Synthesis and Characterization of Polyesters Produced by *Rhodospirillum rubrum* from Pentenoic Acid

Alberto Ballistreri and Giorgio Montaudo*

Dipartimento Scienze Chimiche, Università di Catania, Viale A. Doria 6, 95125 Catania, Italy

Giuseppe Impallomeni

Istituto per la Chimica e la Tecnologia dei Materiali Polimerici, Consiglio Nazionale delle Ricerche, Viale A. Doria 6, 95125 Catania, Italy

Robert W. Lenz, Herbert W. Ulmer,† and R. Clinton Fuller

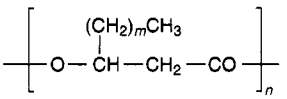
Polymer Science and Engineering Department and Biochemistry Department, University of Massachusetts, Amherst, Massachusetts 01003

Received September 30, 1994; Revised Manuscript Received February 23, 1995®

ABSTRACT: Two different terpolyesters were obtained by fermentation of *Rhodospirillum rubrum* fed either with 4-pentenoic acid alone or with an equimolar mixture of 4-pentenoic and valeric acids. Sequence distributions of these two polyesters were determined by fast atom bombardment mass spectrometry (FAB-MS) of the oligomers prepared by partial methanolysis and separated by HPLC. The distributions in both polymers follow Bernoullian statistics, indicating that both are random terpolyesters.

Introduction

The phototropic, purple, non-sulfur bacterium *Rhodospirillum rubrum* is known to produce intracellular energy and carbon storage products which have been generally described as poly(3-hydroxyalkanoate)s (PHA). These biopolymers have the following general structure:



in which m can vary from 0 to 9 depending on the carbon substrate used for growth and on the bacteria.[1] In the case of *R. rubrum* m can vary from 0 to 3.[2−4]

Recently, it has become of industrial interest to evaluate these polyesters as biodegradable thermoplastics for a wide range of agriculture, marine, and medical applications.[5,6] Because the physical and mechanical properties of these copolymers can change considerably as a function of the monomer's composition and distribution, it is desirable to incorporate different types of repeating units into the polymer in order to produce materials with specific requirements for practical applications. In that regard, PHAs containing functional groups such as phenyl,[7] olefin,[8] chloride,[9] and fluoride[10] have been obtained from the growth of *Pseudomonas oleovorans* on functionalized substrates.

It has also been reported that *R. rubrum* produces a copolymer with unsaturated side chains when it is grown on 4-pentenoic acid.[4] The composition was obtained by ^{1}H-NMR analysis, but this technique was not able to give information on the sequence distribution of the repeating units.[4]

We have previously reported on the use of fast atom bombardment mass spectrometry (FAB-MS) to determine the repeating unit composition and the sequence distribution of repeating units in copolymers produced by *Alcaligenes eutrophus*[11,12] and *Pseudomonas oleovorans*.[13−15] In those determinations the oligomers were obtained by partial methanolysis or partial pyrolysis. In this report we describe how the copolyesters obtained by fermentation of *R. rubrum* can be analyzed for copolymer composition and sequence distribution by FAB-MS of the oligomers formed by the partial methanolysis. Two different samples of PHA produced by *R. rubrum* grown on 4-pentenoic acid were investigated.

Experimental Section

Polyester Production. *Rhodospirillum rubrum* was obtained from the American Type Culture Collection (ATCC 25903). Stock cultures were grown anaerobically in the light in 25 mL screw cap test tubes using the 550 R8AH[15] medium, which contained malate as the carbon source. The same medium was used to grow *R. rubrum* under PHA-producing conditions, but malate and ammonium sulfate were omitted from the medium. Instead of malate, a variety of other carbon sources were used. Cultures were prepared by inoculating a 250 mL screw cap bottle containing 200 mL of the PHA-producing medium (carbon source: 30 mmol of malate) with 2 mL of the stock solution and allowing the cells to grow for 2 days under anaerobic conditions in the light in 1 L screw cap bottles. The PHA-producing medium with the desired carbon source was inoculated with 10 mL of the inoculum and allowed to stand for 5 days with daily stirring. The cells were harvested by centrifugation (Sorvall RC2-B, 5 °C, 10000 *g*)). The whole cell pellets were lyophilized to yield the dry cells. Dry cell weights were determined gravimetrically. The polymer was extracted from the lyophilized cells in a Soxhlet extractor with 300−500 mL of chloroform, filtered through a cotton plug, and precipitated in a 10-fold increase of volume of rapidly stirred methanol. The polymer was centrifuged (Sorvall RC2-B, 4 °C, 10000 *g*) and dried *in vacuo* (1 mmHg) for 16 h. Further purification was done by repeating the same solution and precipitation procedure. The total amount of PHA was determined gravimetrically and calculated as the percentage of cellular dry weight.

Polymer Characterization. The molecular weights were determined by gel permeation chromatography (GPC) with a Waters Model 6000A solvent delivery system, a Model 401 refractive index detector, and a Model 730 data module with 2 Ultrastyragel linear columns in series. Chloroform was used as the eluent at a flow rate of 1.0 mL/min. Sample concentra-

† Permanent address: International Specialty Products, Wayne, NJ.

® Abstract published in *Advance ACS Abstracts,* April 1, 1995.

