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Synthesis of sedoheptulose from 2-*C*-(hydroxymethyl)-D-allose by molybdic acid-catalysed carbon-skeleton rearrangement☆

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Abstract

A new branched-chain aldose, 2-*C*-(hydroxymethyl)-D-allose (**3**), was obtained by a base-catalysed addition of 2,3:5,6-di-*O*-isopropylidene-β-D-allofuranose to formaldehyde followed by acid hydrolysis of the aldol product. On treatment with a catalytic amount of molybdic acid at 90 °C, **3** afforded its equilibrium mixture with sedoheptulose tautomeric and anhydro forms in the ratio 12:1. Sedoheptulose in its 2,7-anhydro form, 2,7-anhydro-β-D-*altro*-heptulopyranose, was obtained from this mixture by treatment with 0.5 M H_2SO_4 and crystallisation (overall 63% yield).

Keywords: Sedoheptulose; Sedoheptulosan; D-*altro*-heptulose; 2-*C*-(Hydroxymethyl)-D-allose; Molybdic acid catalysis; Carbon-skeleton rearrangement

1. Introduction

D-*altro*-Heptulose, sedoheptulose, is a naturally occurring saccharide that plays an important role in the metabolism of both plants and animals. It is present as an intermediate in the photosynthetic cycle in its phosphorylated forms and takes part in the formation of hexoses from lower-carbon fragments both in photosynthesis and in carbohydrate metabolism by animal tissues.

Sedoheptulose was originally found in leaves and stems of *Sedum spectabile* Bor., a common herbaceous perennial plant. It has also been detected in many other succulent plants. *S. spectabile* contains about 1% of this sugar [1] and has been used as a main source for isolation of sedoheptulose from this plant extract. Previously, sedoheptulose was prepared by enzymatic synthesis from derivatives of D-erythrose and triose phosphate, but the yield of this reaction was very low (less than 0.5%) [2]. Slightly higher yields have been obtained by accumulation of sedoheptulose by wild-type strains of *B. subtilis* [3]; *Flavobacterium* sp. and some strains of actinomycetes also produce small amounts of sedoheptulose [4].

The epimerization of aldoses catalysed by molybdic acid is an effective method for preparation of C-2 epimeric aldoses [5–7]. The reaction mechanism of this transformation has been revealed by rearrangement studies with ^{13}C-substituted aldoses [8]. Further studies on the extension of this approach have shown that a similar type of rearrangement is also possible in the case of 2-ketoses which, in

☆ Dedicated to the memory of Professor V. Bílik.

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the presence of molybdic acid, are interconvertable with 2-*C*-(hydroxymethyl) aldoses [9]. The stereospecific transformation of D-(2-^{13}C)-fructose to D-(2-^{13}C)-hamamelose [2-*C*-(hydroxymethyl)-D-(2-^{13}C)-ribose] has proved that the mechanism of this transformation [10] is analogous to that of the Bílik reaction [11]. The thermodynamic equilibria of these interconversions are, however, strongly shifted to the side of 2-ketose formation. This suggests a new, general, and one-step approach to 2-ketoses from 2-*C*-(hydroxymethyl) aldoses, provided that the latter sugars are readily available. The paradigm applied to the synthesis of sedoheptulose is presented in this contribution.

