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# The role of the anion in the reaction of reducing sugars with ammonium salts

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

**Abstract**

Reactions of reducing sugars with ammonia and its compounds are important commercially, particularly in the preparation of flavors and caramel colors. However, such reactions generally produce a complex series of products ranging from simple molecules to complex polymeric materials, particularly since commercial systems generally involve mixtures of sugars as opposed to single sugars. This complexity has made understanding the mechanisms of such reactions difficult. Therefore, investigatory work has generally been focused on model systems. Herein we report one such study with model systems: the effects of the nature of the anion of the reactions of reducing sugars with ammonium salts. D-Glucose was reacted in aqueous solution with each of the following ammonium salts: acetate, bicarbonate, carbonate, chloride, citrate, formate, monohydrogenphosphate (DAP), sulfate, and sulfite. These reactions were carried out in a Parr bomb at 93 °C for 2.5 h. The initial pH of the reaction mixtures was adjusted to pH 8.0 at 25 °C. The resulting mixtures were analyzed by LC–MS, and the results were analyzed by comparing the product yields and distributions with those obtained with DAP. The major reaction product of interest was 2,6-deoxyfructosazine, as it had been shown to be a marker for the polymeric material formed from such reactions. It was found that ammonium salts of weak acids were much more effective in effecting the desired reactions than were those of strong acids; however, none was as effective as DAP. 

*Keywords:* Model systems; D-Glucose; Ammonium salts

The products of the reactions of reducing sugars with ammonia and ammonium salts are important commercially particularly as colorants in the food and beverage industry. For example, the caramel color used in carbonated soft drinks is formed from the reaction of sugars with sulfite and ammonium compounds.[1] The tobacco industry has made extensive use of the reactions of endogenous or added sugars and diammonium phosphate and various ammonia-containing compounds (including endogenous ammonia) for modifying and/or increasing the flavor of the smoke from such tobaccos.[2–9] In both the food-colorant industry and the tobacco industry, the desired reaction product is the polymeric material, not the numerous volatile and semivolatile compounds ($M_W < 400$ g/mol) that are also formed in such reactions.

Despite the commercial importance of such reactions, relatively little is known about the reaction mechanisms, kinetics, and products associated with them, as the complexity of such systems has defied even the most diligent investigators. Thus, most investigators have focused on model systems and then only on selected classes of compounds. With respect to the role of the anion in sugar–ammonia reactions, Jezo showed that the presence of triammonium phosphate altered the ratio of pyrazines to imidazoles, as well as the overall yield of products formed during the aminolysis of sucrose, and that similar effects were found during the aminolysis of other carbohydrates.[10–12] Jezo's work was unique for the time as his analyses included not only the volatile alkyl pyrazines and imidazoles, but also the

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relatively nonvolatile hydroxyalkyl pyrazines and imidazoles.

Wong and Bernhard studied the volatile reaction products from the reactions of ammonium hydroxide, ammonium formate, and ammonium acetate with glucose.[13] A key finding from that research was that the yields of 2,6-dimethylpyrazine relative to 2,5-dimethylpyrazine were much higher when the formate and acetate salts were used as compared to the case when ammonium hydroxide was used.

In a series of papers extending over a decade, Komoto and co-workers at Kobe University studied the factors that affect the products from the reaction of glucose with ammonia and its compounds. Reaction products of interest in their research included 2-(D-*arabino*-1′,2′,3′,4′-tetrahydroxybutyl)-5-(D-*erythro*-2″,3″,4″-trihydroxybutyl)pyrazine (also known as deoxyfructosazine) and its 2,6-isomer (hereinafter abbreviated 2,5-DOF and 2,6-DOF, respectively), as well as the corresponding fructosazines, 2,5-bis-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)pyrazine, and its 2,6-isomer.

Three key findings from their extensive research were that (1) the ratio of fructosazines to deoxyfructosazines (DOFs) was dependent on the acidity of the reaction medium; (2) clarification of the mechanisms for the formation of 2,5- versus 2,6-substitution patterns in the pyrazines produced from the reactions of reducing sugars and ammonium compounds; and (3) the relevance of deoxyfructosazine formation as a marker for the formation of the so-called browning polymers.[14–19]

The research of two industrial scientists, who studied similar reactions, has recently become available. Lynm of the R.J. Reynolds Tobacco Company studied the kinetics of the reaction of DAP with glucose, fructose, and a 50:50 mixture of glucose and fructose. Reaction mixtures were heated briefly at 70 °C and then allowed to age at room temperature for up to 28 days. His research showed that (1) when glucose alone was used, no DOFs were detected at the end of the heating period; and the 2,6-DOF was the predominant DOF isomer at the end of the aging period; (2) when fructose alone was used, both 2,5- and 2,6-DOF were formed initially with the 2,5-isomer being the predominate product; (3) when a 50:50 mixture of glucose and fructose was used, both DOF isomers were formed, but that the 2,5-isomer predominated; and (4) the total amount of DOFs formed was as follows: fructose $>$ 50:50 fructose and glucose $\gg$ glucose.[20,21]

