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Design of a Visualized Sugar Sensing System Utilizing a Boronic Acid-azopyridine Interaction

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The boronic acid-amine (B-N) interaction was applied to the molecular design of a visualized sensing system for saccharides. 3-Nitrophenyl-boronic acid (3b) interacts with the pyridine nitrogen of 4-(4-dimethyl-aminophenyl-azo) pyridine (2) in methanol and changes its color from yellow to orange. Added saccharides form complexes with 3b and enhance the acidity of the boronic acid group. As a result, the B-N interaction becomes stronger and the intensified intramolecular charge-transfer band changes the solution color to red. By combined ^{1}H-NMR and absorption spectroscopic studies, four association constants governing the present ternary complex system have been determined. In case of D-fructose, the 3b·D-fructose complex can interact with 2 240 times more strongly than 3b and the 2·3b complex can associate D-fructose 241 times more strongly than 3b. Clearly, the B-N interaction not only enables the colorimetric saccharide sensing but also enhances the association ability (*i.e.*, sensitivity). Thus, the present paper offers a novel basic concept for molecular design of visualized saccharide sensing systems.

Keywords: Sugar sensing, colorimetry, saccharide, boronic acid, azopyridine

INTRODUCTION

Recognition of neutral organic species by synthetic molecular receptors has been of great interest to many chemists, but some new methodology which is different from recognition of ionic species has to be exploited. Most of the known synthetic molecular receptors utilize hydrogen-bonding interactions in order to recognize and bind with neutral guest molecules [1]. However, these interactions are less effective in aqueous media where guest species are water-soluble and well hydrated. On the other hand, covalent interactions found in the binding between boronic acids and saccharides are stronger than such hydrogen-bonding interactions in aqueous media and therefore, should be more effective. The usefulness of the boronic acid function as a saccharide receptor has been demonstrated in saccharide recognition in rigid matrices, [2, 3] CD detection [4], fluorescence detection [5, 6], molecular assemblies [7], membrane transport [8], *etc.* A potential breakthrough found through these studies is the finding that the acidity of boronic acids is intensified when they form cyclic boronate esters with diols [5, 6, 9, 10]. The tertiary amine bearing an intramolecular boronic acid group changes its basicity upon the saccharide-binding

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through the boronic acid-nitrogen (B–N) interaction: thus, the spectroscopic properties of amine-containing chromophores are also changed by the saccharide-binding [6, 7, 10]. This finding has enabled us to detect the saccharide-binding event with absorption and fluorescence spectroscopic methods [7a, 11, 12]. In Scheme 1, for example, compound **1** has a large dipole from a dimethylamino group (donor: D) to a sulfonate group (acceptor: A) [12]. The free boronic acid intramolecularly interacts with the dimethylamino group in the absence of saccharides whereas the boronate ester-saccharide complex with the intensified acidity interacts with the dimethylamino group more strongly. This difference changes the electron-donation ability of the dimethylamino group and eventually changes the solution color. The basic concept of this color-change system can be illustrated as in Figure 1a.

Here, we considered that the acidity change in the boronic acid moiety would be more efficiently utilized by the interaction with the electron-withdrawing A (as in Fig. 1b) rather than by the interaction with the electron-donating D (as in Fig. 1a). In order to evaluate the working hypothesis in Figure 1b one has to choose some "tertiary amine" with an electron-withdrawing nature. We noticed that the pyridine group can exactly satisfy this requirement. Thus, we synthesized compound **2** and estimated the influence of the intermolecular boronic acid (**3**)-pyridine (A in **2**) interaction on the intramolecular charge-transfer band. As boronic acids, phenylboronic acid (**3a**) and 3-nitrophenylboronic acid (**3b**) were employed. After a through search for the optimum measurement conditions, we have found that the methanol solution containing **2** and **3** sensitively changes its solution color upon addition of saccharides.

