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Conformational Analysis of Oligomers of (*R*)-3-Hydroxybutanoic Acid in Solutions by ^{1}H NMR Spectroscopy

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Oligomers of (*R*)-3-hydroxybutanoic acid (3-HB) with different end groups and chain lengths have been prepared as model compounds of bacterial poly[(*R*)-3-hydroxybutanoate] [P(3-HB)]. They were studied in terms of their conformational behavior generated by rotation about CH_2–CH bond of 3-HB units in solutions. The conformational behaviors of 3-HB oligomers were investigated in various solvents by analysis of vicinal coupling in the 500-MHz ^{1}H NMR spectra. For all 3-HB oligomers studied in this work, 3-HB units without hydroxy group were found, in every solvent, to adopt similar conformational distributions to those of P(3-HB) polymer backbone in solutions, in which *trans* and *gauche* conformers are preferable, while the other *gauche* conformer is strongly disfavored. On the other hand, 3-HB units adjacent to hydroxy terminal group in 3-HB oligomers show different conformational behaviors relative to those of other 3-HB units. In non-polar organic solvents, only *gauche* conformer is predominant, due to the formation of an intramolecular hydrogen bond between hydroxy and carbonyl groups, while in polar organic solvents, the conformer distributions are similar to those of 3-HB units without hydroxy groups. In aqueous solution, for 3-HB units adjacent to hydroxy terminal group, the fractions of *gauche* conformer are still higher than those of *trans* conformer due to the formation of an intramolecular hydrogen bond.

Poly[(*R*)-3-hydroxybutanoate] [P(3-HB)] is an optically active biopolyester synthesized in many bacteria as a storage material of carbon and energy.[1,2] Since its discovery, P(3-HB) and related poly[(*R*)-3-hydroxyalkanoates] have attracted growing interest both in basic research and in industry, because of their biodegradability and biocompatibility which allow them to be used as biodegradable substitutes for conventional plastics.[3—5]

Biosynthesized P(3-HB) is polydisperse and of high molecular weight.[3] In order to obtain more detailed information on this biopolyester, oligomers of (*R*)-3-hydroxybutanoic acid (3-HB) with an uniform chain length have been chemosynthesized as a model compound of P(3-HB). Dimers, trimers, and longer oligomers of 3-HB were used by the groups of Merrick[6] and Masamune[7,8] in understanding the mechanism of enzymatic degradation of P(3-HB), and by Seebach et al.[9—12] in studying solid-state structures and biological functions of P(3-HB).

In previous papers,[13—15] we reported conformational analyses of P(3-HB) in solutions by ^{1}H NMR spectroscopy, showing that the polymer backbone adopts predominantly *trans* and *gauche* conformers around CH_2–CH bond. Here, we have prepared a series of 3-HB oligomers in order to obtain more detailed information on conformational behaviors of P(3-HB). Recently, we reported the conformational analysis of methyl (3*R*)-3-[(3*R*)-3-hydroxybutanoyloxy]butanoate [**16**: H(3HB)$_2$M], a methyl ester of 3-HB dimer, as a model compound of the hydroxy terminal part of P(3-HB), showing that the monomer unit adjacent to the hydroxy group has a different conformational distribution due to the formation of an intramolecular hydrogen bond between hydroxy and carbonyl groups in both chloroform and aqueous solution.[16]

In this paper, we report the conformational analyses of five 3-HB oligomers with different end groups and chain lengths in various non-polar and polar solvents, to investigate the effects of end group, chain length, and solvent on the conformational structures of 3-HB units in the series of 3-HB oligomers.

Results and Discussion

Figure 1 shows the scheme of syntheses of several 3-HB oligomers and the abbreviations used in this paper. The 3-HB oligomers were prepared using the segment-coupling method reported by Seebach's group.[9—12] Figure 2 shows the ^{1}H NMR spectra of five 3-HB oligomers (**9**, **13**, **16**, **18**, and **20**) prepared in this study. The NMR assignments of five oligomers were made by using two-dimentional pulsed field gradient ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC (heteronuclear multiple-bond correlation) spectroscopy as described in our previous paper.[16]

For each 3-HB unit of a 3-HB oligomer, around the CH_2–CH bond there are three possible conformers: *trans* (T), *gauche* (G), and the other *gauche* ($\overline{G}$), as shown in Fig. 3. Taking torsional strain and steric repulsion into consideration, *trans* and *gauche* conformers are more stable than the other *gauche* conformer. As reported in previous papers,[13—16] the distribution of conformers around the CH_2–CH bonds of 3-HB units in solution can be determined by means of ^{1}H NMR spectroscopy. The methylene proton resonances are associated with the methine proton (H_X)

