **4** and **5**. The integrated intensities are summarized in Table I. Assuming a value of one hydrogen bond in **4** and **5**, the IR data suggest that **6** adopts the incipient 3_{10} helical conformation shown in Figure 2d. Further support for this conclusion comes from ^1H NMR studies which suggest that the Aib(2) and Ala(3) NH groups are solvent shielded. Temperature coefficients of NH chemical shifts in dimethyl sulfoxide of 2×10^{-3} ppm/°C for both Ala(3) and methylamide NH groups together with the observed J value of 8.1 Hz for Ala(3) (R. Nagaraj, unpublished experiments) are compatible with the conformation shown in Figure 2d.

In the structures proposed for the tripeptides **4**–**6**, the CO group of the benzyloxycarbonyl moiety is a hydrogen-bond acceptor. From the data in Table I it is clear that the stretching frequency (ν_{CO}) of the urethane carbonyl appears at lower frequency in **4**–**6** (1715–1718 cm^{-1}) as compared to the dipeptide **2** (1724 cm^{-1}) in which the CO group is free. The urethane ν_{CO} in **3** appears at 1714 cm^{-1} while in the model compound (benzyloxycarbonyl)alanylmethylamide (Z-Ala-NHMe) the corresponding ν_{CO} is at 1715 cm^{-1}. This observation strengthens the conclusion that **3** does not possess a significant population of intramolecular hydrogen-bonded, folded structures. It may be noted that the removal of a methyl group at C(α) in Ala as compared to Aib leads to a lowering of the urethane ν_{CO} by 10 cm^{-1}.

Alamethicin Fragments. Figure 4 shows the 1600–1800-cm^{-1} region of the IR spectra of the amino-terminal peptides of alamethicin. As described earlier, a quantitative correlation of the ν_{NH}-bonded intensity with the number of intramolecular hydrogen bonds leads to the conclusion that the tri- (**9**), tetra- (**10**), penta- (**11**), and hexapeptide (**12**) fragments contain one, two, three, and four hydrogen-bonded amide NH groups, respectively (Rao et al., 1979). For a more conclusive determination of the conformation of these peptides, it would be desirable to establish the nature of the hydrogen-bonded carbonyl groups. From Figure 4 it is seen that in Z-Aib-Pro-OMe (**7**) three ν_{CO} bands can be observed at 1741, 1727, and 1633–1640 cm^{-1}, which may be assigned to the ester, urethane, and tertiary amide (Aib-Pro) carbonyl groups, respectively. Conversion of the C-terminal ester to the methylamide in Z-Aib-Pro-NHMe (**8**) results in the disappearance of the ester ν_{CO} and the appearance of the methylamide ν_{CO}

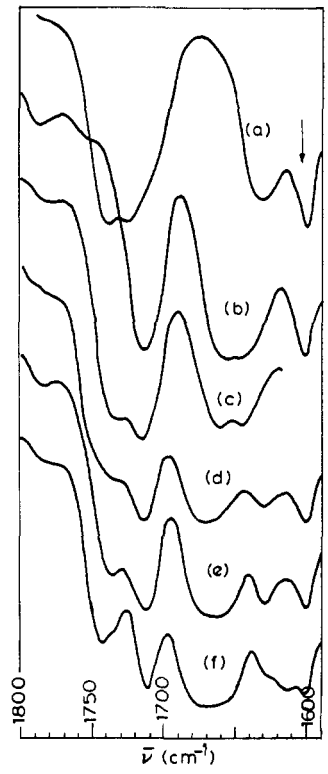

FIGURE 4: ν_{CO} bands of alamethicin amino-terminal fragments in $CHCl_3$. (a) Z-Aib-Pro-OMe (**7**); (b) Z-Aib-Pro-NHMe (**8**); (c) Z-Aib-Pro-Aib-OMe (**9**); (d) Z-Aib-Pro-Aib-Ala-OMe (**10**); (e) Z-Aib-Pro-Aib-Ala-Aib-OMe (**11**); (f) Z-Aib-Pro-Aib-Ala-Aib-Ala-OMe (**12**). The arrow indicates a peak at 1600 cm^{-1} due to the solvent.

