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Syndiotactic poly[(*R,S*)-β-hydroxybutyrate] isolated from methylaluminoxane-catalyzed polymerization

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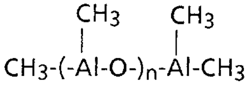
Summary:

Poly[(R,S)-β-hydroxybutyrate] was prepared via the methylaluminoxane-catalyzed polymerization of (R,S)-β-butyrolactone, fractionated, and characterized by ^{1}H and ^{13}C nmr, gel permeation chromatography, differential scanning calorimetry, and x-ray diffraction. All the material isolated from these reactions was identified by nmr as poly(β-hydroxybutyrate), but in each case, ^{13}C nmr showed that a portion of the material contained more syndiotactic than isotactic diads. Although this new syndiotactic material was low in molecular weight, it showed crystallinity by differential scanning calorimetry and x-ray diffraction, with d-spacings different in value and relative intensity from those of the isotactic polymer.

Introduction:

Poly(β-hydroxybutyrate) (PHB) is a bacterial polyester whose thermoplastic properties and biodegradability are currently of great interest (1,2). As made by bacteria, all chiral carbons have an R configuration, leading to a perfectly isotactic polymer with a left-handed helical structure (3,4). The material can also be made synthetically by ring-opening polymerization of β-butyrolactone (5-8). Optically active synthetic PHB can be prepared either by starting with optically active lactone (9,10) or by using a stereoelective catalyst with racemic monomer (11,12). Studies using racemic lactone with a diethyl zinc/water catalyst have produced atactic polymer (7,8), while the trialkylaluminum/water catalyst has a stereoregulating effect (5-8, 13), yielding stereoblock polymer with an x-ray diffraction pattern identical to that of bacterially-produced PHB. Improved results are reported using preformed oligomeric catalysts prepared by the prior reaction and high vacuum treatment of trialkylaluminum and water (7,8,10,14,15).

In this study, racemic β-butyrolactone was polymerized using methylaluminoxane (MAO), an oligomeric catalyst of structure I. The majority of



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$$CH_3\text{-}(\underset{}{\overset{CH_3}{\overset{|}{Al}}}\text{-}O\text{-})_n\text{-}\overset{CH_3}{\overset{|}{Al}}\text{-}CH_3$$

the product was isotactic, ranging from atactic to mainly isotactic diads. Additionally, in contrast to bacterial PHB, a new stereospecific species syndiotactic in character was obtained. Although low in yield and in molecular weight, this material was shown by nmr to have a surplus of syndiotactic diads, and different melting behaviour and x-ray diffraction than bacterial PHB or isotactic synthetic material.

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Experimental:

Materials:

(R,S)-β-butyrolactone was purchased from the Aldrich Chemical Co. and purified before use by heating to 80°C over CaH_2 for four hours and distilling under reduced pressure. The lactone was stored overnight under argon then checked by FTIR before proceeding.

Methylaluminoxane (MAO) was purchased from Ethyl Corp. as a 12 wt% solution in toluene, reported by them to be 5.5 wt% Al and to have a gas:aluminum mole ratio of 1.58. This was used as received without further analysis, having been stored under nitrogen for about six months at the time of the reported reactions.

Polymerization:

Polymerizations were done in thick-walled glass ampoules with side arms by a method modified from that of Bloembergen *et al.* (13). Ampoules and syringes were rinsed with acetone and dried overnight in an oven at 80°C, then cooled to room temperature in a dessicator. Transfers were done by syringe in an argon-filled dry box. In a typical polymerization, (R,S)-β-butyrolactone was added to the MAO solution at -98°C and stirred magnetically until homogeneous. The ampoule was then sealed, removed from the dry box, and attached via the side arm to a vacuum line, where the pressure was reduced to below 10^{-3} torr through a series of freeze/pump/thaw cycles. The ampoule was then re-sealed, removed from the vacuum line, and held at 60°C for seven days, during which time the contents went from pale yellow or colourless liquid to transparent bright yellow solid.

