# A Study of Proton Relaxation Mechanisms, Stereochemistry, and Dynamics of the Decapeptide Tyrocidine A

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**Abstract:** Correlation times and interproton distances for the backbone of the peptide tyrocidine A, an analogue of gramicidin S, have been determined from proton spin-lattice relaxation rate measurements in the monoselective, $R^i(\tilde{i})$, biselective, $R^i(\tilde{i},\tilde{j})$, and nonselective, $R^i(\mathrm{NS})$, modes. The values obtained agree with those determined from NOE measurements. These relaxation parameters were also calculated for some protons from the correlation time and the interproton distances of the tyrocidine A molecule assuming the latter to contain $\beta$I-turn, $\beta$II′-turn, and antiparallel $\beta$-pleated sheet moieties. In every case the calculated relaxation parameters, $R^i(\tilde{i})$ and $R^i(\mathrm{NS})$, agreed with the experimental values.

## Introduction

Although proton relaxation studies of peptides have been reported,[1] quantitation in terms of distance, conformation, and correlation times was unsatisfactory due to neglect of cross-relaxation effects.[2] The latter have been treated for small molecules[3-5] and evaluated in amino acids[3,6] and peptides[7-9] from a combination of NOEs and monoselective spin-lattice relaxation times. Cross-relaxation effects in proteins have been recognized but not evaluated quantitatively.[10-12]

The cross-relaxation rates, $\sigma_{ij}$, have been measured for gramicidin S[7-9] and tyrocidine A[13] via the NOE and used to estimate their interproton distances and correlation times. Here we report a proton relaxation study of tyrocidine A; the $r_\phi$, $r_\psi$, and transannular interproton distances were evaluated and are consistent with the type I$\beta$-turn, type II′$\beta$-turn, and antiparallel $\beta$-pleated sheet conformation[13-15] previously reported. This confirms the conclusion regarding conformation in the NOE study,[13] demonstrates the additivity of spin-lattice relaxation parameters, and proves that all relaxation rates are dominated by dipolar mechanisms principally involving protons but also $^{14}$N nuclei.

## Experimental Section

The tyrocidine A was obtained from Professor Lyman C. Craig and had been purified by counter current distribution. The samples were prepared by dissolving 4.5 mg of tyrocidine A in 1 mL of 100% $Me_2SO$-$d_6$ and were thoroughly deoxygenated. The NMR spectra were taken on a Bruker WH 270 spectrometer equipped with a Nicolet 1180 computer. $(T\text{-}180°\text{-}\tau\text{-}90°\text{-})_n$ pulse sequences were used in the $T_1$ experiments. The nonselective 180° pulse was typically 20 $\mu$s, and 200 FIDs were accumulated; the selective 180° pulse, provided by the decoupler channel, was typically 10 ms and 64 FIDs were accumulated. In the biselective experiments the decoupler frequency was modulated to produce two sidebands which simultaneously inverted the two proton resonances to be studied. The relaxation rates were determined from semilogarithmic plots of $\log(I_0 - I_z)$ vs. delay time $\tau$. Typically, $\tau$ values of up to 200 ms were used. Examples of spectra used to determine monoselective, biselective, and nonselective proton spin-lattice relaxation rates are shown in Figures 1, 2, and 3, respectively. The temperature was kept at 26 ± 1 °C by the Bruker temperature control unit and was calibrated with the methanol and ethylene glycol standard samples.

## Error Analysis

**1. Reproducibility.** Some of the selective relaxation rates have been determined from up to four different experiments, showing a total spread of less than 10%. This indicates that a single measurement can be reproduced to within ±5% of the mean value.

**2. Systematic Errors.** Systematic errors due to imperfect rf pulses and nonresonance conditions have been discussed[16] and are here assumed to be unimportant; furthermore, if they are always the same, they will cancel in the calculation of cross-relaxation rates and distances.

