Monatshefte für Chemie 132, 731–737 (2001)

Monatshefte für Chemie
Chemical Monthly

Stereospecific Synthesis of *D*-Glycero-*D*-ido-oct-2-ulose

Zuzana Hricovíniová*, **Miloš Hricovíni,** and **Ladislav Petruš**

Institute of Chemistry, Slovak Academy of Sciences, SK-84238 Bratislava, Slovakia

Summary. *D*-Glycero-*D*-gulo-heptose reacted with 2,2-dimethoxypropane to give its 2,3:6,7-di-O-isopropylidene derivative. Its base-catalyzed addition to formaldehyde resulted in the formation of 2,3:6,7-di-O-isopropylidene-2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptofuranose. After acid hydrolysis of this aldolization product, a new branched-chain aldose, 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose, was obtained, which was stereospecifically rearranged under the catalytic action of molybdic acid to *D*-glycero-*D*-ido-oct-2-ulose.

Keywords. *D*-Glycero-*D*-ido-oct-2-ulose; Branched-chain aldose; 2-C-(Hydroxymethyl)-*D*-glycero-*D*-gulo-heptose; Mo(VI) Catalysis.

Introduction

Naturally occurring higher sugars, such as *D*-manno-hept-2-ulose, *D*-altro-hept-2-ulose (sedoheptulose), or *D*-glycero-*D*-ido-oct-2-ulose, have been of particular interest in the field of carbohydrate chemistry. The biological activities of these sugars and their phosphate derivatives, especially their role in intracellular communication, have been studied [1, 2]. *D*-Glycero-*D*-ido-oct-2-ulose occurs in *Craterostigma plantagineum*, which belongs to a small group of drought-tolerant higher plants and plays an important role in the metabolism of plants. Chemical analysis revealed that this 8-carbon sugar represents almost 90% of carbohydrates in the fully hydrated leaves. It is metabolized into sucrose during the dehydration process and is converted back to octulose during the rehydration [3]. Due to its exceptional adaptation a number of studies have been initiated to understand the molecular mechanism of desiccation. *D*-Glycero-*D*-ido-oct-2-ulose exists in the free state in plant extracts from which it has been isolated. The configuration of this rare octulose has been determined by MS and ^{1}H NMR [4].

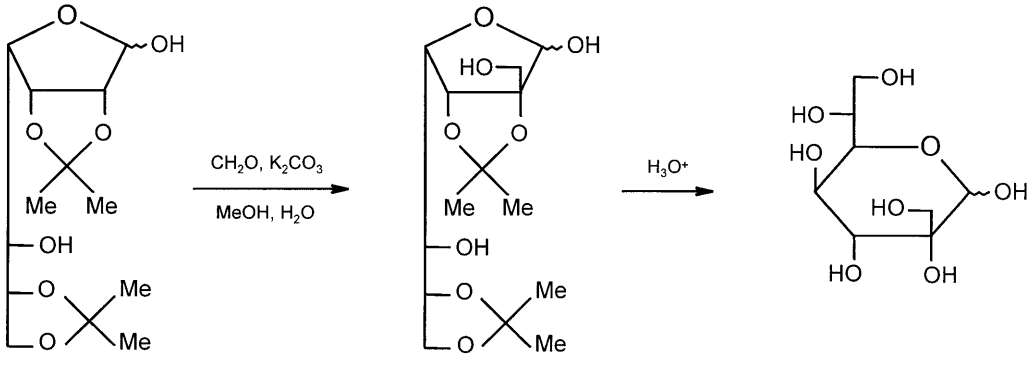
Being aware of the known stereospecifity in the isomerization reactions of reducing sugars in the presence of Mo(VI) [5–8], we have utilized the catalytic effect for the synthesis of some rare saccharides in previous studies [9–12]. It has been shown that 2-ketoses undergo a molybdic acid-catalyzed stereospecific rearrangement under formation of 2-C-(hydroxymethyl)-aldoses and *vice versa*. The thermodynamic equilibrium of these transformations is strongly shifted to the side

* Corresponding author

of 2-ketoses [9, 10]. This approach has been used for preparation of some biologically important saccharides such as hamamelose [10, 11] or sedoheptulose [12]. The one-step synthesis of *D*-glycero-*D*-ido-oct-2-ulose from the 2-C-branched aldose, 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose, is a further extension of the aforementioned method and represents an efficient preparation of this eight-carbon saccharide. The details of the synthesis are given herein.