2. Experimental

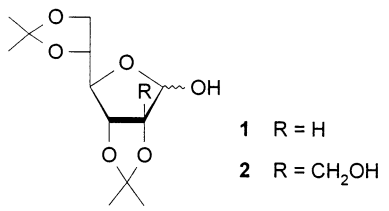
General methods and materials.—Melting points were measured on a Koffler stage. The optical rotations were performed with a Perkin–Elmer polarimeter model 141, at 20 °C. NMR spectra were recorded on a Bruker DPX 300 spectrometer equipped with a 5 mm inverse broadband probe with a shielded *z*-gradient. The experiments were carried out at 40 (D_2O) and 25 °C (acetone), respectively. The ^{1}H and ^{13}C chemical shifts were referenced to internal TSP (D_2O) and Me_4Si (acetone). One-dimensional (1D) spectra were recorded with a spectral width of 1800 Hz and usually 16 scans were accumulated to achieve a good signal/noise ratio. A 5 mm QNP probe was used for measurements of 1D ^{13}C NMR spectra. Two-dimensional (2D) techniques, COSY, HMBC and HSQC, were used to determine ^{1}H and ^{13}C chemical shifts; the 2D HSQC experiment was performed in phase-sensitive pure-absorption mode. The spectral widths in 2D experiments were 1200 (^{1}H) and 5000 Hz (^{13}C); the spectra were zero-filled before the Fourier transformation, giving respective digital resolutions of 1.2 and 5.9 Hz/pt.

Thin-layer chromatography (TLC) was carried out with glass plates precoated with silica gel L (5–40 μm, Lachema) to monitor the reactions and to certify the purity of the reaction products. Detection was effected by spraying the chromatograms with 10% ethanolic sulphuric acid and charring them on a hot plate. Flash chromatography was performed on silica gel (40–100 μm, Lachema) eluted with ethyl acetate–petroleum ether (3:1 (solvent A) or 2:1 (solvent B)). Column chromatography was performed on Dowex 50W X8 in Ba^{2+} form (200–400 mesh), using water as the eluant. Paper chromatography (PC) was performed by the descending method on Whatman No. 1 paper using 8:2:1 ethyl acetate–pyridine–water as the mobile phase. The spots of compounds were detected with alkaline silver nitrate. All concentrations were carried out under reduced pressure at a bath temperature not exceeding 45 °C.

2,3:5,6-Di-O-isopropylidene-β-D-allofuranose (**1**).—The reaction mixture of D-allose (1 g, 5.6 mmol), 1,2-dimethoxyethane (50 mL), toluene-4-sulfonic acid monohydrate (0.1 g) and 2,2-dimethoxypropane (5 mL, 40.5 mmol) was stirred until all components were dissolved. Then Drierite (1.2 g) was added and stirring continued at room temperature (rt) for 24 h until the disappearance of starting material on TLC (solvent B). The reaction mixture was neutralised by addition of $NaHCO_3$ (0.6 g) and then the neutral mixture was filtered with suction and washed with methanol (2 × 25 mL). Concentration of filtrates afforded a syrupy residue which was purified by flash-chromatography on silica gel (solvent B). TLC indicated major product **1** isolated as a syrup, which crystallised from methanol. Yield 1.54 g (71%); mp 66–67 °C; $[\alpha]_D$ −24,5° (*c* 1, chloroform); R_f 0.73 (solvent B). The data are comparable with those published [12]; ^{1}H NMR (acetone, 300.13 MHz): δ 6.07 (H-1), 5.60 (H-3), 5.36 (H-2), 4.93 (H-5), 4.85 (H-6), 4.70 (H-4), 4.64 (H-6'), ^{13}C NMR (acetone, 75.45 MHz): δ 112.49 (2,3 CMe_2), 110.21 (5,6 CMe_2), 103.71 (C-1), 88.50 (C-4), 87.18 (C-2), 83.25 (C-3), 76.90 (C-5), 67.91 (C-6).

2,3:5,6-Di-O-isopropylidene-2-C-(hydroxymethyl)-D-allofuranose (**2**).—A reaction mixture of **1** (0.7 g, 2.7 mmol), potassium carbonate (0.51 g), MeOH (10 mL) and 37% aq soln of formaldehyde (5.5 mL) was refluxed under argon at 85 °C for 48 h until the disappearance of **1** on TLC (solvent A). The reaction mixture was neutralised with

