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# THE INTERACTION OF ARENEBORONIC ACIDS WITH MONOSACCHARIDES

S. A. Barker, A. K. Chopra, B. W. Hatt, and P. J. Somers
*Department of Chemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (Great Britain)*

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## ABSTRACT

The interaction of benzeneboronic acid, 4-methoxybenzeneboronic acid, and 3-nitrobenzeneboronic acid with D-glucose, D-mannose, and D-fructose at various pH values has been investigated by means of optical rotation methods. The effects of (*a*) various molar ratios of sugar and acid and (*b*) overall concentration on the extent of complex formation are reported.

## INTRODUCTION

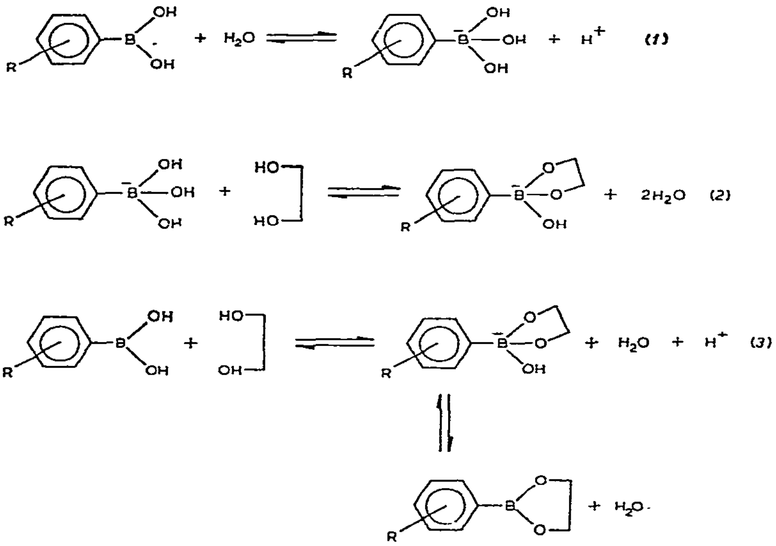
Monosaccharides may be equilibrated with certain of their isomeric forms by general acid and base catalysis in the well-known Lobry de Bruyn–Alberda van Ekenstein transformation[1]. The concentration of the isomeric sugars at equilibrium, or, more correctly, pseudo-equilibrium is governed largely by their stability under the conditions obtaining. The relative concentrations, however, may be displaced by an added reagent if certain of the isomers are more strongly complexed than the others. Thus, by suitable choice of complexing reagent, the equilibrium may be displaced towards one isomer.

It has long been known that boric acid and its anion can form complexes with carbohydrates[2,3], but frequently more than one type of complex may be formed with a particular sugar. Carbohydrates can also form complexes with benzeneboronic acid[4–8]. The complexing of areneboronic acids with polyhydroxy compounds is considerably simpler than for boric acid–borate because complexes with a 2:1 ratio of sugar and acid cannot form. Another advantage is the possibility of affecting the stability of complexes at given pH values by altering substituents in the aromatic ring with regard to their inductive (or steric) effect on the dihydroxyborono group.

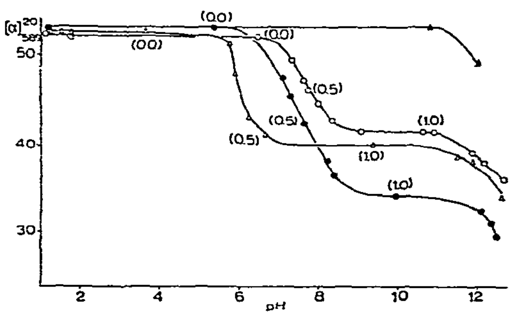
Before using areneboronic acids to displace the equilibria between sugar isomers, the interaction of the acids with certain monosaccharides was investigated.

