Conformational Behavior of Methyl (3*R*)-3-{[(3′*R*)-3′-Hydroxybutanoyl]oxy}butanoate in Solutions: Effect of Intramolecular Hydrogen Bond

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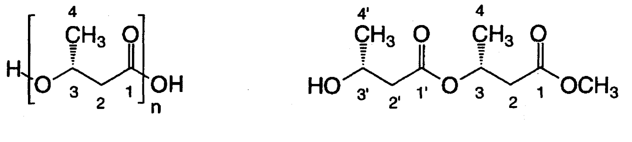
Methyl (3*R*)-3-{[(3′*R*)-3′-hydroxybutanoyl]oxy}butanoate (MHBOB) has been prepared as a model compound of the hydroxy terminal part of poly[(*R*)-3-hydroxybutanoate] [P(3-HB)]; the NMR assignments and conformational analyses of the compound are reported. The NMR assignments of MHBOB were made by means of two-dimensional pulsed field gradient ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC (heteronuclear multiple-bond correlation) spectroscopy. The conformational behavior of MHBOB generated by rotation about two CH_2–CH bonds was investigated in chloroform and in aqueous solution at various temperatures by analysis of vicinal coupling in the 500-MHz ^{1}H NMR spectra. In both chloroform and aqueous solution, the monomer unit adjacent to the methoxy group was found to adopt a similar conformational distribution to that of the P(3-HB) polymer backbone, in which the *trans* and *gauche* conformers were predominant, while the another *gauche* conformer was suppressed to almost zero. On the other hand, the monomer unit adjacent to the hydroxy group had a different conformational distribution relative to that of the other monomer unit, due to the formation of an intramolecular hydrogen bond between hydroxy and carbonyl groups. In chloroform, only the *gauche* conformer was predominant at temperatures between −60 and 57 °C, and even the fraction of another *gauche* conformer was higher than that of *trans* conformer at low temperatures below 0 °C. In aqueous solution, the fraction of *gauche* conformer was also higher than that of *trans* conformer, suggesting the formation of the intramolecular hydrogen bond. However, the formation of the intramolecular hydrogen bond in aqueous solution was not so strong as that in chloroform.

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(*β*-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] In previous papers,[6—8] we reported conformational analyses of P(3-HB) in solutions by ^{1}H NMR spectroscopy, showing that the polymer backbone adopts predominately the *trans* (T) and *gauche* (G) conformers around the CH_2–CH bond. On the other hand, oligomers of (*R*)-3-hydroxybutanoic acid (3-HB) with an uniform molecular weight have been prepared as a model compound of P(3-HB). Dimer, trimer, and longer oligomers of 3-HB were used by the groups of Merrick[9] and Masamune[10,11] in obtaining more detailed information on the mechanism of enzymatic degradation of P(3-HB), and by Seebach et al.[12—15] in studying solid state structures and biological functions of P(3-HB). Here, we prepared methyl (3*R*)-3-{[(3′*R*)-3′-hydroxybutanoyl]oxy}butanoate (MHBOB), a methyl ester of 3-HB dimer, as a model compound of the hydroxy terminal part of P(3-HB) (Chart 1). In this paper, we report the conformational analyses of the model compound in solutions by means of ^{1}H NMR spectroscopy. It is suggested that the hydroxy terminal part of P(3-HB) shows a different conformational behavior in comparison with that of the polymer backbone.



P(3-HB)

MHBOB

Chart 1.

Results and Discussion

NMR Assignments. In the ^{1}H NMR spectrum of MHBOB, the methylene, methine, and methyl protons show signals in pairs (top trace in the COSY spectrum, Fig. 1). In each pair of signals, one signal corresponds to the proton(s) of the monomer unit adjacent to the methoxy group, and the other corresponds to the proton(s) of the monomer unit adjacent to the hydroxy group. In order to make the proton assignments of the spectrum, we first recorded the two-dimensional ^{1}H–^{1}H COSY spectrum of MHBOB using the pulsed field gradient (PFG) technique. The ^{1}H–^{1}H PFG-COSY spectrum is shown in Fig. 1. The correlation peaks are observed between resonances at 2.59 and 5.33 ppm, between those at 2.43 and 4.19 ppm, between those at 1.32 and 5.33 ppm, and between those at 1.23 and 4.19 ppm, respectively, indicating that methylene resonance at 2.59 ppm, methine resonance at 5.33 ppm, and methyl resonance at 1.32 ppm are attributed to one monomer unit, and those at 2.43,

