provides an entire spectrum of possibilities for modes of reaction which should be immensely useful in probing the details of the active sites of enzymes.

Acknowledgment. The authors gratefully acknowledge Dr. W. D. Phillips of the E. I. du Pont de Nemours and Co., and Professor Melvin Calvin and Dr. George Kotowycz of the Laboratory of Chemical Biodynamics, the University of California, Berkeley, for the use of, and expert assistance with, their 220-MHz nmr spectrometers. We are indebted to the National Institutes of Health and the National Science Foundation for grants which partially supported this investigation.

(11) On sabbatical leave from Brooklyn College, Brooklyn, N. Y.

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The Effect of Association on the Nuclear Magnetic Resonance Spectra of Tyrocidine B[1]

Sir:

The tyrocidines are cyclic antibiotic decapeptides of known amino acid sequence which are excellent models[2-8] for the study of many concepts which are currently much discussed in protein chemistry. These concepts are based on interpretations from physical measurements such as viscosity, diffusion, sedimentation, rotatory dispersion, circular dichroism, nuclear magnetic resonance, ultraviolet and infrared spectroscopy, etc. It seems possible to develop a more definitive understanding of the interacting forces which determine the shape (conformation), state of aggregation, solubility, etc., of polypeptides and proteins.

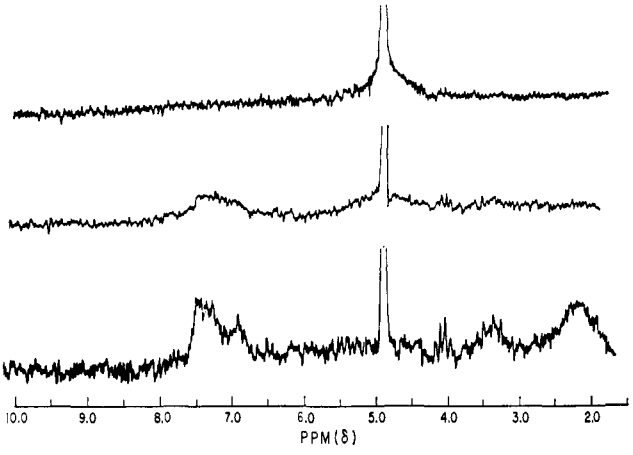

Figure 1. Effect of concentration on 100-MHz nmr spectrum of tyrocidine B in D_2O at 60°. Top pattern = 6%, middle pattern = 2%, bottom pattern = 1%.

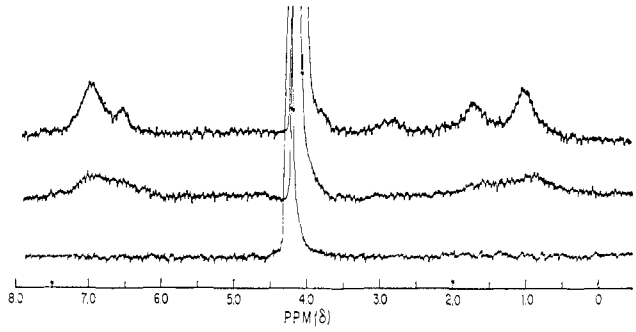

Figure 2. Effect of changing the temperature on the 60-MHz nmr spectrum of 8% tyrocidine B in D_2O. Top pattern = 100°, middle pattern = 80°, lower pattern = 45°.

Tyrocidines A, B, and C all show a strong tendency to aggregate.[3,9] In contrast, gramicidin S-A under similar conditions exhibits little tendency to associate. Nuclear magnetic resonance has been used to establish the conformation of gramicidin S-A in solution,[10] *viz.*, all the Ψ, ϕ, and ω angles were estimated; six of the amino acid residues were involved in an antiparallel β-pleated sheet with four intramolecular hydrogen bonds, and the molecule was unequivocally shown to have a C_2 axis of symmetry perpendicular to the molecular plane. This model agrees in many details with earlier formulations by other workers[11,12] and with proton exchange studies.[10,13]

Since nuclear magnetic resonance can provide much information about structure and interaction of molecules, it became of interest to apply this technique to a study of the self-association and structure of the tyrocidines. This communication describes a preliminary investigation by nmr of the phenomenon of aggregation of tyrocidine B.

When a 6 % (w/w) solution of tyrocidine B in heavy water was examined by high-resolution nmr at ambient temperature no signal was observed (Figure 1). A spectrum could be detected and resolution improved by lowering the concentration of tyrocidine B or by raising the temperature (Figures 1 and 2). The latter phenomenon was reversible. Addition of increasing proportions of methanol also gave nmr spectra of increasing resolution (Figure 3).