After the polymerization period, the ampoule was opened and the rubbery solid was dissolved in a minimum of warm chloroform, to which 5% acetylacetone had been added to remove aluminum products. The solution was then filtered through a coarse glass frit and poured slowly into a six-fold excess of stirred, ice-cold methanol. The resulting white precipitate was separated from the yellow solution by filtration or centrifugation, washed with ice-cold methanol, slurried in distilled water, and freeze-dried overnight before fractionation. The yellow solution was combined with the methanol washings, evaporated to about 50 mL, sealed, and stored at 0°C.

Fractionation:

The initial white precipitate was fractionated according to its solubility in acetone. First, the freeze-dried precipitate was stirred in 50-60 mL reagent grade acetone for 4-5 hours, separating it into acetone-soluble and acetone-insoluble fractions. The acetone solution was evaporated to dryness and cast from chloroform to form a colourless or yellow film, fraction 1a. The component insoluble in acetone at room temperature was extracted overnight with boiling acetone in a Soxhlet extractor. The fraction soluble in boiling acetone was cast into a clear film, fraction 1b, while the solid residue was washed with distilled water and freeze-dried overnight, giving fraction 1c as a white powder.

Additional fractions were obtained from the yellow methanol filtrate from which the original white precipitate had been isolated (Figure 1). After storing this concentrated solution at 0°C for 4-7 days, a white precipitate was isolated from the yellow solution by centrifugation. This was washed with cold methanol, slurried in distilled water, and freeze-dried overnight to yield a white powder, fraction 2. The collected methanol washings were added to the supernatant, then evaporated to about 50 mL, re-sealed, and stored at 0°C for another 1-8 weeks. After this time, a further white precipitate had formed, which was again isolated

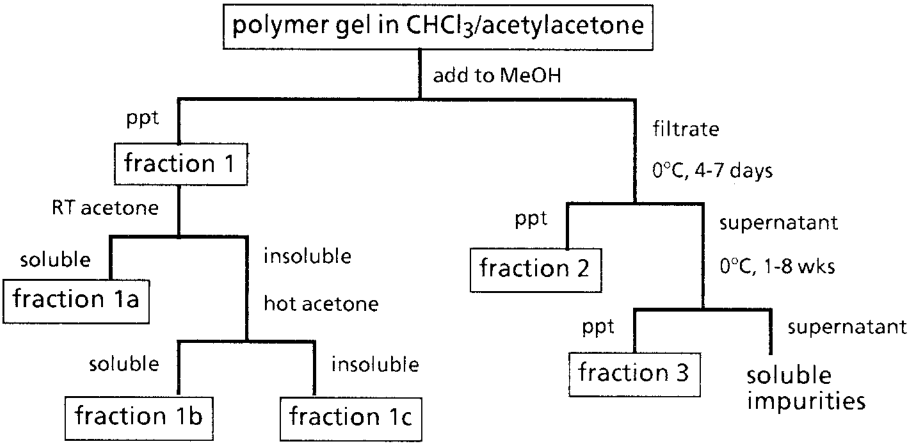

Figure 1: Fractionation of Synthetic PHB

by centrifugation, washed with cold methanol, and freeze-dried from water if the amount was sufficient, giving a white powder, fraction 3.

Characterization:

Proton nmr spectra were recorded on a Varian XL-200 or a JEOL CPF-270 spectrometer for 50-100 transients, using 0.01 g/mL polymer solutions in $CDCl_3$ with TMS as internal standard. Proton-decoupled ^{13}C nmr spectra were recorded on 0.03 g/mL polymer solutions in $CDCl_3$, using either a Varian XL-300 or a JEOL CPF-270 spectrometer, and collecting 1500-2500 transients. The XL-300 was run at 75.4 MHz, with a spectral window of 16500 Hz, acquisition time of 0.970 s, pulse width of 38.8°, and 1.0 Hz line broadening. The CPF-270 ran at 67.8 MHz, with a spectral window of 18050 Hz, acquisition time of 0.908 s, pulse width of 30°, and pulse delay of 0.8 s. All nmr spectra were recorded at ambient temperature.

