Preparation of Predominantly Syndiotactic Poly(β-hydroxybutyrate) by the Tributyltin Methoxide Catalyzed Ring-Opening Polymerization of Racemic β-Butyrolactone

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ABSTRACT: In this report, we have documented the ability of tributyltin methoxide, $SnBu_3OCH_3$, to catalyze the ring-opening polymerization of β-butyrolactone, BL, with a preference for syndiotactic (syn) placement. This has therefore resulted in the chemical synthesis of a new stereoisomeric form of poly(β-hydroxybutyrate), PHB, specifically syn-PHB, which has predominantly alternating *R* and *S* stereocenters. The polymerization of racemic BL catalyzed by $SnBu_3OCH_3$ was carried out at temperatures of 40, 60, 75, and 90 °C. The yields of hexane/ether (1/1) insoluble PHB ranged from 24 to 69%, with the highest yield for the polymerization temperature of 75 °C. An analysis of the stereochemical sequence distribution for the syn-PHB stereoisomers by ^{13}C NMR allowed the degree of syndiotacticity achieved as a function of the polymerization conditions to be quantitated. Thus, it was determined that the racemic (r) diad fractions were 0.70, 0.66, 0.63, and 0.61 for the polymerization temperatures of 40, 60, 75, and 90 °C, respectively. Therefore, the degree of syndiotacticity decreased for higher polymerization temperatures, as was anticipated; however, the degree of change was unexpectedly small over the 50 °C temperature range investigated. The M_n values of the syn-PHB samples ranged between approximately 2500 and 5300 and were determined both by ^1H NMR spectroscopy end group analysis (after derivatization of the products with diazomethane) and by VPO. These M_n values were approximately 72% of that calculated by the assumption that the number of polymer chains is equivalent to the number of moles of catalyst added. In addition, by analysis using ^1H NMR of the number of methyl ester end groups which were present on the syn-PHB samples before and after diazomethane derivatization, it was determined that the ratio of carboxylic acid to methyl ester end groups ranged from 2.5 to 3.1. These results suggest that lactone polymerization by $SnBu_3OCH_3$ is not solely initiated by an insertion mechanism between the tin–methoxide bond. The syn-PHB polymers when isolated by solution precipitation and analyzed by DSC showed three distinct endotherm component peaks with peak temperatures of approximately 47, 62, and 79 °C where the dominant endotherm component was, in all cases, at approximately 62 °C. Dramatic changes in the endothermic melting transitions were observed when the samples were melt annealed as opposed to solution precipitated. The X-ray diffractogram for the 70-syn-PHB sample and the calculated *d*-spacings were compared to those of natural-origin (*R*)-PHB. Specific *d*-spacings measured at 7.52, 5.40, 3.83, and 3.11 Å for the 70-syn-PHB sample were not observed for (*R*)-PHB and are evidence for the existence of a different crystalline structure than that determined for an isotactic PHB stereoisomer. FTIR analysis of the 70-syn-PHB sample revealed spectral features which were unique to the conformational order and crystalline structure of this new PHB stereoisomer. Specifically, the appearance of a new band at approximately 1206 cm^{-1}, with a notable increase in the spectral absorption band at approximately 1103 cm^{-1}.

Introduction

Poly[(*R*)-β-hydroxybutyrate], (*R*)-PHB, is synthesized by a wide variety of bacteria[1–6] and serves as an intracellular carbon and energy reserve material.[1] (*R*)-PHB is itself a highly crystalline and environmentally biodegradable thermoplastic[7] and due to its repeating unit structure is completely isotactic.

Synthetic analogues of natural-origin PHB can be prepared by the ring-opening polymerization of β-butyrolactone, BL, using various organometallic catalysts.[8–16] The ring-opening polymerization of specifically substituted β-lactone rings for the preparation of poly(β-esters) allows for modulation of the polymer chain stereochemistry, which is currently not possible through the biosynthetic route where, to date, only the *R* stereochemistry has been observed. In addition, ring-opening polymerization allows for the introduction of a range of pendant group structural types. The use of synthetic analogues to study the biological degradation of poly(β-esters) of controlled structure has the potential of elucidating fundamental relationships between polymer stereochemistry, crystalline morphology, enzyme–substrate specificity, and biological degradation kinetics under various exposure conditions.[17]

PHB in high optical purity has previously been prepared from (*S*)-BL.[11] It was demonstrated that the mode of ring opening for BL with a diethylzinc ($ZnEt_2$)/H_2O catalyst (1/0.6) was by bond breaking between the carbonyl carbon and the oxygen of the lactone ring (acyl cleavage) with retention of configuration.[11] This catalyst forms an ideal random stereocopolymer from racemic BL, indicating an inability to cause stereoregulation during the polymerization.[16]

In contrast, it was shown for the ring-opening polymerization of BL catalyzed by aluminum-based catalysts that either acyl cleavage with retention of configuration or cleavage occurring between the β-carbon and oxygen bond of the lactone ring (alkyl cleavage) resulting in inversion of configuration may both be operative mechanisms.[11] The dominant mode of ring opening was found to be strongly dependent on the method used for the catalyst preparation.[11] In addition, the use of aluminum-based catalysts derived from trialkylaluminum/water for the polymerization of racemic BL[18–23] has allowed the preparation of semicrystalline PHB as well as copolymers of HB with β-hydroxyvalerate, P(HB-*co*-HV),[24] due to the stereoregulating ability of these catalyst systems to form isotactic block segments. The similarity of the X-ray diffraction patterns for the crystalline synthetic and

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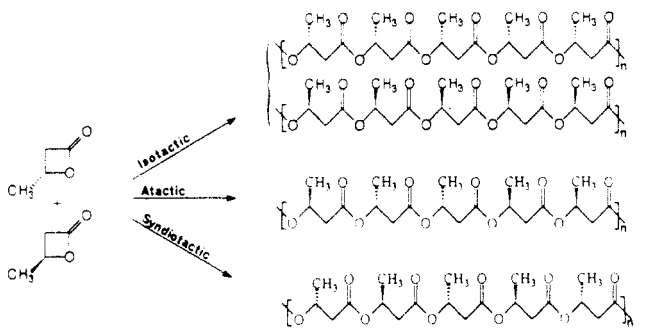
natural-origin isotactic (*R*)-PHB samples was evidence that the use of specific aluminum-based catalyst systems results in isotactic as opposed to syndiotactic chain sequences.[20-22] This was further confirmed by ^{13}C NMR studies which showed that the crystalline synthetic PHB and P(HB-*co*-HV) samples had a high fraction of meso diads.[23-25]

As example of stereoregulation in lactide polymerization was reported by Schindler and co-workers.[26] These researchers claim that the polymerization of *meso*-lactide catalyzed by stannous octoate resulted in a heterotactic polymer structure consisting of alternating meso and racemic diads. Thus far, work in our laboratory has not confirmed their result, and it appears that a considerably more complex polymer stereochemistry results for the polymerization conditions reported.

