

Chromatographic Separation of Anomeric Glycosides

III. Crystalline Methylfuranosides of L-Fucose, D-Ribose, and L-Rhamnose

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Three crystalline methylfuranosides of L-fucose, D-ribose, and L-rhamnose, respectively, have been prepared by column chromatography. The rates of hydrolysis of these and some previously prepared furanosides have been measured at 20° in N hydrochloric acid. The rule seems to exist that furanosides having *cis*-configuration at C₁ and C₂ are more easily hydrolyzed than the anomeric forms with *trans*-configuration.

Since the publication of part II¹ of this series Watkins² has described the isolation of crystalline α -methyl-L-fucofuranoside. Already before his publication appeared we had obtained the same crystalline fucoside, but although our results agree with those of Watkins we find it appropriate to include them here. Barker and Smith³ have described the chromatographic separation on a column of "Solkaflor" of the four isomeric methyl-D-ribosides, but they did not obtain any of the furanosides in the crystalline state. Using a similar procedure as described in part II of this series we succeeded in isolating the crystalline β -methyl-D-ribofuranoside. By the same method we have also prepared the crystalline α -methyl-L-rhamnofuranoside. The melting points and rotations of the new crystalline furanosides are as follows:

α -Methyl-L-fucofuranoside	M.p.	126°	$[\alpha]_D^{20}$	-111.4°	(in water)
β -Methyl-D-ribofuranoside	"	80°	"	-49.8°	"
α -Methyl-L-rhamnofuranoside	"	62°	"	-98.6°	"

The rates of hydrolysis of the furanosides in N hydrochloric acid at 20° have been calculated from measurements of free sugar. As the rates of hydrolysis given in part II were measured only at room temperature we have redetermined them at exactly 20°. For comparison the rates of hydrolysis of a few other methylfuranosides have been included. The results are collected in

Table 1 in which the rate constants are calculated from the equation $k = 1/t \cdot \ln(a/a-x)$ with time in minutes. As some authors, *e. g.* Riiber and Sørensen ⁴ and Isbell and Frush ⁵ have calculated the rates of hydrolysis of glycosides from the equation $k_{10} = 1/t \log(a/a-x)$, we have also given the figures for k_{10} .

Table 1. Rates of hydrolysis in *N HCl* at 20°.

	$[\alpha]_D^{20}$	k	k_{10}	Half life
β -Methyl-D-ribofuranoside	-49.8°	0.0067	0.0029	1 h 44 m
α -Methyl-L-rhamnofuranoside	-98.6°	0.00050	0.00022	23 » 6 »
α -Methyl-D-xylofuranoside	+182°	0.0128	0.0056	0 » 54 »
β - » » »	-89°	0.0075	0.0033	1 » 32 »
α -Methyl-L-arabofuranoside	-127°	0.000206	0.000089	56 » 5 »
β - » » »	+118°	0.00140	0.00061	8 » 15 »
α -Methyl-D-arabofuranoside	+128°	0.000206	0.000089	56 » 5 »
β - » » »	-119°	0.00120	0.00052	9 » 38 »
α -Methyl-D-galactofuranoside	+104°	0.00104	0.00045	11 » 7 »
β - » » »	-112°	0.000123	0.000053	93 » 55 »
α -Methyl-L-fucofuranoside	-111.4°	0.00049	0.00021	23 » 35 »
β - » » » (syrup)	+113°	0.00015	0.000065	76 » 50 »

As the ring in the furanosides is most probably plane a relationship between configuration and physical constants is more likely to be found for these glycosides than in the case of the pyranosides in which the six-membered ring may have different conformations (see, *e. g.*, the discussion recently by Edward ⁶). In the following consideration of this question we have, besides our own experimental data, made use of the figures for rotation and hydrolysis of the α - and β -ethyl-D-glucofuranosides determined by Haworth, Porter, and Wayne ⁷. Their values for k were measured at 55–60° in 0.01 *N* hydrochloric acid.

