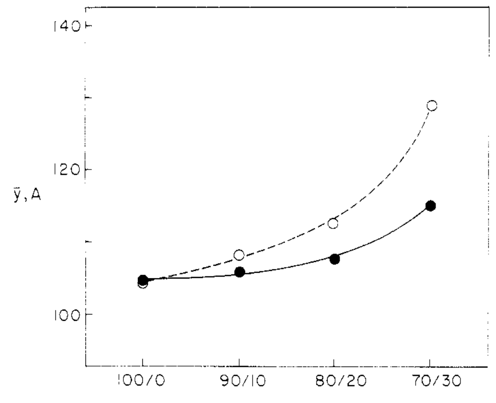


Figure 3. Mean thickness of the amorphous layers, $\bar{y}$. The solid curve is calculated from the improved model, the broken curve from Hosemann's model.

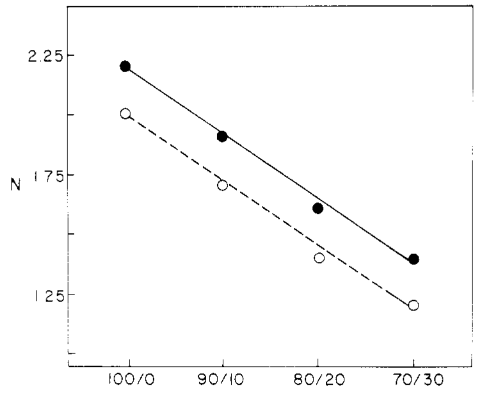


Figure 4. Average number of crystals in a stack calculated for both models (solid curve, improved model; broken curve, Hosemann's model).

lation of the crystals, clusters of crystal lamellae as well as crystals with no correlated neighbors will occur in the sample.

For crystallinities below 50% the shape of the scattering curve is determined primarily by the fluctuation of the interlamellar distance distribution function. To what extent lamellar twisting or similar dislocations of the supermolecular lattice leads to erroneous determinations of structure parameters cannot be concluded from these experiments alone. Evaluations of crystal size parameters from wide-angle X-ray scattering curves[1] yield values of the lamellar thicknesses of the same order of magnitude as the present results, and since there is no reason to assume the two models deal differently with the problem, we feel justified in discussing the parameters obtained at least qualitatively. Lamellar twisting, however, would primarily affect the correlation parameter N. Electron micrographs of iPS/PPO blends, obtained by the defocusing method,[7] show bundles of 3–4 lamellae per cluster. It is difficult to compare morphological parameters derived from electron micrographs to those calculated with a one-dimensional model; it seems, however, that the statistical parameter N does not exactly define the number of the lamellae stacked in a cluster. However, the results do indicate that in this system of imperfectly formed crystalline aggregates both models show, albeit somewhat qualitatively, that blending further lowers the apparent lamellar correlation.

The calculations show that for both models the correlation decreases with increasing PPO content (Figure 4). This is confirmed by electron microscopy, which seems to show an even steeper decrease of this parameter.[7] The results show that the lamellar cluster model has to be used carefully when systems with a very low orientation correlation of the lamellae are investigated. Although the order of magnitude of the correlation parameters and their variation with sample composition can be obtained with some accuracy, no absolute values can be given.

Acknowledgment. F.E.K. thanks AFOSR for support through AFOSR Grant 84-0033.

Registry No. Isotactic polystyrene, 25086-18-4; PPO, 24938-67-8.

References and Notes

(1) Hammel, R.; MacKnight, W. J.; Karasz, F. E. *J. Appl. Phys.* **1975**, *46*, 4199.
(2) Wenig, W.; Karasz, F. E.; MacKnight, W. J. *J. Appl. Phys.* **1975**, *46*, 4194.
(3) Hosemann, R. *Z. Phys.* **1939**, *114*, 10.
(4) Hosemann, R.; Baghi, S. N. "Direct Analysis of Diffraction by Matter"; North-Holland Publishing Co.: Amsterdam, 1965.
(5) Laue, M. V. "Rontgenstrahlinterferenzen"; Akad. Verlagsgesellschaft: Frankfurt/Main, 1960; Chapter 2.
(6) Wenig, W.; Bramer, R. *Colloid Polym. Sci.* **1978**, *256*, 125.
(7) Petermann, J., unpublished results.

