STILBAZOLIUM BORONIC ACID/BORATE - A NEW STILBAZOLIUM BETAINE DYE: AN OPTICAL MOLECULAR SENSOR FOR MONOSACCHARIDES

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Abstract – The Knoevenagel condensation of 1,4-dimethylpyridinium and 1,4-dimethylquinolinium salts with 4-formylbenzeneboronic acid gave new stilbazolium dyes (**2**) and (**3**), respectively.   The UV-VIS absorption behavior of the boronic acid-substituted stilbazolium dyes were studied in acetonitrile-aqueous buffered solutions (1:25 v/v), in the absence and in the presence of various sugars as guest species. The dyes form complexes with monosaccharides at neutral pH together with a bathochromic shift of the intramolecular charge transfer band.

As the important roles of carbohydrates in biology are elucidated, the development of synthetic receptors for carbohydrates is growing into an active research area,[1] in particular, the recognition and sensing of saccharides in aqueous media by boronic acid based molecular receptors have shown tremendous growth during the last decade.[2,3] Arylboronic acids readily form cyclic esters with diols in basic aqueous media and the boronic acid esters change to its hydroxy anion adducts at a lower pH range than free boronic acids.[2]   By taking advantage of the differences in their acidity, the complex formation with saccharides can be detected from the optical or electrochemical response of boronic acid receptors.

On the other hand, stilbazolium betaine (**1**),[4,5] well known as Brooker's merocyanine, has attracted interest because their spectum is very sensitive to the medium polarity,[6] pH,[7] and ion concentration.[8] We thought that the replacement of the phenolate oxygen atom of **1** by boronic acid would give a good chemosensor for saccharides. The   -electronic properties of **2** would be significantly perturbed by

complexation with saccharides to result in a large spectral change, since the neutral form of the boronic acid group acts as an electron-withdrawing group, whereas the anionic form acts as a less or non electron-withdrawing group.[3a,9] Furthermore, electron-withdrawing character of cationic heterocyclic moiety would shift the **pKa** value of **2** to a more acidic region, probably to a neutral area.
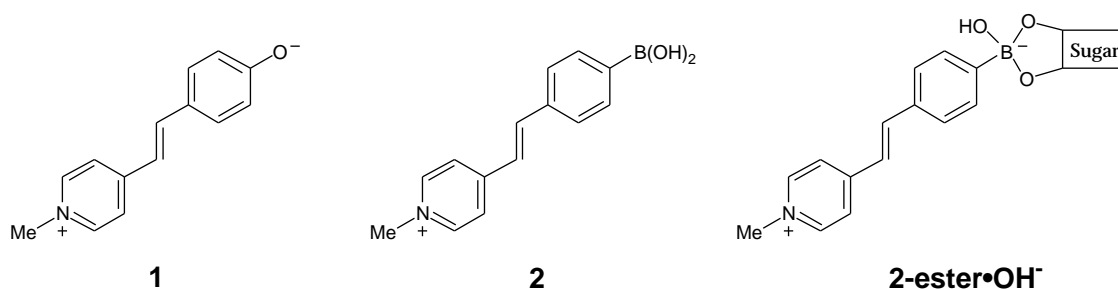

