54. Solid-State CP/MAS ^{13}C-NMR Spectra of Oligolides Derived from 3-Hydroxybutanoic Acid[1])[2])

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The solid-state CP/MAS ^{13}C-NMR spectra (cross-polarization/magic-angle spinning ^{13}C-NMR) of eight lower cyclic and one linear oligomers and several polymers of (R)-3-hydroxybutanoic acid (3-HB) are reported. The polymeric samples of different origin and molecular weight give remarkably similar and well resolved spectra, indicating considerable similarity in the conformations of the molecules and homogeneity in the solid-state environment. The crystalline cyclic oligomers **1–8** containing 3–9 units of 3-HB give very well resolved spectra. The number of nonequivalent positions in the solid state can be identified and is in accord with structures from X-ray diffraction where these were determined. The spectra of the oligolides become increasingly similar to those of the polymer as the ring size increases. This spectral evidence supports the view of a homogeneous and well defined conformation for the polymeric material (as proposed previously, based on other experiments).

Introduction. – The biopolymer P(3-HB) is a polymer of (R)-3-hydroxybutanoic acid (3-HB). P(3-HB) and the related copolymer P(3-HB)/P(3-HV), known under the trade name *BIOPOL* (3-HV = 3-hydroxyvaleric acid), are members of a family of endogenous microbial storage materials known as the poly(hydroxyalkanoates) PHA. P(3-HB) is deposited as an inclusion inside the cells of certain bacteria [3] as a means of storing energy (in the form of accessible acetate) and reducing equivalents (accessible NADH), and it is of commerical importance as a biodegradable and biocompatible polymer (see the review articles [1c] [4a], the monograph by *Doi* [4b], and a congress volume[4])).

Molecular-mechanics calculations were performed on P(3-HB) to calculate likely structures for the polymer. The calculated models were then used to match with fibre

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X-ray diffraction results [5]. The best fitting model was then refined further using the diffraction data, and a final *R* factor of 0.15 was obtained [5c]. The solid-state conformation of the P(3-HB) chain obtained by this method is a left-handed spiral with two molecules per turn (2_1 helix) and *local centres of symmetry* giving one molecule per asymmetric unit.

Cyclic oligomers **1–8** of (*R*)-3-hydroxybutyrate were prepared and characterized[1–3][5]) by *Seebach* and coworkers [1] [2]. The structures of several of these cyclic oligomers were solved by X-ray diffraction techniques [2b–e] (see *Table 1*). It seemed to us that another approach to obtain information about the structure of the P(3-HB)'s could be by comparison of the solid-state CP/MAS ^{13}C-NMR spectra of the polymer[6]) with those of the cyclic oligomers whose structures were already known. Accordingly, we examined the solid-state ^{13}C-NMR spectra of the cyclic (all-*R*)-oligomers **1–7** containing from 3 to 9 (3-HB) units for comparison with the spectra of several different forms of the polymer. In addition, we report the spectrum of *rac*-(*l,u*)-triolide[7]) **8**.

The oligolides **1–7** have average C_3 through C_9 symmetry in solution, at least at ambient temperature, as evident, *e.g.*, from a single ^{13}C-NMR signal[3]) (of different chemical shift) for the 3 through 9 carbonyl C-atoms of these oligolides [1c] [2c]. In the solid state, the molecules of the various oligolides studied so far by X-ray diffraction have C_1 symmetry (space groups $P2_12_12_1$, $P2_1/n$ or $P2_1$) or C_2 symmetry (space group C_2; *Table 1*). Thus, there is either one molecule in the asymmetric unit, or two (in the $P2_1$ form of hexolide **4**), or only half a molecule, where the other half is generated by crystallographic symmetry of the space group $C2$ (**2** and **4**).

