^{11}B NMR Study of *p*-Carboxybenzeneboronic Acid Ions for Complex Formation with Some Monosaccharides

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The ^{11}B NMR spectra of *p*-carboxybenzeneboronic acid (PCBA) ions demonstrated that their chemical shifts depend on the pH. At a lower pH, PCBA exists as a mixture of neutral PCBA and the monoanion with a trigonal structure, and at higher pH as the dianion with a tetrahedral structure. In the intermediate pH region, both the mono- and dianion coexist. The $\text{p}K_{\text{a}}$ of the monoanion of PCBA has been estimated to be 8.53. The complex formation constants of PCBA with several monosaccharides suggest that PCBA-fructose complex is most predominant.

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Boron neutron capture therapy (BNCT) is a form of radiation therapy that has two components. The first is the delivery of boron to a tumor.¹ This can be achieved chemically by boronating a tumor-seeking compound, which is stable *in vivo* and infused intravenously into a subject body. Then, the boronated compound selectively accumulates sufficient amounts in the tumor cells. The second is to irradiate with thermal neutrons.¹ The capture of a thermal neutron by a ^{10}B nucleus in a tumor causes the nucleus to split, releasing an α (^4He)-particle and a lithium (^7Li) nucleus in opposite directions.² A suitable compound for BNCT must have low toxicity, high solubility in water for rapid injection, and a much higher concentration in the tumor cells than in normal cells.³

A vast array of ^{10}B -enriched compounds have been synthesized and tested for BNCT.⁴ Among a large number of boron compounds, ^{10}B -enriched *p*-carboxybenzeneboronic acid (PCBA) was firstly used in clinical trials as a BNCT agent for some brain-tumor patients.³ According to those trials, PCBA was one of the most promising compounds as a BNCT drug from the standpoint of the tumor/brain boron ratio, but was found to have relatively high toxicity.⁵ The solubility of PCBA is so low at physiological pH 7.4 that it is insufficient for medical uses. This compound dissolves in water by a sufficient amount for injection into patients, at pH 9.4.⁶ Therefore, an experiment was carried out as Na-salt of PCBA adjusted as this pH. An injection of this alkaline solution resulted in respiratory depression, seizure, and often the death of some patients.^{6,7} The authors suggested that if the solubility of PCBA increased at the physiological pH, then the above mentioned toxicity should be decreased. It is well-known that boric acid⁸⁻¹⁰ and *p*-boronophenylalanine (*p*-BPA) react with monosaccharide to form a soluble anionic complex.¹¹⁻¹³ PCBA has a boronic acid group. Therefore, it can be expected that the boronic acid moiety of PCBA may react with a monosaccharide to form an easily soluble anionic PCBA-monosaccharide complex, as indicated in Eq. (1), which should be recommended as an injection to patients for the BNCT of both brain tumor and

malignant melanoma.

According to the above view, our interest has become involved with the characterization of PCBA ions in solution, and with the formation of soluble anionic complexes between the PCBA ion and a neutral polyhydroxy-compound of monosaccharides at the physiological pH. However, to the best of our knowledge, ^{11}B NMR studies for both the characterization of PCBA ions and for PCBA complexation with monosaccharides have not been reported so far.

In this paper, we wish to describe the chemical behaviors of PCBA ions at various pH, the complex formation equilibria of the PCBA ion with several monosaccharides at the physiological pH based on an investigation of the ^{11}B NMR spectra, and the possible structure of this complex.

Experimental

Reagents

Unless stated otherwise, all of the chemicals were of analytical-reagent grade and purchased commercially from Nacalai Tesque, Inc., Kyoto, Japan; twice-deionized distilled water was used throughout the experiment. The reference compound, PCBA, was synthesized from semi-anhydride *p*-toluene boronic acid (*p*-TBA) [mixture of 6% acid (monomer) and 94% anhydride (trimer)]¹⁴ as described by Michaelis.¹⁵ Solutions of NaCl (0.16 M), NaOH (0.32 M), and HCl (0.32 M) were prepared by dissolving appropriate amounts of the reagents in water. The amount of the synthesized PCBA was insufficient to complete the present study. Therefore, commercially available PCBA from Lancaster was also used after further recrystallization from hot water.

