For polypropylene or poly(vinyl chloride), $n = 1$ and the ^{13}C chemical shifts are very sensitive to stereostructure. It has also been shown[8,24] for $n = 2$ that the ^{13}C chemical shifts can reflect configurational or conformational structure even though the magnitude of the effect is reduced. However, for the system under consideration $n = 5$ and the ^{13}C chemical shifts are not sensitive to stereostructure.

Past infrared analysis[1,2,4,5] of reported alternating propylene–butadiene copolymers certainly could be used to rule out the presence of long runs of butadiene or propylene. However, it was difficult to use those data to rule out the possibility of some random adjacent monomer and thus was not unequivocal. The 220-MHz proton nmr analyses[3] on the other hand were used to assign an alternating structure. However, the 220-MHz proton interpretation is based on deconvoluting badly overlapping multiplet patterns, calculating theoretical spectra, and simplifying the spectra by examining deuterated copolymers. The ^{13}C nmr method is more satisfying as direct observation of each copolymer can be made and the results appear unequivocal.

Conclusions

Pulsed Fourier transform ^{13}C nmr is shown to be an unambiguous method for determining an alternating monomer sequence distribution in copolymers of propylene and butadiene that were made with either a vanadium or a titanium catalyst. The two copolymers were hydrogenated and the ^{13}C nmr spectra of the resulting polyalkanes can be shown to reflect a regular repeating sequence of five methylene carbons bounded by tertiary carbons bearing methyl groups. This interpretation is based upon comparing the observed ^{13}C chemical shifts with expected values derived from empirical additivity relationships shown to hold for branched alkanes and by a comparison with analogous chemical shifts reported for ethylene–propylene copolymers. The determination of the structure for the hydrogenated copolymers provides a sound basis for interpreting the ^{13}C nmr spectra of the original propylene–butadiene copolymers. A trans or cis olefin bond substituent effect is shown to account for chemical shift differences between spectra of the hydrogenated and original copolymers. The spectra of the original copolymers show that the copolymer made with a vanadium catalyst is alternating with essentially all of the butadiene in a trans configuration. There appears to be less than 2% polybutadiene homopolymer present as an impurity. On the other hand the copolymer made with a titanium catalyst is an alternating copolymer with about 89% of the butadiene in a trans configuration and 11% of the butadiene in a cis configuration. This copolymer has about 7 to 8% *cis*-1,4-polybutadiene homopolymer present as an impurity. In the future, it should be possible to use the proposed interpretation of the ^{13}C nmr spectra of copolymers of propylene and butadiene to determine the extent of alternation without the necessity of also hydrogenating the copolymer and obtaining the ^{13}C nmr spectrum of the resulting polyalkane.