Table II: Infrared Data (ν_{CO}) for Amino-Terminal Alamethicin Fragments in $CHCl_3$

peptide	ν_{CO} (cm^{-1})
Z-Aib-Pro-OMe (7)	1741, 1726, 1633–1640[a]
Z-Aib-Pro-NHMe (8)	1715, 1658, 1645
Z-Aib-Pro-Aib-OMe (9)	1734, 1716, 1662, 1646
Z-Aib-Pro-Aib-Ala-OMe (10)	1738, 1716, 1665–1680,[a] 1632
Z-Aib-Pro-Aib-Ala-Aib-OMe (11)	1736, 1713, 1655–1675,[a] 1631
Z-Aib-Pro-Aib-Ala-Aib-Ala-OMe (12)	1735, 1712, 1650–1680,[a] 1632

[a] Broad, unresolved band.

at 1658 cm^{-1}. The data in Table II show that the urethane ν_{CO} in **8** appears 12 cm^{-1} lower than in **7**. Studies of the ν_{NH} bands have already established the **8** possesses a single C_{10} hydrogen bond, whereas this is not possible in **7**. Further, X-ray studies of **8** have demonstrated the presence of a type III β turn in the solid state, stabilized by a hydrogen bond between the urethane CO and the methylamide NH group (Prasad et al., 1979). Evidence for the persistence of this structure in solution has also been obtained from ^{1}H NMR studies (R. Nagaraj, unpublished experiments). Consequently, the lowering of the ν_{CO} of the urethane group may be considered to unequivocally establish involvement of the CO group in an intramolecular hydrogen bond.

The data for the amino-terminal fragments in Table II show that the urethane ν_{CO} occurs at 1712–1716 cm^{-1} in the peptides **9**–**12**, suggesting that the Aib(1)-Pro(2) β turn is maintained in the series from the tripeptide to the hexapeptide. A comparison of the tertiary amide ν_{CO} [Aib(1) CO] in these peptides shows that in **8** and **9** a value of 1645–1646 cm^{-1} is obtained, whereas there is a distinct lowering in frequency to 1631–1632

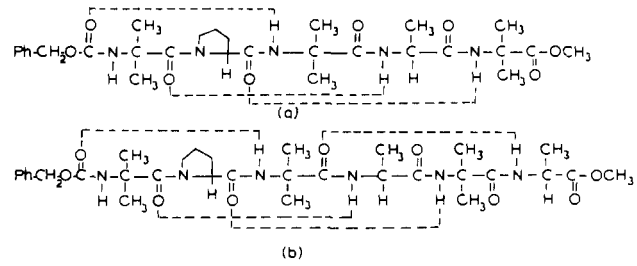

FIGURE 5: Schematic structures indicating the hydrogen-bonding pattern in the pentapeptide **11** (a) and the hexapeptide **12** (b).

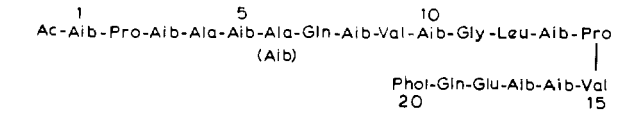

FIGURE 6: Sequence of alamethicin I and II (replacement at position 6, shown in parentheses) proposed by Pandey et al. (1977a).