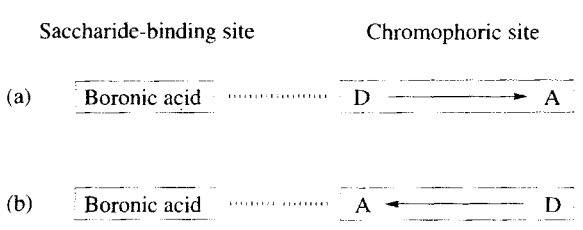
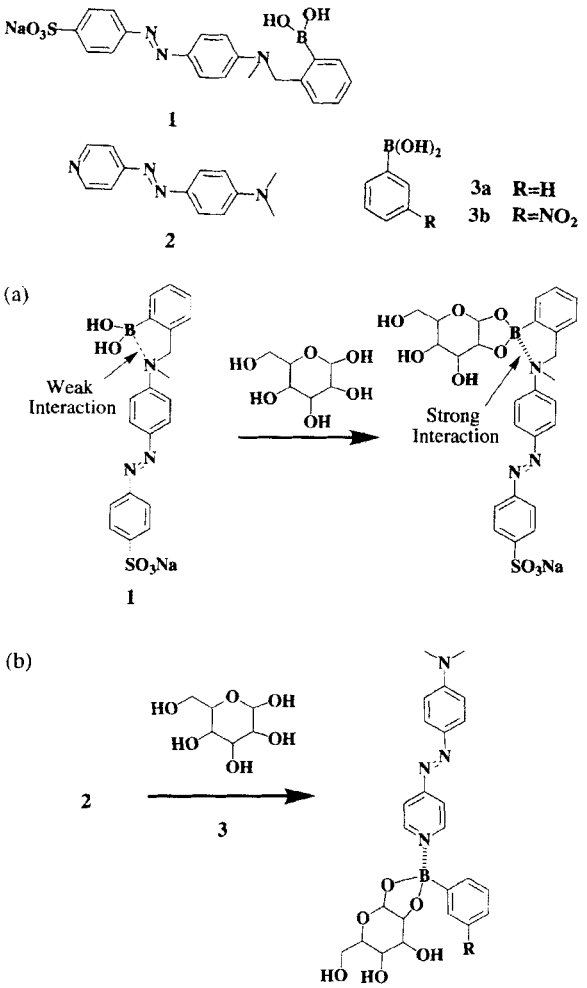


FIGURE 1 Concept of molecular design for colorimetric saccharide sensing: (a) the boronic acid interacts with the electron-donor, (b) the boronic acid interacts with the electron-acceptor.



SCHEME 1

RESULTS AND DISCUSSION

Firstly, saccharide sensing with a **2** plus **3** system was tested in aqueous media. We found, however, that the spectral change induced by the **B** (boron in **3**)-N (pyridine in **2**) interaction over-

laps with that induced by protonation of **2**. In order to estimate the saccharide-binding separately, therefore, one has to offset the pH-dependent spectral change in **2** as a background. This situation makes the saccharide sensing system very complicated. Furthermore, the spectroscopic studies in aqueous media showed that the concentration-absorbance plot deviates from the linearity (Lambert-Beer's law) at high concentration region ($\sim 10^{-4}$M). This implies that **2** tends to aggregate in aqueous media. We thus decided to adopt methanol as a solvent because (i) protonation of **2** can be disregarded, (ii) the B–N interaction is stronger in methanol than in aqueous media and (iii) saccharides are still soluble in methanol in a practical level.

