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Efficient Glucoside Extraction Mediated by a Boronic Acid with an Intramolecular Quaternary Ammonium Ion

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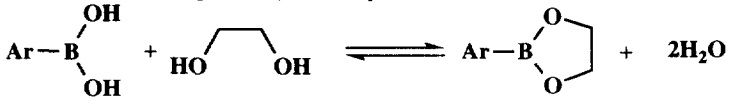
Abstract: To develop an efficient sugar extractant on the basis of the mechanistic view a phenylboronic acid bearing a trioctylammonium group at the ortho position (**4**) was synthesized. To avoid the complexity we employed a simple two-phase solvent-extraction system which corresponds to the first step in membrane transport, *i.e.*, extraction from a donating aqueous phase to an organic liquid membrane phase. The extraction rates and equilibria were estimated using α-*p*-nitrophenyl-D-glucopyranoside as a sugar and 1,2-dichloroethane as an organic phase and compared with those of a 2-methylphenylboronic acid (**5**) / trioctylmethylammonium chloride (TOMAC) 1 : 1 binary system. The extraction rates for **4** were faster by 2.5 - 29 fold than those for **5** + TOMAC. The distribution coefficients were also enhanced by 5 - 8 fold. The results indicate that the intramolecular quaternary ammonium group is very effective to neutralize the anionic charge developed in the boron atom upon sugar-binding and create extractable zwitterionic sugar complexes. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Cross-membrane transport of saccharides is one of the most important biological events necessitated for the maintenance of the life activity. This process is sometimes mediated by proteins such as a Na^+/D-glucose co-transporter protein.[1] In an artificial system, however, it is not so easy to capture very hydrophilic saccharides on the membrane surface and transport them across membranes. In 1986, Shinbo *et al.*[2] reported that phenylboronic acid, which forms cyclic esters with diols (and therefore, also with diol-containing saccharides),[3] can transport D-glucose across a liquid membrane. The transport efficiency observed therein was very low, however. Now, we can learn a lot of strategies for the improvement of the transport-efficiency from crown-ether-mediated membrane transport of metal cations.[4] For example, it is known that introduction of lipophilic groups which make crown ethers more hydrophobic or anionic caps which neutralize the cationic charge of bound metal cations improves the transport efficiency without exception.[4] Thus, hydrophobic boronic acid derivatives were synthesized and tested for solvent extraction and liquid membrane transport.[5-7]

Boronic acids form cyclic esters **1** with diols in aprotic solvents. Most monosaccharides thus form 1 : 2 saccharide / boronic acid complexes using two pairs of diol groups.[3,8] It is known, however, that although these sp^2-hybridized species are neutral and therefore favorable to solvent extraction, they are relatively unstable and easily hydrolyzed in water (and also at the water-solvent interface).[3] In contrast, **2** generated at high pH region is more stable in water but the anionic charge is not necessarily favorable to solvent extraction. To overcome this dilemma hydrophobic quaternary ammonium ions (*e.g.*, trioctylmethylammonium chloride (TOMAC)) were used as a co-extractant to neutralize the anionic charge in **2** by the ion-pair formation.[6,7,9,10]



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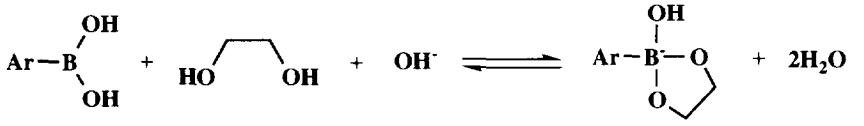