These phenomena are consistent with the known aggregation of the tyrocidines in water solution. The high molecular weight aggregates so formed have poorly resolved nmr spectra due to the well-known phenomenon of dipolar line broadening.[14,15] Dissociation of the aggregates by lowering the tyrocidine concentration, increasing the temperature, or adding so-called denaturing solvents gives smaller "polymers" and monomers in which freer rotation results in

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Table I. 50% Escape Times[a] (Minutes) of Tyrocidine B

	1%[b]	0.4%[b]	0.2%[b]
0.1 *N* acetic acid		(4°) 117	
		(15°) 153	
	(25°) 220	(25°) 155	(25°) 83
	(40°) 182	(40°) 128	(40°) 71
0.1 *N* acetic acid–20 methanol		(25°) 103	
0.1 *N* acetic acid–20% ethanol	(25°) 51	(25°) 33	(25°) 28
		(40°) 14	
0.1 *N* acetic acid–20% propanol		(25°) 28	

[a] All values corrected for temperature and viscosity to 25°. [b] Initial concentration of tyrocidine B in retentate.

reduced dipolar broadening and better resolved spectra. In fact, pure methanol or dimethyl sulfoxide gives nmr spectra sufficiently resolved possibly to perform a full spectral analysis.

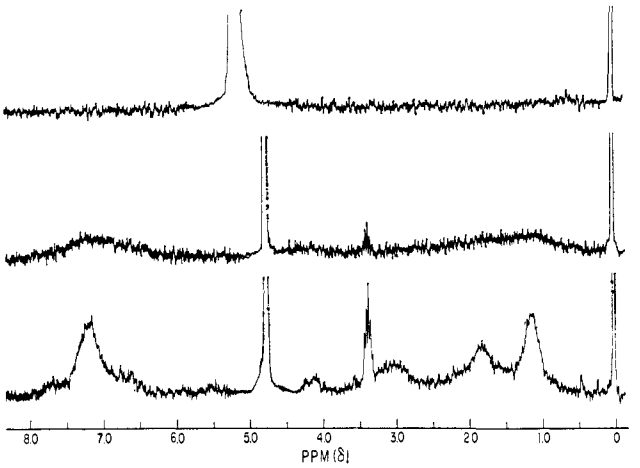

Figure 3. Effect of methanol on 60-MHz nmr pattern of 8% tyrocidine B at 25°. Top = 100% D_2O, middle = 80% D_2O–20% CD_3OD, bottom = 50% D_2O–50% CD_3OD.

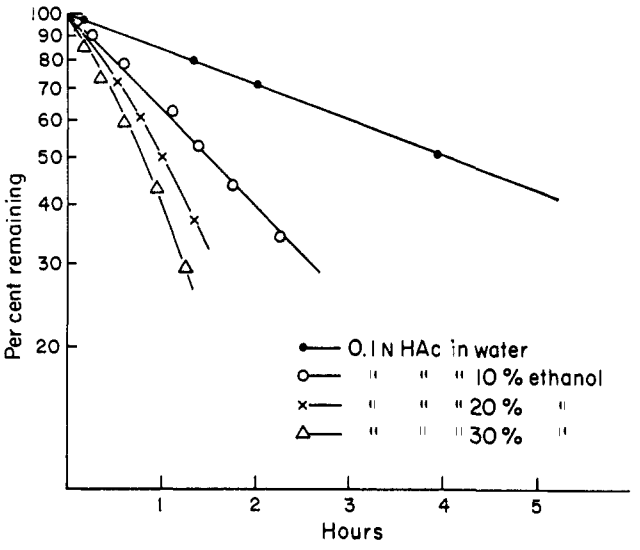

Figure 4. Thin-film dialysis escape patterns of tyrocidine B in various solutions.

The association behavior of tyrocidine B is clearly shown by the thin film dialysis technique.[16] In 50% aqueous 0.1 *N* acetic acid–methanol tyrocidine B dialyzed at a rate near that expected for a peptide in the 1200 molecular weight range. Ethanol was found to have a similar effect but at a lower concentration, as shown in Figure 4 and Table I. The reverse curvature characteristic[16] of a dissociating aggregate is seen in the escape curves with the ethanol solutions.

A comparison of high-resolution infrared spectra of several complex peptides in the fingerprint region (1000–400 cm^{-1}) reveals that the spectra of tyrocidine B and gramicidin S-A are very much alike, and also different from those spectra of the other complex cyclic peptides (Figure 5). This suggests that the conformations of tyrocidine B and gramicidin S-A are *similar*.

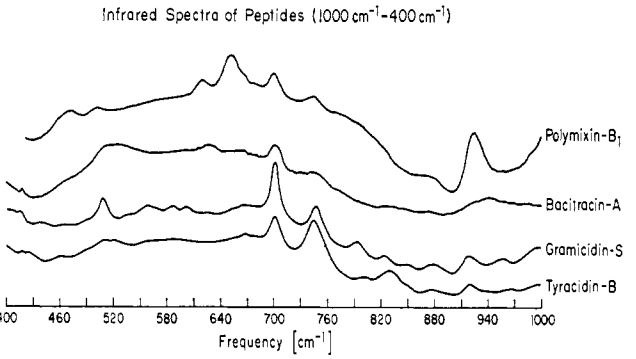

Figure 5. Infrared spectra of cyclic polypeptides, KBr disk.