1 $n = 3$, (all-*R*)
2 $n = 4$, (all-*R*)
3 $n = 5$, (all-*R*)
4 $n = 6$, (all-*R*)
5 $n = 7$, (all-*R*)
6 $n = 8$, (all-*R*)
7 $n = 9$, (all-*R*)

+ enantiomer

rac-**8**

[5]) A first, preliminary report on linear and cyclic oligomers of 3-hydroxybutanoic acid with very little experimental detail was publihsed by *Masamune* and coworkers in 1982 [6].

[6]) Many NMR investigations of P(3-HB), also solid-state spectra, were published [4b] [4c] [7], see also the article by *Sanders* in the congress volume mentioned in *Footnote*[4]).

[7]) *l* = like and *u* = unlike relative configuration, *i.e.* (*R,R,S*)- and (*R,S,S*)-triolides have (*l,u*)-configuration, (*R,R,R*)- and (*S,S,S*)-triolides have (*l,l*)-configuration, see [8].

Table 1. *Some Crystallographic Data of the Oligolides* **1**–**6** *and* **8** [2c–e]. More than one modification or mixtures thereof may be present in a given sample of the higher members of the series.

		Space group	Number of molecules		CSD reference code	Ref.
			in elementary cell (Z)	in asymmetric unit		
(all-R)-Triolide	**1**	$P2_12_12_1$	4	1	– [a])	[2d]
rac-(l,u)-Triolide	**8**	$P2_1/n$	4	1	–	– [b])
Tetrolide	**2**	$C2$	2	1/2	– [a])	[2c]
Pentolide	**3**	$P2_12_12_1$	4	1	SEJWOT	[2b]
Hexolide[c])	**4**	$C2$	2	1/2	SEJWUZ 01	[2b]
	4	$P2_1$	4	2	SEJWUZ	[2b]
Heptolide	**5**	$P2_12_12_1$	4	1	–	– [b])
Octolide[c])[d])	**6**	$P2_12_12_1$	4	1	–	– [b])
	6	$P2_12_12_1$	4	1	–	– [b])

[a]) Data were submitted, but not yet included in the *Cambridge Crystallographic Data File* or assigned a code.
[b]) Structure determination will be published later [2e].
[c]) Two modifications of **4** and of **6** were found so far in different crystallization batches. The m.p. of chemically pure samples may vary or be broad due to the fact that there are a couple of modifications which may occur in mixtures.
[d]) Two different modifications of the octolide could be crystallized (same space group, different cell dimensions).

Solid-State ^{13}C-NMR Spectra. – Typical spectra from this work are shown in *Figs. 1–3*. Chemical shifts are recorded in *Table 2*. The assignment of the resonances in the solid-state ^{13}C-NMR spectra was trivial as they appeared at the expected positions for C-atoms of their types. The NMR spectrum of (all-R)-triolide **1** (*Fig. 1a*) shows 3 carbonyl peaks at *ca.* 170 ppm, 3 CH signals at *ca.* 68 ppm, and 2 peaks each (ratio 2:1) for the CH$_2$ and Me groups at 43 and 22 ppm, respectively. Similarly the spectrum of rac-(l,u)-triolide **8** contains 3 peaks for each of the CO, CH, and Me groups and 2 signals (ratio 2:1) for the CH$_2$ groups. The tetrolide **2** is the only cyclic oligomer examined to show fewer peaks for each type of C-atom (there are 2 peaks) than atoms of that type (there are 4 atoms) in the molecule (*Fig. 1b*). The spectra of both triolides and the tetrolide are the simplest obtained and show clearly what can be inferred from the spectra of the higher oligomers, that the CH$_2$ groups give rise to the broadest lines ($w_{1/2} = 108$ Hz for triolide and 90 Hz for tetrolide), whilst the other C-atoms give remarkably well resolved lines ($w_{1/2} = 43$ Hz for triolide and 29 Hz for tetrolide).

The spectrum of pentolide **3** contains more than 5 lines for each site, and possibly as many as 10, including shoulders, for the CH group. The spectrum of hexolide **4** used for the measurement also shows considerably more than 6 lines and possibly as many as 12 at each spectral position (*Fig. 1c*). Finally, the spectra of heptolide **5**, octolide **6**, and nonolide **7** are in agreement with the presence in the solid state of molecules having each and every C-atom in a different environment. In particular, the methine region of heptolide **5** shows 7 distinct signals (*Fig. 2*).