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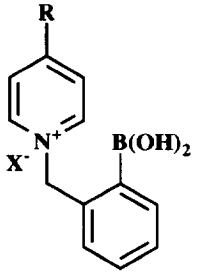
Here, it occurred to us that like an anionic cap neutalizing the cationic charge of crown-bound metal ions, introduction of an intramolecular quaternary ammonium group may be effective to extract **2** into apolar organic media. We have found that the boronic acid in **3** can efficiently bind saccharides in water owing to the intramolecular electrostatic interaction between the boronate anion developed by saccharide complexation and the pyridinium cation introduced into the ortho position.[11-13] We thus synthesized compound **4** which possesses a quaternary ammonium group to neutralize the boronate ester anion at the ortho position of a boronic acid group and three lipophilic octyl groups to retain **4** in the organic phase.[14] The binary **5** + TOMAC system was used as a reference. As a transport guest we chose *p*-nitrophenyl-α-D-glucopyranoside (**6**) in order to monitor the rates and equilibria by a spectroscopic method. It is known that in boronic-acid-mediated glycoside transport across a liquid membrane the rate-determining step is frequently switched from extraction of glycosides from an aqueous donating phase into an organic membrane phase to exit from the membrane phase to an aqueous receiving phase.[7] To avoid this complexity arising from the switching of the rate-determining step in a membrane transport system we here estimated the complexation ability in a single step, that is, the rates and equilibria in extraction from the aqueous phase to the organic phase.



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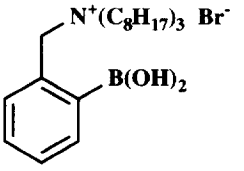

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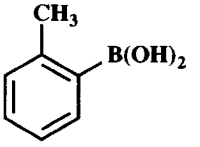

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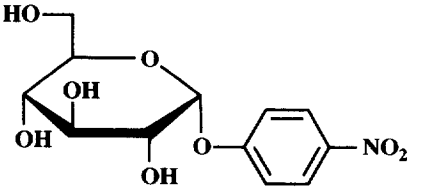

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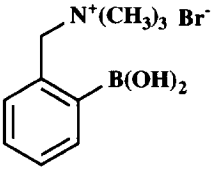

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RESULTS AND DISCUSSION

pK_a Determination

To estimate the pK_a of water-insoluble **4** we synthesized water-soluble **7** as a model compound.

The absorption spectra of **5** and **7** were measured at the range of pH 3.5 ~ 13.0 in the absence and the presence of D-fructose. D-Fructose was chosen because of its largest complexation constant among monosaccharides for monoboronic acids.[3] At [**5**] = [**7**] = 0.88 mM and [D-fructose] = 440 mM, for example, one can regard that the boronic acid group is entirely converted to its D-fructose complex.[3] In all cases the spectra gave tight isosbestic points, indicating that only one functional group is dissociated at this pH range. In Figure 1 the change in the absorbance at 270 nm (A_{270}) is plotted against medium pH. It is seen from Figure 1 that added D-fructose induces a significant shift of the pK_a to lower pH region. The pK_a values thus determined are summarized in Table 1.

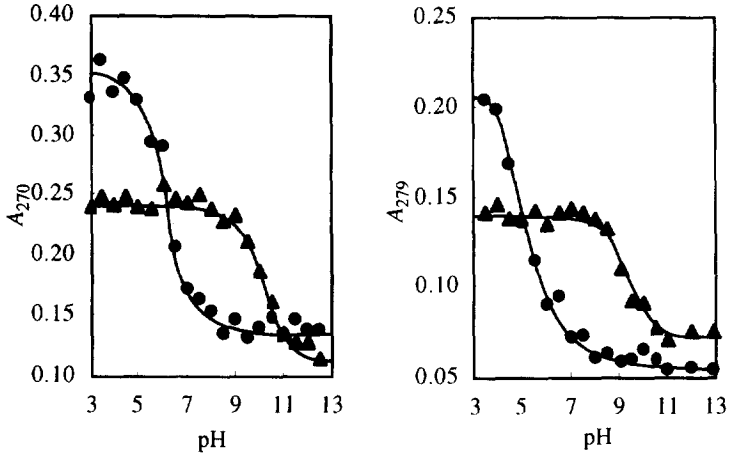

Figure 1. Plots of A_{270} *vs.* pH for **5** (left) and **7** (right): 25 °C, μ = 0.3 with KCl , [**5**] = [**7**] = 0.99 mM in the absence of D-fructose (▲) and 0.88 mM in the presence of D-fructose (●), [D-fructose] = 440 mM.

