JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Synthesis of Carbohydrates in Mineral-Guided Prebiotic Cycles

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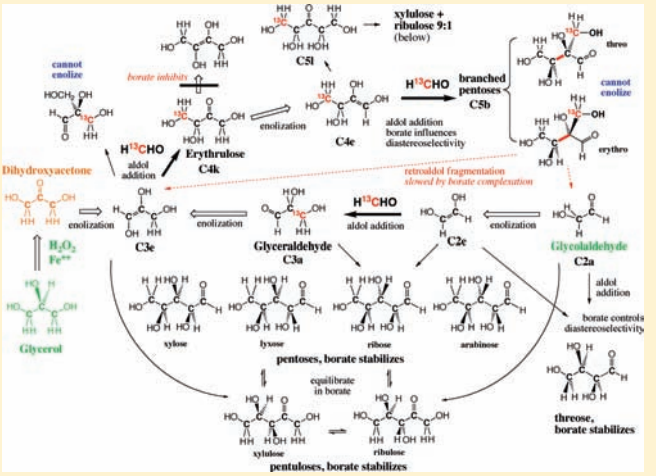
Supporting Information

ABSTRACT: One present obstacle to the "RNA-first" model for the origin of life is an inability to generate reasonable "hands off" scenarios for the formation of carbohydrates under conditions where they might have survived for reasonable times once formed. Such scenarios would be especially compelling if they deliver pent(ul)oses, five-carbon sugars found in terran genetics, and exclude other carbohydrates (e.g., aldotetroses) that may also be able to function in genetic systems. Here, we provide detailed chemical analyses of carbohydrate premetabolism, showing how borate, molybdate, and calcium minerals guide the formation of tetroses ($C_4H_8O_4$), heptoses ($C_7H_{14}O_7$), and pentoses ($C_5H_{10}O_5$), including the ribose found in RNA, in "hands off" experiments, starting with formaldehyde and glycolaldehyde. These results show that pent(ul)oses would almost certainly have formed as stable borate complexes on the surface of an early Earth beneath a humid CO_2 atmosphere suffering electrical discharge. While aldotetroses form extremely stable complexes with borate, they are not accessible by pathways plausible under the most likely early Earth scenarios. The stabilization by borate is not, however, absolute. Over longer times, material is expected to have passed from borate-bound pent(ul)oses to a branched heptulose, which is susceptible to Cannizzaro reduction to give dead end products. We show how this fate might be avoided using molybdate-catalyzed rearrangement of a branched pentose that is central to borate-moderated cycles that fix carbon from formaldehyde. Our emerging understanding of the nature of the early Earth, including the presence of hydrated rocks undergoing subduction to form felsic magmas in the early Hadean eon, may have made borate and molydate species available to prebiotic chemistry, despite the overall "reduced" state of the planet.



INTRODUCTION

Prebiotic chemistry embodies two themes. One, exemplified by Powner et al. and Müller et al.,[1,2] explores prebiotic reactivity by performing sequential steps of chemistry under human control in the laboratory. The other, exemplified by historic work of Löw,[3] Löb,[4] and Miller,[5] seeks to create laboratory conditions that resemble early Earth, hoping to constrain how "chemical evolution" might have proceeded in these environments without continuous human intervention.

Those following the first theme must manage challenges directed against the relevance of their work.[6] Those following the second must also do this, as well as address imperfect models for early Earth environments, the need to observe in days what might have taken millennia to occur naturally, and the propensity of organic species, left unattended, to form unproductive "tars".[7]

Recently, minerals have been found that cause otherwise stable organic precursors to react to yield biomolecules.[8] This *increases* the diversity of organic species that might have been present on the early Earth. In contrast with this have been studies with minerals that *prevent* biomolecules (once formed) from reacting further.[9,10] This, in principle, would *decrease* the diversity of organic species present on early Earth.

Which kinds of minerals prove to be more productive as we struggle to develop our understanding of life's origins depends on which of two competing views of "origins" is more compelling: (a) the view that holds that life is more likely to arise in more complex mixtures,[11] or (b) the view that increasing complexity is

Received: February 25, 2011
Published: May 09, 2011

dx.doi.org/10.1021/ja201769f | J. Am. Chem. Soc. 2011, 133, 9457–9468

likely to increase the number of *inhibitors* of life.[7] The second implies that biofriendly environments must manage reactivity to allow *some* reactions to occur while preventing others.

This paper addresses these two contrasting needs in prebiotic chemistry, for "reaction" and, in the same environment, for "no reaction". We focus on carbohydrates ($C_nH_{2n}O_n$), whose intrinsic reactivity makes this contrast especially severe. Indeed, the intrinsic instability of many carbohydrates under "prebiotic conditions" has encouraged some to *preclude* carbohydrates as parts of the very first genetic molecules.[12]

Here, we show how borate-, molybdate-, and calcium-containing minerals guide the formation of tetroses ($C_4H_8O_4$), heptoses ($C_7H_{14}O_7$), and pentoses ($C_5H_{10}O_5$) in "hands off" experiments. These products include the aldopentose ribose found in RNA, the carbohydrate hypothesized by the "RNA first" model for the origin of life to be important to start Darwinian chemistry.[13] Detailed chemical studies are presented to show how these minerals might have guided prebiotic pathways and cycles to give such carbohydrates as metastable mineral complexes in a "carbohydrate world" (or one of its variant appellations).[14]

RATIONALE, EXPERIMENTAL DESIGN, AND RESULTS

Model for the Prebiotic Environment. We begin by assuming that formaldehyde ($C_1H_2O_1$ or HCHO), the simplest carbohydrate, was generated within a humid CO_2-rich atmosphere by the action of light and electrical discharge.[15] This assumption appears to be robust regardless of whether the atmosphere was dominated by reduced or oxidized carbon (e.g., CH_4 or CO_2), having been observed in many laboratories over the past century.[4,5,15]

Our model further assumes that HCHO rained from the atmosphere onto the surface of early Earth in aqueous streams that eroded igneous rocks containing serpentine minerals (e.g., peridotite). The resulting streams would have been alkaline,[16] although their pH might be lowered through buffering by CO_2 derived from the atmosphere.

If they were also hot (60−80 °C) and contained calcium (Ca^{2+}), these erosion mixtures would have inevitably provided conditions compatible with the conversion of formaldehyde to higher carbohydrates via the "formose process".[3,17] Ca^{2+} appears essential for the first step in the formose process, which converts two molecules of HCHO into glycolaldehyde, which subsequently enolizes and reacts further with formaldehyde to yield, transiently, carbohydrates as intermediates on the way to more complex, tarry mixtures (Figure 1).

Our model also assumes that igneous rocks exposed to erosion contained tourmalines, a mineral that contains borate. Tourmalines appear in basalts as one of their lithologies.[18] Tourmalines are well known to erode easily to deliver borate to an erosion aquifer. Here, borate would have been concentrated by evaporation in the hydrosphere on any early Earth that contained unsubmerged land surfaces.

General Features of the Formose Process. The formose process has been much discussed,[17] and recent work continues to add to its characterization.[19] One feature of the formose process itself is the initial slow conversion of formaldehyde, creating a lag period whose length depends on the concentration of HCHO and temperature (Supporting Information, Figure S1). During this period, the mixture remains uncolored. This lag period is then followed by a rapid loss of HCHO, after which the material turns yellow, then brown. Earlier studies[19] showed that during this lag period, C4, C5, C6, and C7 carbohydrates accumulate. Yellowing occurs only after all HCHO is removed. Further incubation yields mixtures that progressively become more like "tar".

Some have speculated that the formose process requires an impurity in either the HCHO or the $Ca(OH)_2$ to be initiated. Several lines of evidence suggest that this is not the case. In particular, HCHO obtained in two ways (from commercial formalin and via the depolymerization of sublimed paraformaldehyde) and $Ca(OH)_2$ obtained in three ways (commercial, dissolution of calcium metal in water, and double decomposition of $CaCl_2$ and NaOH) gave indistinguishable lag kinetics (data not shown).

During the lag period run in D_2O, the initially formed C4, C5, C6, and C7 carbohydrates incorporate essentially no deuterium. This implies that once a carbonyl compound involved in their formation containing *n* carbon atoms enolizes in the presence of HCHO, it never "ketonizes" to restore the C═O species but is rather carried on to form an $n+1$ carbohydrate. Consistent with this view, only after all of the HCHO is consumed, products containing deuterium begin to be formed.[19] These facts, plus general principles, suggest an overall mechanistic hypothesis for the formose process, represented by the scheme in Figure 1.