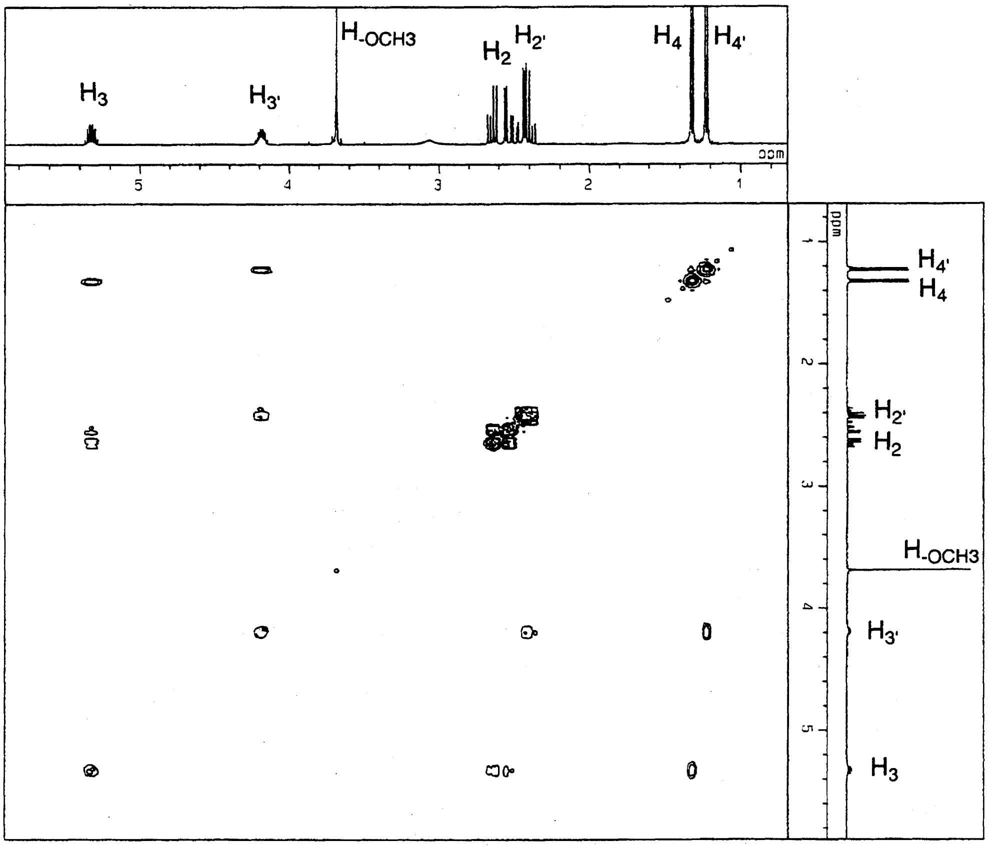


Fig. 1. Two-dimensional ^1H–^1H PFG-COSY spectrum of MHBOB at 25 °C in $CDCl_3$.

4.19, and 1.23 ppm are attributed to the other monomer unit of MHBOB. However, the COSY spectrum can not tell the relationship between the two sets of resonances and the two monomer units of MHBOB.

HMBC (heteronuclear multiple-bond correlation) spectroscopy is a well-accepted NMR technique for elucidating chemical structures by identifying long-range (two- and three-bond) correlation between heteronuclei such as ^1H and ^{13}C.[16—18] A convenient starting point is the proton resonance of $-OCH_3$ at 3.69 ppm, which is expected to show a long-range correlation with ^{13}C resonance of C_1 of the neighboring carbonyl group. Figure 2 shows the two-dimensional ^1H–^{13}C PFG-HMBC spectrum of MHBOB. Herein the PFG technique was used to improve the resolution and S/N ratio of the spectrum.[19—21] In Fig. 2, the correlation peak between the proton of $-OCH_3$ and C_1 is clearly detected, while no correlation peak between the proton of $-OCH_3$ and $C_{1'}$ appears. Consequently, the methylene protons which show correlation peaks with C_1 have been assigned to H_2, and the methylene protons which show correlation peaks with $C_{1'}$ have been assigned to $H_{2'}$.