Results and Discussion

It is known that isomerization of aldoses in aqueous solutions in the presence of a catalytic amount of molybdic acid at enhanced temperatures affords an aldose/epialdose mixture. This transformation proceeds without production of undesirable by-products [5–8] thanks to its unusual mechanism [14, 15]. Similar mutual interconversion was observed in the case of 2-ketoses and 2-C-(hydroxymethyl)-aldoses [9–12]. This evidence extends the application of the Mo(VI) catalysis for the preparation of other rare saccharides, such as *D*-glycero-*D*-ido-oct-2-ulose from an appropriate 2-C-(hydroxymethyl) branched-chain aldose. The first step in the synthesis of the 2-C-(hydroxymethyl)-aldose, which leads to the 2-ketose upon molybdic acid-catalyzed isomerization, was the preparation of a diisopropylidene derivative of *D*-glycero-*D*-gulo-heptose. Thus, transacetalation of *D*-glycero-*D*-gulo-heptose with 2,2-dimethoxypropane in the presence of toluene-4-sulfonic acid in 1,2-dimethoxypropane at room temperature afforded 2,3:6,7-di-O-isopropylidene-β-*D*-glycero-*D*-gulo-heptofuranose (**1**) as the major product (79%). Its alkali-catalyzed addition to formaldehyde gave a 2-C-branched-chain aldose, 2,3:6,7-di-O-isopropylidene-2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptofuranose (**2**) in 86% yield. The isopropylidene groups were removed by acid hydrolysis in the presence of Amberlite IR 120 in the H^+ form. Using this approach, the novel compound 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose (**3**) was prepared in good yield (95%). The synthesis of **3** is shown in Scheme 1. The structures of compounds **1**, **2**, and **3** were confirmed by NMR spectroscopy.



1 **2** **3**

Scheme 1

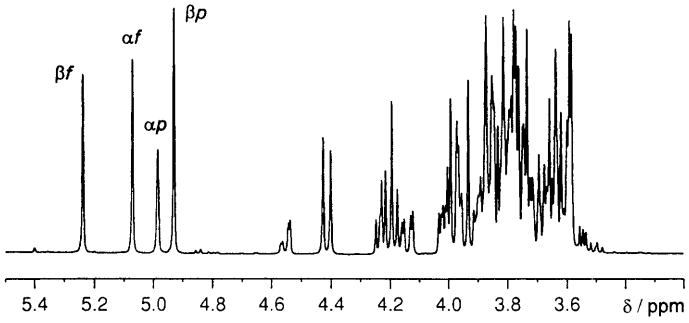
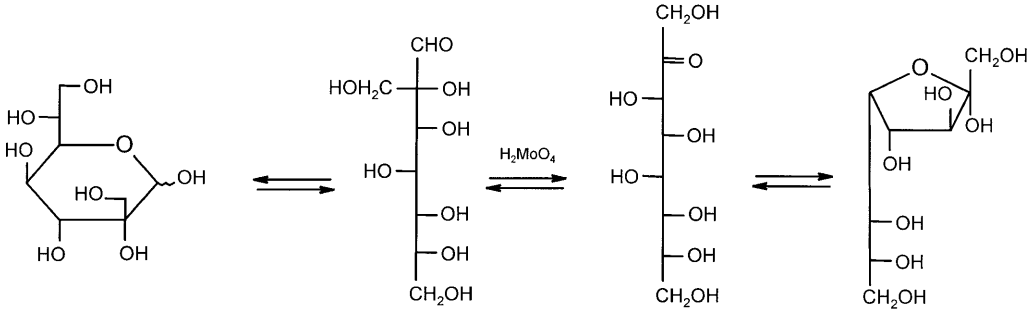