Gel permeation chromatography (GPC) data were recorded at 30°C on a Waters 150CV GPC equipped with a linear Waters µstyragel high temperature column and a differential refractive index detector. Chloroform was used as the eluent at a flow rate of 1.0 mL/min. Sample concentrations were about 0.2%, with an injection volume of 100 µL and duplicate injections of each. Universal calibration was done using polystyrene standards purchased from Scientific Polymer Products and published Mark-Houwink parameters for polystyrene (16) and PHB (17).

Differential scanning calorimetry (DSC) data were obtained using a Perkin-Elmer DSC-7 at a heating rate of 20°C/min from -25 to 140 or 180°C, with baseline subtraction. After quenching at 200°C/min, a second scan was run under the same conditions as the first. Indium and isodecane standards were used for calibration. Samples were stored at room temperature for at least one month prior to analysis, and run as isolated (powder or film), without annealing. Melting temperatures and enthalpies of melting were taken from the first scan, with the peak maximum of the highest melting endotherm used if multiple endotherms were present; glass transition temperatures were taken from the second. After one month at room temperature for recrystallization, the previously melted samples were rerun under the same conditions to verify melting endotherms.

X-ray diffraction patterns were recorded at 40 kV and 20 mA using a Philips PW 1730 x-ray generator with a Ni filter to provide Cu $K\alpha$ radiation ($\lambda = 1.542$Å). Diffractometer scans were recorded for $2\theta = 8$-36° using a Philips powder diffractometer equipped with a graphite monochromator. D-spacings were calculated from x-ray diffraction photographs recorded at room temperature under vacuum using a Statton flat plate camera, 0.015" pinhole collimator and 5 cm film to sample distance. Samples were stored at room temperature for at least one month before running. For diffractometer scans, sample powders were placed in aluminum holders, while photographs were recorded on samples in 1.0 mm glass capillaries, with finely powdered NaF mixed with the sample or adhered to the capillary to calibrate the sample to film distance.

Results and Discussion:

Four reactions were performed with variation in the monomer:catalyst ratio and temperature at which the monomer and catalyst were mixed, as listed in Table 1.

Table 1: Polymerization conditions*, yields, and tacticities

reaction (molar Al: lactone ratio)	mL MAO solution (mmol Al)	T of mon/cat mixing (°C)	P at sealing (torr)	yield (%)	fraction	fractionated yield (% of total yield)	% isotactic diads (± 5%)
1 (1:20)	5.00 (8.66)	25	1.2×10^{-3}	7.4	1a 1b 1c 2 3	15 13 58 5 9	55 58 79 37 39
2 (1:20)	5.00 (8.66)	-98	2.0×10^{-3}	5.6	1a 1b 1c 2 3	20 14 32 12 22	52 56 85 36 40
3 (1:25)	4.00 (6.93)	-98	4.6×10^{-4}	18	1a 1b 1c 2 3	43 9 44 2 2	50 52 81 32 34
4 (1:15)	6.67 (11.6)	-98	5.0×10^{-4}	17	1a 1b 1c 2 3	55 14 20 6 4	50 50 80 34 35

* 14.13 mL (0.173 mol) (R,S)- β- butyrolactone used in each case

Overall yields were low, especially in the first two reactions where less effective vacua were obtained. Comparison of relative yields of the fractions within each reaction shows that the single largest fraction was the hot acetone insoluble one, except in reaction 4 with the 1:15 Al:lactone ratio. Yields of fractions 2 and 3 were much lower than those of fraction 1; obviously, these constitute minor components of the reaction.