Table 1. pK_a of **5** and **7**

Boronic acid	D-Fructose / mM	pK_a
5	0	9.9
5	440	6.0
7	0	9.0
7	440	5.1

It is known that saccharide complexation lowers the pK_a of boronic acids.[3] In **5** the pK_a changes from 9.9 to 6.0, the difference induced by added D-fructose being 3.9 pK units. The pK_a of **7** is somewhat lower than that of **5**. This is either due to the electron-withdrawing nature of the Me_3N^+ group and/or due to the electrostatic stabilization of the generated B^- group by the intramolecular Me_3N^+ group. The pK_a shift induced by added D-fructose is also 3.9 pK units. Taking these pK_a values into consideration, we set up the measurement conditions for two-phase solvent extraction (*vide post*).

Leakage of Boronic Acids into the Aqueous Phase

Prior to two-phase solvent-extraction we carefully checked whether or not **4** and **5** leak into an aqueous phase from an organic (1,2-dichloroethane) phase. The organic phase (10 ml) containing **4** (1.00 mM) or **5** + TOMAC (1.00 mM each) was mixed with the aqueous phase (10 ml, pH 7.5 with

100 mM phosphate buffer or 11.0 with 100 mM carbonate buffer). The mixture was stirred at 25 °C. The time course for the absorbance increase in the aqueous phase showed that the distribution equilibria have been attained after 3 h. The leakage was estimated from the absorbance increase in the aqueous phase. At both pH 7.5 and 11.0 **4** did not leak into the aqueous phase at all, indicating that three lipophilic octyl groups act efficiently to retain **4** in the organic phase. In contrast, **5** did leak into the aqueous phase even in the presence of TOMAC, the fraction being 21% at pH 7.5 and 33% at pH 11.0. The finding becomes serious warning for the use of **5** (or phenylboronic acid) in solvent extraction and membrane transport. Furthermore, the results imply that the extraction mechanism is very different between **4** and **5**: in **4**, extraction of **6**, which is virtually insoluble in the organic phase, occurs at the water-1,2-dichloethane interface whereas **5** is partially partitioned into the aqueous phase, forms an anionic complex with **6**, and the resultant complex is extracted by TOMAC into the organic phase. Of course, **5** may also extract **6** at the interface in parallel with the above-mentioned mechanism, but it is difficult to estimate the relative contribution of these two processes.

Two-Phase Solvent-Extraction

The rate and the extractability (*Ex%*) with **4** and **5** + TOMAC were determined using an organic (1,2-dichloroethane, 10 ml) solution and a buffered aqueous solution (10 ml). The organic phase was stirred at a constant speed (150 ± 5 rpm) at 25°C. Under these conditions the two-phases were cleanly separated. The concentration of **6** transferred into the organic phase was determined from the decrease in the absorbance (at 307 nm) of the aqueous phase. From the time course of the extraction process (Figure 2) we could determine both the rate and the *Ex%* simultaneously.