10% aq sulfuric acid and evaporated. Extraction with chloroform (4 × 15 mL) gave a combined fraction that was dried over anhyd 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** isolated as a syrup. Yield 0.69 g (88%); $[\alpha]_D$ +1.7° (*c* 1, chloroform); R_f 0.42 (solvent A); ^{1}H NMR (acetone, 300.13 MHz): δ 5.30 (H-1α, d, 1.9 Hz), 5.09 (H-1β, d, 9.2 Hz), 4.70 (H-3α, s), 4.68 (H-3β, d, 1.1 Hz), 4.15–4.25 (H-5α, m), 4.10–4.15 (H-5β, m), 4.03–4.10 (H-6α,β, m), 3.88–3.93 (H-4α,β, m), 3.75–3.90 (H-6′α,β, m), 3.65–3.80 (H-2α,β, H-2′α,β, m). ^{13}C NMR (acetone, 75.45 MHz): δ 105.18 (C-1 α), 98.95 (C-1 β), 95.20 (C-2 α), 92.06 (C-2 β), 88.13 (C-4 α), 85.47 (C-3 α), 83.87 (C-3 β), 83.25 (C-4 β), 76.55 (C-5 α), 75.66 (C-5 β), 67.87 (C-6 α), 67.43 (C-6 β), 63.00 (CH_2(C-2) α), 63.00 (CH_2(C-2) β).



1 R = H

2 R = CH_2OH

*2-C-(Hydroxymethyl)-*D*-allose* (**3**).—A mixture of **2** (0.5 g, 23.8 mmol), water (20 mL) and Dowex 50 W X4 resin in the H^+ form (4 mL) was stirred at 70 °C for 5 h. The resin was removed by filtration, washed with water (3 × 6 mL) and the combined filtrate was purified with charcoal and evaporated to dryness to give syrupy **3**. Yield 0.35 g (96%); $[\alpha]_D$ +8.7 → +6.5° (*c* 1, water) (24 h); ^{13}C NMR (D_2O, 75.45 MHz): δ 103.75 (C-1 β*f*), 100.05 (C-1 α*f*), 97.79 (C-1 β*p*), 95.93 (C-1 α*p*), 84.60 (C-4 β*f*), 84.22 (C-4 α*f*), 83.95 (C-2 β*f*), 81.02 (C-2 α*f*), 77.16 (C-5 β*p*), 76.96 (C-2 β*p*), 75.72 (C-2 α*p*), 75.47 (C-5 β*f*), 74.60 (C-3 β*f*), 74.34 (C-5 α*f*), 74.06 (C-3 α*p*), 73.63 (C-3 β*p*), 72.61 (C-3 α*f*), 70.60 (C-5 α*p*), 68.37 (C-4 β*p*), 67.78 (C-4 α*p*), 66.08 (*C*H_2(C-2) α*p*), 65.87 (*C*H_2(C-2) α*f*), 65.27 (*C*H_2(C-2) β*f*), 65.27 (C-6 β*f*), 65.12 (C-6 α*f*), 64.12 (C-6 β*p*), 63.60 (C-6 α*p*), 63.45 (*C*H_2(C-2) β*p*).

D-Altro-heptulose (sedoheptulose, **4**).—A mixture of **3** (0.21 g, 10 mmol) and 0.2% aq molybdic acid (5 mL) was heated at 90 °C for 36 h. The composition of the reaction mixture was examined by paper chromatography (8:2:1 ethyl acetate–pyridine–water) until the equilibrium mixture was reached. The cold reaction mixture was stirred with Amberlite IRA-400 in the HCO_3^- form (10 mL), which was removed by filtration after 15–20 min and washed with water (3 × 5 mL). The filtrates were concentrated to a syrup (0.2 g), which was fractionated by column chromatography.