It has been demonstrated by Kricheldorf and co-workers that tributyltin methoxide is very active in the polymerization of ϵ-caprolactone, L,L-lactide, β-propiolactone, and BL.[12,27] Mechanistic studies indicate that the polymerization of these lactones proceeds by a coordination insertion mechanism where chain growth involves cleavage of the acyl-oxygen bond and yields polyester chains with a methyl ester end group.[12,27] In addition, polymerizations of racemic BL with tributyltin methoxide were carried out with a monomer/catalyst ratio of 100/1 at 50 and 75 °C for 48 h with percentage conversions reported as 50 and 100%, respectively.[12] Other characteristics of the products of the polymerizations with racemic BL were not reported.[12] From the discussion above, it is then possible that the tributyltin methoxide catalyst may polymerize racemic BL to form isotactic, atactic, or sydiotactic polymers (see below).



As an extension of the work by Kricheldorf,[12,27] studies were initiated to further characterize the stereochemistry, molecular weight characteristics, and mechanism of PHB synthesis from racemic BL using various organometallic species, including tributyltin methoxide. In this paper we wish to report on the ring-opening polymerization of racemic BL to form a new PHB stereoisomer, specifically, 50%-(*R*)-PHB with a predominantly syndiotactic placement of repeat units. To our knowledge, this is the first demonstration of a stereospecific ring-opening polymerization where a large preference for syndiotactic placement has been observed. The effects of temperature on the stereoregulating ability of the catalyst were investigated. Characterization of the new materials by gel permeation chromatography (GPC), vapor pressure osmometry (VPO), ^{13}C and ^{1}H nuclear magnetic resonance (NMR) spectroscopy, differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction will be presented to confirm the proposed repeat unit stereochemical sequence arrangement.

Experimental Section

NMR Spectroscopy. Carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded at 67.9 MHz on a Bruker WP-270 SY in 10-mm tubes as $CDCl_3$ solutions, with chemical shifts in parts per million (ppm) referenced relative to chloroform ($CHCl_3$) as an internal reference at 77.00 ppm. Polymer spectral acquisitions were conducted as 4.0% w/w $CDCl_3$ solutions using the following parameters: temperature 308 K, 90° pulse width, 16K data points, acquisition time 0.5 s, delay time 1.0 s, 32000–35000 transients. ^{13}C NMR measurements for the quantification of racemic (r) and meso (m) diads were recorded using the same parameters as above, with the exception that the spectral acquisition window was narrowed to include only the carbonyl resonances giving a spectral resolution of 0.220 Hz/point (compared with 1.890 Hz/point over the 0–200 ppm range above). Consequently, the acquisition time used for this experiment was 4.5 s and the time between pulses was 5.5 s.

Proton (^{1}H) NMR spectra were recorded on a Bruker WP-270 SY spectrometer at 270 MHz and were used to determine the chain end group structure. ^{1}H NMR chemical shifts in parts per million are reported downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. The parameters for the polymer spectra are as follows: 3.5% w/w polymer in $CDCl_3$, temperature 308 K, pulse width 4.9 μs, 32K data points, relaxation delay 0.50 s, 100–200 transients. Peak areas were determined by spectrometer integration.

IR Spectroscopy. Fourier transform infrared (FTIR) spectra were recorded on either a Bruker IFS 113v or a Perkin-Elmer 1600 Series FTIR. Spectra were obtained either as a KBr pellet or as a film on a NaCl plate as indicated. The spectral positions of the sample IR absorbances are given in units of reciprocal centimeters (cm^{-1}). The determination of the effect of crystallinity on IR absorbance peak positions and intensities for the 70-syn-PHB sample was carried out by solution casting of the polymer sample from $CHCl_3$ onto a NaCl plate, subsequent melting of the sample by placing the plate in an oven at 140 °C for 10 min, and annealing first at 37 °C for 24 h and later at 25 °C for up to 7 days. The IR spectra of this sample for various annealing times were recorded.

Thermal Analysis. Differential scanning calorimetry (DSC) was conducted on a Dupont DSC 2910 equipped with a TA 2000 data station, using between 7.0 and 12.0 mg of sample, a heating rate of 10 °C/min, and a nitrogen purge.

Molecular Weight Measurements. Molecular weight determinations by gel permeation chromatography (GPC) were carried out using a Waters Model 510 pump, a Model 410 refractive index detector, and a Model 730 data module with 10^3–10^6-Å Ultrastyragel columns in series. Chloroform was used as the eluent at a flow rate of 1.0 mL/min. Sample concentrations of 0.3% w/v and injection volumes of 125 μL were used. Polystyrene standards with a low polydispersity (Polysciences) were used to generate a calibration curve. Molecular weight determinations by vapor pressure osmometry were carried out in chloroform (fractionally distilled twice over P_2O_5) using a UIC Model 070 vapor pressure osmometer calibrated with biphenyl at 30 °C. Molecular weight determinations by end group analysis were carried out by ^{1}H NMR using spectrometer integration (see above). The relative ^{1}H NMR signal intensities due to the methyl ester end group and the polymer repeat units both before and after reaction of the polymer samples with diazomethane were used for the measurements.[28]

X-ray Diffraction. X-ray diffraction measurements were made on packed powder samples at 25 °C using a Rigaku Geigerflex camera operating at a voltage of 40 kV and a current of 25 mA. Nickel-filtered Cu Kα radiation (λ = 1.542 Å) was used. The sample exposure time was 12 h and the sample-to-film distance was 4.96 cm.