## DISCUSSION

Areneboronic acids are Lewis acids[5] (equation *1*), and their acidity is affected by the electron density on the boron atom. The addition of certain polyhydroxy compounds to a solution of boric acid increases its acidity[2], and Boeseken suggested[9] that a complex was formed between the polyhydroxy compound and the borate anion. Lorand and Edwards[5] invoked similar complexes for benzeneboronic acid (equation *2*). However, since it is the oxygen atoms of the polyhydroxy compound that are involved in the complex, it is probable that the complex is formed by the sequential nucleophilic attack of these oxygen atoms on the boron atom. This attack is less likely to occur if the boron atom carries a negative charge. A more likely series of steps is summarized in equation *3*, where the free acid reacts with the diol to give either the anionic or neutral complex. At equilibrium, however, the two schemes will be indistinguishable and, in both schemes, the formation of the anionic complex is favoured in alkaline solution. The anionic complex should be stabilized by electron-withdrawing groups in the aromatic ring.



Isbell *et al.*[10] have shown that the interaction of borate with sugars and sugar alcohols may be investigated by observing changes in optical rotation. A similar approach has been employed in the present work except that, as the degree of complex formation may be expected to be sensitive to variation in pH, the acidity of the solutions was carefully controlled. Three areneboronic acids were investigated; namely benzeneboronic acid[11] (p$K_a$ 8.86), 3-nitrobenzeneboronic acid[12] (p$K_a$ 7.30), and 4-methoxybenzeneboronic acid[12] (p$K_a$ 9.31). The areneboronic acids were prepared as their anhydrides since the free acids are difficult to prepare pure due to their facile dehydration. The acid is regenerated from the anhydride by the action of water. The interaction of these acids with D-glucose, D-fructose, and D-mannose in aqueous solution was examined as these three sugars may be brought into equilibrium with one another in the presence of alkali. The effect of pH on the optical rotation of the equimolar solutions of an acid and a sugar is shown in Figs. 1–3. The interpretation of the results assumes that a complex of the sugar will have a specific optical rotation different from that of the free sugar. From the graphs, it is seen that, at low pH values, the rotation corresponds to that of the free sugar and thus, as predicted by theory, complexes are not formed at acidic pH values. For D-glucose and D-fructose, the rotation decreases with increase in pH until it reaches a constant value which, presumably, corresponds to that of an anionic complex formed at high pH. For D-glucose, the rotation decreased further when the pH was increased above 11, but interpretation



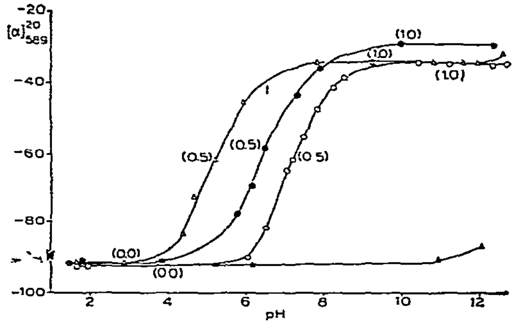
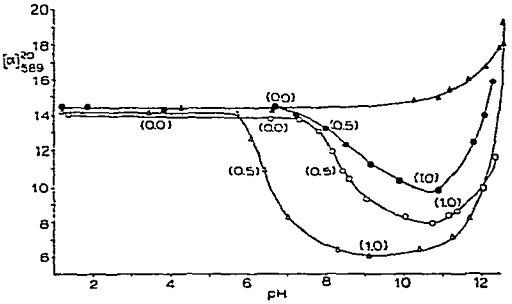


Fig. 1. Graph of optical rotation against pH for solutions equimolar in D-glucose and areneboronic acid. ●—●, Benzeneboronic acid+D-glucose; ○—○, 4-methoxybenzeneboronic acid+D-glucose; △—△, 3-nitrobenzeneboronic acid+D-glucose; ▲—▲, D-glucose.

Fig. 2. Graph of optical rotation against pH for solutions equimolar in D-fructose and areneboronic acid. ●—●, Benzeneboronic acid+D-fructose; ○—○, 4-methoxybenzeneboronic acid +D-fructose; △—△, 3-nitrobenzeneboronic acid+D-fructose; ▲—▲, D-fructose.