Here, a very small amount of glycolaldehyde (**C2a**, $C_2H_4O_2$), formed by the very rare coupling of two HCHO molecules, begins the process of "fixing" many more HCHO molecules. These cycles repeat just three types of reactions characteristic of compounds having a C═O (carbonyl) group: (i) removal of a proton (H^+) from a carbon next to the C═O ("enolization") to give an *enediolate*, (ii) attack of the resulting enediolate nucleophile on a C═O electrophile to form a new carbon−carbon bond ("aldol addition"), and (iii) retroaldol fragmentation of higher species to generate lower carbohydrates.

These data also support four rules that unify all of the data, including new data reported here: (a) Reverse enolization restoring H^+ to an enediolate does not compete with aldol addition of HCHO if HCHO is present. (b) Retroaldol reactions extruding HCHO proceed at negligible rates. (C) Carbohydrates that can form cyclic hemiacetals are less reactive than those that do not. (d) Carbohydrates that can neither enolize nor cyclize are susceptible to reduction via a Cannizzaro reaction with HCHO, with formate being the second product.

The cause of the late rapid decline in HCHO is attributed to the formation of dihydroxyacetone (**C3k**) via retroaldol fragmentation (dotted arrows, Figure 1) of higher carbohydrates that are formed during the lag period. Retroaldol fragmentation is expected to be faster in highly substituted β-hydroxycarbonyl species. Further, under the rules summarized above, dihydroxyacetone cannot be formed in any way other than via retroaldol fragmentation.

The principal competing reaction to consume formaldehyde unproductively is the Cannizzaro reaction, which converts HCHO into essentially unreactive methanol (CH_3OH) and formate ($HCOO^-$) at high pH. On early Earth, methanol and formate were presumably recycled by evaporation and photochemical oxidation to carbon dioxide, which can again be reduced to HCHO.

Mineral Species That Inhibit Undesired Reactions. The formose process exploiting the reactivity of compounds containing C═O units has been extensively discussed as a prebiotic source of carbohydrates, including ribose.[17,19] Unfortunately, the

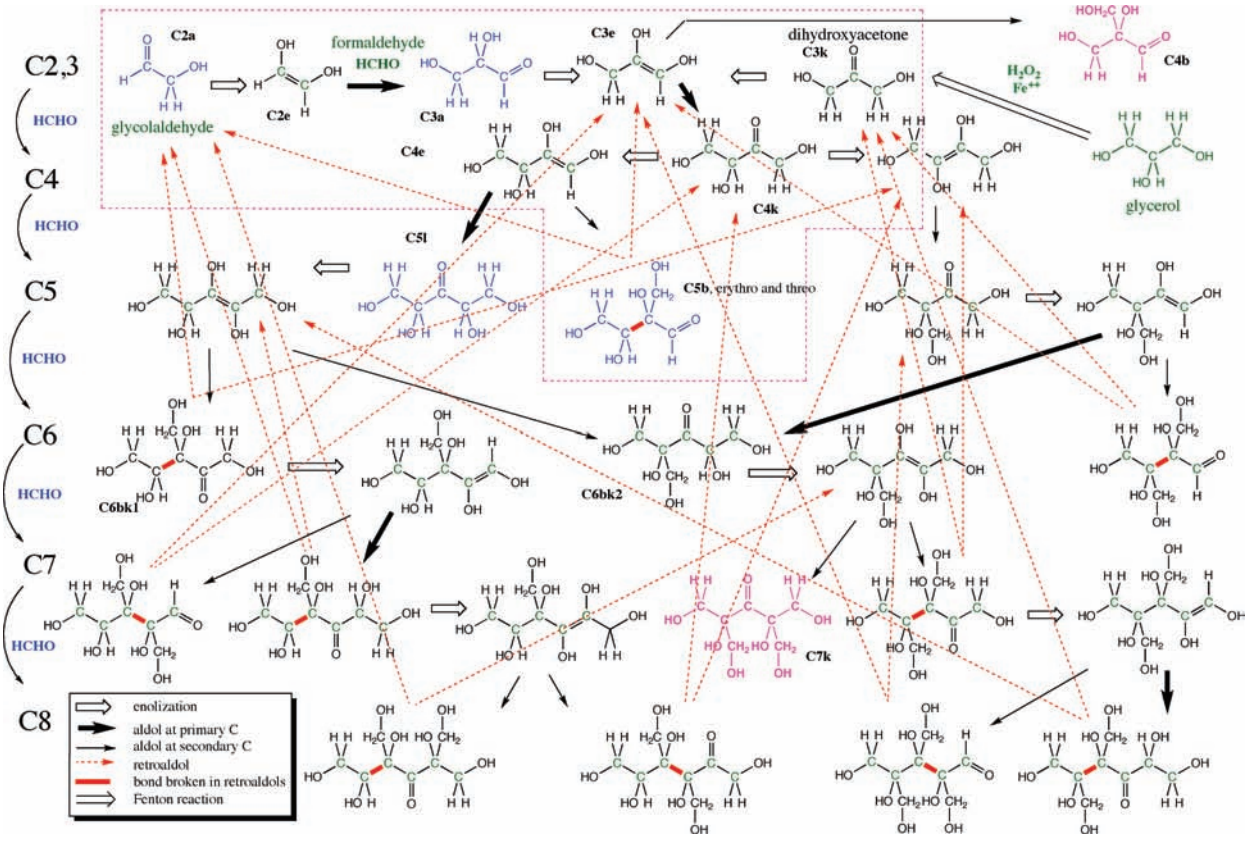


Figure 1. The complexity of the classical formose process arises from just the two reaction types, enolization and aldol addition, repeated again and again, to give a complex manifold of reaction possibilities. To systematize these, carbohydrates (**C**) are labeled by the number of carbon atoms they contain, followed by a letter representing whether they are aldehydes (**a**), ketones (**k**), enediolates (**e**), or branched (**b**) or linear (**l**) isomers. In the classical formose process,[14] two formaldehyde (HCHO) molecules give single molecules of glycolaldehyde (**C2a**, upper left), which initiate a cascade of reactions that fix more HCHO to give higher carbohydrates. Open horizontal black arrows represent enolizations. Heavy vertical or diagonal black arrows represent aldol additions at less hindered centers of an enediolate. Light vertical or diagonal black arrows represent aldol additions at more hindered centers. Dotted red arrows indicate retroaldol reactions that fragment higher carbohydrates to give lower carbohydrates. Compounds in magenta are "dead ends", not capable of either retroaldol reaction or enolization. Compounds in blue and magenta have been prepared separately in authentic form to support the analysis of this complexity. Dihydroxyacetone (**C3k**) is the key to the late, rapid consumption of HCHO. The red dotted box encloses the proposed borate-moderated cycle, shown in detail in Figure 2.

desired carbohydrate products arising from the formose process *also* have C═O groups. This makes them also reactive: unstable against further processing and decomposition at the high pH and high temperatures required to initiate the formose process. Thus, while the high pH's characteristic of serpentinizing solutions are essential for the formose process, highly alkaline conditions cause the destruction of any interesting carbohydrates formed. Therefore, we must find components of prebiotic environment that might confer *non*reactivity selectively on carbohydrates after they are formed, especially pentoses and pentuloses ("pent(ul)oses").

Various mineral species might be considered for this role, including borates and silicates. Both borate[20] and silicate[21] bind to 1,2-diols. This binding is especially tight when the −OH groups are held in a *cis* orientation in a five-membered ring, which is possible for carbohydrates that form cyclic hemiacetals. Cyclic hemiacetals are possible only for carbohydrates that have at least four carbon atoms (Figure S2). As complexes of cyclic hemiacetals have no C═O group, they are essentially unreactive at high pH.

Silicate as a stabilizing species has been examined elsewhere and found to offer relatively little stabilization of carbohydrates that are able to form cyclic hemiacetals.[22] Stabilization of these by borate, in contrast, is significantly greater. Long know known to bind to carbohydrates, borate was shown to stabilize ribose and other pentoses formed by the reaction of glycolaldehyde (**C2a**) and glyceraldehyde (**C3a**).[23,24] To confirm these reports, we quantitated this stabilization (Tables S1 and S2); ribose gives the thermodynamically most stable pentose−borate complex (Figure S3).

Mitigating the Borate Inhibition: Excess Glycolaldehyde. Borate therefore might offer the *non*reactivity needed to capture pentoses once they are formed. The nonreactivity caused by borate minerals has a downside, however. The formose reaction cycles that fix *more* HCHO to give higher carbohydrates have intermediates that *also* have 1,2-dihydroxyl units that might bind to borate and prevent their reaction. Thus, borate at 100 mM prevents formose cycling essentially completely (data not shown). Instead of being fixed, HCHO disproportionates under these conditions via the Cannizzaro reaction to give formate and methanol. Even at 6 mM, borate significantly slows formose cycling.

