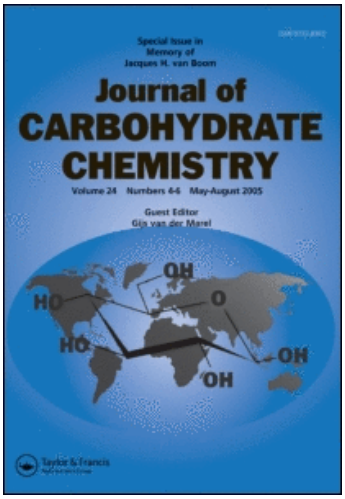

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HPLC ANALYSIS OF THE PRODUCT DISTRIBUTION IN THE IODINE–CATALYZED METHYL GLYCOSIDATION OF PENTOSES AND 6-DEOXYHEXOSES

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ABSTRACT

The product distribution of the iodine–catalyzed methyl glycosidation of four pentoses (D-ribose, D-arabinose, D-xylose, and D-lyxose) and two 6-deoxyhexoses (L-rhamnose, and D-fucose) was studied by HPLC using an APS column (dihydrogen sulphate form) with different acetonitrile–water mobile phases. In agreement with earlier results, a temperature dependent on–column isomerization was observed for all the investigated aldoses, except for ribose. The first-eluted furanosides were followed by pyranosides, and the free sugars were eluted last with the highest retention volumes.

INTRODUCTION

A recently published procedure[1] for the preparation of methyl glycofuranosides of D-fructose and D-glucose reported that the product distribution of the iodine–catalyzed process was governed by kinetic control, and under the applied conditions the furanoside →pyranoside isomerization was rather slow. As pentofuranosides are valuable intermediates in various nucleoside syntheses, we decided to study the applicability of the

reported procedure for the preparation of methyl pentofuranosides. The on–column isomerization of free sugars was investigated, and suitable conditions were elaborated for the separation of the non-isomerizable glycosides. The composition of the fractions was determined by polarimetry and, in some cases, by ^{1}H NMR spectroscopy.

RESULTS AND DISCUSSION

On–Column Isomerization During HPLC: Nishikawa *et al.*[2] have shown that free sugars frequently undergo on–column isomerization on an Aminex HPX-87C (Ca^{2+} form) ion–exchange column, and the rate of isomerization is dependent on the temperature. We observed a similar phenomenon on an amino column, which was converted into the sulphate form. Separation of the isomers (furanose/pyranose) and anomers (α/β) could only be achieved at low temperature. Isomerization was observed in the case of all of the monosaccharides studied, except for D-ribose, which eluted as a single peak—independent of the temperature. As D-ribose is known to present in solution in all of the four possible forms (α-p : β-p : α-f : β-f = 21.5 : 58.5 : 6.5 : 13.5),[3] our observation can only be explained by a very fast on–column isomerization.

The elution profiles changed as a function of the column temperature (Fig. 1a. and 1b.). At 50 °C a broad peak was observed in each case; at 30 °C two peaks with a bridge appeared in the case of arabinose, xylose and rhamnose, two peaks for fucose, and a single peak for lyxose. A small peak appeared preceding the two big peaks in the case of both arabinose and fucose at 0 °C, as these sugars may also undergo α→β and furanose→pyranose transformations. In other cases (lyxose, xylose, and rhamnose) two peaks were observed, which could be assigned to the six-membered pyranose ring-form of α- and β-monosaccharides.

The peaks were identified according to data reported in the literature. Table 1 shows the results of our measurements, and a comparison of these data with those described earlier. Except for arabinose, the α-pyranoses always eluted before the β-pyranoses. The observed retention sequence of the anomers is in agreement with the suggestion of Jäger *et al.*[4] that the more hydroxyl groups located *equatorially* in the molecule the longer the retention time. It is probable that α-D-arabinose adopts the $^1C_4$

