Addition of 2,6-Anhydro-7-deoxy-7-nitro-L-*glycero*-L-*galacto*-heptitol to Formaldehyde and the Conversion of the Adduct to 3,7-Anhydro-D-*glycero*-L-*manno*-octulose

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Dedicated to Dr. Ing. Š. Bauer, DrSc., in honour of his 70th birthday

The reaction of 2,6-anhydro-7-deoxy-7-nitro-L-*glycero*-L-*galacto*-heptitol with formaldehyde in dimethyl sulfoxide in the presence of sodium methoxide afforded a crystalline adduct which, by the ozonolysis in an aqueous solution at room temperature, was transformed to 3,7-anhydro-D-*glycero*-L-*manno*-octulose as a major product and 2,6-anhydro-D-*glycero*-L-*manno*-heptonic acid as a minor product. The products as well as the intermediates, 3,7-anhydro-2-deoxy-2-nitro-D-*threo*-L-*galacto*-octitol and its epimer 2,6-anhydro-7-deoxy-7-nitro-L-*erythro*-L-*galacto*-octitol, were characterized by the ^{1}H and ^{13}C NMR spectrometry.

The nitromethane synthesis is an important method of the carbon chain elongation in aldoses. Its gist is the addition of the nucleophile, generated from nitromethane or nitroethanol in a basic medium, to an aldose [1]. The contribution deals with an opposite approach to the utilization of the reaction, *i.e.* a nitro sugar derivative is used as a nucleophile being added to one-carbon aldehyde.

The addition of the readily available 2,6-anhydro-7-deoxy-7-nitro-L-*glycero*-L-*galacto*-heptitol [2, 3] (*I*, Scheme 1) to formaldehyde in dimethyl sulfoxide in the presence of sodium methoxide gave rise to the crystalline sodium salt of 2,6-anhydro-7-deoxy-7-*aci*-nitro-L-*glycero*-L-*galacto*-octitol (*II*). The compound was hygroscopic and therefore not characterized. Upon its acidification with a mixture of a strong-acidic cation exchanger and carbon dioxide (which significantly inhibits the retroaldol decomposition [3, 4]), *II* was transformed to the mixture of 3,7-anhydro-2-deoxy-2-nitro-D-*threo*-L-*galacto*-octitol (*III*) and its epimer, 2,6-anhydro-7-deoxy-7-nitro-L-*erythro*-L-*galacto*-octitol (*IV*). Using the preparative paper chromatog-
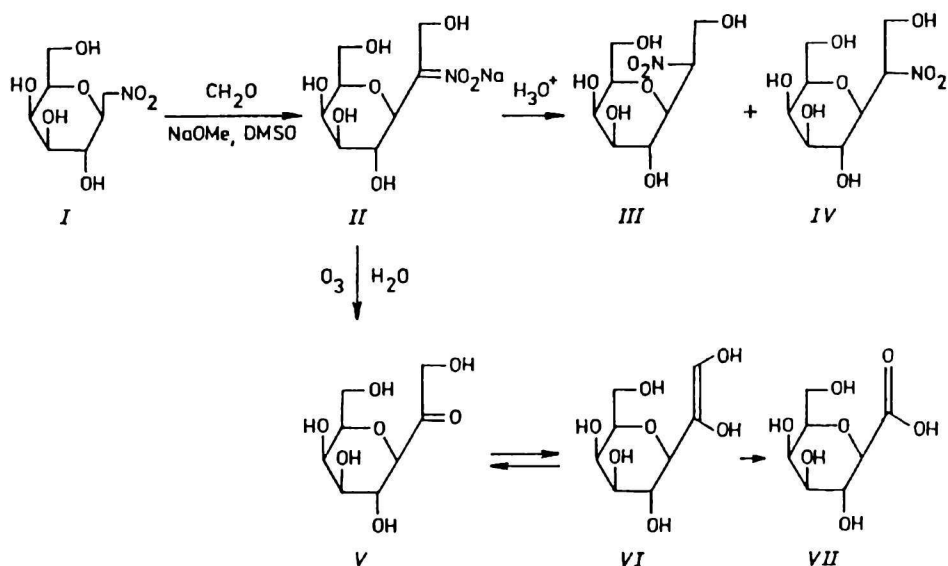
Scheme 1

raphy, *III* and *IV* were resolved only partially (R_f = 0.89 and 0.82). Thus, an enriched mixture *A* containing 82 % of the faster epimer and 18 % of the slower one was obtained. The other mixture *B* from the separation contained 50 % of each epimer. The determination of the composition of the mixtures was made by the ^{1}H and ^{13}C NMR spectrometry. Using the amount of substance ratios obtained, the specific optical rotations of both epimers were calculated. By the ^{1}H NMR spectrometry, however, we did not succeed to ascribe the configurations of the newly created asymmetric centres of both particular epimers.