0024-9297/95/2228-3664$09.00/0

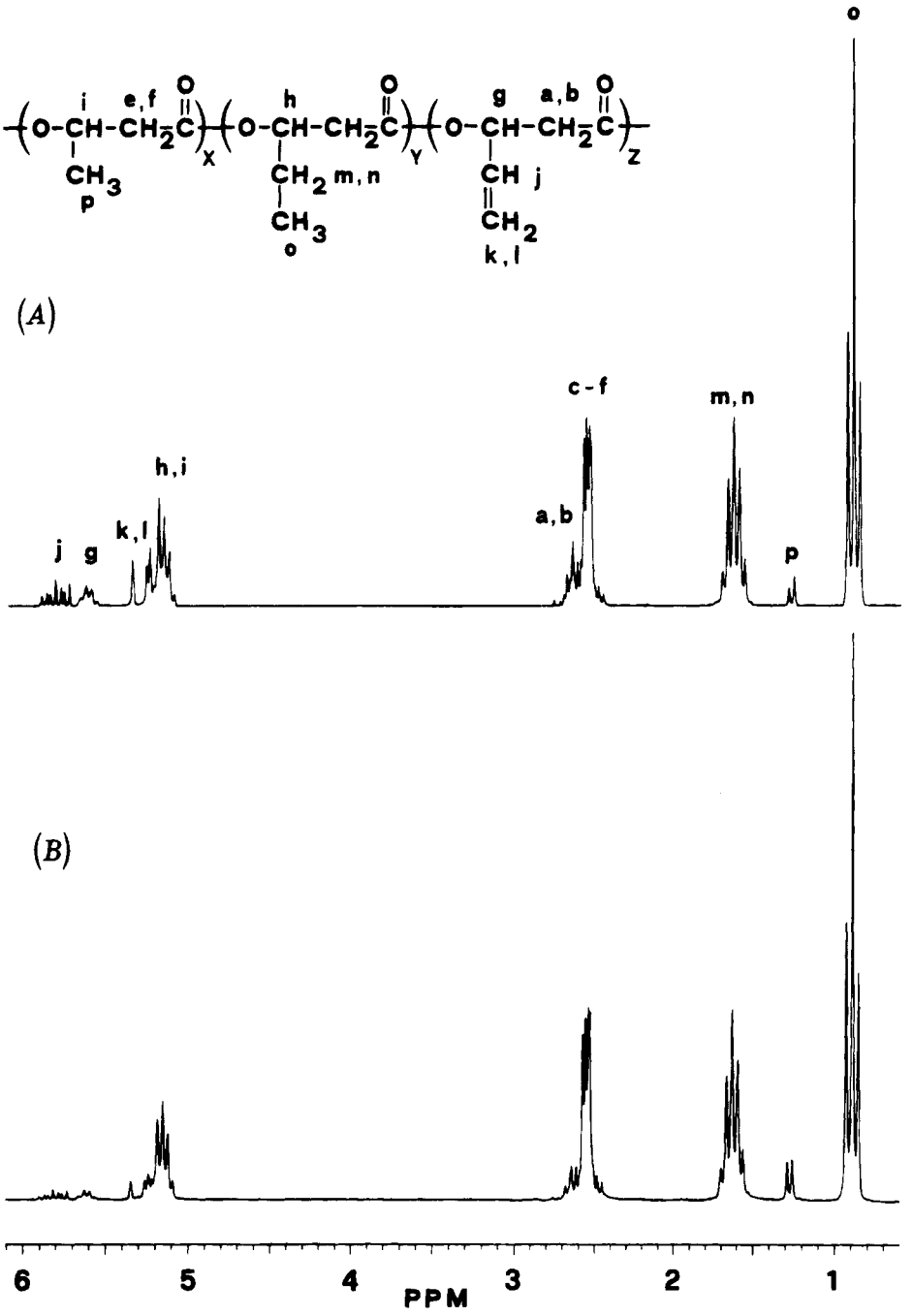


Figure 1. 200 MHz ^{1}H-NMR spectra of samples 1 (A) and 2 (B).

Table 1. Compositions of the PHA Samples Obtained by ^{1}H-NMR and FAB-MS Analyses

sample	technique	HV[a]	HP[a]	HB[a]
1[b]	NMR	77	19	4
	MS	83	14	3
2[b]	NMR	85	8	7
	MS	90	7	3

[a] HV, HP, and HB are 3-hydroxyvalerate, 3-hydroxypentenoate, and 3-hydroxybutyrate units, respectively. [b] For sample 1 the only carbon source available was 4-hydroxypentenoic acid; for sample 2 the carbon source was an equimolar mixture of 4-hydroxypentenoic acid and valeric acid.

tions of 10−15 mg/mL and injection volumes of 100 μL were used. A calibration curve was generated with seven polystyrene standards of low polydispersity, which were purchased from Polysciences. The melting points of the polyester samples were measured by differential scanning calorimetry (DSC) with a Mettler TA 3000 apparatus at a heating rate of 10 °C/min. The data reported are from the first heating cycle.

The 200 MHz ^{1}H-NMR spectra were recorded at 25 °C in $CDCl_3$ (20 mg/mL) on a Bruker AC 200 spectrometer with a 4 s pulse repetition, 2000 Hz spectral width, 16K data points, and 256 scans accumulation. Chemical shifts are expressed in ppm from internal tetramethylsilane.

Partial Methanolysis. A 1 N solution of HCl in dry methanol was prepared by bubbling gaseous HCl through redistilled anhydrous methanol. The amount of HCl dissolved was determined gravimetrically, and the concentration was adjusted by adding an appropriate volume of methanol. About 0.1 g of each sample was dissolved in 20 mL of $CHCl_3$, and 3 mL of a freshly prepared 1 N solution of HCl in methanol was added. The mixture was allowed to react at room temperature for 48 h, after which the solvent was evaporated. The residue was taken up with 2 mL of acetonitrile and transferred for HPLC analysis.