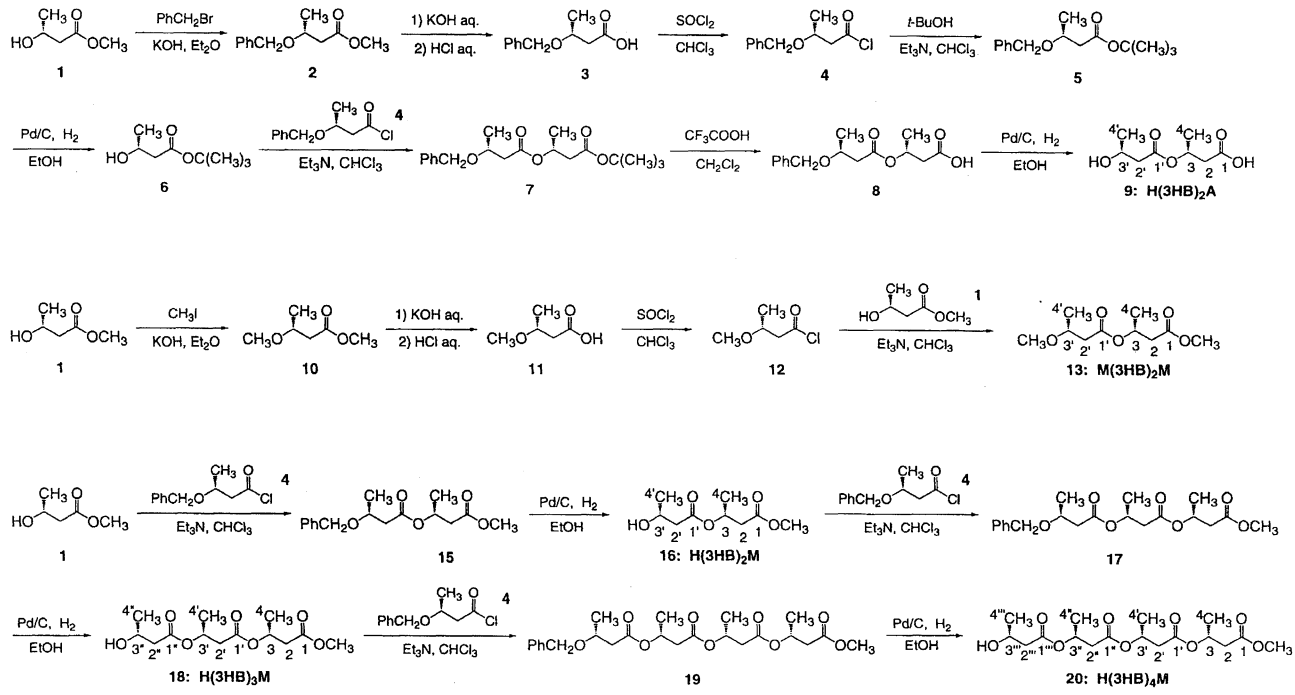

Fig. 1. Scheme of syntheses of 3-HB oligomers.

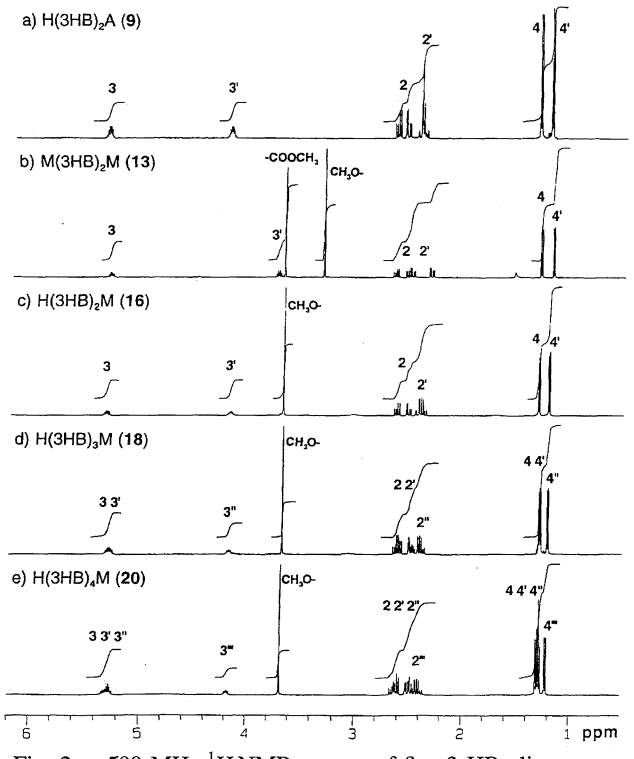

Fig. 2. 500-MHz ^{1}H NMR spectra of five 3-HB oligomers in $CDCl_3$ at 27 °C. The proton assignments are given in Fig. 1.

and are analyzed as an ABX three-spin system with a vicinal coupling of H_A and H_B protons. It is assumed that the flexible molecular chain in solution undergoes a rapid interconversion among the three conformers. Then, the coupling constants J_{AX} and J_{BX} are presented by average values of the

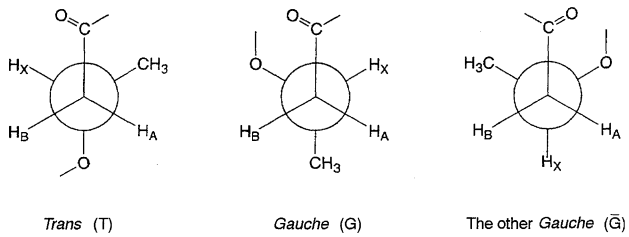

Fig. 3. Newman projections of possible conformers around the CH_2–CH bond in 3-HB oligomers.

component coupling constants in three conformers weighed by their fractional populations P_T, P_G, and $P_{\bar{G}}$, as follows:

$$J_{AX} = P_T J_t + P_G J_g + P_{\bar{G}} J_g \quad (1)$$

$$J_{BX} = P_T J_g + P_G J_t + P_{\bar{G}} J_g \quad (2)$$

$$1 = P_t + P_G + P_{\bar{G}} \quad (3)$$

where J_g and J_t are the *gauche* and *trans* vicinal coupling constants, respectively. Assuming the reasonable values of J_g= 2.1 Hz and J_t=11.0 Hz,[17] we can calculate the fractional populations P_T, P_G, and $P_{\bar{G}}$ for the CH_2–CH bonds under various conditions. The values of J_g (2.1 Hz) and J_t (11.0 Hz) were proposed by Bovey[17] and have been used for the calculation of the fractional populations of conformers for P(3-HB) in solutions.[13–15]