Fractionation on Dowex 50W X8 in the Ba^{2+} form.—The syrupy residue (0.2 g) containing a complex equilibrium mixture of sedoheptulose, sedoheptulosan and remaining 2-*C*-(hydroxymethyl)-D-allose was applied on a column (95 × 1.6 cm) of Dowex 50W X8 (200–400 mesh) in the Ba^{2+} form and eluted with water at a flow rate 7 mL/h. Tubes were combined according to the chromatographic behaviour of their contents (PC test). The paper chromatograms indicated that Fraction 1 (eluting between 90–125 mL) contained both sedoheptulose and sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose) (180 mg, 90%). Fraction 2 (eluting between 140–180 mL) showed the presence of a single spot corresponding to 2-*C*-(hydroxymethyl)-D-allose (15 mg, 7%).

*2,7-Anhydro-β-*D*-altro-heptulose (sedoheptulosan*, **5***) monohydrate.*—A syrupy mixture of sedoheptulose and sedoheptulosan obtained by concn of Fraction 1 (180 mg) was dissolved in aq 0.5 M sulfuric acid (4.5 mL) and heated for 15 h at 80 °C. After cooling, the reaction mixture was treated with Amberlite IRA-400 in the HCO_3^- form (30 mL), which was removed by filtration for 15–20 min and washed with water (3 × 15 mL). The deionized soln was concd to a syrup (168 mg), which was crystallised after its inoculation with seed crystals of authentic sedoheptulosan hydrate. After recrystallization from MeOH, large clear prisms of **5** monohydrate were obtained (113 mg; 63%), mp 103–104 °C, $[\alpha]_D$ −130.5° (*c* 1, water). The obtained data are in accordance with those published [13]. Both ^{1}H and ^{13}C NMR spectra of **5** were identical to those of commercial sedoheptulosan isolated from *S. spectabile* Bor. [14].

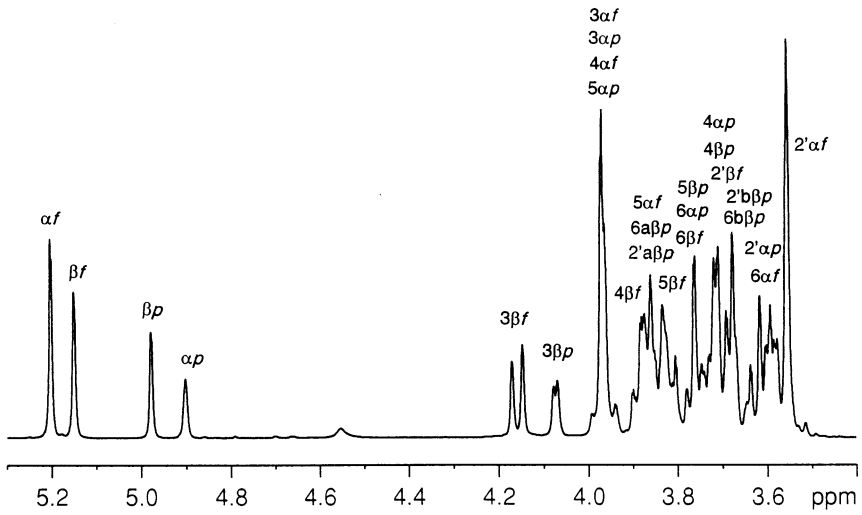
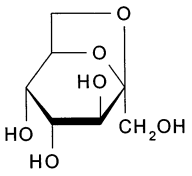


Fig. 1. A 300 MHz ^{1}H NMR spectrum of 2-*C*-(hydroxymethyl)-D-allose in aqueous solution at 40 °C. Four singlet resonances in the anomeric region originate from anomeric protons of four cyclic forms (5.21 ppm, α*f*; 5.17 ppm, β*f*; 4.98 ppm, β*p*; 4.90 ppm α*p*).