HPLC Fractionation. The fractionation of the methanolysis products was performed by HPLC using a Varian Vista 5500 HPLC system equipped with a Rheodyne injector with a 50 μL loop, a Varian 2050 UV detector, and a Waters μ-Bondapack 300 × 4 mm column (C18). A 20 μL aliquot of

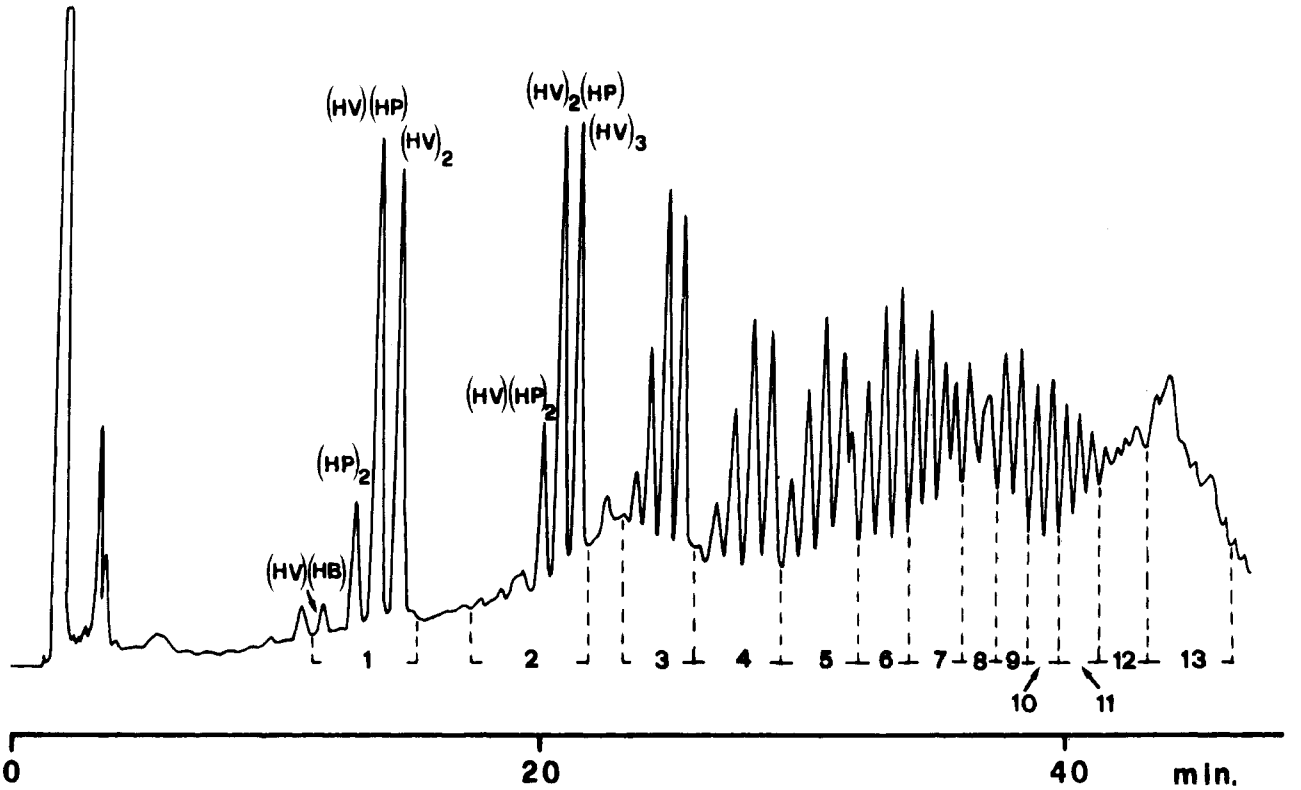


Figure 2. HPLC separation of the methanolysis products from sample 1; oligomers are identified in Table 2.

the acetonitrile solution was injected using an elution gradient starting with a 20/80 acetonitrile/water composition and ending with 100% acetonitrile in 40 min with 1 mL/min flow rate and UV detection at 205 nm.

FAB Mass Spectrometry. A double-focusing Kratos MS 50 equipped with the standard FAB source and a DS 90 data system was used to obtain mass spectra. The instrument was scanned from *m/z* 2000 to *m/z* 60, with a scan rate of 10 s/decade. The accelerating voltage was 8 kV. Cesium and rubidium iodides (50/50 w/w) were used for computer calibration. The resolution was approximately 2500. Lyophilized samples to be analyzed were dissolved in acetonitrile. About 2 μL of the sample solution was placed on the target of the direct insertion probe and mixed with 3-nitrobenzyl alcohol doped with NaCl. Peak intensity values reported in Tables 2 and 3 represent the average of three samples. The spectrum from each sample was averaged over three scans.

Thermospray LC/MS. Thermospray LC/MS was carried out with the MS 50 S instrument interfaced with a Kratos LC/MS thermospray apparatus using the same HPLC set described above. The interface was operated at a vaporizer temperature of 220 °C and at an ion source temperature of 230 °C. Poly(ethylene glycol) (MW 600) was used for computer calibration. A 20 mL aliquot of an acetonitrile solution of the mixture of the methanolysis products was injected, using an elution gradient starting with a 20/80 ratio of acetonitrile/0.1 M ammonium acetate in water and ending with100% acetonitrile in 40 min with a flow rate of 1 mL/min. Another mode of operation consisted of flushing the thermospray interface with a 0.1 M solution of ammonium acetate in water and injecting an HPLC peak or fraction previously collected. This operation mode was therefore equivalent to the FAB-MS analysis of the lyophilized HPLC fractions as described above except for the ionization mode used.

Results and Discussion

The two terpolyesters investigated were produced by fermentation of *Rhodospirillum rubrum*. Sample 1 was obtained from a culture in which 4-pentenoic acid was the only carbon source available to the cells, while sample 2 was obtained from the cells grown on an equimolar mixture of 4-pentenoic acid and valeric acid.

The ^{1}H-NMR spectra of the two polymers are shown in Figure 1. From these spectra it is possible to calculate the copolymer composition[4] in terms of 3-hydroxyvalerate (HV), 3-hydroxypentenoate (HP), and 3-hydroxybutyrate (HB) units (Table 1), but it is not possible to establish whether a single terpolymer, a mixture of copolymers, or a mixture of homopolymers has been obtained. Previously published results have shown that the GPC chromatograms for both samples contained only single peaks.[11] However, the DSC thermograms contained multiple endothermic peaks with the highest temperature peak at approximately 100 °C for both polymers, and it was not known whether these peaks represented melting transitions for different PHAs. In order to address this problem, we decided to analyze the two PHA copolymers by investigating the structure of the oligomers formed by partial methanolysis by means of their FAB-MS spectra.[12-15] The procedure used for the partial methanolysis of the two PHA samples studied and for the HPLC fractionation of the oligomers formed was similar to that previously reported.[12,14]

Sample 1. Figure 2 shows the HPLC trace of the partial methanolysis products from sample 1. In a first run fractions corresponding to each peak were collected, lyophilized, and analyzed by FAB-MS. Each mass spectrum for peaks up to a retention time of 21.6 min obtained from the HPLC trace showed the presence of a single oligomer, as evidenced by the molecular ions MH^+ and MNa^+ in the FAB mass spectra. As noted in our previous studies[12,14] and also in this case, no other peaks, which could have been formed from ion fragmentation of this oligomer, were present down to the lowest possible masses.