Fig. 1. 300 MHz ^{1}H NMR spectrum of 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose in aqueous solution at 313 K; four signals in the anomeric region originate from four cyclic forms present in solution: βf (5.24 ppm), αf (5.07 ppm), αp (4.98 ppm), and βp (4.93 ppm)

The presence of four resonances, with comparable intensities, was observed in the anomeric region of the ^{1}H NMR spectrum of **3** (Fig. 1). These signals originate from four cyclic forms present in aqueous solution. The ratio of these forms was determined from the ^{1}H NMR spectrum and indicates that the β-pyranose form is the most abundant (30%) at 40°C, whereas the other three are nearly equally populated: 21% αp, 25% αf, 24% βf. The assignment of all ^{1}H and ^{13}C NMR signals was deduced from 2D COSY, HSQC, and HMBC spectra.

The key step of the reaction is a skeletal rearrangement of the 2-C-(hydroxymethyl) branched-chain aldose **3** to the desired 2-ketose *D*-glycero-*D*-ido-oct-2-ulose (**4**) (Scheme 2). The isomerization takes place in mild acidic aqueous solution in the presence of a catalytic amount of molybdic acid at 80°C. Under these reaction conditions an equilibrium mixture of the starting branched-chain aldose **3** and the product ketose **4** was formed in a ratio of 1:5 as determined from the ^{1}H NMR spectrum. The formation of the 2-ketose is in accordance with the mechanism of an isomerization reaction studied recently [10, 11]. The mechanism