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References and Notes

(1) J. Furukawa, R. Hirai, and M. Nakaniwa, *J. Polym. Sci., Part B,* **7,** 671 (1969).
(2) A. Kawasaki, I, Maruyama, M. Taniguchi, R. Hirai, and J. Furukawa, *J. Polym. Sci., Part B,* **7,** 613 (1969).
(3) T. Suzuki, Y. Takegami, J. Furukawa, and R. Hirai, *J. Polym. Sci., Part B,* **9,** 931 (1971).
(4) J. Furukawa, H. Amano, and R. Hirai, *J. Polym. Sci., Part A-1,* **10,** 681 (1972).
(5) J. Furukawa, *Angew. Makromol. Chem.,* **23,** 189 (1972).
(6) C. J. Carman and C. E. Wilkes, *Rubber Chem. Technol.,* **44,** 781 (1971).
(7) C. E. Wilkes, C. J. Carman, and R. A. Harrington, *J. Polym. Sci., Part C,* 237 (1973).
(8) C. J. Carman, A. R. Tarpley, Jr., and J. H. Goldstein, *Macromolecules,* **6,** 719 (1973).
(9) D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.,* **86,** 2984 (1964).
(10) J. C. Randall, *J. Polym. Sci., Polym. Phys. Ed.,* **11,** 275 (1973).
(11) W. O. Crain, Jr., A. Zambelli, and J. D. Roberts, *Macromolecules,* **4,** 330 (1971).
(12) A. Zambelli, G. Gatti, C. Sacchi, W. O. Crain, Jr., and J. D. Roberts, *Macromolecules,* **4,** 475 (1971).
(13) D. E. Dorman, M. Jautelat, and J. D. Roberts, *J. Org. Chem.,* **36,** 2757 (1971).
(14) H. J. Harwood, D. D. Russell, J. J. A. Verthe, and J. Zymonas, *Macromol. Chem.,* **163,** 1 (1973).
(15) M. W. Duch and D. M. Grant, *Macromolecules,* **3,** 165 (1970).
(16) V. D. Mochel, *J. Polym. Sci., Part A-1,* **10,** 1009 (1972).
(17) J. Schaefer and D. F. S. Natusch, *Macromolecules,* **5,** 416 (1972).
(18) C. J. Carman and C. E. Wilkes, *Macromolecules,* **7,** 40 (1974).
(19) H. E. Diem, The B. F. Goodrich Co., private communication.
(20) A. Zambelli, D. E. Dorman, A. I. R. Brewster, and F. A. Bovey, *Macromolecules,* **6,** 925 (1973).
(21) C. J. Carman, A. R. Tarpley, Jr., and J. H. Goldstein, *J. Amer. Chem. Soc.,* **93,** 2864 (1971).
(22) C. J. Carman, A. R. Tarpley, Jr., and J. H. Goldstein, *Macromolecules,* **4,** 445 (1971).
(23) C. J. Carman, *Macromolecules,* **6,** 725 (1973).
(24) J. I. Kroschwitz, M. Winokur, H. J. Reich, and J. D. Roberts, *J. Amer. Chem. Soc.,* **91,** 5927 (1969).

The Nature of the Molecular Motions of Poly(L-proline) for the Cis ⇌ Trans Conformational Transitions

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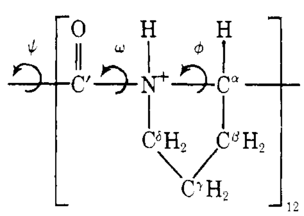
ABSTRACT: Conformational analysis has been used to study the molecular motions in the cis ⇌ trans transitions of poly(L-proline) by assuming cooperative couplings between residues obey Gaussian functions. The results suggest that the free energy barriers to both the cis → trans and trans → cis transitions are minimum when three residues are involved in the cooperative motions. The cis → trans transition prefers to start at the N-terminal end of the chain and proceed inward, while the trans → cis transition nucleates, most probably, at the C-terminal end of the chain and proceeds inward. Only two stable conformations, corresponding to the observed cis and trans helices, have been found for every transition path tested. Also, a single energy barrier separates the cis and trans states.

This paper presents the results of an initial attempt, using conformational analysis, to describe the molecular motions taking place in poly(L-proline) as it undergoes an order-to-order conformational transition.[2-6] This transition is characterized by the cis ⇌ trans (C ⇌ T) torsional interconversion with respect to the imide bond of each residue. In a general vain, an attempt has been made to minimize the transition path free energy, for a wide variety of possible transition paths, so that the global minimum barrier height and, as a consequence, the most probable transition path might be found. The impetus to carry out this study is twofold. First, we wish to know if conformational analysis can be used to semiquantitatively describe molecular motions in chain molecules, and, second, we hope to discover if this type of an approach can demonstrate that a cooperative dynamic motion, as inferred from experiment,[5-7] is prerequisite to minimizing the transition barrier height.

Method

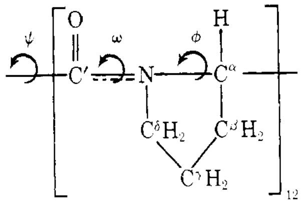
A chain molecule consisting of 12 residues was used in the final computations that are reported here. It is not yet resolved as to the mechanism of $C \rightleftharpoons T$ interconversion about the imide bond. There is a reasonable amount of evidence[2,3] that the mechanism involves protonation of the imide nitrogen which destroys the partial double-bond character of the imide bond. We adopted the structural model consistent with this mechanism as part of our study, and thus carried out a transition analysis for

Model I



where the intrinsic torsional barrier height for rotation about the C′—N⁺ bond was set at zero. However, we also carried out calculations using a trigonal imide nitrogen. Thus the second model considered in our investigation was, essentially, a duo-decamer oligomer of poly(L-proline); *i.e.*