The HPLC fractions corresponding to peaks with retention times of 12.0, 13.2, 14.0, and 14.8 min did not yield FAB mass spectra because they represent the

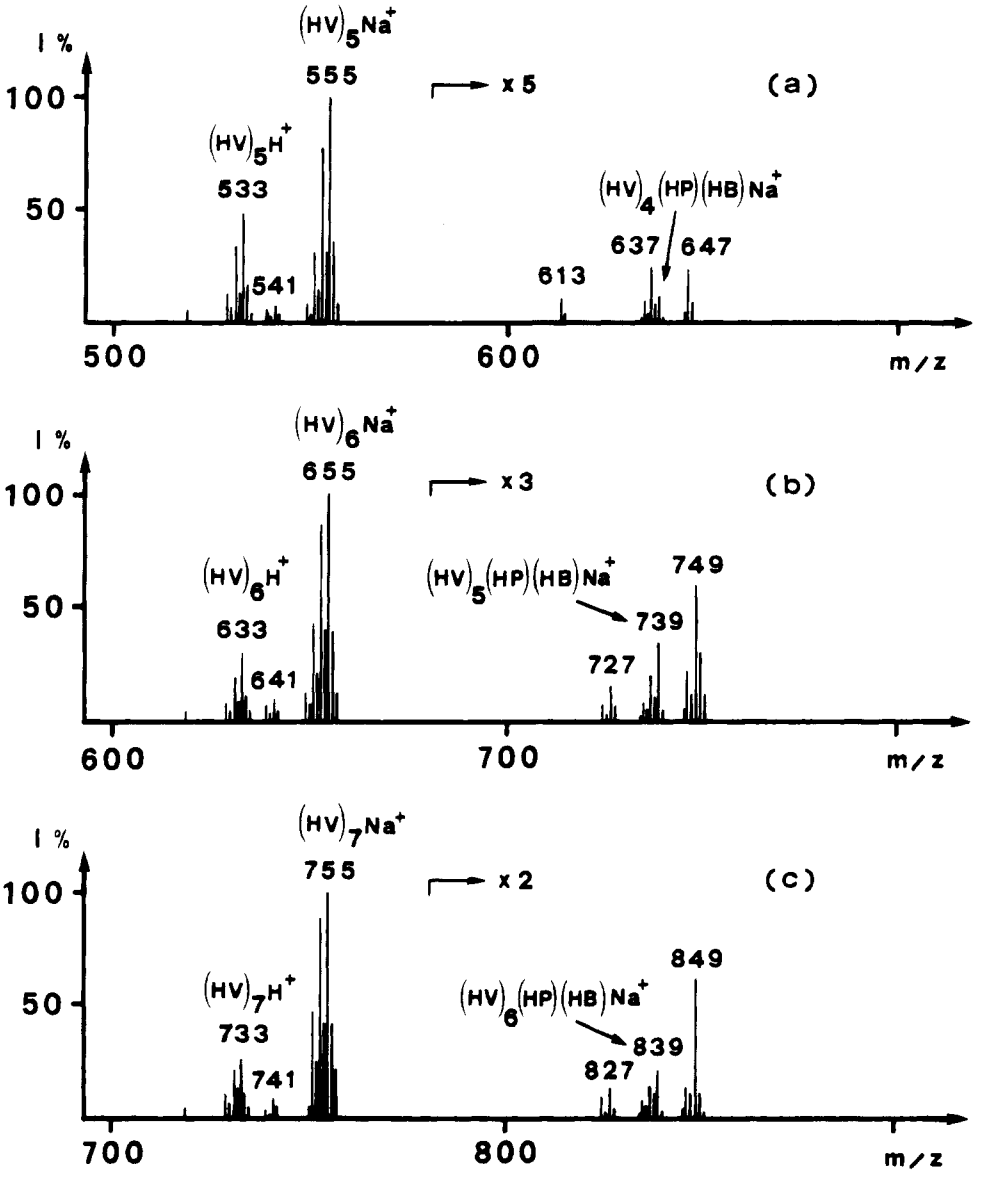


Figure 3. FAB mass spectra corresponding to fractions 4 (a), 5 (b), and 6 (c) in the HPLC trace of the methanolysis products from sample 1.

(HV)(HB), $(HP)_2$, (HV)(HP), and $(HV)_2$ dimers, respectively, which were too volatile and evaporated together with the solvent in the lyophilization process. The identification of these peaks was made instead by using a thermospray probe interfaced to the mass spectrometer and the HPLC to allow the analysis of the HPLC peaks without the isolation of fractions.

As noted above, HPLC peaks up to retention times of 21.6 min contained only a single oligomer, but FAB analysis of peaks with higher retention time revealed that each of these contained several oligomers. The presence in each fraction of oligomers of composition $(HV)_x(HP)_y(HB)_z$ demonstrates that *R. rubrum* is able to synthesize a terpolymer. As we showed previously[12,14] (for instance, in the case of PHAs obtained from *A. eutrophus* and *P. oleovorans*), the FAB mass spectra of fractions containing mixtures of oligomers are suitable for determining the copolymer composition and sequence distribution. That is, because no fragmentation of the pseudomolecular ions occurs and if it is assumed that the same response factor to FAB-MS can be used for the oligomers produced in the partial methanolysis, then the relative intensities of the MNa^+ ions present in the FAB mass spectrum of each HPLC fraction yield the copolymer composition.

