Determination of the Solution Conformations of Cyclic Polypeptides

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The determination of the conformations of cyclic polypeptides and depsipeptides in solution has recently been the subject of a number of experimental studies in this and other laboratories, principally by nmr, optical spectroscopy (ORD and CD), and X-ray diffraction.[†,1-29] The motivation of these investigations stems not only from the challenge provided by the multitude of possible conformations of these relatively complex molecules, but also because many of them possess biological activity, as, for example, hormones, antibiotics, ionophores (*i.e.*, substances capable of complexing and transporting ions), toxins, and antidotes. It is reasonable to suppose that there is a very close relationship between their biological activity and their solution conformations, and that the latter may not necessarily correspond to the conformations determined by X-ray diffraction.

Efforts have been made to establish the conformations of some cyclic polypeptides by potential energy calculations alone, without reference to spectral data or at most using experimentally suggested structures as a starting point. (See the review of Scheraga[30] and references contained therein.) Several conformational structures have been suggested for gramicidin S, a cyclic decapeptide.[1-7,31-35] These in general are in at

L-Val-L-Orn-L-Leu-D-Phe-L-Pro
$\rightleftarrows$
L-Pro-D-Phe-L-Leu-L-Orn-L-Val
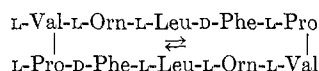
least partial disagreement with each other and with the nmr findings.[1,7] For example, the conformation proposed by Momany, *et al.*,[35] on the basis of *ab initio* calculations without any reference to experimental results (other structures proposed by this group[34,35] are energy-minimization refinements of previously suggested structures) predicts some $-N-C_\alpha-$ dihedral angles (see below) which are not compatible with nmr

† References to the publications of this laboratory are given at appropriate points later in this Account; references to other work are grouped below as follows: gramicidin A, ref 1-7; antamanide (Na$^+$ complex), ref 8; oxytocin, ref 9-11; valinomycin, ref 12-15; nonactin, ref 16, 17; enniatin B, ref 18, 19; ferrichromes, ref 20; actinomycin D, ref 21-24; cyclic sarcosine peptides, ref 25, 26; cyclo-(pentaGly-L-Tyr), ref 27, 28; cyclo(Gly-Cys-Gly-Gly-Pro)₂, ref 29. For recent general reviews of the structure and function of cyclic polypeptides, see: M. M. Shemyakin, Yu. A. Ovchinnikov, and V. T. Ivanov, *Angew. Chem., Int. Ed. Engl.,* **8**, 492 (1969); C. H. Hassall and W. A. Thomas, *Chem. Brit.,* **7**, 145 (1971); D. W. Urry and M. Ohnishi in "Spectroscopic Approaches to Biomolecular Conformation," D. W. Urry, Ed., American Medical Association, Chicago, Ill., 1970. This last includes data on polymyxins E₁ and B₁, vernamycin Bα, and patricin A.

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(25) J. Dale and K. Titlestad, *Chem. Commun.,* 656 (1969).
(26) J. Dale and K. Titlestad, *ibid.,* 1403 (1970).

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measurements and does not predict the intramolecular hydrogen bonds strongly indicated by nmr-monitored peptide NH exchange studies.[1,7] All such proposals are, however, consistent with the C_2 symmetry of the X-ray[36] structure suggested by the repeating sequence of the amino acid residues.

For more complex polypeptides, which in general lack any element of symmetry, *ab initio* energy calculations are virtually impossible because of the enormously large number of possible conformations. Some experimental information is necessary to reduce these to a manageable number. Infrared and electronic spectral data (CD and ORD) can be useful, but nmr is by far the most powerful method at present available for such studies because of the large number of spectral parameters it provides. This information is very rich but in general cannot in itself be completely and unambiguously interpreted in terms of conformation, as we shall see in later discussion. It has been found, however, that by judiciously combining nmr with energy calculations self-consistent structures (or groups of structures) can be arrived at. (The possibility of doing this has been discussed by Gibbons, *et al.*[37]) This is achieved by eliminating from consideration all conformations having one or more residues in a conformation which does not correspond to the observed J coupling of the α-CH and NH backbone protons (see below) or to a low conformational energy. Approximate potential energy estimates are made using the general procedures described in ref 30 and 38 and using standard van der Waals, dispersion, torsional, and electrostatic interactions.

In this Account, we describe how this is done and illustrate some of the results.

cyclo-(Pro-Ser-Gly-Pro-Ser-Gly) and *cyclo*-(Ser-Pro-Gly-Ser-Pro-Gly)

We begin with two cyclic hexapeptides which are sufficiently simple that some of their conformations can be deduced from nmr directly. These compounds illustrate well the typical features of the nmr spectra of

Figure 1. Spectrum (220 MHz) of *cyclo*-(Pro-Ser-Gly-Pro-Ser-Gly) in D_2O and (upper left) in H_2O–CH_3DOOH, 98:2 (v/v); 22°. The H_2O solution is made acidic to retard exchange of NH protons with solvent (see ref 40).

