from the solvent by the side chains of Tyr(29) on one side and Tyr(24) and Tyr(28) on the other. Access of solvent to hydrogen bond ($N_{27}$—$H_{27}\cdots O_{25}$) seems to be hindered partly by $C^{\beta}$ of Thr(26) and also by the phenolic oxygen of Tyr(29) which appears to be within hydrogen-bonding distance of $O_{25}$.

The agreement between the backbone conformational angles and hydrogen bond lengths observed for the "$\gamma$ turn" in thermolysin and those predicted by Némethy and Printz[6] (Table I) is quite good, particularly for the crucial conformation at the second $\alpha$-carbon. Conformations lying in this region of energy diagrams (near $\phi = 60°, \psi = -60°$) were designated as "disallowed" for residues other than glycine in early studies,[12,13] but more recent calculations[14-17] have suggested that this conformation might be allowed, or even favored. The conformational angles of (86°, −57°) observed for Thr(26) in thermolysin may be compared with the conformations at energy minima which have been predicted to occur at (∼60°, ∼−60°),[14,15] (69°, −69°),[16] and (80°, −60°).[17] The conformation is characterized by a bent hydrogen bond ($N_3$—$H_3\cdots O_1$) which contributes favorably to the conformational energy, and by close approaches ($C_2^{\beta}\cdots O_1$) and/or ($N_3$—$H_3\cdots O_1$), which were originally considered too short to be allowed.[12,13] There is precedent for the occurrence of this conformation in globular proteins. For example, in lysozyme,[18] Phe(38) has been reported to have the conformation (112°, −27°), and in ribonuclease S[19] Lys(37) was reported to have a conformation near (77°, −45°). On the other hand, participation of a residue with this conformation in a $\gamma$ turn has not been heretofore reported. It will be noted in Table I that the biggest discrepancy between the observed conformations[20] and those predicted by Némethy and Printz[6] is (−40°, +36°) for ($\Delta\phi_1$, $\Delta\psi_1$). Since these differences are approximately equal and opposite, they tend to offset each other in such a way that the observed course of the polypeptide chain is quite similar to that predicted theoretically (see Figure 1 of ref 6). The predicted values for ($\phi_1$, $\psi_1$) lie just outside the low-energy region of most conformational maps, whereas the observed value for Ser(25) lies inside the "fully allowed" region.[12]

It may be noted that model-building experiments show that another polypeptide chain reversal is possible by utilizing three $\alpha$-carbon atoms, $C_1^{\alpha}$, $C_2^{\alpha}$, $C_3^{\alpha}$, with dihedral angles approximately (70°, −170°), (−86°, 57°), and (−155°, −60°). In this case the hydrogen bonds are ($N_3$—$H_3\cdots O_1$), as in the $\gamma$ turn, and ($N_4$—$H_4\cdots O_0$) [*cf.* $N_1H_1\cdots O_3$], the former arising from conformational angles inverse to those at $C_2^{\alpha}$ in the $\gamma$ turn. This alternative three-$\alpha$-carbon chain reversal might therefore be designated the "inverse $\gamma$ turn," or the "inverse 1–3 turn." Note that the $\gamma$ turn and inverse $\gamma$ turn are not to be confused with the "$\gamma$ helix"[21] which has the conformation (84°, 78°).

The occurrence of a $\gamma$ turn in thermolysin suggests that it may be found in other proteins, and should therefore be considered as a potential structural feature along with $\beta$ turns, $\beta$ structure, and helices. Furthermore, the $\gamma$ turn and conformations near to (60°, −60°) should also be considered as possible conformations in structural predictions for both macromolecules and smaller peptides.

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## Microstructure Analysis of Poly(propylene oxide) by $^{13}C$ Nuclear Magnetic Resonance Spectroscopy

The microstructure analysis of a polymer provides indispensable information on the mechanism of the polymerization reaction. The $^1H$ nmr analysis of the microstructure of poly(propylene oxide-$\alpha$-$d_1$) developed in this laboratory[1] provided some information on the mode of action of catalysts used in the polymerization.[2] Unfortunately, the application of this method is limited to the dyad structure.