Assuming a Bernoullian (random) distribution of repeating units in these copolymers,[17] the probability of finding a given $(HV)_x(HP)_y(HB)_z$ sequence ($P_{x,y,z}$) can be calculated by the Leibnitz formula[17] as follows:

$$P_{x,y,z} = \frac{(x+y+z)!}{x!y!z!} P^x{}_v P^y{}_p P^z{}_b \qquad (1)$$

In this equation P_v, P_p, and P_b are the molar fractions of the HV, HP, and HB units in the terpolymer. The polynomial coefficient in this equation is the number of possible sequence arrangements of the $(HV)_x(HP)_y(HB)_z$ oligomers. It is possible to find a set of P_v, P_p, and P_b which gives an oligomer distribution closest to that experimentally found, and this composition gives the lowest agreement factor (AF), as described in our previous studies.[15,17]

In order to apply these calculations, we collected the fractions as indicated in Figure 2. As an example, three FAB mass spectra corresponding to fractions 4, 5, and 6 are given in Figure 3a–c, respectively. The oligomers identified and the calculations for sample 1 are reported in Table 2. The spectrum corresponding to each fraction shows the presence of oligomers of the general formulas $(HV)_x$, $(HV)_{x-1}(HP)$, $(HV)_{x-2}(HP)_2$, $(HV)_{x-1}(HB)$, and

Table 2. Experimental[a] and Calculated[b] Relative Amounts of the Methanolysis Products from Sample 1

oligomer[c]	MNa^+	obsd from FAB-MS[a]	calcd[b] for HV/HP/HB mole ratio		
			77/19/4	82/15/3	85/13/2
		Fraction 1			
$(HV)_2$	250[d]				
(HV)(HP)	248[d]				
$(HP)_2$	246[d]				
(HV)(HB)	236				
		Fraction 2			
$(HV)_3$	355	53	48	57	63
$(HV)_2(HP)$	353	32	36	31	29
$(HV)(HP)_2$	351	7	9	6	4
$(HV)_2(HB)$	341	8	7	6	4
AF[e]			0.11	0.08	0.18
		Fraction 3			
$(HV)_4$	455	49	36	46	53
$(HV)_3(HP)$	453	32	36	33	32
$(HV)_2(HP)_2$	451	11	13	9	7
$(HV)(HP)_3$	449	2	2	1	1
$(HV)_3(HB)$	441	4	7	7	5
$(HV)_2(HP)(HB)$	439	2	5	4	2
AF[e]			0.24	0.09	0.10
		Fraction 4			
$(HV)_5$	555	44	28	38	45
$(HV)_4(HP)$	553	34	35	35	34
$(HV)_3(HP)_2$	551	14	17	13	11
$(HV)_2(HP)_3$	549	3	4	2	2
$(HV)_4(HB)$	541	3	7	7	5
$(HV)_3(HP)(HB)$	539	2	7	5	3
$(HV)_2(HP)_4$	647				
$(HV)_4(HP)(HB)$	639				
$(HV)_3(HP)_2(HB)$	637				
$(HV)_3(HB)_3$	613				
AF[e]			0.30	0.14	0.07
		Fraction 5			
$(HV)_6$	655	40	25	34	40
$(HV)_5(HP)$	653	35	37	37	37
$(HV)_4(HP)_2$	651	16	23	17	14
$(HV)_3(HP)_3$	649	5	7	4	3
$(HV)_5(HB)$	641	4	8	7	6
$(HV)_4(HP)_3$	749				
$(HV)_3(HP)_4$	747				
$(HV)_5(HP)(HB)$	739				
$(HV)_4(HP)_2(HB)$	737				
$(HV)_5(HB)_2$	727				
$(HV)_4(HP)(HB)_2$	725				
AF[e]			0.31	0.13	0.07
		Fraction 6			
$(HV)_7$	755	38	23	31	37
$(HV)_6(HP)$	753	36	40	40	39
$(HV)_5(HP)_2$	751	22	29	22	18
$(HV)_6(HB)$	741	3	8	8	6
$(HV)_5(HP)_3$	849				
$(HV)_4(HP)_4$	847				
$(HV)_6(HP)(HB)$	839				
$(HV)_5(HP)_2(HB)$	837				
$(HV)_4(HP)_3(HB)$	835				
$(HV)_6(HB)_2$	827				
$(HV)_5(HP)(HB)_2$	825				
$(HV)_5(HB)_3$	813				
AF[e]			0.31	0.17	0.10
		Fraction 7			
$(HV)_8$	855	37	20	27	33
$(HV)_7(HP)$	853	37	39	40	40
$(HV)_6(HP)_2$	851	23	33	25	21
$(HV)_7(HB)$	841	4	8	8	6
$(HV)_7(HP)_2$	951				
$(HV)_6(HP)_3$	949				
$(HV)_5(HP)_4$	947				
$(HV)_7(HP)(HB)$	939				
$(HV)_6(HP)_2(HB)$	937				
AF[e]			0.35	0.20	0.10

[a] Relative intensities of MNa^+ ions in the FAB mass spectrum. [b] Relative intensities of methanolysis products calculated with eq 1 for three copolymer compositions. [c] (HV) = 3-hydroxyvalerate, (HP) = 3-hydroxypentenoate, (HB) = 3-hydroxybutyrate. [d] Identified by thermospray mass spectrometry as MNH_4^+ pseudomolecular ions. [e] $AF = [\sum_i (Iexp - I_{calc})^2 / \sum_i I_{exp}^2]^{1/2}$, where I_{exp} and I_{calc} are the normalized experimental and calculated abundances of partial methanolysis products.