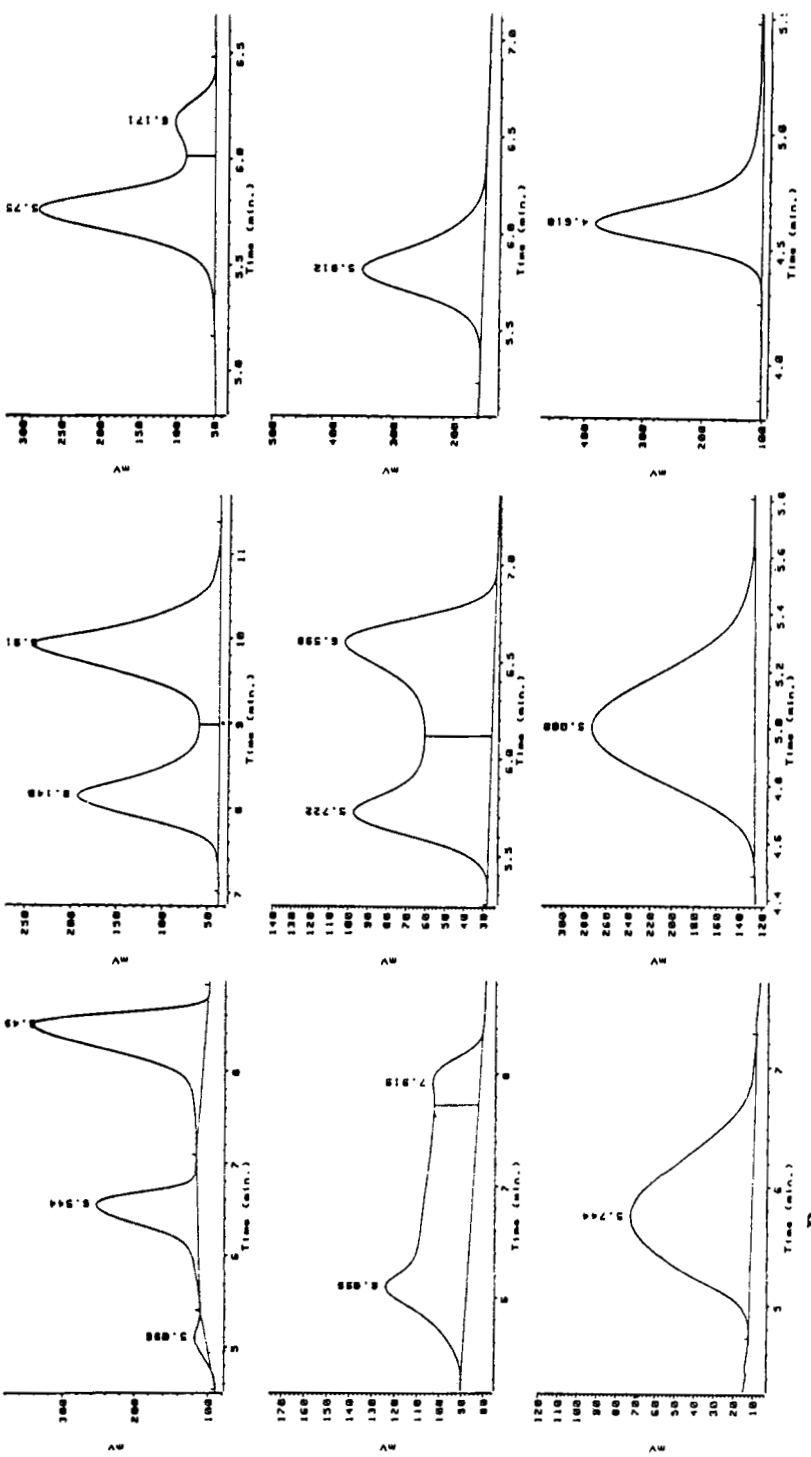

Figure 1a. The elution profiles of the studied sugars. B: D-arabinose, C: D-xylose, D: D-lyxose. The column temperatures are 0 °C, 30 °C, 50 °C, respectively. Column: APS in sulphate form (see Experimental). Eluant: MeCN : water = 9 : 1, flow rate = 0.7 mL/min.

