Quantitative Measurement of Isomer Composition in Polypentenamer Using Carbon-13 Nuclear Magnetic Resonance

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ABSTRACT: Fourier transformed pulsed ^{13}C nuclear magnetic resonance spectroscopy has been used to quantitatively measure the cis and trans content in a series of eight polypentenamers. Multiple measurements on each sample showed that the precision of the ^{13}C nmr analysis is ±0.8% over the concentration range of 6.9–85.5% trans. Spin–lattice relaxation times for the methylene carbons varied from 0.5 to 1.4 sec, depending on the overall trans content of the polymers.

Polypentenamer, $(-CH_2CH_2CH{=}CHCH_2-)_n$, is produced by the ring-opening polymerization of cyclopentene. In the past,[1-10] the determination of the relative concentrations of cis and trans structure was based upon an infrared method developed for analyzing polybutadiene.[11] However, inexact absorption coefficients and problems with band overlap left the resultant analyses open to question.

Carbon-13 nuclear magnetic resonance spectroscopy (^{13}C nmr) seemed ideally suited for determining the isomer composition in polypentenamers. We have used ^{13}C nmr as the primary analytical method to precisely determine the isomer composition of a series of eight polypentenamers. These results and samples will provide standards to determine infrared absorption coefficients, and thus provide a new infrared analysis.[12]

Two recently published books[13,14] clearly show that the flurry of research over the past few years has firmly established carbon-13 nuclear magnetic resonance (^{13}C nmr) as a valuable spectroscopic tool for the organic chemist. Its potential for establishing polymer molecular structure has equally been exciting and encouraging.[15-41] The advantage of carbon-13 over proton nmr has been the dispersion of chemical shifts over a much wider range. This has meant that unique, separate nmr peaks have been obtained which describe molecules with subtle differences in molecular structure. The analytical use of ^{13}C nmr has been suggested and used to a limited extent to quantitatively measure stereoconfiguration[15,16,24] and monomer sequence distribution.[21,27,28] However, no detailed investigation has been reported on precision or accuracy if ^{13}C nmr is used as the primary analytical method for measuring polymer microstructure.

As with proton nmr, measurements of peak areas are necessary to obtain quantitative analyses. Allerhand has predicted[42] that ^{13}C integrated intensities should be valid as a carbon count in spectra of complex molecules. Schaefer[31] subsequently has shown that within a polymer system the carbons undergo equal nuclear Overhauser enhancement (NOE), even though the total NOE of different polymers may not be equal or maximum. Hence one can compare relative areas within a ^{13}C nmr spectrum without fear of inadequately accounting for all of the area of a given structural feature.

Experimental Section

The ^{13}C nmr spectra were obtained in natural abundance from the Fourier transforms (FT) of the proton noise-decoupled ^{13}C free induction decays. The pulsed FT spectra were recorded on a Varian XL-100-15 operating at 25.16 MHz. Spectra were obtained from *o*-dichlorobenzene solutions (10 w/v %) at 60°. Although *o*-dichlorobenzene prevented obtaining olefin carbon chemical shift information, this was the best solvent to dissolve the range of polymers studied. Spectra were obtained using sweep widths of 2500, 1400, 1000, and 300 Hz. But the data shown were obtained