Conformational Analyses. For each monomer unit of MHBOB, there are three possible conformers: *trans* (T), *gauche* (G), and another *gauche* ($\overline{G}$), as shown in Fig. 3. Taking torsional strain and steric repulsion into consideration, the *trans* and *gauche* conformers are more stable than the another *gauche* conformer. As reported in previous papers,[6—8] the distribution of conformers around the CH_2–CH bonds of 3-HB units in solution can be determined by means of ^1H NMR spectroscopy. The methylene proton resonances are associated with the methine proton (H_X) 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 component coupling constants in the three conformers weighted by their fractional populations P_T, P_G, and $P_{\overline{G}}$, as follows:

$$J_{AX} = P_T J_t + P_G J_g + P_{\overline{G}} J_g \tag{1}$$

$$J_{BX} = P_T J_g + P_G J_t + P_{\overline{G}} J_g \tag{2}$$

$$1 = P_T + P_G + P_{\overline{G}} \tag{3}$$

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,[22] we can calculate the fractional populations P_T, P_G, and $P_{\overline{G}}$ for the CH_2–CH bonds under various conditions.

Figure 4 shows the methylene parts of the 500-MHz ^1H NMR spectra for MHBOB in $CDCl_3$ at various temperatures. The resonance patterns of H_2 and $H_{2'}$ are apparently different, indicating that the C_2–C_3 and $C_{2'}$–$C_{3'}$ bonds take different conformational distributions. In addition, the coupling constants for both H_2 and $H_{2'}$ show some variations at different temperatures. We also recorded the 500-MHz ^1H NMR spectra for MHBOB in D_2O at various temperatures. From the ^1H NMR spectra of MHBOB in $CDCl_3$ and

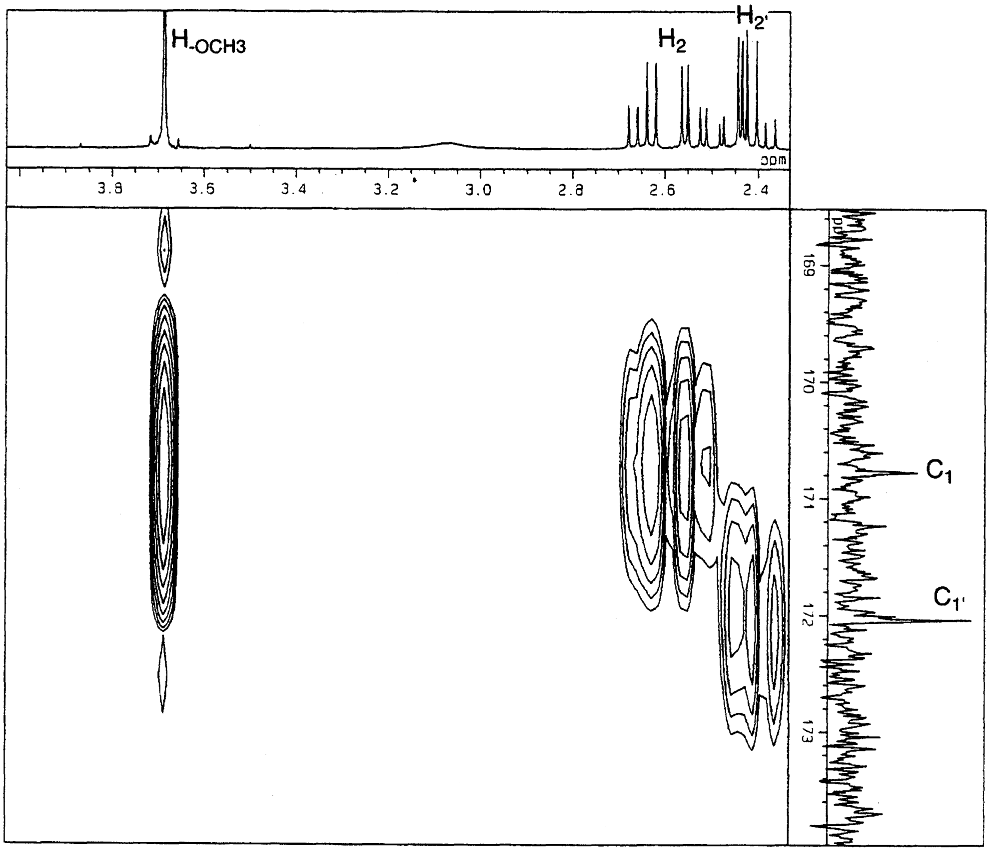
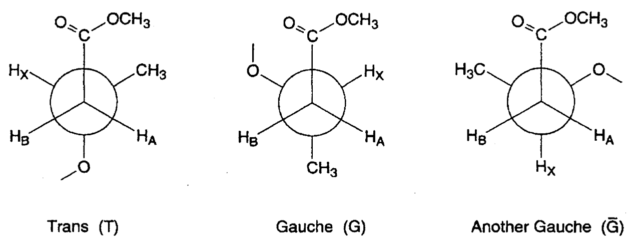


Fig. 2. Expansion of two-dimensional $^1H-^{13}C$ PFG-HMBC spectrum of MHBOB at 25 °C in $CDCl_3$.

