

Reactions of saccharides catalyzed by molybdate ions. XX.*

Preparation of epilactose and epimaltose

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Received 7 July 1976

Hydroxylation of lactal by hydrogen peroxide under catalysis by molybdate ions gives epilactose and lactose in the ratio 9:1. Epimeric disaccharides are formed at the same ratio by hydroxylation of maltal. Nitromethane synthesis with 3-*O*- β -D-galactopyranosyl-D-arabinose followed by oxidative decomposition of nitroalditols gives epilactose and lactose in the ratio 3:2. Under the conditions of molybdate catalyzed epimerization of monosaccharides, disaccharides containing 1 \rightarrow 4 linkage (lactose, epilactose, maltose, epimaltose) were not converted to the corresponding disaccharide epimers. A preparation of 2-deoxylactose is also described in this communication.

Гидрокселированием лактала перекисью водорода при катализе молибдатных ионов возникает эпилактоза и лактоза в отношении 9:1. Эпимеры дисахаридов возникают в этом отношении и гидрокселированием мальтала. Из 3-*O*- β -D-галактопиранозил-D-арабинозы нитрометановым синтезом и последующим окислительным разложением нитроальдитолов возникает эпилактоза и лактоза в отношении 3:2. При условиях эпимеризации моносахаридов, катализированной молибдатными ионами, дисахариды с связью 1 \rightarrow 4 (лактоза, эпилактоза, мальтоза, эпимальтоза) не конвертируют на отвечающие эпимеры дисахаридов. Описывается также приготовление 2-деоксилактозы.

On a series of monosaccharides we have previously shown the advantage of molybdate ions used as a catalytic agent in: a) stereoselective hydroxylation of glycals leading to the formation of aldoses having *cis* arrangement of the hydroxyl groups at carbon atoms C-2 and C-3 [1]; b) epimerization of aldotetroses [2], aldopentoses [3], aldohexoses [4], and aldoheptoses [5]; c) oxidative decomposi-

* For Part XIX see *Chem. Zvesti* **32**, 242 (1978).

tion of 1-deoxy-1-nitroalditols into aldoses [6]. The present paper describes applications of the above-mentioned reactions to disaccharides following the purpose of preparation of epilactose and epimaltose.

Hydroxylation of lactal by aqueous solution of hydrogen peroxide under catalytic action of molybdate ions gave epilactose and lactose in the ratio 9:1, besides trace amounts of 2-deoxylactose and aldohexoses. Epilactose was isolated in 73% yield by fractionation on a Dowex 50 W (Ba^{2+} form) column. A similar ratio of epimeric disaccharides was obtained by hydroxylation of maltal. Contrary to epilactose, epimaltose of sufficient purity could be obtained by direct crystallization of the reaction mixture. This procedure of preparation and isolation of epilactose and epimaltose is substantially simpler than their preparation by hydroxylation with perbenzoic acid [7, 8]. A complicated step in this synthesis remains the preparation of sufficiently pure starting glycals (lactal by fractional crystallization, maltal by chromatography on a cellulose column).

Nitromethane synthesis with 3-*O*- β -D-galactopyranosyl-D-arabinose followed by oxidative decomposition of the formed epimeric nitroalditols gave epilactose and lactose in the ratio 3:2, however, the yield of epilactose was only about 15%. The starting 3-*O*- β -D-galactopyranosyl-D-arabinose was prepared by oxidation of lactose diethyl dithioacetal with hydrogen peroxide under catalytic action of ammonium molybdate (a modified procedure of the oxidation of monosaccharide dithioacetals to sulfones in ammoniacal medium as described in [9]). Due to a large amount of by-products in the reaction mixtures, both lactose diethyl dithioacetal and 3-*O*- β -D-galactopyranosyl-D-arabinose had to be isolated from the corresponding reaction mixtures by chromatographic fractionation on cellulose columns.

The epimerization of aldoses in acidic aqueous solution of molybdate ions leads to an equilibrium mixture of the corresponding epimeric aldoses [2—5]. Under the conditions suitable for the epimerization of aldoses, 1 \rightarrow 4-linked disaccharides (lactose, epilactose, maltose, epimaltose) did not epimerize. After a longer treatment of lactose at a higher concentration of molybdenic acid, only traces of epilactose were observed on paper chromatograms. Under the conditions of epimerization, the disaccharides were partially hydrolyzed to aldoses, which underwent epimerization readily, however. The fact that 1 \rightarrow 4-linked disaccharides are not epimerized, can be interpreted from a view of the present concept of the mechanism of epimerization of aldoses [4]. The epimerization of disaccharides apparently requires a conformational flexibility of the reducing end of the molecule. This is hindered, however, with the bulky substituent at carbon atom C-4. It has been shown by means of circular dichroism measurements that disaccharides having 1 \rightarrow 6 glycosidic bond form complexes with molybdate ions, while lactose, maltose, and cellobiose do not [10]. From the above said it follows that the hydroxyl group at the position C-4 also plays a significant role in the formation of molybdate complexes.