Preparation of solutions for ^{11}B NMR studies

^{11}B NMR solutions of PCBA were prepared at various pH, like 5, 6, 7, 7.4, 8, 9, 10, and 11. We could not obtain a clear

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aqueous solution of PCBA below pH 5. Therefore, our experiments were carried out in the range of pH from 5 to 11, as follows. A weighed amount of PCBA was dissolved in a NaCl (0.16 M) solution, and the pH was kept higher than 10 by adding NaOH (0.32 M). After PCBA had been completely dissolved, the pH of the solution was adjusted with NaOH (0.32 M) or HCl (0.32 M). The concentration of each PCBA solution was 0.01 M, and the ionic strength was 0.16. In the case of PCBA-monosaccharides, appropriate amounts of PCBA and each monosaccharide were dissolved into a NaCl (0.16 M) solution. The pH of each solution was kept at 7.4 by adding NaOH (0.32 M) or HCl (0.32 M). The concentration ratios of each PCBA-monosaccharide solution was 0.01 M:0.01 M. Every ^{11}B NMR sample solution was freshly prepared just before the experiments to avoid the partial decomposition of PCBA into the boric acid. The 1 – 1.5 ml aliquots were transferred into a 5-mm quartz NMR sample tube and sealed. All of these ^{11}B NMR samples were prepared at a room temperature (20 – 25°C).

Apparatus and experimental conditions

The effective pH value of each solution was measured with an HM-30G TOA pH meter. ^{11}B NMR spectra of PCBA at various pH, and of PCBA-monosaccharides solutions at the physiological pH were recorded on a JEOL LA 400NB spectrometer. The operations were performed at 128.15 MHz. The probe temperature was 25°C. The measurements were carried out without sample spinning and field lock. All of the signals were obtained after subtracting the background signals of NaCl solutions (the solution was prepared by twice-deionized distilled water) under the respective conditions. A solution of BF_3OEt_2 was used as an external reference ($\delta = 0.00$ ppm).

Results and Discussion

^{11}B NMR study of PCBA solutions at various pH

In a weak acidic solution, PCBA exists in equilibrium with the carboxylate benzenboronic anion (monoanion) and the hydronium ion as follows:

The charge of this anion is minus one. With increasing pH, the boronic acid group of carboxylate benzenboronic anion (Eq. (2)) begins to become a dianion according to the following equation:

In a strong alkaline solution, the carboxylate benzenboronate anion (Eq. (3)) takes the sp^3 structure. In this way, if a chemical species of PCBA has a charge of minus, it is soluble in water. We can see from the equilibria, (2) and (3), that there are two chemical species of PCBA anions in solution. Among of them, the dianion of PCBA is more soluble than that of the monoanion, especially at high pH.

The ^{11}B NMR spectra of PCBA showed a singlet at various pH. Figure 1 shows the dependence of ^{11}B NMR chemical shift of PCBA on the pH.

Fig. 1 pH dependence of ^{11}B NMR chemical shift of PCBA ion-PCBA solutions (●). The signal of BF_3OEt_2 was used as a reference at $\delta = 0.00$ ppm. The line-width ($W_{0.5}$) is shown by the vertical bar.

We can see from Fig. 1 that although a PCBA solution has only one ^{11}B NMR signal in all pH regions, the chemical shift changes from 29.3 ppm (pH 5) to 3 ppm (pH 11). In addition, the line-width (the width at the half height ($W_{0.5}$)) of the signal changes from 395 Hz (pH 5) to 183 Hz (pH 11), recording the maximum width of 1098 Hz at pH 8. This can be attributed to a change of the chemical species. The dissociation constant (pK_a) of benzoic acid is 4.204.¹⁶ The chemical shift at a high pH above 10 corresponds to that of the tetrahedral benzenboronate anion and to that of a mixture of PCBA and the carboxylate benzenboronic anion below pH 7.^{8,17-19} In this low-pH range, the chemical shift and $W_{0.5}$ are almost constant, because both species have the same trigonal environment around the ^{11}B nucleus. In the intermediate region, pH 7 to 10, both the carboxylate benzenboronic and benzenboronate anions coexist. The mole ratio of the boronic species predominates near pH 7, and the ratio of the boronate species gradually increases with increasing pH (Fig. 1). Above pH 10, both the chemical shift and the line-width are almost constant, indicating that the chemical species is solely restricted to the dianion. The appearance of only one signal at an intermediate chemical shift is indicative of a rapid interconversion between the benzenboronic anion and the boronate anion (Eq. (3)).^{8,17-19} As a result, the ^{11}B NMR signals of boronic and boronate anions are not observed separately. Therefore, if any other boron species does not exist in this pH region, the chemical shift of the signal may be ascribed to the relative concentration of the carboxylate benzenboronic anion and the boronate anion. From the chemical shift at a given pH, the ratio of the concentration of both species can be calculated using the following equation:¹⁹