Obviously, the power of any mineral to stabilize carbohydrates (nonreaction) is useless if the mineral prevents their formation (reaction). Two approaches might resolve this paradox.

dx.doi.org/10.1021/ja201769f |*J. Am. Chem. Soc.* 2011, 133, 9457–9468

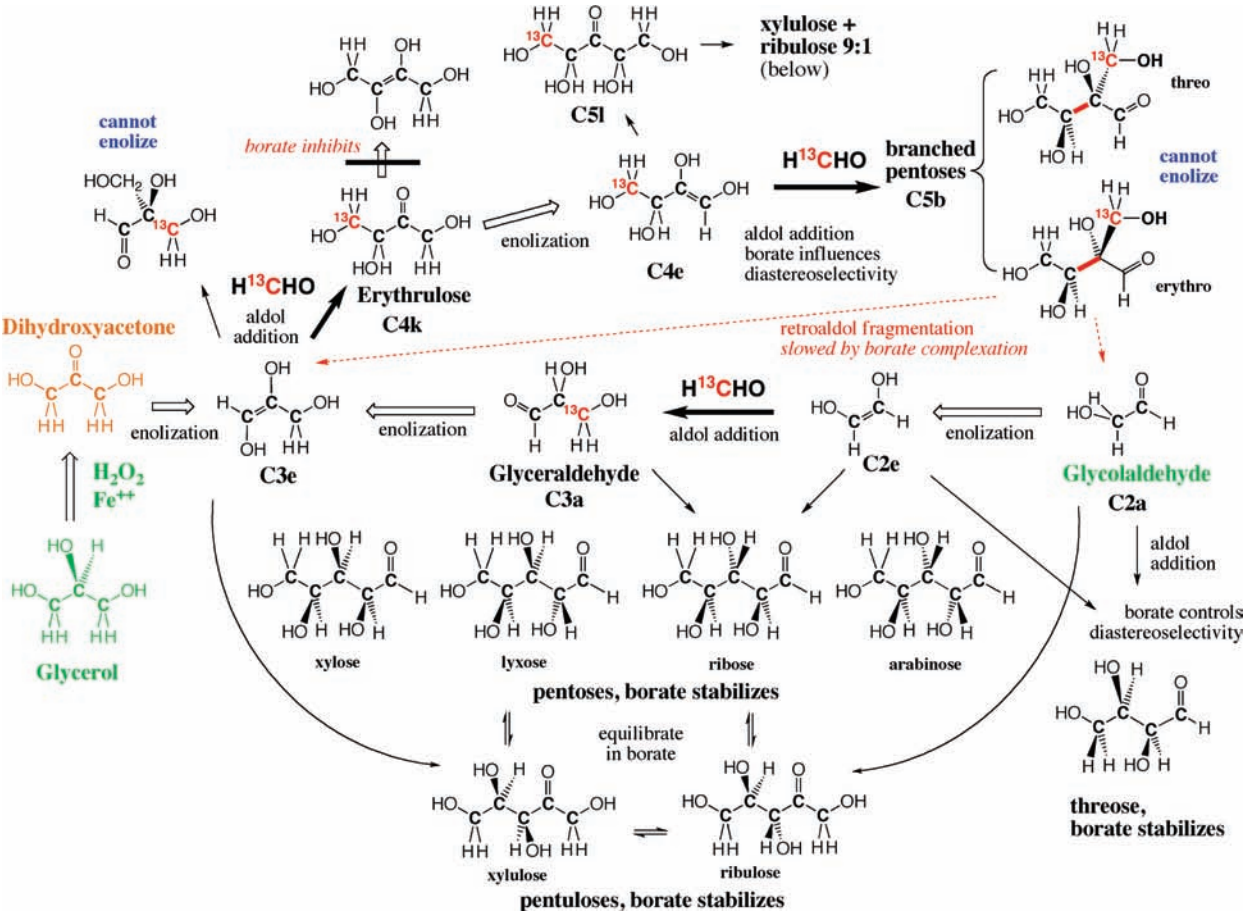


Figure 2. Proposed borate-constrained abiotic metabolic cycle to fix HCHO to form pentoses (including ribose) and pentuloses. Compounds in green are available prebiotically from meteorites, electrical discharge, photochemistry, minerals, or the interstellar nebula. Certain carbon atoms that have been C-13 labeled by chemical synthesis are indicated. Species are labeled on the basis of the number of carbon atoms they contain (e.g., **C2** has two carbon atoms) and whether they are aldehydes (**a**), enediols (**e**), ketones (**k**), branched (**b**), or linear (**l**).

The first reflects the well-known formation of glycolaldehyde by electrical discharge through humid CO_2.[4] We asked whether adding borate to a mixture of HCHO and glycolaldehyde (**C2a**, Figure 2) in a ratio of 1:2 might give borate-stabilized pent-(ul)oses in the absence of cycling. Here, the proposed pathway requires first the enolization of glycolaldehyde to give **C2e**, then the aldol adition of HCHO to **C2e** to give glyceraldehyde (**C3a**), and finally the reaction of glyceraldehyde (as an electophile or as its enolate) with the second molecule of glycolaldehyde (as its enolate or as an electrophile) to give pentoses or pentuloses (respectively).

Indeed, adding 1 equiv of $H^{13}CHO$ to 2 equiv of unlabeled glycolaldehyde in "borate buffer" (1.1 M sodium carbonate, pH 10.4, [boron] = 0.28 M) gave 5-^{13}C-ribose (61.18 ppm), 5-^{13}C-arabinose (63.33 ppm), and 1-^{13}C-xylulose (64.73 ppm) as major products (Figure 3). The formation of ribose was confirmed by superimposition of the signals from authentic material. The ribose and arabinose products arose through the enolization of **C2a** to give **C2e**, the aldol addition of HCHO to give **C3a** (2 + 1 = 3), and the aldol addition of **C3a** with another **C2e** molecule to give the pentoses. These results are consistent with the (3 + 2 = 5) reaction observed by Ricardo et al.[10] Xylulose is formed by the reaction of **C3e** and **C2a** in a (2 + 3 = 5) reaction.

Once formed, the C5 species bind borate, and nonreaction ensues. The analogous reaction with borate replaced by carbonate or silicate (pH 11.9) gave primarily arabinose. Arabinose is the pentose that is the most stable under these conditions. Thus, this result is consistent with the hypothesis that arabinose predominates *not* because of any interaction between it and the mineral species, but because it is simply the most stable pentose.[22b]

These results were then examined without labeling using standard preparative chemistry (Table 1). Ribulose (ca. 3%, not easily observed by NMR), xylulose (ca. 12%), and arabinose (ca. 7%) were isolated as acetonides following incubation of HCHO and glycolaldehyde (1:2) in borate buffer. Also isolated as acetonides were threose and erythrose (ca. 30 and 9%), arising from (2 + 2) aldol reactions. Other species were not identified.

These results provide estimates for the relative rate constants of enolization of glycolaldehyde and glyceraldehyde and the relative reactivity of HCHO and **C2a** as electrophiles under these conditions. Qualitatively, formaldehyde and **C2a** participate approximately equally in product formation, despite HCHO being present overwhelmingly as its hydrate. This illustrates the high electrophilicity of free HCH═O.

Another source of a **C3** species might be glycerol, abundant in meteorites but lacking a C═O unit and therefore quite stable in non-oxidizing environments.[25] Glycerol yields dihydroxyacetone (with a C═O unit, **C3k**, Figures 1 and 2) via oxidation in the presence of iron(II)[26] minerals by H_2O_2, coming via electrical

