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Preparation of 2,5-anhydro di-(hydrido) di-phosphate di-hydrate Mannitol (Glucitol) from Banana Fruit Yields a Possible Fructose 1,6 Bisphosphate Aldolase Inhibitor(s)

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Abstract: With previously unknown chemistry, 2,5-anhydro di-(hydrido)-di-phosphate-di-hydrate mannitol (glucitol) has (have) been prepared by H^- nucleophilic attack of neokestose-1,6-di-phosphate from commercial banana fruit. Negative ion mass spectrometry, Inductively Coupled Mass Spectrometry (ICP-MS), both ms and ms^2, has been used to characterize the product of this reaction. $NaBH_4$ in NH_4OH (pH 11.4, IN) for 18 hours was used to convert the 1,6-di-phospho neokestose to the 2,5 anhydro derivative(s). This molecule(s) are structural analogues of the known inhibitors of fructose-1,6 bisphosphate aldolase. The known inhibitors are 2,5 anhydro mannitol and 2,5 anhydro glucitol. They have K_is in the range of 10^{-3} mM. It is expected that the di-(hydrido) di-phosphate di-hydrate 2,5 anhydro mannitol (glucitol), prepared here, may be tighter binding to fructose bisphosphate aldolase than the known non-phosphorylated inhibitors, because of their structural similarity to the substrate of the enzyme, fructose-1,6-bisphosphate. If the prepared molecule(s) bind to fbp aldolase, their preparation from inexpensive banana fruit would lead to an inexpensive method for treating fast growing cancer cells. The low cost of making the molecule(s) would allow access to cancer treatment by destitute or low income people.

Keywords: Banana Fruit, Fructose-1,6-bisphosphate Aldolase (Fbp Aldolase) Inhibitors, Synthesis, Mass Spectrometry

1. Introduction

Fast growing cancer cells are known to require more energy. Therefore targeting glycolysis, i.e. fructose-1,6-bisphosphate aldolase (fbp aldolase), with fbp aldolase inhibitors, could be effective in the treatment of fast-growing cancer cells, the Warburg effect.[1]

Both 2,5 anhydro mannitol and 2,5 anhydro glucitol are known inhibitors of fructose 1,6-bisphosphate aldolase (fbp aldolase). Their K_is are in the 1.2×10^{-3} to 3.0×10^{-3} range, respectively. [2-4] Because of the possible increase in binding strength of the di-(hydrido) di-phosphate di-hydrate analogue (s) to fbp aldolase, and that this (these) 2,5 anhydro mannitol (glucitol) analogue (s) can be synthesized, simply, from banana fruit, this (these) molecule(s) is an important target for fast growing cancer cells' treatment.

Described herein is a method for the synthesis of the title compound(s) from banana fruit. The first step is the extraction of banana fruit with ethanol (95%). [5] Next a portion of this extract is added to NH_4OH (pH 11.4, 1 N). To this solution is added $NaBH_4$ and the mixture allowed to stand for 18 hours at ambient temperature. The NH_4OH is removed as NH_3 (g), by evaporation and the solution is analyzed by negative ion mass spectrometry (ICP), ms, and ms^2.

Because of the known inhibition of fbp aldolase by 2,5 anhydro mannitol and 2,5 anhydro glucitol, preparation of the di-(hydrido)-di-phosphate-di-hydrate analogues of 2,5 anhydro mannitol and 2,5 anhydro glucitol from an inexpensive source, banana fruit, and via a simple method, one step, with readily available reagents, people with fast growing cancers who are financially unable to cover the cost of expensive cancer treatments, could be treated with these analogues of fructose-1,6-bisphosphate, for cancer.

2. Materials and Methods

2.1. Isolation of Neokestose-1,6-di-phosphate

Ripe banana, commercially available, fruit is placed in a centrifuge tube. Ten volumes of ethanol (95%) is added and the tube shaken. The ethanol phase is collected by decantation. The ethanolic solution is analyzed by ms and ms^2. Negative ion ms and ms^2 were used to characterize the extract as neokestose-1,6-di-phosphate. [5-7]

2.2. Reaction of Neokestose-1,6-di-phosphate with $NaBH_4$ in NH_4OH (pH 11.4, 1N)

To a portion of ethanolic solution, noted above, (10 µL) was added $NaBH_4$ (3 µL, 4N solution) and NH_4OH (pH 11.4, 1N, 1.00 mL). The reaction mixture was allowed to stand at ambient temperature for 18 hours. The reaction mixture was evaporated with a stream of N_2 to no less than 200 µL to remove excess NH_4OH as NH_3 (g). The reaction mixture was cooled to below 0°C while awaiting analysis by negative ion ms and ms^2. For interpretation of ms and ms^2 negative ion Inductively Coupled Plasma ms and ms^2 spectra, principles described in [6] were used.

