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Chromatographic Separation of Anomeric Sugars

IV. Crystalline Methylfuranosides of D-Lyxose and L-Fucose Triacetate

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Crystalline α-methyl-D-lyxofuranoside and β-methyl-2,3,5-*O*-triacetyl-L-fucofuranoside have been obtained by chromatography on cellulose. The ring structures have been confirmed by periodate oxidation. The rate of hydrolysis of the anomeric lyxofuranosides in N hydrochloric acid gives further data for the previously suggested rule for the rate of hydrolysis of furanosides.

Van Ekenstein and Blanksma [1] reported the isolation of crystalline α-methyl-D-lyxopyranoside which crystallized from the mixture after methylation of D-lyxose with anhydrous methanol containing 0.5 % hydrogen chloride. Purification by repeated recrystallizations [2] gave a product with specific rotation $[\alpha]_D^{20} = +59.4°$ and m.p. 108–109°C; the pyranose ring was later confirmed by periodate oxidation [3]. By a similar method Isbell and Frush [4] isolated crystalline β-methyl-D-lyxopyranoside having $[\alpha]_D^{20} = -128.1°$ and m.p. 118°C; also here the pyranose ring was verified by the periodate oxidation method.

In previous papers of this series [5–7] crystalline methylfuranosides have been prepared by separation of the isomeric glycosides on cellulose columns.

We now report the chromatographic isolation of crystalline α-methyl-D-lyxofuranoside besides the previously described crystalline α- and β-pyranosides.

The methylation of the sugar was carried out in hot 0.004 N methanolic hydrogen chloride. By treatment of the syrup on a cellulose column the glycosides were eluted in the order: α-furanoside, α-pyranoside, β-furanoside and β-pyranoside, the ratios being 66:17:7:10, respectively. The melting points and rotations of the isomers were as follows:

α-Methyl-D-lyxopyranoside,	m.p. 107.5°C	$[\alpha]_D^{20}$ + 59.3°	(in water)
β-Methyl-D-lyxopyranoside	» 118°C	» −128°	»
α-Methyl-D-lyxofuranoside	» 96.5–97°C	» + 128°	»
β-Methyl-D-lyxofuranoside	syrup	» − 85°	»

All the three crystalline methyl-D-lyxosides were found by paper chromatography to be pure, using two different solvents: methyl ethyl ketone saturated with water or ethyl acetate-propanol-water (5:3:2). The physical data obtained for the two pyranosides are nearly identical with those recorded previously [2-4]. The syrupy β-methyl-D-lyxofuranoside from the first separation was treated repeatedly on cellulose column. Even though some of the fractions were chromatographically pure (see experimental) no crystallization could be effected neither by treatment with ethyl acetate nor by storing at −20°C for two years.

The rate of hydrolysis of the furaniosdes has been found [7] to depend on the configuration at C_1 and C_2 in such a way that the isomer with *cis*-configuration is hydrolysed much faster than that one with *trans*-configuration. Of the anomeric lyxofuranosides the β-form having *cis*-configuration at C_1-C_2 ought therefore to have a higher rate of hydrolysis than the α-form. Our experiments have confirmed this as we found the values of the rate constants to be $k_\beta = 6.3 \times 10^{-3}$ and $k_\alpha = 1.57 \times 10^{-3}$ corresponding to the half lives 1 h 50 min and 7 h 22 min for the β- and α-anomers, respectively.

Watkins [8] has described the preparation of crystalline α-methyl-L-fucofuranoside later obtained also in our laboratory. The β-anomer has, however, not been reported crystalline. Experiments have therefore been carried out in an attemt to prepare also the β-anomer in the crystalline state. Chromatography of the syrup obtained by methylation in 0.0039 N methanolic hydrogen chloride, followed by repeated chromatography of the fractions containing almost pure β-methyl-L-fucofuranoside, gave a colourless syrup. This syrup which was found, at least by the method used, to be chromatographically pure ($[\alpha]_D^{20} = +113°$); did, however, not crystallize either from solutions in ethyl acetate or on storing at −20°C for one year. In an attempt to obtain a crystalline product, the syrup was transformed into the 2,3,5-*O*-triacetyl derivative which was obtained crystalline $[\alpha]_D^{20} = +75.5°$ ($c = 2.4$, meth.) m.p. 71°C. On deacetylation of the pure triacetate the result was again a colourless syrup having $[\alpha]_D^{20} = +117.5°$ ($c = 2.4$, w.) and the same R_F-value as the β-methyl-L-fucofuranoside. Although the value of the rotation has increased somewhat, the syrup has not yet crystallized on standing at low temperature.