Synthetic Procedures. All glassware was silanized with dichlorodimethylsilane, washed with methanol, oven dried, and subsequently flame-dried under vacuum and purged with argon. Chloroform was washed with distilled water, dried over sodium sulfate, and distilled two times from phosphorus pentoxide under an argon atmosphere. Tributyltin methoxide, $SnBu_3OCH_3$ (purchased from Aldrich Chemical Co., 97% purity), was fractionally distilled [87 °C (0.25 mmHg)] using an argon bleed. Immediately before polymerization, racemic BL (purchased from Aldrich Chemical Co., 98% purity) was dried over calcium hydride with stirring at room temperature for 16 h followed by fractional distillation [74–75.5 °C (25 mmHg)] with an argon bleed. All

Table I
Polymerization of Racemic β-Butyrolactone Using $SnBu_3OCH_3$: Effect of Polymerization Temperature on the Chain Stereochemical Sequence Distribution[a]

temp (°C)	polym time (days)	% yield[b]	stereochem diad (m)[d] (±0.02)	seq anal.[c] (r)[d] (±0.02)
40	55	37	0.30	0.70
60	25	55	0.34	0.66
75	18	69	0.37	0.63
90	13	24	0.39	0.61

[a] Polymerizations were carried out neat. [b] Insoluble material obtained using the polymer purification scheme described in the Experimental Section. [c] Given as the fraction of (m) and (r) diads determined by ^{13}C NMR analysis (see Experimental Section). [d] (m) represents meso and (r) racemic diads. This nomenclature is equivalent to that where (m) is considered isotactic and (r) syndiotactic diad stereosequences.

transfers were carried out by syringe through rubber septum caps under an argon atmosphere.

To each polymerization ampule (10-mL internal volume) was transferred racemic BL (3.0 g, 0.035 mol) followed by 0.35 mL of a 0.825 M $SnBu_3OCH_3$ solution in $CHCl_3$. The ampules were sealed under slight vacuum (approximately 150 mmHg) with an argon bleed. The tubes were then placed into separate constant-temperature baths at 40, 60, 75, and 90 °C for 55, 25, 18, and 13 days, respectively.

The purification procedure involved the dissolution of the ampule contents in chloroform (4 mL), precipitation of this solution into 125 mL of a 2.5/1 hexane/diethyl ether solution, and storage of the resultant mixture in a freezer for several hours. The insolubles were separated by decanting away the supernatant, air-dried, redissolved in 10 mL of acetylacetone (AcAc), and stirred at room temperature for approximately 12 h. The polymers were then reprecipitated into 100 mL of a 1/1 hexane/diethyl ether solution, the supernatant was decanted away, and the remaining insoluble material was triturated twice with 10-mL portions of 1/1 hexane/diethyl ether. The remaining insoluble materials were then dried at 30 °C in vacuo (50 μmHg) for 24 h. The resultant white powders were characterized by both NMR and IR spectroscopy (see above), and the spectra obtained agreed with those previously published.[20,25,29] Molecular weight and stereochemical analyses of these polymeric materials are provided in the Results and Discussion section.

Results and Discussion

Polymer Yields, Molecular Weight Measurements, and End Group NMR Analyses. The polymerization of racemic BL catalyzed by $SnBu_3OCH_3$ was carried out as described in the Experimental Section at temperatures of 40, 60, 75, and 90 °C. As can be observed in Table I, the yields of hexane/ether (1/1) insoluble material (see the Experimental Section for details of the polymer isolation scheme) ranged from 24 to 69%, with the apparent highest yield of isolated purified 50%-(*R*)-PHB at a polymerization temperature of 75 °C. 50%-(*R*)-PHB M_n values determined by GPC measurements ranged from 5000 to 9200, with polydispersity index values (M_w/M_n) of as low as 1.04 (see Table II). The M_n for these samples was also determined by ^1H NMR (after derivatization with diazomethane) and VPO (see Experimental Section). ^1H NMR analysis of the molecular weight was carried out by spectrometer integration of the chain end methyl ester group ^1H NMR signal at 3.67 ppm[12] relative to the signal intensities of the corresponding chain repeat units.[30] The results of VPO and NMR measurements (recorded after diazomethane derivatization) agreed rather well and showed that the synthesized PHB samples had values of M_n which ranged between approximately 2500 and 5300 (see Table II). Theoretical M_n values shown in Table II were calculated from the relationship

$$M_n = \frac{\text{BL (grams)} \times \%\ \text{yield}}{\text{SnBu}_3\text{OMe (moles)} \times 100} \quad (1)$$

Equation 1 assumes that one catalyst species results in the polymerization of a single polymer chain. From observation of Table II, the M_n measurements made by ^1H NMR and VPO are approximately 72% of that calculated by eq 1, with the exception of the polymerization carried out at 90 °C, where the experimental and calculated M_n values are, within experimental error, identical. In addition, the molecular weight distributions determined by GPC (see Table II) for the product PHB samples are very narrow such that M_w/M_n values as low as 1.04 were measured. It may be that the narrow molecular weight distribution is obtained due to fractionation of a relatively broader polymer product, since only the precipitated polymer product was analyzed and not the remaining material which did not precipitate during the polymer purification scheme. However, regardless of the nature of the soluble material from this polymerization, it may be concluded in the absence of monomer degradation to nonpolymeric products that for the polymerization temperatures of 40, 60, and 75 °C, a minimum of approximately 1.4 PHB chains are formed for each molecule of $SnBu_3OCH_3$ added to the polymerization. Another important observation of the molecular weight analysis by ^1H NMR measurements is the large difference noted between the values for the PHB products analyzed before and after reaction with diazomethane (see Table II). The ratio of the NMR molecular weight measurements before and after reaction with diazomethane is reported as the ratio of free acid to methyl ester end groups in the polymer product. This ratio ranged from 2.5 to 3.1 for the different polymerization temperatures investigated (see Table II) and has interesting implications as to the mechanism(s) which is/are operative in the initiation of BL ring-opening polymerization with the $SnBu_3OCH_3$ catalyst.

It is interesting to note that for the polymerization of L,L-lactide at 100 °C with $SnBu_3OCH_3$ carried out by Kricheldorf and co-workers,[12] the experimentally determined (VPO and viscosity measurements) M_n values were significantly lower than those calculated using the above relationship. This result is consistent with that observed above for BL polymerization using the $SnBu_3OCH_3$ catalyst system at 40, 60, and 75 °C polymerization temperatures. These workers doubted the validity of their VPO and viscosity measurements and instead favored the M_n measurements obtained by ^1H NMR spectrometer integration of the methyl ester end groups as described herein. It must be noted that the M_n measurements by ^1H NMR were carried out on the product without derivatization with diazomethane so that the reported values may have been larger than the actual M_n values. The discrepancy between measured M_n values by VPO, viscosity, and NMR in addition to questions as to the validity of the NMR M_n values raises a degree of uncertainty as to the authors' conclusion that eq 1 is obeyed for L,L-lactide polymerization using $SnBu_3OCH_3$ as catalyst. These authors also studied $SnBu_3OCH_3$-catalyzed racemic BL polymerization[12] and reported that the initiation followed a covalent metal alkoxide insertion mechanism as demonstrated by the observation of methyl ester end groups. The results obtained in the present study and shown in Table II suggest that BL polymerization using $SnBu_3OCH_3$ is more complex than originally postulated by Kricheldorf et al.[12,27] Specifically, the observation that the carboxyl chain end group has predomi-

