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Quantitation and test of enantiomeric purity of the L-ketohexoses by liquid chromatography with dual refractive index and laser-based chiroptical detection

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Abstract

Experimental data for the synthetic methods of preparation of the L-ketohexoses have been lacking an analytical method for monitoring the chemical or enzymatic reactions described. Dual refractive index and laser-based chiroptical detection provides an ideal method for following the reactions, since the refractive index detector quantifies the amount of analyte, while the ratio of optical rotation to refractive index response allows the enantiomer mole fraction to be determined. Sulfonated polystyrenedivinylbenzene resin in the Ca form as the stationary phase with H_2O at 80 °C as the eluent gives base-line separation of sorbose, fructose, tagatose, and psicose. Dependent on the complexity of the reaction mixture, analysis times range from 20 to 60 min.

Keywords: Chiroptical detection; Reaction monitoring; Ketohexoses

1. Introduction

The synthesis of L-sugars is of interest in organic chemistry since they are useful as sweetening agents. Unlike the D-sugars, they are not metabolized by the body or are metabolized to a lesser extent than the D-sugars. The sugars may also be used as moisture-imparting agents, crystallization-preventing agents, and gloss-imparting agents.

Four sugars make up the L-ketohexose group: L-sorbose, L-tagatose, L-psicose, and L-fructose. Chemical procedures for L-fructose have been reported by Fischer [1], Wolfrom and Thompson [2,3], and Chen and Whistler [4] as well as others [5,6]. Recently, the enzymatic production of L-tagatose and L-fructose from L-sorbose and L-psicose as substrates has been described [7,8]. The synthesis of L-sugars is of interest in organic chemistry since these sugars are useful as sweetening agents [9]. Unlike the D-sugars, they are not metabolized by the body or are metabolized to a lesser extent than the D-sugars [10]. These features make L-sugars desirable if one wishes to reduce caloric intake. The sugars may also be used as moisture-imparting agents, crystallization-preventing agents, and gloss-imparting agents. To date, obtaining experimental data to support the synthetic method(s) for the preparation of the L-ketohexose group has been hampered by a lack of modern analytical method(s) for monitoring the chemical or enzymatic reactions described [1–10]. Earlier ana-

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lytical methods supporting the enzymatic preparation of L-fructose employed paper chromatography, polarimetry, and derivatives for identification. Isolated crystals from ion-exchange chromatography were identified by HPLC analysis, infrared spectroscopy, specific rotation, and melting point. Reaction mixtures containing monosaccharides and alditols have been analyzed using an anion-exchange resin for separation and refractive index for detection but baseline separation was not obtained [11] and the enantiomeric purity was unknown. The specific rotation [α], has been used to address a variety of analytical problems: to determine the magnitude of racemization, to assess enantiomeric purity for a mixture, to determine the purity of a chromatographic peak, and to demonstrate that a synthetic transformation has occurred. Dual refractive index detection (RI) and laser polarimetry detection offer an ideal method for following the reaction since the RI detector quantifies the amount of analyte (sum of the D- and L-forms) while the ratio of optical rotation to RI response allows the enantiomer mole fraction to be determined. In some cases, the ultraviolet or diode array detector response can be used instead of the RI response.

2. Experimental

2.1. Materials

L-Fructose was obtained from Davos (Englewood Cliffs, NJ, USA). D-Fructose, D-sorbose, L-sorbose, and D-tagatose were obtained from Aldrich (Milwaukee, WI, USA). Psicose was available as a 70% solution from Pfanstiehl (Waukegan, IL, USA).

2.2. Chromatographic conditions

The HPLC system consisted of an Agilent Model G1311A pump and degasser, G1313A autosampler, G1315B diode array detector, and G1316A column thermostat. For primary detection, an Agilent G1362A refractive index detector and PDR-Chiral (Lake Park, FL, USA) Model ALP2000.01, laser based polarimeter with a 22-μl flow-cell and two-level internal calibration was used.

The separation of the L-ketohexoses was obtained using a MetaChem Technologies (Torrance, CA, USA) MetaCarb 87C, 300×7.8 mm, calcium cation-exchange column. The column was thermostated at 80 °C and the flow-rate of the mobile phase, Milli-Q water, was 1 ml/min. The concentration of the samples and standards was approximately 4 mg/ml in Milli-Q water. The injection volume was 30 μl.

