^{11}B-NMR Study of the Complex Formation of Borate with Catechol and L-Dopa

Kazuo YOSHINO,* Masahiro KOTAKA, Makoto OKAMOTO, and Hidetake KAKIHANA

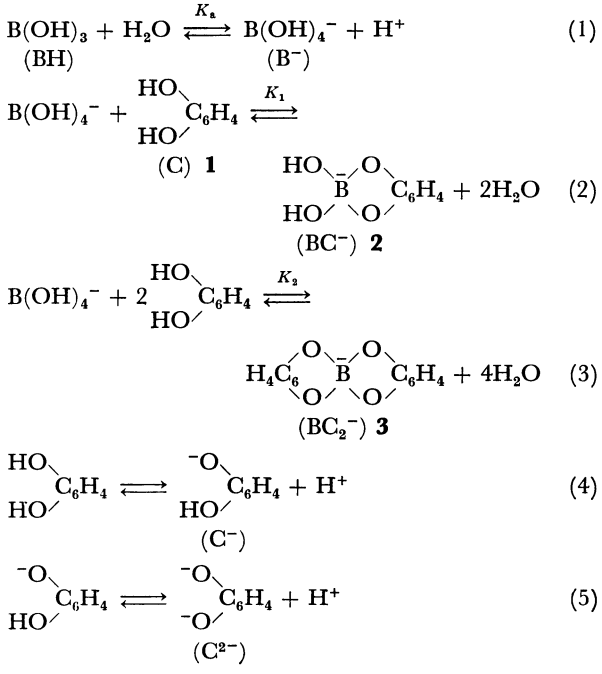
Research Laboratory for Nuclear Reactors, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152

(Received January 30, 1979)

In the solution of catechol and borax at pH 11, the equilibria between catechol and the monomeric borate anion to form the 1: 1 and 2: 1 (catechol: boron) anionic complexes have been demonstrated by the existence of ^{11}B-NMR signals for the two complexes and the monomeric borate anion. In the solution of L-dopa and borax at pH 11, however, the signal for the 2: 1 anionic complex was not observed, though it was observed below pH 7. By the ^{11}B-NMR spectra at pH 6.5, the complex formation constants, log K_1 and log K_2, have been estimated as 3.9 and 4.4 for catechol, and as 4.3 and 5.0 for L-dopa, respectively.

The neutron-capture therapy requires a boron-10-containing compound which concentrates selectively in cancer tissue and does not in normal tissue in order to collect boron-10 selectively in the malignant tumor.[1-3] L-Dopa is one of the precursors of melanine, which is produced in a great quantity in malignant melanoma. Planning to carry the boron-10 into melanoma using L-dopa as a carrier, the compound "dopaborate," which has one boron atom per molecule, has been synthesized. The present authors have investigated the structure and reactivety of this compound in an aqueous solution by means of the UV spectroscopic method.[4] It was thus recognized that the solution of "dopaborate" was just the same as that of a mixture of 1: 1 L-dopa and boric acid at about pH 8.5, and in an aqueous solution the catechol site of L-dopa coordinates to the monomeric borate anion.

In the equilibrium system of catechol and the monomeric borate anion, the equlibria may be represented by the following equations (Equilibria 1—3). The equilibrium constants for these equilibria can be represented by Eq. 6—8. Since catechol itself is an acid, the acidic strength of which is comparable to that of boric acid, its dissociation reactions must also be taken into consideration (Equilibria 4 and 5).



$$B(OH)_3 + H_2O \overset{K_a}{\rightleftharpoons} B(OH)_4^- + H^+ \quad (1)$$
(BH) (B$^-$)

$$B(OH)_4^- + C_6H_4(OH)_2 \overset{K_1}{\rightleftharpoons} \text{(C) } \mathbf{1} \qquad \text{(BC}^-\text{) } \mathbf{2} + 2H_2O \quad (2)$$

$$B(OH)_4^- + 2\,C_6H_4(OH)_2 \overset{K_2}{\rightleftharpoons} \text{(BC}_2^-\text{) } \mathbf{3} + 4H_2O \quad (3)$$

$$C_6H_4(OH)_2 \rightleftharpoons C_6H_4O^-OH + H^+ \quad (4)$$
(C$^-$)