In aqueous medium containing 1% trichloroacetic acid, lactal was converted into 2-deoxylactose which was isolated from the reaction mixture by crystallization in 84% yield. In comparison to the use of sulfuric acid in procedures for preparation of 2-deoxymonosaccharides from glycols [11a], the use of trichloroacetic acid is advantageous because the reaction mixture contains lower amount of by-products and moreover, in the case of lactal, hydrolytic cleavage of the glycosidic bond does not occur.

Experimental

Specific rotation of saccharides was measured with an automatic polarimeter Perkin—Elmer, type 141, and melting points were determined on a Kofler stage. Reaction mixtures of saccharides were fractionated on a Dowex 50 W (X-8, 100/200 mesh, Ba²⁺ form) column (3.5 × 135 cm) using elution with water (flow rate 30 ml/h) or on a cellulose column (3.5 × 130 cm) in the solvent system *n*-butanol—ethanol—water (5 : 1 : 4, v/v) (flow rate 45 ml/h). Purity and mutual ratios of participated saccharides were followed by chromatography on Whatman No. 1 paper using the above-solvent system, and incidentally, by direct scanning of chromatograms visualized with the diphenylamine reagent with an ERI-10 densitometer (Zeiss, Jena).

Preparation of epilactose by hydroxylation of lactal

Lactal (8 g, obtained by modified procedure for preparation of D-galactal [11b], purified by fractional crystallization from methanol, m.p. 190—192°C, $[\alpha]_D^{23} + 27.5^\circ$ (c 2, water); Ref. [7] m.p. 191—192°C, $[\alpha]_D^{23} + 27.5^\circ$ (c 1.6, water) was dissolved in water (50 ml), sodium molybdate (0.4 g) and, in portions, 15% aqueous solution of hydrogen peroxide (30 ml) were added at a rate to keep the temperature of the reaction mixture below 30°C. After standing for 20 h at room temperature, the mixture was treated for 24 h with 5% Pd/C (0.3 g) and then deionized on Wofatit KPS (H⁺ form) and Wofatit SBW (acetate form). The ratio of epilactose and lactose was found to be 9 : 1. The deionized solution was concentrated under reduced pressure and the residue (7.9 g) was fractionated on a Dowex 50 W column and eluted with water. Fraction 1 (elution volume 625—710 ml) contained lactose, fraction 2 (735—875 ml) epilactose (6.4 g, i.e. 73.2%) and traces of galactose. Crystallization from a mixture water (6.5 ml)—methanol (32 ml) gave chromatographically homogeneous epilactose, m.p. 205—207°C, $[\alpha]_D^{23} + 10^\circ$ (2 min) → +26.3° (equilibrium, 2 h) (c 3, water). Ref. [12] gives for epilactose m.p. 195—196°C and $[\alpha]_D^{23} + 18^\circ$ (3 min) → +27.2° (equilibrium) (c 1.2, water).

Preparation of epilactose from 3-O-β-D-galactopyranosyl-D-arabinose by nitromethane synthesis and oxidative decomposition of nitroalditols

Lactose diethyl dithioacetal

A solution of lactose (20 g) in concentrated hydrochloric acid (20 ml) was cooled to ca. 5°C and mixed with ethanethiol (20 ml). After standing for 3 h and addition of ice (ca. 50 g),

the mixture was mixed with formamide (10 ml) and left to stand at room temperature for 20 h. The solution was then concentrated under reduced pressure (temperature below 50°C) to about 1/3 of the original volume, diluted with methanol (100 ml) and left to stand for 24 h. The separated salts were filtered off, the filtrate was evaporated to a sirup which was dissolved in anhydrous ethanol (40 ml) and left to stand for 24 h. After removal of the second portion of separated salts, the filtrate was fractionated on a cellulose column (elution system *n*-butanol—ethanol—water) to obtain chromatographically homogeneous sirup of lactose diethyl dithioacetal (13 g, in the elution volume 1970—2490 ml) having $[\alpha]_D^{23} + 4.5 \pm 0.2^\circ$ (*c* 5, water).

For $C_{16}H_{32}O_{10}S_2$ calculated: 42.87% C, 7.14% H, 14.28% S; found: 42.80% C, 7.20% H, 13.92% S.

3-*O*- β -D-Galactopyranosyl-D-arabinose

A solution of lactose diethyl dithioacetal (12 g) in water (10 ml) was mixed with concentrated aqueous solution of ammonia (10 ml), and ammonium molybdate (0.5 g) was added. After cooling (to *ca.* 5°C), 15% aqueous solution of hydrogen peroxide (40 ml) was added at a rate to keep the temperature of the reaction mixture below 25°C. After standing for 24 h at room temperature, the reaction mixture was treated with 5% Pd/C (0.1 g) for 24 h and then filtered. The solution was concentrated *in vacuo* and the residue was fractionated on a cellulose column (as described in the procedure for preparation of lactose diethyl dithioacetal) to give (in the elution volume 550—950 ml) 3-*O*- β -D-galactopyranosyl-D-arabinose (1.8 g, *i.e.* 21.5%) having $[\alpha]_D^{23} - 61^\circ$ (*c* 2, water). Ref. [13] gives for 3-*O*- β -D-galactopyranosyl-D-arabinose m.p. 166—168°C and $[\alpha]_D^{23} - 54.5^\circ$ (4 min) $\rightarrow -62^\circ$ (equilibrium) (*c* 1.1, water).