c-$(PSG)_2$

c-$(SPG)_2$
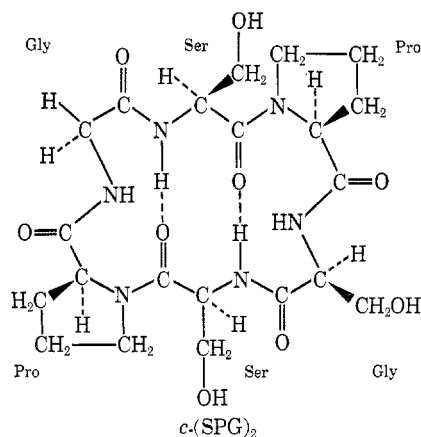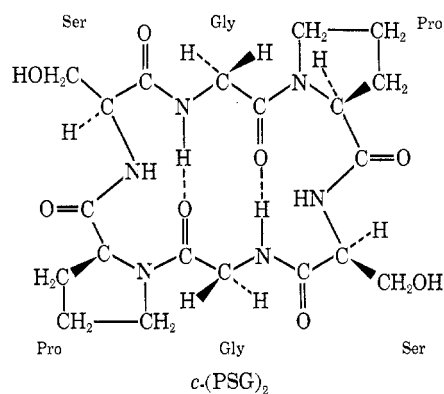
cyclic polypeptides and turn out to exhibit some surprising conformational behavior; the conclusions drawn by nmr are fully supported by energy calculations.[39] In Figure 1 are shown the spectra of *cyclo*-(Pro-Ser-Gly-Pro-Ser-Gly), abbreviated c-$(PSG)_2$, in D_2O and (inset) in H_2O.[40] The principal parameter provided by nmr is the value of the vicinal J coupling, $J_{N\alpha}$, of the α proton and the NH proton in each amino acid residue; this provides a measure of the rotation angle φ about the $-N-C_\alpha-$ bond[41,42] if the dependence of the coupling

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(30) H. A. Scheraga, *Chem. Rev.*, **71**, 195 (1971).

(31) We employ here the standard abbreviations for amino acid residues (see, for example, R. E. Dickerson and I. Geis, "The Structure and Action of Proteins," Harper and Rowe, New York, N. Y., 1969). The arrow indicates the conventional N—Cα—C=O sequence.

(32) R. Schwyzer, *Chimia*, **12**, 53 (1958); R. Schwyzer, *Rec. Chem. Progr.*, **20**, 147 (1959).

(33) A. M. Liquori, P. de Santis, A. L. Kovacs, and L. Mazzarella, *Nature (London)*, **211**, 1039 (1966).

(34) G. Vanderkooi, S. J. Leach, G. Nemethy, R. A. Scott, and H. A. Scheraga, *Biochemistry*, **5**, 2991 (1966).

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(38) P. J. Flory, "Statistical Mechanics of Chain Molecules," Interscience, New York, N. Y., 1969, Chapter VII.
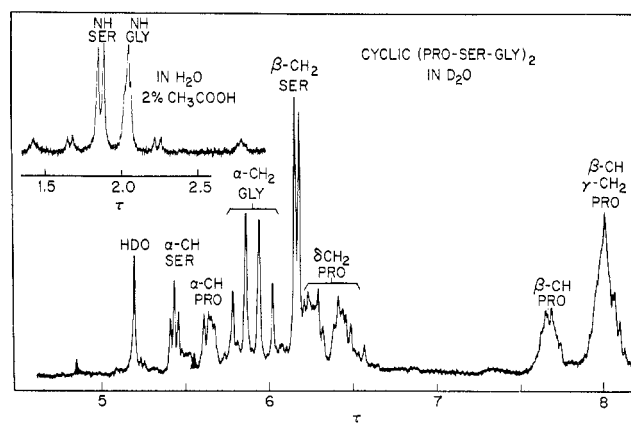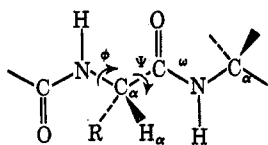
(39) A. E. Tonelli, *J. Amer. Chem. Soc.*, **94**, 346 (1972).

(40) D. A. Torchia, A. di Corato, S. C. K. Wong, C. M. Deber, and E. R. Blout, *ibid.*, **94**, 609 (1972).

(41) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. Ramachandran, and H. A. Scheraga, *Biopolymers*, **4**,

upon this angle is known or can be reasonably assumed. A simple Karplus relation[43,44] seems to be the most reasonable, with coefficients adjusted for the electronegativity of the substituents (eq 1). More elaborate

$$J_{N\alpha} = \begin{cases} 8.5 \cos^2 \varphi' & (0° \leq \varphi' \leq 90°) \\ 9.5 \cos^2 \varphi' & (90° \leq \varphi' \leq 180°) \end{cases} \quad (1)$$

relationships give essentially the same result.[45,46] $J_{N\alpha}$ is given most readily by the spacing of NH resonances (in H_2O), appearing as a "triplet" for the glycine residues and as a doublet for the serine residues. Proline residues, having no NH, do not give a resonance in this position, but this is not important as in this case φ is fixed at *ca.* 120° by the pyrrolidine ring.[47]
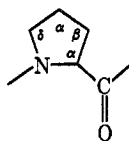
In D_2O, the NH protons are exchanged for deuterium and this coupling cannot be measured, but the α-proton spectrum is now simpler to analyze. The assignments of the glycine and serine α protons are shown in Figure 1; the former appear as an AB quartet in the asymmetric environment of the serine and proline residues; the latter is effectively a triplet, although actually the X part of an ABX system. The proline ring protons give a complex spectrum, the assignments being based on extensive previous study.[48-51]

In dimethyl-d_6 sulfoxide (DMSO-d_6) the spectrum

Figure 2. Temperature dependence of the Gly and Ser NH protons of *cyclo*-(Pro-Ser-Gly-Pro-Ser-Gly) in DMSO-d_6; 22° (see ref 40).

(not shown) of c-(PSG)$_2$ is essentially similar to Figure 1; in this solvent the NH protons are not exchanged and appear in the same relative positions as in Figure 1 (inset). In both H_2O and DMSO-d_6 the temperature dependence of the chemical shifts of the NH protons gives information concerning their participation in hydrogen bonds. Upfield shifts of the resonances of protons capable of forming hydrogen bonds are usually attributed to the breaking of an increasing fraction of such bonds with increasing temperature. This dependence should be small for intramolecular hydrogen bonds but substantial for those capable of forming only external hydrogen bonds to the solvent. In Figure 2, the glycine and serine NH peak positions are plotted *vs.* temperature. The results strongly suggest that the glycine peptide protons participate in internal hydrogen bonds whereas those of the serine residues are externally hydrogen bonded; the relatively greater deshielding of the latter probably reflects principally the strong hydrogen-bond-acceptor properties of DMSO.