Table 3. Experimental[a] and Calculated[b] Relative Amounts of the Methanolysis Products from Sample 2

			calcd[b] for HV/HP/HB mole ratio		
	MNa^+	obsd from FAB-MS[a]	85/8/7	87/9/4	90/7/3
		Fraction 1			
$(HV)_2$	250[d]				
(HV)(HP)	248[d]				
$(HP)_2$	246[d]				
(HV)(HB)	236[d]				
		Fraction 2			
$(HV)_3$	355	70	64	67	74
$(HV)_2(HP)$	353	18	18	21	17
$(HV)(HP)_2$	351	2	2	2	1
$(HV)_2(HB)$	341	10	16	9	7
AF[e]			0.12	0.06	0.07
		Fraction 3			
$(HV)_4$	455	64	54	58	66
$(HV)_3(HP)$	453	21	20	24	21
$(HV)_2(HP)_2$	451	3	3	4	2
$(HV)_3(HB)$	441	10	18	11	9
$(HV)_2(HP)(HB)$	439	2	5	3	2
$(HV)(HB)_6$	671				
$(HV)_2(HB)_4$	599				
$(HV)_2(HP)_2(HB)$	537				
$(HV)_3(HB)_2$	527				
$(HV)_2(HP)(HB)_2$	525				
$(HV)_2(HB)_3$	513				
AF[e]			0.19	0.10	0.04
		Fraction 4			
$(HV)_5$	555	58	51	54	62
$(HV)_4(HP)$	553	26	24	28	24
$(HV)_3(HP)_2$	551	6	4	6	4
$(HV)_4(HB)$	541	10	21	12	10
$(HV)(HB)_8$	843				
$(HV)_2(HB)_6$	771				
$(HV)_3(HB)_4$	699				
$(HV)_4(HP)(HB)$	639				
$(HV)_3(HP)_2(HB)$	637				
$(HV)_4(HB)_2$	627				
$(HV)_3(HP)(HB)_2$	625				
$(HV)_3(HB)_3$	613				
AF[e]			0.21	0.08	0.08
		Fraction 5			
$(HV)_6$	655	55	46	50	57
$(HV)_5(HP)$	653	25	26	30	26
$(HV)_4(HP)_2$	651	7	6	7	5
$(HV)_5(HB)$	641	13	22	13	12
$(HV)_4(HB)_4$	799				
$(HV)_4(HP)_3$	749				
$(HV)_5(HP)(HB)$	739				
$(HV)_4(HP)_2(HB)$	737				
$(HV)_5(HB)_2$	727				
$(HV)_4(HP)(HB)_2$	725				
$(HV)_4(HB)_3$	713				
AF[e]			0.21	0.11	0.05
		Fraction 6			
$(HV)_7$	755	56	41	44	53
$(HV)_6(HP)$	753	25	27	32	28
$(HV)_5(HP)_2$	751	8	8	10	6
$(HV)_6(HB)$	741	11	24	14	13
$(HV)_5(HB)_4$	899				
$(HV)_5(HP)_3$	849				
$(HV)_6(HP)(HB)$	839				
$(HV)_5(HP)_2(HB)$	837				
$(HV)_6(HB)_2$	827				
$(HV)_5(HP)(HB)_2$	825				
$(HV)_5(HB)_3$	813				
AF[e]			0.32	0.23	0.08
		Fraction 7			
$(HV)_8$	855	49	38	40	49
$(HV)_7(HP)$	853	30	28	33	30
$(HV)_6(HP)_2$	851	10	9	12	8
$(HV)_7(HB)$	841	11	25	15	13
$(HV)_6(HB)_4$	999				
$(HV)_6(HP)_3$	949				
$(HV)_7(HP)(HB)$	939				
$(HV)_6(HP)_2(HB)$	937				
$(HV)_7(HB)_2$	927				
$(HV)_6(HP)(HB)_2$	925				
$(HV)_6(HB)_3$	913				
AF[e]			0.30	0.18	0.05

[a] Relative intensities of MNa^+ ions in the FAB mass spectrum. [b] Relative intensities of methanolysis products calculated with eq 1 for three copolymer compositions. [c] (HV) = 3-hydroxyvalerate, (HP) = 3-hydroxypentenoate, (HB) = 3-hydroxybutyrate. [d] Identified by thermospray mass spectrometry as MNH_4^+ pseudomolecular ions. [e] $AF = [\sum_i (I_{exp} - I_{calc})^2 / \sum_i I_{exp}^2]^{1/2}$, where I_{exp} and I_{calc} are the normalized experimental and calculated abundances of partial methanolysis products.