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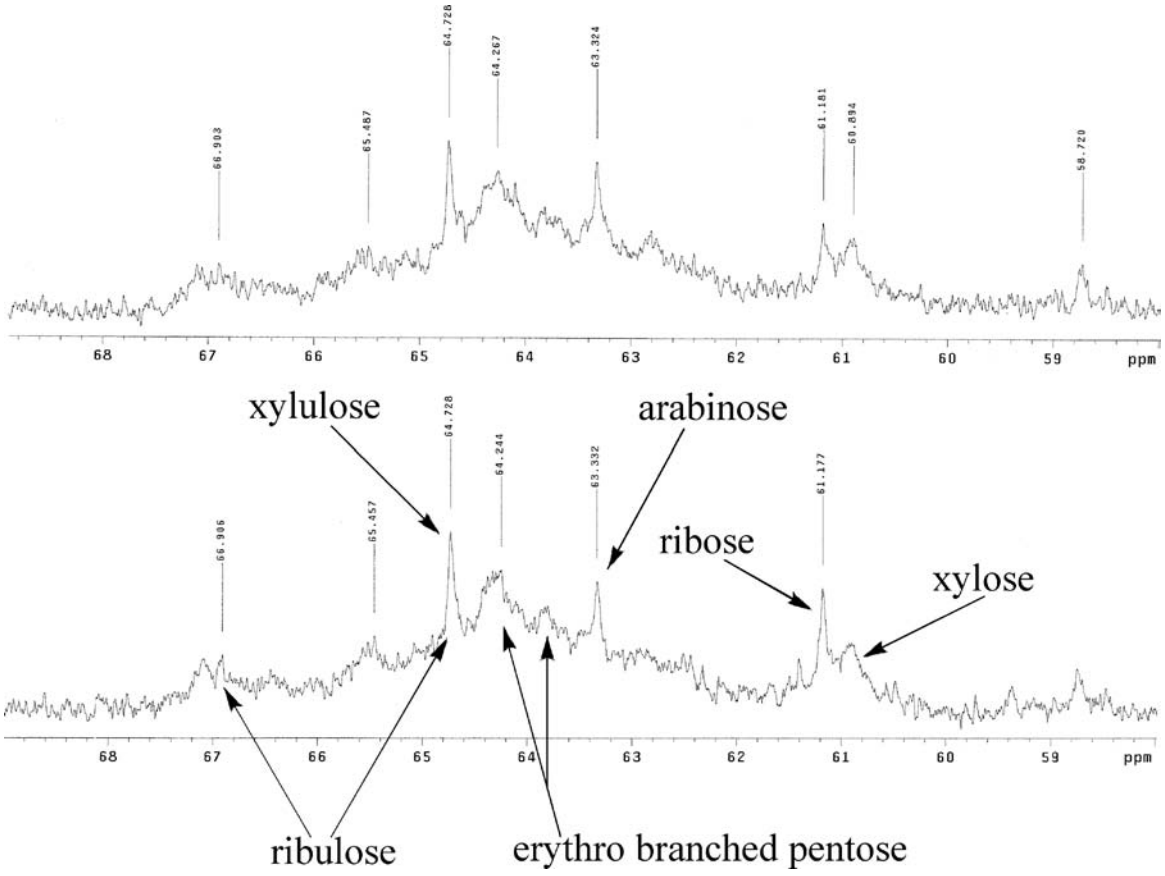
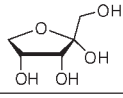
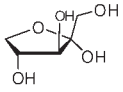
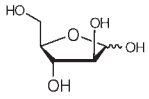
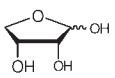
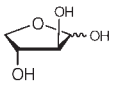


Figure 3. ^{13}C NMR spectra showing the formation of ribose−borate from glycolaldehyde and H^{13}CHO in the presence of borate. Top: Glycolaldehyde (100 mM) was incubated (65 °C, 1 h) with H^{13}CHO (50 mM) in borate buffer (1.1 M sodium carbonate, pH 10.4, [boron] = 0.278 M, D_2O). CH_3OH served as an internal standard (49.50 ppm). Bottom: The same ^{13}C NMR spectrum, with added authentic 5-^{13}C-ribose (Omicron). In separate experiments (data not shown), assignments of arabinose and xylulose were confirmed by superimposition of their signals upon signals from authentic samples.

Table 1. Fraction of Species Isolated (by Their Acetonide Form) from the Reaction of Glycolaldehyde (0.24 g, 4 mmol) and HCHO (0.06 g, 2 mmol) in Borate Buffer at 65 °C for 1 h

	Structure	Yield
Ribulose		3%
Xylulose		12%
Arabinose		7%
Erythrose		9%
Threose		30%

discharge or photochemistry in moist air.[27] As noted above, dihydroxyacetone causes rapid consumption of HCHO in the classical formose process. Of course, the enediol obtained from dihydroxyacetone is the same as the enediol obtained from glyceraldehyde.

Interestingly, without HCHO, borate and glycolaldehyde give tetroses, which are strongly stabilized by borate as their cyclic hemiacetals. Thus, glycolaldehyde (**C2a**) in borate buffer (65 °C for 1 h or room temperature, overnight) gave threose and erythrose in a 3:1 ratio (86% yield by HPLC of their dinitrophenylhydrazones, Figure 4). Consistent with their ability to form cyclic hemiacetals that coordinate borate, threose and erythrose are quite stable in these buffers at pH 10.4. The formation of threose is significant because threose can replace ribose in the backbone of an RNA-like genetic molecule.[28,29]

Demonstration of Cycles in the Presence of Borate. While these studies show the near inevitability of formation of pent-(ul)oses in the presence of borate if glycolaldehyde is produced in a prebiotic atmosphere in large amounts relative to formaldehyde, it is likely that HCHO was far more abundant than glycolaldehyde on the early Earth. Hence, we examined how borate might guide and possibly inhibit cycles that fix HCHO on a large scale, where prebiotic glycolaldehyde would be a catalyst.

dx.doi.org/10.1021/ja201769f |*J. Am. Chem. Soc.* 2011, 133, 9457–9468

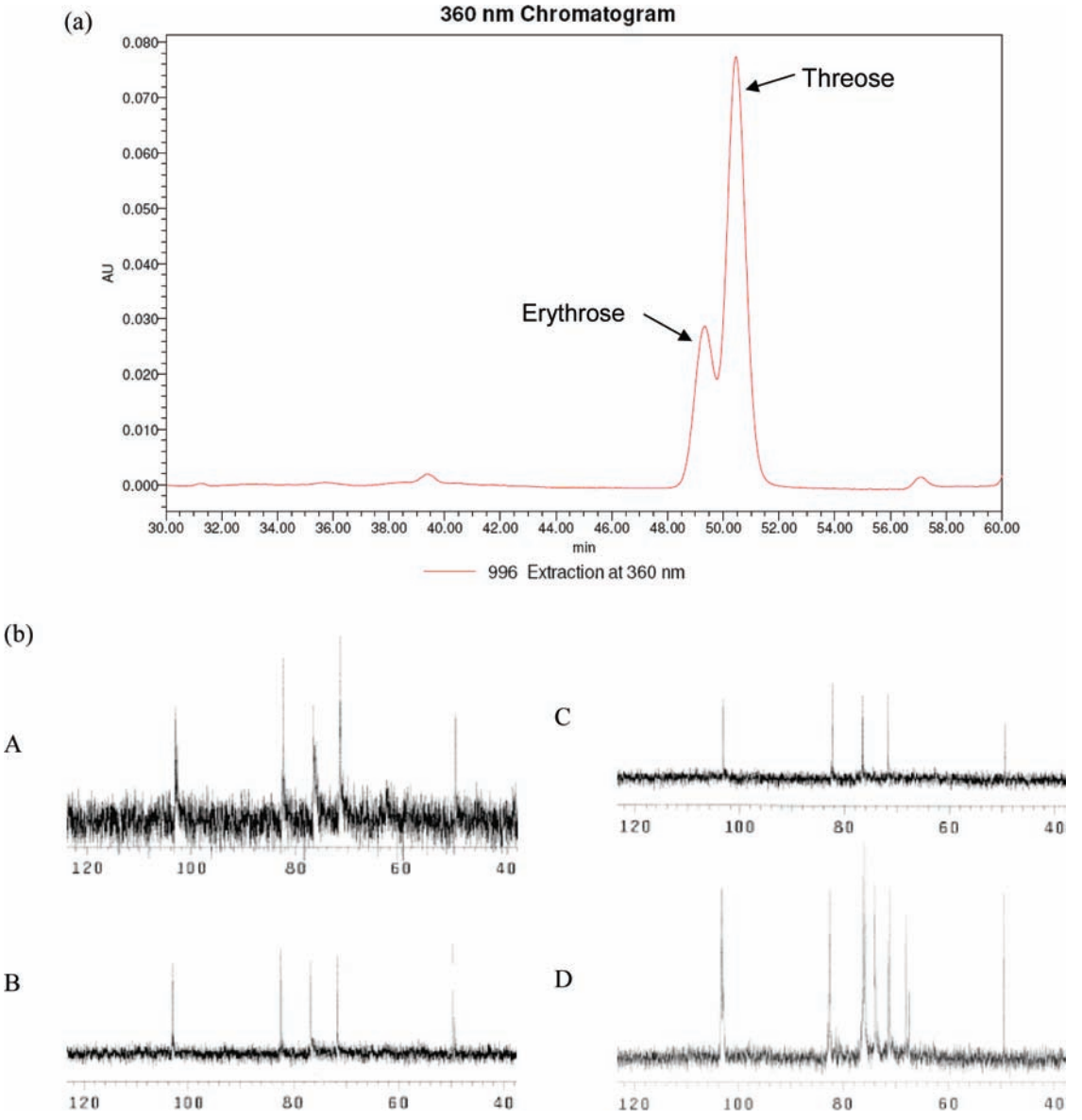


Figure 4. Glycolaldehyde (**C2a**) in borate buffer. (a) HPLC analysis of the reaction of glycolaldehyde in borate buffer in the absence of HCHO (65 °C, 1 h) by dinitrophenylhydrazones derivatization. (b) Natural abundance ^{13}C NMR analysis of the reaction of glycolaldehyde in borate buffer in the absence of HCHO (room temperature, overnight): ^{13}C NMR spectra of (A) reaction products, (B) authentic threose in borate buffer, (C) samples A and B mixed, and (D) authentic erythrose in borate buffer. Signal at 49.50 ppm is reference CH_3OH. Erythrose and threose do not undergo reaction in borate buffer, even after many days.