$$C_6H_4O^-OH \rightleftharpoons C_6H_4O^-O^- + H^+ \quad (5)$$
(C^{2-})

$$K_a = [B^-][H^+]/[BH] \quad (6)$$

$$K_1 = [BC^-]/[B^-][C] \quad (7)$$

$$K_2 = [BC_2^-]/[B^-][C]^2 \quad (8)$$

Two methods have been developed to identify the equilibrium system of catechol and the monomeric borate anion. One is the potentiometric method,[5-8] and the other is the UV spectroscopic method.[9] In the case of potentiometry, the evidence concerning the complex formation is largely indirect. In the case of the UV method, though, since the absorption spectrum of the catechol–borate complex, (**2** or **3**), is different from that of catechol (**1**), the method offers direct evidence concerning the complex formation; however, since the absorption spectrum of the 1: 1 catechol–borate complex (**2**) seems to be the same as that of the 2: 1 catechol–borate complex (**3**), this method does not give directly the concentrations of the two complexes.

In many equilibrium systems of diol and the monomeric borate anion, ^{11}B-NMR spectroscopy demonstrated that the 2: 1 complex has a chemical shift different from that of the 1: 1 complex, and the ^{11}B-NMR spectra give the concentrations of the each boron species directly, which makes it possible to calculate the equilibrium constants.[10,11] Therefore, we have used ^{11}B-NMR spectroscopy in order to elucidate the nature of the equilibria of the catechol–borate and L-dopa–borate systems.

Experimental

Preparation of Solutions for ^{11}B-NMR Studies. Twice-distilled deionized water was used throughout the experiments. Dissolving the weighed amount of borax into water, a weighed amount of L-dopa (or catechol) was slowly added to the solution. Thereby the pH of the solution was kept higher than 8 by adding sodium hydroxide. After the L-dopa (or catechol) had been completely dissolved, the pH of the solution was adjusted to the required value with sodium hydroxide or hydrochloric acid, and the solution was diluted with water to 50 ml. These preparations were carried out in an N_2 atmosphere, because both L-dopa and catechol are oxidized by atmospheric oxygen, especially in an alkaline solution. The 2—4 ml aliquots were put into 8 mmϕ NMR quartz probes and sealed in an N_2 atmosphere to avoid oxidation; these probes were then placed into 10 mmϕ quartz probes which had D_2O as a lock.

^{11}B-NMR Measurements. The FT-NMR spectrometer used was JEOL FX-100. The operation were performed at

Table 1. ^{11}B-NMR spectroscopic data

Diol	Diol-Boron ratio	pH	σ_4	σ_3	σ_2	σ_1	A_4	A_3	A_2	A_1
Catechol	0.5:1	11	—	—	8.9(27)	3.5(17)	—	—	51	49
	1:1	11	—	—	9.0(26)	3.4(45)	—	—	91	9
	2:1	11	—	14.5	9.0(28)	—	—	5	95	—
	1:1*	6.5	20.6(73)	14.4(20)	9.0(23)	—	34	9	57	—
L-Dopa	0.5:1	11	—	—	9.0(50)	3.4(12)	—	—	48	52
	1:1	11	—	—	9.0(57)	3.3(43)	—	—	87	13
	2:1	11	—	—	9.1(74)	—	—	—	100	—
	1:1	12	—	—	9.0(55)	3.1(24)	—	—	79	21
	1:1	10	—	—	9.1(62)	—	—	—	100	—
	1:1	9	—	—	9.2(64)	—	—	—	100	—
	1:1	8	—	13.7(sh)	9.1(63)	—	—	—	100	—
	1:1	7	19.8	14.5	9.1(67)	—	—	—	—	—
	1:1*	6.5	20.4	14.7	9.1(56)	—	28	12	60	—

The total concentration of boron is 0.5 mol dm^{-3}, except in the cases marked with *, which are 0.25 mol dm^{-3}. σ_n, the downfield chemical shift (in ppm) from sodium tetrafluoroborate; the width at half-height ($W_{0.5}$) is shown in brackets in Hz in cases where the values were obtained. A_n, the relative area of the signal at σ_n as % of the total area of the signals. In cases where the signals overlapped, the relative areas of the signals were obtained by computer simulation.[16]

31.96 MHz. The probe temperature was about 27 °C. The number of pulses (the repetition time is 1.0 s) was 100 except in the case of the 0.25 mol dm^{-3} boron concentration in which 400 pulses were needed. The solution of sodium tetrafluoroborate was used as an external reference.