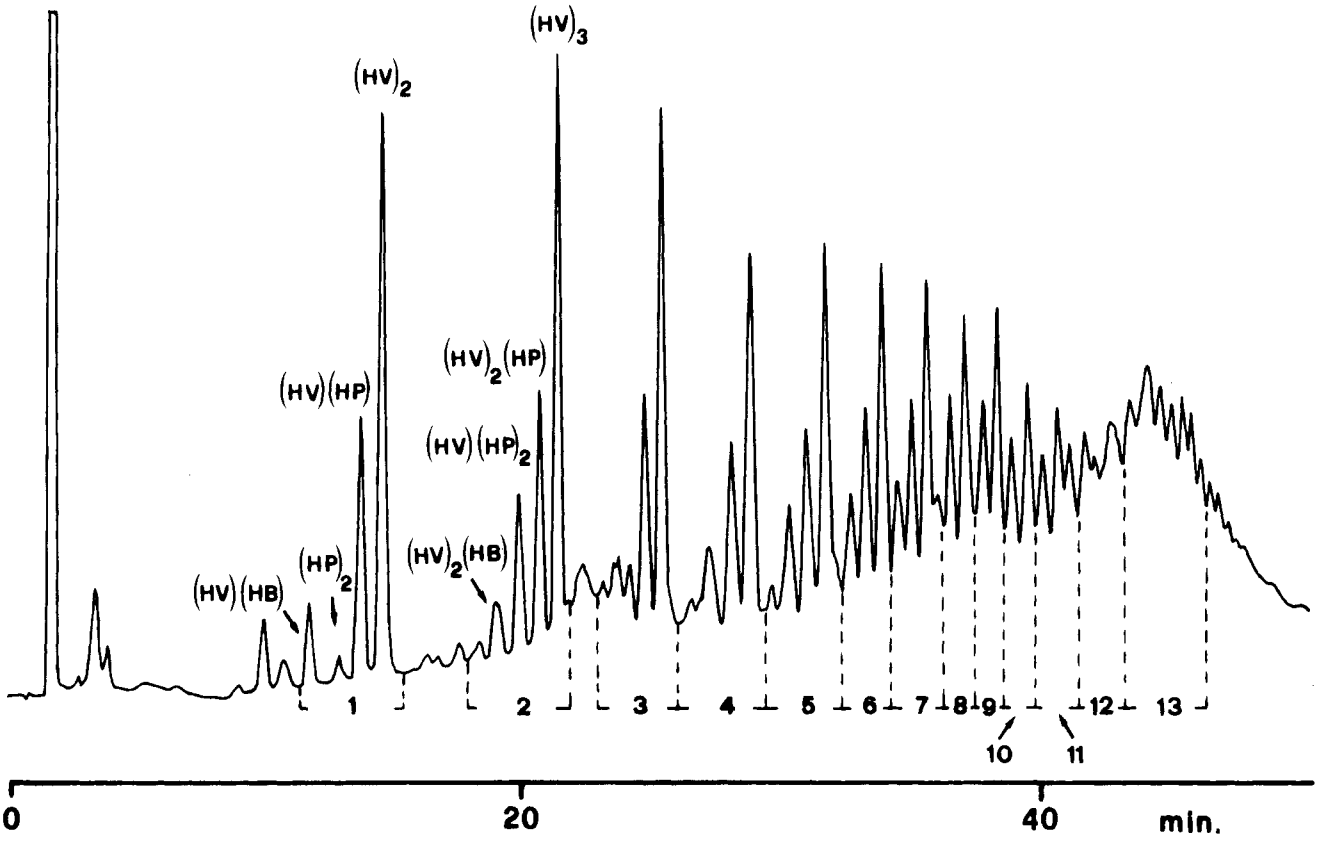


Figure 4. HPLC separation of the methanolysis products from sample 2; oligomers are identified in Table 3.

$(HV)_{x-2}(HP)(HB)$ in higher percentages with respect to the others. In the same spectrum some higher oligomers also appear with lower intensity, implying that the HPLC separation of homolog oligomers is not complete.

In Table 2 we have indicated the mass numbers of these ions but not their relative abundances. These peaks were excluded from the calculations because of possible errors in the measurements of their abundance as a result of their low intensities and because they are shared by adjacent fractions in percentages different for every oligomer. We were also able to identify some of the oligomers contained in the fractions beyond the seventh in Figure 2, but they are not reported in Table 2 because the quality of the mass spectra obtained is not as good as that relative to HPLC fractions of lower retention times. In fact, when examining products of higher degree of polymerization, oligomers with a much larger variety of monomeric composition are encountered, so that FAB matrix discrimination becomes effective and quantitative analysis is not allowed.

The best agreement between the experimental and calculated values was found for a HV/HP/HB ratio varying from 82/15/3 to 85/13/2, which is very close to the 79/19/4 ratio obtained from ^{1}H-NMR data (Table 1).

Sample 2. The HPLC trace of the partial methanolysis products from sample 2 is shown in Figure 4. The number of HPLC peaks for this sample was the same as that for sample 1, but the relative intensities were different. The number of fractions collected corresponds to those of sample 1; thus a direct comparison of mass spectra for each fraction could be performed. In Figure 5a–c are shown the mass spectra of HPLC fractions 4, 5, and 6, respectively. Again, in this case, the identification of oligomers of type $(HV)_x(HP)_y(HB)_z$ established that the sample was a terpolymer.

The experimentally observed intensities were compared with those calculated according to eq 1, and the values are reported in Table 3. The best agreement of the calculated values with the experimental ones was found for a HV/HP/HB composition of 90/7/3, which is very close to that obtained by ^{1}H-NMR of 85/8/7 (Table 1). This result confirms the assumption that sample 2 also possessed a random distribution of units, even though it was grown on a mixture of two different alkanoic acids.

It is of interest to note that each fraction after the second one shows oligomers $(HV)_x(HB)_y$ (see Table 3) with a high content of HB units (e.g., $(HV)_3(HB)_4$ at *m/z* 699, $(HV)_4(HB)_4$ at *m/z* 799, and $(HV)_5(HB)_4$ at *m/z* 899 in Figures 5a–c, respectively), although their peak intensities are very low (<2%). This low amount of oligomers can be correlated to a very small amount (less than 3%) of a copolymer of HV and HB with a high content of HB produced by *R. rubrum* together with the terpolymer. It has already been shown[13,18] that FAB-MS analysis of chemically or thermally degraded samples of PHAs permits one to distinguish between pure copolymers and mixtures of homo- or copolymers. In the case of sample 2 examined here, NMR was unable to reveal the presence of a contaminant copolyester, while HPLC and FAB-MS analyses easily allowed its detection.

Acknowledgment. Partial financial support from the Ministero per l'Università e la Ricerca Scientifica e Tecnologica and from Consiglio Nazionale delle Ricerche (Rome) is gratefully acknowledged. The American authors gratefully acknowledge the support of their work by the Office of Naval Research Biology Program and the Materials Research Laboratory of the University of Massachusetts, which is funded by the Natural Science Foundation.