A more serious source of error may be introduced in the determination of the relaxation rates from the semilogarithmic plots, which assumes that the initial slope approximation is valid for the whole range of $\tau$ values used. When there is cross relaxation among the protons relaxing each other the recovery of the magnetization after the 180° pulse is never purely exponential in the selective experiment and, in the nonselective experiment, the recovery is exponential only when the relaxation rates are the same for all protons relaxing the studied one.[2] We have performed some test calculations, where cross-relaxation effects were taken into account for AX and $AX_2$ systems. These calculations show that under our conditions the selective relaxation rates obtained from the semilogarithmic plots are in error by 5-10% due to the neglect of cross-relaxation effects. The experimental relaxation rates are always slower than the true ones. For the nonselective relaxation rates the situation is more complex and the errors can be appreciable, and in either direction, depending on how much the relaxation rate of the studied proton deviates from that of the proton(s) causing the relaxation. H$\alpha^5$ and H$\alpha^9$ represent the most unfavorable cases for the present molecule because each $\alpha$ proton is mainly relaxed by its $\beta$ protons; however, the error was never more than 15% even though the relaxation rate of the $\beta$ protons was four times that of the $\alpha$ proton. In this case the experimental value proved to be faster than the true one. A combination of the most unfavorable cases for the selective and nonselective relaxation rates can result in an error of as much as 25% in the determined $\sigma$ values; this, however, gives only a 4% error in the distance derived from $\sigma$. Since the errors are systematic the calculated correlation times are probably underestimated by up to 25%. More accurate values have to await a more rigorous treatment of the recovery curves, which is now in progress.

## Results and Discussion

The sequence of tyrocidine A and the proposed conformation are shown in Figure 4.

**1. Proton Microenvironment from Spin-Lattice Relaxation Rates.** The monoselective spin-lattice relaxation rate is defined[2,3] by

$$R^i(\tilde{i}) = 2W_1 + W_2 + W_0 \qquad (1)$$

where $W_0$, $W_1$, and $W_2$ are the transition probabilities for the

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**Table I.** Relaxation Parameters for Backbone Protons of Tyrocidine A[a]

| H($i$) | $R^i$(NS), $s^{-1}$ | $R^i(\tilde{i})$, $s^{-1}$ |
|---|---|---|
| NH(2) | 2.2 | 3.4 |
| NH(3) | 2.7 | 4.0 |
| NH(4) | 2.4 | 3.6 |
| NH(9) | 2.9 | 4.7 |
| NH(10) | 2.4 | 3.4 |
| H$\alpha$(2) | 2.5 | 4.0 |
| H$\alpha$(5) | 0.9 | 1.6 |
| H$\alpha$(7) | 2.3 | 4.0 |
| H$\alpha$(9) | 1.0 | 1.8 |

[a] Temperature, 26 °C; concentration, 15 mg/mL of $Me_2SO$-$d_6$.

zero, one, and two quantum transitions, respectively. $R^i(\tilde{i})$ is sometimes said not to include cross-relaxation effects;[2,17] however, the recovery of the magnetization after a 180° pulse does and $R^i(\tilde{i})$ can only be obtained, using the semilogarithmic plot, from the initial slope of the recovery curve (see Error Analysis).

One approach to evaluating the microenvironments of protons is through the measurement of monoselective spin-lattice relaxation rates. The data for backbone protons in Table I obtained by experiments such as those shown in Figure 1 reveals that $R^i(\tilde{i})$ varies from 1.6 to 4.7 $s^{-1}$ and can be discussed with reference to the conformation of tyrocidine A deduced from scalar coupling constants,[15] NOE ratios,[13] cross-relaxation parameters,[13] $\sigma$ ratios,[13] and hydrogen-bonding studies.[15] This conformation, shown in Figure 4, contains type I $\beta$-turn, type II'$\beta$-turn, and antiparallel $\beta$-pleated sheet conformational moieties.

The relaxation rates, $R^i(\tilde{i})$, of the $\alpha$ protons of $Gln^9$ and $Pro^5$, 1.8 and 1.6 $s^{-1}$, respectively, reflect the relative lack of protons within a 4-Å radius (the microenvironment). The 4.0-$s^{-1}$ rate for $Orn^2H\alpha$ is readily accounted for by efficient relaxation by NH(2), NH(3), H$\alpha$(7), and NH(8) as well as the $Orn^2$ side-chain protons. An equally rich and similar proton microenvironment accounts for the efficient H$\alpha$(7) relaxation.

Each amide proton, NH($i$), is relaxed primarily by the H$\alpha(i)$ and H$\alpha(i-1)$, by the $\beta$ protons of the $i$th and $(i-1)$th residues, and by transannular protons similar to the H$\alpha$(2)-H$\alpha$(7) effects discovered by NOE difference spectroscopy.[18] In general we can write the following additivity relationship for an $\alpha$ or amide proton:

$$R^i(\tilde{i}) = R_\phi + R_\psi + R_\chi + R_0 + R_{TA}$$

where $R_\phi$ is the interaction between the $\alpha$ and NH protons in the same residue, $R_\psi$ is the interaction between NH($i$) and $\alpha(i-1)$ protons, $R_\chi$ is the interaction between an $\alpha$ or NH proton and the side-chain protons, $R_{TA}$ is the transannular interaction, and $R_0$ is the relaxation rate due to mechanisms other than proton-proton dipolar relaxation. For a rigid backbone terms $R_\phi$ and $R_\psi$ will depend only on the correlation time for the overall motion of the molecule and on the distances $r_\phi$ or $r_\psi$, respectively. The term $R_\chi$ may or may not be dependent on internal motion of the side chain in addition to distance and overall motion. $R_{TA}$ can, as well as $R_\chi$, be the sum of several terms; see, for example, H$\alpha$(7) in Table V. The only contribution from other mechanisms that has to be taken into account here is the dipolar relaxation from $^{14}N$ on protons directly bound to the nitrogen, $R_{NH} = 1.4$ $s^{-1}$. Contributions from other mechanisms can safely be assumed to be less than 0.1 $s^{-1}$.

**2. Correlation Times from Cross-Relaxation Rates.** As previously shown[7] $\sigma$ for a pair of protons can be indirectly

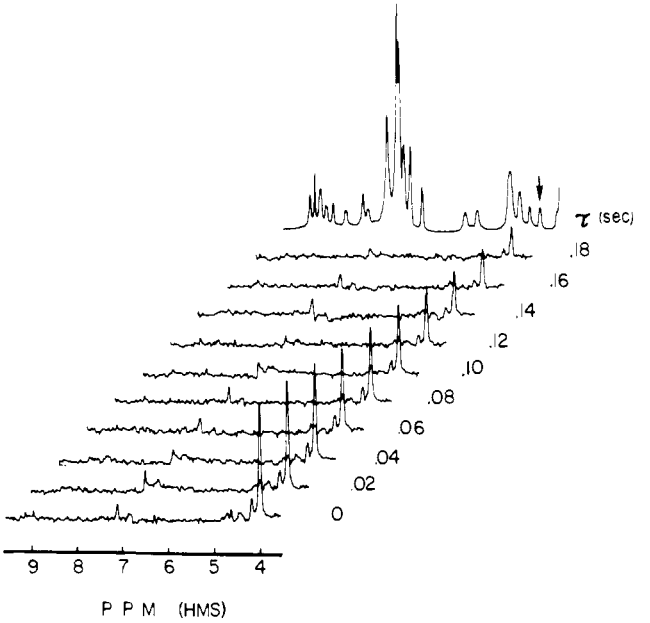


**Figure 1.** Monoselective proton spin-lattice relaxation rate measurements of the $Gln^9$ $\alpha$ proton (↓) of tyrocidine A. The 4–9-ppm region of the NMR spectrum is shown (top). All relaxation spectra were recorded with a selective $(180\text{-}\tau\text{-}90\text{-}T)_n$ pulse sequence. To obtain difference relaxation spectra, the relaxation spectrum for each $\tau$ value was subtracted from the $\tau = \infty$ spectrum. In this manner the individual $\tau$ difference relaxation spectra showed only that proton which was excited by the selective 180° pulse.

calculated from the product of NOEs and $R^i(\tilde{i})$ values or directly from the following equations:[3]

$$R^i(\tilde{i},\tilde{j}) - R^i(\tilde{i}) = \sigma_{ij} \quad (2)$$

$$R^j(\tilde{j},\tilde{i}) - R^j(\tilde{j}) = \sigma_{ji} \quad (3)$$

where $R^i(\tilde{i},\tilde{j})$ is the biselective relaxation rate obtained when resonances $i$ and $j$ are inverted simultaneously and $\sigma_{ij} = W_2^{ij} - W_0^{ij}$, the cross-relaxation rate. These relaxation rates and the calculated $\sigma$'s are shown in Tables I and II, respectively. The biselective relaxation rates of protons $i$ and $j$ were measured as previously described;[6] a typical example is shown in Figure 2.

The $\sigma$'s calculated from eq 2 and 3 and those from the NOEs[13] are shown in Table II. The close resemblance of $\sigma_{ij}$ and $\sigma_{ji}$ calculated from NOEs and relaxation rates for the NH-H$\alpha$ moieties of $Orn^2$ and $Gln^9$ demonstrates the accuracy of both experiments; furthermore, $\sigma$ values for other pairs of protons in tyrocidine A, where measurable by several methods, always agreed (with one exception, Table IV).

Correlation times and/or distances for interproton vectors corresponding to $\sigma_{ij}$ values were calculated[2] from the equation

$$\sigma_{ij} = (\gamma_H{}^4\hbar^2)(1/r_{ij}{}^6)\{3\tau_c{}^{ij}/(5 + 20\omega_0{}^2\tau_c{}^2) - \tau_c{}^{ij}/10\} \quad (4)$$

where $\gamma$ is the proton gyromagnetic ratio, $\hbar$ is Planck's constant, $r_{ij}$ is the interproton distance, $\tau_c{}^{ij}$ is the correlation time for the motion of the vector connecting protons $i$ and $j$, and $\omega_0$ is the spectrometer frequency.