To obtain representative spectra of the polymer P(3-HB), two samples containing 8000–9000 (3-HB) monomer units (molecular weight $7.5 \cdot 10^5$ g/mol) were used; the P(3-HB) had been precipitated by pouring either 1,2-dichloroethane or AcOH solutions
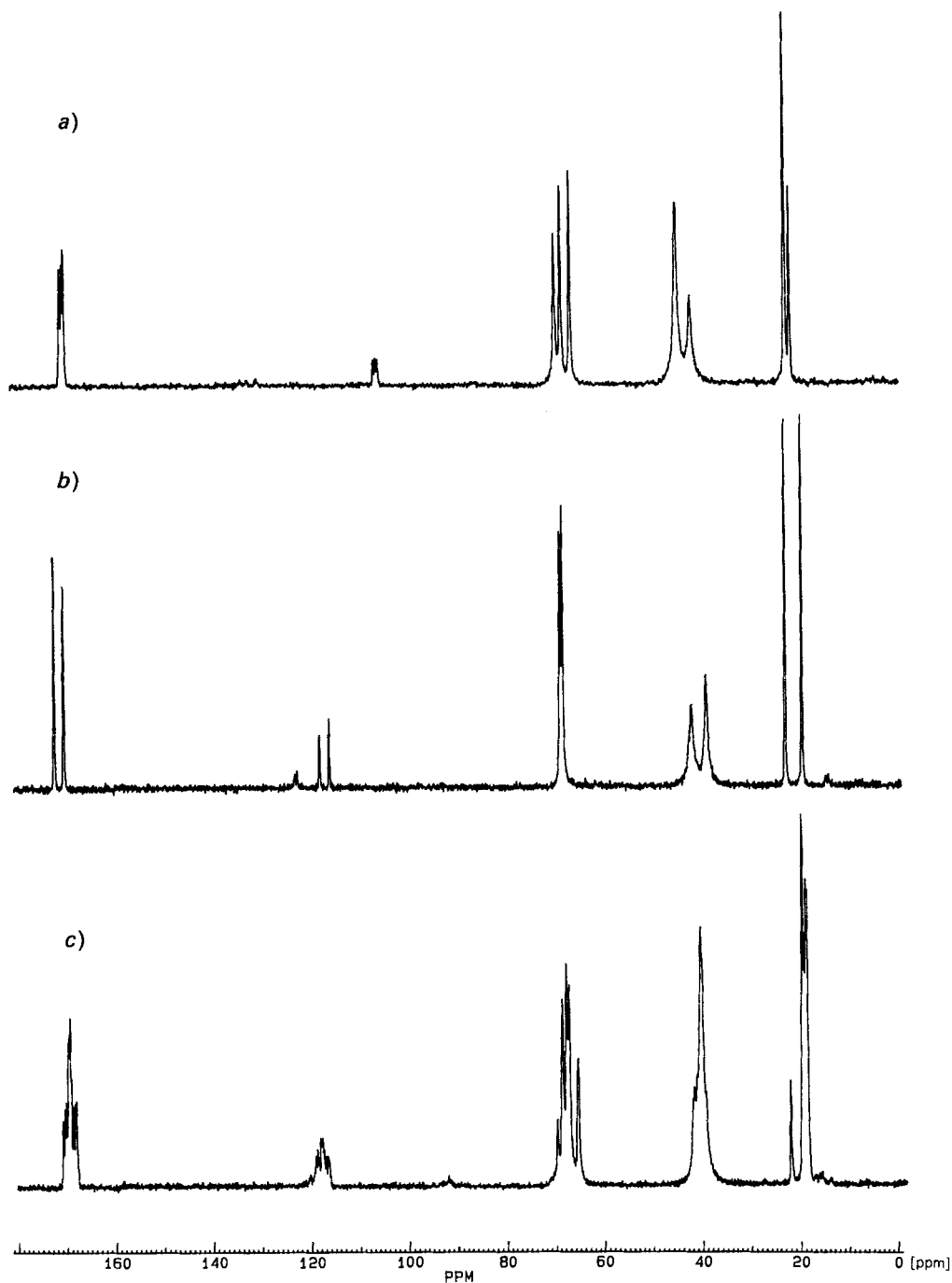
a)

b)

c)