Tafur of Philip Morris USA performed similar studies using ammonium formate (30 min at $\sim$100 °C) and found that the reaction of glucose with ammonium formate produced both DOF isomers, but with the 2,6:2,5 ratio being 4.3:1. When similar reactions were carried out with 2-amino-2-deoxy-D-glucose (glucosamine), glucosamine and glucose, and glucosamine and glucosylamine, the 2,5-DOF was the major product.[22] In further studies, Tafur confirmed the findings of the Kobe group that aldoses produced predominantly the 2,6-isomers while ketoses produced predominantly the 2,5-isomers, and that this finding could be extended to the C-5 sugars. Also, Tafur showed that the reaction of 2-deoxyglucose with ammonium formate did not yield DOFs.[23]

While we were not aware of the studies of Lynm and Tafur at the time, we began the research we are reporting. Our objectives were similar: (1) to understand the role of the anion in sugar–ammonia reactions and the apparently unique role of DAP as an ammonia source; and (2) to understand how the reaction conditions affected the yield and structure of the reaction products formed from such reactions.

Fig. 1 shows the yield of 2,6-DOF for the various ammonia sources under the reaction conditions used. Chromatographic analyses (LC–UV, LC–UV–MS, GC–MS) of the reaction mixtures showed that the other ammoniating agents differed from DAP both in the conversion of glucose to reaction products and the distribution of reaction products. In the case of DAP, the other major nonpolymeric reaction products were shorter chain analogs of 2,6-DOF with molecular weights of 274, 244, and 214 g/mol. There was only a trace of unreacted glucose. When ammonium carbonate was substituted for DAP, the major peaks in the chromatogram were 2,6-DOF, 2,5-DOF, unreacted glucose, and the shorter chain 2,6-DOF analogs with molecular weights of 244 and 214 g/mol. In addition, there was a trace of 2,5-fructosazine, and approximately 5% of unidentified material that had about two times the retention time of 2,6-DOF ($m/z$ 178,179, 222, 342, 227, 306). The reaction with ammonium sulfide yielded some 2,6-DOF and considerable unreacted glucose.

The results of these experiments raised the question as to the role of phosphate in catalyzing the conversion of glucose to 2,6-DOF. Others have reported the catalytic effect of phosphate anions in the Maillard reaction for reactions between sugars and amino acids, proteins, and enzymes.[24–26]

This catalytic effect of phosphate has been rationalized by (1) general base catalysis; (2) facilitating the Amadori rearrangement by catalyzing the abstraction of a proton from carbon-2 of the glycosylamine and the addition of a proton to the α-amino enol intermediate; and (3) formation a covalent bond to sugars or Maillard products. However, these explanations may no longer be satisfactory. A study of the isotope effect of the nonenzymatic glycation of hemoglobin in phosphate buffers indicated that neither the abstraction of a proton from carbon-2 nor the addition of a proton to the α-amino enol intermediate were catalyzed by phosphate.[27] The formation of covalent bonds between phosphate and sugars requires the use of phosphate anhydrides (e.g., metaphosphate or tripolyphosphate

salts, ATP), whether or not the phosphorylation is enzymatically catalyzed. Also, as shown by the work of Reynolds, the reaction of glucose with lysine in a saturated phosphate buffer did not result in the consumption of phosphate.[28]

Our results showed that relative to DAP, the production of 2,6-DOF with the other ammonium salts used ranged from a high of around 25% for carbonate and sulfite to less than 5% with chloride and sulfate. These differences cannot be explained by the differences in the initial pH of the reaction mixtures as all were set to pH 8.0. The differences also cannot be explained by formation of other products. For example, the glucose level in the reaction with ammonium chloride after 2.5 h of heating was almost unchanged from the starting level.

Another potential conclusion that could be drawn from our data is that the ammonium salts of weak acids are more effective in this reaction than are salts of strong acids. If that were true, then the reaction rate constant ($k$) should follow the equation for general base catalysis as shown below, where $A$ and $C$ are constants and $K_a$ is the acidity constant of the acid whose salt is being used.

$$\mathrm{Log}(k) = -A\ \log(K_a) + C$$

If, indeed, this were the case, then most of the salts of the weak acids would show similar levels for the production of 2,6-DOF, and there would be no difference between DAP and the other weak acids.

