1762 *Macromolecules* **1996,** *29,* 1762−1766

Production of Poly(3-hydroxyalkanoates) Containing Aromatic Substituents by *Pseudomonas oleovorans*

Joanne M. Curley,[†] **Baki Hazer,**[‡] **and Robert W. Lenz***

Department of Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts 01003

R. Clinton Fuller

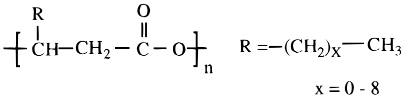
Department of Biochemistry, University of Massachusetts, Amherst, Massachusetts 01003

Received August 14, 1995; Revised Manuscript Received November 29, 1995[⊗]

ABSTRACT: *Pseudomonas oleovorans* was grown separately on 5-(4′-tolyl)valeric acid, 5-(4′-ethylphenyl)-valeric acid, 5-(4′-biphenyl)valeric acid, and 8-(4′-tolyl)octanoic acid either as the sole carbon source or as a coefeed with either nonanoic acid or 5-phenylvaleric acid. For polymer production, 5-(4′-tolyl)valeric acid was the most effective growth substrate of the five. It resulted in the production of poly-3-hydroxy-5-(4′-tolyl)valerate, a crystalline polymer with a glass transition temperature of 18 °C and a melting transition of 95 °C. This poly(3-hydroxyalkanoate) (PHA) is apparently the first example of a crystalline aromatic-containing bacterial PHA. When *P. oleovorans* was cofed an equimolar mixture of 5-phenylvaleric acid and 5-(4′-tolyl)valeric acid, the polymer produced contained 36 mol % of 3-hydroxy-5-phenylvalerate and 64 mol % of 3-hydroxy-5-(4′-tolyl)valerate, and it did not crystallize.

Introduction

Poly(3-hydroxyalkanoates) (PHAs) are a class of polyesters produced as reserve materials by a large number of bacteria when these microorganisms experience metabolic stress, such as a limitation of nitrogen, oxygen, or other essential nutrients in the presence of an excess of carbon source.[1−3] PHAs are an excellent storage material as they are highly reduced and exert no osmotic pressure. These polyesters have the general repeating unit structure shown below, in which the structure of the R group is dependent upon the carbon substrate and the type of bacteria:

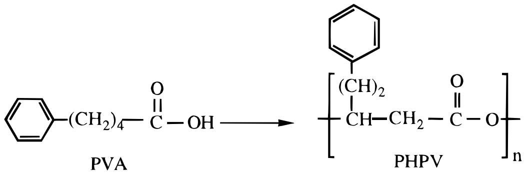


$$\left[\overset{R}{\overset{|}{C}}H-CH_2-\overset{O}{\overset{\|}{C}}-O\right]_n \quad R = -(CH_2)_x-CH_3 \quad x = 0 - 8$$

The microorganisms which produce PHAs can be divided into two classes. The first class are bacteria that store polyesters with short alkyl groups, in which $x = 1-2$ and which are referred to as short chain length PHAs (SCL-PHAs). The second group are those which produce PHAs with longer alkyl groups, in which $x = 3-9$, and which are referred to as medium chain length PHAs (MCL-PHAs).[4]

Pseudomonas oleovorans, which produces MCL-PHAs, has proven to be a versatile organism because this bacterium can produce polyesters when it is grown on a wide variety of carbon substrates, including alkanes, alcohols, and alkanoic acids with chain lengths between 6 and 14 carbons.[5−8] The length of the alkyl group, R, in the resulting polymer can vary between 3 and 9 carbon atoms ($x = 2-8$), and the PHA produced is a random copolymer in which the repeating unit contains the same number of carbons as the feed substrate and can also contain either two carbons less or two carbons more than the feed substrate. The formation of such random copolymers is due to either the removal of a β-ketoacetate group by the β-ketothiolase involved in the monomer synthesis or the addition of an acetyl-CoA molecule to form 3-hydroxyalkanoates repeat units which are either two carbons shorter or two carbons longer than the substrate.