The methanol solution of **2** ($1.00 \times 10^{-6} \sim 5.00 \times 10^{-5}$M) obeyed a Lambert-Beer's law, indicating that **2** is discretely solubilized in methanol. The absorption spectrum of **2** with λ_{max} 455 nm was scarcely changed when **3a** was added (Fig. 2a), whereas a new absorption maximum appeared at λ_{max} 540 nm when **3b** was added (Fig. 2b). The new absorption band with λ_{max} 540 nm is similar to that with λ_{max} 555 nm which is obtained by protonation of **2** in an acidic water-DMSO (1:2 v/v) solution. The result implies that the B–N interaction in a **2** plus **3b** system is as strong as to be comparable to protonation of the pyridine moiety. Figure 3 shows plots of the absorbance (at 540 nm) against the added **3a** or **3b** concentration. The higher sensitivity of **3b** relative to **3a** is due to the higher acidity caused by the electron-withdrawing 3-nitro group. From the analysis of the plot for **3b** using a Benesi–Hildebrand's method [13] the association constant (K_1) was estimated to be 199 M^{-1}. On the other hand, the K_1 value for **3a** was roughly estimated to be 2.1 M^{-1} if one can assume that the saturation value is the same as that of **3b**.

The saccharide-induced color change was evaluated with a **2** plus **3b** system. D-Fructose, which is known to possess the highest complexation ability with monoboronic acids [3–10], was chosen as a saccharide. In Figure 4, the concentration of **3b** was varied while the concentrations of **2** and D-fructose were maintained constant. As expected, the absorbance obtained in the presence of D-fructose increases more efficiently than that obtained in the absence of D-fructose. Further interesting is the fact that the saturation absorbance in the

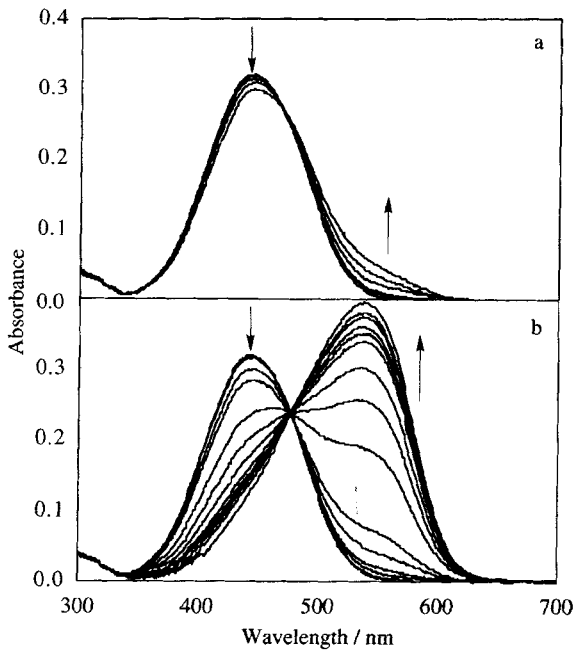


FIGURE 2 Absorption spectra of **2** (1.00×10^{-5}M) in the presence of (a) **3a** ($5.00 \times 10^{-3} \sim 1.00 \times 10^{-1}$M) or (b) **3b** ($1.00 \times 10^{-5} \sim 1.00 \times 10^{-2}$M): 25°C, methanol.

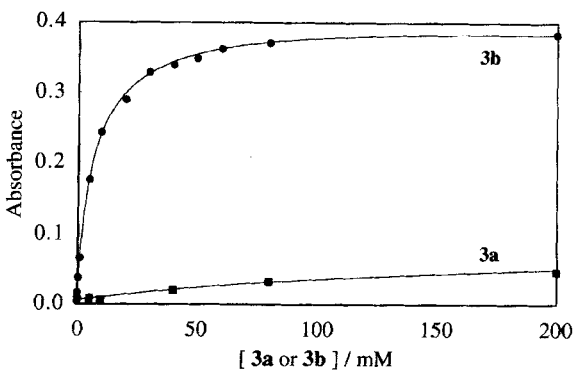


FIGURE 3 Plots of the absorbance (at 540 nm) against the **3a** or **3b** concentration.