$$\frac{[\text{B}^{2-}]}{[\text{B}^-]} = \frac{28.6 - \delta_{\text{obs}}}{\delta_{\text{obs}} - 3.0} \quad (4)$$

where B^{2-} indicates the carboxylate benzenboronate anion, B^- the carboxylate benzenboronic anion and δ_{obs} the chemical shift of the observed signal. The numbers 28.6 and 3.0 represent the chemical shifts at pH 7 and 11, respectively. The high sensitivity of Eq. (3) to the pH in the intermediate region is ascribable to a conformational change of the ^{11}B surroundings from the trigonal boronic anion to tetrahedral boronate. At pH 7, the boronic monoanion almost predominates over all of the

Table 1 Calculated pK_a value of boronic anion of PCBA from the ^{11}B NMR spectra

pH	pK_a
7.40	8.71
8.00	8.56
9.00	8.46
10.00	8.45

other boron species. The dissociation constants (K_a) of the boronic anion for Eq. (3) were calculated from the ^{11}B NMR data at pH 7.4 to 10, using Eq. (4), as shown in Table 1. The mean value of pK_a is 8.53. This value is nearly equal to that of boric acid.⁸

Figure 2 shows the ^{11}B NMR spectra of solutions of PCBA at (A) pH 7, (B) pH 8, and (C) pH 11. The boron concentration of each solution was 0.01 M.

It can be clearly observed from Fig. 2 that the ^{11}B NMR line-width at pH 7 is broader than that at pH 11. In addition, the line-width at pH 8 is much broader than those of at pH 7 and 11. In an acidic solution, the boronic acid group of PCBA and the boronic anion exist as the same trigonal type (Eq. (2)). On the other hand, in an alkaline solution above pH 10, the group exists as a tetrahedral type (Eq. (3)). In the intermediate pH region, the mole ratio of the monoanion and dianion varies. The chemical shift moves with the change of the ratio.²⁰ The boronic acid group of PCBA mainly exists as the trigonal monoanion accompanied by a slight amount of the dianion in a solution of the physiological pH. This may be one of the reasons for the low solubility of PCBA in water. On the contrary, if the pH increases to 11, the boronic acid group becomes a highly soluble tetrahedral species with a charge of minus two.

As already mentioned, in the intermediate pH region the ^{11}B NMR signal arises from an equilibrium mixture of boronic and boronate anions, in which ^{11}B is situated at a trigonal structure in the former and at a tetrahedral structure in the latter, but appeared as a singlet. Pople *et al.*²¹ reported that NMR may often provide additional information on such equilibrium, since the theory of time-dependent effects shows that the properties of a resonance line may be sensitive to the random motions of resonant nuclei among different chemical sites. If the mean lifetime of an atom remaining on one of the two different sites is long compared with the inverse of the chemical shift difference between the two sites, the spectrum will consist of two lines. On the other hand, if the characteristic exchange time is short compared with the difference, a single line with a weighted mean frequency will be observed. In the intermediate pH region, the ^{11}B environment changes many times in the time-scale of the inverse of the chemical-shift difference from trigonal to tetrahedral, and *vice versa*. Therefore, the formation of a singlet may be attributable to a fast intramolecular rearrangement between the two anions, which makes two boron nuclei equivalent. Such a concept has been proposed by Momii *et al.*²² for the NMR equivalence of ^{11}B in $\text{B}_5\text{O}_6(\text{OH})_4^-$. Piper *et al.*²³ and Bennett *et al.*²⁴ also proposed the same concept of rearrangements for the NMR equivalence of ^1H in $(\text{C}_6\text{H}_5)_2\text{Hg}$ and π -cyclopentadienyliron dicarbonyl σ -cyclopentadiene, respectively.