3. Results and discussion

Dual refractive index and laser-based chiroptical detection were used to monitor the L-fructose synthesis procedure of Morgenlie [6]. A chromatogram of the individual ketohexoses plus the reaction mixture from the aldol step is shown in Fig. 1. The ketohexoses elution order was: sorbose 11.7 min, fructose 13.0 min, tagatose 14.6 min, and psicose 18.2 min. The total analysis time for the mixture obtained from the Aldol reaction was 25 min while the elution time for L-fructose obtained from fermentation with the Bakers' yeast was 60 min to allow endogenous substances to completely elute from the column. The response obtained for the refractive index detector was linear, $R^2=0.9999$, from approximately 0.4 to 6.0 mg/ml of L-fructose. Similar results were obtained with psicose and tagatose. With a ketohexose concentration of 2 mg/ml, a linear response was also obtained with injection volumes ranging from 10 to 60 μl, $R^2=0.9979$. The precision of the RI generated peak area for 100% D-fructose, $n=6$, was RSD%=0.02%. The precision obtained for the laser polarimeter generated peak areas, where $n=12$, was RSD%=0.51. The laser polarimeter response was positive or negative depending on the rotation [α], of the individual hexose. This can be illustrated for the mixture of 75% D-fructose:25% L-fructose which has a negative response and 25% D-fructose:75% L-fructose which has a positive response. A calibration curve was prepared for determining the weight percent and enantiomeric excess (ee) of D- or L-fructose (Fig. 2). The series of fructose standards prepared to obtain this calibration were: 100%D, 90%D::0%L, 75%D::25%L, 50%D::50%L, 25%D::75%L, 10%D::90%L, and 100% L. The bold line is the linear regression for the line, $R^2=0.9985$. To improve reliability, a second calibration curve was prepared for a limited con-