Table II
Molecular Weight Measurements on syn-PHB Stereoisomers Obtained from the Polymerization of Racemic BL Using $SnBu_3OCH_3$[a]

sample	M_n calc[b]	M_n VPO[c]	M_n NMR[d]	M_n NMR[e]	M_n GPC[f]	M_w/M_n[f]	CO_2H/CO_2CH_3[g]
70-syn-PHB	3900	3000	2600	6600	5300	1.04	2.5
66-syn-PHB	5900	4500	4100	12000	7700	1.06	2.9
63-syn-PHB	7500	5200	5400	13800	9200	1.12	2.6
61-syn-PHB	2600	2700	2400	7400	5000	1.07	3.1

[a] Polymerizations were carried out in the absence of solvent. [b] Calculated using eq 1 and the percent yield data from Table I. [c] Vapor pressure osmometry. [d] Determined as from *e*, after derivatization of the syn-PHB sample with diazomethane. [e] Determined from ^1H NMR (see Experimental Section) by comparison of the end group methyl ester proton signal intensity to the signal intensities of the methyl, methylene, and methine protons. [f] Determined by gel permeation chromatography relative to polystyrene using 10^3-, 10^4-, 10^5-, and 10^6-Å Ultrastyragel columns (see Experimental Section). [g] Ratio of the carboxylic acid to methyl ester end groups determined from ^1H NMR end group molecular weight analysis (see *e* above) obtained by dividing the apparent M_n values before and after diazomethane derivatization, respectively.

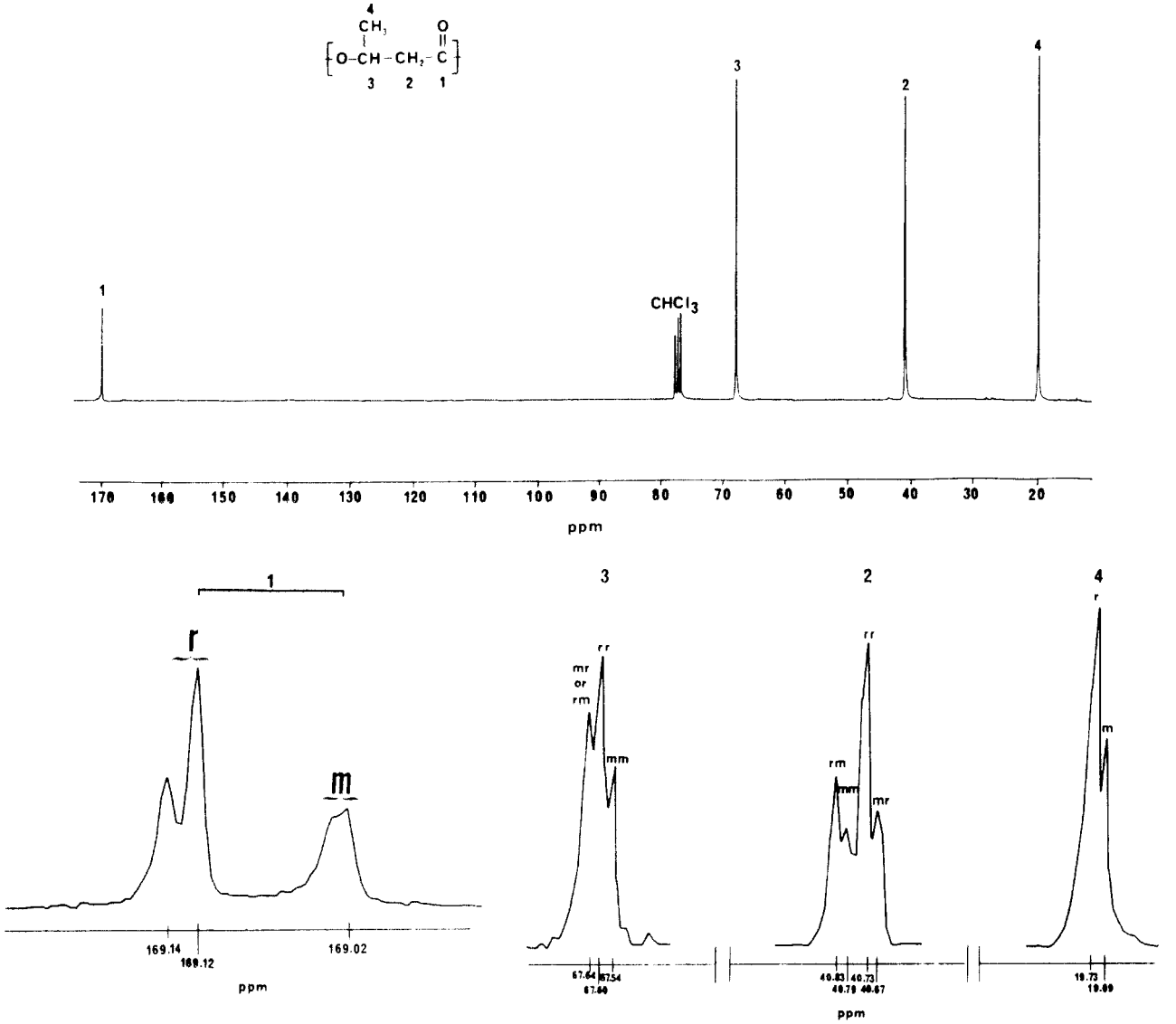


Figure 1. 67.9-MHz ^{13}C NMR spectrum recorded at 35 °C in $CDCl_3$ of (a, top) the 70-syn-PHB sample, (b, bottom left) the carbonyl carbon signal region of the spectrum in (a) obtained by using a narrowed spectral acquisition window (see Experimental Section), and (c, bottom right) expanded regions of the spectrum in (a).

nantly carboxylic acid and, to a lesser extent, methyl ester end groups in combination with the deviation of the molecular weight results from eq 1 suggests that lactone polymerization by $SnBu_3OCH_3$ is not solely initiated by an insertion mechanism between the tin–methoxide bond.