Results and Discussion

The experimental conditions and the obtained data are summarized in Table 1.

A borax solution has only one ^{11}B-NMR signal in all the pH region, but the chemical shift of this signal changes from 20.6 ppm (pH 6.5) to 3.1 ppm (pH 12) with the increase in pH; these values are assigned to boric acid and the monomeric borate anion respectively.[10,12] The appearance of only one signal in the pH region in which both monomeric species exist indicates that Equilibrium 1 is rapidly interconverting.[10] 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 boric acid and the borate anion, and the line-width (the width at the half height) of the signal would be intermediate between those of the monomeric species. Figure 1 shows the ^{11}B-NMR spectra of the solution of borax at pH 7, 9, and 11. The boron concentration of each solution was 0.5 mol dm^{-3}. The signal at pH 11 is assigned to the almost monomeric borate anion. Since the signal at pH 7 has a shoulder peak, and since the line-width of the signal at pH 9 is broader than that of the signal at pH 7 and 11, there may be polyborates.[13,14]

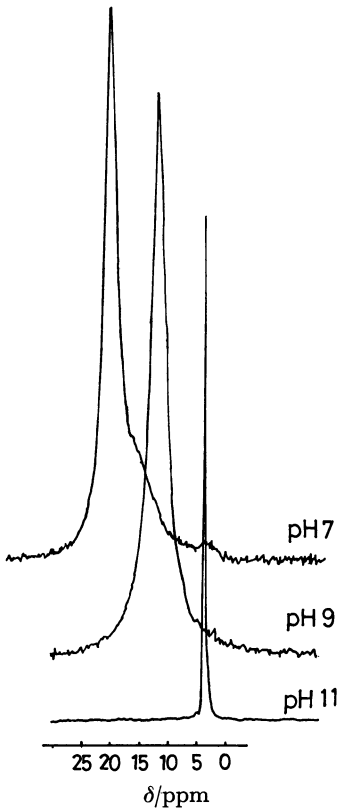


Fig. 1. ^{11}B-NMR spectra of borax solutions at pH 7, 9, and 11. The boron concentration of the solutions is 0.5 M (1 M=1 mol dm^{-3}). The reference signal of sodium tetrafluoroborate at δ 0.0 is not shown.

^{11}B-NMR Spectroscopic Examination at pH *11.*

The spectra of catechol–borax solutions (pH 11) at several molar ratios are illustrated in Fig. 2. The signal at 3.4 ppm corresponds exactly to that of the monomeric borate anion. The change in the signal intensity with the increase in the catechol concentration (the boron concentration is kept at 0.5 mol dm^{-3}) demonstrates that the low-field signals at 9.0 and 14.5 ppm can be assigned to the 1:1 (**1**) and 2:1 (**2**) complexes respectively. The line-width of the 1:1 catechol–borate complex has nearly the same value (27 Hz) in the spectra of three different molar ratios of catechol–boron.

Figure 3 shows the spectra of L-dopa–borax solutions (pH 11) at several molar ratios. The signal at 9.0 ppm,