Figure 4 shows the methylene parts and their assignments of 500-MHz ^{1}H NMR spectra for five 3-HB oligomers in $CDCl_3$. The resonance patterns of methylene protons vary with different 3-HB units, suggesting that CH_2–CH bonds for different 3-HB units take various conformational distributions. We also recorded the 500-MHz ^{1}H NMR spectra for five 3-HB oligomers in D_2O as well as in various non-polar

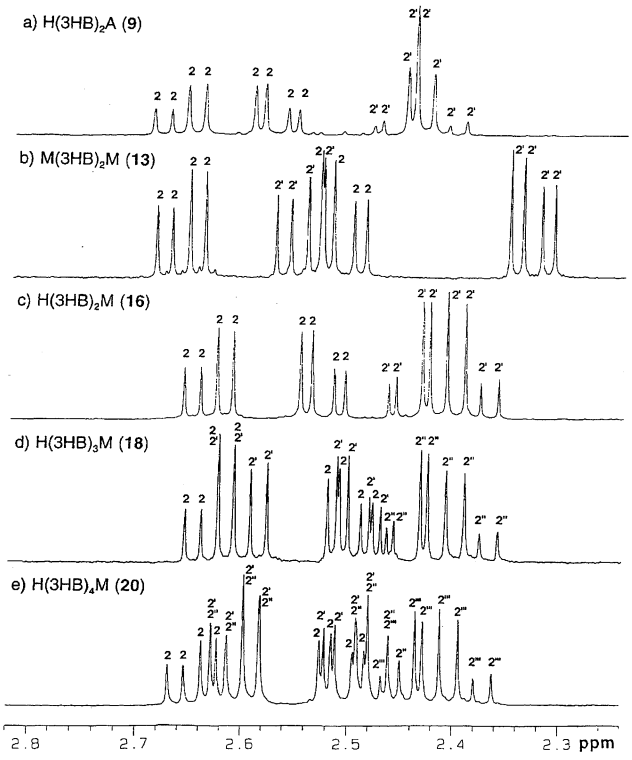

Fig. 4. Expansions and assignments for methylene proton resonances of 500-MHz ^{1}H NMR spectra of five 3-HB oligomers in $CDCl_3$ at 27 °C.

and polar organic solvents. From the ^{1}H NMR spectra of the 3-HB oligomers in various solvents, we determined the relevant NMR parameters such as coupling constants J_{AX}, and J_{BX}, and then calculated the conformer fractions for CH_2–CH bonds in the oligomers by using Eqs. 1, 2, and 3.

Table 1 summarizes the coupling constants of methylene protons and the conformer distributions of CH_2–CH bonds in 3-HB oligomers in $CDCl_3$. Around the CH_2–CH bonds for 3-HB units not adjacent to a hydroxy terminal group in the oligomers in Table 1, i.e., around C2–C3 of **9**, C2–C3 and C2′–C3′ of **13**, C2–C3 of **16**, C2–C3 and C2′–C3′ of **18**, and C2–C3, C2′–C3′, and C2″–C3″ of **20**, the predominant conformer is *trans* (P_T ranges from 0.55 to 0.68), the next preference is *gauche* (P_G ranges from 0.31 to 0.44), and the other *gauche* conformer is suppressed to almost zero (P_G is not higher than 0.02). As described in our previous paper,[16] the result can be accounted for in terms of torsional strain and steric repulsion. In the other *gauche* conformer, both methyl group and oxygen atom are crowed together with carbonyl group, which raises the potential energy of the conformation. The conformer distributions for the above mentioned CH_2–CH bonds are roughly similar to those of 3-HB units in P(3-HB) polymer backbone in $CDCl_3$,[13,14] in which *trans* and *gauche* conformations predominate and the fraction of the other *gauche* conformer is almost zero.

On the other hand, for the 3-HB units adjacent to a hydroxy terminal group, the conformer distributions around CH_2–CH bonds are quite different. As shown in Table 1, around C2′–C3′ of **9**, C2′–C3′ of **16**, C2″–C3″ of **18**, and C2‴–C3‴ of **20**, the preferable conformer is only *gauche* (P_G ranges from 0.68 to 0.75), while the fractions of *trans* conformer drop to be 0.14—0.22, and those of the other *gauche* conformer rise to be about 0.10. The results are difficult to accounted for in terms of only torsional strain and steric repulsion. A careful investigation on the conformational structures around these CH_2–CH bonds suggests that hydrogen bonding plays an important role in governing the conformational structures. As shown in Fig. 5, in both *gauche* and the other *gauche* conformers of 3-HB units with hydroxy terminal group, the proton of hydroxy group and the oxygen of carbonyl group can easily form an intramolecular hydrogen bond. The steric repulsion raises the potential energy, while the hydrogen bonding reduces the potential energy of the conformers. It is apparent that the *gauche*

Table 1. Coupling Constants of Methylene Protons and Conformer Distributions of CH_2–CH Bonds in Five 3-HB Oligomers[a] in $CDCl_3$ at 27 °C