Fig. 1. *CP/MAS ^{13}C-NMR Spectra of* a*) triolide* **1**, b*) tetrolide* **2**, *and* c*) hexolide* **4**. The small peaks between 110 and 120 ppm are first-order spinning side bands on the carbonyl resonances.

Table 2. ^{13}C-NMR Chemical Shifts of the Solid Oligolides **1–8** and of P(3-HB). For comparison, the solution values are also included.

|	C=O		CH$_2$		CH–O		Me	
	solid state	solution (CDCl$_3$)	solid state	solution (CDCl$_3$)	solid state	solution (CDCl$_3$)	solid state	solution (CDCl$_3$)
(R,R,R)-Triolide **1**	170.0, 170.3, 171.5	170.0	41.8, 44.7 (two)	42.2	66.4, 68.3, 69.6	68.9	21.5, 22.5 (two)	20.8
rac-(l,u)-Triolide **8**	168.9, 170.6, 171.0	168.1, 169.2, 170.4	42.3, 43.2 (two)	40.4, 41.8, 42.0	67.8, 69.3, 70.4	68.0, 68.1, 69.2	21.0, 21.6, 22.7	19.3, 19.9, 20.4
Tetrolide **2**	170.6, 172.5	169.7	39.2, 42.1	41.0	68.5, 69.0	67.6	19.5, 22.9	20.0
Pentolide **3**	168.9, 169.2, 170.1, 170.4, 170.5, 170.9	169.4	39.1, 40.9 (several), 43.8	41.0	66.5, 66.7, 67.6 (two), 68.8, 69.3, 70.1 (three), 72.8	67.7	18.0, 20.0 (several), 20.6 (several), 21.5 (several), 22.00	19.8
Hexolide **4**	168.8, 168.9, 169.4, 169.8, 170.1 (two?), 170.5 (two?), 170.8, 171.2, 171.5	169.3	40.0, 40.8, 41.1 (several), 41,9, 42.4, 42.8	40.8	66.2 (two?), 68.0 (three?), 68.6 (three?), 69.4 (three), 70.4	67.5	19.2 (several), 19.6 (several), 19.9, 20.3 (several), 20.6, 22.7, 19.8	19.8
Heptolide **5**	169.5, 169.7 (several), 170.2, 170.7	169.3	40.5, 41.3 (several), 42.3 (several)	40.8	65.1, 66.6, 67.8, 68.3, 69.6, 70.4, 70.6	67.7	20.0, 20.2, 20.6 (several), 21.3	19.8
Octolide **6**	167.7 (two?), 169.1, 169.4, 169.6, 170.3 (several), 170.6, 172.5	169.2	40.6 (several), 41.1 (several), 42.2, 43.4	40.8	66.0, 66.5, 67.2, 68.2 (two?), 69.0, 70.0, 72.6	67.6	18.1, 19.4, 20.2, 20.4, 20.9 (two), 21.6 (two)	19.8
Nonolide **7**	168.1 (two?), 168.9 (two?), 169.0, 169.8, 170.5 (two?), 170.7	169.2	39.1, 40.3, 40.9, 42.2 (two), 43.1 (several), 43.3, 43.9	40.8	66.3, 67.1, 67.7, 68.1 (two), 68.7 (two), 69.3 (two)	67.6	19.1, 19.9, 20.8 (several), 21.2 (several), 21.5 (several)	19.8
P(3-HB)	169.8	169.1	42.7	40.8	68.4	67.6	21.2	19.8
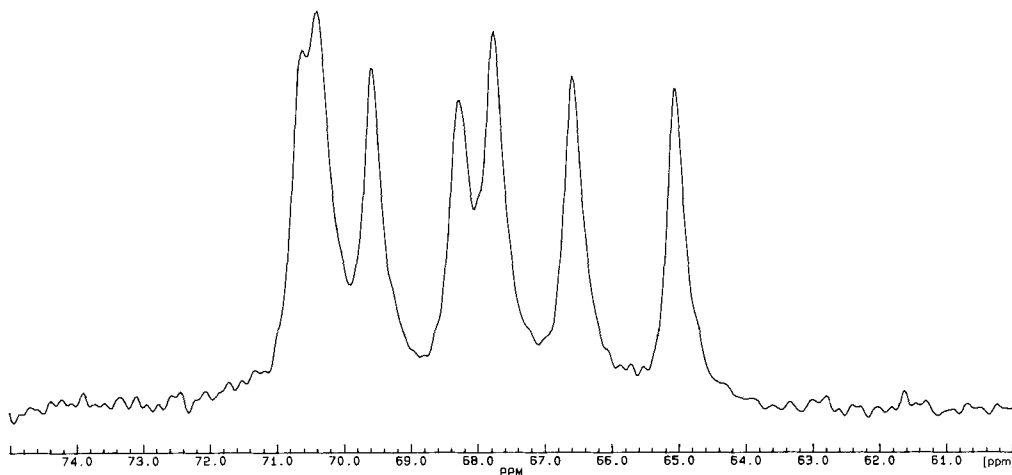
Fig. 2. *The methine region of the CP/MAS* ^{13}*C-NMR spectrum of heptolide* **5** *showing the presence of seven lines*