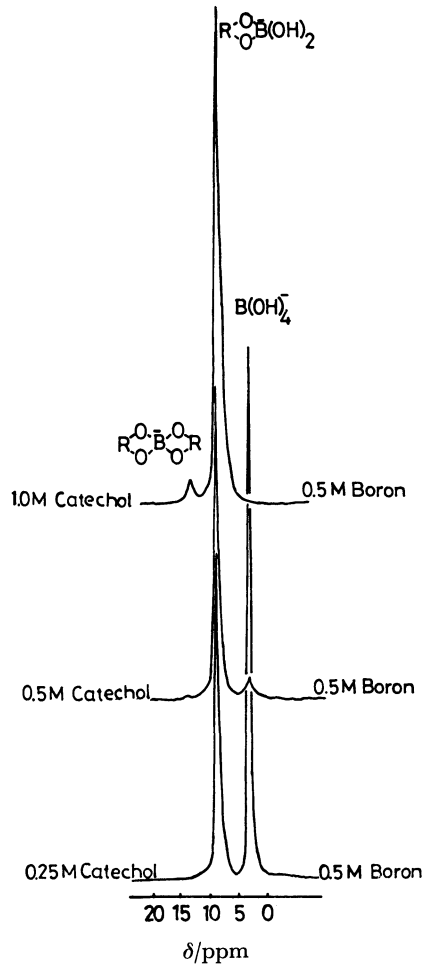


Fig. 2. ^{11}B-NMR spectra of catechol–borax solutions at 2: 1, 1: 1, and 0.5: 1 molar ratios of catechol and boron. In the solutions, the initial concentration of boron is 0.5 M (1 M=1 mol dm^{-3}) and pH is 11. The reference signal of sodium tetrafluoroborate at δ 0.0 is not shown.

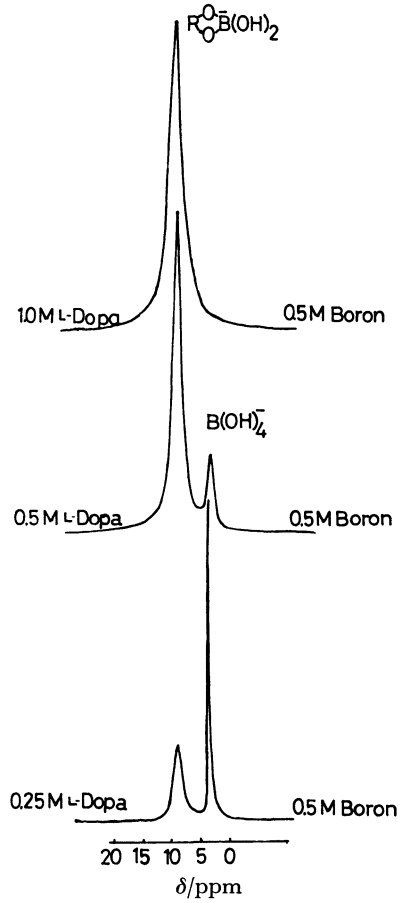


Fig. 3. ^{11}B-NMR spectra of L-dopa–borax solutions at 2: 1, 1: 1, and 0.5: 1 molar ratios of L-dopa and boron. In the solutions, the initial concentration of boron is kept 0.5 M (1 M=1 mol dm^{-3}) and pH is 11. The reference signal of sodium tetrafluoroborate at δ 0.0 is not shown.

which seems to be that of the 1: 1 L-dopa–borate complex, is not so sharp as that of catechol–borate complex, and the broadening of this signal with the increase in the molar ratio of L-dopa–boron is remarkable (the boron concentration is kept at 0.5 mol dm^{-3}). The signal of the 2: 1 L-dopa–borate complex could not be observed separately, not even in the L-dopa–borax solution with a L-dopa–boron ratio of 2: 1.

^{11}B-NMR Spectroscopic Examination for the 1: 1 L-Dopa–Boron Solution at Various pH *Values.* Figures 4 and 5 show the obtained spectra of the 1: 1 L-dopa–boron solutions at pH 12—pH 6.5. The boron concentration is kept at 0.5 mol dm^{-3}, except in the case with pH 6.5, in which the concentration is 0.25 mol dm^{-3}. In the case of pH 12—pH 9, the signal of the monomeric borate anion became smaller and at last disappeared at pH 10 with a decrease in the pH. There may be two reasons for this. One of these is that, in the dissociation equilibria in the catechol part of L-dopa, the amount of un-ionized dihydroxy species, which may coordinate with the monomeric borate anion, increases with the decrease in pH and the formation of the 1: 1 L-dopa–borate complex proceeds; as a result, the monomeric borate anion decreases by the corresponding amount. The other possible reason may be explained as follows. With a decrease in the pH, some monomeric borate anions become boric acid or polyborate anions, as was mentioned above, and the signals of these species overlap with that of the 1: 1 complex. The latter explanation is proved by the change in the line-width for the signal at 9.1 ppm. It becomes larger with a decrease in the pH. The constant chemical shift (9.1 ppm) in this pH region, however, makes it clear that most of the boron species is the 1: 1 L-dopa–borate complex. Figure 5 shows that, at pH 7, two signals (at 19.8 and 14.5 ppm) appear in addition to the signal of the 1: 1 complex. These signals are more clearly observed at pH 6.5. The signals at 19.8 (pH 7) and 20.4 (pH 6.5) ppm are ascribed to the free boron species, as is shown in Fig. 1, and that at 14.5 ppm, to the 2: 1 L-dopa–borate complex. The latter assignment is made by comparing this signal with that of the 2: 1 catechol–borate complex at 14.5 ppm, which was observed in Fig. 2. These assignments are also confirmed by a comparison of these spectra with the spectrum of the 1: 1 catechol–boron solution at pH 6.5, which is shown