Since this is not the case, another mechanism needs to be found to explain the findings. It is known that phosphate catalyzes the oxygen exchange of the anomeric carbon of D-glucose.[29] The structure of the phosphate anion provides bifunctional catalytic properties. It can accept a proton at the same time it can donate a proton via an intramolecular process. The –OH and $–O^-$ moieties in the phosphate anion may allow phosphate to function as both acid and base in catalyzing the ring opening of D-glucopyranose. The situation appears to be analogous to that of 2-pyridinol, which is far more effective than either pyridine or phenol alone in catalyzing mutarotation.[30] An analogous situation is the enhancement of the browning reaction of D-glucose-6-phosphate as compared with glucose alone.[24] In addition, it also has been shown that arsenate is almost as effective as phosphate in catalyzing such reactions, and that disubstituted or trisubstituted phosphates cannot catalyze these reactions.[31]

This proposed explanation of phosphate catalysis is also consistent with the fact that use of D-glucose yields predominately 2,6-DOF, and D-fructose yields predominately 2,5-DOF. Figs. 2 and 3, which were adapted from Ref. 31, show how aldoses yield the 2,6-isomers while ketoses yield the 2,5-isomers.

In summary, we have shown that, among the ammonium salts typically used for ammoniation of glucose, DAP has enhanced reactivity, and this reactivity is likely due to the fact that the monohydrogenphosphate anion can act as both an acid and a base.

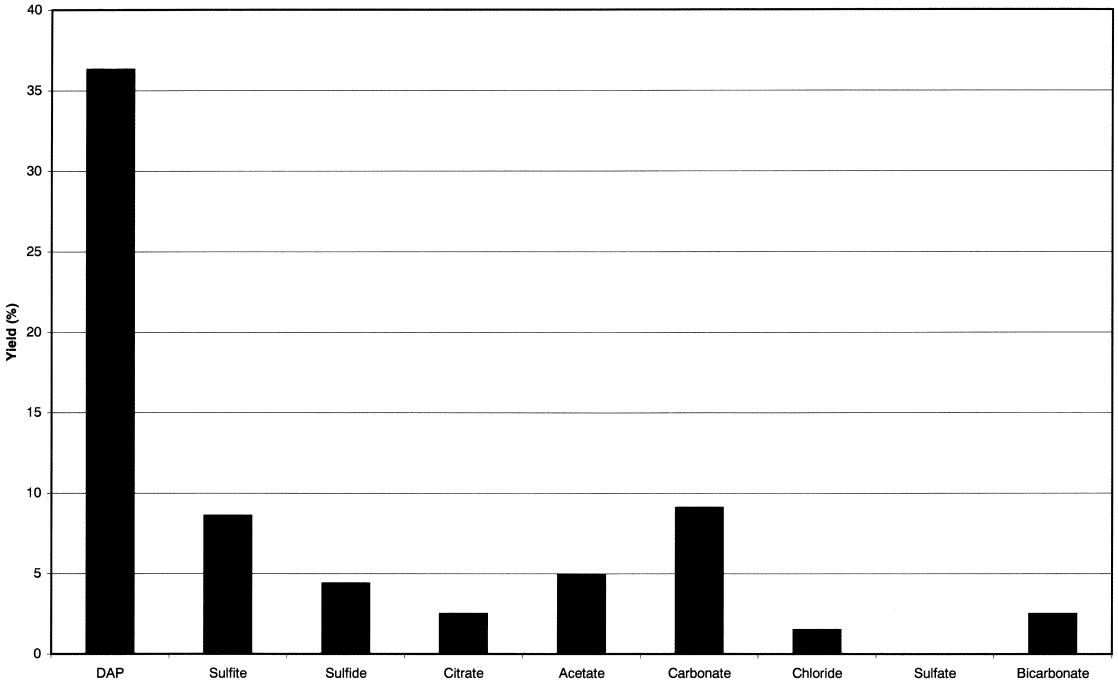


Fig. 1. Yield of 2,6-deoxyfructose with various ammonium salts.