3. Results and Discussion

Shorter reaction times, e.g. 2 hours and 8 hours, produce only the di-(hydrido) Di-phosphate di-hydrate derivatives of neokestose 1,6-di-phosphate, as shown by negative ion ICP MS, Figure 1, ms, and Figure 2, ms^2.

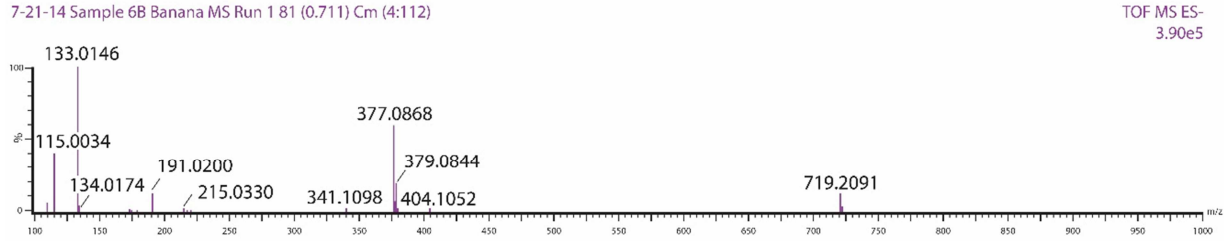

Figure 1. *Mass spectrum of the reaction product of neokestose 1,6 di-phosphate after treatment with $NaBH_4$ in NH_4OH (1N, pH 11.4).*