(1) G. Natta, G. Dall'Asta, and G. Mazzanti, *Angew. Chem.*, **76**, 765 (1964).
(2) G. Natta, G. Dall'Asta, I. W. Bassi, and G. Carella, *Makromol. Chem.*, **91**, 87 (1966).
(3) G. Natta, G. Dall'Asta, and G. Mazzanti, U. S. Patent 3,458,489 (1969).
(4) F. Haas, K. Nützel, G. Pampus, and D. Theisen, *Rubber Chem. Technol.*, **43**, 1116 (1970).
(5) G. Dall'Asta and P. Scaglione, *Rubber Chem. Technol.*, **42**, 1235 (1969).
(6) G. Dall'Asta and G. Motroni, *Angew. Makromol. Chem.*, **16**, 51 (1971).
(7) E. A. Ofstead and N. Colderon, *Makromol. Chem.*, **154**, 21 (1972).
(8) V. A. Kormer, I. A. Poletayeva, and T. L. Yufa, *J. Polym. Sci., Part A-1*, **10**, 251 (1972).
(9) C. E. Wilkes, M. J. P. Peklo, and R. J. Minchak, *J. Polym. Sci., Part C*, in press (1973).
(10) R. J. Minchak and H. Tucker, *Polym. Prepr., Amer. Chem. Soc., Div. Polym. Chem.*, **13**, 885 (1972).
(11) (a) R. S. Silas, J. Yates, and V. Thornton, *Anal. Chem.*, **31**, 529 (1959). (b) C. Tosi, F. Ciampelli, and G. Dall'Asta, *J. Polym. Sci., Part C*, **11**, 529 (1973).
(12) H. E. Diem and C. E. Wilkes, in preparation.
(13) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972.
(14) G. C. Levy and G. L. Nelson, "Carbon-13 NMR for Organic Chemists," Wiley-Interscience, New York, N. Y., 1972.
(15) J. Schaefer, *Macromolecules*, **2**, 210 (1969).
(16) J. Schaefer, *Macromolecules*, **2**, 533 (1969).
(17) M. W. Duch and D. M. Grant, *Macromolecules*, **3**, 165 (1970).
(18) L. F. Johnson, F. Heatley, and F. A. Bovey, *Macromolecules*, **3**, 175 (1970).
(19) J. Schaefer, *Macromolecules*, **4**, 98 (1971).
(20) J. Schaefer, *Macromolecules*, **4**, 105 (1971).
(21) J. Schaefer, *Macromolecules*, **4**, 107 (1971).
(22) J. Schaefer, *Macromolecules*, **4**, 110 (1971).
(23) C. J. Carman, A. R. Tarpley, Jr., and J. H. Goldstein, *J. Amer. Chem. Soc.*, **93**, 2864 (1971).
(24) C. J. Carman, A. R. Tarpley, Jr., and J. H. Goldstein, *Macromolecules*, **4**, 445 (1971).
(25) W. O. Crain, Jr., A. Zambelli, and J. D. Roberts, *Macromolecules*, **4**, 330 (1971).
(26) A. Zambelli, G. Gatti, C. Sacchi, W. O. Crain, Jr., and J. D. Roberts, *Macromolecules*, **4**, 475 (1971).
(27) C. J. Carman and C. E. Wilkes, *Rubber Chem. Technol.*, **44**, 781 (1971).
(28) C. E. Wilkes, C. J. Carman, and R. A. Harrington, *J. Polym. Sci., Part C*, in press (1973).
(29) V. D. Mochel, *J. Polym. Sci., Part A-1*, **10**, 1009 (1972).
(30) V. D. Mochel, *J. Macromol. Sci., Rev. Macromol. Chem., Part C*, **8**, 289 (1972).
(31) J. Schaefer and D. F. S. Natusch, *Macromolecules*, **5**, 416 (1972).
(32) J. Schaefer, *Macromolecules*, **5**, 427 (1972).
(33) D. W. White and G. C. Levy, *Macromolecules*, **5**, 526 (1972).
(34) D. E. Dorman, E. P. Otocka, and F. A. Bovey, *Macromolecules*, **5**, 574 (1972).
(35) J. Schaefer, *Macromolecules*, **5**, 590 (1972).
(36) J. Schaefer, S. H. Chin, and S. I. Weismann, *Macromolecules*, **5**, 798 (1972).
(37) K. Matsuzaki, T. Uryu, K. Osada, and T. Kawamura, *Macromolecules*, **5**, 816 (1972).
(38) N. Oguni, K. Lee, and H. Tani, *Macromolecules*, **5**, 819 (1972).
(39) E. B. Whipple and P. J. Green, *Macromolecules*, **6**, 38 (1973).
(40) D. E. Dorman, D. A. Torchia, and F. A. Bovey, *Macromolecules*, **6**, 80 (1973).
(41) A. Allerhand and R. K. Hailstone, *J. Chem. Phys.*, **56**, 3718 (1972).
(42) A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, **55**, 189 (1971).