The ring form and the configuration at C_1 were examined further by the periodate oxidation technique [9]. A differentiation between a pentopyranoside and a pentofuranoside could be obtained as the former consumes two equivalents of periodate while the latter will consume only one equivalent. The dialdehydes formed from pentopyranosides contain only one asymmetric carbon atom (C_1) and all pentopyranosides will consequently lead to the same pair of enantiomeric dialdehydes. Measurement of the optical rotation of the dialdehydes represents therefore a method for determination of the configuration at C_1 in these glycosides. On the other hand, the dialdehydes obtained by oxidation of pentofuranosides, containing two asymmetric carbon atoms (C_1 and C_4), are the same as those obtained by oxidation of hexopyranosides. The configuration at C_1 of the anomers within the pentofuranose series can therefore be correlated with the anomers within the hexopyranosides [9,10]. Reduction of these dialdehydes [10,11], giving enantiometric diglycols as the

Table 1. Oxidation of some methylglycosides.

Substance	$[\alpha]_D^{20}$	M.p. °C	Equivalents of periodate	Dialdehyde $[\alpha]_D^{20}$	Diglycol $[\alpha]_D^{20}$
α-Methyl-D-lyxopyranoside	+ 59.3°	107.5	2.05	+ 125.5°	− 6.2°
α-Methyl-D-lyxofuranoside	+ 128°	97	1.01 1.06	+ 121.8° + 120.0°	−12.3
α-Methyl-L-fucofuranoside	− 111.4°	126	1.05	− 149°	
β-Methyl-L-fucofuranoside	+ 117.5°	—	0.99	+ 92.8°	

asymmetry at carbon atom 4 is eliminated, enables a correlation to be established also between the D- and the L-series of these glycosides.

6-Desoxyhexopyranosides and furanosides consume two and one equivalent of periodate giving dialdehydes containing two and three asymmetric carbon atoms, respectively. With our present knowledge the method therefore gives no definite information about the configuration at C_1 of this type of furanosides. Nevertheless, the method permits to differentiate between a pyranoside and a furanoside ring in this series too.

α-Methyl-D-lyxopyranoside, α-methyl-D-lyxofuranoside and α- and β-methyl-L-fucofuranoside were oxidized with periodate. The dialdehydes formed from the two first ones were isolated and reduced with sodium borohydride by the method of Smith and co-workers [11]. The results are given in Table 1.

The consumption of periodate as well as the specific rotation of the dialdehydes and the diglycols confirm the conclusion that the two lyxosides examined are α-methyl-D-lyxopyranoside and α-methyl-D-lyxofuranoside, respectively (see Ref.[10]). This was further verified by hydrolysis of the diglycols followed by *p*-nitrobenzoylation,the pyranoside and the furanoside giving glycol-bis-*O*-*p*-nitrobenzoate (m.p. 140—42°C) and glycerol-tris-*O*-*p*-nitrobenzoate (m.p. 196—97°C), respectively. In the case of the fucofuranosides, the fact that only one equivalent of periodate was consumed confirmed the presence of the furanose ring.

EXPERIMENTAL

Paper chromatography was carried out using Whatman No. 1 paper. Methyl ethyl ketone saturated with water (A) or ethyl acetate-propanol-water 5:3:2 (B) was used as eluant and *m*-phenylene diamine hydrochloride served as spray reagent [12].

Methyl-D-lyxosides

In preparing the syrup the same conditions were used which have been found in our laboratory [6] to give highest yield of furanosides. After boiling the lyxose (2 % in 0.004 N methanolic hydrogen chloride, 200 ml) for 2 h, maximum dextrorotation occurred. At this time 99 % of the lyxose were converted to methyllyxoside as found by the Shaffer-Hartmanns method [13]. The syrup (3.3 g) isolated after removing the acid and methanol in the usual way, was chromatographed on a cellulose column (100 × 3 cm), using solvent A as eluant. The effluent leaving the column at a rate of 90 ml/h, was collected automatically in fractions of 11 ml. After examining the fractions polarimetrically they were

evaporated to dryness under reduced pressure (2 mm Hg; 25°C), using an apparatus designed in our laboratory by mag.scient. R. S. Alm for the simultaneous evaporation of up to 100 tubes. Fractions 1–25 contained 1.80 g (54.6 % of the original syrup) of chrystalline *α*-methyl-D-lyxofuranoside (each fraction tested by paper chromatography). Fractions 32–56 gave 0.46 g (14.0 %) of the chrystalline *α*-pyranoside; fractions 60–71 gave 0.10 g (3.0 %) syrup of chromatographically pure *β*-furanoside ($[\alpha]_D^{20} = -84°$); fractions 72–79 gave 0.09 g (2.7 %) syrup of slightly impure *β*-furanoside ($[\alpha]_D^{20} = -109°$) and fractions 90–154 gave 0.24 g (7.9 %) of the chrystalline *β*-pyranoside. The intermediate fractions 26–31, 57–59, and 80–89 together with the fractions 155–208 also contained a small amount of syrup, but these were not worked up. Repeated chromatography of the syrup from the fractions 72–79 together with 0.148 g of syrup ($[\alpha]_D^{20} = -105°$) from the corresponding fractions of another experiment gave 60 tubes containing syrup. Of these, only the 9 first tubes were found to contain chromatographically pure *β*-methyl-D-lyxofuranoside ($[\alpha]_D^{20} = -85°$) while the other fractions beside the *β*-furanoside also contained a small amount of the *β*-pyranoside. The acid hydrolysis was carried out at 20°C using 1 N HCl and *ca.* 0.6 % solution of the furanosides, the free lyxose being determined by Shaffer-Hartmann's method.