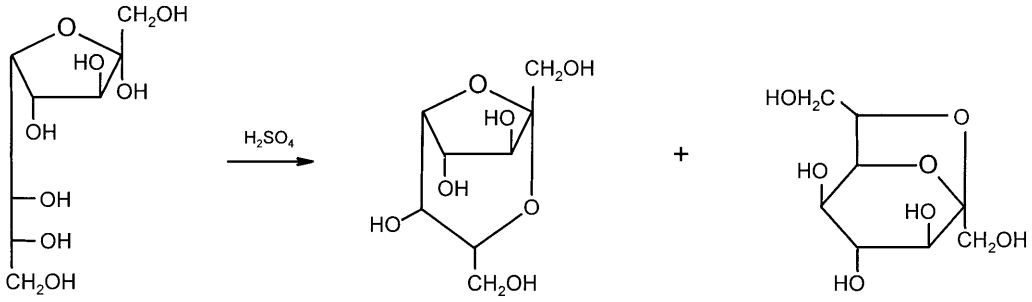
3 **3a** **4a** **4**

Scheme 2

of this transformation proceeds *via* the formation of catalytically efficient dimolybdate complexes of the starting 2-C-branched-chain aldose, yielding the dimolybdate complex of the desired 2-ketose. It has been found that the reaction is reversible as has been shown in a study with saccharides substituted regiospecifically with ^{13}C [10, 11]. Furthermore, the thermodynamic equilibrium of these interconversions is strongly shifted to the side of 2-ketoses. It has been also confirmed that this isomerization proceeds as an intramolecular process under C-1-C-2-C-3 rearrangement of carbon skeleton of the starting 2-C-branched aldose. In the present case, the branched-chain aldose **3** in its acyclic form is bound in a catalytically active dimolybdate complex through its four hydroxyl groups on C-1, C-2, C-3, and C-4 of its hydrated form. The rearrangement occurs in a transition state under the formation of the corresponding dimolybdate complex of acyclic *D*-glycero-*D*-ido-oct-2-ulose (**4**) as shown previously for similar interconversions [11, 12]. During this transformation, bond formation between C-1 and C-3 with simultaneous cleavage of the C-2–C-3 bond takes place. Consequently, the pertinent carbon atoms become the respective C-3, C-2, and C-4 ones in the skeleton of 2-ketose, whereas the branch CH_2OH becomes the C-1 carbon atom. Various forms (cyclic, anhydro, pyranose, furanose) of *D*-glycero-*D*-ido-oct-2-ulose are present in aqueous solution after its release from the complex. In addition to the desired *D*-glycero-*D*-ido-oct-2-ulose and the starting 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose, the reaction mixture obtained after the isomerization contained also *D*-arabinose and 1,3-dihydroxyacetone. Separation of the reaction mixture into its components was achieved by chromatography on a cation-exchange resin column in the Ba^{2+} form. The main component thus isolated in 40% yield was *D*-glycero-*D*-ido-oct-2-ulose. Its ^{13}C NMR assignment is given in the experimental part. The recovery of the starting 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose was 8%. Besides the expected compounds, significant amounts of *D*-arabinose and 1,3-dihydroxyacetone (together 39%) from the reaction mixture were isolated and identified by their NMR spectra.

It is assumed that the partial retro-aldol reaction of *D*-glycero-*D*-ido-oct-2-ulose is a consequence of a relative high abundance of its acyclic form in solution. Thus, the 2-ketose is susceptible for the retro-aldol reaction to *D*-arabinose and 1,3-dihydroxyacetone. A similar process occurs during the enzymatically catalyzed aldolization/retro-aldol reaction of *D*-arabinose-5-phosphate and 1,3-dihydroxyacetone-phosphate to *D*-glycero-*D*-ido-oct-2-ulose 1,8-bisphosphate and *vice versa* [16]. The presence of different forms was evident from ^{13}C NMR spectra in the fraction containing *D*-glycero-*D*-ido-oct-2-ulose. To facilitate the structural assignment the mixture was treated with acid to convert all these forms to the more stable anhydro forms (Scheme 3). Two structures, (2,7-anhydro-*D*-glycero-α-*D*-ido-octulofuranose (**5**) and 2,7-anhydro-*D*-glycero-β-*D*-ido-octulopyranose (**6**)) were identified, the ratio of these forms being 2:1 in favour of the first one. The assignment of the ^{1}H and ^{13}C NMR signals of both forms was performed by analysis of one- and two-dimensional NMR spectra.

The introduced synthetic approach is a possible route to obtain *D*-glycero-*D*-ido-oct-2-ulose in a simple way. It is hoped that the availability of synthetically produced *D*-glycero-*D*-ido-oct-2-ulose may help to elucidate unknown biochemical pathways.



4 **5** **6**

Scheme 3

Experimental

Melting points were measured on a Kofler hotstage microscope. The optical rotations were determined at 20°C with an automatic polarimeter Perkin-Elmer Model 141 using a 10 cm 1 cm^3 cell. NMR spectra were recorded on a Bruker DPX 300 (300.13 MHz) spectrometer equipped with a 5 mm inverse broadband probe with a shielded *z*-gradient. The experiments were carried out in aqueous solution at 40°C and in acetone at 25°C. Chemical shifts were referenced to internal *TSP* (D_2O) and *TMS* (acetone). Presaturation of the residual HDO resonance was achieved by low-power irradiation, and typically 8–16 scans were collected to achieve a good signal/noise ratio in the one-dimensional spectra. A 5 mm QNP probe was used for the measurements of the 1D ^{13}C NMR spectra. Two-dimensional techniques (COSY, HMBC, HSQC) were used to determine the ^{1}H and ^{13}C chemical shifts; the 2D HSQC experiment was performed in the phase-sensitive pure-absorption mode.