**(a) The correlation times** for $\phi$ vectors of residues 2, 3, 4, 9, and 10 calculated from relaxation measurements and NOE/relaxation measurements are shown in Table II, columns 4 and 6, respectively. In the case of $Orn^2$ the four calculated correlation times, 1.24, 1.62, 1.48, and 1.42 × $10^{-9}$ s, are, within experimental error, in agreement with each other and with the $\tau_c$ values for all $\phi$ interproton vectors of other residues. Considering the differences in $^3J_{NHH\alpha}$ values, and hence $r_\phi$ dis-

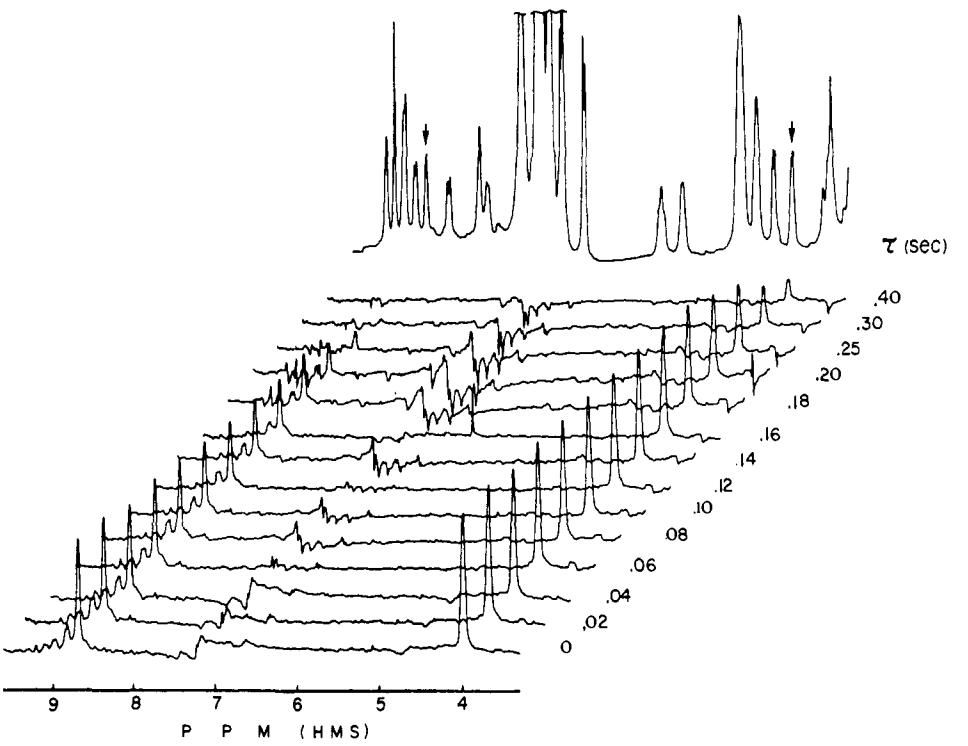


**Figure 2.** Biselective proton spin-lattice relaxation rate measurements: the $Gln^9$ $\alpha$ (↓) and $Gln^9$ amide protons (↓) were simultaneously excited with the selective 180° pulse and the nonselective 90° pulse was applied at different time intervals $\tau$. Incomplete cancellation of the very intense aromatic ring protons can be seen in the difference relaxation spectra around 7.3 ppm.