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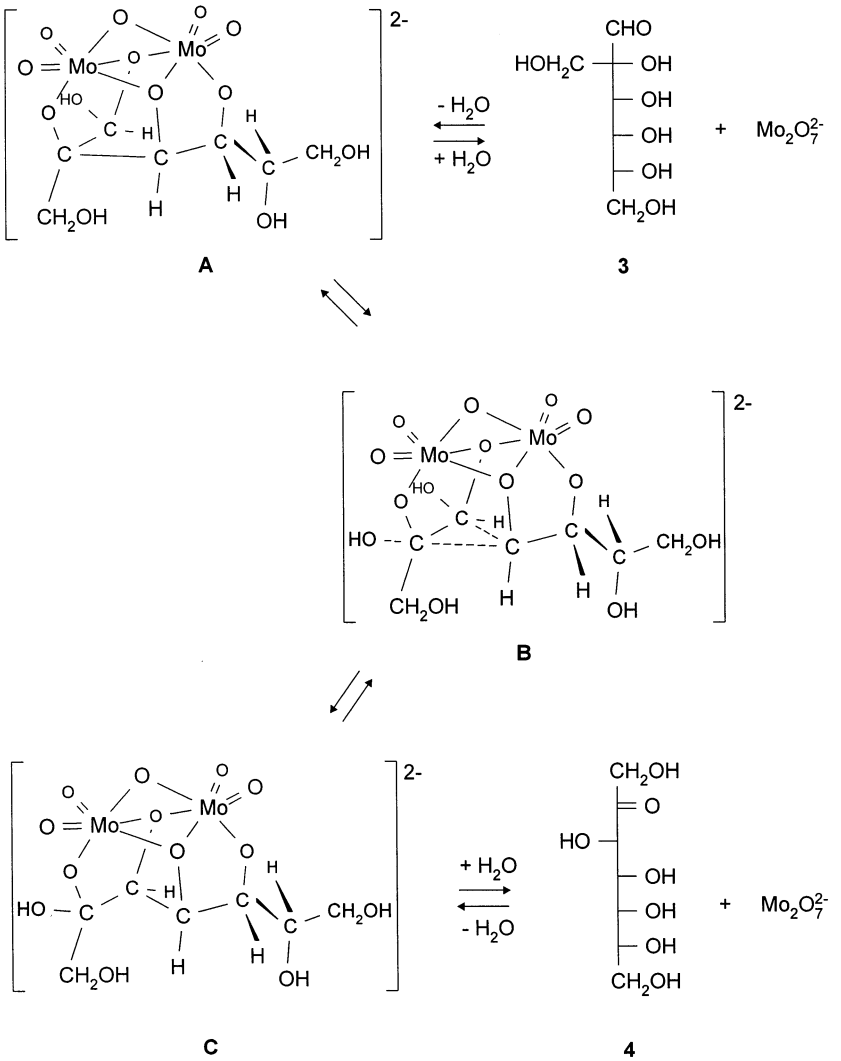
3. Results and discussion

The condensation of D-allose and 2,2-dimethoxypropane in the presence of toluene-4-sulfonic acid in 1,2-dimethoxyethane at room temperature afforded 2,3:5,6-protected D-allofuranose as a major product of the reaction. The substitution anchored the configuration of the sugar at the carbon atom C-2 and left the anomeric centre free for aldolization. The alkali-catalysed addition of 2,3:5,6-di-*O*-isopropylidene-β-D-allofuranose (**1**) to formaldehyde gave the corresponding 2-*C*-branched D-allose **2**, namely 2,3:5,6-di-*O*-isopropylidene-2-*C*-(hydroxymethyl)-D-allofuranose in a 88% yield. Subsequent removal of the isopropylidene groups by acid hydrolysis in the presence of Amberlite IR 120 in the H^+ form gave a new compound, 2-*C*-(hydroxymethyl)-D-allose (**3**) in a 96% yield.

The ^{1}H NMR spectrum of 2-*C*-(hydroxymethyl)-D-allose presented in Fig. 1 partially proved the structure of the branched-chain sugar **3**. Four characteristic singlet resonances in the anomeric region (4.8–5.2 ppm) originate from anomeric protons of four cyclic forms present in aqueous solution at 40 °C. The ratio of these cyclic forms was determined from the resonance intensities in the ^{1}H NMR spectrum (15% α*p*, 37% α*f*, 20% β*p*, 28% β*f*) indicating that in aqueous solution the furanose forms are slightly predominant. Owing to relatively high abundances of four cyclic forms, the ^{13}C chemical shifts of all signals could be determined from the 2D HSQC spectrum together with COSY and HMBC providing further evidence of the branched-chain sugar **3**.