The application of $^{13}C$ nmr spectroscopy, which results in chemical shifts larger than those by $^1H$ nmr has been developed for vinyl polymers. $^{13}C$ nmr analysis of poly(propylene oxide) described in this paper provides new information about the triads of methine carbons in the main chain, and is in disagreement with the analysis made by Schaefer.[3]

Five different samples of poly(propylene oxide) whose microstructures had been analyzed by $^1H$ nmr of their monodeuterio derivatives[1,2] were prepared using three kinds of catalyst: I and II, amorphous polymers prepared with *tert*-BuOK and $Al(C_2H_5)_3$–$H_2O$ (molar ratio 1:1) catalysts, respectively; and the crude (III), crystalline (IV), and amorphous polymers (V) prepared using $Zn(C_2H_5)_2$–$H_2O$ (molar ratio 1:0.8) freeze-dried catalyst. Natural-abundance $^{13}C$ nmr spectra at 25.1 MHz were measured in deuteriobenzene at 30° with proton noise decoupling using a Varian XL-100-15 spectrometer equipped with a standard VFT-100X Fourier transform system. Internal field frequency stabilization of the spectrometer was provided by a lock signal from tetramethylsilane dissolved in the solvent. The peak area ratio was determined by using a Du Pont 310 curve resolver.

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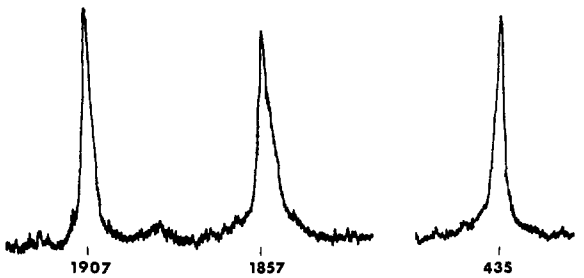


Figure 1. Proton noise-decoupled 25.1-MHz $^{13}C$ nmr spectrum of the main-chain carbons of crystalline poly(propylene oxide). The numbers give the separation in hertz upfield from the proton-decoupled line of tetramethylsilane at 30°. The magnetic field increases from right to left.

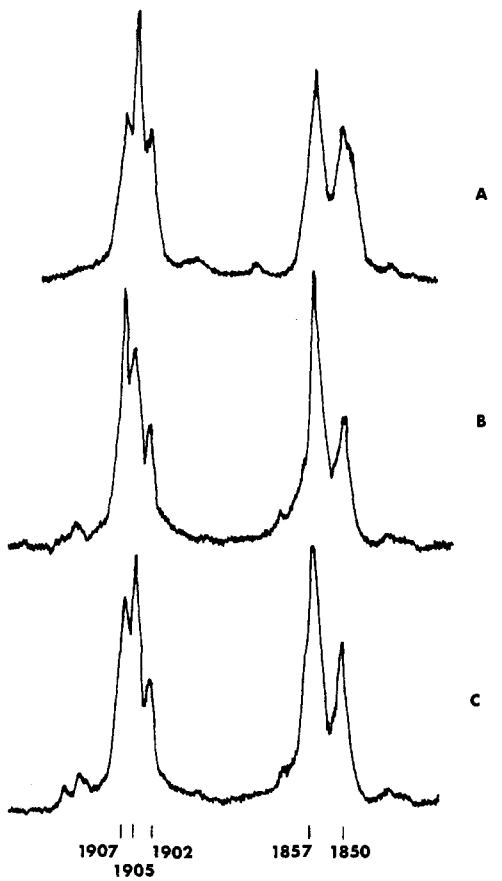


Figure 2. Proton noise-decoupled 25.1-MHz $^{13}C$ nmr spectra in the methylene and methine carbon region of the amorphous polymer (A) prepared with *tert*-BuOK catalyst and of the crude (B) and amorphous (C) polymers prepared with $Zn(C_2H_5)_2$–$H_2O$ (1:0.8) catalyst.