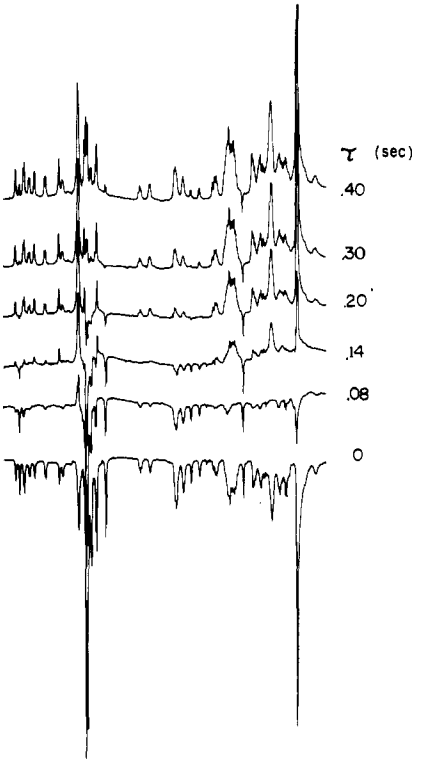


**Figure 3.** Nonselective proton spin-lattice relaxation rate measurements of tyrocidine A.

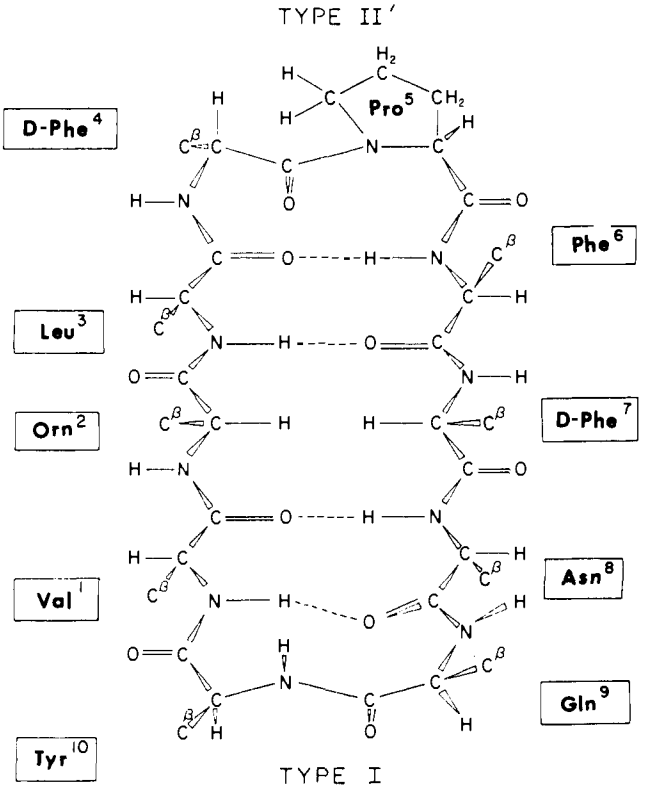


**Figure 4.** Proposed structure of tyrocidine A.

tances, and the uncertainties in the relaxation rates, this agreement is satisfactory and gives an average correlation time for the molecule of $1.3 \times 10^{-9}$ s.

The Hδ1-Hδ2, Hβ1-Hβ2, and Hδ-Hε distances of residues $Pro^5$, $Asn^8$, and $Tyr^{10}$ are independent of motion or conformation and when inserted in eq 4, using the $\sigma$ values of Table II, give $\tau_c$ = 1.10 (1.19), 1.38 (1.41), and 1.16 (1.30) $\times 10^{-9}$ s, respectively; the values in parentheses are NOE-derived correlation times.[13]

**(b) Interproton Distances.** The $\tau_c$, taken as the mean from Table II, and spin-lattice relaxation parameters, Tables II and III, for $Pro^5$Hδ1-$Pro^5$Hδ2 was used to calculate all $r_\phi$'s for residues 2, 3, 4, 9, and 10 and, as expected, the distances agree, Table IV, with those from $^3J_{NHH\alpha}$ and the Karplus curve. This,

**Table II.** Correlation Times for Proton-Proton Vectors of Tyrocidine A

| H($i$)-H($j$) | $R^i(\tilde{i}) + \sigma_{ij}$, $s^{-1}$ | $\sigma_{ij}$, $s^{-1}$ | $r_{ij}$, Å | ${\tau_c}^{ij}$ ($\times 10^9$), s[c] | $(\sigma_{ij}/R^i(\tilde{i})) \times R^i(\tilde{i})$, $s^{-1}$ | ${\tau_c}^{ij}$ ($\times 10^9$), s[d] |
|---|---|---|---|---|---|---|
| NH(2)-Hα(2) | $3.3_3$ | $-0.0_7$ | 2.95[a] | $1.2_4$ | $-0.1_0$ | $1.4_8$ |
| Hα(2)-NH(2) | $3.9_0$ | $-0.1_1$ | 2.95[a] | $1.6_2$ | $-0.0_9$ | $1.4_2$ |
| NH(3)-Hα(3) | $3.9_8$ | $-0.0_8$ | 2.94[a] | $1.2_5$ | $-0.0_7$ | $1.1_9$ |
| NH(4)-Hα(4) | $3.5_0$ | $-0.1_0$ | 2.83[a] | $1.3_0$ | $-0.1_1$ | $1.3_6$ |
| NH(9)-Hα(9) | $4.5_7$ | $-0.0_8$ | 2.86[a] | $1.1_9$ | $-0.0_9$ | $1.2_8$ |
| Hα(9)-NH(9) | $1.7_0$ | $-0.1_0$ | 2.86[a] | $1.3_3$ | $-0.1_1$ | $1.4_0$ |
| NH(10)-Hα(10) | $3.3_5$ | $-0.0_8$ | 2.96[a] | $1.3_2$ | $-0.0_6$ | $1.1_6$ |
| Hδ1(5)-Hδ2(5) | $3.7_0$ | $-1.1_1$ | 1.77[b] | $1.1_0$ | $-1.4_2$ | $1.1_9$ |
| Hβ1(8)-Hβ2(8) | $4.1_8$ | $-1.8_9$ | 1.77[b] | $1.3_8$ | $-1.9_5$ | $1.4_1$ |
| Hε(10)-Hδ(10) | $1.0_0$ | $-0.2_0$ | 2.44[b] | $1.1_6$ | $-0.2_3$ | $1.2_5$ |
| Hδ(10)-Hε(10) | | | 2.44[b] | | $-0.2_6$ | $1.3_4$ |