PHB Stereochemical Chain Sequence Distribution Analysis. Iida et al.[25] and later other investigators[24] published ^{13}C NMR spectra which describe the sensitivity of the carbonyl and methylene regions of PHB to the stereochemistry of the repeat unit sequences. The ^{13}C NMR spectrum of the 50%-(*R*)-PHB polymer sample obtained using $SnBu_3OCH_3$ as catalyst at 40 °C and expansions of the methine, methylene, and methyl regions are shown in parts a and c of Figure 1. A ^{13}C NMR spectrum of the carbonyl region was obtained at higher digital resolution by narrowing the spectral window (see Experimental Section) and is shown in Figure 1b. The upfield and downfield signals in the carbonyl carbon region correspond to the meso, (m), diad sequences (*R*–*R* and *S*–*S*) and the racemic, (r), diad sequences (*R*–*S* and *S*–*R*),

respectively (see Figure 1b).[31] It is interesting to note that the two (r) stereochemical sequences are different due to effects of directionality across the ester linkage and are clearly resolved. A discussion of the rationale in the assignment of the carbonyl upfield and downfield signals has been presented previously.[32]

Doi et al.[33] and Marchessault et al.[34] published work where a quantitative analysis of the comonomer diad sequence distribution for the natural-origin random copolyesters poly(HB-*co*-HV), where HV is hydroxyvalerate, was performed using the corresponding ^{13}C NMR carbonyl carbon resonances. In the NMR experiments carried out by these workers, the time allowed for nuclear relaxation was similar to that used herein (see Experimental Section in this paper and in refs 33 and 34). The fact that these copolyesters are indeed random was further verified by pyrolysis/gas chromatography/mass spectroscopy[35] and methanolysis/high-performance liquid chromatography/fast atom bombardment mass spectroscopy.[36] Furthermore, studies were carried out by Doi et al.[16] on PHB random stereocopolymers of known repeat unit stereochemical composition. These workers found that the carbonyl carbon resonance signal intensities for the respective diad sequences were in agreement with that expected over a range of stereocopolymer compositions investigated.[16] In our laboratory, the quantitative analysis of the carbonyl carbon diad signal intensities for PHB random stereocopolymers containing 50% and 67% *R* repeat units was carried out using an acquisition time of 4.5 s and delay times of 1.0 and 5.0 s (total time between pulses of 5.5 and 10.5 s, respectively). The observed diad fractions were found to be almost identical for spectra obtained using these two delay times. Furthermore, the calculated (based on stereochemical composition for a statistically random stereocopolymer) and observed diad fractions were in good agreement. Based on the above, a quantitative analysis of the stereochemical sequence distribution of various PHB tactic forms can be carried out by observation of the respective carbonyl carbon signal intensities using the NMR parameters described herein. Therefore, we proceeded with such an evaluation for the PHB stereoisomers prepared in this study.

The (r) and (m) diad fractions calculated based on spectral integration of the carbonyl region signals (as shown in Figure 1b) are reported in Table I. The (r) diad fractions were 0.70, 0.66, 0.63, and 0.61 for polymerization temperatures of 40, 60, 75, and 90 °C, respectively (see Table I). It is apparent that the degree of syndiotactic stereoregulation decreases for higher polymerization temperatures as was expected. However, the degree of change is rather small over the 50 °C temperature range investigated. The possibility should be considered that the products of these polymerization reactions were specifically fractionated due to the method used in their isolation (see Experimental Section). Therefore, it may be that an atactic fraction exists but was not isolated in this study.

The effects of PHB stereochemistry on the observed ^{13}C NMR spectral characteristics was further analyzed. Observation of the methylene region expansion in Figure 1c shows four peaks which correspond to triad sensitivity. Assignments of the triad peaks have been made as shown in Figure 1c based on the following: (1) (mm) triads were identified by mixing chemically degraded natural-origin (*R*)-PHB (M_n = 4300 by GPC) with 63-syn-PHB; (2) the (rr) triad was assigned based on its relative peak intensity which corresponded to that expected based on the calculated meso diad fraction determined by evaluation of the carbonyl region; (3) assignment of the (mr) and (rm) triad sequences was made assuming a relatively small chemical shift change between (mm) and (rm), as well as between (rr) and (mr), since there is a closer spatial proximity of the methylene carbon to the repeat unit which is linked to the carboxyl side of the methylene carbon observed in the stereosequence analysis. Thus, one of the diad sequences (underlined above) which comprises the triad stereochemical sequence unit is expected to be dominant in establishing the chemical shift position of the observed methylene in a triad stereochemical sequence. It should be noted that partial resolution of the stereochemical sequences was also observed upon expansion of the methyl (resolved into two peaks) and methine (resolved into three peaks) carbon signal regions. For the methyl and methine carbon signals, diad and triad sequences, respectively, were assigned to the corresponding peaks based on mixing experiments of the 63-syn-PHB sample with chemically degraded natural-origin (*R*)-PHB (M_n = 4300 by GPC) in addition to analysis of the relative peak intensities and comparison of those values to that expected based on the calculated (m) and (r) diad fractions determined by evaluation of the carbonyl region.

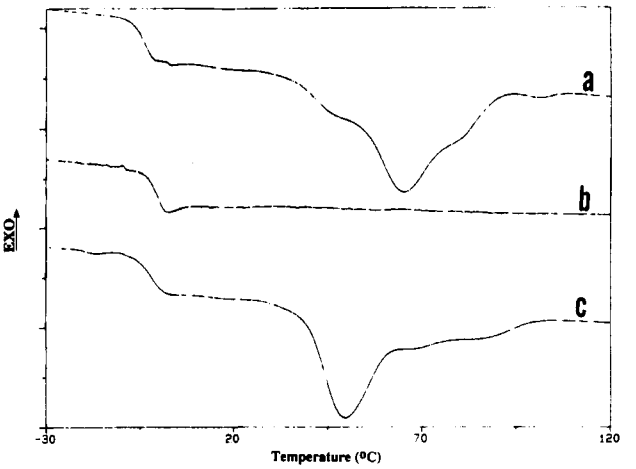


Figure 2. DSC thermograms of the 63-syn-PHB sample obtained using a heating rate of 10 °C/min shown for (a) the first heating scan, (b) the second heating scan which was recorded immediately after a rapid quench from the melt, and (c) the third heating scan after allowing the sample to an anneal for a period of 55 days at 25 °C.

Thermal Analysis. The thermal behavior of the syn-PHB samples was studied by DSC (see Figure 2 and Table III). Representative DSC thermograms recorded for the 63-syn-PHB sample obtained by solution precipitation during the first heating cycle (thermogram a), the second heating scan, which was carried out immediately after quenching from the melt (thermogram b), and also the third heating scan for the same sample annealed from the melt at room temperature for 55 days (thermogram c) are shown in Figure 2. An endotherm is seen for the first heating scan, is not observed after quenching from the melt and rapid recording of a second thermogram, and then reappears after a 55-day annealing period. This determination suggests that the transition corresponds to crystalline melting, and it may be concluded that the crystallization kinetics from the melt were not exceedingly rapid since neither a crystallization exotherm nor a melting endotherm was observed during the second heating scan.