Thin-layer chromatography (TLC) was performed on glass plates precoated with silica gel 60 (Aldrich) to monitor the reactions. Detection was effected by spraying the chromatograms with 10% ethanolic sulfuric acid and heating them to 100°C. Flash column chromatography was performed with silica gel (40–100 µm, Lachema) eluted with ethyl acetate:petroleum ether = 3:1 (solvent A) or 6:1 (solvent B). Separation of the free sugars was accomplished by column chromatography on Dowex 50 W X8 resin in the Ba^{2+} form (200–400 mesh) using water as eluent. Paper chromatography (PC) was performed in the descending method on the Whatman No. 1 paper using ethyl acetate: pyridine:water = 8:2:1 as the mobile phase. The spots were made visible by means of alkaline silver nitrate. All evaporations were carried out under reduced pressure at a bath temperature not exceeding 50°C.

2,3:6,7-Di-O-isopropylidene-D-glycero-β-D-gulo-heptofuranose (**1**; $C_{13}H_{22}O_7$)

A reaction mixture of 1 g *D*-glycero-*D*-gulo-heptose (4.8 mmol), 80 cm^3 1,2-dimethoxyethane, 0.1 g toluene-4-sulfonic acid monohydrate, and 6 cm^3 2,2-dimethoxypropane (49 mmol) was stirred vigorously for 8 h. Then, 1.5 g drierite were added, and stirring was continued at room temperature for 24 h until the disappearance of starting material on TLC (solvent A). The reaction mixture was neutralized by addition of $NaHCO_3$. The neutral mixture was filtered with suction and washed with 2×25 cm^3 MeOH. A syrupy diisopropylidene derivative afforded by concentration of the filtrates was purified by flash chromatography on silica gel (solvent A). TLC indicated **1** as major product which was isolated as a syrup. After 24 h at room temperature, **1** crystallized from its solution in acetone.

Yield: 1.1 g (79%); the spectroscopic data of the light yellow solid matched with previously published data [13]; TLC (solvent A): $R_f = 0.47$; $[\alpha]_D^{20} = -32.5^\circ$ ($c = 1$, $CHCl_3$); m.p.: 98–99°C; ^1H NMR (300 MHz, δ, acetone-d_6): 5.30 (H-1), 4.91 (H-3), 4.58 (H-2), 4.15 (H-5), 4.11 (H-4), 4.03 (H-7), 3.96 (H-6) ppm; ^{13}C NMR (75 MHz, δ, acetone-d_6): 112.69 (2,3 *C*Me$_2$), 109.35 (6,7 *C*Me$_2$), 101.12 (C-1), 86.96 (C-2), 81.90 (C-3), 78.96 (C-4), 76.58 (C-5), 71.09 (C-6), 67.51 (C-7) ppm.

2,3:6,7-Di-O-isopropylidene-2-C-(hydroxymethyl)-D-glycero-D-gulo-heptofuranose (**2**; $C_{14}H_{24}O_8$)

A reaction mixture of 0.9 g **1** (3.1 mmol), 0.82 g K_2CO_3, 18 cm^3 MeOH, and 9 cm^3 37% aqueous formaldehyde (88 mmol) was refluxed under Ar at 85°C for 50 h until the disappearance of **1** on TLC (solvent B). Subsequently, the reaction mixture was neutralized with 10% aqueous H_2SO_4 and evaporated. Extraction with 4×20 cm^3 $CHCl_3$ gave a combined fraction that was dried over anhydrous Na_2SO_4 overnight. The organic layer was then evaporated to give syrupy **2** which was purified on a column of silica gel (solvent A). TLC indicated one major product **2** which was isolated as syrup.

Yield: 0.85 g (86%); TLC (solvent B): $R_f = 0.33$; $[\alpha]_D^{20} = -18.2^\circ$ ($c = 1$, $CHCl_3$); ^{13}C NMR (75 MHz, δ, acetone-d_6): 113.86 (2,3 *C*Me$_2$ $\alpha\beta$), 109.44 (6,7 *C*Me$_2$ $\alpha\beta$), 103.85 (C-1 β), 99.53 (C-1 α), 95.87 (C-2 β), 90.73 (C-2 α), 84.95 (C-3 β), 84.22 (C-3 α), 80.04 (C-4 β), 76.84 (C-5 β), 76.26 (C-4 α), 74.28 (C-6 α), 71.49 (C-6 β), 71.38 (C-5 α), 66.58 (C-7 β), 62.87 (*C*H$_2$(C-2) α), 62.70 (C-7 α), 62.56 (*C*H$_2$(C-2) β) ppm.