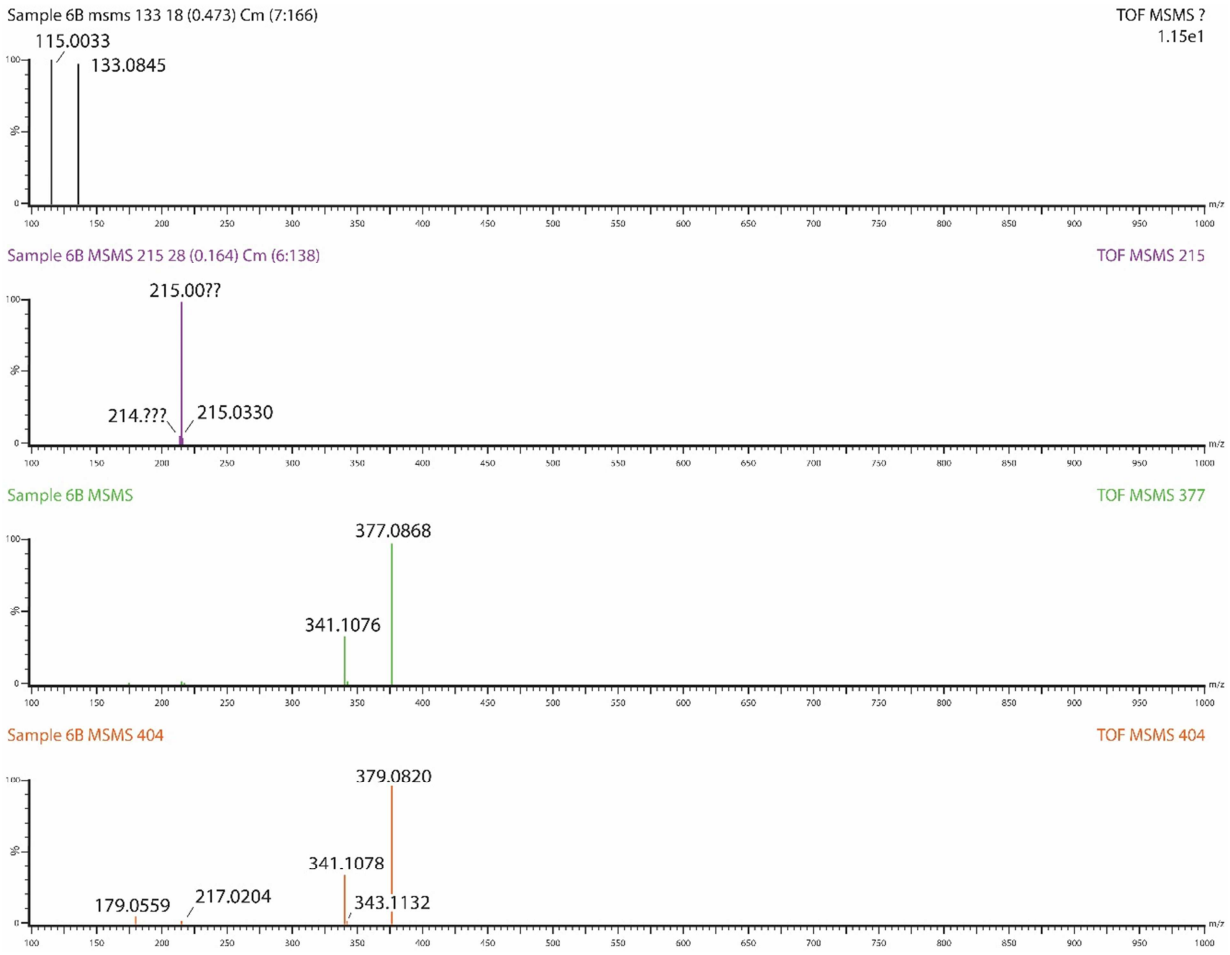

Figure 2. *Tandem mass spectra of four ions noted in Figure 1 of neokestose 1,6 di-phosphate after reaction with $NaBH_4$ in NH_4OH (1N, pH 11.4).*

This chemistry has been documented, for the case of bovine milk and K casein [6-7], respectively. The reason may be that the non-bonding orbital on the anomeric oxygen atom strengthens the P=O double bond with concomitant weakening of the C-1 to O-1 bond. [8-9] Attack of hydride at the anomeric center is facilitated by the leaving group nucleo-fugicity of the O-1-phosphoryl group. This chemistry produces the 1,5-anhydro derivatives of oligosaccharides, noted in previous work. [10-11]

There is evidence for H^- nucleophilic attack of the quaternary carbon, C-2 of fructofuran~~i~~osyl gluco~~fu~~pyranosyl fructofuranoside-1,6,-di-phosphaste, to form the corresponding 2,5-anhydro 1,6- di-(hydrido) di-phosphate di-hydrate mannitol, and the 2,5 anhydro 1,6-di-(hydrido) di-phosphate di-hydrate glucitol. This evidence is mass spectral and tandem mass spectral. Figures 3 through 7 denote fragment ions' structures. They are shown as the mannitol isomer. However, the glucitol isomer is just as likely to be formed.

In Figure 3, there is an ion, m/z 404.13; it is the di-hydrate. It loses two H^+ ions.

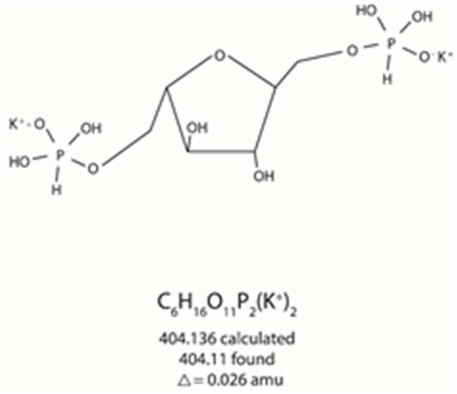

Figure 3. *Ion m/z 404 11 from total ms of neokestose 1, 6 di-phosphate reaction product.*

It is neutralized by K^+.

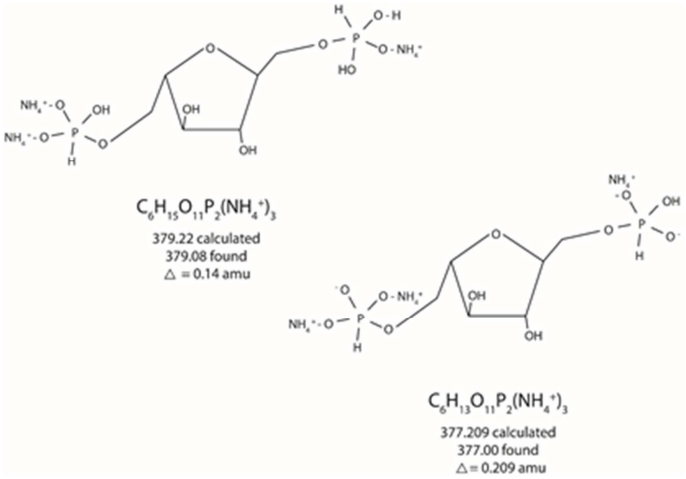

Figure 4. *Ion m/z 379.08 from total ms and ms^2 of neokestose 1, 6 di-phosphate reaction product.*

In Figure 4 the ion m/z 379.08 loses two H^+ ions and is neutralized by NH_4^+ ions with the loss of one additional H^+ ion additional H^+ ion.