Each fraction was examined by both ^{1}H and ^{13}C nmr, revealing the typical spectra of PHB. Tacticities of the fractions can be determined by examining expansions of the methyl peak in ^{1}H nmr (~1.25 ppm), or the carbonyl peak in ^{13}C nmr (~169 ppm) (13); as quantification is easier from the carbonyl peak, this was the method of choice. In all but perfectly isotactic (or perfectly syndiotactic) samples, this peak is divided into two components, corresponding to syndiotactic (racemic) and to isotactic (meso) diads. Integration of these components allows the quantification of tacticity as percent isotactic or syndiotactic diads, with 50% isotactic diads corresponding to an atactic polymer.

Examination of the percent isotactic diads in the materials listed in Table 1 shows that the subfractions from fraction 1 are isotactic, ranging from 50-55% isotactic (essentially atactic) in the cold acetone soluble portions to 79-85% isotactic diads in the hot acetone insoluble portions. However, in fractions 2 and 3, the percentage of isotactic diads falls to 33-40%, corresponding to 60-67% syndiotactic diads (Figure 2). Although this is a lesser degree of stereoregularity than is present in the most isotactic fractions, it corresponds to a new stereospecificity compared to previously isolated material and could be expected to show different physical properties.

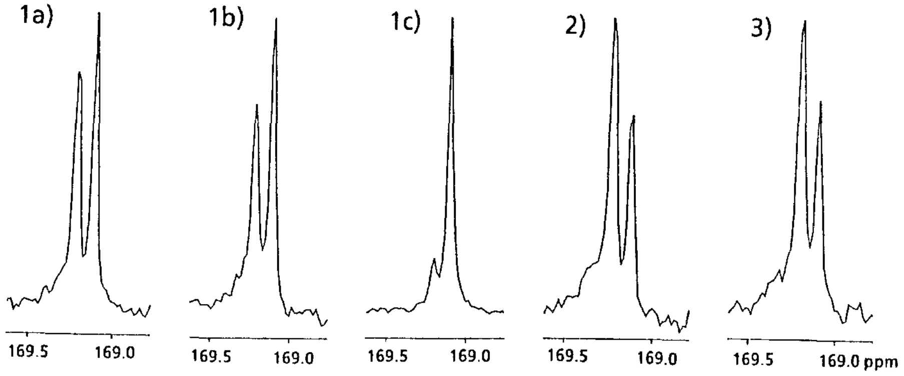

Figure 2: ^{13}C nmr expansions of carbonyl peak from reaction 3, fractions 1a, 1b, 1c, 2, and 3. Downfield component corresponds to syndiotactic diads, upfield to isotactic.

The molecular weights of the fractions are shown in Table 2. As expected, the highest molecular weights are for the hot acetone insoluble (most isotactic) fractions, progressively decreasing for the acetone soluble fractions and fractions 2 and 3. The degrees of polymerization observed for the syndiotactic fractions are $\overline{DP}_w = 27\text{-}50$ and $\overline{DP}_n = 6\text{-}11$, essentially short chains. Polydispersities are high, showing a broad distribution of molecular weights, with the lowest polydispersities occurring in the syndiotactic fractions.

The T_g's of all fractions (Table 2) are similar, and essentially the same as that of bacterial PHB. The presence of a melting peak in each of the samples shows that they are crystalline, with higher values for ΔH_f indicating that the maximum amount of crystallinity occurs in the more isotactic fractions. The T_m of 159-161°C and ΔH_f of 55-62 J/g observed in these samples are slightly lower than the values of 179°C and 105 J/g reported for bacterial PHB, due to the lower percentage of isotactic diads present in the synthetic samples. The syndiotactic fractions also have reasonably high crystallinity, as shown by a melting peak at 74-91 °C with ΔH_f of 33-39 J/g; given the low $\overline{M}_n$ of these samples, the T_m of high molecular weight syndiotactic PHB could be up to 80-100°C higher, as this amount of melting point depression is observed in bacterial PHB of similarly low molecular weight (19).