(a) Newman projections for C_2-C_3 bond



(b) Newman projections for $C_{2'}$-$C_{3'}$ bond

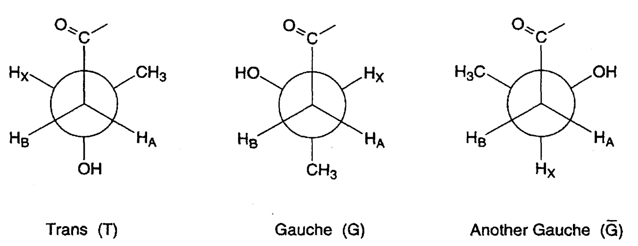


Fig. 3. Newman projections of possible conformers of MHBOB for the C_2–C_3 bond (a) and the $C_{2'}$–$C_{3'}$ bond (b).

in D_2O, we determined the relevant NMR parameters such as coupling constants J_{AX}, J_{BX}, and J_{AB}, and then calculated the conformer fractions for the C_2–C_3 and $C_{2'}$–$C_{3'}$ bonds at various temperatures using Eqs. 1, 2, and 3. The results are given in Table 1.

Figure 5 shows the plots of the conformer fractions of MHBOB for the C_2–C_3 bond and the $C_{2'}$–$C_{3'}$ bond at various temperatures in $CDCl_3$. For the C_2–C_3 bond (Fig. 5a), the conformer fractions at −60 °C are $P_T=0.78$, $P_G=0.22$, and $P_{\bar{G}}=0.00$. Although the conformer fractions show small changes with an increase in temperature, they still remain $P_T=0.59$, $P_G=0.37$, and $P_{\bar{G}}=0.04$ at 57 °C. Over the wide range of temperature, a predominant conformation around C_2–C_3 bond of MHBOB is the *trans* conformer, the next preference is the *gauche* conformer, and the another *gauche* conformer is strongly disfavored in chloroform. The results can be accounted for in terms of torsional strain and steric repulsion. In the another *gauche* conformer, both methyl group and oxygen atom are crowded together with carbonyl group, which raises the potential energy of the conformation. The conformer fractions of 3-HB unit around the C_2–C_3 bond in MHBOB are roughly similar to those of 3-HB units in the P(3-HB) polymer backbone in $CDCl_3$,[6,7] in which the *trans* and *gauche* conformations predominate and almost no another *gauche* conformer exists.

However, the conformer fractions for the $C_{2'}$–$C_{3'}$ bond of MHBOB in $CDCl_3$ (Fig. 5b) are quite different from those for the C_2–C_3 bond. The conformer fractions for the $C_{2'}$–$C_{3'}$ bond are $P_T=0.02$, $P_G=0.85$, and $P_{\bar{G}}=0.14$ at −60 °C. With an increase in temperature, P_G and $P_{\bar{G}}$ decrease, while P_T increases. The conformer fractions become $P_T=0.22$, $P_G=0.69$, and $P_{\bar{G}}=0.09$ at 57 °C. The results are hard to be accounted for in terms of only torsional strain