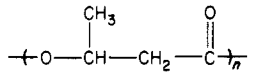
---

^{1}H and ^{13}C NMR Analysis of Poly(β-hydroxybutyrate) Isolated from *Bacillus megaterium*

YOSHIHARU DOI,* MASAO KUNIOKA,
YOSHIYUKI NAKAMURA, and KAZUO SOGA

*Research Laboratory of Resources Utilization,
Tokyo Institute of Technology, Nagatsuta, Midori-ku,
Yokohama 227, Japan.* *Received October 25, 1985*

Poly(β-hydroxybutyrate) (PHB) is a naturally occurring polyester that is synthesized by the condensation of D-(−)-β-hydroxybutyryl coenzyme A in a wide variety of bacteria.[1-3] The polymer is highly crystalline and optically active[4] and functions as both a source of energy and carbon supply for the bacteria.[5] Recently, PHB has attracted industrial attention as a possible candidate of the large-scale biotechnological products.[6] Infrared analysis, elemental analysis, and hydrolysis of the polymer have supported a linear head-to-tail polyester with the following formula:[7-9]



$$\left[O-\overset{CH_3}{\overset{|}{CH}}-CH_2-\overset{O}{\overset{\|}{C}} \right]_n$$

An X-ray fiber diagram of optically active PHB in the solid state showed a fiber repeat of 5.96 Å, corresponding to the length of two monomeric units (2_1 helix).[9,10] The solution properties of PHB in several solvents have been studied by means of intrinsic viscosity, optical rotatory dispersion, and light scattering,[11,12] which indicate retention of the helical conformation in chloroform.

In this paper, we report ^{1}H and ^{13}C NMR spectra of PHB isolated from *Bacillus megaterium* and analyze the possible conformation of PHB in chloroform.

Figure 1 shows a proton-noise-decoupled ^{13}C NMR spectrum at 125 MHz of PHB in chloroform at 27 °C. Every peak of PHB is very sharp and only four lines remain. This result is consistent with that for a PHB containing regular head-to-tail sequences where all methyl, methylene, methine, and carbonyl carbons occupy struc-