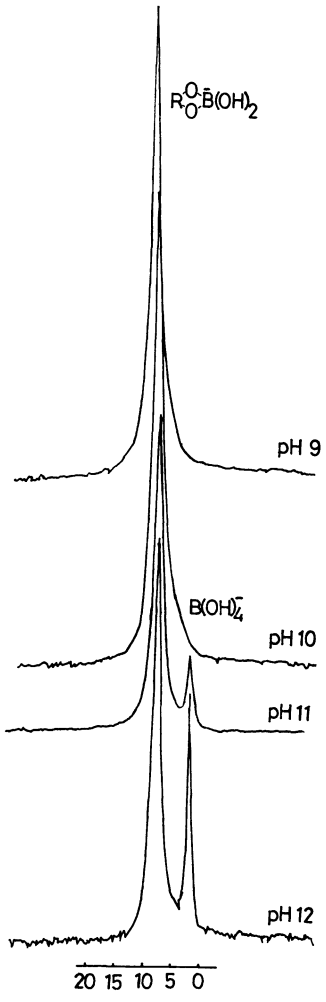


Fig. 4. ^{11}B-NMR spectra of L-dopa–borax solutions at pH 12—pH 9. In the solutions, the ratio of L-dopa and boron is kept 1: 1, and the initial concentration of boron is kept 0.5 M (1 M=1 mol dm^{-3}). The reference signal of sodium tetrafluoroborate at δ 0.0 is not shown.

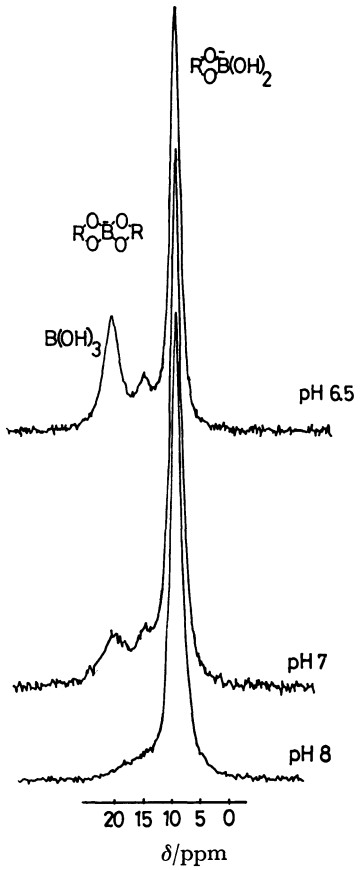


Fig. 5. ^{11}B-NMR spectra of L-dopa–borax solutions at pH 8—pH 6.5. In the solutions, the ratio of L-dopa and boron is kept 1: 1, and the initial concentration of boron is 0.5 M (1 M=1 mol dm^{-3}) except in the case at pH 6.5 in which 0.25 M. The reference signal of sodium tetrafluoroborate at δ 0.0 is not shown.

in Fig. 6 (the boron concentration is 0.25 mol dm^{-3}). The appearance of the 2: 1 L-dopa–borate complex might be explained as follows. As has been mentioned in the introduction, the complex formations may occur between the monomeric borate anion and the un-ionized catechol part of L-dopa. Therefore, under the present experimental conditions, in spite of the same initial concentrations of L-dopa and boron, the concentration of the monomeric borate anion is far less than that of the un-ionized catechol type of L-dopa. Therefore, the equilibrium not only for 1: 1 but also for 2: 1 L-dopa–borate complex formation is shifted to the right.