Using the periodic oxidation method Jackson and Hudson ⁸ have shown that, in the case of the methylpyranosides of aldopentoses and aldohexoses, the α -forms of D-sugars in all cases investigated have the same configuration at C₁, *viz.* the methoxyl group falling to the right in the conventional projection formulae. In a single case they oxidized a furanoside, *viz.* α -methyl-D-arabofuranoside, and found that also this glycoside had the same configuration at C₁ as the α -pyranosides. Assuming now that this hold for the other α -furanosides as well, it will be seen from the simplified projection formulae below that the anomeric form having *cis*-configuration at C₁ and C₂ always is more easily hydrolyzed than the corresponding anomer with *trans*-configuration. In the case of D-fucose it is assumed that enantiomeric glycosides have the same rate of hydrolysis and that their optical rotations differ in sign only. The molecular rotation given are calculated in the usual way as one hundredth of the product of specific rotation and molecular weight.

	$\begin{array}{c} \text{---OCH}_3 \\ \text{---OH} \\ \text{HO---} \\ \text{---CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{---OCH}_3 \\ \text{---OH} \\ \text{HO---} \\ \text{---CHOHCH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{---OCH}_3 \\ \text{---OH} \\ \text{HO---} \\ \text{---CHOHCH}_3 \end{array}$
	β -L-Arabinofurano- side	α -D-Galactofurano- side	α -D-Fucofuran- side
[M] _D	+194°	+202°	+198°
<i>k</i> _{20°}	0.00140	0.00104	0.00049
	$\begin{array}{c} \text{CH}_3\text{O---} \\ \text{---OH} \\ \text{HO---} \\ \text{---CH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_3\text{O---} \\ \text{---OH} \\ \text{HO---} \\ \text{---CHOHCH}_2\text{OH} \end{array}$	$\begin{array}{c} \text{CH}_3\text{O---} \\ \text{---OH} \\ \text{HO---} \\ \text{---CHOHCH}_3 \end{array}$
	α -L-Arabinofurano- side	β -D-Galactofurano- side	β -D-Fucofuran- side
[M] _D	-209°	-218°	-201°
<i>k</i> _{20°}	0.000206	0.000123	0.00015

The molecular rotations of the configurationally corresponding methyl-*glycosides* of the three sugars are as will be seen very nearly the same, indicating that the influence on the optical rotation of the size of the substituent at C₄ is insignificant.

In the case of the methyl-D-xylo- and the ethyl-D-glucofuranosides again the anomers having *cis*-configuration at C₁ and C₂ are hydrolyzed more easily than those with *trans*-configurations, although the ratio *cis:trans* is considerably less than by the three sugars above. The latter fact might be due to the different configuration at C₄. In the case of D-xylose and D-glucose the furanosides which are more easily hydrolyzed have *trans*-configurations of the substituents at C₁ and C₄, while in the case of the three other sugars the more easily hydrolyzable furanosides have the groups at C₁ and C₄ in *cis*-position.

	$\begin{array}{c} \text{---OCH}_3 \\ \text{---OH} \\ \text{HO---} \\ \text{HOCH}_2\text{---} \end{array}$	$\begin{array}{c} \text{---OC}_2\text{H}_5 \\ \text{---OH} \\ \text{HO---} \\ \text{HOCH}_2\text{CHOH---} \end{array}$	$\begin{array}{c} \text{CH}_3\text{O---} \\ \text{---OH} \\ \text{HO---} \\ \text{HOCH}_2\text{---} \end{array}$	$\begin{array}{c} \text{C}_2\text{H}_5\text{O---} \\ \text{---OH} \\ \text{HO---} \\ \text{HOCH}_2\text{CHOH---} \end{array}$
	α -D-Xylofuran- oside	α -D-Glucofuran- oside	β -D-Xylofuran- oside	β -D-Glucofuran- oside
[M] _D	+299°	+210°	-146°	-179°
<i>k</i> _{20°}	0.0128	<i>k</i> _{90°} 0.0230	<i>k</i> _{20°} 0.0075	<i>k</i> _{90°} 0.0124

The experimental material is still too small for a further discussion of these questions, and it must be hoped that additional data will soon be available.