			Coupling constant/Hz		Conformer fraction		
Oligomer	Bond	Probe H	J_{AX}	J_{BX}	P_T	P_G	$P_{\bar{G}}$
9: $H(3HB)_2A$	C2–C3	H2	8.2	4.9	0.68	0.31	0.01
	C2′–C3′	H2′	4.1	8.2	0.22	0.68	0.10
13: $M(3HB)_2M$	C2–C3	H2	7.3	5.8	0.58	0.41	0.01
	C2′–C3′	H2′	7.0	6.1	0.55	0.44	0.01
16: $H(3HB)_2M$	C2–C3	H2	7.6	5.2	0.63	0.35	0.02
	C2′–C3′	H2′	3.7	8.5	0.18	0.73	0.09
18: $H(3HB)_3M$	C2–C3	H2	7.7	5.4	0.62	0.37	0.01
	C2′–C3′	H2′	7.9	5.3	0.64	0.36	0.00
	C2″–C3″	H2″	3.5	8.7	0.16	0.73	0.11
20: $H(3HB)_4M$	C2–C3	H2	7.6	5.5	0.61	0.38	0.01
	C2′–C3′	H2′	7.9	5.2	0.64	0.34	0.02
	C2″–C3″	H2″	7.9	5.4	0.64	0.36	0.00
	C2‴–C3‴	H2‴	3.4	8.8	0.14	0.75	0.11

a) The structures of 3-HB oligomers are given in Fig. 1.

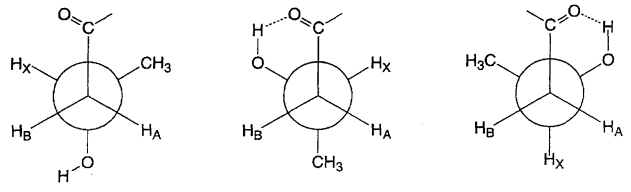

Fig. 5. Conformational structure around CH_2–CH bond of 3-HB unit with a hydroxy terminal group. In both *gauche* and the other *gauche* conformers the proton of hydroxy group forms an intramolecular hydrogen bond with the oxygen of carbonyl group.

conformer is preferred by both steric factor and hydrogen bond, so that it predominantly exists. For the other *gauche* conformer with a large steric repulsion, the intramolecular hydrogen bonding still plays a role in stabilizing the conformation, so the fractions of the other *gauche* conformer 3-HB units with hydroxy terminal are much higher than those for 3-HB units without hydroxy terminal.

Table 2 gives the coupling constants of methylene protons and the conformer distributions of CH_2–CH bonds in five 3-HB oligomers in D_2O. Around the CH_2–CH bonds for 3-HB units without hydroxy terminal, i.e., around C2–C3 of **9**, C2–C3 and C2′–C3′ of **13**, C2–C3 of **16**, C2–C3 and C2′–C3′ of **18**, and C2–C3, C2′–C3′, and C2″–C3″ of **20**, predominant conformers are *trans* and *gauche*, while the other *gauche* conformer is strongly disfavored. Though the conformer distributions for these 3-HB units in D_2O show small differences from those in $CDCl_3$, the conformational behaviors can be still accounted for essentially in terms of torsional strain and steric repulsion. The steric energies of *trans* and *gauche* conformers are much lower than that of the other *gauche* conformer. In addition, the conformer fractions are quite similar to those of P(3-HB) polymer backbone in polar solvents.[14] A difference between conformer distributions for 3-HB units without hydroxy terminal in D_2O and in $CDCl_3$ may result from the difference in the polarity of solvent.[16]

Around CH_2–CH bonds for 3-HB units with hydroxy terminal, i.e., around C2′–C3′ of **9**, C2′–C3′ of **16**, C2″–C3″ of **18**, and C2‴–C3‴ of **20** in D_2O, as shown in Table 2, the fractions of *gauche* conformers are 0.58—0.64, which are much higher than those of *trans* conformers (P_T 0.34—0.38). These 3-HB units still prefer to take *gauche* conformer even in D_2O, indicating that the formation of a hydrogen bond between hydroxy and carbonyl groups stabilizes the *gauche* conformer in aqueous solution. However, the formation of hydrogen bond in D_2O seems to be not so strong as in $CDCl_3$.

From the results of Tables 1 and 2, one may note that, for C2–C3 of H(3HB)$_2$A (**9**), the conformer distribution of 3-HB unit with carboxy terminal in both $CDCl_3$ and D_2O is similar to those of 3-HB units with methoxycarbonyl terminal group. The presence of carboxy terminal group affects conformational behavior of 3-HB units only very slightly. In addition, for M(3HB)$_2$M (**13**), due to methylation of the hydroxy terminal group, the conformer distribution around C2′–C3′ of **13** is similar to those of 3-HB units without hydroxy terminal, suggesting that a methoxy terminal group can not form a hydrogen bond with a carbonyl group. In Table 1, around CH_2–CH bonds for internal 3-HB of 3-HB trimer and tetramer, i.e., around C2′–C3′ of **18** and C2′–C3′, C2″–C3″ of **20**, the conformer distributions are almost same. It has been concluded from the results that a hydroxy terminal group only affects the conformer distribution of the adjacent 3-HB unit.