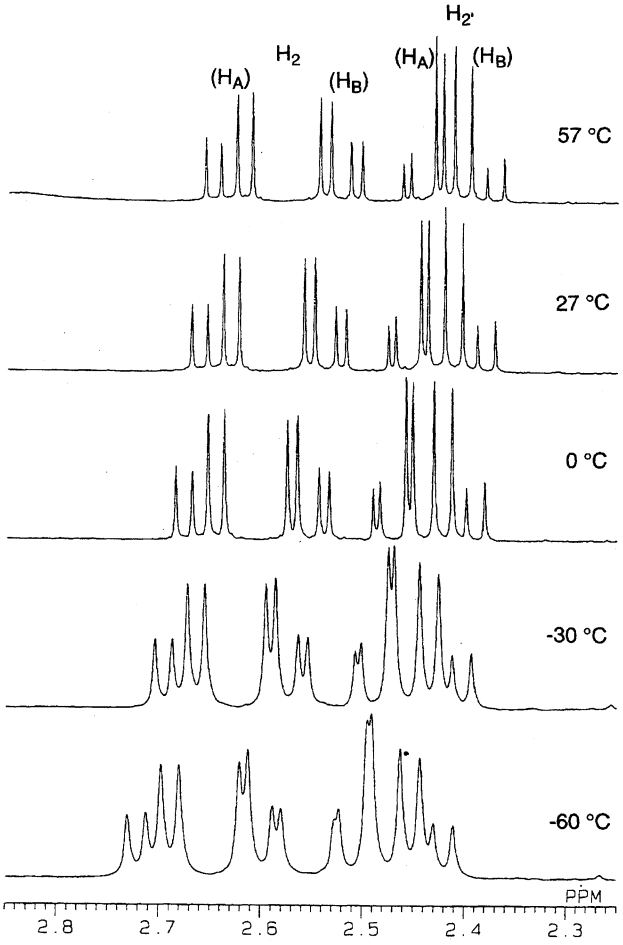


Fig. 4. Expansions for methylene proton resonances of 500-MHz ^{1}H NMR spectra of MHBOB in $CDCl_3$ at various temperatures.

and steric repulsion. A careful investigation on the conformational structures around the $C_{2'}$–$C_{3'}$ bond suggests that the hydrogen bonding plays an important role in governing the conformational structures. As shown in Fig. 6, in both *gauche* and another *gauche* conformers, the proton of hydroxy group and the oxygen of carbonyl group are easy to form intramolecular hydrogen bonds. The steric repulsion raises the potential energy, while the hydrogen bonding reduces the potential energy of the conformers. It is apparent that the *gauche* conformer is preferred by both the steric factor and the hydrogen bond, so that it predominantly exists. For the another *gauche* conformer with a large steric repulsion, the intramolecular hydrogen bonding should stabilize the conformation. Therefore, at a low temperature of −60 °C, $P_{\overline{G}}$ rises to be 0.14, while P_T drops to be 0.02. At high temperatures, the formation of hydrogen bond becomes weak, while the effect of the steric repulsion is relatively greater. As a result, P_T rises to be 0.22 at 57 °C, while $P_{\overline{G}}$ drops to be 0.09. Recently, it has been revealed by several research groups that hydrogen bonding acts as an important driving force which affects conformational structures in monosaccharides,[23] azobenzene derivatives,[24] and calixarenes.[25]

Figure 7 shows the plots of conformer fractions of MHBOB for the C_2–C_3 and $C_{2'}$–$C_{3'}$ bonds at various temperatures in D_2O. For the C_2–C_3 bond (Fig. 7a), the conformer fractions at 5 °C are P_T=0.50, P_G=0.50, and $P_{\overline{G}}$=0.00. The predominant conformations around the C_2–C_3 bond of MHBOB are *trans* and *gauche* conformers, and the conformer fractions show small changes with an increase in temperature. The conformational behavior around the C_2–C_3 bond can be accounted for essentially in terms of torsional strain

Table 1. Parameters of Methylene Proton Resonances (H_2 and $H_{2'}$) in 500-MHz ^{1}H NMR Spectra of MHBOB and Conformational Distribution of CH_2–CH Bonds in MHBOB

			δ/ppm		Coupling constant/Hz				Conformer fraction		
Solvent	Temp/°C	Probe H	H_A	H_B	J_{AB}	J_{AX}	J_{BX}	$(J_{AX}+J_{BX})$	P_T	P_G	$P_{\overline{G}}$
$CDCl_3$	−60	H_2	2.70	2.61	−16.1	9.0	4.0	13.0	0.78	0.22	0.00
		$H_{2'}$	2.50	2.44	−16.5	2.1	9.6	11.8	0.02	0.85	0.14
$CDCl_3$	−30	H_2	2.68	2.58	−15.9	8.5	4.6	13.1	0.73	0.28	0.00
		$H_{2'}$	2.48	2.43	−16.2	2.8	9.2	12.1	0.08	0.80	0.12
$CDCl_3$	0	H_2	2.66	2.56	−15.7	8.0	4.9	12.9	0.67	0.32	0.01
		$H_{2'}$	2.46	2.41	−16.7	3.2	8.9	12.1	0.13	0.76	0.11
$CDCl_3$	27	H_2	2.64	2.54	−15.6	7.6	5.2	12.8	0.63	0.35	0.02
		$H_{2'}$	2.45	2.40	−15.9	3.7	8.5	12.2	0.18	0.73	0.09
$CDCl_3$	57	H_2	2.63	2.53	−15.5	7.3	5.3	12.7	0.59	0.37	0.04
		$H_{2'}$	2.43	2.40	−15.7	4.0	8.2	12.2	0.22	0.69	0.09
D_2O	5	H_2	2.72	2.72	—	6.6	6.6	13.1	0.50	0.50	0.00
		$H_{2'}$	2.53	2.47	−15.1	4.7	8.4	13.1	0.30	0.70	0.00
D_2O	15	H_2	2.72	2.72	—	6.4	6.4	12.8	0.49	0.49	0.02
		$H_{2'}$	2.53	2.48	−15.1	5.0	8.1	13.1	0.33	0.67	0.00
D_2O	27	H_2	2.72	2.72	—	6.4	6.4	12.8	0.49	0.49	0.02
		$H_{2'}$	2.53	2.49	−15.1	5.2	7.8	13.0	0.35	0.64	0.00
D_2O	60	H_2	2.71	2.71	—	6.4	6.4	12.8	0.49	0.49	0.02
		$H_{2'}$	2.51	2.51	−15.2	5.8	7.0	12.8	0.42	0.56	0.02
D_2O	90	H_2	2.71	2.71	—	6.1	6.1	12.2	0.46	0.46	0.09
		$H_{2'}$	2.51	2.51	—	6.4	6.4	12.8	0.49	0.49	0.02