The observation of a single spectrum for each pair of like residues in c-(PSG)$_2$ demonstrates C_2 symmetry. The evidence for glycine NH hydrogen bonding, together with the values of $J_{N\alpha}$ for the glycine (*ca.* 4.0 and 5.0 Hz) and serine (8.5 Hz) residues, is compatible only with the conformations shown in Figure 3 in both water and DMSO. In these structures, all peptide bonds are trans and the glycine and serine residues are in somewhat distorted antiparallel β-type conformations. That designated β_L has glycine angles appropriate for an L-residue β structure, while in the β_D conformation the glycine residues have angles appropriate to a D-residue β structure. Calculations[39] show these conformations to be of nearly equal energy, while the middle-range values of $J_{N\alpha}$ for both the glycine protons indicate an averaging of these couplings corresponding to rapid equilibration of the two forms. The two hydrogen bonds stabilizing this form involve glycine C=O and NH groups only.

The $\beta_D \rightleftarrows \beta_L$ conformations of c-(PSG)$_2$ are the prin-

121 (1966); *J. Biol. Chem.*, **241**, 1004 (1966); *J. Mol. Biol.*, **15**, 399 (1966).

(42) J. C. Kendrew, W. Klyne, S. Lifson, T. Miyazawa, G. Nemethy, D. C. Phillips, G. N. Ramachandran, and H. A. Scheraga, *Biochemistry*, **9**, 3471 (1970); *J. Biol. Chem.*, **245**, 489 (1970); *J. Mol. Biol.*, **52**, 1 (1970). We employ the 1966 convention in this discussion. The angles φ, ψ and ω are defined as 0° in the planar zigzag conformation shown and adopt positive values in the clockwise sense indicated by the arrows. Because of the planar nature of the peptide bond, ω must be either 0° (*trans*) or 180° (*cis*); this is discussed more fully in the text. For α-amino acid residues the trans conformation is strongly preferred, but for α-imino acid residues (*e.g.*, proline) the energy difference is much smaller and both cis and trans may occur.

(43) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959); **33**, 1842 (1960); *J. Amer. Chem. Soc.*, **95**, 2870 (1963).

(44) M. Barfield and M. Karplus, *ibid.*, **91**, 1 (1969).

(45) It should be noted that in equation (1), φ' is the *dihedral* angle, which is related to φ by

$$\varphi = \begin{cases} \varphi' + 240° & (240° \leq \varphi \leq 60°) \\ 240° - \varphi' & (60° \leq \varphi \leq 240°) \end{cases}$$

(46) V. F. Bystrov, S. L. Portnova, U. I. Tsetlin, V. T. Ivanov, and Yu. A. Ovchinnikov, *Tetrahedron*, **25**, 493 (1969); G. N. Ramachandran, R. Chandrasekaran, and K. D. Kopple, *Biopolymers*, **10**, 2113 (1971).

(47) Y. C. Leung and R. E. Marsh, *Acta Crystallogr.*, **11**, 17 (1958).

(48) C. M. Deber, F. A. Bovey, J. P. Carver, and E. R. Blout, *J. Amer. Chem. Soc.*, **92**, 6191 (1970).

(49) C. M. Deber, D. A. Torchia, and E. R. Blout, *ibid.*, **93**, 4893 (1971).

(50) D. A. Torchia and F. A. Bovey, *Macromolecules*, **4**, 246 (1971).

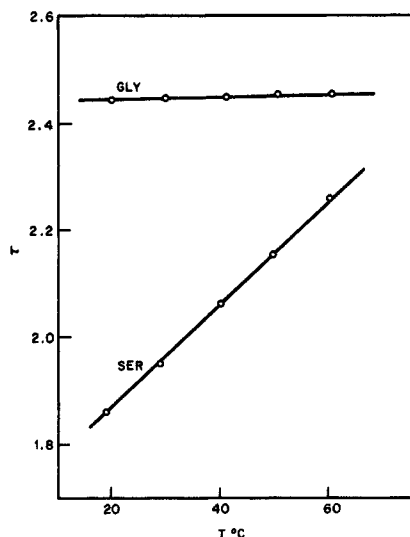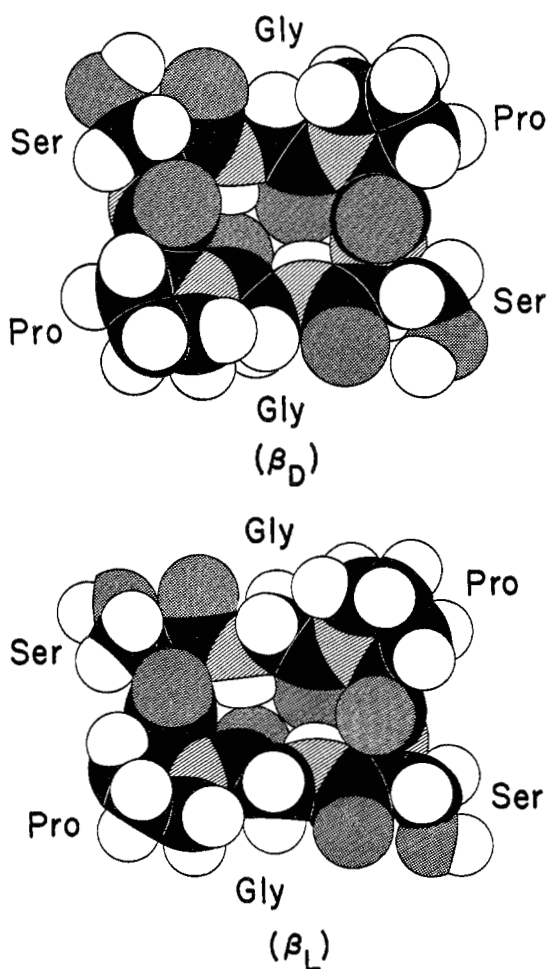(51) D. A. Torchia, *ibid.*, **4**, 440 (1971).