A nucleus with a spin of one or more possesses an electric quadrupole moment (eQ), which produces a broad line. This eQ occurs because of the non-spherical charge distribution of a nucleus, and interacts with the electric-field gradient when the nucleus is situated at a site of lower symmetry than the

Fig. 2 ^{11}B NMR spectra of PCBA solutions at (A) pH 7, (B) pH 8, and (C) pH 11. The boron concentration of each solution was 0.01 M. The reference signal of BF_3OEt_2 at $\delta = 0.00$ ppm is not shown.

tetrahedral environment.^{25,26} ^{11}B is a quadrupolar nucleus with a spin of 3/2, and is situated at the trigonal symmetry below pH 7. Therefore, the signal width is influenced by the interaction between eQ and the electric-field gradient (eq) at the ^{11}B nucleus, as evidenced by one of the present authors.¹⁹ This causes the ^{11}B NMR signal line-width of boronic anion at pH 7 to broaden by 367 Hz compared to that of the boronate anion at pH 11. Although the line broadening of the ^{11}B NMR signal of the boronic anion at pH 7 depends on the factor of the quadrupole effect, the line-width of ^{11}B NMR signal at pH 8 cannot be explained by such a type of factor, due to the different environments.

Several researchers have proposed that the line broadening of the ^{11}B in the intermediate pH region is due to the formation of polyborates.^{18,22,27} The detection of polyborate species by ^{11}B NMR depends on two main factors, *i.e.* the stability of the polyborate ion in the solution and the exchange rate of boron nuclei between different chemical sites in the molecule.²⁷ At pH 8, there are two chemical species, boronic and boronate anions, which undergo a conformational change of ^{11}B . Comparisons between the exchange rates in different systems are often stated in terms of the conditions (temperature, pH, *etc.*) for the coalescence of signals.²⁸ When the exchange rate is slow, two signals appear at the two different chemical shifts. On the contrary, in the case of an extremely high exchange rate, a sharp signal is observed at the middle of the above two signals. In the intermediate rate a broad singlet is recorded at the same position as in the case of an extremely high exchange rate.²⁹ An observation at pH 8 would be applicable to this intermediate case, which will tend to broaden by 548 and 915 Hz compared to that of the signals at pH 7 and 11, respectively.

On the other hand, if the ^{11}B compound has the high symmetrical structure around the ^{11}B nucleus, the signal is sharp.¹⁹ The signal at pH 11 is assigned to the benzenboronate anion, in which ^{11}B is situated at the tetrahedral environment. Therefore, the signal of the benzenboronate anion at pH 11 is very sharp.

Complex formation between the boronic acid moiety of PCBA and monosaccharides at the physiological pH

As mentioned above, the boronic acid group of the boronic

Fig. 3 ^{11}B NMR spectra of PCBA-monosaccharides solutions of (A) PCBA-fructose, (B) PCBA-mannitol, (C) PCBA-galactose, (D) PCBA-mannose, and (E) PCBA-glucose at the physiological pH. The boron-monosaccharide concentration ratio of each solution was 0.01 M:0.01 M. The reference signal of BF_3OEt_2 at $\delta = 0.00$ ppm is not shown.

anion exists mainly as a trigonal species in a solution of the physiological pH. This causes a low solubility of PCBA in water. We also mentioned that the low solubility of PCBA at the physiological pH is insufficient for medical uses as a BNCT drug. To overcome this problem, we investigated the ^{11}B NMR spectra of PCBA-monosaccharides at the physiological pH. As for the monosaccharides, we chose glucose, mannose, galactose, mannitol, and fructose.

The minimum pH, in which PCBA could be dissolved without and with monosaccharides, was determined by the following method:^{11,12} PCBA of 100 mg was mixed with 3 ml of a NaCl solution (0.16 M) or 3 ml of a 0.3 M monosaccharide solution (prepared by NaCl solution (0.16 M)) in a beaker. To this mixture, a NaOH solution (0.32 M) was added dropwise to obtain a clear solution, while measuring pH. At pH 8.06, PCBA was completely dissolved without monosaccharide. With monosaccharide, PCBA was dissolved at pH 6.74 (galactose), 6.24 (glucose), 6.16 (mannose), 5.88 (mannitol), and 5.60 (fructose). Every monosaccharide gave a lower pH of dissolution than that of without a monosaccharide. This indicates the formation of an anionic complex of PCBA with a monosaccharide.