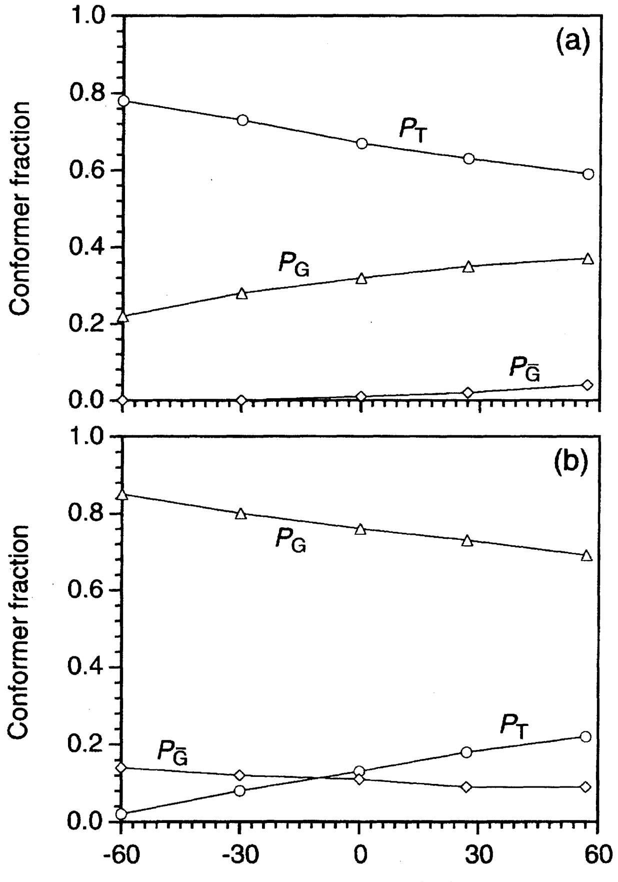


Fig. 5. Conformer fractions of MHBOB for the C_2–C_3 bond (a) and the $C_{2'}$–$C_{3'}$ bond (b) as a function of temperature in $CDCl_3$.

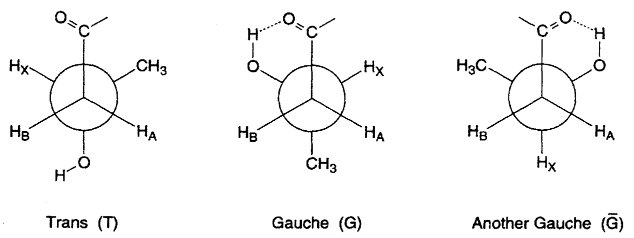


Fig. 6. Conformational structures of MHBOB around the $C_{2'}$–$C_{3'}$ bond. In both *gauche* and another *gauche* conformers the proton of hydroxy group forms an intramolecular hydrogen bond with the oxygen of carbonyl group.

and steric repulsion, and the conformer fractions are quite similar to those of the P(3-HB) polymer backbone in polar solvents.[7] It is of interest to note that the conformer fractions for the C_2–C_3 bond in D_2O show some differences from those in $CDCl_3$. For example, at 27 °C, the conformer fractions in D_2O are $P_T=0.49$, $P_G=0.49$, and $P_{\bar{G}}=0.02$, while those in $CDCl_3$ are $P_T=0.63$, $P_G=0.35$, and $P_{\bar{G}}=0.02$. This phenomenon may result from a difference in the induced dipole moments of the ester linkages by solvents. It is known that the induced dipole moment increases as the polarity of solvent is increased. Apparently, the induced dipole–dipole interaction between the two ester linkages in the *gauche* conformer for the C_2–C_3 bond in polar aqueous solution is much stronger than that in non-polar chloroform. Since the induced dipole–dipole interaction stabilizes the *gauche* conformer, the fraction of the *gauche* conformer for the C_2–C_3 bond in D_2O is larger than that in $CDCl_3$.