2-C-(Hydroxymethyl)-D-glycero-D-gulo-heptose (**3**; $C_8H_{16}O_8$)

A mixture of 0.55 g **2**, 15 cm^3 H_2O, and 4 cm^3 Dowex 50 W X4 resin in the H^+ form was stirred at 75°C for 6 h. The resin was filtered, washed with 3×5 cm^3 H_2O, and the combined filtrate was purified with charcoal and evaporated in vacuum to afford syrupy **3**.

Yield: 0.39 g (95%); $[\alpha]_D^{20} = -16.3 \rightarrow -17.0^\circ$ ($c = 1$, H_2O) (24 h); ^{13}C NMR (75 MHz, δ, D_2O): 103.53 (C-1 βf), 99.40 (C-1 αf), 99.15 (C-1 βp), 95.65 (C-1 αp), 83.24 (C-2 βf), 82.06 (C-2 αf), 81.38 (C-4 αf), 80.13 (C-4 βf), 75.90 (C-5 βp), 75.33 (C-2 βp), 74.73 (C-3 αf), 74.52 (C-3 βf), 74.22 (C-2 αp), 73.95 (C-3 αp), 73.95 (C-3 βp), 73.95 (C-6 βf), 73.95 (C-6 βp), 73.03 (C-5 αf), 72.23 (C-6 αp), 71.85 (C-4 αp), 71.47 (C-4 βp), 71.47 (C-5 βf), 71.47 (C-6 αf), 68.94 (C-5 αp), 66.30 (*C*H$_2$(C-2) αp), 65.97 (C-7 βf), 65.79 (C-7 αf), 65.79 (*C*H$_2$(C-2) αf), 65.66 (C-7) βp), 65.33 (C-7 αp), 65.33 (*C*H$_2$(C-2) βf), 64.18 (*C*H$_2$(C-2) βp) ppm.

D-Glycero-D-ido-oct-2-ulose (**4**; $C_8H_{16}O_8$)

A mixture of 0.35 g **3** and 18 cm^3 0.3% aqueous molybdic acid was heated at 85°C for 8 h. The composition of the reaction mixture was examined by paper chromatography until the equilibrium mixture was reached. The cold reaction mixture was stirred with 20 cm^3 Amberlite IRA-400 in the HCO_3^- form, which was removed by filtration after 15 min and washed with 3×15 cm^3 H_2O. The filtrates were concentrated to a syrup that was separated by column chromatography.

The syrupy residue (0.25 g) containing a complex equilibrium mixture of *D*-glycero-*D*-ido-oct-2-ulose and remaining 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose was applied on a column (95 cm×1.5 cm) of Dowex 50 W X8 (200–400 mesh) in the Ba^{2+} form and eluted with water at a flow rate 10 cm$^3 \cdot$ h^{-1}. Tubes were combined according to the chromatographic behaviour of their contents (PC test). The paper chromatograms indicated that fraction 1 (eluting between 105 and 130 cm^3) contained 1,3-dihydroxyacetone together with *D*-arabinose (35 mg, 14%). Fraction 2 (eluting between 130 and 140 cm^3) contained chromatographically pure *D*-arabinose (62 mg, 25%). Fraction 3 (eluting between 140 and 150 cm^3) contained *D*-arabinose and *D*-glycero-*D*-ido-oct-2-ulose (15 mg, 6%). Fraction 4 (eluting between 150 and 180 cm^3) consisted of chromatographically