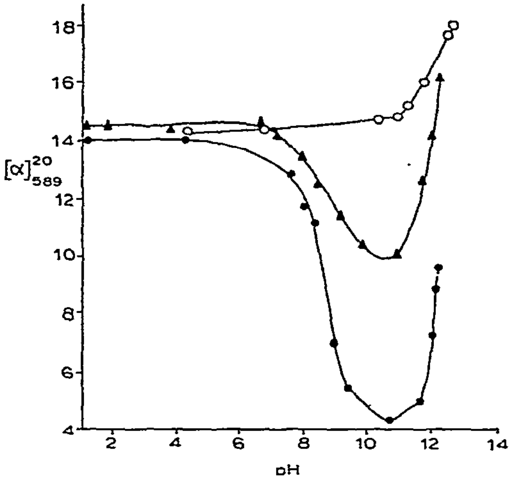


Fig. 3. Graph of optical rotation against pH for solutions equimolar in D-mannose and benzeneboronic acid. ●—●, Benzeneboronic acid+D-mannose; ○—○, 4-methoxybenzeneboronic acid+D-mannose; △—△, 3-nitrobenzeneboronic acid+D-mannose; ▲—▲, D-mannose.

Fig. 4. Graph of optical rotation against pH for solutions of D-mannose, and D-mannose with various molar equivalents of benzeneboronic acid. ○—○, D-Mannose; ▲—▲, D-mannose+benzeneboronic acid (1:1); ●—●, D-mannose+benzeneboronic acid (1:2).

in this region is complicated by the possibility of isomerisation to D-fructose and D-mannose, and by the fact that the sugar hydroxyl groups will ionize significantly and probably alter the optical rotation. Fig. 1 shows that the specific rotation of D-glucose in aqueous solution decreases when the pH is increased above 11. For these reasons, only the region below pH 11 has been interpreted. From the rotation values of uncomplexed D-glucose and D-fructose and those of their anionic complexes with the areneboronic acids, the percentage complex formation at various pH values may be calculated, and are shown in Fig. 7.

For D-mannose (Fig. 3), up to pH ~11, the curves are similar to those in Figs. 1 and 2, but above pH 11 the rotation rises rapidly. Thus, it is not possible to assign specific rotations to the D-mannose–areneboronic acid complexes which are necessary for the calculation of percentage complex formation at intermediate pH values. The specific rotation of D-mannose in aqueous solution rises sharply above pH 11 (Fig. 4) and this effect, if due to ionization of hydroxyl groups, could also cause the change in rotation of the complexes with areneboronic acid. Fig. 4 also shows the change in optical rotation with pH of a solution of D-mannose containing two molar equivalents of benzeneboronic acid, and the graph is similar to that obtained using one molar equivalent of the acid. However, it is significant that the

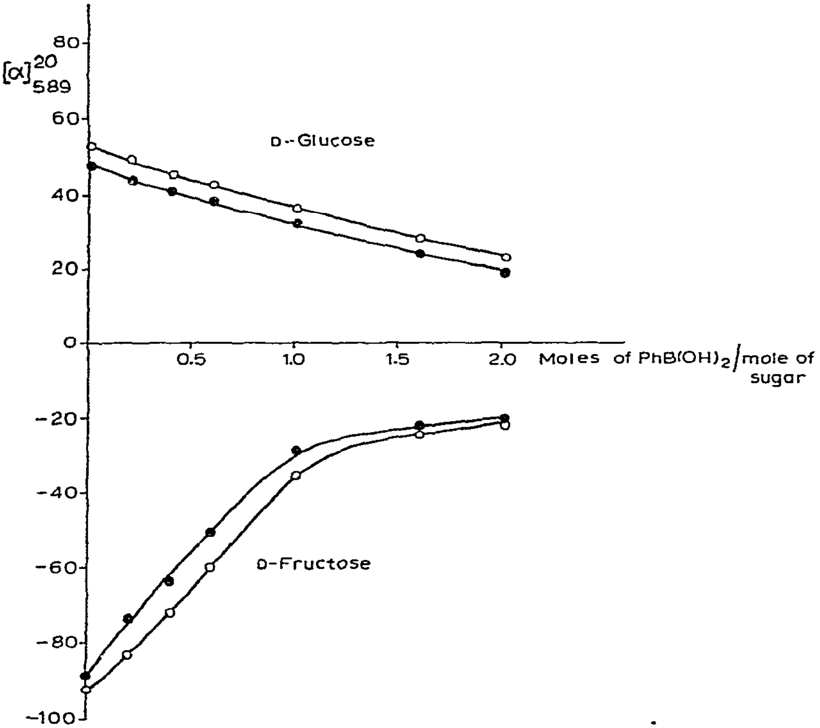


Fig. 5. Graph of optical rotation against molar ratio of benzeneboronic acid to sugar for solutions of D-glucose and D-fructose containing benzeneboronic acid. ○—○, Half-neutralised solution; ●—●, adjusted to pH 12.0.

