Carbohydrate Research 431 (2016) 1–5

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Synthesis of 6-phosphofructose aspartic acid and some related Amadori compounds

Alexandar L. Hansen [a, **], Edward J. Behrman [b, *]

[a] Campus Chemical Instrument Center, The Ohio State University, 496 W. 12th Avenue, Columbus, OH 43210, USA
[b] Department of Chemistry & Biochemistry, The Ohio State University, 484 W. 12th Avenue, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 14 April 2016
Received in revised form
5 May 2016
Accepted 7 May 2016
Available online 14 May 2016
We dedicate this paper to the memory of Derek Horton.

Keywords:
Fructose-asparagine
Fructose-aspartic acid
6-Phosphofructose-aspartic acid
Salmonella
Amadori rearrangement

ABSTRACT

We describe the synthesis and characterization of 6-phosphofructose-aspartic acid, an intermediate in the metabolism of fructose-asparagine by *Salmonella*. We also report improved syntheses of fructose-asparagine itself and of fructose-aspartic acid.

1. Introduction

Fructose-asparagine [N-(1-deoxy-D-fructose-1-yl)-L-asparagine, CAS 34393-27-6] is an important nutrient for *Salmonella*. The proposed pathway for its metabolism involves deamination followed by phosphorylation to 6-phosphofructose aspartic acid [N-(1-deoxy-6-phospho-D-fructose-1-yl)-L-aspartic acid [1]. These molecules are Amadori rearrangement products resulting from the reaction of a sugar with an amine. Their chemistry and other properties have been ably reviewed [2]. Standard syntheses are conducted by heating the components in an appropriate solvent, most frequently methanol. Attempts to make the title compound failed completely in a variety of solvents because of the insolubility of glucose-6-phosphate. Prof. V. V. Mossine of the University of Missouri suggested to me a procedure that has solved the solubility problem for this molecule and has increased the yield for the synthesis of other Amadori products. One dissolves the components in water, adds glycerol or ethylene glycol, removes the water by rotary evaporation, and finally carries out the reaction by heating. Remarkably, all of the components remain in solution at every stage. Following the reaction, the glycol is separated from the product by the addition of 1- or 2-propanol which are miscible with both glycerol and ethylene glycol but in which the Amadori product is insoluble. Reactions are faster in ethylene glycol than glycerol perhaps due to the viscosity difference [3,4]. Purification of the crude material presented another problem. Standard procedures for Amadori products use a sulfonic acid ion exchange column to retain the product but not the excess sugar and other non-cationic molecules. Washing with ammonium hydroxide then elutes the product and excess amine. The title compound was so weakly bound to the resin that it was eluted with a water wash but still after the non-ionic components. This results in a better purification than is usual because the product is not contaminated with excess amine that remains bound to the column. Energy minimization calculations using the MM2 force field within ChemDraw reveals a possible reason for this interesting behavior. Shown in Fig. 1 are models for both the α- and β-anomers at a pH corresponding to that on the Dowex column. Under these conditions, the secondary amine is protonated and the phosphate group is a mono-anion. The sugar residue is bent in a macrocycle for both anomers so that the phosphate anion is close to the $-NH_2^+$- group thus weakening its

* Corresponding author.
** Corresponding author.
E-mail addresses: Hansen.434@osu.edu (A.L. Hansen), Behrman.1@osu.edu (E.J. Behrman).

http://dx.doi.org/10.1016/j.carres.2016.05.003
0008-6215/

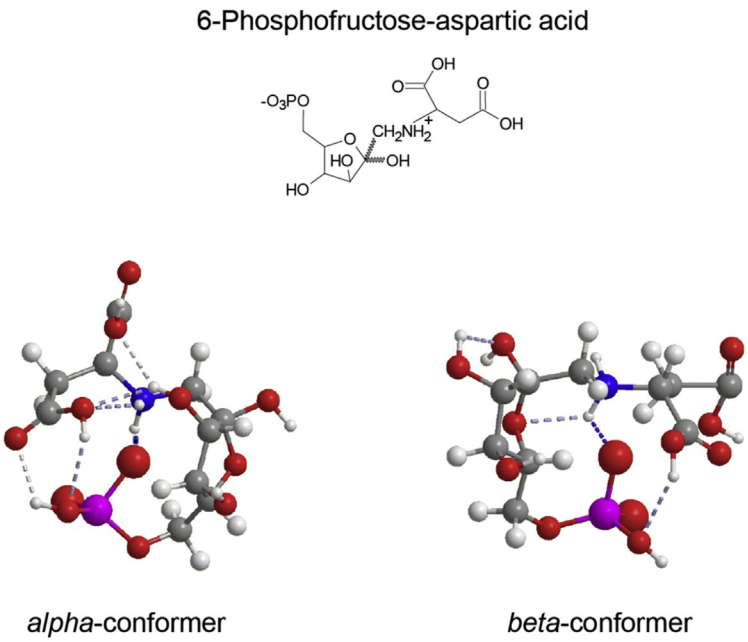

Fig. 1. Low pH conformations of 6-phosphofructose-aspartic acid.