Model II



For this model the intrinsic torsional potential about the imide bond was taken from other works[8,9] to be

$$\begin{aligned} T(\omega) &= (A/2)(1 - \cos 2\omega) \\ A &\approx 20 \text{ kcal/mol} \qquad (1) \\ \omega &= 0^\circ \text{ corresponds to the cis state} \end{aligned}$$

The bond lengths and angles for model II were taken from X-ray work[10] while the geometry about the N^+ atom in model I was based upon "standard" bond lengths and angles.[6] The conformational potential energy functions and fluctuation techniques to compute entropy given in reference six were adopted in the analyses. Solvent contributions were not considered.

Prerequisite to describing the total spatial motion of a chain molecule is understanding how a motion in one unit of the chain produces motion in other nearby units of the chain. Qualitatively, a motion at some location along the chain, induced, say, by thermal fluctuations, generates a force field in which close-by chain segments find themselves. These close-by chain segments react to the force field by undergoing motions in search of a new equilibrium state. The extent of motion in these close-by chain segments coupled with the number of close-by chain segments undergoing motion characterize the *cooperativity* of the motion. Most often the motions of the nearby chain segments are very similar to that of the nucleating fluctuation motion, and the motions are indeed of a cooperative nature. If, on the other hand, the motions of the nearby units are not similar to the initial fluctuation motions, then these motions can be considered non- or anticooperative. When the nucleation fluctuation motion and subsequent cooperative motions lead to a conformational transition in the chain molecule, the change in stable conformational states is termed a cooperative conformational transition.

The major problem which faced us in our attempt to elucidate the motions in poly(L-proline) was determining a suitable means of characterizing the cooperativity between residue units. The most fundamental way to do this would be to carry out dynamical fluctuation calculations similar, perhaps, to those attempted by Simon.[11] However, it was our opinion that we could not generate a suitably realistic force field between residues especially due to the fact that solvent terms were neglected. Moreover, the computer time needed for a dynamical calculation of this type is quite substantial.

Cooperativity was introduced into the transition analyses through what we call a *Gaussian coupling function*

$$\Delta\theta_{j+n,k} = \Delta\theta_{j,k} \exp[(n^*/n)^2 \ln \epsilon] \qquad (2)$$

where $\Delta\theta_{j,k}$ is the magnitude and direction (positive or negative torsional rotation) of the nucleating fluctuation taking place for a rotation, *i.e.*, ϕ, ψ, or ω, in residue j for the kth conformational transition state. $\Delta\theta_{j+n,k}$ is the magnitude and direction of the induced cooperative fluctuation of an equivalent rotation in residue $j + n$ for the kth conformational transition state. n^* and ϵ define the degree of cooperativity in that ϵ is the fractional fluctuation value that a rotation n^* residues from the nucleation site experiences during a transition fluctuation. ϵ was set equal to 0.01 in our analyses.

The choice of a Gaussian function to describe cooperative motion between residues is based upon the following reasoning. As the number of intervening residues increases between any two reference residues the distance between pairs of atoms composed of one atom from each of the two reference residues also increases. The magnitude of the force between a pair of atoms drops off as a function of distance in a manner very similar to a decreasing Gaussian for any reasonable pair-potential function. Since the magnitude of the cooperative motion of a residue is a direct function of the force field in which it exists, it should follow that the magnitude of the cooperative motion also falls off in an approximate Gaussian fashion as a function of distance, *i.e.*, number of intervening residues, from the nucleation site of the fluctuation motion. The parameters n^* and ϵ are not mutually independent, but are meant to serve as markers which define the number of nearby residues involved in a cooperative motion. If ϵ were assigned a value different from 0.01 the n^* scale would be expanded or contracted relative to the scale based upon $\epsilon = 0.01$. Since, however, our study is based upon scanning over a wide range of n^* for a fixed ϵ, it is not critical what value is assigned to ϵ. $\epsilon = 0.01$ is convenient because it tells us residues n^* units from the nucleation site undergo $1/100$ the motion of the nucleating residue.