0024-9297/86/2219-1274$01.50/0

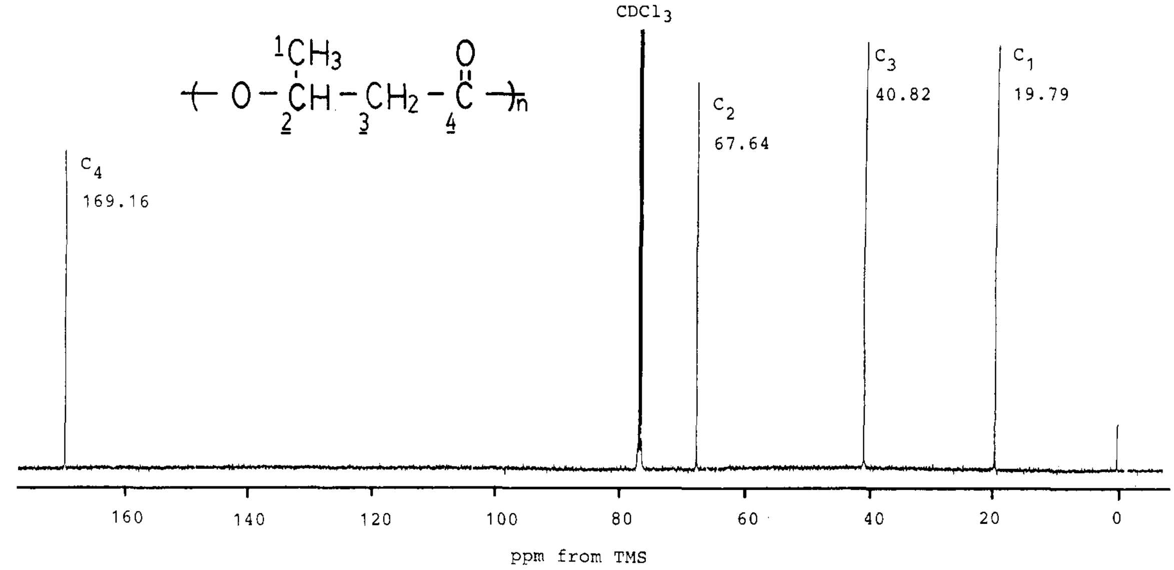


Figure 1. Proton-noise-decoupled ^{13}C NMR spectrum at 125 MHz of PHB in chloroform at 27 °C. Chemical shifts are in ppm downfield from Me_4Si.

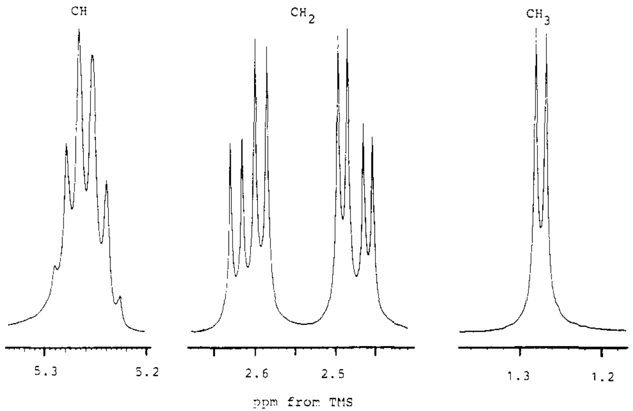
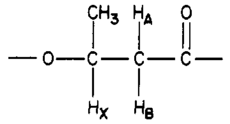


Figure 2. ^1H NMR spectrum at 500 MHz of PHB in chloroform at 27 °C. Chemical shifts are in ppm downfield from Me_4Si.

turally identical environments. In addition, a ^{13}C NMR spectrum without proton decoupling of PHB was recorded for assignment of the four peaks. The observed spectrum led to the chemical shift assignment for the ^{13}C resonances as shown in Figure 1.

Figure 2 shows a ^1H NMR spectrum at 500 MHz of PHB in chloroform at 27 °C. The doublet resonance (J_{H-H} = 6.4 Hz) at 1.274 ppm is assignable to methyl protons, and the multiplet resonance at 5.26 ppm is assigned to methine proton. The methylene proton resonance at 2.45–2.65 ppm is associated with methine proton (H_X) and is analyzed as ABX type with a vicinal coupling of H_A and H_B protons. From the spectrum in Figure 2, we have the following parameters:



$$-O-\overset{CH_3}{\underset{H_X}{C}}-\overset{H_A}{\underset{H_B}{C}}-\overset{O}{\overset{\|}{C}}-$$

H_A, 2.474 ppm; H_B, 2.607 ppm

J_{AB} = -15.5 Hz, J_{AX} = 5.7 Hz, J_{BX} = 7.3 Hz

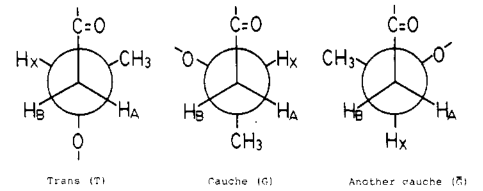


Figure 3. Newman projections of the three rotational isomeric states.