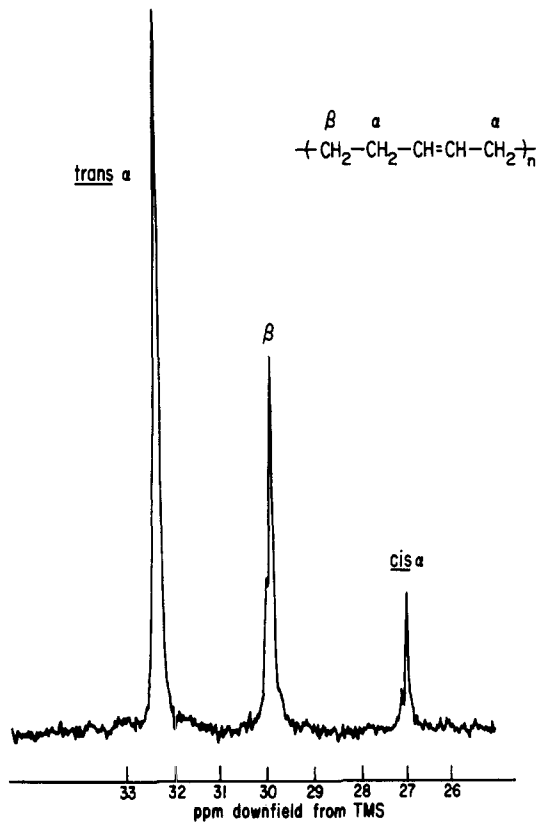

Figure 1. ^{13}C pulsed (FT) nmr spectrum of the methylene carbons from a high trans polypentenamer (85.5% by ^{13}C nmr); 10% w/v in *o*-dichlorobenzene at 60°.

using 1400 Hz. This produced sufficiently high resolution and had no solvent "fold-over." The data were obtained using a pulse repetition rate of 15 sec (2.8-sec acquisition time, delay time of 12.2 sec) in case long spin-lattice relaxation times (T_1) were present. However, later we determined the value of T_1 and the quantitative analysis were not altered by eliminating the 12.2-sec delay time for the highest trans polymer (sample A). The other spectrometer conditions were 43-μsec 90° pulse, 8030 data points, and 300 transients/spectrum. Peak areas were measured by both electronic integration and with a compensating polar planimeter. The latter had a higher precision by a factor of two or three. Future improvements in the computer program for plotting the integrals could improve the precision of electronic integration. Spin-lattice relaxation times (T_1) were measured for the methylene carbons by the inversion-recovery method described by Freeman and Hill.[43]

The polypentenamers were prepared by the ring-opening polymerization of distilled cyclopentene using tungsten hexachloride-triethylaluminum catalysts following previously published procedures.[9,10] Polymers of inherent viscosities in the range of 1.5–6.0 dl/g (toluene, 25°) contained, according to infrared analysis, only cis and trans main-chain double bonds and no vinyl groups. Approximate compositions were determined by an infrared method for cis and trans analysis in polybutadienes.[11] After the approximate infrared analyses, samples were categorized in order of increasing T_g, T_{cr}, and T_m by differential scanning calorimetry[9] and from these we selected eight samples for the development of the ^{13}C nmr method.

Results

The methylene carbon resonances in a ^{13}C nmr spectrum of a high trans polypentamer are shown in Figure 1. The assignments were made based on the ^{13}C chemical shifts reported for *cis*- and *trans*-1,4-polybutadiene. As can be seen in Figure 1, there is fine structure displayed in the cis α and especially in the β resonances. These small resonances reflect configurational sequence information and are a function of trans or cis concentration. We have found, using sufficient resolution, that the trans α resonance reflects similar information. The intent of this report is not to discuss the structural implications of the configurational sequences[44] but to obtain an accurate analysis of total cis and trans composition in polypentamer. Consequently such an analysis can be obtained from area measurements of the cis and trans α resonances. Such measurements would include the entire resonance region assigned to either the cis or trans methylene without consideration of how the fine structure details relate to configurational sequences. Table I gives the ^{13}C chemical shifts of the methylene resonance from polypentenamer. Figure 2 shows a horizontal expansion of the spectrum from Figure 1. These resonance areas were measured using both a planimeter and electronic integration. The mole fractions of cis and trans isomers in the polypentenamer were calculated from the relative areas of the cis and trans α-methylene resonances. The ^{13}C nmr determined composition of a series of eight polypentenamers are given in Table II. Twice the β-methylene resonance area must be equal to the sum of the cis and trans α-methylene areas. Consequently, as a check on internal consistency, the ratio of (trans α + cis α)/2β is also given in Table II. The average deviation from the mean and the number of measurements made on each sample are also shown. Table III shows the peak area measurements for sample F. These data are typical for the observed spread in peak areas measurements. Table IV reports the spin-lattice relaxation times measured for high and low trans polypentenamer. Obviously, the trans content affects the value of T_1. However, the quantitative analytical results shown in Table II will not be affected because a sufficiently long pulse repetition rate was used to preclude attenuation of the resonance areas.