interaction with the resin. The experimental results can be accounted for by a significant contribution of these conformations to the equilibria. This is one possibility; a reviewer has suggested another namely that both 6-PF-Asp and F-Asp are fully protonated on the Dowex column and that their separation is due to a sieving effect, the more extensive hydration of the phosphorylated molecule increasing its effective molecular weight. We favor the first explanation because we think that a molecule with a net charge of +1 will be more strongly bound than one with a net charge of zero Also, pKa values of molecules within the resin matrix are not well established. Perhaps both effects contribute. 6-Phosphofructose asparagine was made in the same way as 6-phosphofructose aspartic acid. Its behavior on the Dowex column was also the same implying the same protected conformation under acidic conditions, which were confirmed with additional modeling calculations.

The ^{1}H NMR spectra of 6-PF-Asp and 6-PF-Asn are complex due to about equal concentrations of the α- and β-furanose anomers for both molecules (Fig. 1). The ^{13}C spectra show the expected 20 resonances for a 1:1 mixture of the alpha and beta anomers. The 5′ and 6′ carbons show splitting by phosphorus. In addition, we have worked out improved conditions for the synthesis and purification of fructose asparagine and fructose aspartate.

2. Experimental

All specific rotations were at 25 °C in water. Exact masses were measured using a Bruker SolariX, 15 T, FT-ICR instrument. IR spectra were taken as Nujol mulls on a Shimadzu Affinity-1S instrument. NMR experiments were performed on a Bruker Avance IIIHD 800 MHz spectrometer equipped with a QCI-P cryoprobe at 25 °C. ^{13}C NMR spectra were recorded on a Bruker Avance IIIHD 700 MHz instrument equipped with a TXO cryoprobe at 25 °C. Accurate chemical shifts and scalar couplings were determined using CLIP/CLAP-HSQC experiments with a 1 s acquisition time, with or without ^{31}P decoupling [5]. Data were processed using TopSpin 3.5 and the nmrPipe suite of programs [6]. Proton and carbon chemical shifts are referenced indirectly to DSS based on the temperature dependence of the water resonance. Phosphorus chemical shifts were indirectly referenced to 85% phosphoric acid. Stereospecific assignment of the diastereotopic methylene protons of the fructose residues were not performed and therefore the assignments in the Tables may be reversed. Errors in the measurements are approximately 1 ppb for proton, 10 ppb for carbon and phosphorus, and 0.2 Hz for scalar couplings.

2.1. Fructose asparagine (F-Asn) [7,8]

Method A

D-glucose (4.1 g, 22 mmol), L-asparagine (0.5 g, 3.7 mmol), sodium bisulfite (0.2 g, 2 mmol), and malonic acid (0.2 g, 2 mmol) were dissolved with gentle heating in 5 mL water. Ethylene glycol (15 mL) was then added to form a homogeneous solution. About 80% of the water was removed by rotary evaporation at about 50 deg. The solution was placed in an incubator, uncovered, at 70° for about 17 h to yield a pale yellow still homogeneous solution. Longer heating results in the formation of brown degradation products which make the purification difficult. Ethylene glycol was removed by precipitation of the crude product by the addition of 1-propanol. The precipitated material was dissolved in a small amount of water and applied to 15 g of a Dowex 50 (200–400 mesh, hydrogen ion form) column (2.5 × 8 cm). The column was washed with about 500 mL of water and then the product together with some asparagine was eluted with 0.4 M ammonium hydroxide. The relevant fractions were evaporated first at RT to remove excess ammonia. This minimizes the formation of diglucosyl amine. Then the temperature was increased to 50° to remove water with a final drying by azeotroping with ethanol. A relatively large flask previously treated with dichlorodimethylsilane facilitates removal of the product. The yield is about 60–70% of an off-white free-flowing powder. It has the approximate composition F-Asn.0.25 NH_4^+ and contains about 10% asparagine as measured by proton NMR using the ratio of the intensity of the 3′-doublet at δ4.1 to the integration of the resonances from δ2.7–2.9 due to the beta protons of asparagine and the asparagine residue of F-Asn.