Although a number of dye-boronic acid compounds have been synthesized in the last few years,[3] there is no report concerned with stilbazolium boronic acids or stilbazolium betaine type zwitterionic salts containing borate anion.[10,11] We report here the preparation, acid-base equilibria, and sugar-binding feature (in a neutral pH solvent) of the stilbazolium boronic acid (**2**) and a styrylquinolinium analog (**3**) in order to evaluate the annulation effects of benzene ring.
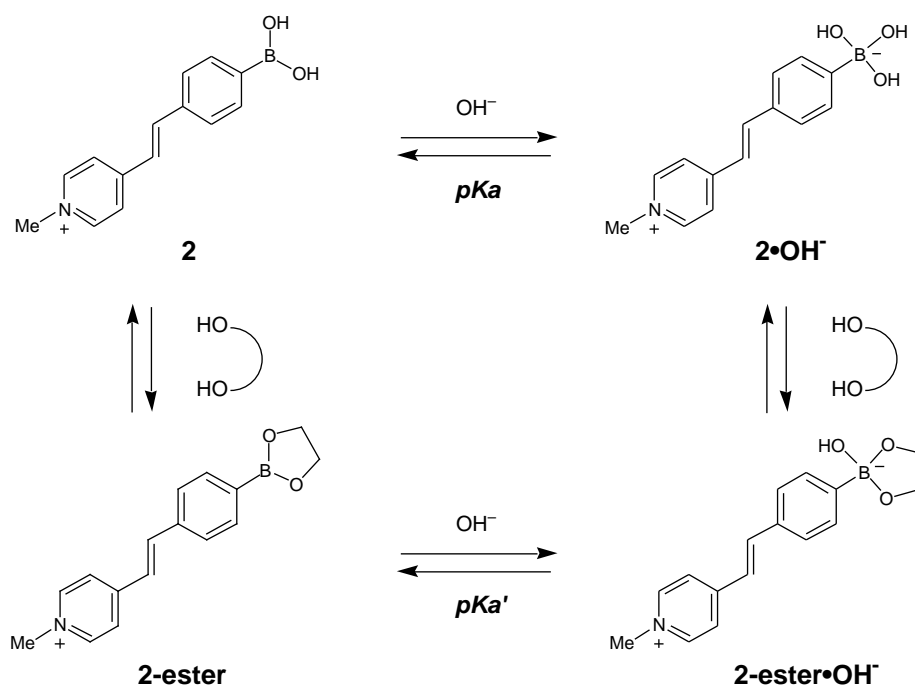
Scheme 1

The synthetic route of the dyes (**2** and **3**) is shown in Scheme 2.   The stilbazolium dye (**2**) was obtained by the Knoevenagel condensation[12] of 1,4-dimethylpyridinium iodide with 4-formylbenzeneboronic acid[13] in the presence of piperidine as a catalyst.   After counter ion exchange, recrystallization from MeCN-

H_2O gave $\textbf{2} \cdot PF_6^-$ as bright yellow needles in good yield (55%).[14] The FAB-MS spectrum of a *m*-nitrobenzyl alcohol (as a matrix) solution of the product showed a peak at m/z 510 corresponding to the M^+ ion of bis(nitrobenzyl)ester of $\textbf{2}$ $[M+2NBA-2H_2O-PF_6^-]^+$. The ^{1}H-NMR spectrum in DMSO-d_6 showed three sets of doublets and two singlet peaks. The broad singlet peak at 8.17 ppm disappeared by an addition of D_2O. Thus, this peak was assigned as hydroxy protons of the boronic acid. Similarly, the reaction of 1,4-dimethylquinolinium iodide with 4-formylbenzeneboronic acid afforded the corresponding styrylquinolinium dye $\textbf{3}$ in 51% yield.

Scheme 2
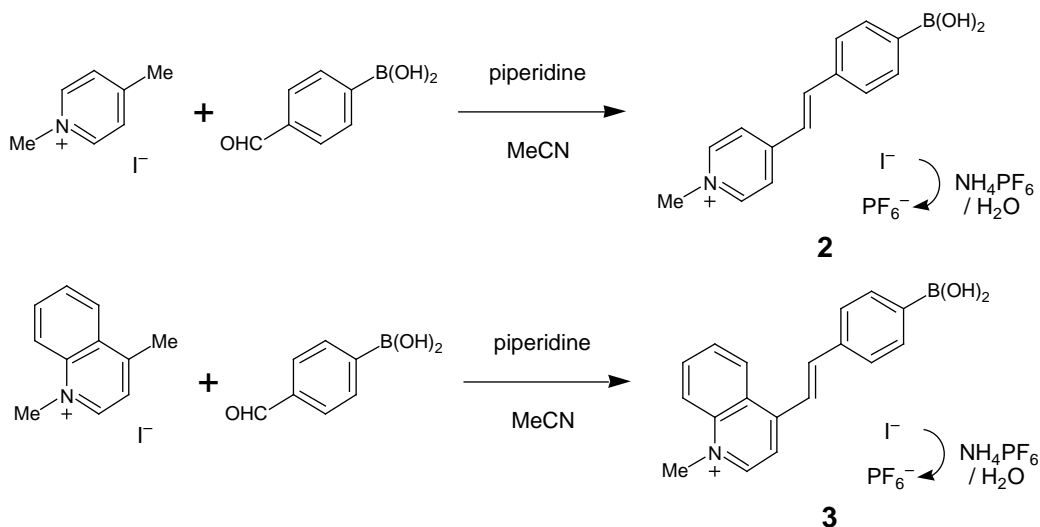
The acid-base equilibria of the PF_6^- salts of $\textbf{2}$ and $\textbf{3}$ have been investigated in pH titration experiments by UV-VIS spectroscopy.[15]   As shown in Figure 1, the increase of pH of the solution (buffer/acetonitrile 25:1 (v/v) was used) induced bathochromic shifts (λ_{max} shifts from 344 to 364 nm) of the intramolecular charge transfer band of $\textbf{2}$ with an isosbestic point at 355 nm.   These bathochromic shifts observed for $\textbf{2}$ can be ascribed to a formation of the zwitter ion $\textbf{2} \cdot OH^-$.[16]   The pKa value of $\textbf{2}$ was calculated as 8.08, which is smaller value than that of stilbeneboronic acid (pKa = 8.86 in 33% methanol)[3a] due to electron-withdrawing character of the cationic heterocyclic moieties.