One exception to this general observation of copolymer formation was found when *P. oleovorans* was grown on 5-phenylvaleric acid (PVA).[8,9] In that case, a homopolymer, poly(3-hydroxy-5-phenylvalerate) (PHPV) was produced. That is, this PHA contained only 3-hydroxy-5-phenylvalerate units (HPV), as shown below:



The glass transition temperature of PHPV (19 °C) is the highest reported so far for all of the PHAs produced by *P. oleovorans*. Although it is a homopolymer, it does not exhibit crystallinity, which is unusual because the polymer has a highly ordered, isotactic structure; i.e., the chiral center at the 3-position in all repeating units is in the *R* configuration.[9] This stereoregularity should generally provide for an ordered packing structure in the solid state, and the absence of a crystalline melting point is therefore surprising.

Other groups have produced PHAs containing phenoxy,[10] nitrophenoxy, and cyanophenoxy groups[11] in the side chain. None of these polymers exhibited any crystallinity. One main objective of this study was to obtain a crystalline aromatic-containing PHA, and two separate approaches were undertaken to achieve this goal, as follows: (1) various different annealing procedures were performed on PHPV to try to induce crystallinity, and (2) modified phenyl-containing substrates were synthesized and fed to *P. oleovorans* either as a sole carbon source or in conjunction with a “good” feed

---

* To whom correspondence should be addressed.

[†] Permanent address: Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

[‡] Permanent address: Department of Chemistry, Karadeniz Technical University, Trabzon 61080, Turkey.

[⊗] Abstract published in *Advance ACS Abstracts,* February 1, 1996.

0024-9297/96/2229-1762$12.00/0

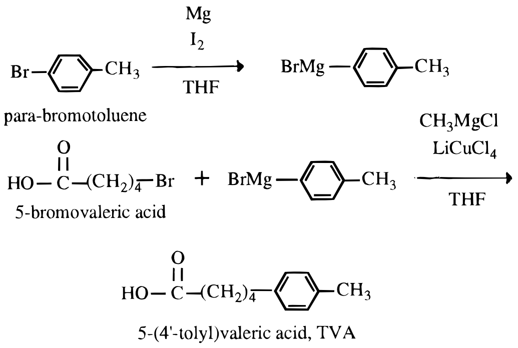
source in an attempt to produce a PHA which would crystallize. A "good" substrate is one which supports both cell growth and polymer production (e.g., nonanoic acid), while a "poor" substrate is one that either does not support cell growth or supports cell growth but not polymer production.

The second approach was based upon the results obtained by Lenz and co-workers[12−14] for an entirely different family of polymers. They observed that a polymer formed by the cationic polymerization of *p*-methyl-α-methylstyrene had a high degree of crystallinity while the polymer with the same degree of tacticity obtained from the unsubstituted monomer, α-methylstyrene, was not crystalline. For this reason, aryl-containing substrates with methyl, ethyl, and phenyl substitutions in the *para* position of the phenyl group located at the 5-position on the valeric acid were synthesized and fed to *P. oleovorans* both as the sole carbon source and as a cofeed with nonanoic acid.

Previous results obtained in this laboratory[15,16] have shown that cofeeding *P. oleovorans* with a combination of "poor" and "good" substrates can in many cases result in the incorporation of a sizable portion (up to 50 mol %) of the "poor" substrate into the PHA to form random copolymers. However, in another study in this laboratory,[17] when *P. oleovorans* was cofed nonanoic acid (NA) and 5-phenylvaleric acid (PVA), a physical mixture of two PHAs was produced, a homopolymer from PVA and the usual copolymer from NA. That is, the homopolymer contained only 3-hydroxy-5-phenylvalerate units while the copolymer contained the long-chain 3-hydroxyalkanoate units of the same composition as the MCL-PHA which is produced when nonanoic acid is the sole carbon source. Nevertheless, when the substrate mixture was used, the yield of the phenyl-containing PHA from cofeeding was greater than in the case when PVA was the sole carbon source.