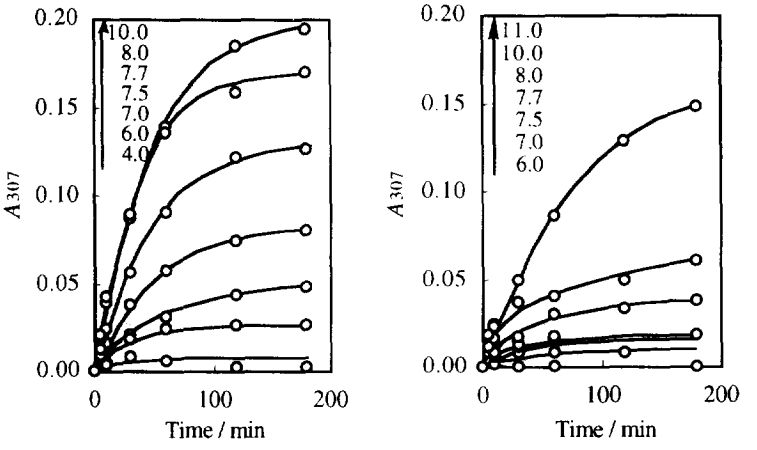

Figure 2. Plots of the extracted fraction of **6** (computed from the decrease in A_{307}) against the extraction time: organic phase (10 ml), [**4**] = 1.00 mM (left), [**5**] = [TOMAC] = 1.00 mM (right) ; aqueous phase (10 ml), [**6**] = 1.00 mM. The numbers in the figures show pH of the aqueous phase.

The time course of the A_{307} increase obeyed the first-order kinetics. We calculated the apparent first-order rate constants (k). Figure 3 shows plots of log k *vs.* pH. The k for **4** increases linearly up to pH 8 and is saturated above this pH. Figure 4 shows plots of $Ex\%$ (at the equilibrium) *vs.* pH. It is seen from Figure 4 that extraction with **4** begins at around pH 4 and increases efficiently at basic pH region. From these curvatures one can judge that the pK_a of **4** is at around 8. Although the pK_a 8 is somewhat higher than that of **7** in water in the presence of D-fructose, such a shift can take place in the event at the water-1,2-dichloroethane interface.[15] On the other hand, the significant extraction with **4** could not be detected below pH 4. The result establishes that the neutral saccharide complex with a sp^2-hybridized boron atom, the contribution of which is proposed in certain membrane transport systems,[6] is virtually negligible as an extraction species in the present extraction system. These results consistently support the view that **4** extracts **6** forming the OH^- adduct of the boronate ester and neutralization by the intramolecular quaternary ammonium cation facilitates the extraction process.

In contrast, Figure 3 shows that the extraction rate with **5** + TOMAC is slower by about one order of magnitude than that with **4** and the clear break point for the pK_a is not observed. The similar tendency is also observed for $Ex\%$ (Figure 4): the significant extraction takes place only above pH 8 and the sigmoidal pH dependence does not appear. The differences clearly indicate the inefficiency of the binary extraction system and the mechanistic complexity arising from the leakage of **5**. These results clearly support the superiority of a boronic-acid extractant with an intramolecular quaternary ammonium group over a simple boronic acid extractant coupled with an intermolecular one.

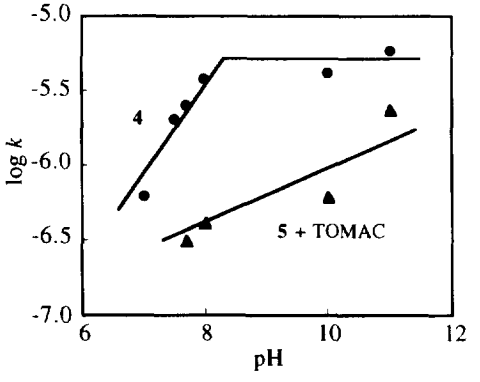

Figure 3. Plots of log k *vs.* pH. The plots were made from the analysis of the data in Figure 2.

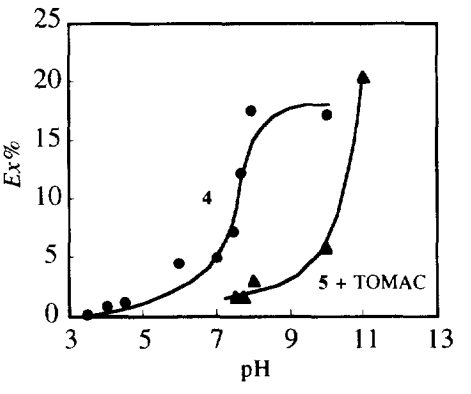

Figure 4. Plots of $Ex\%$ *vs.* pH. The plots were made from the analysis of the data in Figure 2.

