NOTES

Assignment of Non-equivalent Methylene Protons of Poly(3-hydroxybutyrate)

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Poly(3-hydroxybutyrate) is a biodegradable polyester which is produced by bacteria.¹ There have been increasing interests on the property and structure of this polymer. ¹H and ¹³C nuclear magnetic resonance spectroscopies have been used for the study of the structure. The most important problem in studying structure by means of NMR is to assign each resonance peak to each proton or carbon. The polymer has a methylene group of which two protons are non-equivalent. The stereospecific assignment of the two non-equivalent protons of the methylene is needed for studying the conformation of this polymer.

In a previous paper,² the non-equivalent protons of methylene of poly(3-hydroxybutyrate) have been assigned on the basis of the proton NOE between the methyl and the methylene. In this paper, we present an alternative method of assignment based on the three-bond coupling constant ³J_{C–H} between the methyl carbon and the methylene protons by the help of the previous population results.

Poly(3-hydroxybutyrate) of a molecular

weight of 800,000 was dissolved in CDCl₃ at a concentration of 0.04 g/ml. NMR spectra were obtained by JEOL JNM-A600 and JEOL JNM-EX400 spectrometers at 26°C.

Kamiya, *et al.*,² observed the ¹H spectra to obtain vicinal proton coupling constants ³J_{H–H} between methine and methylene. The results were analyzed by the rotation isomer model. They found that the population of *trans*, *gauche*, and *gauche prime* (*P_T*, *P_G*, *P_{G'}*) is either of (0.58, 0.42, 0) or (0.42, 0.58, 0), depending on the assignment of the two non-equivalent protons. We confirmed their results.

To obtain the three-bond coupling constant between the methyl carbon and methylene protons, we carried out the long-range ¹³C *J*-resolved two-dimensional NMR spectroscopy.

Figure 1. Figure 1. Newman Projections of rotation isomers.

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copy.³ It is found that $^3J_{C-H}$ is 3.5 Hz for the high-field methylene proton and 4.7 Hz for the low-field methylene proton. These values are not accurate because of the imperfect selective proton irradiation to the methine proton, but we believe that the relative order of $J_{\text{low-field}} > J_{\text{high-field}}$ is correct. We apply the rotation isomer model to the observed three-bond coupling constant between the methyl carbon and the methylene protons.

$$P_T J_{GC} + P_G J_{GC} + P_{G'} J_{TG} = J_{C-Ha}$$

$$P_T J_{TC} + P_G J_{GC} + P_{G'} J_{GC} = J_{C-Hb}$$

$$P_T + P_G + P_{G'} = 1$$

Because of $P_{G'} = 0$, we obtain

$$J_{TC} - J_{GC} = (J_{C-Hb} - J_{C-Ha}) / P_T$$

It is found that $J_{TC} > J_{GC}$.⁴ Therefore, we can assign the high-field peak to Ha and the low-

field peak to Hb. This is in agreement with the previous results.

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