For the analysis of the ^{1}H NMR spectra, the 1D COSY experiment [5] was used. Despite of the optimization of the fixed time delays in the pulse sequence, with respect to coupling constants, the selective polarization transfer from H-1′ to H-1 was not successful, apparently due to a too low difference between $J_{1,1'}$ and $J_{1',2''}$. The values of $J_{1,1'}$ for both *III* and *IV* were not too different as well and implied a *gauche* orientation of the protons H-1 and H-1′ The ^{1}H and ^{13}C NMR data of *III* and *IV* are given in Tables 1 and 2, respectively.

The treatment of an aqueous solution of *II* with ozone [6] afforded a mixture of 3,7-anhydro-D-*glycero*-L-*manno*-octulose (*V*, a major product) and 2,6-anhydro-D-*glycero*-L-*manno*-heptonic acid (*VII*, a minor product). The ratio of both products in the final reaction mixture was dependent on how *II* was added. If added in one portion, the mass ratio of *V* and *VII* was 8 : 1. The addition of *II* in 3—4 portions however, changed the ratio up to 2 : 1 without a significant difference in the total mass yield of *V* and *VII*. The highest yield of the major product was up to 75 % and that of *VII* did not exceed 25 %. The attempts to prepare *V* using the other known procedures of the transformation of the *aci*-nitro group to the carbonyl group [7, 8] were not successful.
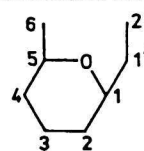
Table 2. The ^{13}C NMR Data of the Compounds Prepared

Compound	Chemical shift δ							
	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′
I	80.1	69.0	75.0	70.2	77.8	62.4	77.8	—
III	80.5	69.7	75.2	70.2	79.3	62.4	90.3	59.7
IV	80.5	68.8	75.4	70.1	77.8	62.4	90.1	61.1
V	82.2	69.2	75.1	70.4	80.3	62.5	210.0	67.7
VII	80.9	70.4	75.6	70.6	79.6	62.6	178.0	—

Apparently, the increased alkalinity of the reaction mixture after the readdition of another portion of *II* enhanced the extent of the isomerization of *V*, already present in the reaction mixture, to the enediol structure *VI*. The ozone cleavage of the enediol double bond thus explains the formation of *VII* together with formic acid (found in the reaction mixture by the ^{1}H and ^{13}C NMR spectrometry as well).

The reaction mixture after the ozone treatment of an alkaline solution of the starting *I* did not contain the acid *VII*. Probably the transformation provided highly reactive 2,6-anhydroaldehydo-D-*glycero*-L-*manno*-heptose as a primary product (similarly as the ozonolysis of a per-O-acetylated silyl nitronate [9]).

The only ascribable protons of the compound *V* directly from its ^{1}H NMR spectrum were H-1, H-3, H-4, and H-6′. Chemical shifts and coupling constants of the other protons were ascribed using 1D COSY experiment with time delays optimized for the particular coupling constants. The protons of the CH$_2$OH group neighbouring to the carbonyl group were recorded as a singlet (δ = 4.62).

Table 1. The ^{1}H NMR Data of the Compounds Prepared^a

Compound	Chemical shift δ									
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	H-1′	H-2′	H-2″
III	b	b	b	3.96	b	3.74	b	5.03	4.34	4.12
IV	b	b	b	3.93	b	b	b	5.09	4.30	4.10
V	3.95	3.75	3.66	3.96	3.73	3.78	3.71	—	4.62	4.62
VII	b	b	3.66	3.95	b	3.77	b	—	—	—

Compound	Coupling constant J/Hz									
	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	$J_{1',2'}$	$J_{1',2''}$	$J_{2',2''}$	$J_{1,1'}$
III	b	b	3.2	0.8	8.5	b	12.1	8.9	3.2	13.1	4.1
IV	b	b	4.5	1.4	b	b	b	9.5	4.1	12.8	4.4
V	9.3	9.0	3.1	1.2	8.7	1.5	11.9	—	—	c	—
VII	b	9.0	3.7	0.2	8.5	b	11.4	—	—	—	—

a) The numbering of the hydrogen atoms corresponds to that of the carbon atoms (Table 2). *b)* Not ascribed or not resolved. *c)* Protons H-2, H-2′ (magnetically equivalent) observed as a singlet.