into MeOH. Furthermore, a linear oligomer of *ca.* 30 monomer units, prepared by pyrolysis of P(3-HB) and, therefore, containing crotonate end groups [9], was measured. Then, the spectrum of the synthetic uniform linear hexadecamer, a non-crystalline solid[3]), was obtained. Finally, we also measured the solid-state NMR spectrum of *BIOPOL*, a high-molecular-weight P(3-HB)/P(3-HV) copolymer containing 19% (3-HV) units. All ^{13}C-NMR spectra obtained show relatively sharp lines (typical $w_{1/2}$ values: 65 Hz for C=O, 97 Hz for C–O, 126 Hz for CH$_2$, and 75 Hz for Me of the P(3-HB) sample (1,2-dichloroethane/MeOH) at 8.04 kHz) with the chemical shifts of the 4 C-sites in each sample being identical within experimental error. This points to a structure of the linear oligomeric and polymeric materials being independent of chain lenght, method of precipitation, or incorporated ethyl side chains[8]).

Discussion. – The measurements described in the previous section warrant some comments. In general, the results are compatible with the conclusions from crystal-structure determinations (where available): in most cases, the number of nonequivalent C-atoms seen equals that of the number of (3-HB) units in the corresponding oligolide. There are, however, three exceptions: tetrolide **2**, pentolide **3**, and hexolide **4**.

Tetrolide **2** shows half the number of signals expected from the number of C-atoms, due to equivalence as a consequence of crystallographic C_2 symmetry. With the higher oligolides, we observed polymorphism. Depending on the conditions, one or another form, or mixtures of different forms may crystallize, with all the confusing consequences: the melting points differ or broad melting ranges are observed with samples which are pure by all other standards – ^{1}H- and ^{13}C-NMR spectra and MS, osmometric molecular-weight determination, specific rotation, *etc.* Thus, we isolated two forms (different conformers in the crystal) of the hexolide [2b] and of the octolide [2e] (*Table 1*). The

