

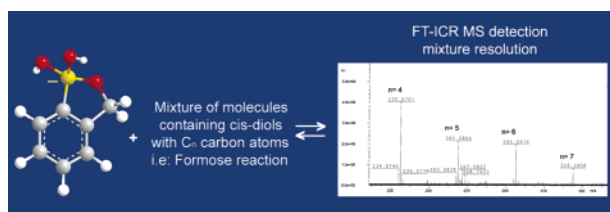
2-Hydroxymethylboronate as a Reagent To Detect Carbohydrates: Application to the Analysis of the Formose Reaction

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2-Hydroxymethylphenylboronate is described as a reagent that converts neutral 1,2-diols, as found in simple carbohydrates, into 1:1 anionic complexes that are easily detected by Fourier transform ion cyclotron resonance mass spectrometry. The value of this reagent was demonstrated through its application to analyze complex mixtures of carbohydrates formed in the formose process, often cited as a way that biologically significant carbohydrates might have been generated from formaldehyde under prebiotic conditions. Coupled with isotope studies, the reagent shows that the simplest autocatalytic cycle for the consumption of formaldehyde in this process cannot account for the bulk consumption of formaldehyde.

As part of our work seeking to understand how borate minerals might moderate the formose reaction to generate carbohydrates possibly useful in the prebiotic synthesis of RNA,¹ we needed to analyze complex mixtures of carbohydrates. The formose reaction generates these via the conversion of formaldehyde in a calcium hydroxide suspension to (presumably) glycolaldehyde via a reaction whose mechanism is unknown but which is very slow.^{2,3} Once formed, the enolizable glyco-

laldehyde reacts rapidly with more formaldehyde to generate triose products that themselves can act as both nucleophiles and electrophiles in subsequent aldol reactions. The consequence is a cascade of aldol reactions and catalytic cycles (Figure 1, expanded figure in the Supporting Information) that generates a brown tar that has never been fully characterized. As the figures suggest, there are many potential products.

The detection of carbohydrates in aqueous solution is, of course, a major challenge for biological chemistry and in other areas.^{4a–g} Several groups have suggested the use of boronic acids for this purpose, on the basis of the ability of boronate to form complexes with the 1,2-diol units. For example, borate itself complexes two 1,2-diol units to form a 2:1 complex that has four boron–oxygen bonds. This complex is anionic and therefore easily visible by electrospray ionization mass spectrometry run in negative ion mode; the parent carbohydrates are not.^{5a,b}

For the purpose of mass spectrometric analysis of carbohydrates in complex mixtures, the anionic complex formed by borate itself is problematic, as the two carbohydrates complexed to a single boron atom need not be the same, and the number of complexes scales with the square of the number of carbohydrates in the mixture. We reasoned that a phenylboronate might be a preferred complexing reagent, especially if it contained a 2-hydroxymethyl substituent, as the 2-hydroxymethyl substituent would itself donate the fourth bond to boron (Figure 2). This is reminiscent of the 2-dialkylaminophenylboronates discussed in 1982 by Wulff as reagents to capture carbohydrates.⁶ Further, after our work was completed, Dowlut and Hall proposed 2-hydroxymethylphenylboronate, available from ASDI Inc. (Newark, DE), for the analysis of simpler mixtures of carbohydrates using NMR. They measured the binding constants of this reagent to several biologically important carbohydrates.⁷

To illustrate the application of this reagent to analyze complex mixtures arising from the formose reaction, a solution containing formaldehyde, CaCl₂, and sodium hydroxide was incubated at 65 °C. Aliquots from this mixture were then treated with a solution of 2-hydroxymethylphenylboronic acid, and the anionic complexes were analyzed by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). Carbohydrates were assigned by identifying peaks having masses of 117 + (*n* × 30 – 2); this formula includes the mass of the reagent core with the mass of the carbohydrate, with *n* indicating the number of carbons in the carbohydrate, minus the two protons removed when the complex is formed. The isotopic signature character-

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(*m/e* of the complex = 325.109) carbohydrates dominate the mixture at times approaching the yellowing point. The observation of the C7 species is particularly important, as one C7 species (1,2,4,5-tetrahydroxy-2,4-dihydroxymethylpentan-3-one) was isolated as a major component from a formose mixture by Shigemasa et al.^{9a,b} and analyzed by ¹³C and ¹H NMR and periodate degradation. We repeated the synthesis and found it reproducible in every detail and consistent with our mass spectrometry results.^{9c,d} The dominance of C6 and C7 species late in the pre-yellowing formose process was observed with the 2-hydroxymethylphenylboronate reagent at all of the conditions examined. This suggests that the quantitation, although not precise, is not wrong by orders of magnitude. Further, after the yellowing was well under way, a mixture of C6, C7, C8, and C9 carbohydrates is seen in an approximate ratio of 1:2.5:1:0.3.

The reagent also allowed us to rule out the simplest autocatalytic cycle for the consumption of formaldehyde, which involves the conversion of glyceraldehyde to a tetulose, the isomerization of the tetulose to a tetrose, followed by its retroaldol cleavage to form two glycolaldehyde molecules (see Supporting Information). To do this, the reaction was repeated at 65 °C in D₂O and *in situ* generated Ca(OD)₂ as the base. Under these conditions, yellowing began at 39 min, indicating a solvent deuterium kinetic isotope effect (compare to the report of Huskey and Epstein).¹⁰ Nevertheless, after 32 and 34 min, the peak at 326, corresponding to the C7 sugar complex that incorporated a single deuterium, was only about 10% of the whole (after correction for ¹³C natural abundance). This shows that, before yellowing, few of the intermediate enols protonate, and very few retroaldol reactions expel formaldehyde.

This rules out the simplest autocatalytic cycle, where the tetulose is converted to the isomeric tetrose, which suffers a retroaldol reaction to give two glycolaldehydes. This would require the introduction of two deuteriums from solvent,

necessarily generating deuterated C6 and C7 species. These were not observed.

Experimental Section

Reagents and Materials. 2-Hydroxymethylphenylboronic acid was purchased from a commercial supplier; sodium deuterioxide was purchased as a solution (10 M, D₂O) from a commercial supplier. All other reagents were purchased from a commercial supplier in their highest purity.

Analysis of Carbohydrates Formed during the Formose Process. In a typical reaction, a solution containing formaldehyde (350 mM, generated from paraformaldehyde), CaCl₂ (50 mM), and sodium hydroxide (60 mM) was incubated at 65 °C. From this, aliquots (0.50 mL, every 2 min) were removed and added to a solution of 2-hydroxymethylphenylboronic acid (0.20 mL, 0.1 M, pH adjusted to 9–10 with NaOH), followed by acidification with Amberlite resin (CG-50 H⁺ form). After acidification, a portion of the mixture (0.40 mL) was removed and added to 4-methyl morpholine buffer (0.40 mL, 50 mM, pH 8.7). The samples were diluted 10-fold in Milli-Q water, mixed 1:1 with acetonitrile, and injected into the FT-ICR instrument.

Deuterium incorporation experiments were done as described above, except that deuterium oxide was used instead of water, and sodium deuterioxide was used instead of the hydroxide.

Instrumentation. All FT-ICR analysis were done in a Bruker APEX II FT-ICR MS. Samples were diluted 10-fold in Milli-Q water and mixed 1:1 with acetonitrile before injection into the instrument.

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Supporting Information Available: Mass spectrometry data, additional figures, and a detailed mechanistic scheme. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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