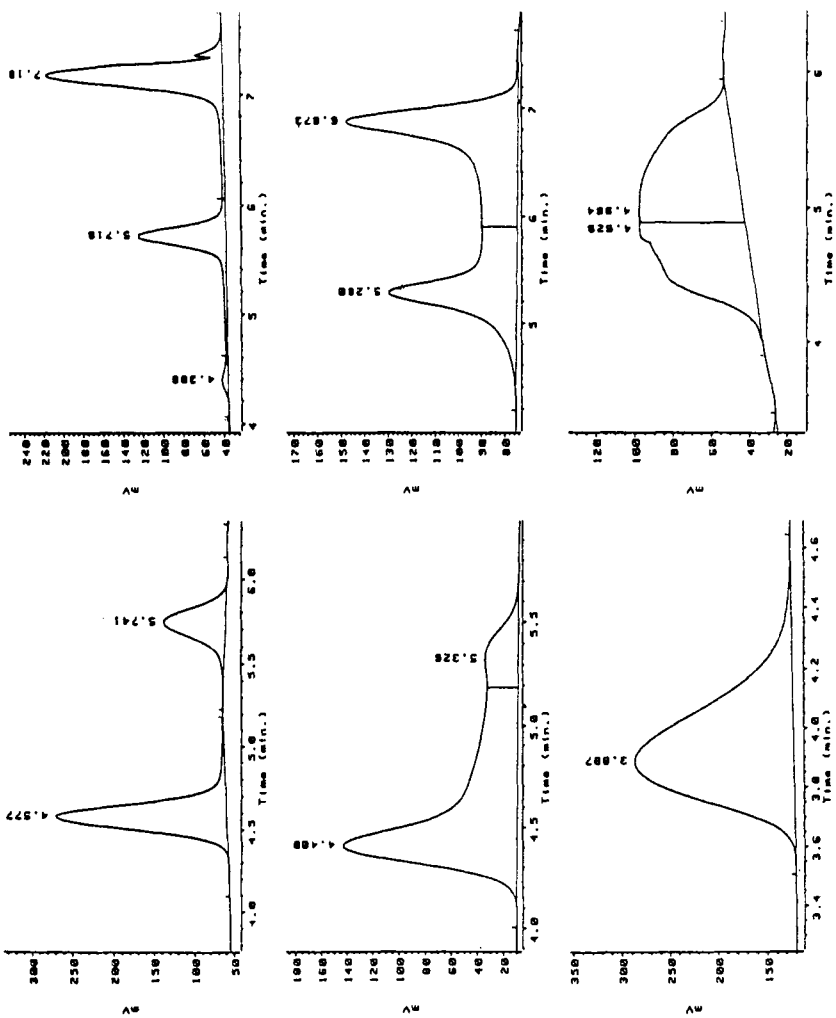

Figure 1b. The elution profiles of the studied sugars. E: L-rhamnose, F: D-fucose. The column temperatures are 0 °C, 30 °C, 50 °C, respectively. Column: APS in sulphate form (see Experimental). Eluant: MeCN : water = 9 : 1, flow rate = 0.7 mL/min.

Table 1. α-Pyranose contents in equilibrium aqueous solution

Sugars	α-Pyranose %		
	Our results	HPLC[4]	NMR[3]
D-Ribose	1 peak	no data	21.5
D-Arabinose	63.7	61[a]	60
D-Xylose	38.9	36	36.5
D-Lyxose	81.2	79	70
L-Rhamnose	67.9	66	60
D-Fucose	31.0	30	28

a. Refers to L-arabinose.

conformation and has one *axial* OH group, but β-D- arabinose has two *axial* OH groups regardless of whether the $^1C_4$ or $^4C_1$ conformation is preferred.

Separation and Identification of the Methyl Glycosides: Separation of the reaction products and the starting material was successfully achieved using an aminopropylsilica (APS) column (dihydrogen sulphate form) with acetonitrile : water = 9 : 1 as the eluant at 0 °C. Identification of the peaks was carried out by measuring the optical rotation of the fractions collected. The retention time values for the peaks of the studied sugars and the sign of the optical rotation of the corresponding fractions are summarized in Table 2. Table 2 also contains some chemical shifts concerning the anomeric hydrogen atom. In the case of methyl furanosides, the 1,2- *trans* anomers were eluted first.

Evaluation of the Iodine–catalyzed Glycosidation Reaction: The data shown in Fig. 2 illustrate that in the case of pentoses, furanosides are the kinetic products, and the reaction times are 5–7 h, except for rhamnose. With ribose (A) no pyranosides can be detected. Arabinose (B) reacts very quickly with methanol; after 4 h no free sugar can be detected in the reaction mixture, but a β-furanoside→β-pyranoside ring expansion starts. To isolate arabinofuranosides the reaction must be quenched after 4 h. A very similar composition was observed also with D-xylose (C), but in this case the pyranosides are present from the very early stage of the reaction. The furanoside→pyranoside

Table 2. Retention time values and selected ^{1}H NMR data for sugars and glycosides

Glycoside	Furanosides		Pyranosides		Aldose	Furanoses	Pyranoses	
	Rt/sign of α_D		Rt/sign of α_D			Rt/x[a]	Rt/x[a]	
	(H-1 δ)							
Methyl D-riboside	3.3/- (4.89)	3.6/+ (4.99)	4.7/+	5.1/-	D-ribose	--	5.5/ α+β	
Methyl D-arabinoside	3.0/+	3.4/-	4.0/-	4.9/+	D-arabinose	5.6/α+β	6.1/β	7.5/α
Methyl D-xyloside	3.4/- (4.90)	3.6/+ (4.98)	4.0/-	4.6/+	D-xylose	--	5.9/α	6.6/β
Methyl D-lyxoside	3.1/+ (4.95)	3.9/- (4.89)	4.5/-	5.4/+	D-lyxose	--	6.5/α	7.4/β
Methyl D-fucoside	3.1/-	3.6/+	4.4/+		D-fucose	5.9/α+β	7.9/α	10.6/β
Methyl L-rhamnoside	2.7/-	3.1/+	3.3/-	4.2/+	L-rhamnose	--	5.2/α	6.0/β

a. anomer type on the basis of anomer ratio reported in the literature. See Table 1.