cm^{-1} in **10**–**12**. Earlier NMR and X-ray studies have established the involvement of the Aib(1) CO group in the formation of a second hydrogen bond, stabilizing the Pro-Aib β turn, in the tetrapeptide **10** (Nagaraj et al., 1979). The IR band position of Aib(1) CO suggests that this β turn is also conserved in the penta- (**11**) and hexapeptides (**12**). Together with the earlier results, based on an analysis of ν_{NH} intensities, that there are three and four hydrogen-bonded groups in **11** and **12** (Rao et al., 1979), these results strongly support the view that a sequential development of type III β turns results, giving rise to a 3_{10} helical fold. The hydrogen-bonded structures obtained are schematically illustrated in Figure 5. With increasing length of the peptide chain the intensity of the ν_{CO} (amide I) band between 1660 and 1685 cm^{-1} increases, resulting in a broad, unresolved envelope in the higher peptides. Addition of each residue after the tetrapeptide (**10**) results in the conversion of a free secondary amide CO to a hydrogen-bonded state, with the concomitant generation of a new free secondary amide CO group. ν_{CO} bonded and ν_{CO} free are not expected to differ by more than 10–15 cm^{-1}. Further, small differences in ν_{CO} result from C(α) substitution when Aib and Ala residues are compared. Hence, a detailed interpretation of this band in this sequence is not feasible at present.

Having established a 3_{10} helical folding of the amino-terminal segment, it is of interest to examine the secondary structure of the remaining regions of the polypeptide chain of alamethicin. An inspection of the proposed sequences (Figure 6) shows an almost regular alternation of Aib residues from 1 to 10 of alamethicin I and II, with only a single replacement of Ala(6) by Aib in alamethicin II (Pandey et al., 1977a). However, the presence of Gly, Leu, and Pro residues at positions 11, 12, and 14 raises the question as to whether the initial 3_{10} helical conformation will indeed be maintained throughout the polypeptide chain. While Pro residues have been implicated in termination of helical segments in proteins, it should be noted that the ϕ and ψ values required for the 3_{10} or α-helical conformations are indeed easily accessible for Pro (Matsuzaki, 1974; Matsuzaki & Iitaka, 1971). The lack of an NH group will, however, result in the loss of the intramolecular hydrogen bond. For a determination of the conformational preference of the 11–16 segment of alamethicin, IR studies of model synthetic fragments were carried out and the results are summarized in Figure 7 and Table III. The spectra in Figure 7 show the absence of significant amounts of hydrogen-bonded species in the tripeptide Boc-Gly-Leu-Aib-OMe (**13**). However, the fragments Boc-Leu-Aib-Pro-Val-Aib-OMe (**14**), Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe (**15**), and Boc-Gly-Leu-Aib-Pro-Val-Aib-Aib-OBz (**16**) show large

Table III: Infrared Data (ν_{NH} and ν_{CO}) for the Central Fragments of Alamethicin and Model Peptides in $CHCl_3$

peptide	ν_{NH} (cm^{-1})[a]	no. of H bonds	ν_{CO} (cm^{-1})
Boc-Leu-Aib-OH	3432		1750, 1717, 1680
Boc-Ile-Aib-OMe	3433		1737, 1706, 1677
Boc-Val-Aib-OMe	3434		1738, 1707, 1680
Boc-Leu-Aib-Aib-OMe	3431, 3410 (sh),[c] 3366 (w)		1737, 1670-1710
Boc-Gly-Leu-Aib-OMe (**13**)	3451, 3433, 3400 (sh)		1735-1720, 1670-1682[b]
Boc-Gly-Leu-Aib-Aib-OMe	3451, 3430 (sh), 3415, 3359		1735, 1722, 1665-1695[b]
Boc-Leu-Aib-Pro-Val-Aib-OMe (**14**)	3435, 3412 (sh), 3329 (2.24)	2	1735, 1689, 1662, 1633
Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe (**15**)	3444, 3428, 3325 (3.4)	3	1735, 1700, 1689, 1655 (sh), 1630
Boc-Gly-Leu-Aib-Pro-Val-Aib-Aib-OBz (**16**)	3444, 3428, 3326 (3.16)	3	1730, 1701, 1686, 1673, 1654, 1630
Boc-Gln-Aib-Val-Aib-Gly-Leu-Aib-OMe (**17**)	3409, 3313 (6.39)	5	1735, 1692, 1650-1690

[a] Values in parentheses are integrated peak intensities. Units are mol^{-1} L cm^{-2} ($\times 10^{-4}$). [b] Unresolved band. [c] sh, shoulder.