(β_D)

(β_L)

Figure 3. The β_D and β_L conformations of *cyclo*- (Pro-Ser-Gly-Pro-Ser-Gly). Angles: all $\omega = 0°$; β_D: $\psi_{Pro} = 290°$; $(\varphi, \psi)_{Ser} = 240°, 210°$; $(\varphi, \psi)_{Gly} = 340°, 180°$. β_L: $\psi_{Pro} = 280°$; $(\varphi, \psi)_{Ser} = 240°, 210°$; $(\varphi, \psi)_{Gly} = 20°, 350°$.

cipal but not the only ones present. The peptide proton spectrum (Figure 1, inset) shows minor resonances appearing as *equal pairs* of glycine NH triplets and serine NH doublets. (Related resonances can be seen upon close inspection of the α-CH region, *e.g.*, near τ 5.5). Variation of temperature and solvent does not alter the relative intensities of these resonances, but does alter somewhat their ratio to the principal spectrum. They therefore correspond to an *asymmetric* conformation, designated CT, with one proline residue now cis and the other trans (see ref 42), in equilibrium with the major symmetric conformation. Their temperature dependence is consistent with one intramolecular glycine NH hydrogen bond, the other glycine and both serine NH protons being exposed to solvent. A proposed structure for this conformation is shown in Figure 4. It can be separately observed because of the *ca.* 24-kcal barrier to rotation about the Gly–Pro peptide bond between cis and trans conformations.[48,50] Its observation is particularly significant because it tells us that structures which appear to have C_2 (or higher) symmetry from the structural formula alone need not actually have such symmetry.

The isomeric hexapeptide *cyclo*-(Ser-Pro-Gly-Ser-Pro-Gly), designated *c*-(SPG)$_2$, shows different confor-
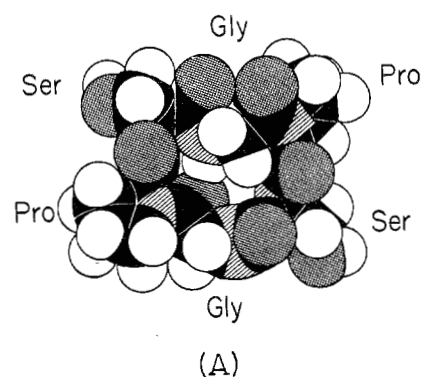

(A)

Figure 4. The CT conformation of *cyclo*-(Pro-Ser-Gly-Pro-Ser-Gly). Angles: Gly (NH H-bonded): $\omega = 180°$; all other $\omega = 0°$.

mational behavior from *c*-(PSG)$_2$. In H$_2$O, the nmr spectrum indicates a conformation similar to the β_L
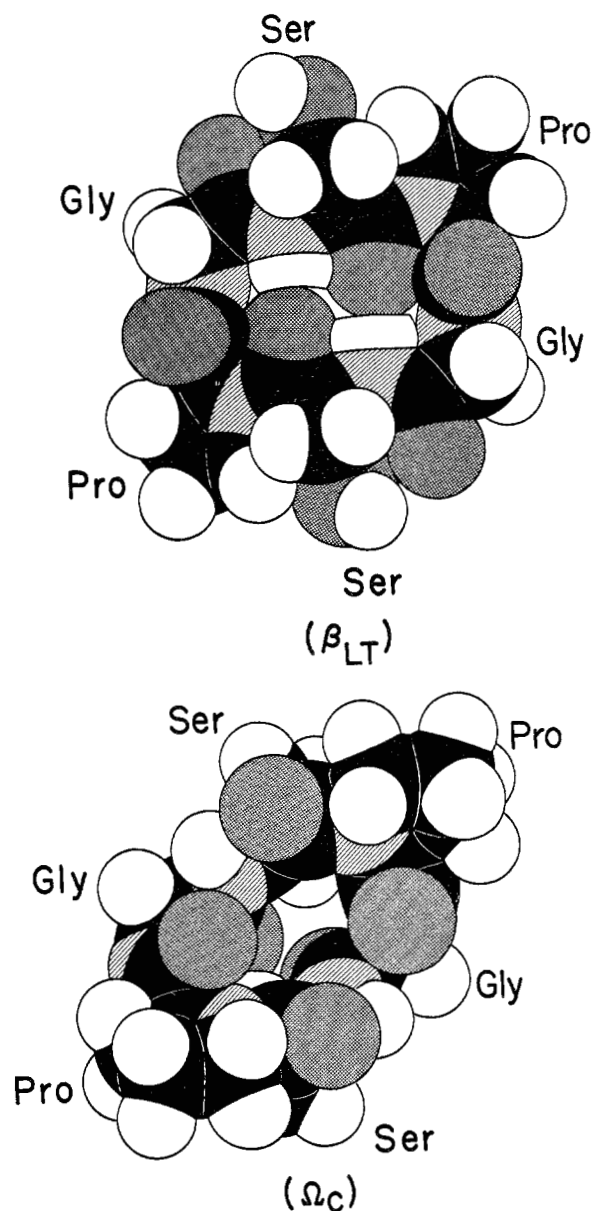

(β_{LT})

(Ω_C)