Under the same conditions, the styrylquinolinium dye ($\textbf{3}$) showed similar spectral changes from the $B(OH)_2$ form (λ_{max} = 382 nm) to the zwitter ion form (λ_{max} = 404 nm) and the pKa value of $\textbf{3}$ is 8.06. Generally, annulation of benzene ring to stilbazolium betaine ($\textbf{1}$) is effective for shifting the absorption band to longer wavelength and increase acidity of phenolic OH of conjugate acids.[6c] Although the absorption maximum of $\textbf{3}$ is observed at ca. 40 nm longer wavelength compared to that of $\textbf{2}$, the λ_{max} between the $B(OH)_2$ and $B(OH)_3^-$ forms of $\textbf{2}$ and $\textbf{3}$ are almost same (20 and 22 nm, respectively). And also the replacement of pyridinium ring to quinolinium ring has a little effect on pKa value within experimental errors.
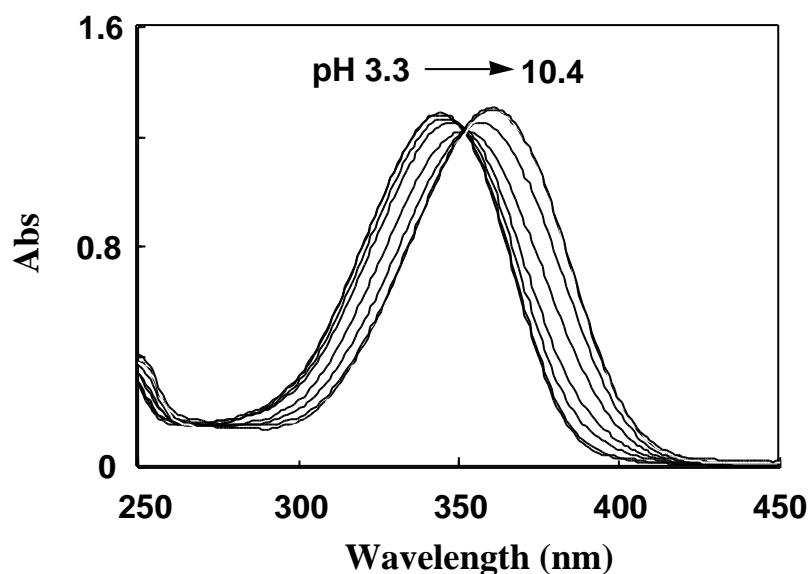
Figure 1. Absorption spectra of buffer/acetonitrile 25:1 (v/v) solutions of dye
2 (4.2 x 10^{-5} mol/L) at different pH.