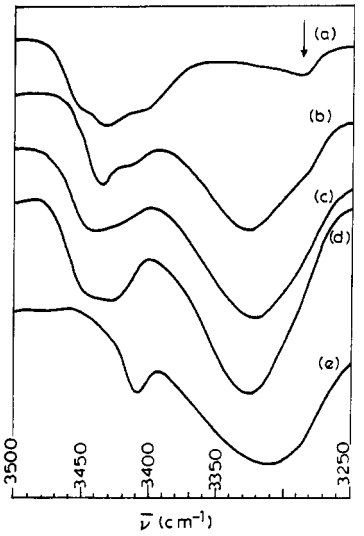

FIGURE 7: ν_{NH} bands of central alamethicin fragments in $CHCl_3$. (a) Boc-Gly-Leu-Aib-OMe (**13**); (b) Boc-Leu-Aib-Pro-Val-Aib-OMe (**14**); (c) Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe (**15**); (d) Boc-Gly-Leu-Aib-Pro-Val-Aib-Aib-OBz (**16**); (e) Boc-Gln-Aib-Val-Aib-Gly-Leu-Aib-OMe (**17**). The arrow indicates a band due to the solvent.

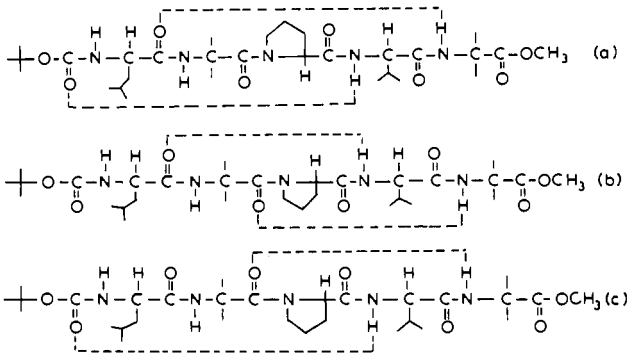

FIGURE 8: Schematic hydrogen-bonded structures for Boc-Leu-Aib-Pro-Val-Aib-OMe (**14**). (a) Two 5 → 1 (C_{13}) hydrogen bonds; (b) two 4 → 1 (C_{10}) hydrogen bonds; (c) 5 → 1 and 4 → 1 hydrogen bonds.

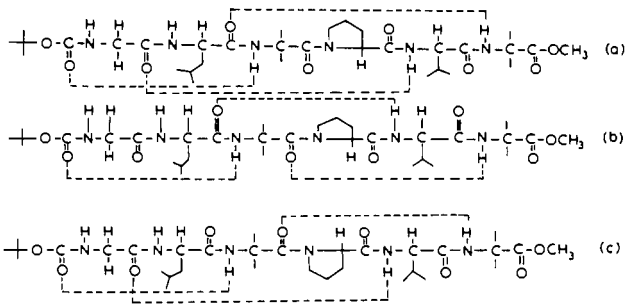

FIGURE 9: Schematic hydrogen-bonded structures for Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe (**15**). (a) 4 → 1, 5 → 1, 5 → 1; (b) 4 → 1, 4 → 1, 4 → 1; (c) 4 → 1, 5 → 1, 4 → 1.

ν_{NH}-bonded intensity at ~3330 cm^{-1}. This may be attributed to the presence of C_{10} hydrogen-bonded β turns.