Kanie *et al.*³⁰ reported the formation of 1,2,6-orthoester of mannose by protection of the primary alcohol of mannose 1,2-orthoester glycosylation. Dahlhoff *et al.*³¹ suggested that the electrophilic boron atom in the 2,3-*O*-ethylboranediyl group exerts a directive effect during glycosylation due to an interaction between the boron and the methoxide ion to form an intermediate borate-type species. The electronic configuration of boron is $1s^2 2s^2 2p$, indicating that the boron in a trivalent compound has one vacant orbital, which is available for the formation of a fourth covalent bond with an electron donor atom, giving a tetrahedral adduct.^{32,33} This can be exemplified by phenylboronic acid in an aqueous solution, as evidenced by Yoshino *et al.*,¹⁹ as follows:

Table 2 ^{11}B NMR spectroscopic data of PCBA-monosaccharides solutions at pH 7.4

PCBA (M)	MS (M)	δ_1	δ_2	A_1	A_2	$\log K$
0.01	(F. 0.01)	27.7	7.8	35	65	2.9
0.01	(F. 0.02)	28.1	7.8	12	88	2.9
0.01	(F. 0.03)	ND	7.8	ND	100	ND
0.01	(*M. 0.01)	27.7	7.8	49	51	2.5
0.01	(G. 0.01)	27.4	7.8	81	19	1.6
0.01	(*M. 0.01)	27.0	8.0	90	10	1.2
0.01	(*G. 0.01)	27.4	7.8	96	4	0.8

M = molar concentration, MS = monosaccharide, F = fructose, *M = mannitol, G = galactose, *M = mannose, *G = glucose, δ_1 = chemical shift of the free mixture of boronic and boronate anions of PCBA in ppm downfield from BF_3OEt_2 , δ_2 = chemical shift of PCBA-monosaccharide complex in ppm downfield from BF_3OEt_2 , A_1 = relative resonance area (%) of the free mixture of boronic and boronate anions of PCBA, A_2 = relative resonance area (%) of PCBA-monosaccharide complex, $\log K$ = equilibrium constant of complex formation, and ND = not determined due to overlap the signal of the free mixture of boronic and boronate anions with that of the complex.

PCBA is a trigonal compound, which shows the above behavior by ionizing in an aqueous solution, and gives the tetrahedral dianion (Eq. (3)). To determine the interaction between boronic acids and carbohydrates in aqueous media, Torssell³⁴ showed the complex formation equilibria between phenylboronic acid and D-fructose by single step equation, as follows:

From the above evidence, the complexation equilibria between boronate anion of PCBA and monosaccharide (MS) can be shown by the following equation:

However, the complexations of PCBA with monosaccharides were confirmed by ^{11}B NMR spectroscopy. Figure 3 shows the ^{11}B NMR spectra of PCBA-monosaccharide solutions of (A) PCBA-fructose, (B) PCBA-mannitol, (C) PCBA-galactose, (D) PCBA-mannose, and (E) PCBA-glucose at the physiological pH. The concentration ratio of each solution was 0.01 M:0.01 M (ionic strength of 0.16).

We can see from Fig. 3 that the ^{11}B NMR spectra of a mixture of PCBA and monosaccharides at the physiological pH exhibit two signals at about 27.4 ppm and 7.8 ppm, respectively. The difference between the chemical shifts is due to the electronic environment around the boron nucleus. The chemical shift of the boron nucleus, arising from the free mixture of boronic and boronate anions at pH 7.4, is 27.4 ppm (Fig. 1). Therefore, the low-field signals at about 27.4 ppm are those of a mixture of boronic and boronate anions, and the high-field signals at about 7.8 ppm are attributable to the complex of boronate anions. The area of each signal corresponds to the concentration of each species. The complex formation constants ($\log K$) were

Fig. 4 Conformations of monosaccharides. (A) fructose, (B) mannitol, (C) galactose, (D) mannose, and (E) glucose.

calculated from the signal area ratios and the initial concentration of PCBA and monosaccharides. The ^{11}B NMR data of all the spectra are summarized in Table 2. It can also be clearly observed from Fig. 3 that the signal intensities of a mixture of boronic and boronate anions of PCBA gradually decrease, and the signal intensities of the complex gradually increase while changing the monosaccharides from (E) to (A). This is due to the difference in the complex formation affinity of the boronate anion of PCBA with various kinds of monosaccharides. The line-widths of the 1:1 PCBA-monosaccharides complex have nearly the same value (ca. 384 Hz), irrelevant of the kinds of monosaccharides at the physiological pH. At higher concentrations of monosaccharides, the signal intensity of the free mixture of boronic and boronate anions of PCBA disappears, and at last all of the boron species form complexes at the physiological pH. However, it is clear from Fig. 3 that the formation of the PCBA-fructose complex is predominant. Table 2 shows the decreasing order of the amount of the complex formation ($\log K$) is fructose, mannitol, galactose, mannose, and glucose, which is nearly the same order as that of the minimum pH values for dissolving PCBA with monosaccharides.