The ion, m/z 377.08, base peak, loses two more H^+ ions from the 379.04 ion.

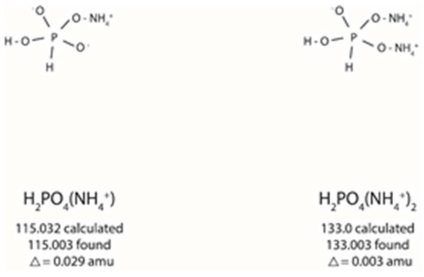

Figure 5. *The two ions m/z 133 and m/z 115.003 are from the total ms and ms^2 of neokestose 1,6-di-phosphate reaction product.*

The ion, m/z 133.19, is also derived from the m/z 379.08 ion. This ion loses two H^+ ions and it is partially neutralized by one Na^+ ion. The formula for this structure produces an ionic mass and it has three charges and is, therefore, divided by three. The fourth ion, m/z 115.03, is a fragmented (hydrido) phosphate hydrate less two H^+ ions.

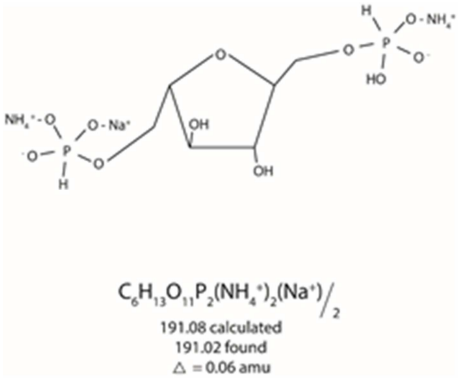

Figure 6. *Ion m/z 191.02 is from the total ms of neokestose 1,6 di-phosphate reaction product.*

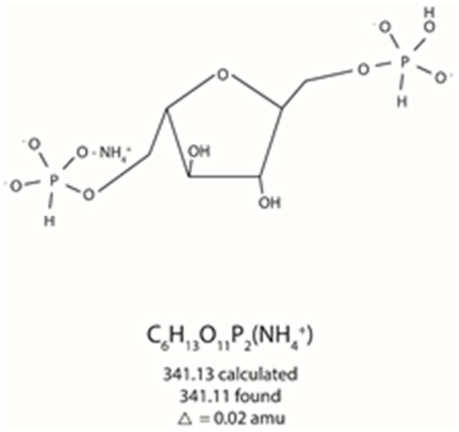

Figure 7. *Ion m/z 341.11 from the ms and ms^2 of the reaction of neokestose 1, 6 di-phosphate with $NaBH_4$ in NH_4OH (1N, pH 1.4).*

Ion, m/z 341, Figure 7, could possibly come from a cleaved glucopyranosyl fructofuranoside of the neokestose-1,6-di-(hydrido) di-phosphate-di-hydrate but not from the parent ion.

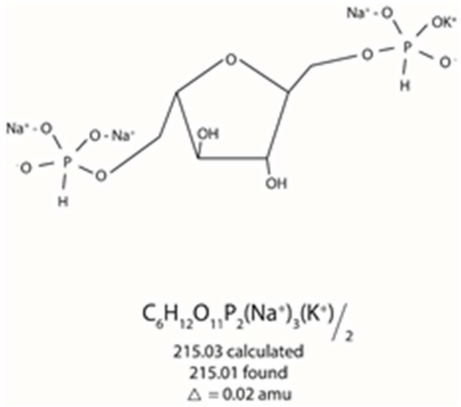

Figure 8. *Ion m/z 215.01 from the ms and ms^2 of the reaction of neokestose 1, 6 di-phosphate with $NaBH_4$ in NH_4OH (1N, pH 1.4).*

Ion, m/z 215.01, is shown in Figure 8. It produces no fragment ions from ms^2. It is drawn as the mixed, $NH_4^+$and Na^+, salt of the partially ionized hydrate, 2,5 anhydro 1,6-di-(hydrido)-di-phosphate di-hydrate mannitol (glucitol).