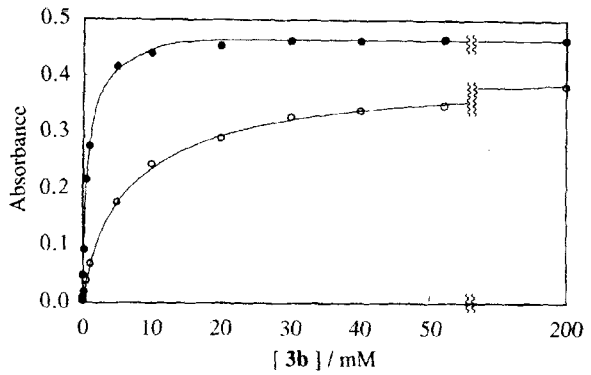


FIGURE 4 Plots of the absorbance (at 540 nm) *vs.* **3b** in the absence (○) and the presence (●) of D-fructose: 25°C, methanol, [2] = 1.00×10^{-5} M, [D-fructose] = 0.100 M.

presence of D-fructose is higher than that in the absence of D-fructose. These results are both rationalized in terms of the "intensified acidity" of the boronic acid group caused by D-fructose complexation. As shown with a color photograph (Fig. 5), it is clearly seen that the solution color changes from yellow to red with increasing D-fructose concentration. The similar concentration-dependent color change was also observed for D-arabinose although the change was not so vivid as that for D-fructose. The results imply that this system is useful for visual sensing of certain saccharides.

In Figure 6, the concentration of various saccharides was varied while the concentrations of **2** and **3b** were maintained constant. The absorbance in the low concentration region increased in the order of D-fructose > D-xylose > D-arabinose > L-fucose > D-mannose (for the structure of the saccharides see Fig. 7). This order is approximately consistent with the order of the association constants with boronic acids determined in aqueous media [9]. At high concentration region, the absorbance for D-xylose has been saturated whereas that for D-arabinose continuously increases: thus, the order of the absorbance at 0.10 M becomes D-fructose > D-arabinose > D-xylose > L-fucose > D-mannose. Figure 8 shows a photograph of **2**, **3b** and saccharide systems at [saccharide] = 0.10M. It is seen from Figure 8 that the difference in the affinity with **3b** is clearly reflected by the difference in the solution color. This means that if the solution color is compared at the same conditions, one can identify the bind of saccharide from the degree of a color change from yellow to red.

Two saccharides showed exceptional behaviors. The solution color of a **2** plus **3b** system was scarcely changed by the addition of L-rhamnose (0 ~ 0.10 M: the plot is not shown in Fig. 6 for the sake of clarity). On the other hand, the addition of D-ribose rather decreased the absorbance at 540 nm (Fig. 6). What is the origin of their unusual behaviors? It has been well established that the primary boronic acid binding site is the 1,2-*cis*-diol group in monosaccharides [6,7,9,10,14,15]. It is likely, therefore, that if 3-OH has the *cis*-configuration to 2-OH, it exists in the same side as the complexed boronic acid to the pyranose plane. Hence, the 3-OH should interfere with the interaction between the pyridine nitrogen in **2** and the vacant *p*-orbital in the boronate ester (at least, one side is sterically blocked). Examination of Figure 7 reveals that D-mannose, L-rhamnose and D-ribose have such a 2,3-*cis*-diol structure. As described above, 9% of **2** interact with **3b** even in the absence of saccharides. Thus, D-mannose only slightly increases the absorbance 540 nm (Fig. 6), L-rhamnose has no effect on it and D-ribose, in which 2-OH, 3-OH and 4-OH are all "down", rather hampers the interaction and decreases the percentage of the **2**–**3b** interaction by the **3b**·D-ribose complexation.

