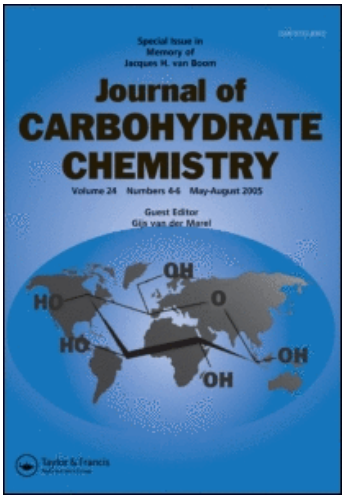

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COMMUNICATION

CONVENIENT CHEMO-ENZYMATIC SYNTHESIS OF D-TAGATOSE

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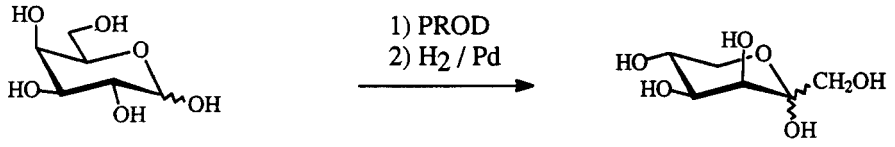
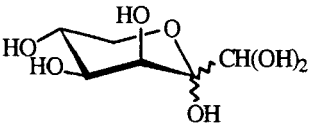
The ketohexose D-tagatose is a rare sugar that is of interest as a potent noncaloric sweetener. The synthesis of D-tagatose has been accomplished by microbiological conversion of dulcitol[1,2] as well as by chemical syntheses in low yields originating from D-galactose[3] or D-fructose.[4] Recently, some patents concerning the synthesis of D-tagatose have been published.[5,6,7] We here present an alternative approach to the preparation of D-tagatose by a combined chemo-enzymatic synthesis starting from D-galactose. Enzymatic oxidation of D-galactose (**1**) leads to the 2-oxidised product, D-galactosone, which in turn is reduced chemically to D-tagatose (**2**). The target sugar is thus available in a one-pot / two-step procedure in a yield of 30 %.



1 **2**

The selective oxidation of the 2-hydroxy group of D-galactose may be accomplished utilising pyranose 2-oxidase (PROD) in combination with a H_2O_2 destroying enzyme, catalase. The enzyme PROD is available from different sources;[8-12] the natural substrate of this enzyme is D-glucose, but activity with D-galactose has been claimed in several cases.[9,10a,12,13] The latter oxidation was, however, never reported as being carried out in a preparative scale, nor was the enzymic reaction product characterised chemically, which is presumably due to the complex composition of solutions of ketohexoses. We used an immobilised pyranose 2-oxidase from *Peniophora gigantea* to obtain D-galactosone (**3**) in yields of 60-80%.[14] Solutions of the aldosulose D-galactosone are complex mixtures; in water at least 15 different isomeric forms exist and a detailed analysis of the NMR spectra including the unequivocal determination of the ratio of different isomeric forms is currently under investigation. However, the mixture of isomers has as a main component (37 %) the hydrated derivatives of a α, β-D-pyranose **3**, formed by cyclisation of the keto group.



3

A chemical reduction of pure D-galactosone with hydrogen and Pd/C mainly leads to D-tagatose (**2**, 76 %), beside minor proportions of two products formed through reduction of the keto group, D-galactose (16 %) and D-talose (8 %). When the hydrogenation is carried out without intermittent isolation of D-galactosone the product D-tagatose (**2**) may be isolated in a yield of 30% based on D-galactose (**1**).

D-Tagatose (**2**) was identified by comparison with authentic samples and NMR analysis. The ketohexose D-tagatose forms a mixture of isomeric forms with the pyranose forms prevailing. The isomeric composition had previously been characterised by ^{13}C NMR spectroscopy;[15-17] we now additionally identified the different forms by ^{1}H NMR analysis.

The ratio of different isomers found in the ^{1}H NMR spectra was nearly identical to what has been deduced from integration of the ^{13}C NMR signals by Breitmeier et. al.[17] (Table 3). The proportion of the acyclic form increases at

TABLE 1. ^{1}H NMR data of isomeric forms of D-tagatose (**2**).

	α-pyranose		ß-pyranose		ß-furanose		α-furanose		acyclic	
H-1	3.516	$J_{1,1'}$ = 11.9	3.494	11.8	3.396	12.0	n.d.	n.d.	4.436	19.5
H-1′	3.317	$J_{3,4}$ = 2.6	3.301	3.5	3.353	4.8	n.d.	4.7	4.336	7.5
H-3	3.696	$J_{4,5}$ = n.d.	3.739	4.1	4.007	3.8	3.992	4.9	4.158	2.1
H-4	3.652	$J_{5,6e}$ = 5.1	3.819	2.1	4.136	7.1	4.265	4.2	3.585	n.d.
H-5	3.649	$J_{5,6a}$ = 10.6	3.721	3.1	3.891	4.9	4.106	6.2	n.d.	n.d.
H-6e	3.556	$J_{6e,6a}$ = 11.0	3.980	13.2	3.677	11.7	3.600	12.2	n.d.	n.d.
H-6a	3.422		3.402		3.589		3.518		n.d.	

n.d. not determined.