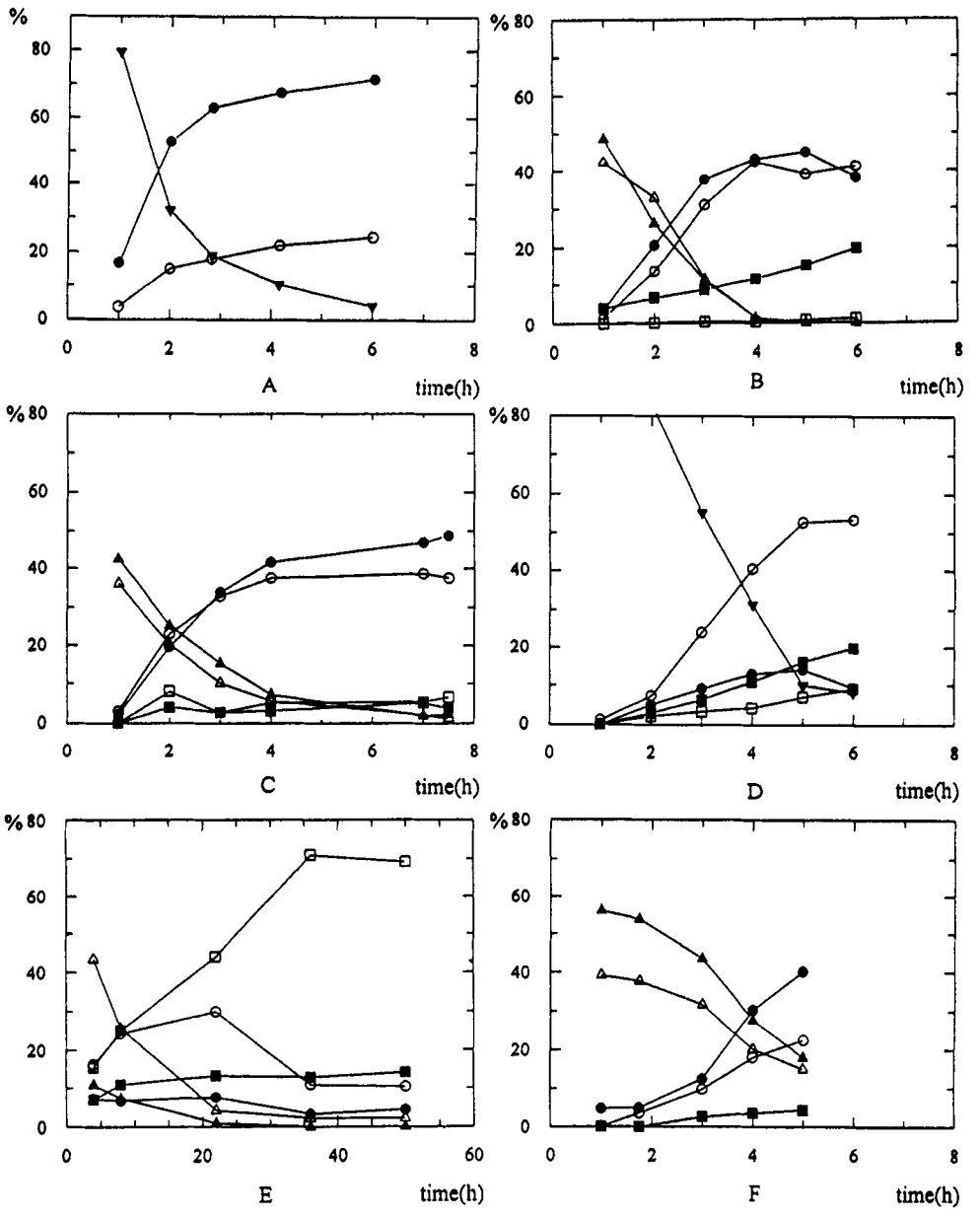

Figure 2. Composition of the reaction mixtures of the different sugars investigated in one hour intervals. Symbols of the Me-glycosides: o α-furanoside, ● β-furanoside, □ α-pyranoside, ■ β-pyranoside. Symbols of the free sugars Δ α-pyranose, ▲ β-pyranose, ▼(α+β)–pyranose