The above parameters may be interpreted in terms of chain conformation.[13] The observed vicinal couplings are averaged over the three possible conformers; trans (T), gauche (G), and another gauche ($\bar{G}$), as shown in Figure 3. Then, the observed coupling constants J_{AX} and J_{BX} are represented by average values of the component coupling constants in the three conformers weighted by their fractional populations P_T, P_G, and $P_{\bar{G}}$ as

$$J_{AX} = P_TJ_t + P_GJ_G + P_{\bar{G}}J_{\bar{G}} \quad (1)$$

$$J_{BX} = P_TJ_g + P_GJ_t + P_{\bar{G}}J_{\bar{G}} \quad (2)$$

$$1 = P_T + P_G + P_{\bar{G}} \quad (3)$$

where J_g and J_t are the gauche and trans vicinal coupling constants. Assuming the reasonable values of J_g = 2 Hz and J_t = 11 Hz,[13] we find from eq 1–3 that P_T = 0.41, P_G = 0.59, and $P_{\bar{G}}$ = 0.00. The calculated populations suggest at least the qualitative conclusion that the G and T conformers are predominantly present in chloroform and the population of $\bar{G}$ conformer is substantially small. This conclusion is in good agreement with the energy calculation by Cornibert and Marchessault,[10] who have indicated a left-handed 2_1 helix of G conformer units for the solid state of PHB. In addition, the predominance of the G conformer in chloroform is consistent with the previous observation[11] by means of intrinsic viscosity and optical rotatory dispersion, which suggested that PHB retains the helical conformation in chloroform.

Experimental Section

The PHB sample was isolated from *B. megaterium* KM, which was grown in a medium containing 2.0% (w/v) glucose and 0.1 M sodium acetate as described by Macrae and Wilkinson.[5] The procedure of PHB isolation involved centrifugation of cells, drying from acetone, and finally extraction of PHB with hot chloroform in a Soxhlet apparatus. The extract was evaporated to dryness, the residue was redissolved in chloroform, and PHB was precipitated with ether. The ^1H and ^{13}C NMR analyses of the PHB sample were carried out on a JEOL GX-500 spectrometer in the pulse Fourier transform (FT) mode. The solution of PHB was made up in $CDCl_3$ to 2.0% (w/v) without degassing. The spectra were recorded at 27 °C with 3.0 s of pulse repetition in $CDCl_3$ solution with Me_4Si as an internal reference.

Acknowledgment. We thank Professors I. Karube and Y. Inoue for helpful discussions and Dr. E. Tamiya for experimental guidance in the preparation of the PHB sample.

Registry No. PHB (SRU), 26744-04-7; PHB (homopolymer), 26063-00-3.

References and Notes

(1) Merrik, J. M.; Doudoroff, M. *Nature (London)* **1961**, *189*, 890.
(2) Senior, P. J.; Dawes, E. A. *Biochem. J.* **1971**, *125*, 55.
(3) Saito, T.; Fukui, T.; Ikeda, F.; Tanaka, Y.; Tomita, K. *Arch. Microbiol.* **1977**, *114*, 211.
(4) Ellar, D.; Lundgren, D. G.; Okamura, K.; Marchessault, R. H. *J. Mol. Biol.* **1968**, *35*, 489.
(5) Macrae, R. M.; Wilkinson, J. F. *J. Gen. Microbiol.* **1958**, *19*, 210.
(6) King, P. P. *J. Chem. Technol. Biotechnol.* **1982**, *32*, 2.
(7) Lemoigne, M. *Ann. Inst. Pasteur, Paris* **1925**, *39*, 144.
(8) Williamson, D. H.; Wilkinson, J. F. *J. Gen. Microbiol.* **1958**, *19*, 198.
(9) Okamura, K.; Marchessault, R. H. "Conformation of Biopolymers"; Ramachandran, G. N., Ed.; Academic Press: London, 1967; p 709.
(10) Cornibert, J.; Marchessault, R. H. *J. Mol. Biol.* **1972**, *71*, 735.
(11) Marchessault, R. H.; Okamura, K.; Su, C. J. *Macromolecules* **1970**, *3*, 735.
(12) Cornibert, J.; Marchessault, R. H.; Benoit, H.; Weill, G. *Macromolecules* **1970**, *3*, 741.
(13) Bovey, F. A. "High Resolution NMR of Macromolecules"; Academic Press: New York, 1972; Chapter IX.