Besides the highly preponderant acyclic form, also the signals of cyclic forms (δ = 97.3, 97.0, 91.0, and 90.4) were found in the ^{13}C NMR spectrum of V. The rather low content of the cyclic forms (ca. 10 %) however did not allow to identify their structures more closely, i.e. whether they were intramolecular poloacetals or dimeric structures analogical e.g. to glycolaldehyde.

The ^{1}H NMR spectrum of VII contained the signals of the protons of the anhydro ring only, two of them (H-4, H-6) being ascribable directly. A selective excitation of H-4 identified the proton H-3. The polarization transfer was not achieved for the other protons. The ^{1}H and ^{13}C NMR data of V and VII are given in Tables 1 and 2, respectively.

EXPERIMENTAL

Specific optical rotations were measured on a Perkin—Elmer 141 polarimeter. Elemental analyses were done with an automatic Perkin—Elmer 240 analyzer. The ^{13}C NMR spectra (75.46 MHz) were recorded with a Bruker AM 300 FT NMR spectrometer in D_2O (internal standard methanol, δ = 50.15) at 298 K using the DEPT pulse sequence. The ^{1}H NMR spectra (300.13 MHz) were recorded at 298 K using a 5 mm ^{1}H probe (internal standard sodium 3-(trimethylsilyl)propionate, δ = 0.00) with the digital resolution 0.24 Hz/point. For the signal assignment, the 1D COSY experiment was used. The Gaussian soft pulse duration was 40 ms. An ozone generator 502 (Fischer) was used for the preparation of ozone from gaseous oxygen.

Addition of 2,6-Anhydro-7-deoxy-7-nitro-L-glycero-L-galacto-heptitol (I) to Formaldehyde

Gaseous formaldehyde (0.3 g; 10.0 mmol, generated by the thermal decomposition of dry paraformaldehyde) was introduced into a solution of I (1.1 g; 4.9 mmol) in dimethyl sulfoxide (15 cm^3). Then, a sodium methoxide solution (0.5 g of sodium, 30 cm^3 of methanol) and 1-butanol (15 cm^3) were added to the solution at 20 °C. After 1 h, the precipitate of II was filtered, washed with 1-butanol (3 x 5 cm^3), and used for the isolation of III and IV, or for the preparation of V.

3,7-Anhydro-2-deoxy-2-nitro-D-threo-L-galacto-octitol (III) and 2,6-Anhydro-7deoxy-7-nitro-L-erythro-L-galacto-octitol (IV)

The washed precipitate of II was added at 10 °C to the stirred suspension of cation ex-changer Amberlit IR 120 in the H-form (20 cm^3, 150—300 μm) and crashed dry ice (10 g). After the dismissal of the carbon dioxide, the cation exchanger was filtered off, washed with water (2 x 10 cm^3), and the combined filtrate and washings were evaporated under diminished pressure to a sirup (1.2 g). The paper chromatography (Whatman No. 1, elution system S_1 1-butanol—ethanol—water, volume ratio = 5 : 1 : 4) revealed the presence of traces of the starting material (R_I = 1.00), two main products III and IV (R_I = 0.89 and 0.82) and small amounts (< 5 %) of two other compounds (R_I = 0.49 and 0.35). The sirup was chromatographed on a Whatman No. 3 paper (0.2 g/sheet) at 25 °C for 60 h. The elution zone containing III and IV was visualized in a dark room with the 254 nm UV light. The front third of the elution zone and its rest were cut off, extracted with water, and extracts were evaporated separately. From the faster zone, the sirupy mixture A (0.2 g), [α](D, 20 °C, ρ = 20 g dm^{-3}, water) = + 28.5° containing 82 % of the faster nitro derivative and 18 % of the slower nitro derivative (^{13}C NMR analysis) was obtained. Working up the slower zone afforded the sirupy mixture B (0.6 g), [α](D, 20 °C, ρ = 20 g dm^{-3}, water) = + 26.3° containing both isomers in the ratio 1 1. For the faster-moving epimer E_1, calculated [α](D, 20 °C, ρ = 20 g dm^{-3}, water) = + 29.7°, and for the slowlier-moving epimer E_2, [α] (D, 20 °C, ρ = 20 g dm^{-3}, water) = + 22.9°.