The foregoing results indicate that the chromogenic saccharide-binding process in the present system takes place according to Scheme 2. For D-fructose the K_1 value has been determined to be 199 M^{-1}(*vide supra*). To obtain a quantitative insight into the complexation mode among **2**, **3b** and D-fructose we attempted to estimate K_2, K_3 and K_4. The K_4 is an association constant between **3b** and D-fructose. Unfortunately, the absorption spectral change was scarcely induced

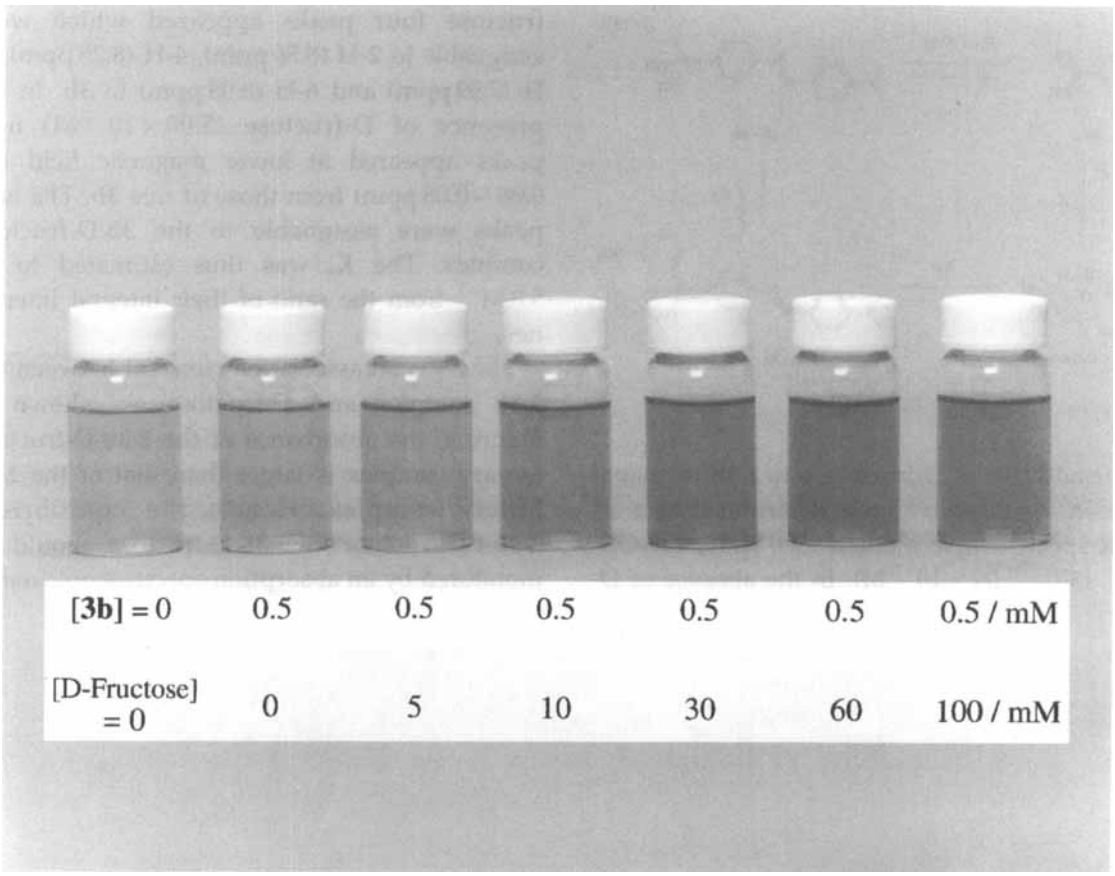


FIGURE 5 A change in the solution color of a **2** (1.00×10^{-5} M) plus **3b** (5.00×10^{-4} M) system with increasing D-fructose concentration. (See Color Plate I at the back of this issue).