X-ray diffraction data (Figure 3) show significant differences between syndiotactic and isotactic PHB. Calculated d-spacings (Table 3) show that apart from two weak reflections missing in the synthetic isotactic due to its lower crystallinity, this crystal structure is the same as that of bacterial PHB. On the other hand, the diffraction of syndiotactic PHB is substantially different in terms

Table 2: Molecular weights and thermal behaviour

reaction	fraction	$\bar{M}_w/\bar{M}_n$	T_g (°C)	T_m (°C)	ΔH_f (J/g)
1	1a	9500/480 (19.8)	4	72	18
	1b	14 000/750 (18.7)	6	129	6
	1c	130 000/16 000 (8.1)	5	160	61
	2	3500/550 (6.4)	5	86	14
	3	4300/590 (7.3)	5	88	36
2	1a	4000/510 (7.8)	2	91	11
	1b	6300/440 (14.3)	6	113	3
	1c	91 000/5800 (15.7)	5	159	55
	2	3200/740 (4.3)	3	84	33
	3	2700/610 (4.4)	2	83	35
3	1a	8300/360 (23.1)	5	70	10
	1b	14 000/430 (32.6)	7	76	20
	1c	160 000/11 000 (14.5)	5	161	62
	2	3700/920 (4.0)	1	91	34
	3	2800/620 (4.5)	2	74	39
4	1a	1700/260 (6.5)	9	80	6
	1b	2400/360 (6.7)	10	84	8
	1c	43 000/1900 (22.6)	6	157	23
	2	2700/530 (5.1)	4	84	37
	3	2300/540 (4.2)	4	78	38
bacterial (1,13, 18)	-	$\bar{M}_w$ = 5000-1 500 000	5	179	105

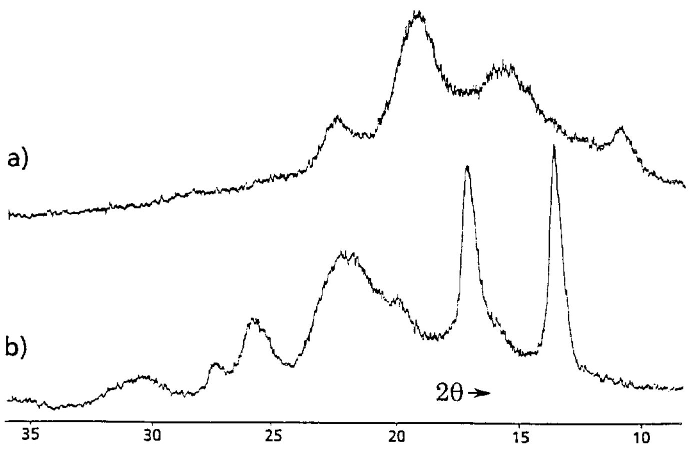

Figure 3: X-ray diffractometer scans for product of reaction 2:
a) syndiotactic and b) isotactic PHB

Table 3: D-spacings of natural, synthetic isotactic, and synthetic syndiotactic PHB

bacterial (20)		synthetic isotactic		synthetic syndiotactic	
(Å)	intensity	(Å ± 0.05)	intensity	(Å ± 0.10)	intensity
6.55	S	6.55	S	7.66	W
5.60	W	-		-	
5.25	S	5.20	S	5.49	M
4.48	W	4.45	W	4.46	S
3.98	M	3.99	M	3.85	M
3.53	M	3.48	M	-	
3.27	W	3.27	W	-	
2.94	W	2.95	W	-	
2.55	W	-		-	

of both d-spacings and intensities. Efforts are currently underway to further examine the crystal structure of the syndiotactic material.