The absorption-pH changes observed for the addition of monosaccharides to **2** are shown in Figure 2. The
pKa' value in the presence of an excess amount of D-fructose shifted to lower side over two **pKa** units
(see Table 1). From the comparison between the pH titration curves, pH = 7.0 was appropriate for
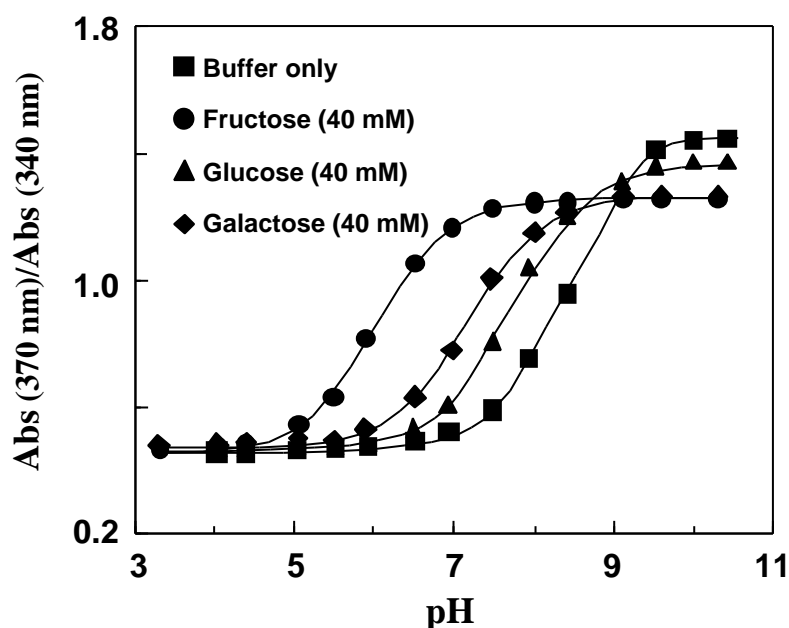following sugar titration study to give a maximum response.

Figure 2. Absorption-pH profile of dye (**2**) (4.2 x10^{-5} mol/L) with and without
sugar in buffer pH 3.3-10.4/acetonitrile 25:1 (v/v).

The stability constants (K)[17] of the boronic acid-saccharide complexes for the dyes (**2**) and (**3**) at pH of
7.0 were calculated from the UV-VIS absorption vs. sugar concentration profiles at least four different

wavelengths, respectively.   Examples of the titration curves of **2** monitored at 375 nm are shown in Figure 3 and the average of the calculated stability constants (**K**), together with **pKa'** values, are listed in Table 1.   The dyes (**2** and **3**) showed a similar selectivity for three monosaccharides (D-fructose > D-galactose > D-glucose). The order is consistent with a general observation for monoboronic acid derivatives.[2,3,18]
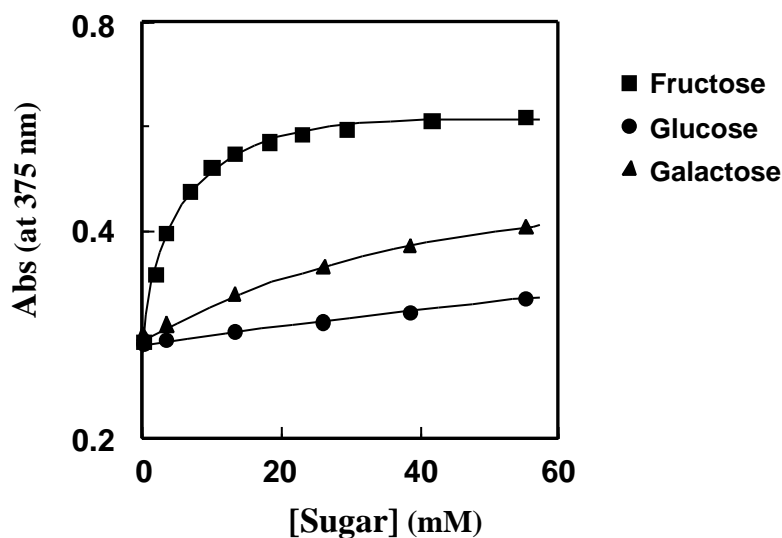

Figure 3. Plots of the absorbance (at 375 nm) of dye (**2**) (4.2×10^{-5} mol/L) against the concentration of D-fructose, D-glucose, and D-galactose in phosphate buffer pH 7.0/acetonitrile 25:1 (v/v).

Table 1. Stability constants (**K**) at pH = 7.0 and **pKa'** values of the dyes (**2** and **3**) with sugars.

Dye	(**pKa**)[b]	K^a / M^{-1} (**pKa'**)[b]		
		D-fructose	D-glucose	D-galactose
2	(8.08)	220 (5.96)	4 (7.48)	14 (7.06)
3	(8.06)	280 (6.04)	6 (7.64)	17 (6.99)

[a] Determined in phosphate buffer (pH = 7.0)/acetonitrile (25:1) at 298 K, errors are estimated to be <10%.