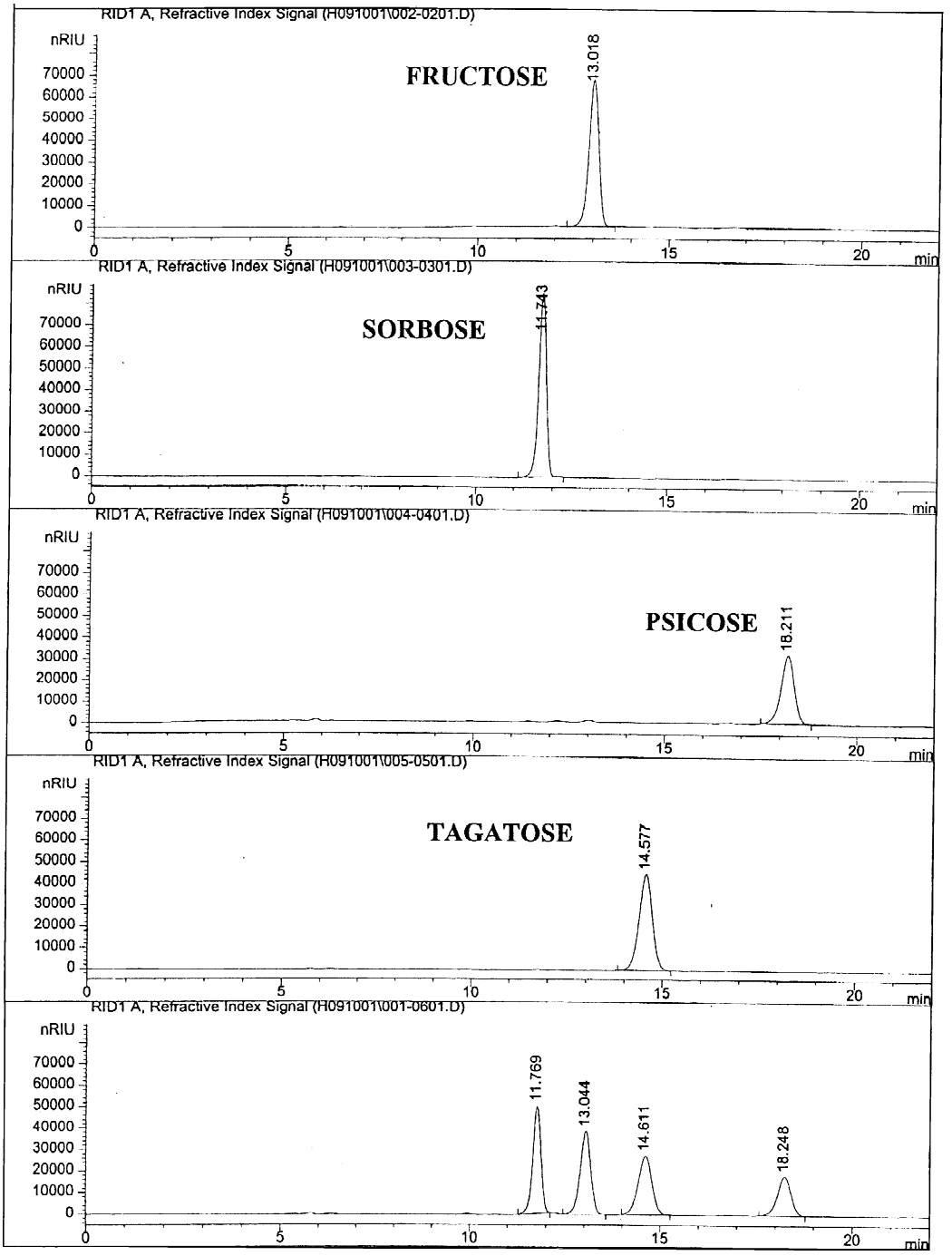

Fig. 1. Representative chromatograms for the separation of the L-ketohexose sugars. Separation with calcium cation exchange column, 300×7.8 mm, at 80 °C, 1 ml/min Milli-Q water, elution order: sorbose, fructose, tagatose, and psicose.

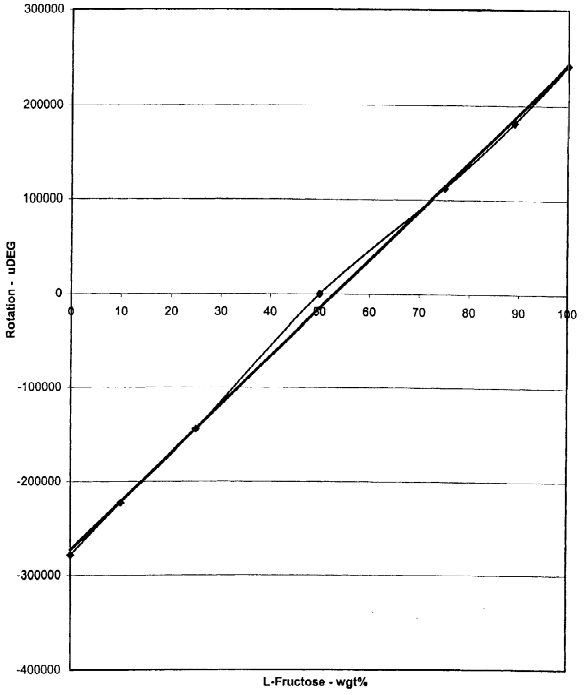

Fig. 2. Laser polarimeter calibration curve for weight percent mixtures of D- and L-fructose, polarimeter's peak area response versus L-fructose wt%. The bold line is the calculated linear regression.

centration range, 80–100% L-fructose, which was the desired ketohexose. The standards used for this calibration were: 80%L::20%D, 90%L::10%D, 94%L::6%D, 96%L::4%D, 98%L::2% D, and 100% L. The linear regression for the line had a $R^2=0.9973$. Using a second calibration curve (Fig. 3), the limit of detection for the fructose was 0.1% (w/w) and the ee limit of quantitation is 0.5%. The enantiomeric excess in a sample was determined using an achiral column.