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[8])   It is known that P(3-HB) and P(3-HV) are isodimorphic, *i.e.* that the Et and the Me groups can replace each other randomly in the crystalline helical domains of the polymer, see ref. in [4b] and a recent investigation [10]
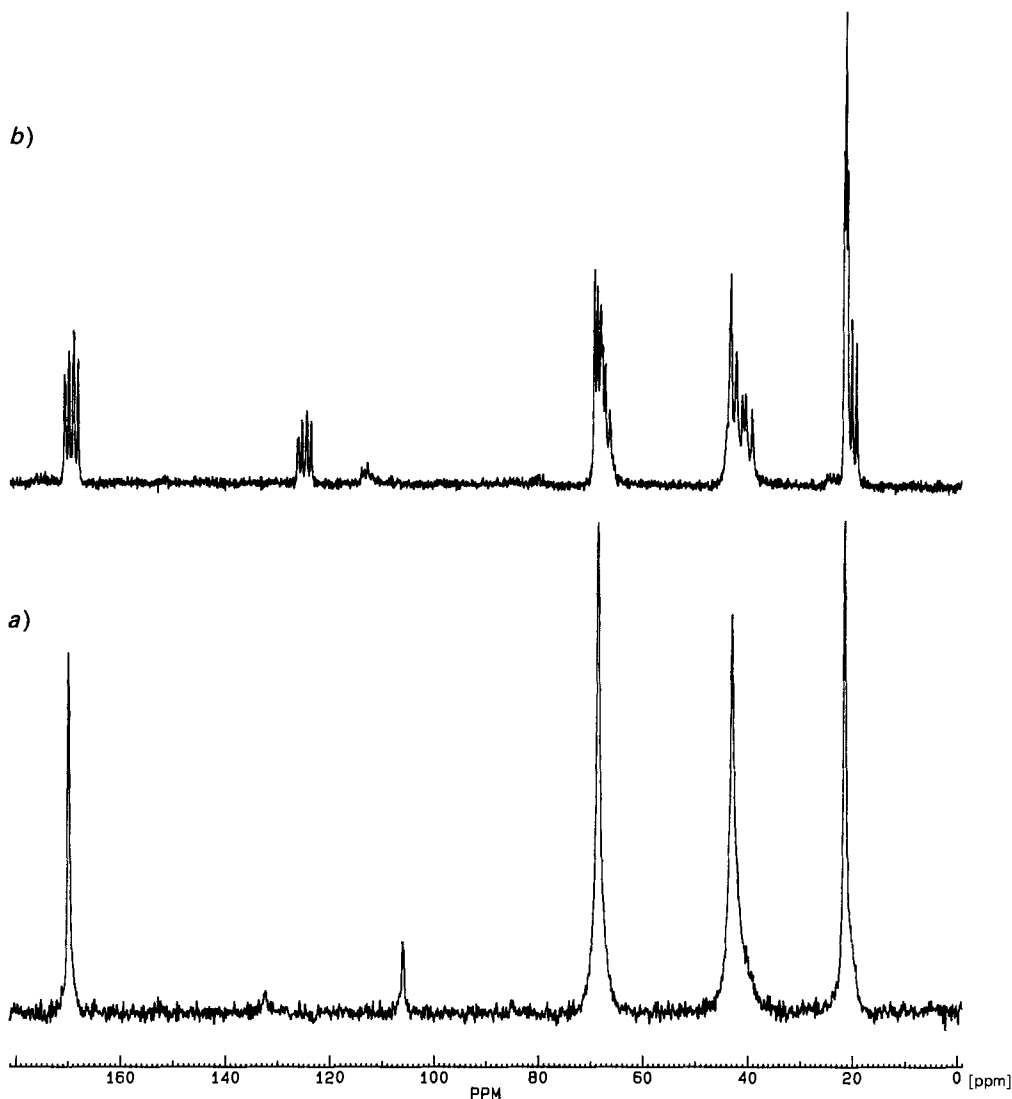
Fig. 3. *CP/MAS ^{13}C-NMR Spectra of* a*) the polymer precipitated from methanol and* b*) of the nonolide* 7. Note the chemical-shift correspondence between the regions of most lines in the nonolide and the line positions of the polymer.

samples for the CP/MAS ^{13}C-NMR measurements were carefully purified and identified chemically, but not by crystallographic means. We might, therefore, have identified a new form of pentolide **3** by the ^{13}C-NMR measurement with two molecules in the asymmetric unit or have been observing a mixture of two polymorphs. In the case of hexolide **4**, the $P2_1$ modification is expected to give more than one set of ^{13}C-NMR signals, so we might have accidentally subjected a sample of that modification to the ^{13}C-NMR measurement.