We began with the hypothesis of a borate-constrained premetabolic cycle that fixes HCHO, consisting of the reactions within the red dotted box in Figure 1, extracted in Figure 2. Here, the enediol **C3e** obtained via enolization of glyceraldehyde or dihydroxyacetone should fix a single HCHO molecule to give erythrulose (**C4k**, 3 + 1 = 4). Erythrulose cannot form a cyclic hemiacetal (and is therefore reactive) but can bind borate weakly through its 3,4-diol unit. This binding should direct the enolization of erythrulose *away* from the borate to give the 1,2-enediol (**C4e**), proceeding clockwise around Figure 2. This enediol should fix a second HCHO to give either the linear (**C5l**) or branched (**C5b**) C5 species at the top and top-right of Figure 2 by reaction (respectively) at the less hindered or more hindered enediol carbon of **C4e** (4 + 1 = 5 reactions).

The branched pentoses **C5b** do not have enolizable hydrogens and, therefore, cannot react further as nucleophiles. They can, however, undergo retroaldol fragmentation to generate glycolaldehyde **C2a** and the enediol of glyceraldehyde **C3e** (5 = 2 + 3). The enediol of glycolaldehyde (**C2e**) should react with another molecule of HCHO to form **C3a** (2 + 1 = 3 reaction), which should enolize to form a second molecule of **C3e**, continuing the cycle two-fold. With each cycle, the amount of HCHO would be fixed, leading to large amounts of fixed carbon.

To obtain experimental support for this cycle, we synthesized (Figure S4) or obtained each of the compounds shown in blue and magenta in Figure 1. We then incubated these in borate buffer at 65 °C (except as noted), following the progress of the reaction using carbon-13 label, introduced from $H^{13}CHO$, ^{13}C-glycolaldehyde, ^{13}C-glyceraldehyde, and/or

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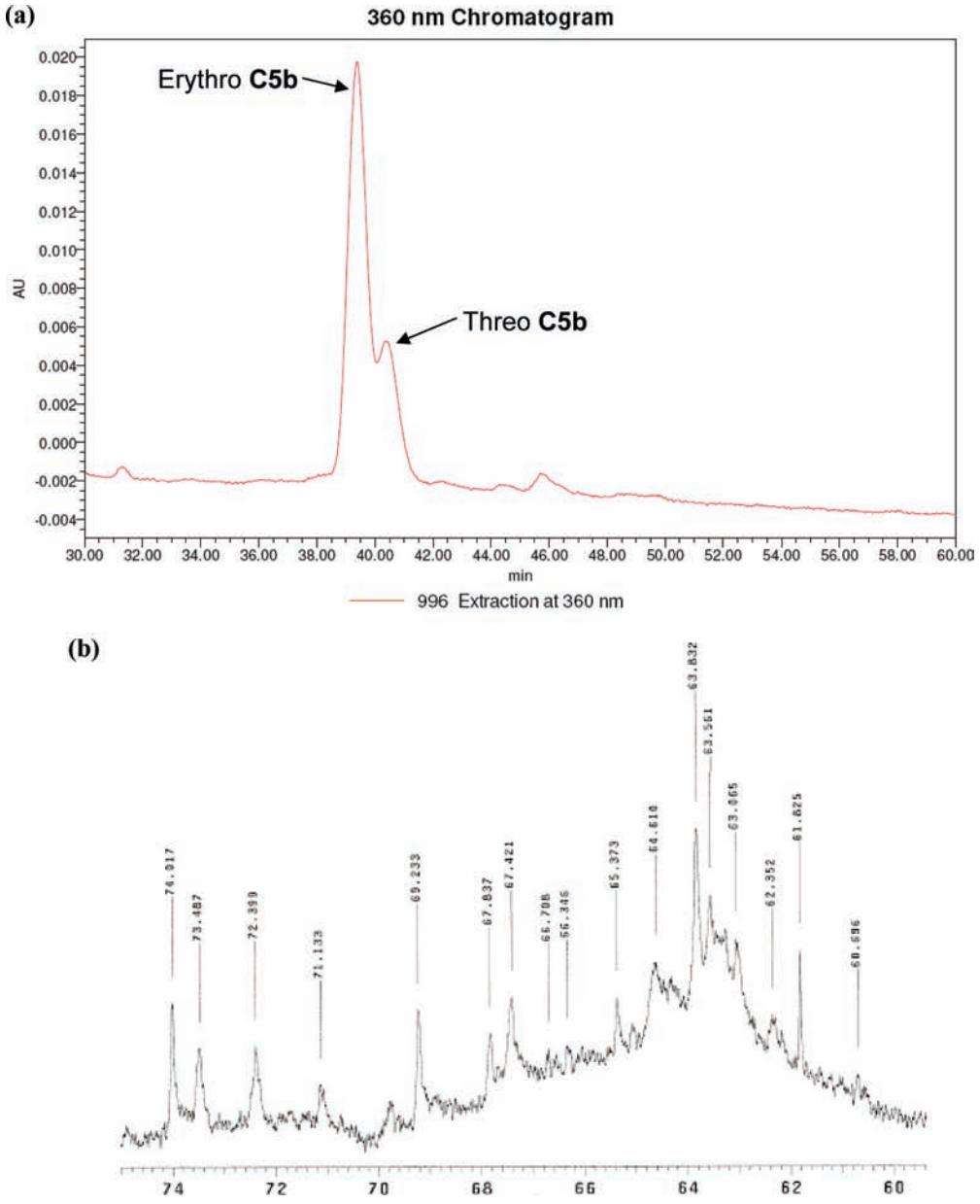


Figure 5. (a) HPLC analysis of the reaction of dihydroxyacetone (33 mM) with HCHO (100 mM) in borate buffer (65 °C, 3 days) by derivatization of dinitrophenylhydrazones. (b) Incubation of dihydroxyacetone (**C3k**) with HCHO. Dihydroxyacetone (33 mM) was incubated with $H^{13}CHO$ (100 mM) for 3 days at 65 °C in borate buffer (1.1 M sodium carbonate, [boron] = 0.278 M, pH 10.4). Peak assignments: 2-hydroxymethylerythrose (**C5b**) of three different borate complexes, 74.01 (C4), 69.233 (C4), and 63.832 ppm (C2′); 2-hydroxymethylthreose, 73.487 ppm (**C5b**); glycolate, 61.825 ppm (C2).

various ^{13}C-labeled compounds synthesized in this work. The chemical shift of various carbohydrates was influenced by borate, requiring us to make ^{13}C NMR measurements in borate buffer. Alternatively, dinitrophenylhydrazone, acetonide, and acetate derivatives were prepared and resolved by HPLC or column chromatography.

With Excess HCHO, Glyceraldehyde Gives Branched Tetrose, Two Stereoisomeric Branched Pentoses, and (Presumably) Linear 3-Ketopentulose. With these tools, each step in the cycle was demonstrated. First, incubation of dihydroxyacetone **C3k** with HCHO gave **C5b** branched pentoses in the presence of borate (3 + 1 + 1 = 5 reaction, Figure 5) in about 11% yield, as determined by preparative chemistry and HPLC of the dinitrophenylhydrazone derivatives (Table 2 and Figure 5a). This was essentially the same result as observed starting with glyceraldehyde **C3a**, which gives the same enediol **C3e** intermediate as dihydroxyacetone **C3k**. A parallel experiment with $H^{13}CHO$ generated labeled **C5b** (*erythro* > *threo*, Figure 5b).

The analogous procedure with silicate or carbonate without borate gave primarily "dead end" carbohydrates **C4b** and **C7k** (or their reduction products), which cannot enolize.[22b] This implied that productive carbohydrates either are not formed or decompose in the absence of guiding or stabilizing borate.