Table I
Carbon-13 Chemical Shifts[a] for Polypentenamer Methylene Carbon Atoms

Methylene Carbon	δc
Cis α	27.0
Trans α	32.4
β	29.9

[a] Parts per million downfield from Me_4Si, chemical shift is for the largest resonance within a resonance region for a 85.5% trans polymer (see Figure 1).

Table II
Polypentenamer Composition[c] Determined by ^{13}C Nmr

Sample	% Trans[a] (Mean Value)	Δ (% Trans)[b]	No. of ^{13}C Measurements
A	85.5	0.4	4
B	82.9	0.8	4
C	80.9	0.7	5
D	68.6	1.6	5
E	62.0	0.7	5
F	28.8	1.1	3
G	16.8	0.5	3
H	6.9	0.4	3

[a] % trans = 100(trans α area)/(trans α area + cis α area). [b] Average deviation of % trans from the mean value. [c] As a check on internal consistency of ^{13}C nmr data, overall mean value of (trans α + cis α)/2β = 1.015 ± 0.016.

Discussion

The reported ^{13}C chemical shifts for the methylene carbon α to the double bond in 1,4-polybutadiene are 27.7

(43) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, 54, 3367 (1971).

(44) C. J. Carman and C. E. Wilkes, in preparation.

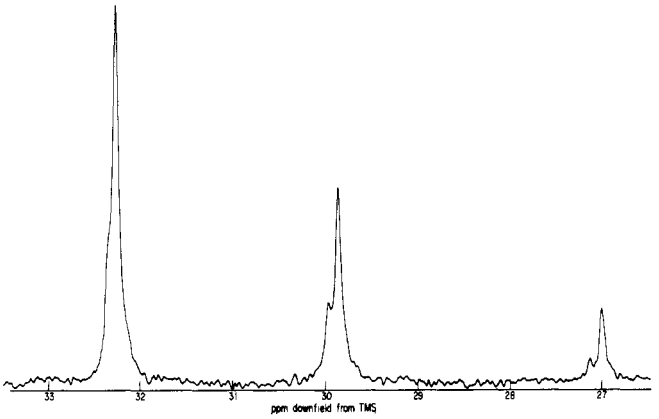

Figure 2. Horizontal expansion of ^{13}C nmr spectrum shown in Figure 1.

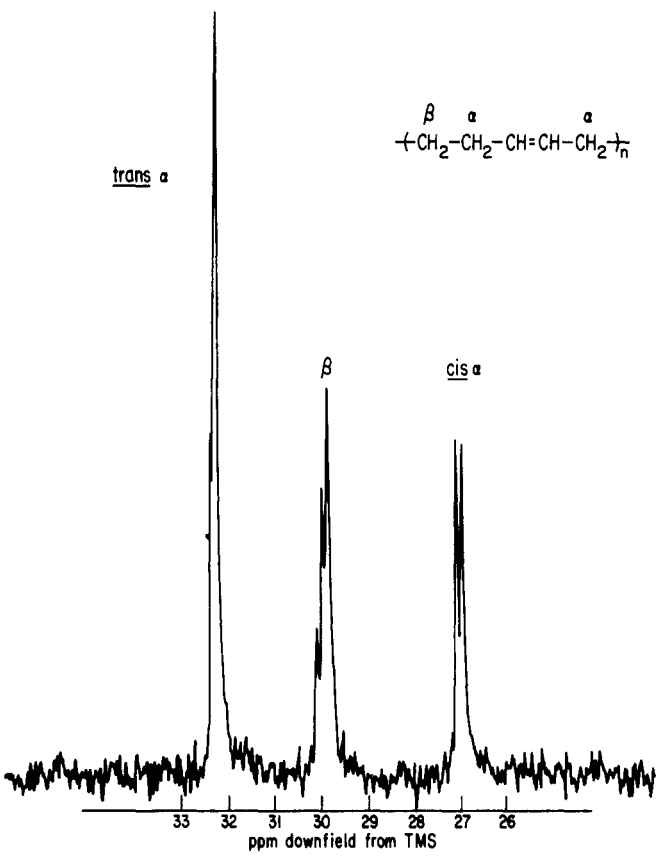

Figure 3. ^{13}C pulsed (FT) nmr spectrum of methylene carbons from a 62% trans polypentenamer; 10% (w/v) in *o*-dichlorobenzene at 60°.