difference in rotation of the free sugar and the minimal rotation obtained (at pH ~ 10.5) in the 1:2 D-mannose–benzeneboronic acid case is twice that obtained with the 1:1 ratio. This suggests that D-mannose may form a 1:2 sugar–benzeneboronic acid complex, and that this and the 1:1 complex both require one equivalent of hydroxide ion for their formation.

Fig. 5 shows the change in optical rotation of D-glucose and D-fructose when the molar ratio of sugar to benzeneboronic acid is increased from 1:0 to 1:2 by using (*a*) a half-neutralized solution of benzeneboronic acid and (*b*) solutions which had been adjusted to pH 12. The discontinuity in both curves for D-fructose at a molar ratio of 1:1 is consistent with the formation of a 1:1 complex. However, for D-glucose, the graphs are both approximately linear up to a bimolar ratio with benzeneboronic acid, which suggests that, as with D-mannose, D-glucose can form a 1:2 complex with benzeneboronic acid. Several possible complexes between areneboronic acid and D-glucose, D-fructose, and D-mannose may be postulated which have the general form of the anionic complex in equation *3*, but other types of complex might also exist. For example, Angyal and McHugh[13] postulated a "tridentate" complex between *scyllo*-quercitol and borate, and, theoretically, this type of complex could also be formed by benzeneboronic acid. There is also the interesting possibility that benzeneboronic acid could form a complex with α-D-fructofuranose at HO-1, HO-6, and the ring-oxygen atom. Complexes of a similar type have been shown to occur with the benzeneboronates of alditols[7]. Such a complex would be similar to that formed between areneboronic acids and diethanolamine, where the nitrogen atom acts as a third ligand, giving an ester which is very stable towards hydrolysis[14].

If uncharged complexes of benzeneboronic acid with sugars exist in aqueous solution, consideration of the equilibrium equations derived from equation *3* shows that their formation would be favoured by high, overall concentration (*i.e.*, low concentration of water) and by high concentrations of hydrogen ion. A series of experiments was performed at pH 8, 10, and 12 to examine the effect on specific optical rotation of overall concentration of solutions of D-glucose and D-fructose in the presence of an equimolar amount of benzeneboronic acid. Fig. 6 shows that, for concentrations greater than 2 g/100 ml for the solution of a single sugar, the specific rotation of D-glucose and D-fructose at each pH value is essentially constant providing no evidence for the existence of neutral complexes. At low concentration and at low pH values, the rotation does change significantly and the only species that is favoured, in theory, by these factors is the free sugar.

The effect of concentration on the specific optical rotation of solutions containing D-glucose, D-fructose, and benzeneboronic acid, where all solutes are present in equimolar amounts, was examined (Fig. 6). In these solutions, D-glucose and D-fructose must compete for the benzeneboronic acid. This experiment was performed at pH 8, 10, and 12, and the results are shown in Fig. 6, the ordinate value being normalized by dividing the rotation observed by the concentration (*c*, g/100 ml) of each individual sugar. It is clear that the composition of the solutions at each pH value changes with overall concentration. As it is reasonable to assume that, at pH 10