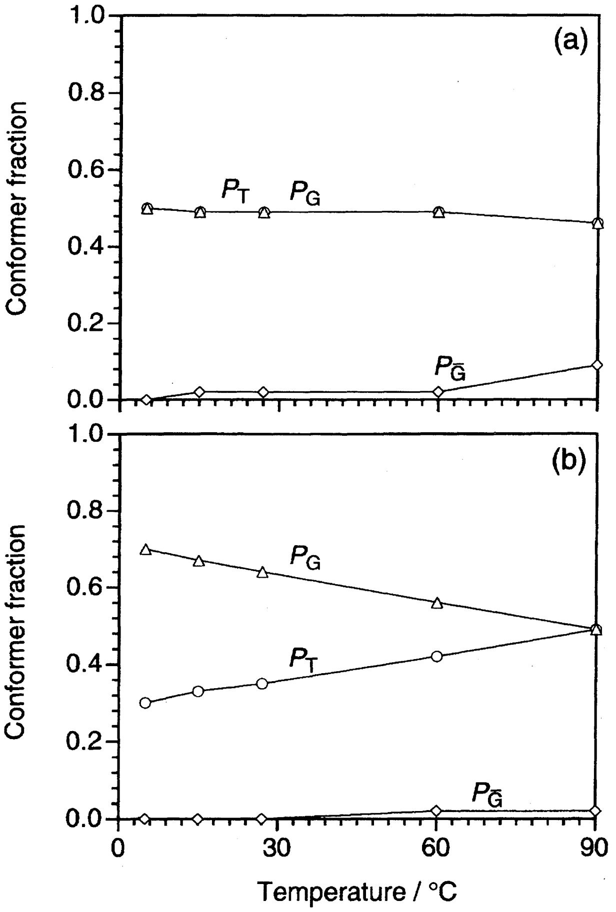


Fig. 7. Conformer fractions of MHBOB for the C_2–C_3 bond (a) and the $C_{2'}$–$C_{3'}$ bond (b) as a function of temperature in D_2O.

For the $C_{2'}$–$C_{3'}$ bond at 5 °C in D_2O, the conformer fractions are $P_T=0.30$, $P_G=0.70$, and $P_{\bar{G}}=0.00$ (Fig. 7b). Compared with those of the C_2–C_3 bond, P_G is much higher than P_T, indicating that the formation of hydrogen bond between hydroxy and carbonyl groups stabilizes the *gauche* conformer even in aqueous solution. However, the formation of the hydrogen bond in D_2O seems to be not so strong as that in $CDCl_3$. The conformational distribution for the $C_{2'}$–$C_{3'}$ bond at 90 °C becomes similar to that for the C_2–C_3 bond, indicating that the hydrogen bond in the *gauche* conformer is completely broken at 90 °C.

In conclusion, the two monomer units of MHBOB show quite different conformational behaviors both in chloroform and in aqueous solution. For the monomer unit adjacent to the methoxy group, the *trans* and *gauche* conformers predominate and the another *gauche* conformer is suppressed to almost zero. The conformer fractions are similar to those of the P(3-HB) polymer backbone. On the other hand, the monomer unit adjacent to the hydroxy group has a different distribution of conformers because of the formation of intramolecular hydrogen bond between hydroxy and carbonyl groups. In the conformational structures of this monomer unit, only the *gauche* conformer predominates, and the another *gauche* conformer also shows relatively high fractions in chloroform at low temperatures. It has been suggested from the above results that the hydroxy terminal unit of a high molecular weight P(3-HB) also has such unusual conformational behavior.

Experimental

Measurements. The ^{1}H NMR spectra of methyl (3*R*)-3-{[(3′*R*)-3′-hydroxybutanoyl]oxy}butanoate were recorded at 500 MHz on a JEOL GX-500 NMR spectrometer. The measurements were carried out at various temperatures in $CDCl_3$ or D_2O with 5.3 s pulse repetition, 5000 Hz spectral width, 32768 data points, and 64 or 128 accumulations. Chemical shifts were referred to TMS as internal reference and DSS as external reference in $CDCl_3$ and D_2O, respectively. The two-dimensional ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC spectra were obtained in $CDCl_3$ at room temperature on a JEOL α-400 spectrometer using pulsed field gradient technique.[19—21] Mass spectra were obtained on a JEOL HX-110 mass spectrometer by the fast atom bombardment (FAB) method.