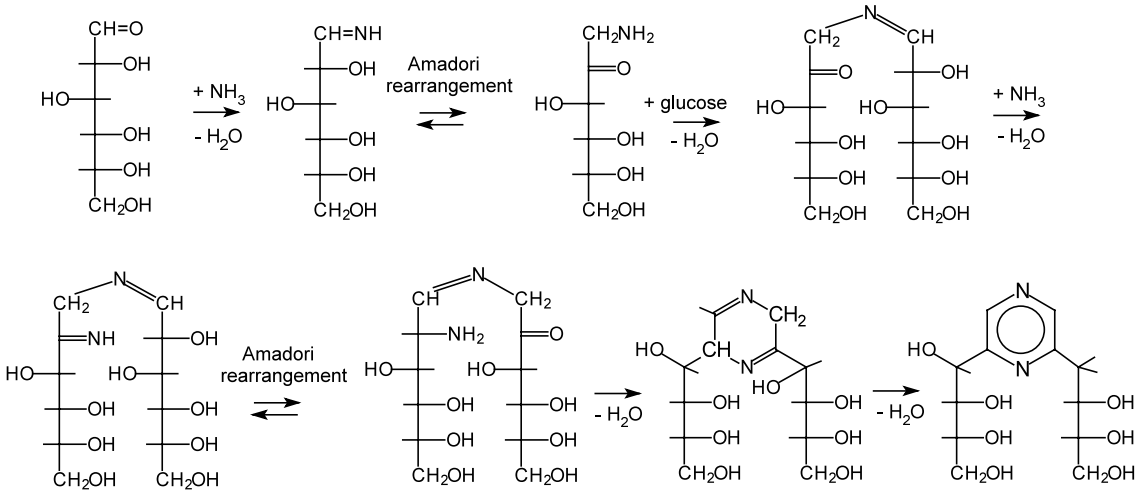


Fig. 2. Mechanism of 2,6-DOF formation.

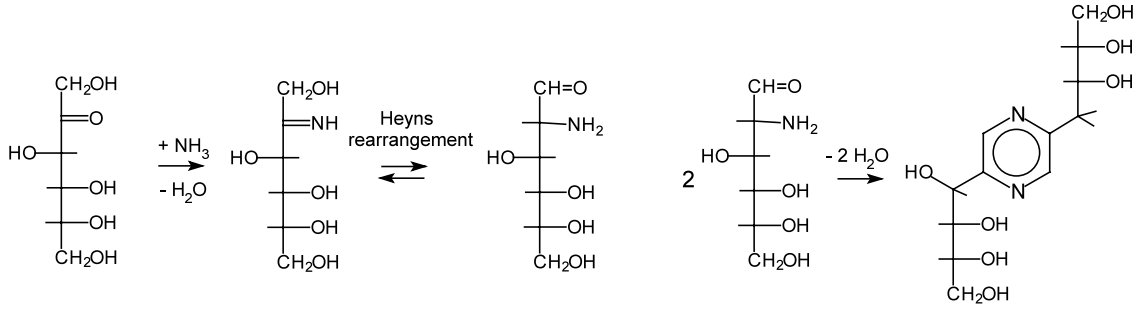


Fig. 3. Mechanism of 2,5-DOF formation.

## 1. Experimental

All reagents and solvents were purchased from commercial sources. The reaction mixtures were prepared by dissolving 0.5 mol of glucose and 0.5 mol of the ammonium salt in water (25 mL). Each salt was considered to have only one active ammonium for the purpose of this reaction. After dissolution of the reagents, the pH of the solution was adjusted to pH 8.0, as that value had been found to be the optimum pH for the reaction of glucose with DAP.[32] In cases where the initial pH of the reaction was less than 8.0, diluted aq NaOH was added dropwise until pH 8.0 was reached. In the case of ammonium carbonate, the initial pH was 9.1, and it was reduced to 8.0 by using ammonium bicarbonate (total ammonia available for reaction kept the same as for the other reactions). After pH adjustment, each solution was placed in a Parr bomb and heated for 2.5 h at 93 °C. The reaction mixtures were cooled to 25 °C in an ice bath and were analyzed immediately (LC–MS) or were lyophilized for later studies (GC–MS, PY–GC–MS).

LC–MS determinations were performed on a VG Instruments 20-253 quadrupole mass spectrometer with either a thermospray or a plasmaspray LC–MS interface. The LC was operated isocratically with two Amino Spheri 5 columns (Brownlee, $4.6 \times 250$ mm) in series. The solvent system was 4:1 MeCN–water at 1.0 mL/min. The injection volume was 10 μL. Detailed instrumental conditions have been reported previously.[33] Identifications were confirmed by known standards and/or comparison with GC–MS data as described below.

GC–MS determinations were done on freeze-dried portions of the reaction mixtures. Approximately 50 mg of the lyophilized reaction mixtures was silylated with BSTFA (800 μL) and DMF (400 μL) at 75 °C for 0.5 h. The derivatized mixture (1 μL) was injected onto a J&W DB-5 column (60 m × 0.32 mm × 0.25 μm), and the temperature was ramped from 50 to 300 °C at 2 °C/min. The GC was a HP 5890 interfaced with to a VG 20-253 quadrupole mass spectrometer using a capillary direct interface. The manufacturer's standard conditions for EI(+) 70 eV spectra were used. Identifications of the spectra of the silylated compounds was done by comparisons with data in literature references.[34,35]

## 2. Note

US Patents can be obtained at http://www.uspto.gov/patft/index.html

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