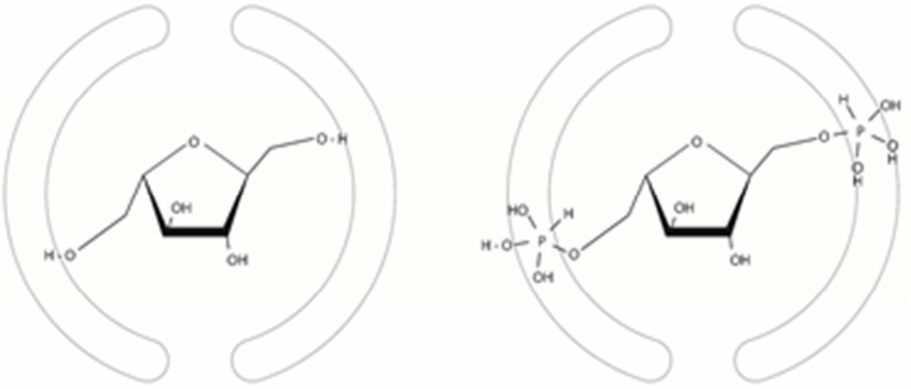

Figure 9. *The two 2,5 anhydro alditols with the mannitol configuration.*

Figure 10. *The 2,5 anhydro alditols with the glucitol configuration.*

In Figures 9 and 10 structures are drawn as a representation for the placement of 2,5-anhydro mannitol and its di-(hydrido) di-phosphate di-hydrate at 1 and 6 positions, Figure 9, and 2,5 anhydro glucitol and its 1,6 di-(hydrido) di-phosphate di-hydrate, Figure 10, in fbp aldolase. Note that, as stated previously, the placement of the primary hydroxyl groups of each molecule. The molecule with the primary hydroxyl groups farthest apart has a K_i higher for the unsubstituted 2,5 anhydro alditols than the molecule with the primary groups closer to each other. Note the Figures 9 and 10 have the di-(hydrido) diphosphate di-hydrate on the right of each Figure and represents the di-(hydrido) di-phosphate di-hydrate; both 2,5 anhydro mannitol and 2,5 anhydro glucitol molecules placed in fbp aldolase. The mannitol isomer may be a better inhibitor of fbp aldolase compared to the glucitol derivative. But both molecules, however, may be

effective inhibitors of this enzyme, since both unsubstituted 2,5 anhydro alditols are both inhibitors of fbp aldolase.

A mechanism for H^- attack of an anomeric center of neokestose-1, 6-di-(hydrido) di-phosphate di-hydrate is shown in Figure 11.

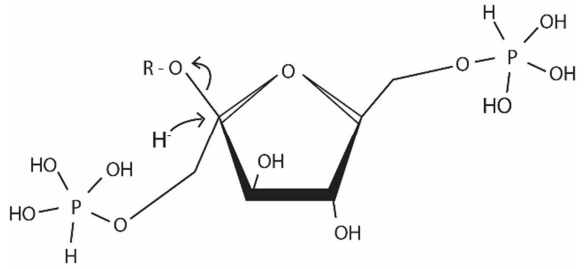

Figure 11. *Proposed mechanism for the H^- attack of neokestose in NH_4OH (1N, pH 11.4).*

Anomeric oxygen, O-2, and C-1 are in the same plane. O-5 and carbons C-2, C-3 and C-4 are in a plane 107° away, presumed to be tetrahedral, with respect to anomeric O-2. The available, unfilled, anti-bonding orbitals, σ^*, from the C-2-O-5 σ bond and the C-2-C-3 σ bond, as well as ring strain of the furanosyl ring, make nucleophilic attack, under an extended reaction time, 18 hours, at C-2 by H^-, possible.

Workers have discovered a method to analyze linkage positions for carbohydrates to include furctofuranosides via reductive cleavage. [12-13]. Their conditions require anhydrous conditions and silylation and treatment with a strong base. Here the 2,5 anhydro mannitol (glucitol) molecules in aqueous conditions under conditions that do not remove the phosphate derivatives, (hydrido) phosphate hydrates, are made.

4. Conclusions

Neokestose-1,6-bisphosphate, found in ripe banana fruit, can be converted, with, to date unknown chemistry, to mannitol (glucitol)-1,6-di-(hydrido) di-phosphate di-hydrate. The reaction conditions are; $NaBH_4$ in NH_4OH (pH 11.4, 1N) for 18 hours. This mannitol or glucitol di-phosphate analogue may be a better inhibitor than 2,5 anhydro mannitol or 2,5 anhydro glucitol, which are known inhibitors of fructose 1,6 bisphosphate aldolase, because of the phosphate derivatives at the 1 and 6 positions of either the mannitol or the glucitol derivative. Preparation of these potential inhibitors could yield inexpensive treatments for destitute or low income cancer patients.

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