For calculating the enantiomeric excess, software supplied by PDR-Chiral was employed. It consisted of an enantiomeric excess Excel worksheet. To perform the calculation, the system was calibrated first using the RI detector followed by the laser polarimeter using known amounts of the pure enantiomer. Measuring the response of the racemic mixture with the RI detector and the optical rotation using the laser polarimeter, the percentage of each enantiomer contained in the sample can be calculated

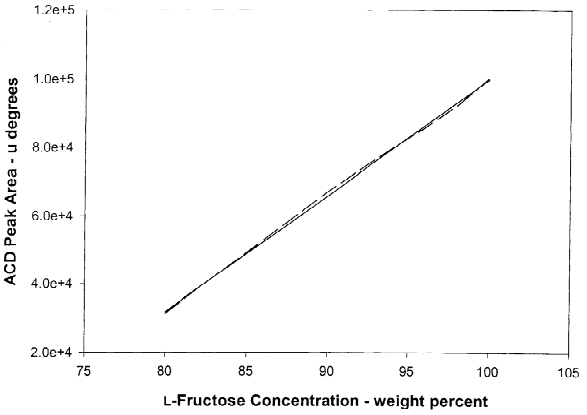

Fig. 3. Laser polarimeter calibration curve for the weight percent mixtures of L-fructose for the concentration range 80–100 wt%. The dotted line is the experimental data and the linear regression is the solid line.

and subsequently used to determine the enantiomeric excess. The measurable %EE for unresolved enantiomers is limited by the degree to which the RI detector's effective linear range overlaps the laser polarimeter's effective linear range. The refractive index detector's effective linear range is limited by the capacity of the column, the detector' linear range and the chromatographic peak volume. The polarimeter's effective linear range is constrained by the specific rotation of the desired compound, the detector's linear dynamic range and the chromatographic peak volume. If the concentration overlap is two orders of magnitude, the minimum %ee will be 1% (50.5/49.5) and the maximum measurable would be 99% (99.5/0.5).

The reaction rate monitored by RI and the laser polarimeter for the fermentation reaction is shown in Fig. 4. In the top figure, the refractive index peak area for the racemic fructose decreases as the D-fructose is consumed in the fermentation process. The rotation goes from essentially zero to a maximum positive rotation in approximately 1 h as the L-fructose concentration increases and the D-fructose concentration decreases, bottom (Fig. 4). L-Fructose's %ee determined for the fermentation reaction with and without yeast preincubation was determined. With yeast preincubation, L-fructose %ee was 85% after 2 h fermentation compared to a 76 ee% without yeast preincubation.

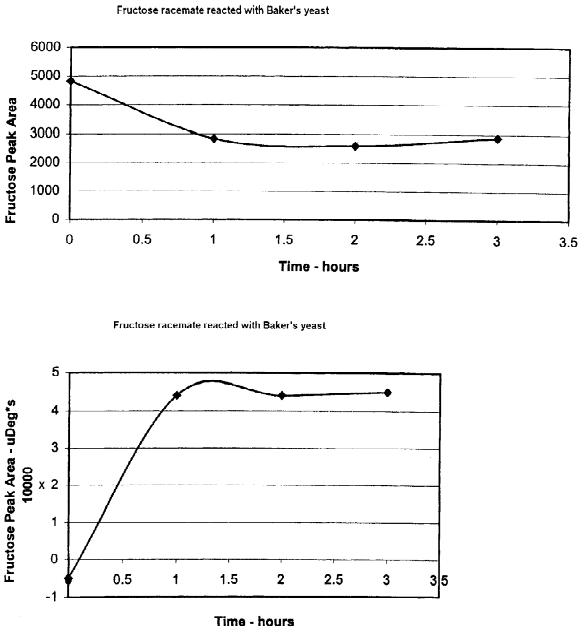

Fig. 4. Top: fermentation reaction monitored using refractive index detector. Bottom: fermentation reaction monitored using the laser polarimeter.

4. Conclusions

Dual refractive index and laser-based polarimetric detection is an ideal method for following reactions for the synthesis of L-ketohexoses and other sugars. The RI detector quantifies the amount of analyte while the ratio of optical rotation to absorbance or RI response allows the enantiomer mole fraction to be determined. With this technology, a real-time assessment of enantiomeric excess can be obtained to optimize and guide the enantioselective separation or reaction process. The Ca form of the stationary phase was found to be a reliable and stable for over 6 months (also see Makkee et al. [11]). It provided reasonable analysis times depending on the complexity of the reaction mixture. The chromatography was very reproducible with RSD$<$1% and it does not require pre- or post-column derivatization.

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