Experimental Section

Synthesis of Substituted Aryl-Containing Substrates. A Grignard reaction similar to that used by Fritzsche and co-workers was used to prepare these substrates.[18,19] A typical example is the synthesis of 5-(4′-tolyl)valeric acid (TVA) as shown by the reaction scheme below:



5-(4′-Tolyl)valeric Acid (TVA): Yield 4.04 g (17.4%). Mp 76−78 °C. ^{1}H NMR (200 MHz, acetone-d_6, TMS) $\delta = 1.5-1.7$ (m, 4H, H-2, H-3), 2.2−2.4 (m, 4H, H-5, H-1), 2.6 (t, 2H, H-4), 7.05 (s, 4H, H_a, H_b, H_c, H_d).

5-(4′-Biphenyl)valeric Acid (BPVA). This substrate was prepared as described using 32.6 g (0.14 M) of 4-bromobiphenyl instead of 4-bromotoluene. Yield 4.11 g (17.6%). ^{1}H NMR (200 MHz, acetone-d_6, TMS) $\delta = 1.5-1.7$ (m, 4H, H-2, H-3), 2.25−2.4 (t, 2H, H-1), 2.6−2.8 (t, 2H, H-4), 7.2−7.6 (m, 9H, H_a, H_b, H_c, H_d, H_e, H_f, H_g, H_h, H_i).

5-(4′-Ethylphenyl)valeric Acid (EPVA). This substrate was prepared as described above using 25.9 g (0.14 M) of 4-(bromoethyl)benzene instead of 4-bromotoluene. Yield 4.75 g (19.2%). ^{1}H NMR (200 MHz, acetone-d_6, TMS) $\delta = 1.1-1.5$ (t, 3H, H-6), 1.55−1.8 (m, 4H, H-2, H-3), 2.25−2.5 (t, 2H, H-1), 2.5−2.9 (m, 4H, H-4, H-5), 7−7.5 (s, 4H, H_a, H_b, H_c, H_d).

8-(4′-Tolyl)octanoic Acid (TOA). This substrate was prepared as described above using 26.8 g (0.12 M) of 8-bromooctanoic acid instead of 5-bromovaleric acid. Yield 3.9 g (13.8%). Bp 147−149 °C/11 Torr. ^{1}H NMR (200 MHz, acetone-d_6, TMS) $\delta = 1.45-1.6$ (m, 8H, H-3, H-4, H-5, H-6), 1.65−1.9 (m, 4H, H-2, H-7), 2.25 (s, 3H, H-9), 2.35−2.5 (t, 2H, H-1), 2.7−2.85 (t, 2H, H-8), 7.3−7.5 (m, 4H, H_a, H_b, H_c, H_d).

Monomer Characterization. The melting points of the substrates synthesized were determined using an Electrothermal melting point apparatus. ^{1}H NMR spectroscopy was performed on a Bruker 200 MHz at 17 °C and 200 MHz. ^{13}C NMR spectroscopy was performed on a Varian 300 MHz at 17 °C and 70.5 MHz. The deuterated solvents used were chloroform-*d* and acetone-d_6. The polymer concentrations for ^{1}H and ^{13}C NMR spectroscopy were 10 and 30 mg/mL, respectively. The ^{1}H NMR spectra were referenced to tetramethylsiloxane (TMS), and the ^{13}C NMR spectra were referenced to chloroform.

PHA Biosynthesis. Stock cultures of *P. oleovorans* (ATCC 29347) were used in all growth and polymer production experiments. *P. oleovorans* was grown and the resulting polymer was extracted using methods previously developed in this laboratory.[20] The substituted phenyl-containing substrates were used both as a sole carbon source, at a concentration of 10 mM, and also as a cofeed with nonanoic acid, so that the total carbon substrate concentration was either 10 or 20 mM.