Table 3 shows the coupling constants of methylene protons and the conformer distributions of CH_2–CH bonds in H(3HB)$_2$M (**16**) in various non-polar and polar organic solvents. In non-polar solvents such as dichloromethane, cyclohexane, and hexane, the conformer distributions of CH_2–CH bonds for two 3-HB units are similar to those in chloroform. In these cases, for the 3-HB unit adjacent to hydroxy terminal group, an intramolecular hydrogen bond between

Table 2. Coupling Constants of Methylene Protons and Conformer Distributions of CH_2–CH Bonds in Five 3-HB Oligomers in D_2O at 27 °C

			Coupling constant/Hz		Conformer fraction		
Oligomer	Bond	Probe H	J_{AX}	J_{BX}	P_T	P_G	$P_{\bar{G}}$
9: H(3HB)$_2$A	C2–C3	H2	6.6	6.6	0.50	0.50	0.00
	C2′–C3′	H2′	5.5	7.3	0.38	0.58	0.04
13: M(3HB)$_2$M	C2–C3	H2	6.4	6.4	0.48	0.48	0.04
	C2′–C3′	H2′	5.8	7.0	0.42	0.54	0.04
16: H(3HB)$_2$M	C2–C3	H2	6.4	6.4	0.48	0.48	0.04
	C2′–C3′	H2′	5.2	7.8	0.34	0.63	0.03
18: H(3HB)$_3$M	C2–C3	H2	—	—	—	—	—
	C2′–C3′	H2′	—	—	—	—	—
	C2″–C3″	H2″	5.2	7.9	0.34	0.64	0.02
20: H(3HB)$_4$M	C2–C3	H2	—	—	—	—	—
	C2′–C3′	H2′	—	—	—	—	—
	C2″–C3″	H2″	—	—	—	—	—
	C2‴–C3‴	H2‴	5.2	7.9	0.34	0.64	0.02

Table 3. Coupling Constants of Methylene Protons and Conformer Distributions of CH_2–CH Bonds in H(3HB)$_2$M (**16**) in Various Organic Solvents at 27 °C

Solvent	Bond	Probe H	Coupling constant/Hz		Conformer fraction		
			J_{AX}	J_{BX}	P_T	P_G	$P_{\bar{G}}$
CD_2Cl_2	C2–C3	H2	7.8	5.4	0.64	0.37	0.00
	C2′–C3′	H2′	3.4	8.5	0.14	0.72	0.14
C_6D_{12}	C2–C3	H2	7.2	5.8	0.57	0.41	0.02
	C2′–C3′	H2′	3.2	8.6	0.10	0.72	0.18
C_6D_{14}	C2–C3	H2	7.3	5.8	0.58	0.41	0.01
	C2′–C3′	H2′	3.0	8.8	0.11	0.75	0.14
THF-d_8	C2–C3	H2	7.3	6.1	0.57	0.43	0.00
	C2′–C3′	H2′	7.3	5.8	0.58	0.41	0.01
Acetone-d_6	C2–C3	H2	7.6	5.5	0.61	0.38	0.01
	C2′–C3′	H2′	7.3	5.8	0.58	0.41	0.01
DMF-d_7	C2–C3	H2	6.6	6.6	0.50	0.50	0.00
	C2′–C3′	H2′	7.0	6.1	0.55	0.44	0.01

hydroxy and carbonyl groups resulted in a predominant formation of *gauche* and the other *gauche* conformers. On the other hand, in polar solvents such as tetrahydrofuran, acetone, and *N*,*N*-dimethylformamide, the conformer distributions are quite different from those in non-polar solvents, Particularly, for the 3-HB unit adjacent to hydroxy group, a predominant conformer is not a *gauche* one any more in polar solvents, and the conformer distributions for two 3-HB units (C2–C3 and C2′–C3′) become similar. The result indicates that an intramolecular hydrogen bond between hydroxy and carbonyl groups is not stabilized in *gauche* and the other *gauche* conformers in polar solvents. It is suggested that polar organic solvents act as a strong hydrogen bond acceptor which inhibits the hydroxy group from forming an intramolecular hydrogen bond with carbonyl group of 3-HB unit.

Conclusions

A series of 3-HB oligomers were prepared and studied in terms of their conformational behavior generated by rotation about CH_2–CH bond of 3-HB units in both non-polar and polar solvents. For five 3-HB oligomers studied in this work, all 3-HB units without hydroxy terminal were found, in every solvent, to adopt similar conformational distributions to those of P(3-HB) polymer backbone in solutions, in which *trans* and *gauche* conformers are preferable, while the other *gauche* conformer is strongly disfavored. On the other hand, 3-HB units with a hydroxy terminal group show different conformational behaviors relative to those of other 3-HB units. In non-polar organic solvents, only *gauche* conformer is predominant due to the formation of an intramolecular hydrogen bond between hydroxy and carbonyl groups, while in polar organic solvents, the conformer distributions are similar to those of 3-HB units without hydroxy group, because the hydroxy group can not form an intramolecular hydrogen bond with the carbonyl group any more in polar organic solvents. In aqueous solution, for 3-HB units adjacent to a hydroxy terminal group, the fractions of *gauche* conformer are also higher than those of *trans* conformer, and the hydroxy group forms an intramolecular hydrogen bond with a carbonyl group. However, the formation of an intramolecular hydrogen bond in aqueous solution is suggested to be not so strong as in non-polar organic solvents. In addition, the presence of a carboxy terminal group does not affect the conformer distributions of the adjacent 3-HB unit and other 3-HB units in an oligomer. Internal 3-HB units in a 3-HB oligomer takes almost the same conformer distributions, and such 3-Hb units do not receive the effects of the hydroxy terminal group on their conformational behaviors, no matter how close to the hydroxy terminal they are.