From observation of Figure 2a and Table III it appears that the melting temperature range for the solution-precipitated samples is composed of three distinct transition endotherm components with peak temperatures of

Table III
Thermal Analysis Obtained by DSC Measurements[a] for Solution-Precipitated and Melt-Annealed PHB Stereoisomers

	from solution precipitate			annealed at 25 °C from melt		
sample	T_g[b] (°C)	T_m[c] (°C)	ΔH_f[d,e] (cal/g)	T_g (°C)	T_m[c] (°C)	ΔH_f[e]
70-syn-PHB	−8.2	39.0	0.5	−3.5	**50.4**[f]	[9.28 ± 0.03]
		58.5[f]	5.6		97.0	
		75.3	2.5			
			[8.52 ± 0.12]			
66-syn-PHB	−6.0	47.3	0.9	−1.9	**48.9**[f]	[7.75 ± 0.15]
		64.7[f]	5.9		68.8	
		80.8	1.8		88.4	
			[8.57 ± 0.03]			
63-syn-PHB	−2.5	48.0	1.1	−1.4	**49.5**[f]	[6.54 ± 0.03]
		65.3[f]	4.7		69.0	
		79.8	1.0		85.7	
			[6.84 ± 0.09]			
61-syn-PHB	−9.0	47.4	1.7	−7.4	**52.1**[f]	[7.15 ± 0.22]
		59.8[f]	4.2		88.2	
		79.8	1.5			
			[7.44 ± 0.07]			

[a] A scanning rate of 10 °C/min was used. [b] Determined during the first heating scan. [c] Listing of observed melting transitions. The values reported are the average of three determinations and have a standard deviation of approximately 0.4 °C. [d] Measured for each peak in the melting endotherm by a Lorentzian curve approximation and subsequent cutting and weighing of the component endotherm transitions. [e] The summation of the melting endotherms and the corresponding standard deviations are given in brackets and are the average of three determinations. These values were obtained by DSC spectrometer integration over the entire melting endotherm temperature range. [f] The component melting endotherm which has the largest contribution to the summed ΔH_f is in bold print.

approximately 47, 62, and 79 °C. In all cases, the most dominant component is the endotherm transition at approximately 62 °C (see Table III). In addition, the contributions of the component endotherm transitions to the total enthalpy of fusion have been determined by peak-fitting approximations and are reported in Table III. From this analysis it was observed that the enthalpy of fusion value for the lower temperature endotherm component increases from 0.5 to 1.7 cal/g for the 70- and 61-syn-PHB samples, respectively. In addition, the higher melting component at approximately 79 °C shows enthalpy of fusion values which range from 2.5 to 1.0 cal/g for the 70- and 63-syn-PHB samples, respectively. Indeed, these comparative results for the 70-, 63-, and 61-syn-PHB samples would be anticipated since the 70-syn-PHB sample should favor a relatively more ordered crystalline phase. However, it should be noted that anomalous thermal behavior was observed when directly comparing the 61- and 63-syn-PHB samples (see Table III), which may have its origin in the significantly higher M_n value (5200 vs 2700 by VPO; see Table II) of the 63-syn-PHB sample.

Melt annealing of the solution-precipitated samples resulted in two major changes in the observed melting endotherm relative to that observed for the solution-precipitated crystalline phase: (1) the largest component endotherm melting transition peak was observed at approximately 50 °C as opposed to 62 °C; (2) the highest temperature component endotherm peak shifted to a higher temperature (from 75.3 to 97.0 °C for the 70-syn-PHB sample). Therefore, the predominant melting transition of the melt-annealed samples occurred at a temperature which corresponds to that of the lowest component melting transition of the solution-precipitated samples (approximately 47 °C). The relationship between the observed thermal transitions and the crystalline structure is currently being studied.

It is of interest to compare the thermal properties recorded in Table III to those reported for ideal random stereocopolymers synthesized in our laboratory using a diethylzinc/water (1.0/0.6) catalyst system with % *R* compositions ranging from 95% *R* to 50% *R*.[17] In this way, it is possible to compare a series of stereocopolymers which have a similar degree of stereoregularity to those reported herein but differ in that the stereosequence regularity favors (m) as opposed to (r) diads. For instance, 81%-(*R*)- and 77%-(*R*)-PHB random stereocopolymers have (m) diad fractions of 69 and 65%, respectively. They have melting endotherm components at 64/87/99 °C and 73/88 °C and enthalpy of fusion values of 11.2 and 8.3 cal/g, respectively (the underlined component melting peak has the largest contribution to the total melting transition). If we compare the thermal properties of these samples to the 70- and 66-syn-PHB samples, it appears that the predominantly (r) syn-PHB samples have considerably lower predominant component peak melting temperatures, similar highest temperature component peak melting transitions, and similar enthalpy of fusion values. This comparison begins to address the fundamental question of the relative thermodynamic stability and crystalline structure of perfectly syndiotactic and isotactic PHB polymer chains. The crystalline structure[37] and thermal properties[38] for isotactic PHB are of course well known since the isotactic polymer is made quite easily by bacterial synthesis,[1-7] but the comparative data on a perfectly syndiotactic PHB sample are unfortunately not yet available. It is interesting to note that Marchessault et al. in 1978 considered the possibility that an α,α-disubstituted β-propiolactone could exist in a predominantly syndiotactic form and surmized that due to the nature of the general 2_1 helix for substituted polyesters based on the poly(β-propiolactone) backbone, the syndiotactic stereochemical sequence is sterically as favorable as the isotactic stereochemical sequence.[39] This contention by Marchessault et al. is of course quite relevant in the present inquiry and will be more readily addressed when the details of the crystalline structure for predominantly syndiotactic PHB are understood.