[a] Interproton distances estimated from ${}^3J_{NHH\alpha}$ values.[13] [b] Interproton distances calculated from standard bond angle and bond distance.[21] [c] Correlation times calculated from $\sigma$ values obtained by purely relaxation rate measurements. [d] Correlation times calculated from a combination of NOEs and monoselective relaxation rates.

**Table III.** Spin-Lattice Relaxation Parameters of the Side-Chain Protons

| H($i$) | $R^i$(NS) | $R^i(\tilde{i})$ |
|---|---|---|
| Hγ2(5) | 2.4 | 3.9 |
| Hδ1(5) | 2.9 | 4.8 |
| Hβ1(8) | 2.9 | 6.1 |
| Hδ(10) | 0.9 | 1.5 |
| Hε(10) | 0.8 | 1.2 |

in certain circumstances, provides a method of removing part of the fourfold $\phi$ degeneracy obtained from ${}^3J_{NHH\alpha}$ values.[19]

The $r_\psi$ distances for residues 8 and 2, Table IV, calculated from the two $\sigma$ values between NH(9)-Hα(8) and NH(3)-Hα(2), are equal (2.6 and 2.1 Å) and the same as those derived from the NOE.[13]

The Hα(4)-Hδ$_1$(5) distance, 2.2 Å, is equal to that derived from NOE ratios[13] and to the corresponding distance of gramicidin S[7,8] (Table IV).

The interproton distance between NH(9) and the β protons of residue 8 provide the only case where the disagreement between distances derived here and those previously derived[13] is more than 0.2 Å.

**3. Calculation of $R^i$(NS) and $R^i(\tilde{i})$ Values.** If (a) the conclusion that the relaxation of Hα's in tyrocidine A are fully explained by dipolar mechanisms is correct, (b) the backbone correlation time is $\tau_c = 1.3 \times 10^{-9}$ s, and (c) if all $\phi$, $\psi$, and transannular distances are those of the β turns and antiparallel β-pleated sheet, we can calculate $R^i$(NS) and $R^i(\tilde{i})$ and compare them with the experimental values. This comparison is shown in Table V.

If an additivity relationship holds, both the observed and calculated $R^i$(NS) and $R^i(\tilde{i})$ values should agree, respectively; this would be additional evidence that the proton relaxation mechanisms are fully dipolar. We can write[2]

$$R^i(\tilde{i}) = (\gamma_H{}^4\hbar^2) \sum_{i \neq j} \left( \frac{3{\tau_c}^{ij}}{1 + 10\omega_0{}^2({\tau_c}^{ij})^2} + \frac{{\tau_c}^{ij}}{10} + \frac{{\tau_c}^{ij}}{5 + 20\omega_0{}^2({\tau_c}^{ij})^2} \right) \left( \frac{1}{r_{ij}} \right)^6 \quad (5)$$

