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Production of rare sugars from common sugars in subcritical aqueous ethanol

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ABSTRACT

A new isomerization reaction was developed to synthesize rare ketoses. D-Tagatose, D-xylulose, and D-ribulose were obtained in the maximum yields of 24%, 38%, and 40%, respectively, from the corresponding aldoses, D-galactose, D-xylose, and D-ribose, by treating the aldoses with 80% (v/v) subcritical aqueous ethanol at 180 °C. The maximum productivity of D-tagatose was *ca.* 80 g/(L h). Increasing the concentration of ethanol significantly increased the isomerization of D-galactose. Variation in the reaction temperature did not significantly affect the production of D-tagatose from D-galactose. Subcritical aqueous ethanol converted both 2,3-threo and 2,3-erythro aldoses to the corresponding C-2 ketoses in high yields. Thus, the treatment of common aldoses in subcritical aqueous ethanol can be regarded as a new method to synthesize the corresponding rare sugars.

1. Introduction

Rare sugars have attracted much attention in food, chemical, and fuel industries. There are many rare ketoses such as D-tagatose, D-xylulose, and D-ribulose. Because D-tagatose has low energy (almost 0 kJ/g of food energy) and can be used as a drug to control type-2 diabetes and obesity, it can be used as a sweetener substitute for sucrose (Levin, Zehner, Saunders, & Beadle, 1995; Lu, Levin, & Donner, 2008). Besides this, dietary restriction of energy by the intake of D-tagatose reduces the incidence of neoplastic lesions and significantly extends the maximum life span (Levin et al., 1995). D-Xylulose is not only an energy resource for growth but can also be used in the pharmaceutical chemistry (Beerens, Desmet, & Eoetaert, 2012) and to synthesize furfural as a safe flavour ingredient in food (Adams et al., 1997). There are few reports on the physiological effects of D-ribulose because of the difficulty in its production and its high cost. D-Ribulose is an important material for the synthesis of nucleosides (Marugg, Tromp, Kuyl-Yeheshiely, van der Marel, & van Boom, 1986). However, among the possible isomers, only seven pentoses and hexoses (D-glucose, D-mannose, D-fructose, D-xylose, D-galactose, D-ribose, and L-arabinose) are found in sufficient quantities in nature to satisfy commercial needs (Gunther et al., 2012). The conversion of these abundant common sugars to rare sugars has become necessary and attracted much attention.

The isomerization of common sugars to rare sugars has mainly been performed by alkali, metal, and enzymatic catalyses. In alkali-catalyzed isomerization reactions, glucose-type monosaccharides (2,3-threo-type) have been shown to be easily isomerized to the corresponding C-2 ketoses such as glucose to fructose isomerization; however, by the same method, mannose-type monosaccharides (2,3-erythro-type) were isomerized to the C-2 ketoses in low yields, such as mannose to fructose isomerization (Khadem, Ennifar, & Isbell, 1989). Using alkali-catalyzed isomerization, D-tagatose, D-xylulose, and D-ribulose have been obtained in low yields from the corresponding glucose-type aldoses (Khadem et al., 1989; Mendicino, 1960; Tipson & Brady Jr, 1969). However, alkali-catalyzed isomerization usually results in many by-products due to various side reactions, and this process often requires tedious purification steps.

The product distribution of metal-catalyzed isomerization reactions has a complicated dependence on the type of metal ions, substrate saccharides, cosolvent, and support of metal ions. By using *N,N,N′,N′*-tetramethylenediamine and calcium chloride together in a methanolic solution, Ca^{2+} converted D-galactose to D-tagatose in 48% yield (Yamauchi, Fukushima, Yanagihara, Osanai, & Yoshikawa, 1990). However, D-galactose concentration was too low for practical applications. Recently, D-xylulose was synthesized in *ca.* 30% yield from D-xylose with an initial concentration of 10 wt% using a tin-containing zeolite as the catalyst (Choudhary, Caratzoulas, & Vlachos, 2013). The preparation of tin-containing zeolite takes many days, and the reaction mixture may contain heavy metals. The synthesis of D-ribulose using metal

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catalysts has not been satisfactory because of the low yield or troublesome postprocessing. An expedient synthesis of D-ribulose was performed by treating D-arabinose with a Pd/C catalyst, leading to an overall yield of 20% (Vourinen & Serianni, 1990). However, D-arabinose is an expensive rare sugar.

Biotransformations of abundant saccharides to rare saccharides have been performed primarily using aldose–ketose isomerases, epimerases, and polyol dehydrogenases (Izumori, 2006). L-Arabinose isomerase (EC 5.3.1.4) has been commonly used to directly synthesize D-tagatose from D-galactose on a large scale (Jørgensen, Hansen, & Stougaard, 2004; Lim, Kim, & Oh, 2007). By utilizing an L-arabinose isomerase, 370 g/L D-tagatose was obtained from 500 g/L D-galactose with a reaction time of 24 h (Lim et al., 2007). Xylose isomerase (EC 5.3.1.5), which is generally used in the production of high-fructose corn syrup from glucose, can also synthesize D-xylulose directly from D-xylose. However, the chemical equilibrium is evidently not favourable for D-xylulose in the enzymatic isomerization because D-xylulose was obtained only in *ca.* 25% overall yield at room temperature (Chiang, Hsiao, Ueng, & Tsao, 1981). Attempts to prepare D-ribulose to allow commercial production have not been successful even by utilizing enzymes because of the low yield and complicated pretreatment and postpurification.