ppm for the cis isomer and 33.1 ppm for the trans isomer.[17,29] The assignments of the α-methylene carbon resonances to cis or trans configurations in polypentenamer were made by comparing the observed chemical shifts in Table I to the data for polybutadiene.[17,29] The very close agreement between the ^{13}C chemical shifts of the α-methylene carbon in these two polymer systems seems more than coincidental and gives us confidence in our assignments. The resonance at 29.9 ppm can be assigned to the β peak. This peak is predicted[45-47] to be at 30.0 ppm. Also its area relative to the assigned α-methylene carbon resonances satisfies a necessary critereon. Consider the β-

(45) C. J. Carman, A. R. Tarpley, Jr., J. H. Goldstein, *Macromolecules*, 6, 719 (1973).
(46) L. P. Lindeman and J. Q. Adams, *Anal. Chem.*, 43, 1245 (1971).
(47) D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, 86, 2984 (1964).

Table III
Representative ^{13}C Nmr Area Measurements[a]

Expt No.[b]	Resonance Areas: Trans α	β	Cis α	% Trans[c]	R[d]
1	0.88	1.60	2.30	27.7	0.994
2	0.82	1.39	2.10	28.2	1.050
3	0.90	1.55	2.05	30.5	0.952

[a] Data were those used to analyze polypentenamer sample F in Table II. [b] Experiments 1, 2, and 3 were run on three successive weeks. [c] % trans = 100(trans α area)/(trans α area + cis α area). [d] R = (trans α + cis α)2β.

Table IV
Spin-Lattice Relaxation Times

Sample	% Trans	T_1 (msec)[a]: Trans α	β	Cis α
A	85.5	525	556	539
G	16.8	1370	1220	1290

[a] Measured at 39°C using a 300-Hz sweep width, 12.0-sec acquisition time, and 13.0-sec delay tlme.

methylene carbon in a polypentenamer chain.

$$\underset{\alpha}{-CH=}\underset{\alpha}{CHCH_2}(-\underset{\beta}{CH_2}\underset{\alpha}{CH=CHCH_2}-)_n$$

The β-methylene resonance area will be half the area of the total methylene carbons which are α to the double bond. As shown in Table II, the relationship

$$(\text{cis } \alpha + \text{trans } \alpha)/2\beta = 1$$

holds regardless of composition. (This of course assumes no vinyl content, which is verified by infrared study.) Not only does this equation substantiate the assignment of the resonance at 29.9 ppm, but it also provides a method to check for internal consistency within a spectrum.

Of the eight polymers studied, only sample D showed evidence of a resonance at 30.7 ppm due to some impurities. This impurity could come from cyclic structures previously suggested,[11b] or some small molecule impurities. The area of the resonance at 30.7 ppm was 3.3% of the total methylene areas. However, the presence of the impurity does not seem to affect the α or β resonances used for the composition analysis. The ratio of (cis α + trans α)/2β was 0.984 for an average of five measurements.

One must consider the effects of both spin-lattice relaxation (T_1) and nuclear Overhauser enhancement (NOE) on quantitative analysis of ^{13}C nmr spectra. Schaefer and Natusch have shown that within a given polymer system all carbons undergo equal NOE.[31] Even though maximum[48,49] theoretical NOE may not be achieved, one can use integrated peak areas of carbon spectra to perform quantitative analyses. Certainly the trans and cis α-methylene carbons have equal NOE as there would be lack of consistency relative to the β peak otherwise. We are concerned that perhaps T_1 would be different for the cis and trans α methylene resonances. During the initial investigation we used a delay time of 12.2 sec in conjunction with a 2.8-sec acquisition time. Using such a long repetition time between pulses assured us that the measured areas should not be attenuated if one of the carbons had a long T_1. Subsequent measurement of T_1, using the inversion recovery technique,[43] found that, within experimen-

(48) K. F. Kuhlmann and D. M. Grant, *J. Amer. Chem. Soc.*, 90, 7355 (1968).
(49) K. F. Kuhlmann, D. M. Grant, and R. K. Harris, *J. Chem. Phys.*, 52, 3439 (1970).

tal error, T_1's for the α-methylene carbons did not differ from each other or from the β carbon, at a given isomer composition. (The values for T_1 are given in Table III.) The average T_1 of 530 msec for a 85.5% trans polymer is close to values reported for similar carbons in high molecular weight polymers.[31,32,41,42] An increase in T_1 to 1.29 sec for the high cis polymer is noteworthy and possibly reflects an increase in segmental motion.[50] Until we do a more detailed study of the effect of isomer composition on T_1, we do not feel it is advisable to comment further on the increase in T_1. However, as mentioned in the Discussion section, the data were obtained under instrument conditions that allowed for the longer value in T_1. Consequently, the analytical results in Table II are reliable. Sample A was reanalyzed using only an acquisition time of 2.8 sec (the 12.2-sec delay time was eliminated). The same analysis for trans content was obtained, and the analysis time was reduced from 1.2 hr to 14 min.