pure title compound (101 mg, 40%). The ^{1}H NMR spectrum of **4** was identical with that of *D*-glycero-*D*-ido-oct-2-ulose isolated from a natural source [4]; ^{13}C NMR (75 MHz, δ, D_2O): 78.97 (C-3), 78.64 (C-4, C-5), 73.87 (C-7), 72.08 (C-6), 66.11 (*C*H_2 (C-1)), 65.70 (*C*H_2 (C-8)) ppm. Fraction 5 (eluting between 180 and 280 cm^3) contained *D*-glycero-*D*-ido-oct-2-ulose together with 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose (7 mg, 3%), fraction 6 (eluting between 280 and 440 cm^3) chromatographically pure 2-C-(hydroxymethyl)-*D*-glycero-*D*-gulo-heptose (20 mg, 8%).

2,7-Anhydro-D-glycero-α-D-ido-octulofuranose (**5**; $C_8H_{14}O_7$) *and 2,7-anhydro-D-glycero-β-D-ido-octulopyranose* (**6**; $C_8H_{14}O_7$)

A syrupy mixture obtained by concentration of fraction 4 (100 mg) was dissolved in 3 cm^3 aqueous 0.5 *M* H_2SO_4 and heated at 80°C for 16 h. After cooling, the reaction mixture was treated with 20 cm^3 Amberlite IRA-400 in the HCO_3^- form which was then removed by filtration and washed with 3×10 cm^3 H_2O. The deionized solution was concentrated to a syrup (91 mg; 91%) containing a mixture of compounds **5** and **6** in a ratio of 2:1 (by NMR).

^{13}C NMR (75 MHz, δ, D_2O): **5**: 110.85 (C-2), 85.17 (C-7), 79.32 (C-6), 78.02 (C-4), 76.44 (C-3), 74.96 (C-5), 63.06 (C-1), 61.92 (C-8) ppm; **6**: 100.67 (C-2), 79.90 (C-4), 74.49 (C-3), 74.43 (C-5), 72.98 (C-7), 66.16 (C-8), 65.45 (C-1), 64.85 (C-6) ppm.

Acknowledgements

This research was supported in part by VEGA grants No. 2/4144/98 and 2/6037/99 awarded by the Slovak Academy of Sciences.

References

[1] Webber JN (1962) Adv Carb Chem **17**: 45
[2] Frankle FP, Kapucinsky M, Macleod JM, Williams JF (1984) Carbohydr Res **125**: 177
[3] Bianchi G, Gamba A, Murelli C, Salamini F, Bartels D (1991) Plant J **1**: 355
[4] Howarth OW, Pozzi N, Vlahov G, Bartels D (1996) Carbohydr Res **289**: 137
[5] Bílik V (1972) Chem Zvesti **26**: 183
[6] Bílik V (1972) Chem Zvesti **26**: 187
[7] Bílik V (1972) Chem Zvesti **26**: 372
[8] Bílik V, Voelter W, Bayer E (1972) Liebigs Ann Chem **759**: 189
[9] Hricovíniová Z, Hricovíni M, Petrušová M, Matulová M, Petruš L (1998) Chem Papers **52**: 238
[10] Hricovíniová-Bíliková Z, Hricovíni M, Petrušová M, Serianni AS, Petruš L (1999) Carbohydr Res **319**: 38
[11] Hricovíniová Z, Hricovíni M, Petruš L (1998) Chem Papers **52**: 692
[12] Hricovíniová-Bíliková Z, Petruš L (1999) Carbohydr Res **320**: 31
[13] Brimacombe JS, Tucker LCN (1968) J Chem Soc C 562
[14] Bílik V, Petruš L, Farkaš V (1975) Chem Zvesti **29**: 690
[15] Hayes ML, Pennings NJ, Serianni AS, Barker R (1982) J Am Chem Soc **104**: 6764
[16] Paoletti F, Williams JF, Horecker BL (1979) Arch Biochem Biophys **198**: 614

Received October 17, 2000. Accepted December 4, 2000