3,7-Anhydro-D-glycero-L-manno-octulose (V)

The washed precipitate II was added at 25 °C to a solution of sodium hydroxide (20 cm^3, c = 0.02 mol dm^{-3}) saturated with ozone. To the reaction mixture, an ozone stream (20 mg min^{-1}) was introduced for 10—15 min until the neutral reaction of the solution was reached. The evaporation of the reaction mixture afforded a sirupy residue (1.2 g) which was resolved on a column (75 cm x 3.2 cm) of the Whatman cellulose using elution system S_1 at the flow rate 10 cm^3 h^{-1} The evaporation of the fraction 1 afforded V hydrate (0.8 g, 68 %), an amorphous hygroscopic material with [α](D, 20 °C, ρ = 20 g dm^{-3}, water) = + 19.2°, R_I = 0.42 (S_1). For $C_8H_{14}O_7$ H_2O (M_r = 240.21) w_i(calc.): 40.00 % C, 6.71 % H; w_i(found): 39.68 % C, 6.78 % H. Fraction 2 contained sodium nitrate.

The evaporation of the fraction 3 yielded sodium 2,6-anhydro-D-glycero-L-manno-heptonate hydrate (0.1 g, 8 %), an amorphous hygroscopic material, [α](D, 20 °C, ρ = 10 g dm^{-3}, water) = + 21.2°, R_I = 0.15 (S_1). For $C_7H_{11}O_7Na$ H_2O

(M_r = 248.16) w_i(calc.): 33.88 % C, 5.28 % H; w_i(found): 33.60 % C, 5.35 % H.

The same procedure, but with the addition of the precipitate *II* in three portions, afforded *V* hydrate (0.6 g, 51 %) and sodium salt of *VII* hydrate (0.3 g, 24 %).

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Production of Extracellular Polysaccharides by *Candida mucifera*

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Candida mucifera, CCY 29-170-1, when cultured in liquid media with various carbon sources, produced D-mannose-dominating extracellular polysaccharides; the only exception was the medium with beer wort, where the dominating sugar component was D-glucose. All the polysaccharides were heterogeneous upon electrophoresis. The protein moiety in the polysaccharides varied from 3 to 16 % and the content of phosphorus from 0.5 to 1.1 %. The lowest limiting viscosity number (14 cm^3 g^{-1}) was found with the D-glucose-dominating polysaccharide and the highest one (67 cm^3 g^{-1}) with the D-mannose-dominating polysaccharide.

In an earlier report *Kocková-Kratochvílová* and *Slávíková* [1] described a new yeast, named *Candida mucifera*. It was originally isolated from frog liver in Amazonian virgin forest, near the city Manaus (Brasil). The ability to utilize methanol as the only carbon source and produce large amounts of extracellular polysaccharides, when grown in liquid medium containing sugar, was considered an important characteristics of this species.

Therefore, it was of interest to characterize the composition and some physicochemical properties of the extracellular polysaccharides produced in liquid media containing various carbon sources, which is the subject of the present work.

EXPERIMENTAL

All evaporations were conducted under diminished pressure at 40—45 °C. Paper chromatography was performed by the descending method on Whatman No. 1 paper with the following systems: S_1, ethyl acetate—pyridine—water (φ_r = 8 : 2 : 1); S_2, ethyl acetate—acetic acid—formic acid—water (φ_r = 18 : 3 : 1 : 4); S_3, ethyl acetate—pyridine—acetic acid—water (φ_r = 5 : 5 : 1 : 3). Reducing sugars were detected by spraying with anilinium hydrogen phthalate and heating the papers for 5 min at 105 °C, and alditols with alkaline silver nitrate reagent. Quantitative deter-