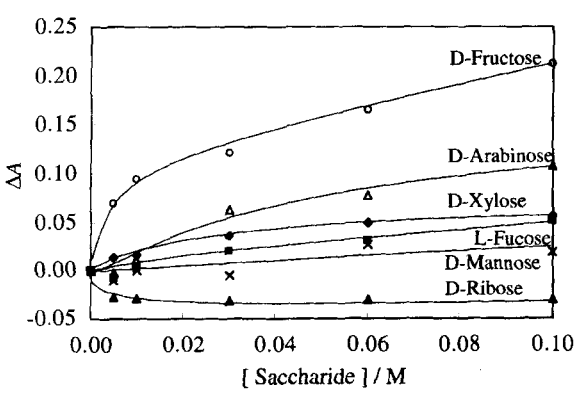


FIGURE 6 Plots of the absorbance difference (ΔA)(at 540 nm) *vs.* [saccharide]: 25°C, methanol, [**2**] = 1.00×10^{-5} M [**3b**] = 5.00×10^{-4} M.

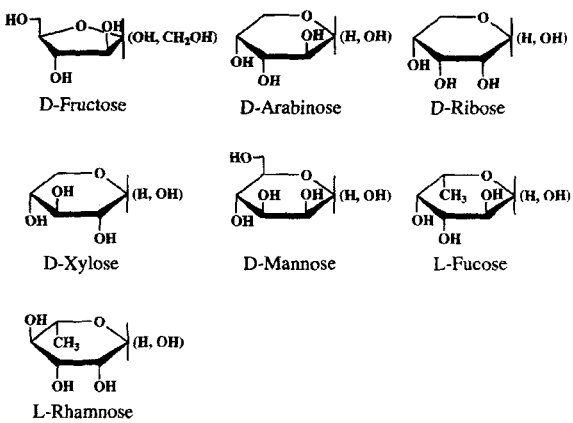
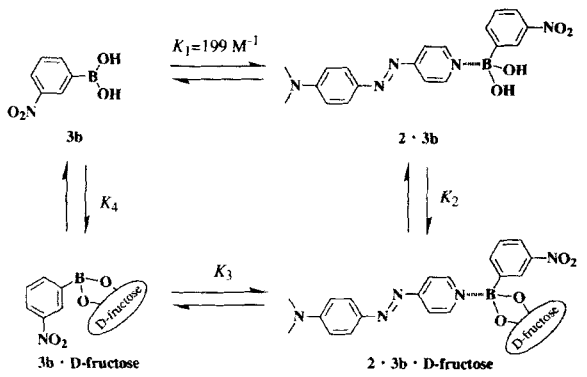


FIGURE 7 Structure of monosaccharides used in this study.



SCHEME 2

by the addition of D-fructose into a **3b** methanol solution. Hence, the K_4 was determined by a ^{1}H NMR spectroscopic method (500 MHz, CD_3OD, 25°C, [**3b**] = 5.00×10^{-2} M). In the absence of D-fructose four peaks appeared which were assignable to 2-H (8.56 ppm), 4-H (8.25 ppm), 5-H (7.59 ppm) and 6-H (8.08 ppm) in **3b**. In the presence of D-fructose (5.00×10^{-2} M) new peaks appeared at lower magnetic field (by 0.06 ~ 0.08 ppm) from those of free **3b**. The new peaks were assignable to the **3b**·D-fructose complex. The K_4 was thus estimated to be 5.8 M^{-1} from the ratio of their integral intensities.

The K_2 is an association constant between the **2·3b** complex and D-fructose. As shown in Figure 4, the absorbance of the **2·3b**·D-fructose ternary complex is larger than that of the **2·3b** binary complex. Hence, the equilibrium **2·3b** + D-fructose ⇆ **2·3b**·D-fructose should be monitored by an absorption spectroscopic meth-