We indicated that in Figures 1 and 2, the trans α and cis α resonances are each actually composed of two resonances, and the β resonances, three resonances. We suggest that each of the three major kinds of methylene carbons may also reflect linkage of cis and trans monomers. Figure 3 shows how the appearance of the spectrum changes at a higher cis concentration. The interpretation of the spectra in terms of sequences of configurational linkages is not needed for the analytical determination of cis and trans configuration. However, we do point out that there is no resonance due to cis–trans linkages intermediate in chemical shift to the trans at 33.1 ppm and cis at 27.7 ppm. Such an intermediate peak was expected for cis–trans linkages in polybutadiene.[29] If such a peak had been present in polypentenamer the analytical problem would have been considerably more complicated. An interpretation of the ^{13}C spectra of polypentenamers in terms of configurational sequence distribution will be the topic of a later report.[44]

The simplicity and internal consistency of these data demonstrate that polypentenamer is the very regular, repeating structure, $(-CH{=}CHCH_2CH_2CH_2-)_n$. There is no apparent isomerization during polymerization forming sequences having two and four methylene groups between olefinic carbons. If $-CH{=}CHCH_2CH_2CH{=}CH-$ were present, we would observe resonances analogous to polybutadiene at 27.7 ppm for cis α-CH_2 carbons or at 33.1 ppm for trans α-CH_2 carbons. These were not detected. If a significant amount of $-CH{=}CHCH_2CH_2CH_2CH_2CH{=}CH-$ were present, we would not have observed the consistency in the relationship, (cis α + trans α)/2β = 1, as a function of composition. The consistency observed for this relationship precludes a significant contribution from the sequence of four methylene groups between a pair of olefinic carbons.

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(50) G. C. Levy, *Accounts Chem. Res.*, 6, 161 (1973).

Influence of Conformational Isomers on the Circular Dichroism of Poly(L-prolylglycine)[1]

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ABSTRACT: The circular dichroism of poly(L-prolylglycine), which has previously been shown to be in a statistical conformation, has been measured in 2:1 (v/v) ethylene glycol–water over the temperature range 50 to −132°. Only negative circular dichroism is observed in the spectral range covered. The minimum near 202 nm changes from −6.0 cm^2/mmol at 50° to −9.3 cm^2/mmol at −132°, and an isosbestic point is observed near 213 nm. There is a one-to-one correlation between the circular dichroism at the minimum and the area of the two resonances observed for the α proton of the L-prolyl residue and the amide proton of the glycyl residue in the 220-MHz pmr obtained by Torchia. Consequently the same configurational change must be responsible for the effects seen in the proton nmr and CD. These effects have previously been attributed to cis–trans isomerization about the glycyl-L-prolyl peptide bond. However, the possibility that isomerization may also occur about the C^α—C′ bond in the L-prolyl residue cannot be eliminated.

The statistical conformation of a polypeptide chain is determined by its conformational energy map[3-5] and the concomitant statistical mechanical averaging.[4,6] The usual considerations of torsional potentials, attractive and repulsive nonbonded interactions, and dipolar interactions, which determine the conformational energy as a function of rotational angle, are influenced by the side group on the α-carbon atom and the solvent medium. Isomerization about the peptide bond will, in addition, also affect the conformational energy and thus the average chain structure. We can, therefore, expect that different polypeptide chains, or the same chain in different thermodynamic environments, despite being in statistical conformations and displaying the properties expected for disoriented chain molecules, will reflect different populations of allowed rotational states characteristic of the single bonds. A randomly coiled polypeptide chain can thus re-

(1) This work was supported by a contract with the Division of Biology and Medicine, Atomic Energy Commission.
(2) Recipient of Public Health Service Postdoctoral Fellowship from the National Institute of General Medical Sciences.
(3) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, 87, 2788 (1965).
(4) P. J. Flory, "Statistical Mechanics of Chain Molecules," Wiley, New York, N. Y., 1969, Chapter VII.
(5) G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, 23, 283 (1968).
(6) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, 87, 2791 (1965).