Experimental

Materials. Methyl (*R*)-3-hydroxybutanoate (**1**) was supplied by Kaneka Chemical Ind. Other chemicals for synthesis were purchased from Kanto Chemical Co., Inc. Chloroform-d_1 ($CDCl_3$, Merck, 99.95%), deuterium oxide (D_2O, Merck, 99.95%), *N*,*N*-dimethylformamide-d_7 (DMF-d_7, Merck, 99.5%), acetone-d_6 (Merck, 99.95%), methanol-d_4 (CD_3OD, Merck, 99.95%), tetrahydrofuran-d_8 (THF-d_8, Aldrich, 99.95%), dichloromethane-d_2 (CD_2Cl_2, Aldrich, 99.95%), cyclohexane-d_{12} (C_6D_{12}, Acros, 99.5%), and hexane-d_{14} (C_6D_{14}, Acros, 99.5%) were used as solvents in the NMR measurements.

Measurements. The ^{1}H NMR spectra of 3-HB oligomers were recorded at 500 MHz on a JEOL GX-500 NMR spectrometer. The measurements were carried out with 5.3-s pulse repetition, 5000-Hz spectral width, and 32768 data points. Chemical shifts were referred to DSS and TMS (δ = 0.00 ppm) as internal references for D_2O and C_6D_{14}, respectively. In other solvents, chemical shifts were referred to the solvent values of δ = 7.26 for $CDCl_3$, δ = 8.03 for DMF-d_7, δ = 2.00 for acetone-d_6, δ = 1.73 for THF-d_8, δ = 5.30 for CD_2Cl_2, δ = 4.87 for CD_3OD, and δ = 1.38 for C_6D_{12}. Mass spectra were obtained on a JEOL JMS-HX 110 mass spectrometer by the fast atom bombardment (FAB) method in positive ion mode with glycerol matrix, 5.0—10.0 kV acceleration voltage, and 1000 resolution. Mass calibration was carried out with CsI spectrum.

Preparation of 3-HB Oligomers. (*R*)-3-(Benzyloxy)butanoic acid (**3**), (*R*)-3-(benzyloxy)butanoyl chloride (**4**), methyl (3*R*)-3-[(3*R*)-3-(benzyloxy)butanoyloxy] butanoate (**15**), and methyl (3*R*)-

3-[(3*R*)-3-hydroxybutanoyloxy] butanoate [**16**: H(3HB)$_2$M] were prepared according to the procedures described in our previous paper.[16] Column chromatography was carried out on an Eyela medium-pressure liquid chromatography system, with delivery pump EFC-1000, differential refractive index detector RI-71, and recorder SS-100F. A column of silica gel 60 (Kanto, Spherical, mesh size 40—100 μm) with Et_2O/hexane solvent was used.

***t*-Butyl (*R*)-3-(Benzyloxy)butanoate (5).** A solution of **4** (9.84 g, 46.3 mmol) in 6 ml $CHCl_3$ was added dropwise to a solution of *t*-butyl alcohol (43 ml) and triethylamine (19 ml) in 24 ml $CHCl_3$ which was cooling in an ice-water bath. The mixture was then stirred overnight at room temperature, and diluted with $CHCl_3$, washed with NH_4Cl aqueous solution (15%), dried, and evaporated. The product was purified by column chromatography (Et_2O/hexane 1 : 9). Yield: 4.23 g (37%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ = 7.32 (m, 5H, Ar-H), 4.53 (m, 2H, $PhCH_2O$), 3.98 (m, 1H, C(3)H), 2.30—2.61 (m, 2H, C(2)H_2), 1.45 (s, 9H, *t*-Bu), 1.25 (d, J = 6.3 Hz, 3H, C(4)H_3). FAB-MS *m/z* (rel intensity) 249.1 (M^++1; 25), 193.1 (100).

***t*-Butyl (*R*)-3-Hydroxybutanoate (6).** To a solution of **5** (4.20 g, 16.8 mmol) in ethanol (20 ml) was added 10% Pd/C (0.50 g); then the mixture was stirred under hydrogen at room temperature overnight. After a TLC test showed that the reaction was completed, the mixture was filtrated and dried. Removal of the solvent and the side-product by vacuum gave the pure product. Yield: 2.30 g (86%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ = 4.13 (m, 1H, C(3)H), 3.10 (broad, 1H, OH), 2.28—2.45 (m, 2H, C(2)H_2), 1.47 (s, 9H, *t*-Bu), 1.20 (d, J = 6.3 Hz, 3H, C(4)H_3).

***t*-Butyl (3*R*)-3-[(3*R*)-3-(Benzyloxy)butanoyloxy]butanoate (7).** A solution of **4** (3.57 g, 16.8 mmol) in 5 ml $CHCl_3$ was added dropwise to a solution of **6** (2.30 g, 14.4 mmol) and triethylamine (2.4 ml) in 5 ml $CHCl_3$ which was cooling in ice-water bath. The mixture was then stirred overnight at room temperature, and diluted with Et_2O, washed with NH_4Cl aqueous solution (15%), dried, and evaporated. The product was purified by column chromatography (Et_2O/hexane 2 : 8). Yield: 2.32 g (48%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ = 7.32 (m, 5H, Ar-H), 5.26 (m, 1H, C(3)-H), 4.53 (m, 2H, $PhCH_2O$), 3.99 (m, 1H, C(3′)H), 2.35—2.67 (m, 4H, C(2)H_2 and C(2′)H_2), 1.42 (s, 9H, *t*-Bu), 1.27 (d, J = 6.3 Hz, 3H, C(4)H_3), 1.26 (d, J = 6.3 Hz, 3H, C(4′)H_3). FAB-MS *m/z* (rel intensity) 337.2 (M^++1; 24).