The boronate anion of PCBA forms complexes with a *cis* OH pair of monosaccharides. To understand the decreasing order, it is reasonable to consider the structure of monosaccharides. Figure 4 shows the conformations of monosaccharides. We can see that α -fructopyranose, mannitol, and α -galactopyranose have two pairs of *cis* OH. On the other hand, mannose and glucose have only one pair of *cis* OH. A larger complex formation constant can be expected with a monosaccharide having more complex-making sites. The obtained results are in agreement with this tendency.

We confirmed that fructose is the only monosaccharide that has a suitable ability to form a complex with PCBA at the physiological pH. Figure 5 shows the ^{11}B NMR spectra of

Fig. 5 ^{11}B NMR spectra of PCBA-fructose solutions for various molar concentrations of fructose at the physiological pH. (A) 0.03 M, (B) 0.02 M, and (C) 0.01 M. The boron concentration of each solution was the same (0.01 M). The reference signal of BF_3OEt_2 at $\delta = 0.00$ ppm is not shown.

PCBA-fructose solutions at various molar concentrations of fructose at the physiological pH. These ^{11}B NMR samples were also prepared by the same procedure as described previously, and the ionic strength was set at 0.16.

We can see from Fig. 5 that at constant pH 7.4, the signal of the free mixture of boronic and boronate anions of PCBA become weaker, and at last disappear with increasing concentration of fructose. A possible reason may be that the dihydroxyl part of fructose is in a complex formation equilibrium (7). The amount of neutral dihydroxy species, which may coordinate with the boronate anion of PCBA, increases with increasing concentration of fructose. Thus, the formation of the PCBA-fructose complex proceeds, resulting in a decrease of the free mixture of boronic and boronate anions of PCBA by a corresponding amount.¹⁸ The constant chemical shift (7.8 ppm) at a concentration of 0.01 M:0.03 M PCBA-fructose solution (Fig. 5(A)) makes it clear that most of the boron species is the PCBA-fructose complex. The ^{11}B NMR data of these spectra are also summarized in Table 2.

As mentioned above, the boronic acid group of PCBA exists in an aqueous solvent of the trigonal or tetrahedral type. In water, fructose exists as a complex mixture of five isomeric forms, in which β -D-fructopyranose predominates over β -D-fructofuranose and α -D-fructofuranose. Small amounts of α -D-fructopyranose and the acyclic *keto* form have also been reported.³⁵ The *cis* OH pairs of fructose may make a five-membered ring complex with PCBA. The hydroxy methyl group and the nearby hydroxy group may make a six-membered ring complex with PCBA.

Norrild *et al.*³⁵ have identified the complex formation bonding sites of *p*-TBA with fructose by using the $^1\text{J}_{\text{CC}}$ coupling method at pH values of 11 to 12. Kitazawa *et al.*³⁶ have reported on the possible structure of the *p*-BPA-fructose complex by using the $^1\text{J}_{\text{CC}}$ coupling constants. According to their studies, the $^1\text{J}_{\text{C2C3}}$ value of the complex is smaller than the corresponding values of α - and β -D-fructofuranoses, the $^1\text{J}_{\text{C1C2}}$ value is larger than the corresponding values of α - and β -D-fructofuranoses, and the $^1\text{J}_{\text{C5C6}}$ value is smaller than the corresponding value of β -D-fructofuranose. From the above studies, they concluded that *p*-

Structure 1 Possible structure of β -D-fructofuranose 2,3,6-PCBA complex in water at the physiological pH.

TBA or *p*-BPA formed a complex with three OH groups of β -D-fructofuranose. Shull *et al.*³⁷ also recently reported on the basis of the ^{13}C NMR chemical shift and the $^1\text{J}_{\text{C}}$ coupling method that *L-p*-BPA formed a complex with β -D-fructofuranose. From the above evidence, it is easy to expect that the boronic acid moiety of PCBA may complex with β -D-fructofuranose, in which the boron atom bound to the three oxygen's at the sites of C2, C3 and C6 of the fructose ring at the physiological pH 7.4, as shown in Structure 1.

In conclusion, the chemical behaviors of PCBA ions in solution have been clarified at various pH values based on the ^{11}B -NMR spectra. ^{11}B NMR studies have proven that PCBA forms a complex with several monosaccharides at the physiological pH, and that the complex formation increased the solubility of PCBA. The complex formation affinity of PCBA is larger with fructose than that of other monosaccharides. More investigations are required to understand the detailed structure and reactivity of this PCBA-fructose complex to use as an actual drug for BNCT.

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