Information concerning the role of the side chains in aggregation has come from chemical studies.[17] It has been found that the intact ring is required since splitting the peptide ring at the Phe–Pro bond removed the association tendency. Aromatic side chains are not required since complete hydrogenation of all the aromatic residues did not reduce the association tendency. Methylation of the tyrosyl hydroxyl, succinylation of the single basic group on the ornithine residue, and substitution of methoxyl for an amide on the Asn or Gln residues failed to eliminate the association property. It therefore was postulated that the major driving force to produce aggregation was hydrophobic interaction of some kind, perhaps strengthened by hydrogen bonding and the rigidity of the molecule.[17]

From all the experimental data available thus far it would appear that the association tendency arises chiefly from interactions between the side chains but requires the rigid backbone structure of the ring to

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maintain a definite stereochemical relationship between the side chains of the interacting monomers. The tyrocidines constitute an excellent example in which the line broadening in the nmr spectra is clearly shown to result from self-aggregation. In proteins which have both tertiary and quaternary structure this distinction cannot be made as clearly.

A more rigorous analysis of the effect of aggregation on line widths and other nmr spectral parameters is now being carried out. Hopefully these may shed further light on the actual mechanism of aggregation and on the role played by the various side chains.

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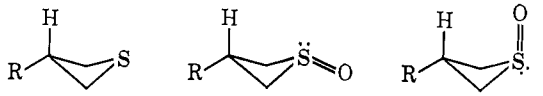
Equilibration of 3-Substituted Thietane 1-Oxides[1,2]

Sir:

The significant stereochemical consequences of lone electron pairs on heteroatoms have come under scrutiny only in recent times. The sulfoxide group, which retains its stereochemical integrity at room temperature, provides an intriguing example for studies of the competitive conformational requirements of an oxygen *vs.* an electron pair. The axial preference exhibited by sulfinyl oxygen in six-membered rings is now well established.[3-5] We report here our observation that sulfinyl oxygen in a four-membered ring exerts a pseudoequatorial preference.

Stereochemistry was assigned to *cis*- and *trans*-3-*p*-chlorophenylthietane 1-oxide,[6] **1a** and **1b**, from their dipole moments.[7a] The dipole moments of **1a** and **1b** in benzene solution were found to be 3.22 ± 0.09 and 2.80 ± 0.03 D, compared with calculated values of 2.99 and 2.47 D, respectively. The theoretical moments were obtained from values of 1.95 D for the



1, R = *p*-ClC_6H_4
2, R = $(CH_3)_3C$

1a **2a** **1b** **2b**

p-chlorophenyl[7b] and 4.19 D for the sulfinyl[8] moieties and a 37° angle of ring puckering.[9]

The stereochemical assignment was extended to the 3-*t*-butylthietane 1-oxide system by observing the deshielding effect of the S=O bond on the β-hydrogen when they are *cis* (*i.e.*, *trans* sulfoxide). First observed for cyclic sulfites,[10] the deshielding effect of the S=O bond has been utilized for the assignment of stereochemistry to five- and six-membered ring sulfoxides.[11-13] The pronounced deshielding of a β-hydrogen which is *syn* and axial to the S=O bond, as in certain six-membered ring systems, has been attributed to a proximity effect[12a,14] and/or acetylenic type anisotropy of the S=O bond.[12] The observed chemical shift difference of the β-hydrogen between *cis*- and *trans*-3-*p*-chlorophenylthietane 1-oxide, **1a** and **1b**, was 65 Hz and the analogous difference for the *t*-butyl system, **2a** and **2b**, was 68 Hz. In the *trans* isomers of these puckered[9,15] thietane oxides, **1b** and **2b**, the S=O bond and β-hydrogen may approach a *syn*-axial relationship.

The isomeric sulfoxides were separated by elution chromatography over silica gel; *cis* isomers eluted prior

Table I. Equilibration of 3-Substituted Thietane 1-Oxides

Compounds	Method (°C)	*cis*/*trans* composition: Starting material	*cis*/*trans* composition: Equilibrium	Method of analysis
2a + **2b**	HCl–dioxane (25)	15/25	85/15	Vpc
2a + **2b**	HCl–benzene (25)	55/45	86/14	Vpc
1a + **1b**	HCl–dioxane (25)	Predominantly *trans*	Predominantly *cis*	Nmr
1a-d_4 + **1b**-d_4	HCl–dioxane (25)	46/54	87/13	Nmr
2a + **2b**	Decalin (170–175)	63/37	82/18	Vpc
2a + **2b**	Decalin (170–175)	35/65	85/15	Vpc
2	N_2O_4 (0)	Sulfide	82/18	Vpc

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