Reaction of $H^{13}CHO$ with Erythulose Gives Product Pentose Mixtures Similar to Those Obtained with Glyceraldehyde or Dihydroxyacetone. Erythrulose is the presumptive

dx.doi.org/10.1021/ja201769f |*J. Am. Chem. Soc.* 2011, 133, 9457–9468

intermediate between **C3e** and **C5b**. To confirm this, erythrulose (**C4k**) was incubated with $H^{13}CHO$ at 65 °C in borate buffer. By NMR, the same mixture of diastereomeric *erythro* and *threo* branched pentoses (**C5b**) was observed. This provides evidence that erythrulose is a common intermediate between glyceraldehyde and dihydroxyacetone, establishing nearly all of the cycle.

Search for Leakage Products C4b, C5l, C6b1, and C7k (Figures 1 and 2). In addition to producing **C5b**, the proposed cycle (Figure 2) suggests two paths that might remove material from the cycle. The first involves addition of HCHO to the more hindered center of **C3e** to give branched tetrose **C4b**. Authentic branched tetrose was synthesized (Figure S4e), and found to be difficult to observe by NMR in borate buffer, presumably because it forms many complexes undergoing dynamic exchange. Further, **C4b** was found to be sensitive to Cannizzaro reduction, which removes it C═O unit and makes it undetectable as a hydrazone. This sensitivity is consistent with its inability to form a cyclic hemiacetal.

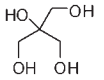
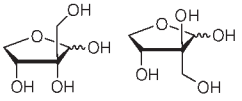
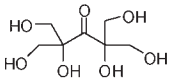
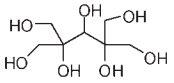
Preparative studies therefore recovered reduced **C4b** product as its acetate. This allowed detection of reduced **C4b** (Table 2), whose structure was confirmed by comparison with a synthetic compound (Figure S4e). This implies that about 10% of the material proceeding through the cycle might be diverted to **C4b**. Absent a retroaldol reaction to extrude HCHO, this is a dead end. Following Cannizzaro reduction, it is a dead end in any case.

A second leakage product might arise from the attack of the enediol of erythrulose (**C4e**) at its less hindered center on HCHO to give **C5l**. To explore the fate of material that leaked from the cycle in this way, synthetic 1-^{13}C-**C5l** was prepared (Figure S4d)[30] and incubated in borate buffer at room temperature without HCHO. Consistent with the fact that **C5l** cannot form a cyclic hemiacetal to bind borate, it reacted with a half-life of 4−5 days at room temperature to give 1- and 5-^{13}C-xylulose (90%, both sites are labeled due to the symmetry of the intermediate) and 1- and 5-^{13}C-ribulose (10%, Figure 6). These pentuloses are stabilized by binding to borate; ribulose is further converted to ribose in the presence of borate.[20,31] Thus, this leakage was "productive": it gave linear pent(ul)oses.

In the presence of HCHO, a second leakage product, heptulose **C7k**, was detected via acetate derivatization (Table 2), with its structure being confirmed by the comparison with a synthetic compound (Figure S4f). This heptulose is also known as a product of formose reaction cycles without borate.[32] Absent a retroaldol reaction to extrude HCHO, **C7k** is also a dead end. Unable to form a cyclic hemiacetal, **C7k** also proved to be sensitive to Cannizzaro reduction, generating a polyol. **C7k** presumably arises via enolization of **C5l**, followed by reaction with HCHO at its less hindered center to give **C6bk2**, which enolizes and reacts again with HCHO at its less hindered center (Figure 1).

These observations led us to seek another product expected from the enolization of **C5l**, this arising from attack of HCHO on the other, slightly more hindered secondary center to give **C6bk1** (Figure 1).[33] Preparative reaction of HCHO and

Table 2. Fraction of Species Isolated (by Their Acetate Form) from the Reaction of Dihydroxyacetone (0.18 g, 2 mmol) and HCHO (0.18 g, 6 mmol) in Borate Buffer at 60−65 °C for 2 Days

	Structure	Yield
C4b reduced		10%
C5b (erythro + threo)		12.5%
C7k		14%
C7k reduced		6.5%

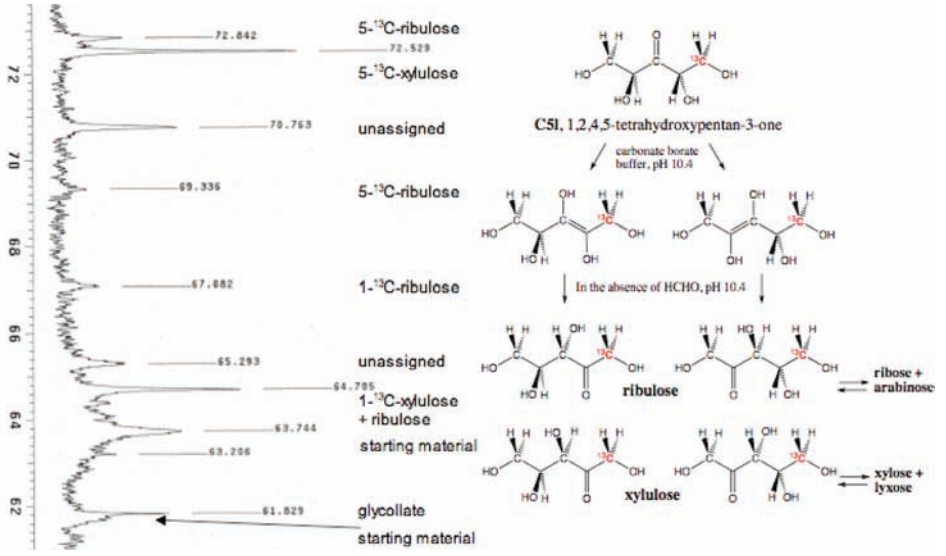
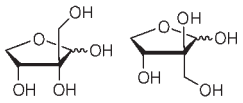
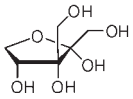


Figure 6. ^{13}C NMR spectrum showing conversion of 1-^{13}C-labeled 1,2,4,5-tetrahydroxypentan-3-one (linear 3-pentulose, **C5l**) to ribulose and xylulose upon incubation for 14 days (25 °C) in borate buffer. In the presence of borate, ribulose equilibrates with ribose and arabinose, while xylulose equilibrates with xylose and lyxose.

dx.doi.org/10.1021/ja201769f |*J. Am. Chem. Soc.* 2011, 133, 9457–9468

Table 3. Fraction of Species Isolated (by Their Acetonide Form) from the Reaction of Dihydroxyacetone (0.18 g, 2 mmol) and HCHO (0.18 g, 6 mmol) in Borate Buffer at 60−65 °C for 2 Days

	Structure	Yield
C5b (erythro + threo)		10%
C6bk1		5%

dihydroxyacetone and analysis of its product by acetonide derivatization allowed detection of some **C6bk1** (~5% yield, Table 3). The structure of **C6bk1** was unequivocally established by a comparison with the synthetic compound (Figure S4g).

Branched Pentose C5b Is Stabilized by Borate. Material that does not leak from the cycle in Figure 2 ends up as branched pentoses **C5b**, which can complete the cycle if they undergo retroaldol cleavage to generate **C2a** and **C3e**. The retroaldol cleavage was readily demonstrated in the absence of borate; ca. 90% of **C5b** is gone in 1 h at 65 °C in 200 mM carbonate (pH 11.8), giving product mixtures too complex to analyze. The branched pentoses **C5b** are only slightly stabilized by 200 mM silicate (also pH 11.8), with ca. 75% gone in the same time.[22b]

However, consistent with the ability of **C5b** to form cyclic hemiacetals that can bind borate (Figure S2), both stereoisomeric branched pentoses were stabilized by borate, with stability increasing with borate concentrations (Table S2). At standard borate concentrations (278 mM), the rate of retroaldol reaction was too slow to conveniently measure. With just 20 mM borate in the presence of $H^{13}CHO$ to give (65 °C, 1 day) the retroaldol products, reduced ^{13}C-labeled **C4b** and **C7k** (and its Cannizzaro-reduced alcohol) were seen. These structures were proven by comparison with authentic material obtained by direct chemical synthesis (Figure S4). The non-dead-end products presumably moved to more complex products at these low concentrations of borate.

Mineralogical Solutions to the "Dead End Problem". Borate stabilization of the branched pentoses **C5b** creates a problem for the cycle. As long as it is bound to borate, **C5b** does not fragment to produce **C2a** and **C3e** that might capture more HCHO and continue around the cycle. Further, we were unable to find concentrations of borate that were low enough to allow the cycle to proceed via fragmentation of the branched pentoses yet high enough to allow for product analysis (other than of the dead end species **C4b** and **C7k**) over periods of time convenient for laboratory study.

Accordingly, we looked for other ways to allow these branched species to be processed productively. Two were found, both involving mineral species, and both related to the Bilik reaction,[34] which rearranges a branched carbohydrate (such as **C4b** and **C5b**) to give a linear carbohydrate (**C4k** and **C5k**, respectively).