^1H NMR studies of the solvent- and temperature-dependence of the amide NH chemical shifts of peptides **14–16** were carried out and the results are summarized in Table IV. In **14** the Val(4) NH and one of the Aib NH groups [tentatively assigned to Aib(5)] are solvent shielded or intramolecularly hydrogen bonded. This follows from the small change in chemical shift on going from a non-hydrogen-bonding solvent like $CDCl_3$ to a strongly hydrogen-bond accepting solvent like $(CD_3)_2SO$ (Nagaraj et al., 1979) and also the comparatively low values for the temperature coefficients in $(CD_3)_2SO$ (Wüthrich, 1976). By a similar argument, the Val(5) NH and two Aib NH groups are solvent shielded in the hexapeptide **15**, while in **16** the Val(5) NH and three Aib NH groups are shielded. The corresponding NMR parameters for the tetrapeptide **10** are also provided for comparison. In **10** a structure involving two intramolecular hydrogen bonds has already been demonstrated unambiguously (Nagaraj et al., 1979). While intramolecular hydrogen bonds are likely to be strongly favored in chloroform solution, it is possible that in $(CD_3)_2SO$ equilibria between folded and partially unfolded structures may exist.

Using a value of three for the number of intramolecular hydrogen bonds in **15** and the integrated intensities of ν_{NH} bonded in Table III, one obtains values of two and three intramolecular hydrogen bonds in **14** and **16**, respectively. The underlying assumption is that these peptides exist predominantly in a single conformation in solution. The presence of two Aib and one Pro residue in the sequence suggests that conformational flexibility would be minimal, with the possible exception of the Gly-Leu segment. For **14** two possible structures may be proposed involving 13-atom (C_{13}, 5 → 1) and 10-atom (C_{10}, 4 → 1) hydrogen bonds. These are schematically illustrated in Figure 8. In both cases the same amide NH groups, Val(4) and Aib(5), participate in hydrogen bonding. The distinction between these structures is therefore not possible by ^1H NMR. The proposed C_{13} hydrogen bond is well established in α-helical polypeptides and proteins (Ramachandran & Sasisekharan, 1968) but has yet to be demonstrated unambiguously in a small acyclic peptide. 5 → 1 hydrogen bonds have, however, been observed in cyclic peptides (Karle, 1975; Karle et al., 1976). The strategic position of Pro at the center of the sequence in **14–16** leads to a disruption of the continuous chain of C_{10} hydrogen bonds. Consequently, these peptides can adopt conformations incorporating both C_{10} and C_{13} structures without any change in the number of intramolecular hydrogen bonds. Some of the

Table IV: Amide NH NMR Chemical Shifts.[a] Solvent Dependence and Temperature Coefficients for Aib-Containing Peptides

peptide	amide NH	chemical shift (δ) $CDCl_3$	chemical shift (δ) $(CD_3)_2SO$	$\Delta\delta$[b]	$(\Delta\delta/\Delta T) \times 10^3$ (ppm/°C)[c]
Boc-Leu-Aib-Pro-Val-Aib-OMe (**14**)	Leu(1)	4.98	6.87	1.89	6.23
	Aib(2)	7.20	8.43	1.23	4.95
	Val(4)	7.46	7.42	−0.04	1.88
	Aib(5)	7.04	7.55	0.51	3.50
Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe (**15**)	Gly(1)	5.97	7.06	1.09	
	Leu(2)	6.88	7.94	1.06	5.56
	Aib(3)	7.63	8.29	0.66	4.48
	Val(5)	7.55	7.35	−0.20	2.56
	Aib(6)	7.23	7.51	0.28	3.13
Boc-Gly-Leu-Aib-Pro-Val-Aib-Aib-OBz (**16**)	Gly(1)	5.65	7.12	1.47	5.11
	Leu(2)	6.73	7.97	1.24	4.68
	Aib(3)	7.60	8.38	0.78	3.78
	Val(5)	7.50	7.62	0.12	3.65
	Aib(6)	7.19	7.48	0.29	4.32
	Aib(7)	7.10	7.20	0.10	2.80
Z-Aib-Pro-Aib-Ala-OMe (**10**)	Aib(1)	5.83	7.93	2.10	5.47
	Aib(3)	7.21	7.75	0.54	4.92
	Ala(4)	7.52	7.49	−0.03	2.96
Z-Aib-Pro-Aib-OMe (**9**)	Aib(1)	5.53	8.04	2.51	5.97
	Aib(3)	7.39	7.56	0.17	2.24