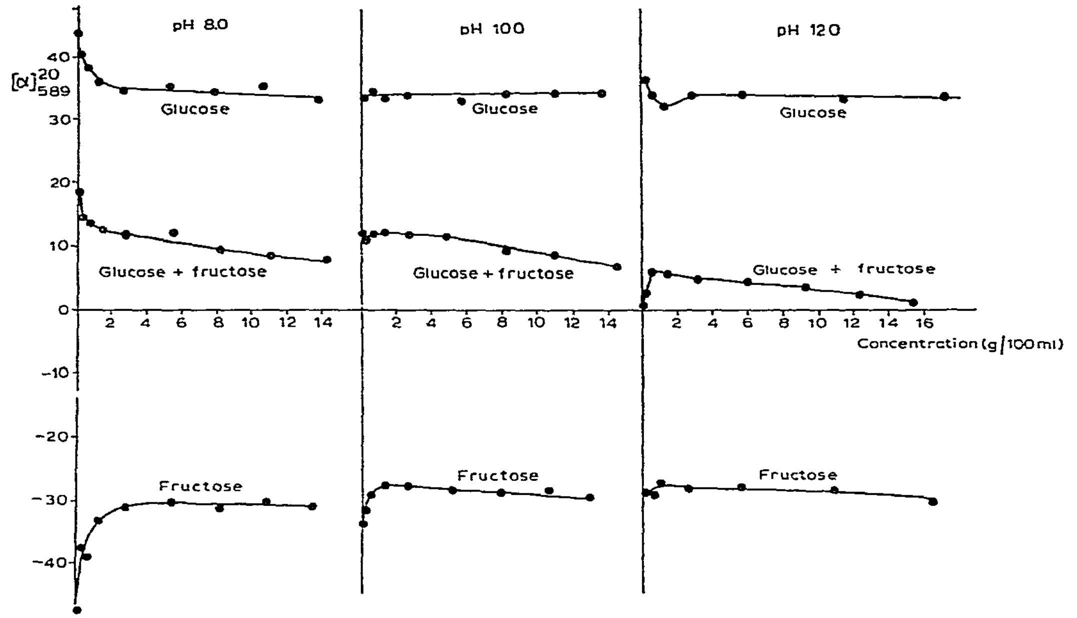


Fig. 6. Graphs of optical rotation against concentration for solutions of D-fructose, D-glucose, or D-fructose–D-glucose mixtures, containing equimolar concentrations of benzeneboronic acid, at various pH values.

and 12, essentially all the benzeneboronic acid is involved in complexes (see Fig. 7), an equation relating the normalized observed rotation ($[\alpha]_{obs}$), the fraction of D-fructose complexed ($x$), and the specific rotation of D-glucose, D-fructose, and their complexes with benzeneboronic acid ($[\alpha]_G$, $[\alpha]_F$, $[\alpha]_{GB}$, and $[\alpha]_{FB}$, respectively) may be formulated as follows:

$$[\alpha]_{obs} = x.[\alpha]_G + (1-x).[\alpha]_{GB} + (1-x).[\alpha]_F + x.[\alpha]_{FB}.$$

This equation was solved for chosen conditions: pH 12 $c=2$, $x=0.76$; $c=15$, $x=0.73$; and pH 10 $c=2$, $x=0.84$; $c=13$, $x=0.81$. These results show that, as the overall concentration increases at both pH values, the preferential complexing of D-fructose with respect to D-glucose diminishes. Comparison of the two sets of results also shows that this preference is diminished on increasing the pH from 10 to 12.

Fig. 7 shows the percentage of complex formed with pH, calculated, in the manner described above, from the graphs in Figs. 1 and 2. Comparison of the pH values at 50% complex formation for D-glucose and D-fructose with the three areneboronic acids is in good agreement with the qualitative predictions based on the relative strengths of the acids. The pH values for 100% formation of complex of D-glucose and D-fructose with benzeneboronic acid and with 4-methoxybenzeneboronic acid are all close to pH 9, whereas the corresponding values for 3-nitrobenzeneboronic acid are close to pH 7. This treatment cannot be reliably applied to

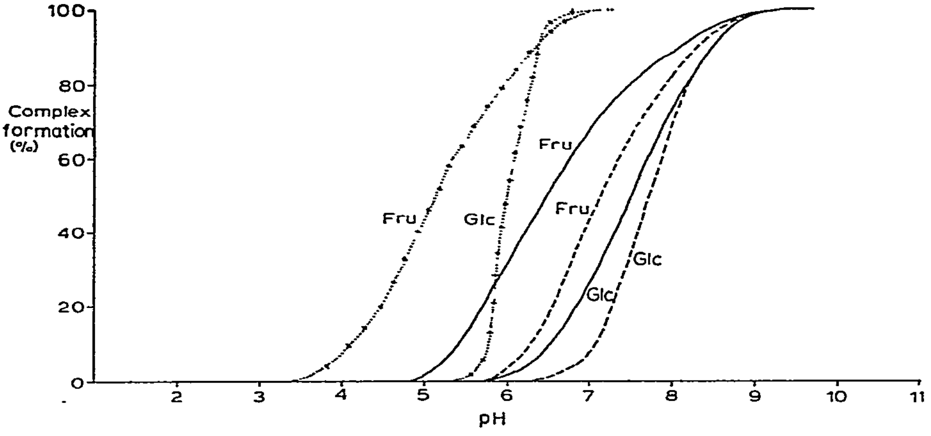


Fig. 7. Graph of percentage complex formation against pH for D-fructose and D-glucose and areneboronic acids. ———, Benzeneboronic acid; – – – – – –, 4-methoxybenzeneboronic acid; +···+, 3-nitrobenzeneboronic acid.