The spectrum of nonolide **7** resembles that of the polymers (*Fig. 3*): the regions of the spectrum from the Me and CH_2 groups have a wedge shape with maximum intensity of the signals coming at the position of the resonance from the polymer. We believe that it is likely that **7** has a solid-state conformation in which there are regions coiled in a similar manner to the polymer in which the chemical shifts are similar to those of the polymer, joined by shorter linking regions of different chemical shift, structural features which start emerging in the octolide crystal conformations [2e][3].

Experimental Part

Oligolides. Compounds **1** [2d], **2** [2c], **3**, and **4** [2a] [2b] were prepared and purified as described before. The *rac-* (*l,u*)-triolide **8** was obtained from *rac-β*-butyrolactone by acid treatment (*cf.* preparation of **1** from the polymer [2d]). The larger macrocycles **5–7** were isolated by careful column chromatography from oligolide mixtures obtained as described before [2a] [2b][3]; a full account with all details is in preparation [2e]. All oligolides **1–7** contain (*R*)-3-hydroxybutyrate moieties only. The lower members of the series (**1–4** and **8**) were recrystallized from CH_2Cl_2/C_5H_{12}, the larger ring compounds **5–7** from Et_2O/C_5H_{12}. The purity of **1–8** was determined by secondary-ionization (SI)-MS (*VG-ZABZ-SEQ*; 3-nitrobenzyl alcohol as matrix) and by ^{1}H-NMR spectroscopy (*Bruker AM-400*, CDCl$_3$).

Linear Oligomers. For purification of P(3-HB), a sample of *ICI* lot No. MBL 100/1241 (5 g) was dissolved in 1,2-dichloroethane (50°) or in 50 ml of boiling AcOH. The resulting slightly yellow, clear solns. were poured into MeOH (*ca.* 300 ml) with vigorous stirring; the fluffy colourless materials thus precipitated were filtered off and dried (50°/0.05 Torr). A sample of P(3-HB/HV) (*BIOPOL*) of lot No. MBL 700/1242 was used as provided by *ICI*. The open-chain hexolide was synthesized following the principle outlined before [1b] [2a] [6][3]; details will be published in a forthcoming paper. The thermally depolymerized P(3-HB) was prepared and characterized by *H. M. Bürger*, ETH Zürich, following known procedures (*cf.* [9]).

Solid-State ^{13}C-NMR Measurements. All solid-state ^{13}C-NMR spectra were obtained at r.t. (*ca.* 20°) and typically required acquisition of between 16 and 200 transients with a 5-s recycle time for adequate signal to noise ratios. Sample sizes of 50–100 mg of the oligomers as crystalline powders were used for the measurements. The solid-state spectra were run in St Andrews on a *Bruker-MSL-500* spectrometer at 125.758 MHz in the CP/MAS mode using 4-mm o.d. rotors. A contact time of 1 ms and a recycle time of 5 s were used throughout. Spectra were referenced to the chemical shift (38.56 ppm) of the CH_2 group of an external sample of adamantane. Although spinning side bands could be readily identified by inspection and are seen in the *Figs.* between 100 and 130 ppm, all samples were examined at at least two spinning speeds, typically between 4 and 9 kHz.

REFERENCES

[1] a) D. Seebach, S. Roggo, J. Zimmermann, in 'Stereochemistry of Organic and Bioorganic Transformations', 'Workshop Conferences *Hoechst*', Eds. W. Bartmann and K. B. Sharpless, VCH, Weinheim, 1987, Vol. 17, p. 85–126; b) D. Seebach, 'Program and Abstracts, 32nd National ACS Organic Chemistry Symposium', University of Minnesota, Minneapolis, June 16–20, 1991, p. 181–195; c) H.-M. Müller, D. Seebach, *Angew. Chem.* **1993**, *104*, in press.