[a] Chemical shifts are expressed as δ (ppm) downfield from internal $(CH_3)_4Si$. Assignments of resonances were made by using decoupling experiments for all residues except Aib. The reported assignments for Aib NH protons in peptides **14–16** are tentative and have been made by comparison with related fragments. Assignments in peptides **9** and **10** have been described earlier (Nagaraj et al., 1979). [b] $\Delta\delta$ is the difference in NH chemical shifts in $CDCl_3$ and $(CD_3)_2SO$. [c] Temperature coefficients have been determined in $(CD_3)_2SO$.

structural possibilities for **14** and **15** are schematically illustrated in Figures 8 and 9. It may be noted that these conformations are distinguishable if the identity of the hydrogen-bonded CO groups is established.

Figure 10 shows the ν_{CO} region of peptides **14–16**. A comparison of the ν_{CO} values in the model peptides Boc-Leu-Aib-OH, Boc-Ile-Aib-OMe, Boc-Val-Aib-OMe, and Boc-Leu-Aib-Aib-OMe (Table III) permits an assignment of the band at 1706–1716 cm^{-1} to the free carbonyl stretching frequency of the Boc-Leu moiety. From the spectrum of **14** it is clear that this band is shifted to significantly lower frequency and overlaps with the secondary amide ν_{CO} at 1685–1690 cm^{-1}. This suggests the involvement of the urethane CO group in hydrogen bonding. The low-frequency position of the tertiary amide CO [Aib(2) CO], 1633 cm^{-1}, also suggests its participation in a hydrogen bond. Together with the earlier result that only two NH groups are hydrogen bonded, it appears that a conformation involving a C_{13} and C_{10} hydrogen-bonding scheme fits the observed data (Figure 8b). In the model compounds Boc-Gly-Leu-Aib-OMe and Boc-Gly-Leu-Aib-Aib-OMe, only very weak bands were detectable in the ν_{NH}-bonded region (3390–3330 cm^{-1}), implying that these molecules largely exist in conformations that are not stabilized by intramolecular hydrogen bonds. The band at 1720–1722 cm^{-1} may be assigned to the urethane carbonyl stretching mode of the Boc-Gly group. In the spectra shown in Figure 10 there is considerable overlap of the ester and urethane bands, leading to errors in the determination of peak positions. In the hexapeptide **15** the band at 1720 cm^{-1} is absent and the band at 1700 cm^{-1} is assigned to the urethane CO group. The shift to lower frequency is again indicative of hydrogen-bonded formation. Further, the tertiary amide [Aib(3)] CO at 1630 cm^{-1} is also suggestive of hydrogen bonding. The results of NMR experiments mentioned earlier suggest the involvement of Aib(3), Val(5), and Aib(6) NH groups in hydrogen-bond formation. A structure as in Figure 9c is proposed for **15** which is compatible with the spectroscopic data. In the proposed conformation an amino-terminal 3_{10} helical (C_{10}) structure expands to one turn of an α helix (C_{13}) and is again tightened to a 3_{10} structure. The ready transformation of 3_{10}