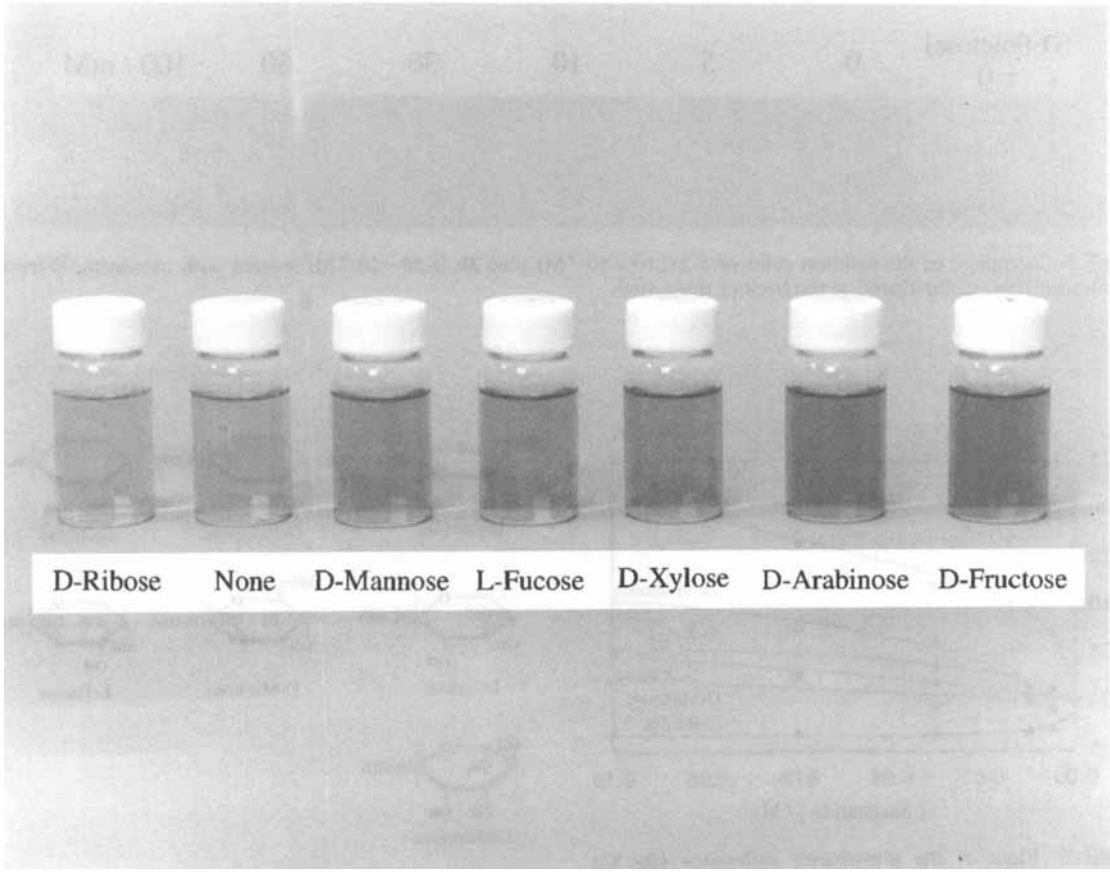


FIGURE 8 Color of the solutions containing **2** (1.00×10^{-5} M), **3b** (5.00×10^{-4} M), and saccharide (0.100 M). (See Color Plate II at the back of this issue).

od. It is seen from Figure 3 that **2** is totally converted to the **2·3b** complex at $[\mathbf{2}] = 1.00 \times 10^{-5}$ M and $[\mathbf{3b}] = 0.200$ M. One can thus consider that added D-fructose is associated either with free **3b** or with the **2·3b** complex where $[\mathbf{3b}]_{free} = [\mathbf{3b}]_{total} - [\mathbf{2{\cdot}3b}] = [\mathbf{3b}]_{total}$(because $[\mathbf{3b}]_{total} \gg [\mathbf{2}]$). Since the K_4 has been determined to be $5.8\,M^{-1}$, one can calculate [**3b**·D-fructose], the concentration of D-fructose consumed for the association with free **3b**. Taking this concentration into account, we made a plot of $\Delta A (= A_{obs}$ in the presence of D-fructose $- A$ for the **2·3b** complex) *versus* $[\text{D-fructose}]_{free} (= [\text{D-fructose}]_{total} - [\mathbf{3b}{\cdot}\text{D-fructose}])$ as in Figure 9 This plot corresponds to an equilibrium for **2·3b** + D-fructose ⇆ **2·3b**·D-fructose. From the analysis of this plot we obtained $K_2 = 1.40 \times 10^3\,M^{-1}$. From a relation $K_1 \cdot K_2 = K_3 \cdot K_4$, the K_3 is now computed to be $4.78 \times 10^4\,M^{-1}$.