Therefore, it is necessary to develop a new method to synthesize these rare sugars simply and efficiently, preferably by one-step isomerization. It was reported that the isomerization of monosaccharides occurred in subcritical water, in which aldoses easily isomerized to the corresponding C-2 ketoses (Lü & Saka, 2012; Usuki, Kimura, & Adachi, 2007). The kinetic analysis showed that among the isomerization reactions of glucose, mannose, and fructose, mannose most easily isomerized to fructose (Usuki et al., 2007). However, the yields of the derived monosaccharides were still low despite varying the reaction temperature (Usuki et al., 2007). However, the reaction equilibrium constants of the monosaccharide isomerization of aldoses to ketoses became larger with increasing reaction temperature (Moliner, Roman-Leshkov, & Davis, 2010). Adding an organic solvent often changes the apparent chemical equilibrium and reaction rate. Among the organic solvents investigated, ethanol can be safely used in food manufacturing processes. Vourinen and Sjostrom (1982) reported that when the isomerization of glucose and fructose was carried out in aqueous ethanol in the presence of sodium hydroxide, the rate constants of glucose to fructose isomerization, and *vice versa*, significantly increased with increasing ethanol concentration, and the yield of fructose in 70 wt% ethanol almost tripled compared to that obtained in water (Vourinen & Sjostrom, 1982). Similar effects of ethanol were also observed in enzymatic isomerization, and the equilibrium yield of fructose from glucose became *ca.* 60% in 90 wt% aqueous ethanol (Visuri & Klibanov, 1987). Furthermore we have previously reported that the addition of ethanol promoted the isomerization of glucose to fructose during the hydrolysis of sucrose in subcritical aqueous ethanol (Gao, Kobayashi, & Adachi, 2014).

In this context, the current study aimed to investigate the isomerization of two glucose-type aldoses, D-galactose and D-xylose, and a mannose-type aldose, D-ribose, in subcritical aqueous ethanol for efficiently producing the corresponding rare C-2 ketoses, D-tagatose, D-xylulose, and D-ribulose.

2. Materials and methods

2.1. Materials

D-Xylose, D-lyxose, D-galactose, D-tagatose, D-talose, and D-arabinose were purchased from Wako Pure Chemical Industries (Osaka, Japan). D-Xylulose and D-ribose were purchased from Sigma–Aldrich Japan (Tokyo, Japan). Because this study focussed on the isomerization of only the D-enantiomers, the prefix, D-, of all the saccharides is omitted hereafter.

2.2. Isomerization of common sugars to rare sugars in subcritical aqueous ethanol

The isomerization reactions were carried out in a coiled stainless steel tubular reactor (0.8 mm I.D. × 1.0 m length) heated in a silicone oil bath. To terminate the reaction, the reactor effluent was directly introduced to a stainless steel tube immersed in an ice bath. The residence time was set in the range 30–500 s. The reaction temperature was set at 180 °C for the treatment of xylose and ribose and at 160–200 °C for the treatment of galactose. Xylose and ribose were treated in 80% (v/v) aqueous ethanol, and the ethanol concentration was changed from 0 to 80% (v/v) for the treatment of galactose. Each monosaccharide was dissolved in distilled water, and the resulting solution was mixed with ethanol to obtain a solution at the predetermined concentration. The concentrations of both xylose and ribose were adjusted at 0.5% (w/v), and the galactose concentration was 0.5–8.5%. The pressure inside the reactor was regulated at 10 MPa using a back-pressure valve (Upchurch Scientific Inc., Oak Harbor, WA, USA).

The residence time was calculated according to the method reported in our previous study (Gao et al., 2014).

2.3. HPLC analysis

The reactor effluent was collected into a test tube, and the liquid samples were analyzed by high-performance liquid chromatography (HPLC). The HPLC system was equipped with an LC-10ADVP pump (Shimadzu, Kyoto, Japan), a COSMOSIL Sugar-D column (4.6 mm I.D. × 250 mm length, Nacalai Tesque, Kyoto, Japan), and a refractometer (RI-101, Showa Denko, Tokyo, Japan). A mixture of water and acetonitrile (20:80, v/v) was used as the eluent at a flow rate of 1 mL/min. The column temperature was regulated at 30 °C using a CTO-10A VP column oven (Shimadzu). Lyxose was analyzed by a combination of two columns, a COSMOSIL Sugar-D column and a Ca^{2+} ion-exchange column, SUPELCOGEL CA (7.8 mm I.D. × 300 mm length, Sigma–Aldrich, Tokyo, Japan), under the same conditions as for the other saccharides.

2.4. Purification and NMR confirmation of rare sugars

The reactor effluent (*ca.* 200 mL) was concentrated to *ca.* 1 mL using a rotary evaporator. The concentrated solution was treated using a Strata C18-E cartridge column (55 μm, 70 Å, Shimadzu) as pretreatment. A solution of 50% (v/v) aqueous methanol was used as the eluent. The solvent in the effluent from the cartridge was evaporated using a rotary evaporator. The purification of the target saccharide was performed using a COSMOSIL Sugar-D column (10 mm I.D. × 250 mm length, Nacalai Tesque) using 95% (v/v) acetonitrile as the eluent at a flow rate of 3 mL/min.

The ^{1}H NMR analyses of the purified saccharides were carried out using an Ascend 400 MHz NMR spectrometer (Bruker Japan, Osaka, Japan) with D_2O as the solvent. Acetonitrile (δ_H = 2.06 ppm) was used as the internal standard. The ^{1}H NMR spectra of the purified tagatose, xylulose, and ribulose were compared with those of the commercial samples or with literature data (Vourinen & Serianni, 1990). Confirmation of arabinose, lyxose, and talose was performed by comparing their retention times in the HPLC chromatograms with the commercial ones, as the low yields of purified products obtained prevented NMR analysis.