(3*R*)-3-[(3*R*)-3-(Benzyloxy)butanoyloxy] Butanoic Acid (8). To a solution of **7** (1.25 g, 3.72 mmol) in 8 ml CH_2Cl_2 was added 2.8 ml of CF_3COOH and the mixture was stirred for 2 d at room temperature. The mixture was diluted with Et_2O, washed with NH_4Cl aqueous solution (15%) and HCl aqueous solution (1 M, 1 M = 1 mol dm^{-3}), dried, and evaporated. The product was purified by column chromatography (Et_2O/hexane 6.5 : 3.5). Yield: 0.807 g (77%). ^{1}H NMR (CD_3OD, 500 MHz) δ = 7.36 (m, 5H, Ar-H), 5.27 (m, 1H, C(3)H), 4.56 (m, 2H, $PhCH_2O$), 4.03 (m, 1H, C(3′)H), 2.45—2.66 (m, 4H, C(2)H_2 and C(2′)H_2), 1.30 (d, J = 6.4 Hz, 3H, C(4)H_3), 1.27 (d, J = 6.1 Hz, 3H, C(4′)H_3

(3*R*)-3-[(3*R*)-3-Hydroxybutanoyloxy] Butanoic Acid [9: H(3HB)$_2$A]. To a solution **8** (0.118 g, 0.421 mmol) in ethanol (1 ml) was added 10% Pd/C (0.02 g); then the mixture was stirred under hydrogen at room temperature overnight. After a TLC test showed that the reaction was completed, the mixture was filtrated. Removal of the solvent and the side-product by high vacuum gave the pure product. Yield: 0.075 g (94%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 5.34 (m, 1H, C(3)H), 4.21 (m, 1H, C(3′)H), 2.55—2.69 (m, 2H, C(2)H_2), 2.39—2.48 (m, 2H, C(2′)H_2), 1.33 (d, J = 6.4 Hz, 3H, C(4)H_3), 1.23 (d, J = 6.4 Hz, 3H, C(4′)H_3). FAB-MS *m/z* (rel intensity) 191.3 (M^++1; 100), 105.6 (57). Anal. Calcd for $C_8H_{14}O_5$: C, 50.52; H, 7.42%. Found: C, 49.80; H, 7.44%.

(*R*)-3-(Methoxy)butanoic Acid (11). To a vigorously stirred suspension of powdered KOH (86%, 40 g) in Et_2O (250 ml) was added dropwise **1** (5.90 g, 50.0 mmol) under dried nitrogen at room temperature. After the mixture was stirred under reflux for 12 h, iodomethane (50 g, 350 mmol) was added dropwise, and the mixture was stirred under reflux for another 24 h. After the excess iodomethane was removed by evaporation, water (120 ml) and Et_2O (130 ml) were added to the reaction mixture, followed by vigorous stirring for 12 h. The aqueous phase was removed, and the organic layer was extracted with KOH solution. The aqueous extracts were combined with the aqueous phase, acidified to pH 2 with HCl, then extracted with Et_2O. The Et_2O extracts were dried with $MgSO_4$, evaporated and the remaining syrup was purified by column chromatography (Et_2O/hexane 3 : 7) to give pure **11**. Yield: 2.04 g (35%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 3.78 (m, 1H, C(3)H), 3.37 (s, 3H, CH_3O), 2.45—2.61 (m, 2H, C(2)H_2), 1.24 (d, J = 6.1 Hz, 3H, C(4)H_3).

(*R*)-3-(Methoxy)butanoyl Chloride (12). To a solution of **11** (0.592 g, 5.00 mmol) in $CHCl_3$ (3 ml) was added thionyl chloride (1.4 ml) and DMF (0.1 ml); then the mixture was stirred at 50 °C for 4 h. The excess thionyl chloride was removed under vacuum, and the crude product was used for succeeding syntheses without further purification.

Methyl (3*R*)-3-[(3*R*)-3-(Methoxy)butanoyloxy] Butanoate [13: M(3HB)$_2$M]. A solution of **12** (0.684 g, 5.00 mol) in 2 ml $CHCl_3$ was added dropwise to a solution of **1** (0.95 g, 8.03 mmol) and triethylamine (1.5 ml) in $CHCl_3$ (1 ml) which was cooling in an ice-water bath. The mixture was then stirred for 4 h at room temperature, and diluted with Et_2O, washed with NH_4Cl aqueous solution (15%), dried, and evaporated. The product was purified by column chromatography (Et_2O/hexane 2 : 8). Yield: 0.651 g (60%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 5.30 (m, 1H, C(3)H), 3.74 (m, 1H, C(3′H), 3.68 (s, 3H, $COOCH_3$), 3.33 (s, 3H, CH_3O), 2.31—2.69 (m, 4H, C(2)H_2 and C(2′)H_2), 1.31 (d, J = 6.4 Hz, 3H, C(4)H_3), 1.19 (d, J = 6.1 Hz, 3H, C(4′)H_3). FAB-MS *m/z* (rel intensity) 219.1 (M^++1; 100). Anal. Calcd for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31%. Found: C, 54.84; H, 8.40%.