Figure 5. The β_{LT} and Ω_C conformations of *cyclo*-(Ser-Pro-Gly-Ser-Pro-Gly). Angles for β_{LT}: all $\omega = 0°$; $(\varphi, \psi)_{Ser} = 30°, 330°$; Pro $\psi = 300°$; $(\varphi, \psi)_{Gly} = 290°, 150°$. Angles for Ω_C: $(\varphi, \psi, \omega)_{Ser} = 25°, 325°, 180°$; $(\psi, \omega)_{Pro} = 300°, 0°$; $(\varphi, \psi, \omega)_{Gly} = 240°, 90°, 0°$.
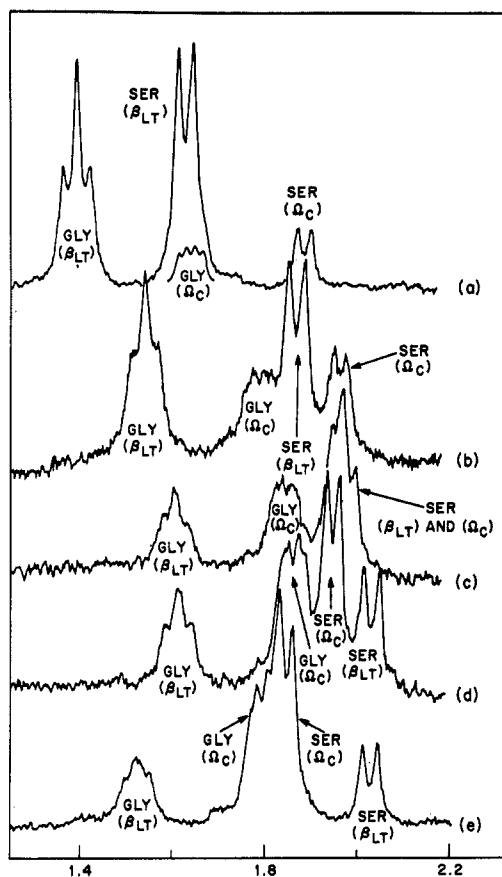
Figure 6. The 220-MHz nmr spectrum of the NH protons of cyclo-(Ser-Pro-Gly-Ser-Pro-Gly): (a) in H_2O–CH_3COOH, 98:2 (v/v); (b, c, and d) in DMSO-d_6: H_2O–CH_3COOH (98:2); (e) in DMSO-d_6. In (a) through (e), the mole fraction of DMSO-d_6 is 0.0, 0.5, 0.7, 0.8, and 1.0. Each spectrum is the result of 15–30 accumulated CAT scans (see ref 52).

Figure 7. The temperature dependence of the chemical shifts of the NH resonances of cyclo-(Ser-Pro-Gly-Ser-Pro-Gly) in (a) H_2O–CH_3COOH, 98:2 (v/v); (b) in DMSO-d_6 (see ref 52).

structure of the latter.[52] The β_D structure is excluded as its energy is substantially higher. The serine and glycine residues have now reversed roles, the serine NH being internally hydrogen bonded, as shown by its small temperature dependence (Figure 7), whereas the glycine NH protons are exposed to solvent. This conformer is designated β_{LT} (Figure 5). A minor fraction of another symmetrical conformer can be detected in the spectrum. When the compound is examined in DMSO-d_6 solution it is found that this becomes the major conformation. Figure 6 shows the appearance of the NH spectrum as a function of solvent composition. The NH temperature dependence demonstrates that this second form has no internal hydrogen bonds (Figure 7); this observation and the $J_{N\alpha}$ values are consistent with a conformation, designated Ω_C, in which both proline residues are cis (Figure 5). It is strongly folded rather than planar like β_{LT}; the reason for its presence may be the formation of external serine NH hydrogen bonds to the DMSO, but no such simple explanation is very satisfactory in view of the fact that the symmetric and asymmetric conformers of c-$(PSG)_2$ have about the same ratio in both DMSO and H_2O. In any event, energy calculations are at present unable to deal with
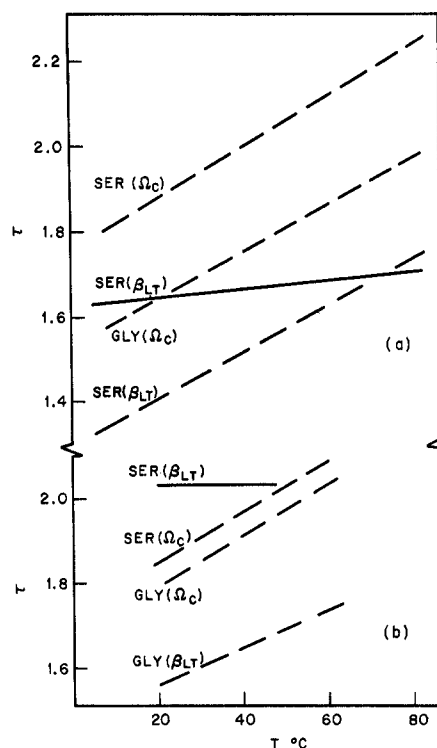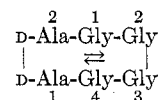
solvent interactions, and so even when dramatic changes such as those of c-$(SPG)_2$ occur, the most that can be concluded is that both the β_{LT} and the Ω_C conformations represent low-energy forms.[39]

cyclo-(Gly-Gly-D-Ala-D-Ala-Gly-Gly)

The structure of this hexapeptide seems at first sight to be simpler than that of the hexapeptides just considered, but the spectrum is in fact more complex because of the lack of symmetry. In addition, the ab-

$$
\begin{array}{c}
\overset{2}{\text{D-Ala}}\text{-}\overset{1}{\text{Gly}}\text{-}\overset{2}{\text{Gly}} \\
| \quad\rightleftarrows\quad | \\
\text{D-Ala}\text{-}\text{Gly}\text{-}\text{Gly} \\
1 \quad 4 \quad 3
\end{array}
$$

sence of proline or any bulky side chains suggests that there will be a substantial number of low-energy conformations between which equilibration will be rapid. One expects (and observes) separate spectra for each of the six residues, the interpretation of which is made somewhat ambiguous by the fact that each represents an average over several conformations, and also by the difficulty of specifically assigning each residue to its place in the sequence. Nevertheless, it is of particular interest to interpret the spectrum of this polypeptide in terms of its solution conformation because its conformation in the crystal is known.[53] Calculation[54] shows that the latter has a very high intramolecular energy, which, however, is more than counterbalanced by the presence of nine external hydrogen bonds to

(52) D. A. Torchia, S. C. K. Wong, C. M. Deber, and E. R. Blout, J. Amer. Chem. Soc., 94, 616 (1972).