X-ray Diffraction Analysis. The X-ray diffractograms were recorded for powders (see the Experimental Section) of natural-origin (*R*)-PHB degraded to an M_n of 4300 by acid-catalyzed methanolysis and the 70-syn-PHB sample. From these diffractograms, *d*-spacings were calculated using the Bragg equation, and their values are compiled in Table IV along with *d*-spacing values previously published by Alper et al.[37] for a natural-origin PHB sample. The *d*-spacings for (*R*)-PHB reported by Alper et al.[37] are in excellent agreement with those measured herein for chemically degraded low molecular weight (*R*)-

Table IV
Interplanar *d*-Spacings (Å) Measured from X-ray Diffraction Patterns of Isotactic (*R*)-PHB and 70-syn-PHB

(*R*)-PHB (natural origin)[a]		(*R*)-PHB ($M_n \sim 4300$)[b,c]		70-syn-PHB[c]	
lit. *d*-spacing (Å)	intensity[d]	*d*-spacing (Å)	intensity[d]	*d*-spacing (Å)	intensity[d]
		13.97 (±0.01)	VW		
		7.17 (±0.01)	VW	**7.52 (±0.04)**[f]	S
6.55	S	6.56 (±0.02)	S		
5.60	W	5.65 (±0.03)	W	**5.40 (±0.02)**[f]	S
5.25	S	5.18 (±0.01)	S		
4.48	W	4.44 (±0.00)	M	4.42 (±0.03)	S
		4.10 (±0.01)	S		
3.98	M	3.93 (±0.05)	VW	**3.83 (±0.02)**[f]	M
3.53	M	3.47 (±0.01)	M	3.44 (±0.01)	W
3.27	W	3.24 (±0.01)	M	**3.11 (±0.01)**[f]	W
2.94	W	2.98 (±0.01)	W		
2.55	W	2.58 (±0.01)	VW		
		2.47 (±0.01)	VW		
		2.35 (±0.02)	VW	2.35 (±0.01)[e]	W
		2.22 (±0.02)	VW		
		2.06 (±0.01)	VW	2.06 (±0.01)[e]	W

[a] The *d*-spacings for natural-origin PHB were those reported by Agostini et al.[22] for a sample derived from *Bacillus cereus*. [b] Natural-origin (*R*)-PHB degraded by acid-catalyzed methanolysis to M_n = 4300 (measured by GPC). [c] X-ray diffraction patterns were taken on packed powder samples, using 12-h exposures at room temperature at a film-to-sample distance of 4.96 cm (see Experimental Section). [d] S = strong, M = medium, W = weak, and V = very. [e] *d*-Spacings determined using a film-to-sample distance of 3.00 cm and a 12-h exposure period on a packed powder sample. [f] Boldfaced *d*-spacings for the 70-syn-PHB sample represent values which are distinctly different from those measured for the (*R*)-PHB samples.

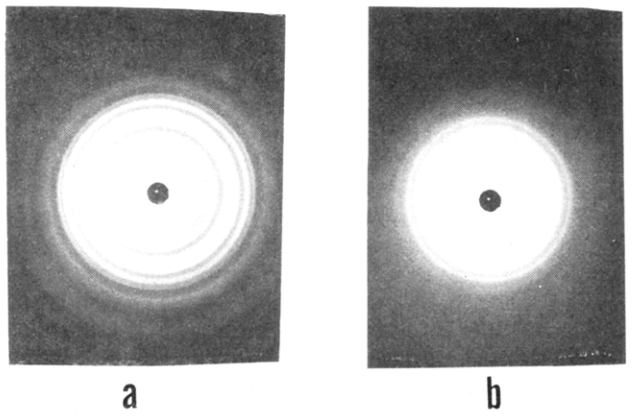


Figure 3. X-ray diffractograms for packed powder samples taken using Ni-filtered Cu Kα radiation (λ = 1.542 Å) at a film-to-sample distance of 4.96 cm and with a 12-h exposure time period: (a) natural-origin (*R*)-PHB (M_n of 4300 measured by VPO) chemically degraded by acid-catalyzed methanolysis; (b) the 70-syn-PHB sample.

PHB (see Table IV).[22,38] The X-ray diffractograms recorded on the 70-syn-PHB as well as on (*R*)-PHB (M_n of 4300) are shown in Figure 3. From comparison of the diffractograms and the corresponding *d*-spacings, it is quite apparent that the 70-syn-PHB sample is indeed crystalline and has distinctly different crystalline diffraction planes than those observed for isotactic PHB. The *d*-spacings which are most unique to the 70-syn-PHB sample are those at 7.52, 5.40, 3.83, and 3.11 Å. Work is currently in progress to determine the crystalline structure for syn-PHB on oriented samples.

FTIR Analysis. It has previously been demonstrated by Bloembergen et al.[29] that the FTIR spectra for natural-origin PHB dissolved in chloroform-*d* and PHB film which is semicrystalline obtained by solution casting have distinctly different IR absorption bands. In Figure 4a, the FTIR spectra of 70-syn-PHB (spectrum a), 50%-(*R*)-atactic-PHB (spectrum b), and natural-origin (*R*)-PHB (spectrum c) recorded in the solid state as KBr pellets are shown. The FTIR spectrum of the 50%-(*R*)-atactic-PHB sample (see Figure 4a) appears almost identical to that reported for the natural-origin (*R*)-PHB sample dissolved in chloroform-*d*.[29] This result is not surprising since the conformational state of isotactic PHB in chlorinated solvents is not highly ordered and may be best described as approximating a random coil.[40,41] The two spectra of atactic and natural-origin PHB in Figure 4a show the identical differences in spectral features as was previously reported.[29] Specifically, significant changes in the absorption bands at 1279, 1228, and 1185 cm^{-1} were observed where the band at 1385 cm^{-1} was considered crystalline insensitive (see Figure 4a).[29]

From a study where the FTIR spectra of 70-syn-PHB samples which had been annealed for various time periods were compared (see the Experimental Section), it was determined that, similar to isotactic PHB, the band at 1385 cm^{-1} appears to be fairly insensitive to the effects caused by ordering of the 70-syn-PHB sample in the crystalline solid state. In addition, it was observed that 70-syn-PHB films which were melt annealed at 37 °C gave regions of the films which were comparatively more opaque than films produced by melt annealing 70-syn-PHB at 25 °C. Since it was suspected that melt annealing of 70-syn-PHB at 37 °C (as opposed to 25 °C) may result in higher sample percent crystallinity, an FTIR spectrum of the 70-syn-PHB was recorded by passing the IR beam through the most opaque region of the 37 °C annealed sample. This spectrum is compared to that for a 70-syn-PHB film which was analyzed by FTIR immediately after melting the solution-cast film (see Figure 4b). The observed intensities of the absorption bands for spectra a, b, and c in Figure 4a as well as the two spectra in Figure 4b were compared by invoking a baseline approximation method described by Koenig[42] et al. and by using the crystalline-insensitive band at 1385 cm^{-1} to normalize for differences in sample concentrations. From this analysis the following conclusions were made as to the most notable spectral features of the crystalline syndiotactic form which allow it to be distinguished from its atactic and isotactic stereoisomers: (1) the appearance of a band at 1206 cm^{-1} (see Figure 4a,b), with a concurrent decrease in intensity at approximately 1180 cm^{-1} (see Figure 4b); (2) significant increases in intensity of the band at 1103 cm^{-1} (see Figure 4a,b). Obviously, additional subtle changes in the IR absorption intensities may be observed by further inspection of Figure 4a.