Polymer Characterization. NMR Spectroscopy. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker 200 MHz and on a Varian 300 MHz, respectively. The deuterated solvents used were chloroform-*d* and acetone-d_6. The polymer concentration for ^{1}H NMR spectroscopy was 10 mg/mL, and the spectra were run using an angle of 90°, a pulse width of 4.3 μs, and a delay of 2 s, and the peaks were referenced to tetramethylsiloxane. The polymer concentration for ^{13}C NMR spectroscopy was 30 mg/mL, and the spectra were run using a pulse angle of 90°, a pulse width of 6.3 μs, and a delay time of 3 s. The peaks were referenced to chloroform. The spectra were assigned by referring to publications by Fritzsche[18] and Gross[20] and by calculating theoretical peak positions.[21]

Gas Chromatography. Methanolysis−gas chromatography was used to determine the composition of the polymers produced. The polymer was converted into its constituent 3-hydroxyalkanoate units, in the form of their methyl esters, by a previously described procedure.[22] The methyl esters were characterized using a Perkin-Elmer 8500 GC equipped with a Durabond Carbowax megabore capillary column (15 m × 0.54 mm, carrier gas He, flow rate 20 mL/min). The program used was as follows: after injection, the sample was maintained at 80 °C for 4 min, then heated at 8 °C/min to 160 °C, and then held for 11 min before cooling to 80 °C.

Thermal Analysis. A DuPont differential scanning calorimeter 2910 (DSC) was used to determine the glass transition temperatures (T_g), the melting transitions (T_m), and the enthalpies of fusion (ΔH_m) of the PHAs produced. Samples were heated from −100 to +250 °C in a nitrogen atmosphere at a rate of 20 °C/min, quenched, and heated a second time using the same range and heating rate. The T_g reported was the inflection temperature in the thermogram of the second scan, and the T_m was the peak temperature in the thermogram of the first scan.

Wide-Angle X-ray Scattering. Wide-angle X-ray scattering (WAXS) patterns were carried out under reduced pressure using a Statton camera with a Siemens K710H generator operating at 40 kV and 30 mA. Nickel-filtered Cu Kα radiation ($\lambda = 0.1542$ nm) was used. Polymer samples of approximately 0.5 mm thickness were exposed to X-rays for 8 h.

Results and Discussion

Attempted Crystallization of PHPV. Several different solvent treatments and annealing procedures

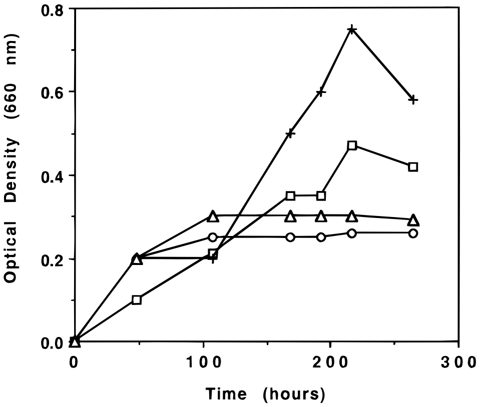


Figure 1. Growth curves obtained when *P. oleovorans* was grown on substituted aryl-containing substrates as the sole carbon source: (□) 5-(4′-tolyl)valeric acid (TVA); (+) 8-(4′-tolyl)-octanoic acid (TOA); (○) 5-(4′-ethylphenyl)valeric acid (EPVA); (△) 5-(4′-biphenylyl)valeric acid (BPVA).

were carried out on PHPV. In the first method, a 10% solution of the polymer in chloroform was evaporated slowly in a chloroform-saturated environment. In the second method, samples of solid PHPV were annealed in a DSC apparatus at two temperatures, 75 and 160 °C, for 16 h. In the third method, solid PHPV samples were heated for 16 h in nonsolvents, that is, in solvents in which they became swollen but did not dissolve. The solvents and temperatures used for that purpose were hexane at 60 °C and 1-butanol at 100 °C. After each of these treatments, analysis of the PHPV samples by both wide-angle X-ray scattering (WAXS) and differential scanning calorimetry (DSC) showed that none of the treatments resulted in a crystalline polymer.