The conformational transition path energy was explicitly minimized as a function of ψ and ω. This was done by starting from either the preferred cis, or trans, conformation, k

Table I
Minimum Transition Barrier Heights (kcal/mol/residue) for the T → C and C → T Transitions of Poly(L-proline) for Different Nucleation Sites

	N end inward	C end inward	Middle of Chain
		Model I	
T → C	19.4	13.6	19.4
C → T	10.8	16.9	16.9
		Model II	
T → C	35.4	21.8	36.2
C → T	23.5	28.8	31.4

= 0, and determining the minimum conformational path energy ρ degrees away according to path generator functions

$$\omega_k = \rho \sin \nu_k \tag{3}$$

$$\psi_k = \rho \sin \nu_k \tag{4}$$

where ν_k goes from 0 to 360°.

Using the conformational state with the minimum path energy, *i.e.*, $k = 1$, as a new "jump-off point," the above procedure was iteratively repeated, *i.e.*, $k = 2, 3, \ldots,$ until a stable minimum energy conformation was obtained. In order to prevent the transition from turning around and returning to the starting point, each previous conformational state along the path was assigned a high energy value. In other words, our path energy minimization procedure reaches a point on the (ψ,ω) plane in the kth iterative step and "looks around," this is the ν_k rotation, to find the minimum conformational energy ρ degrees away where

$$\rho = \sqrt{(\omega_k - \omega_{k-1})^2 + (\psi_k - \psi_{k-1})^2}$$

The $(k + 1)$ step is then made to this newly found energy minimum and the entire procedure repeated. A practical and yet reasonable value for ρ was found to be 5° while ν_k was best set at 15° for all k. Torsional fluctuations, ϕ, were allowed about the N-C$^\alpha$, or N$^+$-C$^\alpha$, bond for each intermediate conformational state during the (ω,ψ) energy minimization. Thus ϕ was also implicitly considered in the minimization procedure. This entire method of path energy minimization was repeated for $n^* = 1, 2, 3, 4, 5, 6,$ and ∞. An intrinsically gratifying result, which was necessary in order to perform these calculations, was that the cis and trans conformations, observed experimentally,[10] were the *only* stable conformations of the chain molecule. Thus we could be sure that our simulation calculations would undergo the (C $\rightleftharpoons$ T) conformational transitions and not "get stuck" in some stable intermediate state.

Results

For each direction of the transition, *i.e.*, C → T or T → C, three initial nucleation sites for the transitions were considered: the N-terminal end, the C-terminal end, and the middle of the chain. The barrier heights of the transitions for each of the three sites are given in Table I. For both structural models it can be seen that the T → C transition prefers nucleating from the C-terminal end of the chain while the C → T transition prefers originating from the N-terminal position. Neither transition shows an energetic preference for initiating in the middle of the chain.

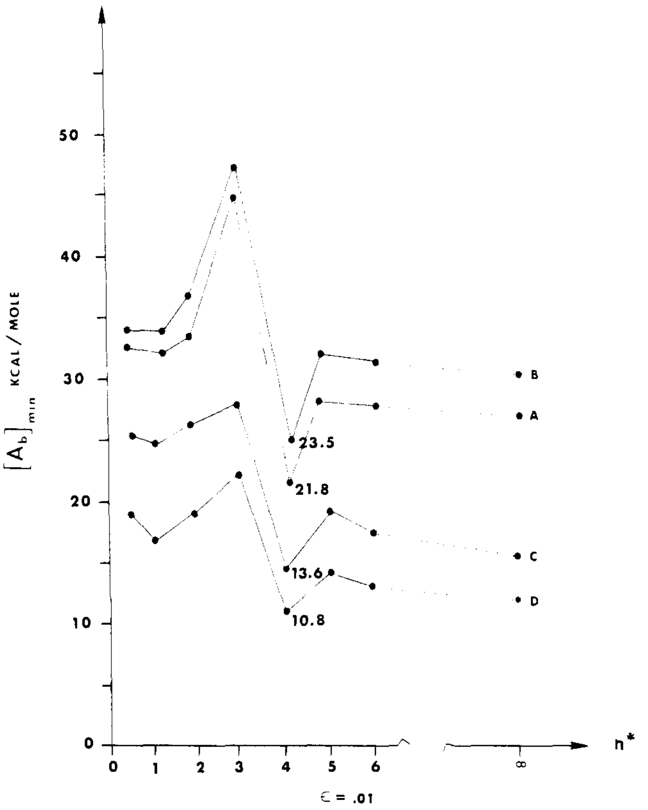


Figure 1. Minimum free energy barrier height as a function of the degree of cooperativity n^*: (A) ... TTTT ... TTTC ... TTCC (Model II); (B) CCCC... TCCC... TTCC... (Model II); (C) ...TTTT ...TTTC ...TTCC (Model I); (D) CCCC... TCCC... TTCC... (Model I).