The Estimation of the Equilibrium Constants for the 1: 1 and 2: 1 Complex Formations. In the case of the catechol–borate complex formation, the equilibria which seem to take place in this experiment have already been summarized in Equilibria 1—5. In the case of L-dopa–borate complex formation, however, the equilibria are much more complicated, because L-dopa has an amino group, the acidic strength of which seems

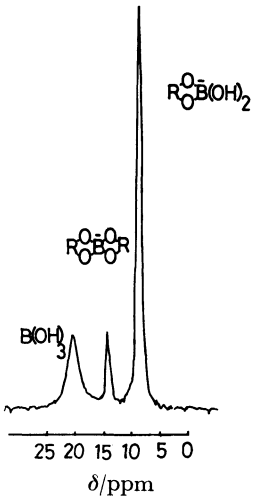
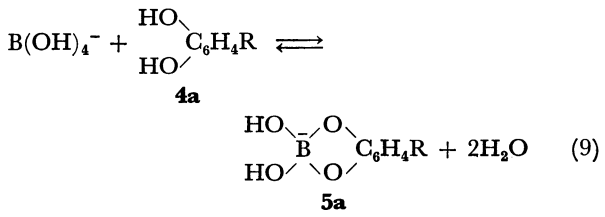


Fig. 6. ^{11}B-NMR spectrum of catechol–borax solution at pH 6.5. The ratio of catechol and boron is 1: 1, and the initial concentration of boron is 0.25 M (1 M =1 mol dm^{-3}). The reference signal of sodium tetrafluoroborate at δ 0.0 is not shown.

to be comparable to that of catechol or boric acid. Therefore, L-dopa has many dissociation equilibria

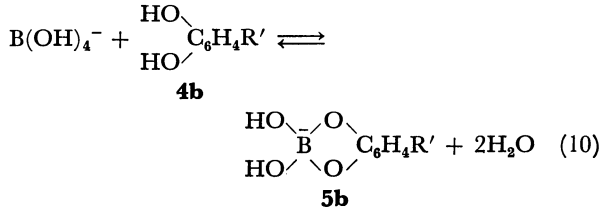
which must be taken into consideration; the equilibria for L-dopa–borate complex formation may be represented as follows in the experimental pH region:



4a

(9)

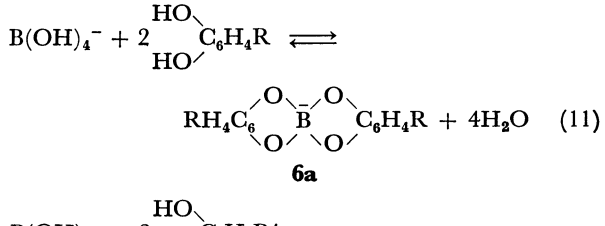
5a



4b

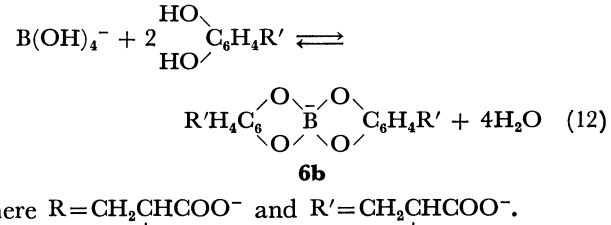
(10)

5b



(11)

6a



(12)

6b

where R=CH_2CHCOO^- (NH_3^+) and R′=CH_2CHCOO^- (NH_2).

In order to estimate the equilibrium constants by using the ^{11}B-NMR spectra, the concentrations of the monomeric borate anion ($B(OH)_4^-$) and un-ionized dihydroxy catechol (**1**) or un-ionized dihydroxy L-dopa (**4a**) and (**4b**) should be obtained. At pH 11, the free boron species is regarded as almost entirely the monomeric borate anion, and its concentration is calculated directly from the signal area of the ^{11}B-NMR spectra. However, the concentration of (**1**) cannot be obtained without using the dissociation constants of Equilibria 4 and 5, and the concentrations of (**4a**) and (**4b**) are hard to obtain, because the dissociation constants of L-dopa, which are needed to calculate the concentrations of (**4a**) and (**4b**), have not yet been determined. On the other hand, at pH 6.5, since free catechol and free L-dopa are regarded as (**1**) and (**4a**) respectively, these concentrations can be obtained from the ^{11}B-NMR spectra and the ionization constant of boric acid (K_a).