PHAs from Various Aryl-Containing Substrates. *P. oleovorans* was grown on 5-(4′-tolyl)valeric acid (TVA), 5-(4′-ethylphenyl)valeric acid (EPVA), 5-(4′-biphenylyl)valeric acid (BPVA), and 8-(4′-tolyl)octanoic acid (TOA), in each case as the sole carbon source. The first three substrates are 5-substituted valeric acids, so that the R substituent in the substrate and the PHA desired would be CH_2CH_2Ar and Ar would be 4-tolyl, 4-ethylphenyl, or 4-biphenylyl for TVA, EPVA, and BPVA respectively. The fourth substrate is an 8-substituted octanoic acid, and the R group in the PHA desired would be phenylpentyl. The growth curves for *P. oleovorans* grown on each of these modified phenyl-containing substrates as the sole substrate are shown in Figure 1. Cell growth was slow with each of the novel substrates used. The maximum OD achieved was 0.75, in the case where TOA was the sole carbon substrate, but no polymer was obtained when these cells were extracted. No attempts were made to extract the cultures which were grown on either TVA, BVA, or PEBVA as the sole carbon source because the final OD values achieved were very low.

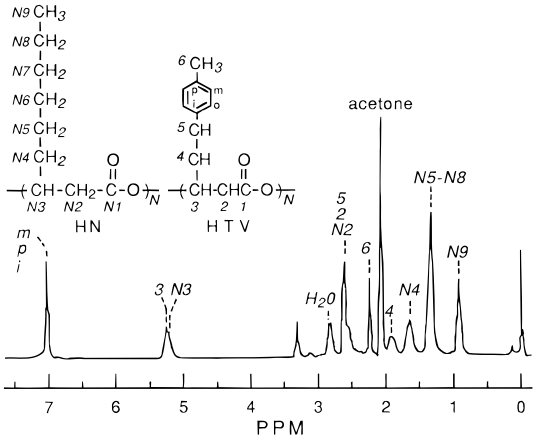


Figure 2. 200 MHz ^{1}H NMR spectrum of the PHA obtained when *P. oleovorans* was grown on TVA.

Cofeeding of Substituted Aryl-Containing Substrates. When *P. oleovorans* was grown on each of the four substituted aryl-containing substrates as the sole carbon source, the maximum OD achieved was low, so each of the substrates was cofed with a “good” carbon source, i.e., a substrate on which the bacteria grew rapidly and produced polymer. The objective of cofeeding the substituted aryl-containing substrates with the good substrate, in this case nonanoic acid (NA), was to increase the yield and to produce PHAs with other aryl groups.

All of the modified aryl-containing substrates were cofed with NA, and in addition TVA was cofed with PVA. The harvest time, OD at harvest, cell yield, polymer yield (based on cell dry weight), and the mol % aryl-containing units in the PHAs produced are given in Table 1. The mol % aryl groups were calculated by comparing the area under the peak due to the phenyl ring to the area under the peak due to the methine in the backbone. When either TOA or PEPVA and NA were cofed to *P. oleovorans,* the bacteria only metabolized the NA, resulting in the production of PHN; that is, the PHA produced was the same polymer as that obtained when NA is the sole carbon source. When BPVA was cofed with NA, phenyl groups were detected in the polymer produced, but subsequent reprecipitation of this polymer reduced the amount of phenyl groups produced from 18 to 5 mol %, indicating that the phenyl groups present were probably not in the PHA but in the BPVA feed substrate which remained associated with the PHA.

Table 1. Growth Conditions and Results Obtained When *P. oleovorans* Was Cofed Substituted Aryl-Containing Substrates with either NA or PVA

					mol % aryl-containing repeating units	
substrates[a]	harvest time, h	OD at harvest	cell yield, g/L	polymer yield, % DW[b]	after 1st precipitation	after 2nd precipitation
5 mM TVA:15 mM NA	21	3.4	1.4	15	3.0	3.8
5 mM TVA:5 mM NA	19	1.5	0.68	7.0	27	27
5 mM TVA:5 mM PVA	46	1.2	0.45	23	64	
5 mM BPVA:5 mM NA	23	1.7	1.0	13	18.0	5.0
5 mM TOA:5 mM NA	19	1.5	0.62	5.6	0	
5 mM EPVA:5 mM NA	25	2.8	1.52	5.2	0	
10 mM TVA[c]	54	1.2	0.58	0.03	40	

[a] TVA is 5-(4′-tolyl)valeric acid, NA is nonanoic acid, BPVA is 5-(4-biphenylyl)valeric acid, and TOA is 8-(4′-tolyl)octanoic acid, EPVA is 5-(4′-ethyl)phenylvaleric acid. [b] Polymer yield based on cell dry weight. [c] PHN was also present from the preculture, which contained NA as a carbon substrate.