[b] Determined in buffer (pH = 3.3-10.4)/acetonitrile (25:1) with and without sugars (40 mM) at 298 K.

In summary, we have shown that the stilbazolium dyes in resonance with boronic acid group could lead an absorption change depending on pH. This spectral change is due to the change of the boron atom center between its neutral (sp^2-B(OH)$_2$) and anionic (sp^3-B$^-$(OH)$_3$) forms. Although the spectral change is not so large compare to stilbazolium betain (**1**), the new dyes (**2** and **3**) form esters with monosaccharides at neutral pH solvent (pH = 7.0) and show a D-fructose selectivity over D-galactose and D-glucose. In

addition, the ionic dyes (**2** and **3**) have a high solubility for aqueous solution. These properties are favorable for a fructose-sensing probe. By modification of N-alkyl substituent, the new stilbazolium dyes would be applicable to sugar-sensing devices.

Further investigation on this and related systems are ongoing in order to obtain better optical responses and specific selectivity for sugars and other biological important species.

ACKNOWLEDGEMENTS

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14. Some analytical data for **2** PF_6^-; ^{1}H NMR (270 MHz, DMSO-d_6):   4.26 (s, CH$_3$, 3H), 7.56 (d, J = 16.5 Hz, *trans*-CH=CH, 1H), 7.71 (d, J = 7.9 Hz, Ph, 2H), 7.89 (d, J = 7.9 Hz, Ph, 2H), 7.99 (d, J = 16.5 Hz, *trans*-CH=CH, 1H), 8.17 (s, B(OH)$_2$, 2H), 8.22 (d, J = 6.4 Hz, Py-3,5, 2H), 8.86 (d, J = 6.4 Hz, Py-2,6, 2H); ^{13}C NMR (67.8 MHz, DMSO-d_6):   46.9, 123.6, 123.7, 127.0, 134.7, 134.7, 136.4, 140.6, 145.1, 152.4; FAB-MS (*m*-NBA): m/*z* 510 [M+2NBA-2H$_2$O-PF$_6^-$]$^+$; *Anal*. Calcd for C$_{14}$H$_{15}$NO$_2$BF$_6$P: C, 43.67; H, 3.93; N, 3.64. Found: C, 43.63; H, 3.81; N, 3.55.

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    pKa = pH – log {(R – R$_A$) / (R$_B$ – R)} – log {Abs$_A$($_2$) / Abs$_B$($_2$)}

    where R$_A$ = $_A$ ($_1$) / $_A$ ($_2$), R$_B$ = $_B$ ($_1$) / $_B$ ($_2$), and R = Abs ($_1$) / Abs ($_2$)

16. It was reported (ref. 9) that 4'-*N,N'*-dimethylaminostilbene-4-boronic acid aggregates at neutral form in water-DMSO (30:1) solution with absorption spectral change. In the case of dyes (**1** and **2**), no spectral changes were observed in the concentrate range (0.2-40 x 10^{-5}M) at both acidic (pH = 5) and basic (pH = 10) solutions. Furthermore, similar pH-depended bathochromic shifts of dyes (**1** and **2**) were observed in different compositions of buffer-acetonitrile (1:1 and 250:1 v/v). We believe that the dyes (**1** and **2**) are present as monomer under the experimental conditions.

17. *K* [Sugar] = {(Abs – Abs$_0$) / (Abs$_{lim}$ – Abs) }

    where Abs$_0$ = $_{Dye}$ l [Dye], Abs$_{lim}$ = $_{Ester-OH}$ l [Ester-OH$^-$]

    At a given pH the (conditional) stability constant *K* is a constant.

18. J. P. Lorand and J. O. Edwards, *J. Org. Chem.,* 1956, **24**, 769. See also references 2 and 3. Stability constants (*K*) of phenylboronic acid (ref. 18) esters are 4370, 276, and 110 M^{-1} and stilbene-

4-boronic acid (ref. 3a) esters are 290, 170, and 90 M^{-1} (pH = 8.0 in 33% methanol) for D-fructose, D-galactose, and D-glucose, respectively.