The foregoing results allow us to propose an extraction mechanism depicted in Figure 5. As the free boronic acid scarcely picks up OH^- from the aqueous phase,[15] the reaction path including the reaction between **6** and boronate anion ($-B^-(OH)_3$) of **4** at the interface is very unlikely. As a conceivable path, **6** would form a neutral complex with a sp^2-hybridized boronate ester at the water-1,2-dichloroethane interface. Since this species is an unextractable cationic species and unstable in aqueous solution, it is reversibly hydrolyzed back to **4** and **6**. This view is compatible with the finding that **6** cannot be extracted at acidic pH region. However, the pK_a of the boronic acid-saccharide complex is lowered and the formation of the OH^- adduct is facilitated. Thus, the zwitterionic, apparently-neutral species is easily formed by the reaction with OH^- at the interface and eventually transferred into the organic phase, like an action of TOMAC as a phase transfer catalyst.

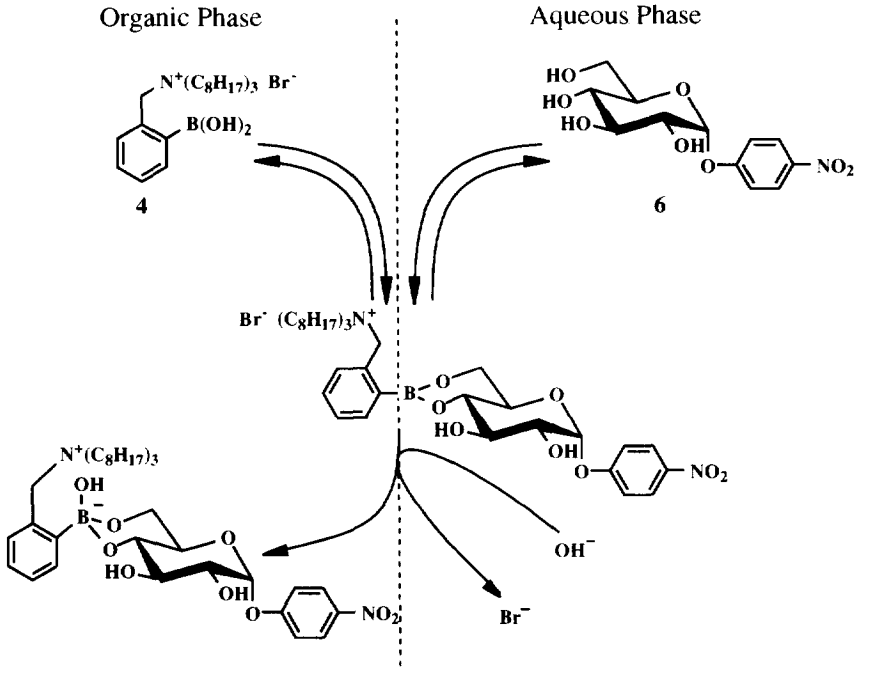

Figure 5. Extraction mechanism proposed for **4**.

CONCLUSION

To extract hydrophilic saccharides into an organic phase two different methods are now known: one method utilizes hydrogen-bonding interactions and the other utilizes complexation with boronic acids.[2,5-10,14] The former method is very simple and the well-designed lipophilic hydrogen-bond acceptors frequently show selectivity toward saccharides.[16-20] Because of the weakness of hydrogen-bonding interactions at the interface, however, the concentration of sugars in the aqueous phase must be enhanced up to 0.5 ~ 1.0 M in order to obtain the measurable concentration of saccharides extracted into the organic phase. This implies that the D (D: distribution coefficient = saccharide in the organic phase / saccharide in the aqueous phase) in this method is 0.1 ~ 1.0%, very low values from the practical viewpoint. In contrast, the latter method using lipophilic boronic acids has the much higher D values (5 ~ 21%) and therefore is more practical: they can extract saccharides even from the low concentration aqueous solution. However, the mechanism is more complicated. In particular, one has to take the neutralization of the anionic charge developed on boron into consideration. Present two-phase solvent-extraction established that the ortho $R_3N^+CH_2$-group in phenylboronic acid acts very efficiently from both kinetics and equilibrium viewpoints. Clearly, the role of this group is to neutralize the anionic charge and to facilitate the extraction of the zwitterionic species into the organic phase.