(53) I. L. Karle, J. W. Gibson, and J. Karle, ibid., 92, 3755 (1970).
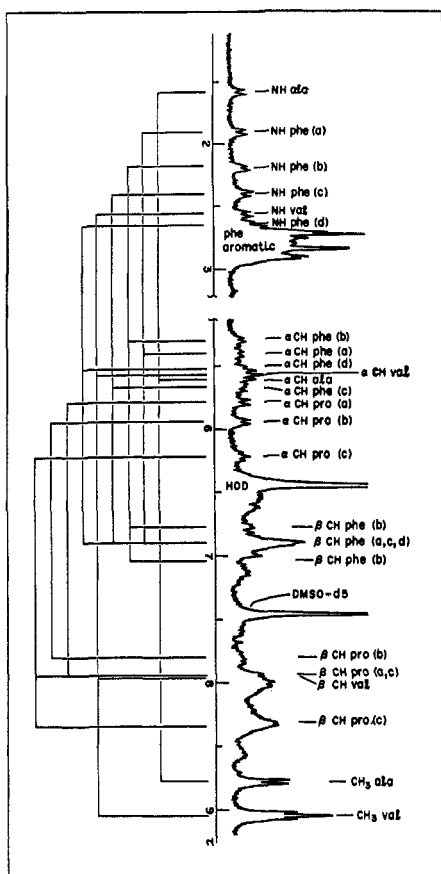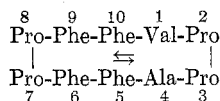(54) A. E. Tonelli and A. I. Brewster, J. Amer. Chem. Soc., in press.

Figure 8. The 220-MHz spectrum of antamanide in dioxane (5% w/v; 23°). The lines below the spectrum connect multiplets shown by double resonance to be coupled (see ref 56 and D. J. Patel, unpublished observations.)

three H_2O molecules of crystallization (per peptide molecule), one intermolecular $NH\cdots O{=}C$ hydrogen bond, and two Gly_1-Gly_4 intramolecular hydrogen bonds. There are 25 low-energy solution conformations consistent with the observed values of $J_{N\alpha}$, all of which have energies about 15 ± 1 kcal less than that of the crystal conformation. Eight of these are calculated to have one $(NH)_{Gly_3}\cdots(O{=}C)_{Ala_2}$ hydrogen bond. It is observed that, while all the glycine residues exchange peptide NH protons rapidly, one of them has a temperature coefficient of chemical shift about one-third as great as the others. This may be Gly_3. It appears that no single conformation describes this hexapeptide in solution, but rather that it is, within definable limits, a flexible molecule.

Antamanide

The all-L decapeptide antamanide was isolated from the mushroom *Amanita phalloides* by Wieland, *et al.*[55] It acts as an antidote for the toxins amanitin and phalloidin (see below) secreted by this species. The amino acid sequence is

```
        8    9   10    1    2
      Pro-Phe-Phe-Val-Pro
        |          ⇆         |
      Pro-Phe-Phe-Ala-Pro
        7    6    5    4    3
```
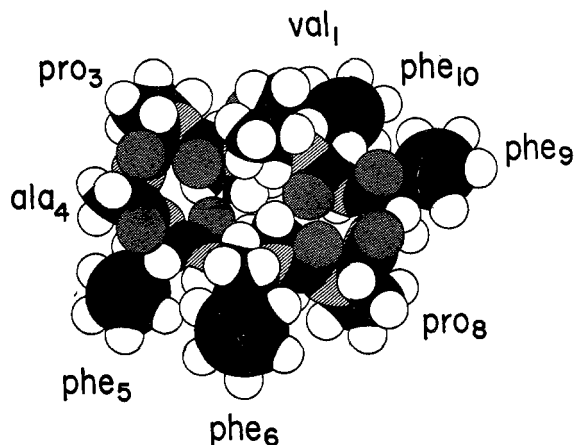

Figure 9. The conformation of antamanide. The Pro_2 and Pro_7 residues project to the rear and are largely obscured. Angles: $\varphi_{Pro_2} = \varphi_{Pro_7} = 102°$; $\psi_{Pro_2} = \psi_{Pro_7} = 310°$; $\varphi_{Pro_3} = \varphi_{Pro_8} = 122°$; $\psi_{Pro_3} = \psi_{Pro_8} = 125°$; $(\varphi, \psi)_{Phe_9} = (\varphi, \psi)_{Ala_4} = 90°, 120°$; $(\varphi, \psi)_{Phe_{10}} = (\varphi, \psi)_{Phe_5} = 90°, 330°$; $(\varphi, \psi)_{Val_1} = 90°, 270°$; $(\varphi, \psi)_{Phe_6} = 90°, 300°$.

Although most biologically active polypeptides contain at least one proline (or hydroxyproline) residue, the proportion of this residue, as well as that of phenylalanine, in antamanide is probably unique.