As stated earlier, different values of n^* in eq 2 yield different degrees of cooperativity for a fixed ϵ. The larger the value of the n^* the longer the range of cooperativity. Figure 1 contains plots of minimum free energy barrier heights, $[A_b]_{min}$, as a function of n^* for both structural models considered in this work. The most significant observation from this figure is that the global free energy minimum barrier height is achieved for $n^* = 4$ in both the C → T and T → C transitions for both structural models. This suggests the range of cooperativity may involve at least three complete residues which is, interestingly, the repeat unit of the trans helix and approximately the repeat unit of the cis helix, *i.e.*, 3.3 residues/turn for the cis helix. There is also some experimental evidence that helix formation in both the cis and trans oligomers takes place for the tetramer in aqueous media.[12] Deber, *et al.*,[13] have found that L-proline oligomers prefer form II, all-trans conformer, abruptly at the pentamer in chloroform. The low energy barrier height for infinite cooperativity, that is all residues simultaneously undergoing the transition uniformly, is also an interesting finding. One might speculate from the data in Figure 1 that all modes of cooperative motion having $n^* \geq 4$ may be taking place in the order-to-order transitions of poly(L-proline).

The last aspect to describing the transition motions is to state the direction of the rotations ψ and ω during the transition. In Figure 2 is an energy map of ψ and ω for infinite cooperativity in model I. The minimum energy transition routes for the C → T and T → C transitions are superimposed on the energy map. The direction of the rotations for the T → C is positive, relative to standard convention, for both ψ and ω. The opposite is true for the C → T transition. It is to be noted that the C → T and T → C transition paths are slightly different. This is due to the fact that each intermediate transitional state along the transition path is dependent upon its path history. Seeking the minimum free energy routes starting from two *different* points on the

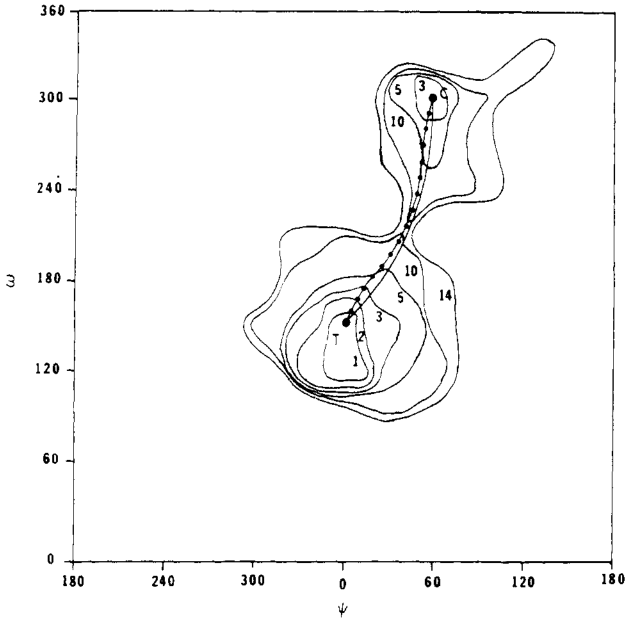


Figure 2. The ψ *vs.* ω conformational energy map of poly(L-proline) model I subject to infinite cooperativity, that is, the equivalence condition. The energy contours (kcal/mol/monomer) are at the indicated values above the global minimum. The location of the trans helix is denoted by T while the cis helix is located at the position indicated with a C. (·–·–·) indicates the T → C minimum free energy path while the (——) indicates the same for the C → T transition.

energy surface leads to different history-dependent paths. The directions of the rotations described in Figure 2 are preserved for $n^* = 4, 5, 6, \ldots \infty$ for both models I and II.

Discussion

The results of our investigation suggest the following properties of the C ⇌ T transitions in poly(L-proline).