Although a detailed study of the experimental conditions required to prepare syndiotactic PHB remains to be done, it should be noted that the preparation of this material is not restricted to the MAO catalyst system. Second fractions have been isolated from earlier reactions in this laboratory (21,22), using either 1:1 $Me_3Al:H_2O$ or 1:1 $(iBu)_3Al:H_2O$ catalysis; these have also been shown by ^{13}C nmr to contain more syndiotactic than isotactic diads, although to a lesser extent than the samples just described. These are of higher molecular weight than the samples isolated from the reactions with MAO, and show DSC melting peaks as well as the characteristic syndiotactic x-ray diffraction pattern. Thus, at least three of the alkylaluminum catalyst systems are capable of producing syndiotactic PHB.

Conclusions:

This study has shown that although only isotactic PHB has been isolated from natural sources, it is also possible to form a predominantly syndiotactic backbone with this unit, and to isolate such material from a reaction intended to produce isotactic material. This syndiotactic material has been shown by ^1H and ^{13}C nmr to be pure, and to contain 60-67% syndiotactic diads. Molecular weights of these samples were low, but a definite melting peak is observed and x-ray diffraction patterns display d-spacings and relative intensities significantly different from those of isotactic PHB.

Acknowledgements:

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References:

1. Holmes, P.A. (1988) "Biologically Produced (R)-3-Hydroxyalkanoate Polymers and Copolymers". In Basset, D.C. (ed) *Developments in Crystalline Polymers.* Elsevier, New York (vol 2, pp 1-65)
2. Anderson, A.J. and Dawes, E.A. (1990) *Microbiological Reviews* **54**:450

3. Cornibert, J. and Marchessault, R.H. (1972) *J. Mol. Biol.* **71**:735
4. Yokouchi, M., Chatani, Y., Tadokoro, H., Teranishi, K., and Tani, H. (1973) *Polymer* **14**: 267
5. Agostini, D.E., Lando, J.B., and Shelton, J.R. (1971) *J. Polym. Sci., Polym. Chem. Ed.* **9**:2775
6. Tani, H., Yamashita, S., and Teranishi, K., (1972) *Polym. J.* **3**: 417
7. Teranishi, K., Iida, M., Araki, T., Yamashita, S., and Tani, H. (1974) *Macromolecules* **7**:421
8. Iida, M., Araki, T., Teranishi, K., and Tani, H. (1977) *Macromolecules* **10**:275
9. Kemnitzer, J.E., McCarthy, S., and Gross, R.A. (1990) *Polym. Preprints* **31**:435
10. Zhang, Y., Gross, R.A., and Lenz, R.W. (1990) *Macromolecules* **23**:3206
11. Takeichi, T. Hieda, Y., and Takayama, Y., (1988) *Polym. J.* **20**:159
12. Le Borgne, A. and Spassky, N., (1989) *Polymer* **30**:2312
13. Bloembergen, S., Holden, D.A., Bluhm, T.L., Hamer, G.K., and Marchessault, R.H. (1989) *Macromolecules* **22**:1656
14. Gross, R.A., Zhang, Y., Konrad, G., and Lenz, R.W. (1988) *Macromolecules* **21**:2657
15. Benvenuti, M. and Lenz, R.W. (1991) *J. Polym. Sci., Polym Chem. Ed.* **29**:793
16. Dawkins, J.V. (1968) *J. Macromol. Sci., Phys. Ed.* **2**:623
17. Akita, S., Einaga, Y., Miyaki, Y., and Fujita, H. (1976) *Macromolecules* **9**:774
18. Barham, P.J., Keller, A., Otun, E.L., and Holmes, P.A. (1984) *J. Mat. Sci.* **19**:2781
19. Marchessault, R.H., Coulombe, S., Morikawa, H., Okamura, K., and Revol, J.F. (1981) *Can. J. Chem.* **59**:38
20. Alper, R., Lundgren, D.G., Marchessault, R.H., and Cote, W.A. (1963) *Biopolymers* **1**:545
21. Jesudason, J.J., Marchessault, R.H., and Saito, T. *Journal of Environmental Polymer Degradation* accepted Oct. 1992
22. Hocking, P.J. and Jesudason, J.J. unpublished results

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