Assignment of absorption bands observed in the spectrum of the crystalline polymer (IV) was performed by the off-resonance decoupling method. Three peaks observed at 435, 1857, and 1907 Hz downfield from tetramethylsilane can be identified as arising from methyl, methylene, and methine carbons, respectively (Figure 1). These chemical shifts are in the same order as the assignments by Schaefer.[3] Sample I, prepared with *tert*-BuOK catalyst, is interpreted as an almost completely atactic polymer because it contains equal amounts of isotactic and syndiotactic dyads and practically no head-to-head and tail-to-tail linkages in the polymer main chain.[1,2,4]

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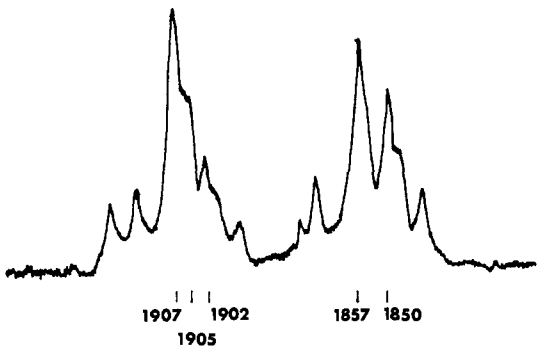


Figure 3. Proton noise-decoupled 25.1-MHz $^{13}C$ nmr spectrum of the methylene and methine carbon region of the amorphous polymer prepared with $Al(C_2H_5)_3$–$H_2O$ (1:1) catalyst.

The spectrum of the sample I (A in Figure 2) shows three peaks in the methine carbon region and two main peaks in the methylene carbon region. It is reasonable to assign two of the methylene carbon peaks (observed peak area ratio 50:50) to isotactic and syndiotactic dyads (i and s), and three methine carbon peaks (observed peak area ratio 25:50:25) to isotactic, heterotactic, and syndiotactic triads (I, H, and S). Two peaks observed at 1857 and 1907 Hz in the spectrum of the crystalline isotactic polymer sample (IV) are therefore assigned to isotactic dyad and triad, respectively. These assignments lead naturally to those of syndiotactic dyad (1850 Hz), syndiotactic triad (1902 Hz), and heterotactic triad (1905 Hz). Correctness of these assignments is confirmed by comparing the spectra of the crude (III) and amorphous (V) samples prepared with the $Zn(C_2H_5)_2$–$H_2O$ catalyst. The former shows higher intensities of isotactic dyad and triad and lower of syndiotactic dyad than the latter (B and C in Figure 2). In both spectra, the equations $i = I + H/2$ and $s = S + H/2$ are also satisfied.

Poly(propylene oxide) contains a true asymmetric carbon atom in each monomeric unit, so that there should be two "heterotactic" triad sequences, destinguishable at least in principle. Nevertheless, the spectra described above show a singlet for the heterotactic triad, possibly because of the strong magnetic shielding effect of oxygen.

The occurrence of head-to-head and tail-to-tail linkages should produce a complicated nmr spectrum in which the presence of 4 (dyad) and 16 (triad) lines is expected, corresponding to the structural and configurational isomeric monomeric units. While Schaefer observed 3 peaks in the methine carbon region and four peaks in the methylene carbon region, in this work 13 visible peaks are observed in the spectrum of sample II (Figure 3), possibly due to the presence of these abnormal linkages. This problem should be solved by examination of the spectra of a series of optically active poly(propylene oxide) samples differing in their degrees of specific rotation, and this work is now in progress.

In the wide-sweep spectrum of sample I, splitting of the downfield peak in the methylene proton region is observed, very possibly originating from different tetrad sequences.

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