The further studies have currently been continued in this laboratory: that is, the possible extensions are (i) introduction of two structural units of **4** into one molecule, which should show high selectivity for certain saccharides as diboronic acids generally do,[3,21] (ii) kinetics and equilibrium studies on the saccharide release from an organic phase to a receiving aqueous phase, and (iii) construction of an efficient membrane transport system composed of a donating aqueous phase, an organic phase, and a receiving aqueous phase.

EXPERIMENTAL

Materials

The synthesis of 2-(2-bromomethylphenyl)-1,3-dioxaborinane (**8**) was reported previously.[22]

2-[(*N*, *N*, *N*-Trioctylmethylammonium)methyl]phenylboronic acid bromide (4). A chloroform solution (70 ml) containing **8** (1.00 g, 3.93 mmol) and trioctylamine (3.4 ml, 7.86 mmol) was refluxed under a nitrogen atmosphere. The progress of the reaction was monitored by a TLC method (silica gel, chloroform : methanol = 15 : 1 v/v). After two days the solution was concentrated to dryness. The oily residue was stirred with a two-phase mixture of chloroform (30 ml) and 24% HBr solution (30 ml) for one day at room temperature. The organic layer was separated, dried over $MgSO_4$, and subjected to a column chromatography separation (silica gel, chloroform: methanol = 20 : 1 v/v). We thus obtained **4** as slightly yellow oil : yield 82 %; IR (neat)

ν_{OH} 3200-3600 cm^{-1}, ν_{B-O} 1350 cm^{-1}; ^1H NMR (250MHz, $CDCl_3$, 25°C) δ 0.87 (CH_3, t(J = 5.8Hz), 9H), 1.25 ((CH_2)$_5$, br s, 30H), 1.64 ($NCCH_2$, br s, 6H), 3.30 (NCH_2, br s, 6H), 5.06 ($ArCH_2$, s, 2H), 7.45, 8.36 (ArH, m, d respectivity, 3H, 1H respectivity), 7.66 (OH, s, 2H). Anal.Calcd for $C_{31}H_{59}BBrNO_2$: C,65.48; H,10.48; N,2.46%. Found: C,65.79; H,10.36; N,2.44%.

2-[(*N*, *N*, *N*-Trimethylammonium)methyl]phenylboronic acid bromide (7). This compound was synthesized by bubbling trimethylamine gas into a benzene solution (50 ml) containing **8** (1.10 g, 4.31 mmol) for 1 h. The white precipitate was collected by filtration and confirmed to be pure by TLC (silica gel, chloroform : methanol = 15 : 1 v/v, R_f = 0.0): mp=199.6-203.2°C, yield 87%; IR (KBr) ν_{B-O} 1320cm^{-1}; ^1H NMR (250MHz, $CDCl_3$, 25°C) δ 3.37 (CH_3, s, 9H), 5.14 ($ArCH_2$, s, 2H), 7.47, 7.67, 7.96 (ArH, m, d (J = 6.1Hz), d (J = 6.2Hz) respectivity, 2H, 1H, 1H respectivity). Anal. Calcd for $C_{13}H_{21}BBrNO_2$•1/4H_2O: C,49.01; H,6.82; N,4.40%. Found: C,48.86; H,6.54; N,4.40%.

Phototitration of 7

The phototitration was carried out at 25 °C in 100 mM buffered solutions. The solution pH was adjusted with acetate for pH 3.5 - 5.5, phosphate for pH 6.0 - 8.5, carbonate for pH 9.0 - 11.0, and KOH for 11.5 - 13.0. The absorption maxima used were 270 nm for **5** and 279 nm for **7**.

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