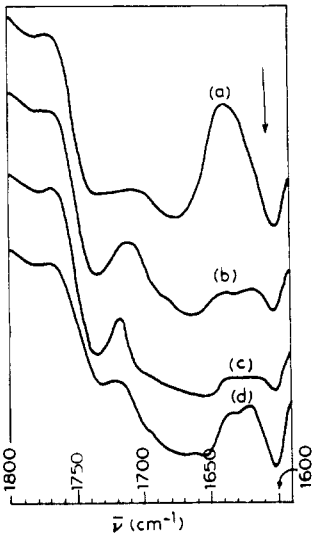

FIGURE 10: ν_{CO} bands of central alamethicin fragments in $CHCl_3$. (a) Boc-Gly-Leu-Aib-OMe (**13**); (b) Boc-Leu-Aib-Pro-Val-Aib-OMe (**14**); (c) Boc-Gly-Leu-Aib-Pro-Val-Aib-OMe (**15**); (d) Boc-Gly-Leu-Aib-Pro-Val-Aib-Aib-OBz (**16**). The arrow indicates a band due to the solvent.

helices to α helices by only small changes in φ and ψ angles has been noted in theoretical studies of polypeptide helices (Ramachandran et al., 1966). It is interesting that Pro(4) now occupies a position which is stereochemically favorable, but the absence of an NH group does not affect the number of intramolecular hydrogen bonds formed. A similar conformation is also likely in the heptapeptide **16**, where the addition of another Aib residue does not alter the conformation. The integrated intensity of the ν_{NH}-bonded peak yields a value of three intramolecular hydrogen bonds in **16**. Further, the urethane and tertiary amide CO bands at 1701 and 1632 cm^{-1}, respectively, argue for the involvement of the Boc CO and Aib(3) CO groups in hydrogen bonds. A conformation similar to that in **15** is proposed for **16**, with the C-terminal Aib residue exerting little influence. The absence of a β turn with Val(5) and Aib(6) at the corners, stabilized by a Pro(4)

CO---Aib(7) NH hydrogen bond, is in agreement with the low tendency of Val residues to occur at the $i + 1$ position in β turns (Chou & Fasman, 1977).

The results presented in this paper provide strong support for the presence of well-defined solution conformations in peptides containing Aib residues. It has been demonstrated that β-turn (C_{10}) formation is preferred to C_5 and C_7 structures wherever possible. However, C_5 and C_7 species may be detected in protected dipeptide esters lacking the NH group for stabilizing the C_{10} hydrogen bond. The rigidity imposed by Aib residues greatly limits conformational flexibility and hence reduces the problems that arise due to the presence of different conformations, permitting a correlation between X-ray, NMR, and IR results. This has been clearly demonstrated by the excellent quantitative correlation obtained for the ν_{NH}-bonded intensities with the number of hydrogen bonds detected by NMR and X-ray methods. IR studies of alamethicin fragments suggest that a 3_{10} helical structure is likely to be maintained from residues 1–6 of the sequence. In the fragments related to the residues 11–17, the presence of the Gly-Leu-Pro sequence leads to the possibility of obtaining structures with C_{13} hydrogen bonds, as a consequence of the interruption of the 3_{10} helix by Pro. The IR data support structures for the 11–16 hexapeptide and 11–17 heptapeptide which involve expansion of the 3_{10} helix to a single turn of an α helix followed by tightening of the backbone to a 3_{10} structure.

While crystal structures of the peptides **4**, **8**, and **10** are in agreement with the conformations deduced from solution studies, ongoing structure determination of peptides **6**, **12**, and **14** in our laboratory will serve to test the conformational conclusions of these IR investigations. For further development of the stereochemistry of alamethicin, preliminary IR studies of the 7–13 heptapeptide Boc-Gln-Aib-Val-Aib-Gly-Leu-Aib-OMe (Figure 7e) and the 1–13 tridecapeptide Z-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-OMe have been carried out in dilute $CHCl_3$ solution. Both peptides show a substantial intensity of the ν_{NH}-bonded peak at 3320–3340 cm^{-1}, suggesting the presence of folded structures stabilized by intramolecular hydrogen bonds. Detailed studies on these fragments and on the synthetic 20-residue sequence of alamethicin are in progress, and the results will form a subsequent report.

References

Aubry, A., Protas, J., Boussard, G., Marraud, M., & Neel, J. (1978) *Biopolymers 17*, 69–89.