TABLE 2. Complete ^{13}C NMR data of the isomeric forms of D-tagatose (**2**).

	α-pyranose[15]	ß-pyranose[15]	ß-furanose[15]	α-furanose[15]	acyclic[a)]
C-1	64.15	63.73	62.86	62.64	66.93
C-2	98.35	98.54	102.71	105.10	213.28[17)]
C-3	70.04	63.96	70.90	76.92	73.68
C-4	71.12	71.12[a)]	71.27	71.43	70.53
C-5	66.55	69.44	80.24	79.41	71.51
C-6	62.44	60.35	61.20	60.29[a)]	62.72

a) this work.

TABLE 3. Composition of D-tagatose (**2**) in D_2O by ^1H NMR and ^{13}C NMR analysis.

Temperature	α-pyranose (%)	ß-pyranose (%)	ß-furanose (%)	α-furanose (%)	acyclic (%)
^1H NMR:					
27 °C	78	14	5	2	<0.5
31 °C	75	16	6	2	<0.6
60 °C	66	17	10	4	3
^{13}C NMR:					
27 °C[15]	79	16	4	1	
30 °C[16]	71	15	9	5	
31 °C[17]	71.3	18.2	7.6	2.6	<0.3

elevated temperatures and it was thus possible to assign the ^{13}C NMR resonances of this form in addition to the complete assignment of the ^1H NMR resonances of all forms (Tables 1 and 2).

EXPERIMENTAL

Catalase (EC 1.11.16) was purchased from NOVO Nordisk Industri, Copenhagen, Denmark. Pyranose 2-oxidase (EC 1.1.3.10) was isolated and immobilised as described.[12b]

Reactions were monitored by TLC on silica gel plates ($60F_{254}$, Merck) using phenol/water 4:1 as eluent. Spots were detected by staining with ethanolic sulphuric acid and charring. For chromatography a DOWEX 50W X8 column (600 x 26 mm, loaded with Ca^{2+}) was used at a flow rate of 10 mL/min at medium pressure with water as eluent at 71 ^{0}C and a RI detector for monitoring. Ultrafiltration was performed with Amicon YM 10 membranes.

NMR spectra were recorded in D_2O on a Bruker ARX 500 at 500.14 MHz for the ^{1}H and 125.76 MHz for the ^{13}C nucleus. Chemical shifts are referenced to internal acetone (δ = 2.030 and 30.50 ppm). 2D spectroscopy (H/H-COSY and C,H-COSY) was applied using the standard Bruker software.

α- and β-D-*Lyxo*-hex-2-ulose (D-tagatose) (2). D-Galactose (500 mg, 2.8 mmol), immobilised pyranose 2-oxidase (46 U), and catalase (0.1 mg, 5000 U) were suspended in water (200 mL). Gentle stirring and aeration (0.01 L/min) through a porous sintered glass tube was maintained for 6 d and the pH was kept constant at 7.0 by automatic titration with NaOH (0.1 N). The mixture was filtered through sintered glass funnels (16-40 μm) to remove the immobilised enzyme; soluble catalase was removed by ultrafiltration. The mixture was lyophilised and the brownish foam was redissolved in water (6 mL), Pd/C was added (78 mg) and the mixture was stirred under an atmosphere of hydrogen (1.5 atm) for 5 d. The mixture was filtered over celite, the catalyst was washed with hot water (3 mL, 100 ^{0}C) and the combined filtrates were concentrated. In order to obtain pure D-tagatose, the product was separated twice on a chromatographic column, which first eluted D-galactose, followed by D-tagatose and D-talose and yielded 149 mg (30 %) of crystalline D-tagatose, mp 129 ^{0}C (Lit[18] 134-135 ^{0}C from ethanol).

α- and β-D-*Lyxo*-hexos-2-ulose (D-galactosone) (3). D-Galactose (**1**) (1.0 g, 5.55 mmol) was oxidised as described above using 58 U of pyranose 2-oxidase for 2.5 d. Filtration and lyophilisation yielded a product that contained 80% of D-galactosone as shown by ^{1}H NMR spectroscopy. In order to obtain pure D-galactosone the product was purified twice by column chromatography which afforded 252 mg (23 %) of D-galactosone (**3**), mp 104 ^{0}C (Lit[19] 89.9 ^{0}C); $[\alpha]_D^{20}$ +8.7^0 (*c* 0.12 in H_2O); MS (70 eV) *m/z* (%): 179 (13) [M+1], 161 (100) [M+1 -H_2O], 133 (18) [M+1 - H_2O - CO], 115 (19) [M+1 - 2 H_2O - CO].

Hydrogenation of purified D-galactosone. To D-galactosone (**3**) (89 mg, 0.45 mmol) dissolved in water (5 mL), was added Pd/C (25 mg) and the mixture was stirred for 10 d under a hydrogen atmosphere (1.5 atm). The mixture was filtered through celite and the filtrates concentrated. According to

^{1}H and ^{13}C NMR spectral analysis, the reaction mixture consisted of D-tagatose, D-galactose, and D-talose[20] in a ratio of 76:16:8.

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