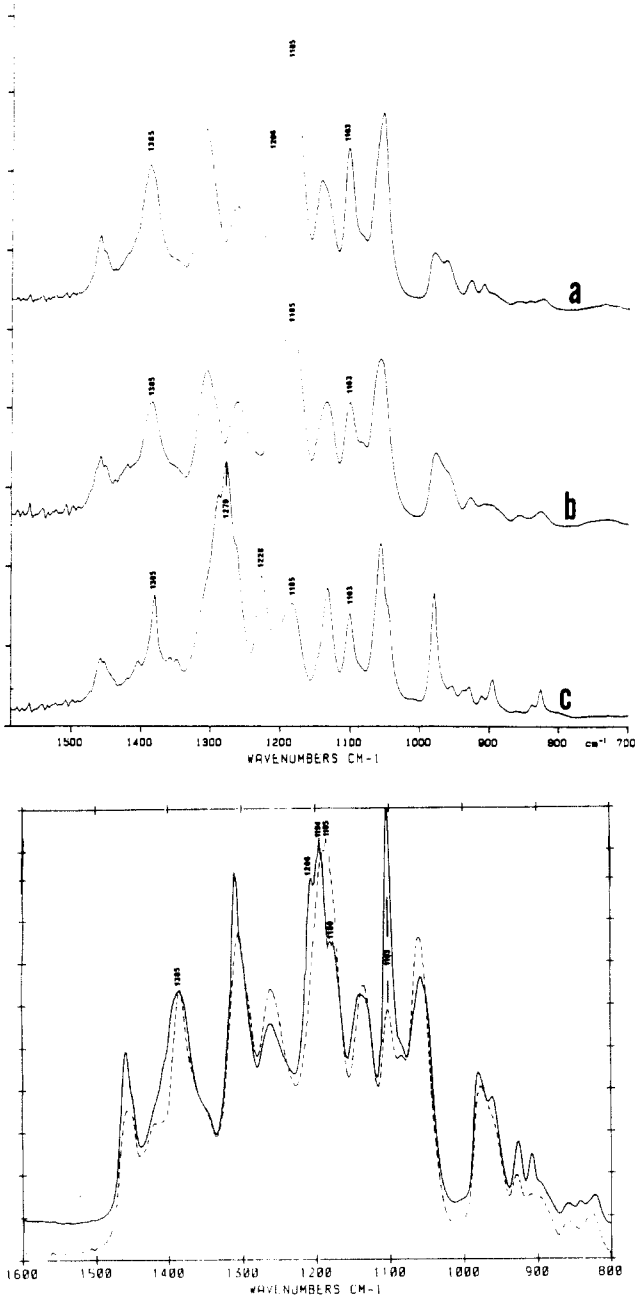


Figure 4. FTIR spectra recorded at room temperature: (a, top) using KBr pellets of the 70-syn-PHB sample (spectrum a) crystallized by solution precipitation, atactic PHB (spectrum b) obtained from racemic BL ring-opening polymerization using a $ZnEt_2/H_2O$ (1/0.6) catalyst system, and isotactic natural-origin (*R*)-PHB (spectrum c); (b, bottom) using a film of the 70-syn-PHB sample which was solution cast onto a NaCl plate, then melted and immediately observed (the dashed line) and subsequently recorded after annealing the film first at 37 °C for 24 h followed by 7 days at 25 °C (solid line).

Melt annealing of 70-syn-PHB at 37 °C simply amplified the changes observed at 1206, 1180, and 1103 cm^{-1} (see Figure 4a,b) most likely due to a significant increase in the degree of crystallinity relative to the solution-precipitated sample. It is interesting to note that crystallization of both the isotactic and the predominantly syndiotactic samples results in decreased intensity of the band at approximately 1180 cm^{-1}; however, for the isotactic and syndiotactic stereoisomers new bands arise in distinctly different spectral positions, specifically 1226 and 1103 cm^{-1}, respectively. Clearly, it has been demonstrated in this study that IR spectroscopy can be utilized for distinguishing between the crystalline structures of isotactic and syndiotactic PHB, in addition to the atactic amorphous PHB stereoisomer.

Summary of Results

In this report we have documented the ability of $SnBu_3OCH_3$ to catalyze the ring-opening polymerization of BL with a preference for syndiotactic placement. From ^{13}C NMR measurements it was determined that the fraction of (r) diad stereosequences was 0.70 for a polymerization temperature of 40 °C. The degree of syndiotacticity decreased for higher polymerization temperatures, as was anticipated; however, the degree of change was unexpectedly small over the 50 °C temperature range investigated. Indeed, the fraction of (r) diad stereosequences was 0.61 for a 90 °C polymerization temperature. The yields of hexane/ether (1/1) insoluble syn-PHB ranged from 24 to 69%, with the highest yield at a polymerization temperature of 75 °C. The M_n of the PHB products had values ranging between approximately 2500 and 5300, determined both by ^1H NMR spectroscopy end group analysis (after derivatization of the products with diazomethane) and by VPO. The M_n values measured experimentally were approximately 72% of that calculated by the assumption that the number of polymer chains is equivalent to the number of moles of catalyst added. In addition, end group analysis by ^1H NMR, both before and after derivatization of the chain ends with diazomethane, showed that the ratio of carboxylic acid to methyl ester end groups for the synthesized PHB chains ranged from 2.5 to 3.1. These results suggest that lactone polymerization by $SnBu_3OCH_3$ is not solely initiated by an insertion mechanism between the tin–methoxide bond. For the solution-precipitated syn-PHB samples, three distinct transition endotherm component peaks with peak temperatures of approximately 47, 62, and 79 °C were measured by DSC, where the dominant component is that at approximately 62 °C. Dramatic changes in the endothermic melting transitions resulted when the samples were obtained by melt annealing as opposed to solution precipitation. The X-ray diffractogram for the 70-syn-PHB sample and the calculated *d*-spacings were compared to those of natural-origin (*R*)-PHB. Specific *d*-spacings measured at 7.52, 5.40, 3.83, and 3.11 Å for the 70-syn-PHB sample were not observed for (*R*)-PHB and are evidence for the existence of a different crystalline structure than that determined for an isotactic PHB stereoisomer. FTIR analysis of the 70-syn-PHB sample revealed spectral features which were unique to the conformational order and crystalline structure of this new PHB stereoisomer: specifically, the appearance of a new band at approximately 1206 cm^{-1}, with a notable increase in of the spectral absorption band at approximately 1103 cm^{-1}.

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