Materials. Methyl (*R*)-3-hydroxybutanoate was supplied by Kaneka Chemical Ind. Other chemicals for synthesis were purchased from Kanto Chemical Co., Inc. $CDCl_3$ and D_2O used as solvent in the NMR measurements were obtained from Merck.

Methyl (3*R*)-3-{[(3′*R*)-3′-hydroxybutanoyl]oxy}butanoate was prepared according to the method reported by Seebach's group.[12—15]

(*R*)-3-(Benzyloxy)butanoic Acid. Methyl (*R*)-3-hydroxybutanoate (11.81 g, 0.10 mol) was added to a vigorously stirred suspension of powdered KOH (86%, 47 g) in Et_2O (450 ml) under dried nitrogen at room temperature. After the mixture was stirred under reflux for 12 h, benzyl bromide (120 g) was added dropwise, and stirred under reflux for another 24 h. Water (200 ml) was added to the reaction mixture, followed by vigorous stirring for 12 h. After removal of the aqueous phase, 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 evaporated and the remaining syrup was purified by flash chromatography on silica gel 60 column (Et_2O/hexane 1 : 1) to give pure (*R*)-3-(Benzyloxy)butanonic acid. Yield: 9.6 g (50%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ=7.32 (m, 5H, Ar-H), 4.56 (m, 2H, $PhCH_2O$), 4.01 (m, 1H, C(3)H), 2.59 (m, 2H, $C(2)H_2$), 1.30 (d, *J*=6.3 Hz, 3H, $C(4)H_3$).

(*R*)-3-(Benzyloxy)butanoyl Chloride. To a $CHCl_3$ solution (13 ml) of (*R*)-3-(Benzyloxy)butanoic acid (4.66 g, 0.024 mol) was added thionyl chloride (7.0 ml), then the mixture was stirred overnight at room temperature. The excess thionyl chloride was removed under high vacuum, and the crude product was used for succeeding synthesis without further purification.

Methyl (3*R*)-3-{[(3′*R*)-3′-(Benzyloxy)butanoyl]oxy}butanoate. A solution of (*R*)-3-(benzyloxy)butanoyl chloride (5.10 g, 0.024 mol) in 8 ml $CHCl_3$ was added dropwise to a solution of methyl (*R*)-3-hydroxybutanoate (2.98 g, 0.025 mol) and triethylamine (2.67 g, 0.026 mol) 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 flash chromatography on silica gel 60 column (Et_2O/hexane 2 : 8). Yield: 4.50 g (64%). ^{1}H NMR ($CDCl_3$, 270 MHz) δ=7.32 (m, 5H, Ar-H), 5.29 (m, 1H, C(3)H), 4.53 (m, 2H, $PhCH_2O$), 3.99 (m, 1H, C(3′)H), 3.65 (s, 3H, OCH_3), 2.36—2.69 (m, 4H, $C(2)H_2$ and C(2′)-H_2), 1.29 (d, *J*=6.3 Hz, 3H, $C(4)H_3$), 1.25 (d, *J*=5.9, 3H, C(4′)H_3). FAB-MS *m*/*z* (rel intensity) 295.1 (M^++1; 100), 181.1 (32). Anal. Cacld for $C_{16}H_{22}O_5\cdot 0.2H_2O$: C, 64.50; H, 7.58%. Found: C, 64.27; H, 7.34%.

Methyl (3*R*)-3-{[(3′*R*)-3′-Hydroxybutanoyl]oxy}butanoate. To a solution of methyl (3*R*)-3-{[(3′*R*)-3′-(benzyloxy)butanoyl]oxy}butanoate (4.40 g, 0.015 mol) in ethanol (15 ml) was added 10% Pd/C (0.54 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: 2.97 g (97%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ=5.29—5.36 (m, 1H, C(3)H), 4.16—4.22 (m, 1H, C(3′)H), 3.69 (s, 3H, OCH_3), 3.05 (s, broad, 1H, OH), 2.51—2.67 (m, 2H, $C(2)H_2$), 2.37—2.48 (m, 2H, C(2′)H_2), 1.32 (d, *J*=6.1 Hz, 3H, $C(4)H_3$), 1.23 (d, *J*=6.4 Hz, 3H, C(4′)H_3). FAB-MS *m*/*z* (rel intensity) 205.0 (M^++1; 100), 119.2 (44). Anal. Cacld for $C_9H_{16}O_5\cdot 0.4H_2O$: C, 51.13; H, 8.01%. Found: C, 50.83; H, 7.68%.

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