Calculation of Equilibrium Constants. *Catechol*: The three signal areas of the ^{11}B-NMR spectrum at pH 6.5 correspond to $[BC^-]$, $[BC_2^-]$, and $[B]+[B^-]$ respectively. The combination of these concentrations with the following equations gives the concentrations of all the species:

$$[B]_0 = [BH] + [B^-] + [BC^-] + [BC_2^-]$$
$$[C]_0 = [C] + [BC^-] + 2[BC_2^-]$$
$$K_a = [B^-][H^+]/[BH]$$

where $[B]_0$ is the initial concentration of boron and $[C]_0$ is the initial concentration of catechol. 9.00 was used for log K_a.[15]

L-Dopa: The method is the same as that used in the case of catechol, but in this case [C], $[BC^-]$, and $[BC_2^-]$ correspond to the concentrations of (**4a**), (**5a**), and (**6a**) respectively, and $[C]_0$ is the initial concentration of L-dopa.

Table 2. Comparison of equilibrium constants (K_1 and K_2) calculated from this ^{11}B-NMR study for catechol and L-dopa with the values obtained from other methods

Diol	Method	Temp	log K_1	log K_2	Remarks
Catechol	pH-Change[8]	25 °C	3.97	4.26	
	UV[9]		4.3	—	pH 7.8
	^{11}B-NMR	27 °C	3.9	4.4	pH 6.5
L-Dopa	pH-Change		—	—	
	UV[9]		4.2	—	pH 7.8
	^{11}B-NMR	27 °C	4.3	5.0	pH 6.5

The values thus obtained, log K_1 and log K_2, are shown in Table 2, along with the values of the other methods. The values obtained for catechol are consistent with the values obtained by potentiometry. Among the results obtained, it is found for the first time that the 2:1 L-dopa–borate complex is formed between L-dopa and the monomeric borate anion.

The authors wish to thank Professor Atsuo Nishioka and Dr. Isao Ando of the Tokyo Institute of Technology for their helpful discussion, and also JEOL for measuring the ^{11}B-NMR spectra.

References

1) Y. Mishima, *Pigment Cell.* **1**, 215 (1973).
2) H. Kakihana and K. Yoshino, *Gendai Kagaku*, **71**, 42 (1977).
3) H. Hatanaka, *Shinkei Shinpo*, **22**, 142 (1978).
4) K. Yoshino, H. Kakihana, M. Okamoto, M. Kotaka, and T. Iizuka, 36th National Meeting of the Chemical Society of Japan, Osaka, April 1977, Abstr, No. 3U17.
5) G. L. Roy, A. L. Laferriere, and J. O. Edwards, *J. Inorg. Nucl. Chem.*, **4**, 106 (1957).
6) P. J. Antikainen and A. Kauppila, *Suom. Kemistil. B*, **31**, 255 (1958).
7) J. M. Conner and V. C. Bulgrin, *J. Inorg. Nucl. Chem.*, **29**, 1953 (1967).
8) P. J. Antikainen and I. P. Pitkanen, *Suom. Kemistil. B*, **41**, 65 (1968).
9) K. T. Yasunobu and E. R. Norris, *J. Biol. Chem.*, **227**, 473 (1957).
10) W. G. Henderson, M. J. How, G. R. Kennedy, and E. F. Mooney, *Carbohydr. Res.*, **28**, 1 (1973).
11) G. R. Kennedy and M. J. How., *Carbohydr. Res.*, **28**, 13 (1973).
12) M. J. How, G. R. Kennedy, and E. F. Mooney, *Chem. Commun.*, **1969**, 267.
13) R. K. Mommi and N. H. Nachtrieb, *Inorg. Chem.*, **6**, 1189 (1969).
14) H. D. Smith and R. J. Wiersema, *Inorg. Chem.*, **11**, 1152 (1972).
15) N. Ingri, *Acta Chem. Scand.*, **17**, 573 (1963).
16) Y. Kato, I. Ando, and A. Nishioka, *Kobunshi Ronbunshu*, **32**, 200 (1975).