Comparison of four association constants reveals that **3b**·D-fructose complex interacts with **2** 240 times more strongly than **3b** (as shown by K_3/K_1) and (ii) the pyridine-coordinated boronic acid in **2·3b** complex can associate D-fructose 241 times more strongly than the boronic acid in **3b** (as shown by K_4/K_2). As mentioned above, factor (i) is rationalized in terms of the "intensified acidity" of the boronic acid by D-fructose complexation [5, 6, 9, 10, 12]. It is known that the involvement of a sp^2-hybridized atom as a ring member increases the ring strain [4]–[7]. Thus, conversion of **3b** to the cyclic **3b**·D-fructose complex would accompany an unfavorable increase in the ring strain. In contrast, the boron atom in the **2·3b** complex has already been changed into sp^3-hybridized one through coordination of the pyridine, so that conversion of the **2·3b** complex to the cyclic **2·3b**·D-fructose complex is less affected by the ring strain. Because of this stereochemical difference the **2·3b** complex has an advantage over **3b** in the saccharide binding: that is, the B-N interaction not only enables the colorimetric saccharide sensing but also enhances the association ability (*i.e.*, sensitivity) for saccharides.

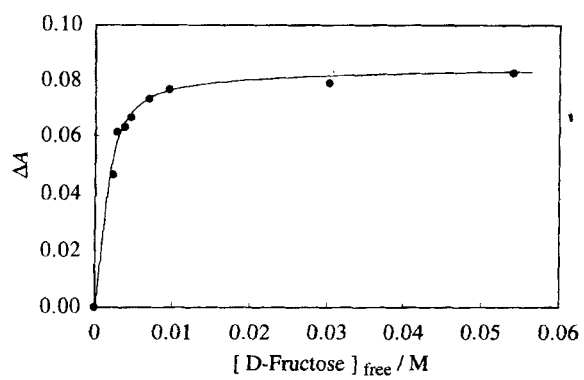


FIGURE 9 Plot of ΔA (at 540 nm) *vs.* $[\text{D-fructose}]_{free}$: $[\mathbf{2}] = 1.00 \times 10^{-5}$ M, $[\mathbf{3b}] = 0.200$ M, methanol, 25°C.

CONCLUSION

The present paper has demonstrated a basic principle for visual color sensing of saccharides using the interaction between boronic acids and nitrogen-containing chromophores. It has been shown that a nitrogen acting as an acceptor site in the chromophore is more suitable to this purpose and the method is actually applicable to colorimetry of D-fructose and D-arabinose. Further studies (including the molecular design of chromophores bearing an intramolecular boronic acid and glucose-selective bis (boronic acid)s) are currently continued in this laboratory. We believe that those efforts eventually lead to the sensitive and selective colorimetric sensing of saccharides.

EXPERIMENTAL

Materials

Phenylboronic acid and 3-nitrophenylboronic acid were purchases from Ardrich. The synthesis of 4-(4-dimethylaminophenylazo) pyridine was previously reported [16].

Miscellaneous

^{1}H-NMR spectra were measured with a Brucker AMX500 spectrometer using tetramethylsilane as reference. UV-Vis spectra were measured with a Shimadzu UV-160A spectrometer.

Acknowledgments

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