the results for D-mannose (Fig. 3) for reasons that have been discussed, but it is clear that benzeneboronic and 4-methoxybenzeneboronic acids start to complex with D-mannose at about pH 7, whereas 3-nitrobenzeneboronic acid begins to complex below pH 6; the results are again in agreement with the strengths of the acids.

## EXPERIMENTAL METHODS

*Materials and general methods.* — Benzeneboronic acid was prepared as its anhydride by the method of Washburn and Levens[15] and recrystallised twice from benzene. 4-Methoxybenzeneboronic acid was prepared as its anhydride by the same method. 3-Nitrobenzeneboronic acid was prepared by the method of Seaman and Johnson[16].

Optical rotations were measured in a 1-dm cell (20.0°, 589 nm) with a Perkin–Elmer 141 polarimeter.

*Effect of areneboronic acids on the optical rotation of sugars at different pH values.* — To samples (10 ml) of an aqueous solution of benzeneboronic acid (0.2M) and sodium hydroxide (0.1M) were added aliquots (2 ml) of aqueous solutions (M) of D-glucose, D-fructose, and D-mannose. To the equimolar solutions of benzeneboronic acid and sugar was added a known volume of 0.5M hydrochloric acid or 0.5M sodium hydroxide. The volume of each sample was made up to 20 ml with water and, after 2 h at room temperature, the pH and optical rotation (equilibrium value) of the sample were measured. Any precipitate that had formed was redissolved by shaking. Figs. 1–3 show the specific optical rotation (*c* 1.8) of the samples plotted against pH, points being annotated with the molar ratio of total hydroxide ion added to sugar (and to benzeneboronic acid) in the solution. The experiment was repeated with D-mannose and benzeneboronic acid in a 1:2 molar ratio and with D-mannose alone ($c$ 0.9). The results are shown in Fig. 4. The experiment was repeated with 4-methoxybenzeneboronic acid, using 0.2M sodium hydroxide. Some precipitation occurred in the solutions of lower pH, and the supernatant liquid was used to measure the optical rotation. The experiment was also repeated with 3-nitrobenzeneboronic acid, and Figs. 1–3 show both these sets of results.

*Effect of the ratio of benzeneboronic acid to sugar on the optical rotation at pH 12.* — Aliquots (0–5 ml) of a solution of benzeneboronic acid (0.4M) and potassium hydroxide (0.4M) were added to samples (1 ml) of a solution of D-glucose (M) and of D-fructose (M). The volume of each sample was made up to 10 ml with distilled water and the optical rotation ($c$ 1.8) measured. An aliquot (8 ml) of each sample was then adjusted to pH 12.0 with a known volume (*ca.* 1.6 ml) of 0.5M potassium hydroxide and its optical rotation re-measured. Fig. 5 shows the change in optical rotation with the molar ratio of benzeneboronic acid to sugar for both experiments.

*Effect of increasing concentration on equimolar mixtures of benzeneboronic acid and sugars at constant pH.* — Benzeneboronic anhydride (1.44 g) and sodium hydroxide were dissolved in distilled water (10 ml), followed by anhydrous D-glucose (2.25 g) and/or D-fructose (2.5 g). The pH of the solution was adjusted to 8, 10, or 12 with concentrated, aqueous sodium hydroxide. A special glass electrode for alkaline solutions (205 HA) and a sleeve reference electrode (305 W), both supplied by Pye-Unicam, were used. Weighed aliquots were then diluted to different extents, maintaining the pH at 20° by addition of aqueous sodium hydroxide. The optical rotation ($c$ 0.1–15) of each solution was measured, and the results are shown in Fig. 6.

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