Avignon, M., Huong, P. V., Lascombe, J., Marraud, M., & Nee, J. (1969) *Biopolymers 8*, 69–89.

Burgess, A. W., & Leach, S. J. (1973) *Biopolymers 12*, 2599–2605.

Chou, P. Y., & Fasman, G. D. (1977) *J. Mol. Biol. 115*, 135–175.

Irmscher, G., Bovermann, G., Boheim, G., & Jung, G. (1978) *Biochim. Biophys. Acta 507*, 470–484.

Jung, G., Konig, W. A., Liebfritz, D., Ooka, T., Janko, K., & Boheim, G. (1976) *Biochim. Biophys. Acta 433*, 164–181.

Karle, I. L. (1975) *Peptides: Chemistry, Structure and Biology*, (Walter, R., & Meienhofer, J., Eds.) pp 61–84, Ann Arbor Science Publishers, Ann Arbor, MI.

Karle, I. L., Karle, J., Wieland, Th., Burgermeister, W., & Witkop, B. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1782–1785.

Konig, W., & Geiger, R. (1970) *Chem. Ber. 103*, 2024–2033.

Marshall, G. R., & Bosshard, H. E. (1972) *Circ. Res., Suppl. 30 and 31*, 143–150.

Martin, D. R., & Williams, R. J. P. (1976) *Biochem. J. 153*, 181–190.

Matsuzaki, T. (1974) *Acta Crystallogr., Sect. B 30*, 1029–1036.

Matsuzaki, T., & Iitaka, Y. (1971) *Acta Crystallogr. Sect. B 27*, 507–516.

Mueller, P., & Rudin, D. O. (1968) *Nature (London) 217*, 713–719.

Nagaraj, R., Shamala, N., & Balaram, P. (1979) *J. Am. Chem. Soc. 101*, 16–20.

Pandey, R. C., Carter Cook J., Jr., & Rinehard, K. L., Jr. (1977a) *J. Am. Chem. Soc. 99*, 8469–8483.

Pandey, R. C., Carter Cook, J., Jr., & Rinehart, K. L., Jr. (1977b) *J. Am. Chem. Soc. 99*, 5205–5206.

Pandey, R. C., Meng, H., Carter Cook, J., Jr., & Rinehart, K. L., Jr. (1977c) *J. Am. Chem. Soc. 99*, 5203–5205.

Prasad, B. V. V., Shamala, N., Nagaraj, R., Chandrasekaran, R., & Balaram, P. (1979) *Biopolymers 18*, 1635–1646.

Ramachandran, G. N., & Sasisekharan, V. (1968) *Adv. Protein Chem. 23*, 283–438.

Ramachandran, G. N., Venkatachalam, C. M., & Krimm, S. (1966) *Biphys. J. 6*, 849–872.

Ramsay, D. A. (1952) *J. Am. Chem. Soc. 74*, 72–80.

Rao, Ch. P., Nagaraj, R., Rao, C. N. R., & Balaram, P. (1979) *FEBS Lett. 100*, 244–248.

Shamala, N., Nagaraj, R., & Balaram, P. (1977) *Biochem. Biophys. Res. Commun. 79*, 292–298.

Shamala, N., Nagaraj, R., & Balaram, P. (1978) *J. Chem. Soc., Chem. Commun.*, 996–997.

Smith, G. D., Duax, W. L., Czerwinski, E. W., Kendrick, N. E., Marshall, G. R., & Mathews, F. S. (1977) *Peptides: Proceedings of the Fifth American Peptide Symposium* (Goodman, M., & Meienhofer, J., Eds.) pp 277–279, Wiley, New York.

Tsuboi, M., Shimanouchi, T., & Mizushima, S. (1958) *J. Am. Chem. Soc. 80*, 1406–1411.

Wüthrich, K. (1976) *NMR in Biological Research: Peptides and Proteins*, pp 73–95, North-Holland Publishing Co., Amsterdam and Oxford.