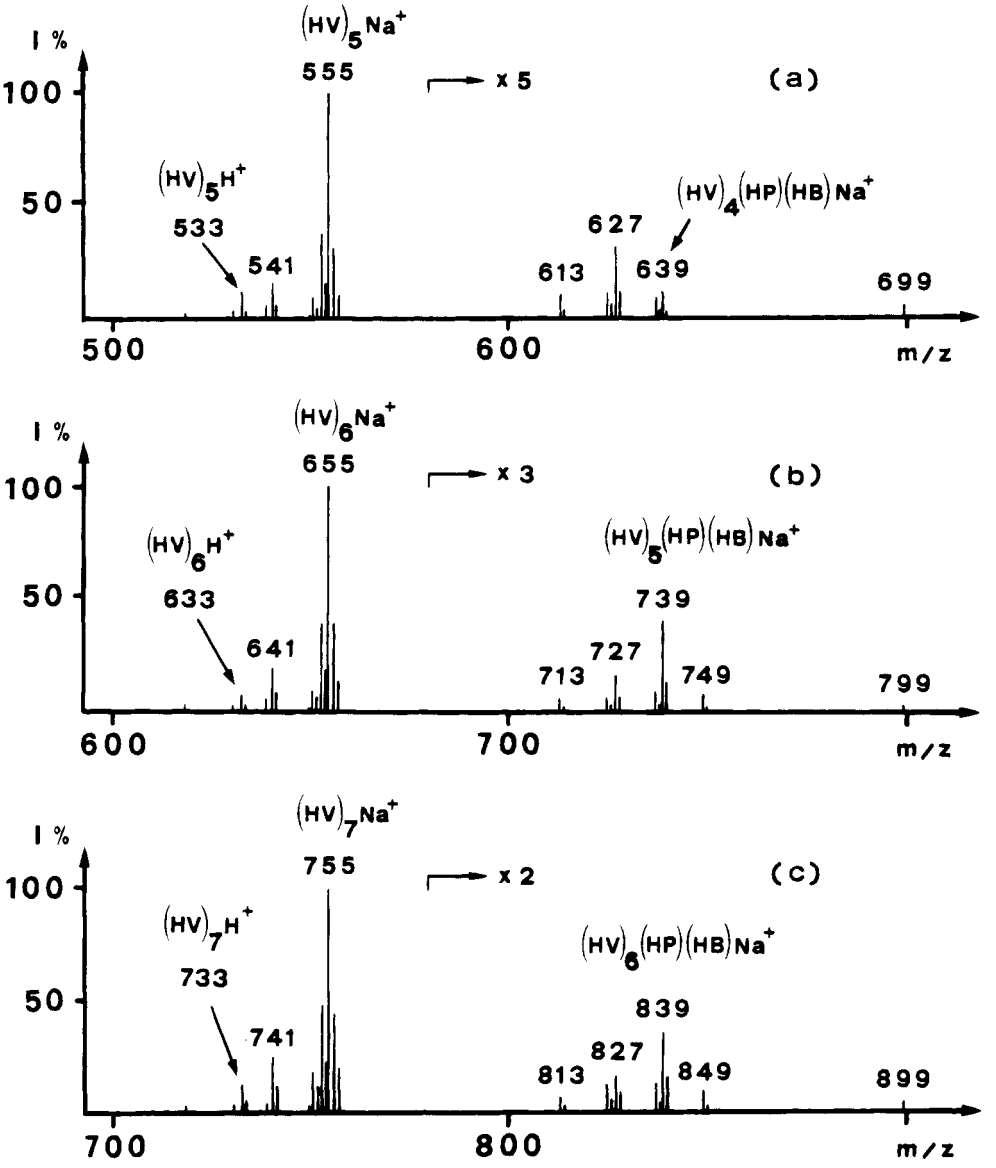


Figure 5. FAB mass spectra corresponding to fractions 4 (a), 5 (b), and 6 (c) in the HPLC trace of the methanolysis products from sample 2.

References and Notes

(1) Dawes, E. A.; Senior, P. J. *Adv. Microb. Physiol.* **1973**, *10*, 135.
(2) Merrick, J. M. In *Photosynthetic Bacteria*; Clayton, R. K., Sistrom, W., Eds.; Plenum Press: New York, 1978; p 199.
(3) Brandl, H.; Knee, E. J., Jr.; Fuller, R. C.; Gross, R. A.; Lenz, R. W. *Int. J. Biol. Macromol.* **1989**, *11*, 49.
(4) Gross, R. A.; Brandl, H.; Ulmer, H. W.; Posada, M. A.; Fuller, R. C.; Lenz, R. W. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1989**, *30*, 492.
(5) Holmes, P. A. *Phys. Technol.* **1985**, *16*, 32.
(6) Brandl, H.; Gross, R. A.; Lenz, R. W.; Fuller, R. C. *Adv. Biochem. Eng. Biotechnol.* **1990**, *41*, 77.
(7) Fritzche, K.; Lenz, R. W.; Fuller, R. C. *Makromol. Chem.* **1990**, *191*, 1957.
(8) Preusting, H.; Nijenhuis, A.; Witholt, B. *Macromolecules* **1990**, *23*, 4220.
(9) Doi, Y.; Abe, C. *Macromolecules* **1990**, *23*, 3705.
(10) Abe, C.; Taima, Y.; Nakamura, Y.; Doi, Y. *Polym. Commun.* **1990**, *31*, 404.
(11) Ulmer, H. W.; Gross, R. A.; Posada, M.; Weisbach, P.; Fuller, R. C.; Lenz, R. W. *Macromolecules* **1994**, *27*, 1675.
(12) Ballistreri, A.; Garozzo, D.; Giuffrida, M.; Impallomeni, G.; Montaudo, G. *Macromolecules* **1989**, *22*, 2107.
(13) (a) Ballistreri, A.; Garozzo, D.; Giuffrida, M.; Montaudo, G. In *Novel Biodegradable Polymers*; Dawes, E. A., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1990; pp 49–64. (b) Ballistreri, A.; Montaudo, G.; Garozzo, D.; Giuffrida, M.; Montaudo, M. S. *Macromolecules* **1991**, *24*, 1231.
(14) Ballistreri, A.; Montaudo, G.; Impallomei, G.; Lenz, R. W.; Kim, Y. B.; Fuller, R. C. *Macromolecules* **1990**, *23*, 5059.
(15) Ballistreri, A.; Montaudo, G.; Giuffrida, M.; Lenz, R. W.; Kim, Y. B.; Fuller, R. C. *Macromolecules* **1992**, *25*, 1845.
(16) *American Type Culture Collection Catalogue of Cell Lines and Hybridomas*, 5th ed.; American Type Culture Collection: Rockville, MD, 1985.
(17) Montaudo, M. S.; Ballistreri, A.; Montaudo, G. *Macromolecules* **1991**, *24*, 5051.
(18) Nedea, M. E.; Morin, F. G.; Marchessault, R. H. *Polym. Bull.* **1991**, *26*, 549.

MA946116V