Periodate oxidation. The procedure described by Berner and Kjølberg [10] was used.

α-Methyl-D-lyxofuranoside (1.54 m mole) was oxidized in 7 ml of water with periodic acid (2.31 m mole). After 30 min the rotation became constant + 2.34° in a 1 dm tube equivalent to $[\alpha]_D^{20} = +120.0°$, but the oxidation was continued for further 30 min before precipitation of excess periodate and isolation of the dialdehyde. Analysis showed that 1.06 mole periodic acid had been consumed per mole furanoside. Reduction of the dialdehyde, together with that one from another experiment (2.5 % dialdehyde in water; 15 ml) gave a solution with $\alpha_D^{20} = -0.31°$ equivalent to $[\alpha]_D^{20} = -12.3°$. The solution was de-ionized using a mixture of cation exchange resin IR-120 (20 g) and anion exchange resin IR-45 (20 g) and evaporated to dryness. For complete hydrolysis the diglycol was treated with 2 N HCl at room temperature for 1 h (no optical rotation could be detected after 30 min). After neutralization of the hydrolysate and evaporation at reduced pressure, glycerol-tris-*O*-*p*-nitrobenzoate was prepared as described by Smith *et al.*[11] using *ca.* 200 % of the theoretical amount of *p*-nitrobenzoyl chloride. Recrystallization three times from ethyl acetate gave m.p. 197–98°C alone or in admixture with an authentic specimen. (Found: C 53.5; H 3.23; N 6.80. Calc. for $C_{24}H_{17}O_{12}N_3$: C 53.4; H 3.2; N 7.8.)

α-Methyl-D-lyxopyranoside (1.50 m mole) was oxidized in 20 ml of water with H_5IO_6 (4.06 m mole). The rotation became constant + 1.23° (tube: 1 dm) after 45 min which corresponds to $[\alpha]_D^{20} = +125.5°$. Analysis after 75 min showed that 2.05 mole of periodate had been reduced per mole lyxoside. The dialdehyde was isolated (165 mg) and reduced (in 10 ml) giving a diglycol with optical rotation −0.10° (tube: 1 dm) equivalent to $[\alpha]_D^{20} = -6.2°$. Isolation of the diglycol, hydrolysis and *p*-nitrobenzoylation of the glycol was carried out in a similar way to that described for glycerol. Recrystallization from ethyl acetate gave m.p. 140–142°C and no depression was observed on mixing with ethylenglycol-bis-*O*-*p*-nitrobenzoate.

Methyl-L-fucofuranoside

The syrup containing the four isomeric methyl-L-fucosides was prepared as described by Augestad and Berner [7]. The syrup (4.00 g) was resolved on cellulose column using solvent A as eluant giving 1.45 g (36 %) with chromatographically pure *β*-methyl-L-fucofuranoside ($[\alpha]_D^{20} = +113°$) and 0.66 g (17 %) of crystalline *α*-methyl-L-fucofuranoside (m.p. 126°, $[\alpha]_D^{20} = -111°$). (The fractions containing the pyranosides were not worked up.)

The pure syrupy *β*-furanoside (1.0 g) was acetylated using sodium acetate (0.5 g) and acetic anhydride (5 ml). The acetylated product was extracted 5 times with petroleum ether (b.p. 90–100°C) giving crystalline triacetate (1.8 g) by evaporation of the solvent under reduced pressure. The product had m.p. 71°C and $[\alpha]_D^{20} = +75.5°$ ($c = 2.4$, meth.) after recrystallization three times from petroleum ether (b.p. 90–100°C). (Found: C 51.8; H 6.57; OCH_3 11.6. Calc. for $C_{13}H_{20}O_8$: C 51.3; H 6.63; OCH_3 10.2).

A colourless syrup was again obtained $[\alpha]_D^{20} = +117.5°$ ($c = 2.4$, w) and + 122° ($c = 2.4$, meth.) after deacetylation of the triacetate using Zemplén's method [13].

Periodate oxidation. α-Methyl-L-fucofuranoside (80 mg; 5 ml) or β-methyl-L-fucofuranoside (133 mg; 10 ml) was oxidized with periodic acid (2.2 mole per mole of furanoside). The rotation was constant −2.36° and + 1.23° after 135 and 210 min, respectively, corresponding to $[\alpha]_D^{20} = -149°$ and + 92.8°. The oxidation was carried out for 21 h without change of the rotation. Analysis showed that α-methyl-L-fucofuranoside had consumed 1.05 and 1.05 equivalents of periodate after 4 and 20 h, respectively, and β-methyl-L-fucofuranoside had consumed 0.99 and 1.01 equivalents after the same oxidation periods.

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