At high pH, Ca^{2+} catalyzes a Bilik reaction. Thus, treating 2′-^{13}C-2-hydroxymethylerythrose (**C5b**) with $Ca(OH)_2$ in the absence of borate gave (presumably without retroaldol reaction) xylulose (Figure 7). Adding borate slowed the rate.

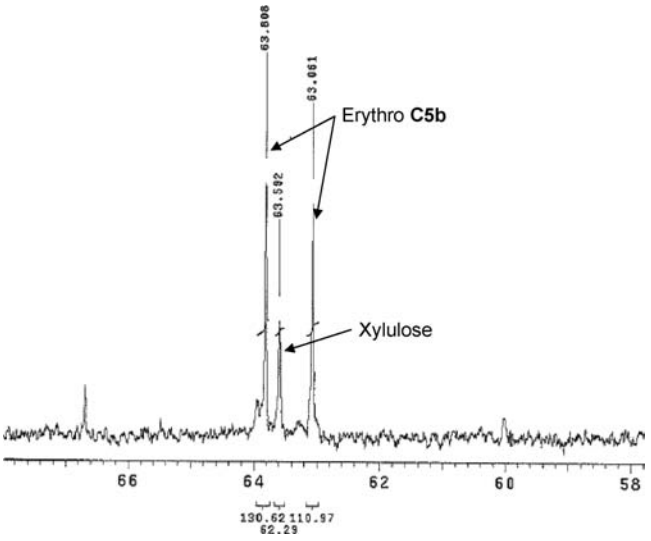


Figure 7. Bilik reaction of branched pentose (**C5b**) catalyzed by calcium. ^{13}C NMR spectrum of the reaction of 20 mM **C5b** (erythro, 5-^{13}C labeled) in the presence of 20 mM calcium chloride and 20 mM sodium hydroxide at room temperature for 30 min.

At lower pH, the Bilik reaction can be catalyzed by molybdate minerals. Thus, incubating (65 °C, 24 h) labeled **C5b** (*erythro*, 50 mM) in the presence of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$, 2 mM) at pH 5.9 leads to an equilibrium mixture of **C5b** starting material and linear xylulose (a desired product), with some linear pentulose 1,2,4,5-tetrahydroxypentan-3-one **C5l**. The rearrangement was stereospecific: *threo* **C5b** gave ribulose (Figure 8). Xylulose and ribulose equilibrate slowly with Mo^{6+} to give xylose and ribose.[31] Thus, molybdate minerals can generate free ribose and xylose from **C5b** branched pentoses.

DISCUSSION

These results establish the cycle in Figure 2 as a laboratory reality. Further, they provide an indication of how borate is able to direct organic species derived from HCHO and glycolaldehyde along just a few of many alternative paths that are conceivable (Figure 1). For example, borate helps direct attack of HCHO at the more hindered center of the enediol of eryrthulose, **C4e**, to give the branched pentoses **C5b**. Further, these study show that borate hinders the retroaldol reaction of **C5b**, permitting it to accumulate.

Since HCHO and glycolaldehyde are generated by electrical discharge through humid CO_2 atmospheres, and since some borate must be present in leaching igneous rocks, it appears hard to avoid the conclusion that any subaerial evaporite region on early Earth exposed to a humid CO_2 atmosphere suffering electrical discharge accumulated some branched pentose **C5b** as its borate complex. Only the amount is at issue. This amount would be determined by the excess of carbon over boron, the pH, and the temperature, factors not well constrained in models for early Earth.

These results also support rules that allow the interpretation of such processes generally. In the preparative work reported here, approximately 50% of the carbon originating in HCHO can be accounted for in various products. Further, as long as HCHO is present, the mixture never turns yellow. Thus, as long as HCHO is present to capture carbon fragments arising from retroaldol

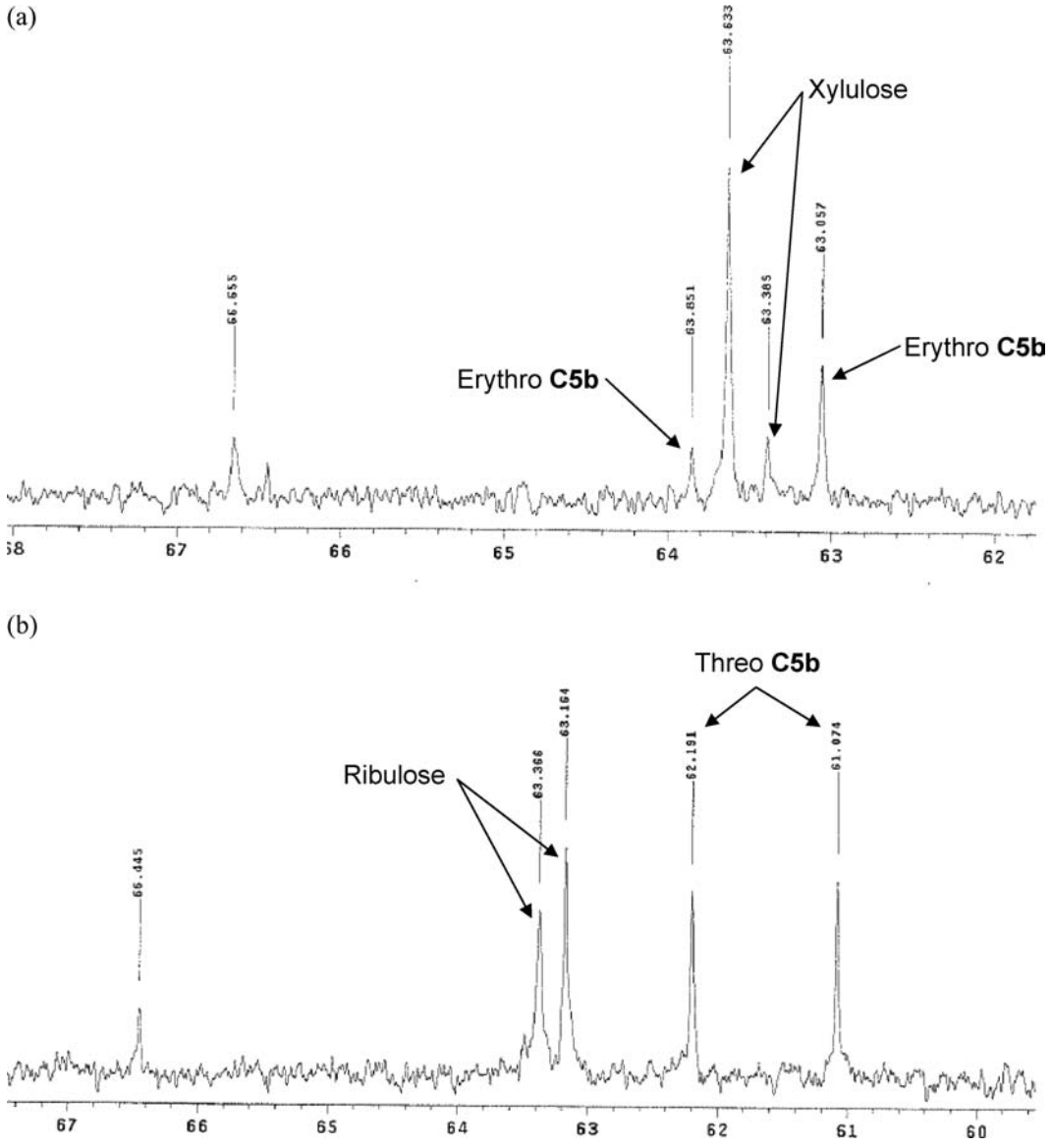


Figure 8. Bilik reaction of branched pentoses (**C5b**) catalyzed by molybdate. (a) ^{13}C NMR spectrum of the reaction of 50 mM **C5b** (*erythro*, 5-^{13}C-labeled) in the presence of 2 mM sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$, pH 5.9) at 65 °C for 24 h. (b) ^{13}C NMR spectrum of the reaction of 50 mM **C5b** (*threo*, 5-^{13}C-labeled) in the presence of 2 mM sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$, pH 5.9) at 65 °C for 24 h.

fragmentation of **C5b** that escapes its borate complex, pent(ul)oses will accumulate, simply because they are the first products accessible in this system that are able to form cyclic hemiacetals that can coordinate borate strongly.

These results also suggest a choice among mineral species that might have been productively involved in the prebiotic transformation of HCHO and its derived carbohydrates. For example, Lambert and co-workers have suggested that silicate might be useful. In the Earth's crust, silicate is far more abundant than borate.[22a] In aqueous solution, however, especially at neutral pH and below, silicate precipitates to give SiO_2 (opal or quartz), while borate remains in solution. Further, borate stabilizes cyclic pentoses more than silicate, whose stabilizing effect, although detectable, is small.