(A) Minumum free energy barriers for the transitions occur for cooperativity extending over three residues. Higher degrees of cooperativity, extending to infinity, are also probable.

(B) The C → T transition prefers to proceed from the N-terminal end of the chain inward. Torchia and Bovey[14] have suggested that the C → T transition originates at the C-terminal end of the chain and proceeds, sequentially, inward. This model is based upon the findings from an extensive nmr study of this transition in D_2O. These findings are, obviously, in direct conflict with our results. However, Torchia and Bovey believe their C → T transition model may not hold for nonaqueous solutions. Since our calculations are for a free-space medium, we suggest solvent effects, in accordance with Torchia and Bovey, may be the key to resolving these discrepancies. Still we do not rule out that our potential energy functions are in error and/or that we have not explored all possible transition paths as possible reasons for our conflicting findings. Our calculations suggest that the T → C transition originates at the C-terminal end of the chain. Torchia and Bovey have not reported on this transition.

(C) The directions of the rotations ψ and ω for the C → T transitions are both negative, while, in contrast, both rotations are positive for the T → C transition.

(D) There are no stable intermediate states for the C ⇌ T transitions when the degree of cooperativity involves three or more residues. The cis and trans helices observed in the solid state[10] are the only stable conformational states. Torchia and Bovey[14] discuss the observation that conformations such as CCCC . . . CCTT . . . T can be observed on the nmr time scale for poly(L-proline) in concentrated aqueous salt solutions. This again would be in conflict with our findings. However, interactions between the solution ions and the biopolymer may drastically modify the conformational behavior of poly(L-proline) as compared to our free-space approximation. Thus we feel a comparison between these findings should be taken with caution.

(E) Structural models I and II have very similar transitional properties. The geometry and topology about the imide nitrogen does not appear to have a significant influence on the motions involved in the C ⇌ T transitions.

(F) For the most probable transition paths the major contributions to the transition barrier come from steric and electrostatic repulsions between first and second nearest neighbor carbonyl oxygens. This is the case for both structural models. There are also some bad contacts among the pyrollidine hydrogens on adjacent residues.

These calculations have not taken the effect of solvent–solute interactions into consideration in computing the minimum free energy paths. These types of interactions could modify barrier heights considerably. Thus, one can at best state that the magnitude of the barrier heights is only qualitatively meaningful. The barrier-height values should not be used in quantitative thermodynamic discussions. Moreover, we are well aware that the structural models used in the conformational analyses may be inadequate. For example, the carbonyl carbon or oxygen may be protonated as part of the transition mechanism. Most workers feel this is unlikely, but it cannot be ruled out.

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References and Notes

(1) Sloan Research Fellow.
(2) I. Z. Steinberg, A. Berger, and E. Katchalski, *Biochem. Biophys. Acta*, **28**, 647 (1958).
(3) I. Z. Steinberg, W. F. Harrington, A. Berger, M. Sela, and E. Katchalski, *J. Amer. Chem. Soc.*, **82**, 8263 (1960).
(4) H. Strassmair, J. Engel, and G. Zundel, *Biopolymers*, **8**, 237 (1969).
(5) L. Mandelkern, "Poly-Amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, and references therein.
(6) A. J. Hopfinger, "Conformational Properties of Macromolecules," Academic Press, New York, N. Y., 1973.
(7) F. Gornick, L. Mandelkern, A. F. Diorio, and D. E. Roberts, *J. Amer. Chem. Soc.*, **86**, 2549 (1964).
(8) F. K. Winkler and J. D. Dunitz, *J. Mol. Biol.*, **59**, 169 (1971).
(9) W. A. Hiltner and A. J. Hopfinger, *Biopolymers*, **12**, 1197 (1973).
(10) V. Sasisekharan, *Acta, Crystallogr.*, **12**, 897 (1959).
(11) E. M. Simon, *Biopolymers*, **10**, 973 (1971).
(12) H. Okabayashi, T. Isemura, and S. Sakakibara, *Biopolymers*, **6**, 323 (1968).
(13) C. M. Deber, F. A. Bovey, J. P. Carver, and E. R. Blout, *J. Amer. Chem. Soc.*, **92**, 6191 (1970).
(14) D. A. Torchia and F. A. Bovey, *Macromolecules*, **4**, 246 (1971).