A: D-ribose, B: D-arabinose, C: D-xylose, D: D-lyxose, E: L-rhamnose, F: D-fucose

Table 3. Composition of the reaction mixtures after 4 h reaction time

Sugar	Methyl glycosides (%)				Aldose
	α-furanoside	β-furanoside	α–pyranoside	β-pyranoside	(%)
D-ribose	22.0	67.6	-	-	10.4
D-arabinose	42.4	43.1	11.4	0.4	2.8
D-xylose	41.2	37.5	4.1	5.2	12.0
D-lyxose	40.5	13	10.3	4.2	31.3
L-rhamnose	16.2	7.2	15.4	7.0	54.2
D-fucose	18.0	30.4	3.6	-	48.0

isomerization is not a fast reaction. D-Lyxose (D) reacts with a considerably high rate, the α-furanoside is the major product, but all the other three glycosides are also present. In general, pentoses require 4–5 h to reach a nearly complete conversion into glycosides, the major (and in some cases the exclusive) products are furanosides, and the anomer-selectivity is rather low.

Of the two 6-deoxyhexoses L-rhamnose showed the lower rate of conversion; after 6 h 40% of the starting sugar was unchanged. Until 8 h reaction time approximately equal amounts of the α-furanoside and α-pyranoside were produced. After 8 h an α-furanoside→α-pyranoside ring expansion started and the α-pyranoside became the major product.

The composition of the reaction mixtures after a 4 h reaction time is summarized in Table 3.

There are many reports on the methyl glycosidation of sugars with methanolic hydrogen chloride[6,7,8] or in the presence of cation–exchange resins.[9] When studying the reaction of D-xylose with methanolic hydrogen chloride Bishop *et al.*[6] found that the β-xylofuranoside was present in a larger quantity than the α-xylofuranoside (α/β=1/1.7) after 48 h reaction time at 25 °C. On the other hand, Ferrier and coworkers[7] observed that the α/β-furanoside ratio was 1 : 1.2 under the same reaction conditions.

We obtained similar results: the iodine–catalyzed methyl glycosidation of D-xylose resulted in a 1 : 1.29 α-D-xylofuranoside/β-D-xylofuranoside mixture. The 1,2-*trans*-furanoside product was more preferred in the case of D-ribose, D-lyxose, and L-rhamnose, but no such preference was observed in the other cases. The most important result of our present experiments is the recognition that under the applied conditions methyl pentofuranosides can be selectively prepared under kinetic control, and this might be well used in the synthesis of nucleosides.

EXPERIMENTAL

General Methods: A Hewlett-Packard 1090 series II Liquid Chromatograph equipped with a refractive index detector, an automatic sampler, and a ChemStation were used for the separation experiments. The separation was performed on a Hypersil APS (aminopropylsilica, 5 μm, 4,6 x 100 mm) column, which was converted into the sulphate form by the method of Kahle et al.[4] No sufficient separation of all of the isomers of the studied aldoses and methyl glycosides could be achieved on an APS column and a reverse-phase column. Different acetonitrile : water mixtures were used as the eluants. The column temperature was controlled by a column oven, and the 0 °C temperature was assured with an ice–water bath.

The chemicals used were AR grade, and gradient grade solvents were employed for HPLC. Purified water was obtained from a laboratory purification system equipped with both ion-exchange and carbon filters (Millipore, Bedford, MA, USA).

For the isomerization studies of the sugars the sample was 10 mg/mL sugar in water. The percentage compositions of the mixtures resulting from the glycosidation reactions were determined by HPLC analysis after removal of the rest of I_2 with a saturated aqueous sodium thiosulphate solution. The injected sample-volume was 15 μL. The peaks of the chromatograms were identified by comparison of the calculated anomeric ratio with data reported in the literature for the free sugars. Identification of the peaks was carried out by measuring the optical rotation value of the fractions collected for the reaction mixture. Optical rotation was measured with a Perkin-Elmer polarimeter Model 241 at 25 °C. The ^1H NMR spectra were recorded in D_2O with a Bruker AM-360 instrument at room temperature.

Reaction of the Sugar and Methanol in the Presence of I_2 Catalyst: A stirred suspension of the sugar (1000 mg) in methanol (50 mL) was treated with iodine (70 mg), the mixture was heated under reflux for 6–8 hours, and samples were taken at each hour. The cooled samples were treated with a saturated aqueous sodium thiosulphate solution until colorless, and were injected into the APS column.

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