Added in proof: The α - and β -methyl-D-mannofuranosides have been isolated by means of column chromatography, the β -form, however, only as a syrup. The rates of hydrolysis in N hydrochloric acid at 20° were found to be in agreement with the results in the case of the other anomeric furanosides described above: α -Methyl-D-mannofuranoside, m. p. 120°, $[\alpha]_D^{20} = +109^\circ$, $k = 0.000327$ ($k_{10} = 0.000142$), half life 35 h 21 m. β -Methyl-D-mannofuranoside (syrup) $[\alpha]_D^{20} = -86.5^\circ$, $k = 0.00471$ ($k_{10} = 0.00205$), half life 2 h 27 m.

EXPERIMENTAL

Methyl-L-fucofuranosides

Watkins² using the method described by us succeeded in preparing crystalline α -methyl-L-fucofuranoside with m. p. 125–126° and $[\alpha]_D -108^\circ$ ($c = 2$ in water). The corresponding β -furanoside was obtained as a clear syrup with $[\alpha]_D +113^\circ$.

In the present case a 2 % solution of L-fucose in methanol containing 0.014 % HCl (0.0039 N) was refluxed for 2 hours. After removing the hydrogen chloride with silver carbonate the solution was evaporated to a syrup. On leaving the syrup from 3.1 g fucose in a vacuum desiccator for a week some crystalline material (0.37 g) had separated which was found to be α -methyl-L-fucopyranoside having m. p. 156° and $[\alpha]_D -190^\circ$ (water). This separation of the α -pyranoside was found to be advantageous for the subsequent separation on the cellulose column. As mobile phase was used a mixture of butanol-1, methyl ethyl ketone, and water (1:1:1) and the effluent was collected automatically in tubes. At certain intervals paper chromatograms were run (using methyl ethyl ketone saturated with water) in order to check the degree of separation. The pyranosides necessary for this purpose were prepared according to Minsaa³. The tubes 1–19 contained only β -furanoside, and the solution on evaporation gave a clear syrup (1.08 g) which has not yet crystallized; $[\alpha]_D^{20} +113^\circ$ ($c = 2$ in water). From the tubes 28–39 was obtained the pure α -methyl-L-fucofuranoside (0.5 g) which recrystallized from ethyl acetate had m. p. 126° and $[\alpha]_D^{20} -111.4^\circ$ ($c = 3$ in water). (Found: C 47.33; H 7.71. Calc. for $C_7H_{14}O_5$: C 47.19; H 7.92.)

 β -Methyl-D-ribofuranoside

Barker and Smith³ prepared a mixture of the four ribosides by treatment of ribose at room temperature with methanol containing 1 % hydrogen chloride. The separation of the ribosides was effected on a column of cellulose using butanol-water as eluant. The ribosides finally distilled in a high vacuum were obtained as syrups except in the case of the β -pyranoside which had also previously been prepared in the crystalline state.

In the present investigation a syrup rich in furanosides was obtained by refluxing for 70 min. a methanolic solution containing 2.6 % D-ribose and 0.0108 % HCl (0.003 N). Under these conditions the sugar was transformed completely into glycosides. After removing the hydrogen chloride in the usual way and evaporating the methanol a clear syrup was obtained. The syrup (4.04 g) dissolved in a small quantity of the eluant was placed on top of a column of powdered cellulose and eluted with a mixture of ethyl acetate, propanol-1, and water (5:3:2) which left the column at a rate of 28 ml per min, 7 ml being collected in each tube. The following fractions were obtained:

Fraction	Tube No.	Weight
A	2–12	1.44 g
B	13–18	1.30 »
C	19–25	0.73 »
D	26–46	0.40 »
		3.87 g (95 % recovered)

On a new column, using butanol-1 saturated with water as eluant, fraction A was separated into two fractions A₁ and A₂ of 0.73 and 0.57 g respectively. Fraction A₁ was dissolved in 30 ml dry ethyl acetate, treated for one hour at room temperature with charcoal, filtered and then 25 ml of the solvent was removed by distillation. After the concentrated solution had been kept for a few days above calcium chloride at –20° the β -methyl-D-ribofuranoside had crystallized. On inoculating A₂ with the new crystalline riboside also this fraction crystallized. Fractions B and C were each fractionated a second time on cellulose columns, using butanol-1 saturated with water as eluant. In both cases

the first fractions on inoculation gave some crystalline β -furanoside. The yield of crystalline β -furanoside was in all 2.34 g (58 %). After recrystallization from ethyl acetate, m. p. 80° and $[\alpha]_D^{20} -49.8^\circ$ ($c = 4.2$ in water).

The second fractions of B and C had the specific rotations +100° and +121°, respectively, but the quantities were too small for further treatment.

α -Methyl-L-rhamnofuranoside

Of the L-rhamnosides the α - and β -pyranosides have been known in the crystalline state since the days of Emil Fischer. They also represent the pair of anomeric glycosides which was for the first time separated by a chromatographic method; see Hough, Jones, and Wadman¹⁰. In the present case a mixture of methylrhamnosides rich in furanosides was prepared by refluxing for 4 1/2 hours a solution of anhydrous L-rhamnose (10 g) in methanol (500 ml) containing 0.02 % hydrogen chloride (0.0055 N). After treatment first with lead carbonate and then with silver carbonate the solvent was evaporated at room temperature. The syrup was redissolved in some methanol and shaken with charcoal in order to remove traces of colloidal silver. Finally the filtered clear solution on evaporation in a vacuum above calcium chloride gave a syrup with $[\alpha]_D -39^\circ$ (in water).

The first fractionation of the syrup (10 g) was carried out on three columns of powdered cellulose, each loaded with about 3 g. The eluant was a mixture of butanol-1, ethyl acetate, and water (7:5:2) and the general procedure by the separation as described before. As an example may serve the fractionation in one of the columns:

Fraction	Tube No.	Weight
A	1-15	1.92 g
B	16-20	0.27 »
C	21-25	0.15 »
D	36-60	0.29 »
E	61-71	0.07 »

Paper chromatograms showed that A contained mainly α -furanoside, and actually the tubes 1-6 from one of the columns on evaporation gave a syrup which crystallized spontaneously. In order to achieve a better separation the rest of fraction A from the same column and the whole of the fractions A from the other two columns were put together and the syrup obtained from them fractionated for a second time on a column. The new first fraction (AII) together with the above tubes 1-6 gave 3.05 g which were dissolved in ethyl acetate (20 ml), treated with charcoal and filtered. After distilling off half of the solvent the solution was placed at -20° overnight and then inoculated whereby the glycoside separated in crystals. On recrystallization from benzene this new α -methyl-L-rhamnofuranoside was obtained in needles with m. p. 62° and $[\alpha]_D^{18} -98.6^\circ$ ($c = 3.8$ in water). A paper chromatogram gave a single spot only.

The fractions B and C from the first and BII and CII from the second fractionation contained mixtures of the other rhamnosides. The fractions D were rich in β -pyranoside and on evaporation crystallized partly. In order to obtain more of the β -pyranoside all fractions B and C were collected and their content subjected to a new fractionation. The β -methyl-L-rhamnopyranoside thus isolated was recrystallized from ethyl acetate and obtained in needles with m. p. 138° and $[\alpha]_D^{22} +96.4^\circ$ ($c = 2.2$ in water). On the paper chromatogram a single spot only appeared.

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