Epilactose

3-*O*- β -D-Galactopyranosyl-D-arabinose (1.8 g) was dissolved in methanol (10 ml), which was followed by addition of nitromethane (5 ml), methanolic solution of sodium methanolate (0.3 g of sodium in 10 ml of methanol) and *n*-butanol (10 ml). The reaction mixture was then left to stand for 20 h at room temperature. The sodium salts were filtered off, dissolved in water (50 ml), and, after addition of sodium molybdate (0.25 g), mixed with 15% aqueous solution of hydrogen peroxide (7 ml) added in portions. After standing at room temperature for 24 h, the solution was diluted with water, deionized on added ion exchangers (catex, anex), and evaporated. Epilactose, lactose, and monosaccharides in the distillation residue (0.6 g) were in the ratio 3:2:1. Epilactose (0.29 g), $[\alpha]_D^{23} + 26.5^\circ$ (3 h) (*c* 1, water) was isolated by chromatography on Whatman No. 3 paper.

Stability of 1 \rightarrow *4*-linked disaccharides under the epimerization conditions

A disaccharide (1 g of lactose, epilactose, maltose or epimaltose) was dissolved in water (25 ml) and after addition of molybdenic acid (0.5 g) heated at 95°C for 8 h. Paper chromatography of the solutions deionized on ion exchangers (Wofatit SBW in acetate

form) showed in all cases the presence of starting saccharide (maltose — R_{malt} 1.00, epimaltose 1.49, lactose 0.79, epilactose 1.29) and small amounts of aldohexoses formed on hydrolysis of disaccharides and the aldohexose epimers. The complementary epimeric disaccharide has never been found in the reaction mixture.

Epimerization under modified reaction conditions

A solution of lactose (10 g) and molybdenic acid (6 g) in water (100 ml) was heated at 95°C for 24 h. The reaction mixture was then deionized, evaporated and crystallized from methanol to separate a portion of lactose (more than 50%). Paper chromatographic examination showed that the crystalline product contains exclusively lactose. In the mother liquor, besides lactose as the main component, small amounts of aldohexoses formed on hydrolysis of lactose, their epimers and traces of epilactose were detected.

Preparation of epimaltose

Maltal (9 g of sirup, $[\alpha]_{\text{D}}^{23} + 109 \pm 2^\circ$ (c 2, water), prepared by modified procedure for preparation of D-galactal[11b] and purified by chromatography on a cellulose column) was subjected to hydroxylation under the conditions used for preparation of epilactose. The deionized solution was evaporated *in vacuo* and the residue was crystallized from methanol to give the first crop of crystalline epimaltose (2.3 g). The mother liquor was fractionated on a Dowex 50 W column. Three fractions were collected: fraction 1 containing maltose (2 g in elution volume 460–630 ml), fraction 2 containing maltose and epimaltose in the ratio 1:1 (1.5 g, 630–750 ml), and fraction 3 containing epimaltose (4.3 g, 750–1050 ml). Crystallization from a mixture methanol–water afforded epimaltose, m.p. 212–216°C, $[\alpha]_{\text{D}}^{23} + 96.3^\circ$ (3 min) $\rightarrow +97.1^\circ$ (5 min) $\rightarrow +100.7^\circ$ (10 min) $\rightarrow +110.3^\circ$ (1 h) $\rightarrow +110.3^\circ$ (equilibrium) (c 2, water). Ref. [8] gives for epimaltose m.p. 215–216°C and $[\alpha]_{\text{D}}^{17} + 97^\circ \rightarrow +115^\circ$ (60 min) (c 1, water).

Preparation of 2-deoxylactose

A solution of lactal (5 g) in 1% aqueous trichloroacetic acid (30 ml) was kept at room temperature for 24 h, then deionized (Wofatit SBW in OH⁻ form) and concentrated under reduced pressure. The resulting residue was crystallized from a mixture water (5 ml)—methanol (20 ml) to give the first portion of crystalline 2-deoxylactose (3.0 g). Crystallization of concentrated mother liquor gave the second portion of the product (1.4 g). The overall yield 84.5%. Recrystallization from a mixture water—methanol afforded 2-deoxylactose, m.p. 213–215°C, $[\alpha]_{\text{D}}^{23}$ (+33.1°, extrapolation) +34.6° (3 min) $\rightarrow +35.8^\circ$ (5 min) $\rightarrow +37.4^\circ$ (8 min) $\rightarrow +38.5^\circ$ (10 min) $\rightarrow +41.4^\circ$ (1 h) $\rightarrow +42.3^\circ$ (equilibrium) (c 3, water).

For C₁₂H₂₂O₁₀ (326.26) calculated: 44.17% C, 6.80% H; found: 44.08% C, 6.71% H.

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Translated by P. Biely