These results also show how other minerals might determine the fate of **C5b**. Both Ca^{2+} minerals and molybdate minerals can convert **C5b** to linear pent(ul)oses via the Bilik reaction. Ca^{2+} was almost certainly available to prebiotic chemistry. Calcium borate minerals (colemanite and ulexite, for example) are relatively soluble in water. Indeed, the special need for Ca^{2+} in the formose process (see above) may arise because it can catalyze Bilik-like rearrangements.

The disadvantage of Ca^{2+} as a prebiotic Bilik catalyst is that it operates only at high pH, conditions where the carbohydrate products require borate stabilization. This is not true for molybdate, which catalyzes the formation of linear pent(ul)oses at near-neutral pH and moderate temperatures. The pent(ul)oses formed do not require borate stabilization under these conditions; indeed, at lower pH, the borate complexes are less stable, making their carbohydrates accessible to molybdate.

While the model that we have used here for the prebiotic atmosphere is currently the consensus, what is the likelihood that the other constraints are met? Borate minerals are known from 3.8 Ga old rocks in the Isua supracrustal belt of western

Greenland,[35] where they may have arisen from evaporite basins of the sort needed for the cycles proposed here. Borate minerals are also associated with stromatolites in the Barberton greenstone belt in South Africa, where they seem almost certain to have arisen from evaporites.[18] While some have questioned whether the early Earth was sufficiently differentiated to have allowed borate concentration in the geosphere,[36] borate is enriched in the residual melt of any igneous species, from which borates are easily weathered, allowing them to be concentrated in the hydrosphere even if they are not concentrated in the lithosphere. This makes it difficult to argue against borate-rich evaporates in any early Earth scenario that includes dry land.

Molybdate presents more of a challenge to the prebiotic chemist, as it is oxidized relative to the redox state of early Earth, as presently modeled ($MoO_2 + H_2O + 2Fe^{3+} \rightarrow MoO_3 + 2H^+ + 2Fe^{2+} = +236$ mV). However, a "planetary redox potential" is unlikely to be relevant to the existence of such minerals. Emerging models for early Earth suggest that continents and their associated subduction zones were present as early as 4.4−4.5 Ga.[37] These would have generated felsic magmas that would have included minerals that are more oxidized than the terran surface as a whole, including sulfate, borate, and molybdate (S. J. Mojzsis, personal communication).

These results highlight the potential of minerals to provide simultaneously both the desired reactivity and the desired nonreactivity within a "prebiotic soup". Further, they offer a "vestigiality" explanation for why pent(ul)oses, including ribose, are found in genetic material. With excess HCHO, pentoses are the first species that can be formed by a cycle with excess HCHO that have available a hemiacetal form that can be bound and stabilized by borate. Once stabilized, they react no further to give hexoses, heptoses, and higher sugars, even with excess HCHO. While aldotetroses can form cyclic ligands that are extremely stable in borate, they are not accessible by the pathway where HCHO is in excess, and therefore they are not formed.

Further, this work illustrates how fluctuating conditions might support transformations of prebiotic organic molecules, including changes in pH or the relative amounts of stabilizing mineral species and organic species needing stabilization. For example, for those concerned that it might be difficult to release ribose from its borate complex,[38] simply lowering the pH through buffering from atmospheric CO_2 can do this. At pH 7, ribose is released from borate to nearly neutral conditions, where it is quite stable against enolization and aldol reactions that lead to the destruction of carbonyl compounds at high pH. There, ribose is available to be phosphorylated by prebiotic mechanisms developed in other laboratories.[39]

As a final word, although the work here is driven by the "RNA-first" hypothesis for the origin of life, the cycles described here share some of the features proposed for cycles hypothesized for "metabolism-first" models.[40] Although "genetics-first" and "metabolism-first" models for the origin of life are currently being presented as adversaries,[41] no logic compels them to be. It is nearly certain that chemical processes that might be likened to metabolism occurred on Earth before genetics was established in its macromolecular form. These processes may have provided the components of whatever genetic system did first emerge. While it is difficult to know whether borate-moderated formaldehyde-fixation cycles meet criteria required by advocates of a "metabolism-first" scenario, Figure 2 represents a metabolic cycle resembling those found in contemporary terran life.

EXPERIMENTAL SECTION

General Methods. ^{13}C-labeled carbohydrates (arabinitol, arabinose, lyxose, ribose, xylose, ribulose, xylulose, glycolaldehyde, glyceraldehydes) were obtained from Omicron Bio. H^{13}CHO and labeled paraformaldehyde were obtained from Cambridge Isotopes. All other reagents were obtained from Sigma-Aldrich and were used without purification. Flash column chromatography was carried out using Merck 9385 silica gel 60 (230−400 mesh). NMR spectroscopy was carried out on a Varian Mercury 300 NMR spectrometer.

Typical Procedure for Dinitrophenylhydrazone Formation and Analysis by HPLC. A mixture of dihydroxyacetone (0.18 g, 2 mmol) and formaldehyde (0.18 g, 6 mmol) in borate buffer (1100 mM carbonate and 278 mM borate, made by dissolving 4.68 g of Na_2CO_3 and 0.688 g of H_3BO_3 in 40 mL of H_2O) was stirred at 60−65 °C for 2 days under an Ar atmosphere.

To 20 μL of the above reaction mixture were added 300 μL of TFA solution (2% TFA in MeOH, v/v) and 200 μL of 2,4-dinitrophenylhydrazine solution (1.5% DNP in dimethoxyethane, w/v). The mixture was heated at 65 °C for 90 min and then cooled to room temperature, and 400 μL of acetone was added. After evaporation to dryness, the residue was treated with 400 μL of 5% triethylamine in methanol and evaporated. This residue was dissolved in 80 μL of 1,2-dimethoxyethane and treated with 500 μL of water, and the resultant suspension was centrifuged (10 000 rpm, 2 min). The aliquot was injected into the HPLC (column, Waters Nova-Pak HR C18 6 μm, 60 Å, 7.8 × 300 mm Prep Column; eluents, A = 0.02% TFA in water, B = CH_3CN, gradient from 15 to 25% B in 60 min, flow rate 1 mL/min). The peaks of the DNP−sugar derivatives eluted were detected by their absorbance at 360 nm.

Typical Procedure for Acetate Derivatization. A mixture of dihydroxyacetone (0.18 g, 2 mmol) and formaldehyde (0.18 g, 6 mmol) in borate buffer was heated to 60−65 °C for 2 days under Ar atmosphere. After cooling to room temperature, the mixture was neutralized by acidic resin (Dowex), filtered, and lyophilized. The resulting solid was dissolved in methanol (20 mL), evaporated on a rotary evaporator (repeated three times), and further dried under high vacuum to give a reddish brown solid. It was then treated with acetic anhydride (3 mL), DMAP (50 mg), and pyridine (20 mL) and stirred at room temperature for 24 h. It was evaporated and separated by silica gel column chromatography (EtOAc:Hex = 1:2 to 2:1) to give 11 crude fractions. After evaporation, each fraction was weighed and characterized by ^{1}H NMR. The identity of the each fraction was confirmed by comparison with the authentic material.

Typical Procedure for Acetonide Derivatization. A mixture of dihydroxyacetone (0.18 g, 2 mmol) and formaldehyde (0.18 g, 6 mmol) in borate buffer was heated to 60−65 °C for 2 days under Ar atmosphere. After cooling to room temperature, the mixture was neutralized by acidic resin (Dowex), filtered, and lyophilized. The resulting solid was dissolved in methanol (20 mL), evaporated on a rotary evaporator (repeated three times), and further dried under high vacuum to give a reddish brown solid. It was then treated with acetone (50 mL) and sulfuric acid (1 mL) and stirred at room temperature for 1.5 h. It was neutralized by sodium bicarbonate and evaporated and separated by silica gel column chromatography (EtOAc:Hex = 1:2 to 2:1) to give five crude fractions. After evaporation, each fraction was weighed and characterized by ^{1}H NMR. The identity of the each fraction was confirmed by comparison with the authentic material.

ASSOCIATED CONTENT

Supporting Information. Relative stability of pent(ul)oses (Tables S1 and S2 and Figure S3), consumption of formaldehyde in formose reaction (Figure S1), and synthetic

details of various suspected carbohydrate intermediates with ^{13}C label at specific sites (Figure S4). This material is available free of charge via the Internet at http://pubs.acs.org.

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ACKNOWLEDGMENT

This paper is dedicated to the memories of Leslie Orgel and Stanley Miller. We are indebted to the NASA Astrobiology/ Exobiology program (NNX-08AO23G), the National Science Foundation Chemical Bonding Program (CHE-0434507), and the John A. Templeton Foundation for support of this work.

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