where the meaning of the parameters is the same as in eq 4. Assuming only one ${\tau_c}^{ij}$ exists, all the distances, $r_{ij}$, between Hα(7) and each of the protons NH(7), NH(8), Hα(2), NH(3), Hβ1(7), and Hβ2(7) were used in the $\Sigma\, 1/r_{ij}{}^6$ term of eq 5. The calculated $R^i(\tilde{i})$, 3.5 $s^{-1}$, agrees with the observed value, 4.0, especially considering that the effect of one proton, Hδ(7), was not accounted for. The distance between Hδ(7) and Hα(7) depends on the $\chi^1$ and $\chi^2$ torsion angles of the D-Phe[7] residue. Since $\chi^1(7) = +60°$ and $\chi^2(7) = 90 \pm 30°$, this distance can range between 2.5 and 3.6 Å. Adding this contribution makes the selective relaxation rate of Hα(7) equal to $3.8 \pm 0.2$ $s^{-1}$. The nonselective relaxation rate, $R^{H\alpha(7)}$(NS), is the sum of $R^i(\tilde{i})$ and $\Sigma\sigma_{ij}$. From eq 4, $\Sigma\sigma_{ij}$ can be calculated, and therefore the $R^{H\alpha(7)}$(NS) (Table V). In a similar way the selective and nonselective relaxation rates of NH(4) and Hα(5) were calculated. For NH(4) the contributions for Hβ(3), Hγ(3), and Hδ(3) were ignored, and for Hα(5) the contributions from Hγ(5) and Hδ(5) were also ignored. The results also agree with the observed values. Unfortunately, enough data were not available to do this for other protons, but these examples suffice to illustrate the principle and that the mechanisms are dipolar.

**Table IV.** Interproton Distances Calculated from Cross-Relaxation Parameters[a]

| H($i$)-H($j$) | $R^i(\tilde{i}) + \sigma_{ij}$, $s^{-1}$ | $\sigma_{ij}$, $s^{-1}$ | $r_{ij}$,[b] Å | $(\sigma_{ij}/R^i(\tilde{i})) \times R^i(\tilde{i})$, $s^{-1}$ | $r_{ij}$,[c] Å |
|---|---|---|---|---|---|
| Hα(2)-NH(3) | $3.4_2$ | $-0.5_9$ | $2.1_3$ | $-0.6_0$ | $2.1_2$ |
| NH(3)-Hα(2) | $3.4_6$ | $-0.6_0$ | $2.1_2$ | $-0.4_9$ | $2.1_9$ |
| Hα(2)-Hα(7) | $3.6_4$ | $-0.3_7$ | $2.3_0$ | $-0.2_6$ | $2.4_4$ |
| Hα(7)-Hα(2) | $3.6_4$ | $-0.3_3$ | $2.3_4$ | $-0.2_8$ | $2.4_1$ |
| Hα(7)-NH(7,8) | $3.3_4$ | $-0.6_6$ | | $-0.6_4$ | |
| Hδ1(5)-Hα(4) | $4.2_4$ | $-0.5_7$ | $2.1_4$ | $-0.4_6$ | $2.2_2$ |
| NH(9)-Hα(8) | $4.4_7$ | $-0.1_8$ | $2.5_9$ | $-0.1_3$ | $2.7_4$ |
| NH(9)-Hβ1(8) | $4.0_7$ | $-0.5_8$ | $2.1_3$ | $-0.5_8$ | $2.1_3$ |
| Hβ1(8)-NH(9) | $5.4_0$ | $-0.6_7$ | $2.0_8$ | $-0.7_8$ | $2.0_3$ |
| NH(9)-Hβ2(8) | $4.3_8$ | $-0.2_7$ | $2.4_2$ | $-0.0_6$ | $3.1_0$ |

[a] The latter was derived from relaxation[b] and from NOE[c] experiments. [b] Interproton distances calculated from $\sigma$ values obtained by purely relaxation rate measurements. [c] Correlation times calculated from a combination of NOEs and monoselective relaxation rates.

## Conclusions

In this report we measured the nonselective, $R^i$(NS), biselective, $R^i(\tilde{i},\tilde{j})$, and monoselective, $R^i(\tilde{i})$, spin-lattice relaxation rates for tyrocidine A to obtain the cross-relaxation rates, $\sigma_{ij}$. The latter agreed with $\sigma$'s calculated from NOE measurements.[13] Correlation times for NH, Hα, and side-chain protons, calculated from $\sigma_{ij}$ parameters, agree with those measured previously in NOE studies.[13] The correlation times for Hδ-Hε, Hδ1-Hδ2, and Hβ1-Hβ2 vectors of three side chains were, within experimental error, the same as the correlation time for the backbone protons; the latter was in satisfactory agreement with the correlation time for the backbone of gramicidin S (an analogue) calculated from $^{13}C$ $T_1$ data.[20]