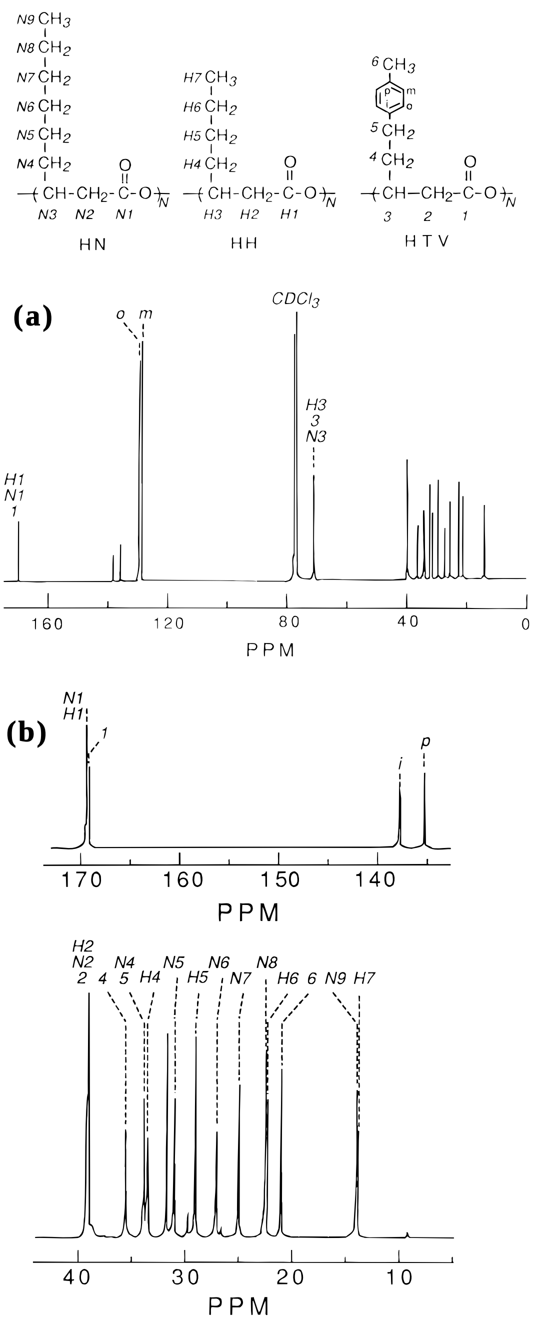


Figure 3. 74.5 MHz ^{13}C NMR spectrum of the PHA obtained when *P. oleovorans* was grown on TVA: (a) complete spectrum; (b) expanded portions of spectrum.

An interesting result was obtained when a 1 L culture of TVA was inoculated with 100 mL of a preculture grown on NA. The purpose of the experiment was to produce 100% PHTV, but PHN homopolymer was also present in the preculture as it was grown on NA before it was used to inoculate the 1 L culture. ^{1}H NMR analysis of the polymer produced (Figure 2) showed that 40% of the repeating units obtained were 3-(hydroxytolyl)valerate (HTV) units. The peaks in the ^{13}C NMR spectrum which were assigned to the HTV units are shown in Figure 3. The DSC thermogram of the polymer produced (Figure 4) clearly shows two glass transition temperatures, T_g, one at −37 °C, which corresponds to the T_g of PHN, and one at 17 °C, which was assigned to the T_g of PHTV. The thermogram also exhibited two melting transitions, T_m, one at 48 °C, which corresponds to the T_m of PHN, and the other at 95 °C, which was assigned as the T_m of PHTV, indicating that PHTV was a crystalline polymer. It appears that the presence of the *p*-methyl group on the phenyl ring allows the chains to pack into a conformation which is unavailable to the corresponding unsubstituted polymer. In this case, the PHA produced was a physical mixture of two polymers: PHTV and PHN; solvent fractionation was not carried out due to a lack of material.