Methyl (3*R*)-3-[(3*R*)-3-{(3*R*)-3-(Benzyloxy)butanoyloxy}butanoyloxy]Butanoate (17). A solution of **4** (4.30 g, 20.2 mmol) in 8 ml $CHCl_3$ was added dropwise to a solution of **16** (4.00 g, 19.6 mmol) and triethylamine (3.0 ml) in 2 ml $CHCl_3$ which was cooling in ice-water bath. The mixture was then stirred overnight at room temperature, and diluted with Et_2O, washed with NH_4Cl aqueous solution (15%), dried, and evaporated. The product was purified by column chromatography (Et_2O/hexane 3 : 7). Yield: 4.00 g (54%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ = 7.33 (m, 5H, Ar-H), 5.27 (m, 2H, C(3)H and C(3′)H), 4.53 (m, 2H, $PhCH_2O$), 3.99 (m, 1H, C(3″)H), 3.67 (s, 3H, CH_3O), 2.36—2.67 (m, 6H, C(2)H_2, C(2′)H_2, and C(2″)H_2), 1.24—1.28 (m, 9H, C(4)H_3, C(4′)H_2, and C(4″)H_3). FAB-MS *m/z* (rel intensity) 381.2 (M^++1; 42).

Methyl (3*R*)-3-[(3*R*)-3-{(3*R*)-3-Hydroxybutanoyloxy}butanoyloxy]Butanoate [18: H(3HB)$_3$M]. To a solution of **17** (4.00 g, 10.5 mmol) in ethanol (13 ml) was added 10% Pd/C (0.44 g); then the mixture was stirred under hydrogen at room temperature overnight. After a TLC test showed that the reaction was complete, the mixture was filtrated. After removal of the solvent by evaporation, the product was purified by column chromatography (Et_2O/hexane 7 : 3). Yield: 2.15 g (70%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 5.26—5.33 (m, 2H, C(3)H and C(3′)H), 4.18—4.20 (m, 1H, C(3″)H), 3.67 (s, 3H, CH_3O), 3.03 (broad, 1H, OH), 2.37—

2.66 (m, 6H, C(2)H_2, C(2′)H_2, and C(2″)H_2), 1.22—1.31 (m, 9H, C(4)H_3, C(4′)H_3, and C(4″)H_3). FAB-MS *m/z* (rel intensity) 291.1 (M^++1; 39), 205.2 (39), 187.3 (29), 173.3(45). Anal. Calcd for $C_{13}H_{22}O_7$: C, 53.78; H, 7.64%. Found: C, 53.68; H, 7.77%.

Methyl (3*R*)-3-[(3*R*)-3-{(3*R*)-3-[(3*R*)-3-(Benzyloxy)butanoyloxy]butanoyloxy}butanoyloxy]Butanoate (19). A solution of **4** (2.35 g, 11.0 mmol) in 7 ml $CHCl_3$ was added dropwise to a solution of **18** (1.83 g, 6.30 mmol) and triethylamine (1.5 ml) in 2 ml $CHCl_3$ which was cooling in an ice-water bath. The mixture was then stirred overnight at room temperature, and diluted with Et_2O, washed with NH_4Cl aqueous solution (15%), dried, and evaporated. The product was purified by column chromatography (Et_2O/hexane 1 : 1). Yield: 2.54 g (86%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ=7.31 (m, 5H, Ar-H), 5.27 (m, 3H, C(3)H, C(3′)H, and C(3″)H), 4.53 (m, 2H, $PhCH_2O$), 3.99 (m, 1H, C(3‴)H), 3.67 (s, 3H, CH_3O), 2.37—2.65 (m, 8H, C(2)H_2, C(2′)H_2, C(2″)H_2), and C(2‴)H_2), 1.25—1.29 (m, 12H, C(4)H_3, C(4′)H_3, C(4″)H_3, and C(4‴)H_3). FAB-MS *m/z* (rel intensity) 467.1 (M^++1; 15), 273.1 (13), 187.1 (27), 155.1 (69).

Methyl (3*R*)-3-[(3*R*)-3-{(3*R*)-3-[(3*R*)-3-Hydroxybutanoyloxy]butanoyloxy}butanoyloxy]Butanoate [20: H(3HB)$_3$M]. To a solution of **19** (2.40 g, 5.14 mmol) in ethanol (10 ml) was added 10% Pd/C (0.23 g); then the mixture was stirred under hydrogen at room temperature overnight. After a TLC test showed that the reaction was completed, the mixture was filtrated. After removal of the solvent by evaporation, the product was purified by column chromatography (Et_2O/hexane 8 : 2). Yield: 1.64 g (85%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 5.24—5.33 (m, 3H, C(3)H, C(3′)-H, and C(3″)H), 4.18 (m, 1H, C(3‴)H), 3.68 (s, 3H, CH_3O), 2.36—2.67 (m, 8H, C(2)H_2, C(2′)H_2, C(2″)H_2, and C(2‴)H_2), 1.27—1.31 (m, 9H, C(4)H_3, C(4′)H_3, and C(4″)H_3), 1.22 (d, J = 6.4 Hz, 3H, C(4‴)H_3). FAB-MS *m/z* (rel intensity) 377.2 (M^++1; 86), 291.1 (332), 173.3 (52), 155.4 (79). Anal. Calcd for $C_{17}H_{28}O_9$: C, 54.25; H, 7.50%. Found: C, 54.36; H, 7.61%.

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