Communications to the Editor

Homo- and Heteroadduct Polymers Derived from Unsymmetric Diolefin Crystals

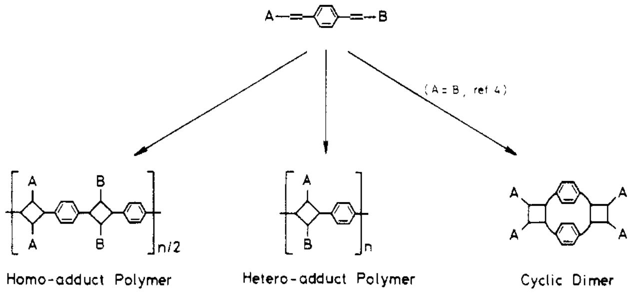
Addadi and Lahav[1,2] prepared optically active dimer and oligomers from a series of α-cyano-1,4-phenylenediacrylic ester crystals via a topochemical photoreaction. Recently, we reported that some 4-(3-oxo-3-phenyl-1-propenyl)cinnamate and 1,4-phenylenediacrylate derivatives photopolymerize into crystalline linear polymers with high molecular weight in high yields.[3] According to previous results,[1-4] various types of photoadducts, including homo- and heteroadduct polymers or cyclic dimer, can be anticipated from the topochemical reaction of unsymmetrically substituted diolefinic compounds as shown in Scheme I. More recently, we found a series of photoreactive crystals of 4-[2-(2- or 4-pyridyl)vinyl]cinnamate derivatives.

In the present communication, we report the photopolymerization behavior of two diolefin derivatives: methyl 4-[2-(4-pyridyl)vinyl]cinnamate (**1**) and ethyl α-cyano-4-[2-(4-pyridyl)vinyl]cinnamate (**2**).[5]

Fine crystals of **1**, recrystallized from methanol (mp 149 °C), were dispersed in water–ethanol (9:1 volume ratio) and, with stirring, irradiated with a 500-W high-pressure mercury lamp through a uranium filter under a nitrogen atmosphere at room temperature for 13 h.

The IR spectrum of the photoproduct showed the disappearance of the carbon–carbon double bond (1630 and 975 cm^{-1}) and a shift of the carbonyl group to higher wavenumber (1710 → 1730 cm^{-1}), indicating the formation of saturated ester. In the ^{1}H NMR spectrum, olefinic peaks were absent and four new broad signals were observed at δ 3.10, 4.35, 6.9–7.1, and 8.15. The broad peak at δ 4.35 (4 H) is characteristic of protons bonded to a cyclobutane, and other peaks at δ 3.10, 6.9–7.1, and 8.15 are assigned to methoxy protons (3 H), protons at the 3- and 5-positions of 4-pyridyl (2 H) and 1,4-phenylene (4 H), and protons at the 2- and 6-positions of 4-pyridyl (2 H), respectively.

Scheme I



Comparing these spectroscopic data with those of other cyclobutane-containing polymers,[6] we assign the photoproduct to linear polymer **3** with recurring cyclobutane and aromatic rings alternating in the main chain. From the continuous change of the X-ray diffraction pattern during the reaction, the monomer crystal was gradually transformed into a nearly amorphous polymer. Although the final photoproduct is amorphous, it is obvious that the polymer is produced under topochemical control because a single configuration of the cyclobutane is formed exclusively.

Polymer **3** had an inherent viscosity of 0.47 dL/g at 0.30 g/dL in *m*-cresol at 30 °C and was soluble in *N*,*N*-dimethylformamide, dimethyl sulfoxide, and strong acids such as concentrated HCl, indicating electrolyte character of the polymer chain.

0024-9297/86/2219-1276$01.50/0