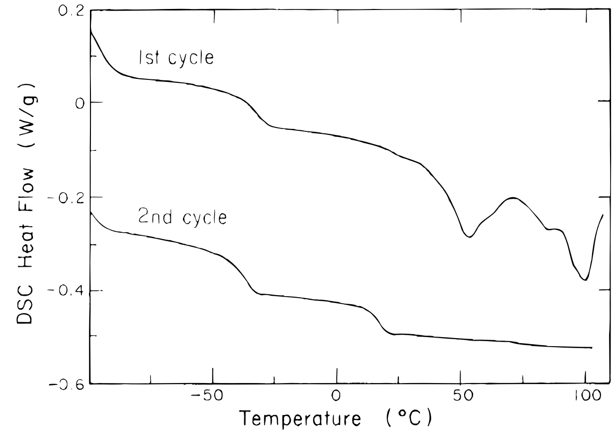


Figure 4. DSC thermograms of the polymer obtained when *P. oleovorans* was grown on TVA: (1) first heating cycle; (2) second heating cycle.

Table 2. Interplanar Spacings *d* from WAXS Patterns of PHN and PHN/PHTV

	diffraction maxima, Å					
polymer	d_1	d_2	d_3	d_4	d_5	d_6
PHN	19.1			4.6		4
PHN/PHTV		8.6	5.3	4.8	4.3	4

A comparison of the WAXS diffraction pattern obtained from PHN with that obtained from a blend of PHN and PHTV showed that, while PHTV and PHN exhibited some crystallinity, they crystallized separately to form two distinct crystalline phases. The diffraction maxima (*d*), which represent the interplanar spacings for PHN and PHPV, were calculated using Bragg's law and are reported in Table 2. The *d* spacings of PHN in Table 2 agree with those reported by Marchessault and co-workers.[23]

When *P. oleovorans* was cofed an equimolar mixture of PVA and TVA, the PHA produced contained 36 mol % HPV units and 64 mol % HTV units. Analysis of this polymer by WAXS and DSC showed that it was completely amorphous. The lower temperature DSC thermogram (Figure 5a) of the polymer produced showed only one T_g at 21 °C, and a higher temperature DSC thermogram (Figure 5b) did not contain a melting endotherm. These results indicate that the PHA product was an amorphous copolymer, but it is also possible that the bacteria produced a blend of PHPV and PHTV, and the two PHAs were miscible, so that crystallization could not occur.

To better evaluate the properties of PHTV, attempts were made to separate the PHPV and PHTV by solvent fractionation. This procedure had been previously used to fractionate a blend of PHN and PHPV using chloroform and hexane because PHPV was insoluble in hexane and could be removed by centrifugation.[12] However, this type of fractionation was not successful with a blend of PHPV and PHTV because no solvent was found which dissolved one polymer in preference to another.

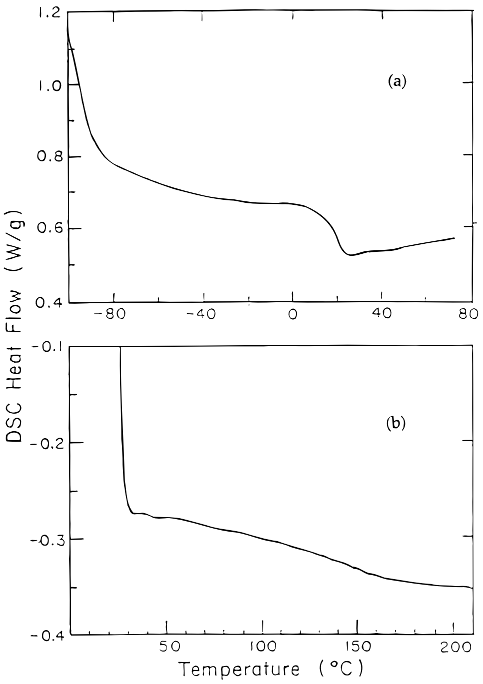


Figure 5. DSC thermograms of the polymer produced when *P. oleovorans* was cofed an equimolar mixture of PVA and TVA: (a) lower temperature thermogram (−100 to +100 °C); (b) higher temperature thermogram (25 to 225 °C).