The treatment of 2-*C*-(hydroxymethyl)-D-allose with a catalytic amount of molybdic acid at 90 °C for 36 h afforded a complex equilibrium mixture of tautomeric cyclic and anhydro forms of sedoheptulose and the remaining starting sugar in the ratio 12:1.

This is in accordance with the mechanism of the isomerization of D-(2-^{13}C)-fructose [10], according to which the branched-chain aldose **3** in its dimolybdate tetradentate complex rearranges to the straight-chain D-*altro*-heptulose (**4**) (Scheme 1). The essential step of the interconversion is the formation of catalytically efficient complex of the 2-*C*-(hydroxymethyl)-D-allose (**A**) via its four hydroxyl oxygen atoms at C-1, C-2, C-3 and C-4 of its hydrated form. In the transition state (**B**) a



Scheme 1.

new bond formation between C-1 and C-3 occurs with a simultaneous cleavage of the C-2–C-3 bond; consequently the dimolybdate complex of D-*altro*-heptulose (**C**) is formed. The interconversion is obviously reversible and its thermodynamic equilibrium is strongly shifted to the side of 2-ketose as a consequence of different steric demands of both mutually interconvertable sugars. Previously, under the mild acidic conditions of the interconversion, a part of heptulose **4** was converted into its 2,7-anhydro form seen in the ^{1}H NMR spectrum of the reaction mixture as a characteristic crown-shaped triplet at 4.68 ppm. This is in agreement with the fact that D-*altro*-heptulose is isolated and commercially available in its 2,7-anhydro form, which is a highly predominant form of the sugar in acidic solutions [13].

Separation of the reaction mixture on a column of cation-exchange resin in the Ba^{2+} form afforded two chromatographically pure fractions. The first contained both sedoheptulose and sedoheptulosan. The presence of different forms of sedoheptulose was evident from its ^{13}C NMR spectrum in the region between 94 and 105 ppm where five resonances were detected. The second fraction contained 2-*C*-(hydroxymethyl)-D-allose. The syrupy fraction containing the mixture of sedoheptulose forms was converted, under strongly acidic conditions, by treatment with 0.5 M sulphuric acid into its anhydro form, 2,7-anhydro-β-D-*altro*-heptulopyranose (**5**),

obtained by crystallisation in a 63% yield with respect to the starting 2-*C*-(hydroxymethyl)-D-allose. Both ^{1}H and ^{13}C NMR spectra of the final product were identical to those of the commercial 2,7-anhydro-β-D-*altro*-heptulopyranose [14].

D-Allose, which is a practical starting material for the presented synthesis of D-*altro*-hep-tulose, is easily available by the secondary process accompanying the molybdic acid-catalysed epimerization[1] of aldoses. Under more demanding reaction conditions of the Bílik reaction, i.e., at enhanced temperature with a higher concentration of the catalyst, the secondary process becomes significant and equilibrium mixtures of the C-2, C-3 diastereoisomeric aldoses are formed [15,16]. By such a procedure 10% of D-allose can be easily prepared in one step from D-glucose. Also, the other steps towards preparation of sedoheptulose presented in this paper suggest a simple synthetic approach and thus, an alternative to its isolation from natural sources.

To conclude, the 2-ketose approach appears a useful way to prepare such a compound as sedoheptulose. The method is simple to perform and the achieved yield is good. Other possibilities for the application of molybdic acid-catalysis for preparation of similar compounds are currently being studied.

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[1] In spite of the C-1–C-2–C-3 rearrangement mechanisms of the reaction of aldoses catalysed by molybdic acid, this stereospecific reaction formally causes only the C-2 epimerisation of the isotopically uniform aldoses.