In Figure 8 is shown the spectrum of antamanide in dioxane.[56] We do not propose to discuss it in detail but show it as illustrative of the spectra of the more complex polypeptides which lack symmetry. The main parameters sought here are the values of $J_{N\alpha}$, each assigned if possible to a specific residue in the sequence. An essential procedure is the association of the NH resonances (at τ 1.5–3.0) with their corresponding α protons, (τ 5.0–6.2) and the association of these with the side-chain β protons at higher field, and so on. This is done by double resonance. The irradiation of an α-CH multiplet causes the associated NH doublet to collapse to a singlet; the assignment may be confirmed by reversing the procedure and observing the resulting perturbation of the α region of the spectrum. This operation is then extended to the β, γ, etc., protons of the side chain. The lines in Figure 8 connect multiplets corresponding to coupled groups of spins. From the form of the α-CH and side-chain multiplets the residues can be assigned by type but not to specific sequence position. All the $J_{N\alpha}$ had relatively large values of 6.0–8.5 Hz; 137 all-trans cyclic conformations were generated which were consistent with this information. Of these, one was conspicuously lower in energy than the rest, and is shown in Figure 9. This structure has no internal hydrogen bonds.

Antamanide is known to complex Na^+ quite strongly[8,57] (stability constant *ca.* 2500), more strongly in fact than K^+, behavior which is unique among ionophoric polypeptides and depsipeptides. Ivanov, *et al.*,[8] have suggested a folding of the structure to form a cavity in which six carbonyl groups can bond strongly

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(56) A. E. Tonelli, D. J. Patel, M. Goodman, F. Naider, H. Faulstich, and T. Wieland, *Biochemistry*, **10**, 3211 (1971).

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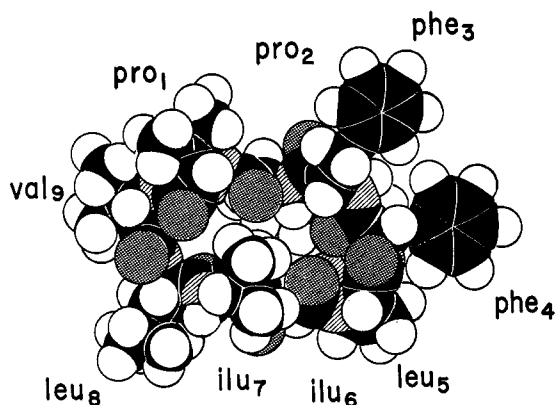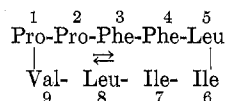
Figure 10. The conformation of cyclolinopeptide A. Angles: all ω = 0°; $(\varphi, \psi)_{Pro_1}$ = 102°, 310°; $(\varphi, \psi)_{Pro_2}$ = 122°, 125°; $(\varphi, \psi)_{Phe_3}$ = 90°, 330°; $(\varphi, \psi)_{Phe_4}$ = 100°, 270°; $(\varphi, \psi)_{Leu_5}$ = 90°, 0°; $(\varphi, \psi)_{Ile_6}$ = 90°, 120°; $(\varphi, \psi)_{Ile_7}$ = 90°, 120°; $(\varphi, \psi)_{Leu_8}$ = 90°, 330°; $(\varphi, \psi)_{Val_9}$ = 100°, 310°.

to Na$^+$. This appears, however, to be a very high energy conformation (probably at least 50 kcal above that of the uncomplexed molecule) and implies an improbably high energy of interaction of the carbonyl groups with the cation. The sodium complex is currently under study in this laboratory.

Cyclolinopeptide A

The nonapeptide designated cyclolinopeptide A was synthesized with the object of imitating the action of antamanide and learning the structural requirements of this action.[58] It has the sequence

$$
\begin{array}{ccccc}
1 & 2 & 3 & 4 & 5 \\
\text{Pro-} & \text{Pro-} & \text{Phe-} & \text{Phe-} & \text{Leu} \\
| & & \rightleftarrows & & | \\
\text{Val-} & \text{Leu-} & \text{Ile-} & \text{Ile} \\
9 & 8 & 7 & 6
\end{array}
$$

It thus resembles antamanide in size and in having a Pro-Pro-Phe-Phe sequence, but it is not at present known whether it has antidote activity. Because it has fewer like residues than antamanide, it is possible to assign the resonances and therefore the values of $J_{N\alpha}$ for each residue (other than Pro) by correlation of nmr observations and calculated conformational restrictions.[59,60]

The conclusion drawn from this study is that cyclolinopeptide A probably exists in four closely related conformations, the relative populations of which change with solvent and temperature. This conclusion appears to be consistent with CD measurements.[61] The predominant conformer is believed to be that shown in Figure 10. In this structure there are no internal hydrogen bonds. Substantial positive temperature coefficients are observed for the chemical shifts of all the peptide NH resonances except that of Phe$_3$, which is exceptional in showing a negative slope. The calculated structures (including that in Figure 10) have the
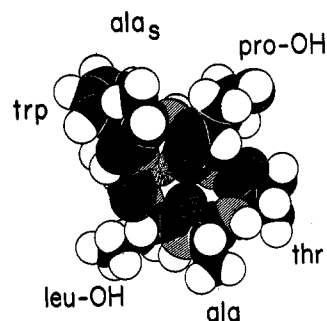

Figure 11. The conformation of phalloidin. Angles: all ω = 0°; $(\varphi, \psi)_{Ala_5}$ = 100°, 120°; $(\varphi, \psi)_{Trp}$ = 60°, 120°; $(\varphi, \psi)_{Leu(OH)}$ = 110°, 0°; $(\varphi, \psi)_{Ala}$ = 100°, 330°; $(\varphi, \psi)_{Thr(OH)}$ = 270°, 30°; $(\varphi, \psi)_{Cys}$ = 100°, 300°; $(\varphi, \psi)_{Hyp}$ = 122°, 125°.

NH of Phe$_3$ buried in the interior of the highly hindered structure. It becomes more buried as the temperature is raised, i.e., as the populations of the higher energy structures are increased. This NH also exhibits slower deuterium exchange than the others.