**Table V.** Data Used to Calculate the Monoselective and Nonselective Relaxation Rates of Protons, $i$, in Tyrocidine A

| | | | | | | | | relaxation rates | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | H($j$) | | | | | | $R^i(\tilde{i})$ | | $R^i$(NS) | |
| H($i$) | | NH(8) | NH(7) | Hα(2) | NH(3) | Hβ1(7) | Hβ2(7) | calcd | obsd | calcd | obsd |
| Hα(7) | $r_{ij}$, Å[a] | 2.1 | 3.0 | 2.4 | 3.1 | 3.0 | 2.4 | | | | |
| | $R$, s$^{-1}$ [b] | 1.6 | 0.2 | 0.7 | 0.2 | 0.2 | 0.7 | 3.6 | 4.0 | 2.1 | 2.3 |
| | | Hα(3) | Hα(4) | Hβ1(4) | Hβ2(4) | $^{14}$N | | | | | |
| NH(4) | $r_{ij}$, Å[a] | 2.3 | 2.8 | 2.4[c] | 2.5[c] | 1.02 | | | | | |
| | $R$, s$^{-1}$ [b] | 1.0 | 0.3 | 0.7 | 0.5 | 1.4 | | 3.9 | 3.6 | 2.9 | 2.4 |
| | | Hβ1(5) | Hβ2(5) | NH(6) | | | | | | | |
| Hα(5) | $r_{ij}$, Å[a] | 2.8 | 2.3 | 3.0 | | | | | | | |
| | $R$, s$^{-1}$ [b] | 0.3 | 0.8 | 0.2 | | | | 1.3 | 1.6 | 0.8 | 0.9 |

[a] These distances are between H($i$) and all the H($j$) protons in its microenvironment. It is assumed that the conformation of tyrocidine A is known.[13-15] [b] The spin-lattice relaxation rate contributions of H($j$) to the relaxation of H($i$) calculated from $r_{ij}$. [c] Mean distances calculated by assuming rotamer populations of 0.68, 0.26, and 0.06 for $\chi_1$ = 180, +60, and −60°, respectively.[14]

The $r_\phi$ distances calculated from $\sigma_\phi$ values of the Pro$^5$ Hδ1-Hδ2 vector agree with those from $^3J_{NHCH}$ measurements and present a method of removing the $\phi$ angle degeneracy of Karplus curves; they agree also with $r_\phi$ values from NOE measurements. The $r_\psi$ and transannular distances support the type Iβ-turn/type II′β-turn/antiparallel β-pleated sheet conformation. The transannular distances prove that the hydrogen bonds exist and provide a method of delineating donor and acceptor groups. The calculated Hα-Hβ and NH-Hβ distances for certain side chains agree with the side-chain conformations determined from scalar coupling constants and proton-chromophore distance measurements.[14]

The selective spin-lattice relaxation rate, $R^i(\tilde{i})$, was calculated for one amide and two α protons based upon the measured/known distances from each proton in their microenvironment (<4 Å) and assuming the proposed tyrocidine A conformation to be correct. The agreement between experimental and calculated $R^i(\tilde{i})$ values was good. This conclusion substantiated the solution conformation of tyrosidine A but more importantly proved the dipolar nature of all the proton relaxation mechanisms. Thus the additivity equation $R^i(\tilde{i}) = R_\phi{}^i + R_\psi{}^i + R_\chi{}^i + R_{TA}{}^i + R_{^{14}N}{}^i + R^*$ was established; other mechanisms such as scalar relaxation or non-$\phi$,$\psi$,$\chi$,TA and $^{14}$N contributions are included in $R^*$. By adding all cross-relaxation parameters, $\sigma$, to the calculated $R^i(\tilde{i})$ values it was found that $R^i(\text{NS})_{calcd} = R^i(\text{NS})_{obsd}$. Again this confirms the structure, additivity, and mechanisms.

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## References and Notes

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(17) In fact also $R^i(\tilde{i})$ contains cross-relaxation effects, $W_2$ and $W_0$; however, there is no effect from $\sigma = W_2 - W_0$, which is often called the cross-relaxation parameter. $R^i(\tilde{i})$ is the same relaxation rate that should be observed if all the protons causing the relaxation of the $i$th proton were saturated during the time that the spin $i$ is allowed to relax toward its equilibrium magnetization value after, e.g., an 180° pulse. We prefer to keep the nomenclature[2,3] and call $\sigma$ the cross-relaxation rate and $\rho$ or $R^i(\tilde{i}) = \sum \rho_{ij}$ the relaxation rate not including cross-relaxation effects. Kalk and Berendsen[11] have used a slightly different nomenclature with $R_{1i} = 2W_1 + 2W_2$ and the cross-relaxation parameter $R_{itj} = W_0 - W_2$, which transforms into the other through $R^i(\tilde{i}) = R_{1i} + \sum_i R_{itj}$ and $\sigma_{ij} = -R_{itj}$.
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