[2] a) D. Seebach, U. Brändli, P. Schnurrenberger, M. Przybylski, *Helv. Chim. Acta* **1988**, *71*, 155; b) D. Seebach, U. Brändli, H.-M. Müller, M. Dobler, M. Egli, M. Przybylski, K. Schneider, *ibid.* **1989**, *72*, 1704; c) H.-M. Müller, M. Dobler, P. Zbinden, D. Seebach, *Chimia* **1991**, *45*, 376; d) D. Seebach, H.-M. Müller, H. M. Bürger, D. A. Plattner, *Angew. Chem.* **1992**, *104*, 443; *ibid. Int. Ed.* **1992**, *31*, 434; e) D. A. Plattner, A. Brunner, M. Dobler, H.-M. Müller, W. Petter, P. Zinden, D. Seebach, *Helv. Chim. Acta*, in preparation.

[3] M. Lemoigne, *Ann. Inst. Pasteur (Paris)* **1927**, *41*, 148, and earlier work cit. therein.

[4] a) A. Steinbüchel, *Nachr. Chem. Techn. Lab.* **1991**, *39*, 1112; b) Y. Doi, 'Microbiol. Polyesters', VCH, Weinheim, 1990; c) Y. Doi, M. Konioka, Y. Nakamura, K. Soga, *Macromolecules* **1986**, *19*, 1274.

[5] a) M. Yokouchi, Y. Chatani, H. Tadokoro, K. Teranishi, H. Tani, *Polymer* **1973**, *14*, 267; b) J. Cornibert, R. H. Marchessault, *J. Mol. Biol.* **1972**, *71*,735; *Macromolecules* **1975**, *8*, 296; c) S. Brückner, S. V. Meille, L. Malpezzi, A. Cesaro, L. Navarini, R. Tombolini, *ibid.* **1988**, *21*, 967; d) W. J. Orts, R. H. Marchessault, T. L. Blum, G. K. Hamer, *ibid.* **1990**, *23*, 5368.

[6] T. Tanio, T. Fukur, J. Shirakura, T. Saito, K. Tomita, T. Kaio, S. Masamune, *Eur. J. Biochem.* **1982**, *124*, 71; Y. Shirakura, T. Fukui, T. Saito, Y. Okamoto, T. Narikawa, K. Koide, K. Tomita, T. Takemasa, S. Masamune, *Biochem. Biophys. Acta* **1986**, *880*, 46.

[7] S. Bloembergen, D. A. Holden, T. L. Blum, G. K. Hamer, R. H. Marchessault, *Macromolecules* **1989**, *22*, 1656; S. B. Amor, T. Rayment, J. K. M. Sanders, *ibid.* **1991**, *24*, 4583.

[8] D. Seebach, V. Prelog, *Angew. Chem.* **1982**, *94*, 696; *ibid. Int. Ed.* **1982**, *21*, 654.

[9] F. E. Küng, US-Patent, 1944, 2361036 (*CA:* **1944**, *38*, 6301); B. Hauttecoeur, M. Jolivet, R. Gavard, *C. R. Acad. Sci., Ser. D* **1975**, *280*, 2801; S. Coulombe, P. Schauwecker, R. H. Marchessault, B. Hauttecoeur, *Macromolecules* **1978**, *11*, 279; H. Morikawa, R. H. Marchessault, *Can. J. Chem.* **1981**, *59*, 2306; N. Grassie, E. J. Murray, P. A. Holmes, *Polym. Degrad. Stab.* **1984**, *6*, 47, 95, 127; M. Kunioka, Y. Doi, *Macromolecules* **1990**, *23*, 1933.

[10] M. Scandola, G. Ceccorulli, M. Pizzoli, M. Gazzano, *Macromolecules* **1992**, *25*, 1405.