Phalloidin

As we have seen, phalloidin is one of the toxins of the mushroom *Amanita phalloides* (amanitin has a similar structure). It was isolated by Lynen and Wieland[62] and its covalent structure was shown by T. Wieland[63,64] to be
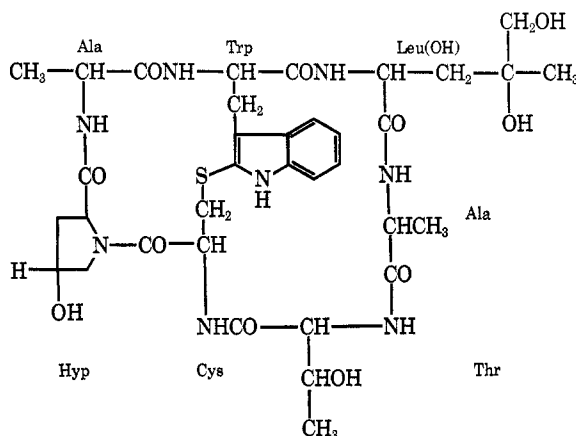
All the residues are L except threonine. It is exceptional among the structures reported here in being bicyclic. The 220-MHz spectrum shows the same general pattern as that of the other complex polypeptides discussed.[65] By suitable minor variation of solvent, all six NH resonances can be discriminated and values of $J_{N\alpha}$ measured. Two features of the spectrum deserve mention: (a) the tryptophan peptide NH shows a markedly smaller rate of deuterium exchange, which is fast for the rest; (b) one of the two alanine methyl groups is strongly shielded, by ca. 0.8 ppm, compared to the other and to the normal alanine methyl group in peptides. Energy calculations[65] appropriate to this bicyclic structure provide a very plausible ex-

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(65) D. J. Patel, A. E. Tonelli, P. Pfaender, H. Faulstich, and T. Wieland, *J. Mol. Biol.,* in press.

planation for both of these observations. The lowest energy structure (Figure 11), as well as six others of very similar conformation and only slightly higher calculated energy, all show the tryptophan NH to be internally buried and the methyl group of the alanine residue marked Ala₈ in Figure 11 to be in the shielding region of the tryptophan ring. It appears that phalloidin is a relatively rigid molecule in solution.

Conclusion

A number of other cyclic polypeptides are currently under study in our laboratory in collaboration with other groups. Complete conformational structures are not yet available for these molecules, which include the neurohypophyseal hormones oxytocin[66,67] and lysine-vasopressin[68,69] and related precursors and partial structures.

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Chemical Applications of Optical Pumping

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The chemist is a guest at the physicist's table, and he frequently dines well, *viz.*, X-rays, nuclear magnetism, induced emission, recoilless γ-rays, ... The optical pumping phenomenon, that is, the orientation of atomic electronic spins through the use of circularly polarized light, may, by analogy, also be interesting to chemists: optical pumping is a method for studying a special class of free radicals. Free radicals have been studied by an array of techniques, *e.g.*, their catalysis of the conversion of ortho- to para-hydrogen (1920's), removal of a metallic mirror (1930's), and electron spin resonance. The latter method is so powerful that one may wonder what else could possibly be added. The answer is that esr is of limited sensitivity. For example, chemically induced nuclear spin polarization is one way of measuring the effects of radicals which live for only 10^{-9} sec. By optical pumping the hyperfine structure of special radicals can be measured with greater precision and the radical can be detected at much lower concentrations than with esr.

Optical pumpers (scientists who optically pump) measure two types of properties—those of the excited states of atoms and molecules inferred from their fluorescence, and the properties of the ground state. We want to concentrate here on the generation, stability, and usefulness of a ground-state ensemble of spin-oriented atoms. A gas-phase atom is characterized by a total angular momentum $\vec{J}$, the sum of the electronic orbital and spin angular moments and a quantized component, J_z. The spin angular momentum is in general reoriented only by magnetic fields, but the orbital an-

gular momentum is easily reoriented by the electric fields which act during a collision. To prevent destruction of the spin orientation within a few collisions the ground state of the atom must have $J = {}^1/_2$ (groups Ia, Ib, and IIIa of the periodic table) or if $J > {}^1/_2$, L must be 0 (groups V, VIIb, and VIb except for W). For experimental convenience most work has been done with the alkali atoms which have ${}^2S_{1/2}$ ground states.

Generation of a Gas of Spin-Oriented Atoms

A particular example will suffice to show what optical pumping is. Consider a hypothetical atom with zero nuclear spin and a single valence electron. Its energy levels in a small magnetic field are shown in Figure 1. Let us apply a beam of right circularly polarized light in the same direction (z) as a magnetic field; the wavelength of the light is chosen so that it excites only the ${}^2P_{1/2}$ state. The light wave carries angular momentum in the z direction, and when it is absorbed the angular momentum of the atom must increase, *i.e.*, $\Delta M = +1$ where M is the magnetic quantum number. Only the $-{}^1/_2 \rightarrow {}^1/_2$ transition is allowed. The excited $M = {}^1/_2$ state can undergo radiative or nonradiative (collisional quenching) transitions to the ground state. If the atom returns to the ground state $M = -{}^1/_2$ state nothing is gained or lost. If it goes to the ground state $M = {}^1/_2$ state, it will remain there for some time until spin relaxation takes place. This results in a large excess of atoms in the $M = {}^1/_2$ state. In favorable cases the net spin orientation of the gas can exceed 90%. As an atom in the $M = {}^1/_2$ state cannot absorb the right circularly polarized light, the spin orientation can be inferred from optical absorption experiments. One does not need to perform electron spin resonance to determine the spin orientation.

Richard Bersohn was trained as a theorist at Harvard University (Ph.D., 1950). His interest in optical pumping was first generated by spending part of a sabbatical leave from Cornell at the laboratories of Professors Brossel and Kastler in Paris. His identical twin brother, Malcolm Bersohn, is also a chemistry professor (at Toronto).