Acknowledgment. Support for this work was provided by Eastman Chemical Co., Kingsport, TN, and by the National Science Foundation (Grant No. MCB-9202419). The use of the facilities of the Materials Research Laboratory of the University of Massachusetts, which is supported by the National Science Foundation, is also gratefully acknowledged. The authors wish to thank Dr. L. Charles Dickenson for assistance with the nuclear magnetic spectroscopy. The authors gratefully acknowledge the support from the Fullbright Commision (Ankara) for a fellowship for B.K.

References and Notes

(1) Byrom, D. *Trends Biotechnol.* **1987**, *5*, 246.
(2) Dawes, E. A.; Senior, P. J. In *Advances in Microbial Physiology*; Rose, E. A., Tempest, D. W., Eds.; Academic Press: New York, 1973; Vol. 10, p 135.
(3) Merrick, J. M. In *Photosynthetic Bacteria*; Clayton, R. K., Eds.; Plenum Press: New York, 1987; Chapter 11.
(4) De Koning, G. J. M.; van Bislen, H. M. M.; Lemstra, P. J.; Hazenberg, W.; Witholt, B.; Preusting, H.; van der Galien, J. G.; Schirmer, A.; Jendrossek, D. *Polymer* **1994**, *35* (10), 2090.
(5) Wallen, L. L.; Rohwedder, W. K. *Environ. Sci. Technol.* **1974**, *8*, 57.
(6) Doi, Y. *Microbial Polyesters*; VCH Publishers: New York, 1990.
(7) Langeveen, R. G.; Huisman, G. W.; Preusting, H.; Ketelaar, P.; Eggink, G.; Witholt, B. *Appl. Environ. Microbiol.* **1988**, *54* (12), 2924.
(8) Fritzsche, K.; Lenz, R. W.; Fuller, R. C. *Makromol. Chem.* **1990**, *191*, 1957.
(9) Safwat, A.; Grizzi, I.; Lenz, R. W.; Fuller, R. C. *Chirality*, **1991**, *3*, 492.
(10) Ritter, H.; von Spee, H. G. *Macromol. Chem. Phys.* **1994**, *195*, 1665.
(11) Ohyoung, K.; Gross, R. A.; Rutherford, D. R. *Can. J. Microbiol.* **1995**, *41*, 32.
(12) Lenz, R. W.; Regel, W.; Westfelt, L. *Makromol. Chem.* **1975**, *176*, 781.
(13) Lenz, R. W.; Westfelt, L. C. *J. Polym. Sci.* **1976**, *14*, 2147.
(14) Lenz, R. W.; Sunderland, J. E.; Westfelt, L. C. *Makromol. Chem.* **1976**, *177*, 653.
(15) Kim, Y. B.; Lenz, R. W.; Fuller, R. C. *Macromolecules* **1992**, *25*, 1852.
(16) Kim, Y. B., Ph.D. Thesis, Department of Polymer Science and Engineering, University of Massachusetts, Amherst, Massachusetts 1991.
(17) Kim, Y. B.; Lenz, R. W.; Fuller, R. C. *Macromolecules* **1991**, *24*, 5256.
(18) Fritzsche, K.; Lenz, R. W.; Fuller, R. C. *Int. J. Biol. Macromol.* **1990**, *12*, 92.
(19) Hazer, B.; Lenz, R. W.; Fuller, R. C. *Macromolecules* **1994**, *27*, 45.
(20) Gross, R. A.; DeMello, C.; Lenz, R. W.; Brandl, H.; Fuller, R. C. *Macromolecules* **1989**, *22*, 1106.
(21) Wilhelm, S.; Clerk, T. *Structural Analysis of Organic Compounds by Spectroscopic Methods*; McDonald & Co.: London, 1971.
(22) Brandl, H.; Gross, R.; Lenz, R. W.; Fuller, R. C. *Appl. Environ. Microbiol.* **1988**, *54*, 1987.
(23) Marchessault, R. H.; Monasterois, J.; Morin, F. G.; Sundararajan, P. R. *Int. J. Biol. Macromol.* **1990**, *12*, 158.

MA951185A