Reactions of saccharides catalyzed by molybdate ions. XV.* Mechanism of the epimerization reaction

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Epimerization reaction catalyzed by molybdate ions was studied with D-glucose-1-^{3}H and D-mannose-2-^{3}H. A possible mechanism of the epimerization reaction is discussed, where the change of configuration involves mutual exchange of hydrogen atoms in the positions C-1 and C-2. The hydrogen transfer is mediated by a transitient state with formation of tricentric bond between the carbon atoms C-1 and C-2 of the aldose.

Эпимеризационная реакция протекающая при каталитическом действии ионов молибденовой кислоты была изучена на D-глюкозе-1-^{3}H и D-манозе-2-^{3}H. Обсуждается механизм реакции, по которому взаимным обменом водородных атомов, находящихся на углеродных атомах C-1 и C-2, через переходную трехцентровую связь происходит одновременное изменение конфигурации на приведенных углеродных атомах альдозы.

In diluted mineral acids the saccharides undergo mainly intermolecular and intramolecular dehydration reactions. These reactions, when carried out at increased temperatures and in more concentrated acids, are accompanied by further elimination reactions leading to furane derivatives [1]. Consecutive reactions lead to aromatic compounds [2] and, to a lesser extent, to the isomerization of saccharides [3, 4]. After treatment of glucose by sulfuric acid (concentration of acid up to 1.5 N, 2 hrs at 100°C) the reaction mixture was found to contain arabinose and fructose (about 1%) and small amounts of mannose and xylose [3]. *Harris* and *Feather* [4] studied the effect of sulfuric acid on labelled hexoses (D-glucose-1-^{3}H, D-mannose-2-^{3}H, D-fructose-1-^{3}H) and concluded that the isomerization is an intramolecular process, involving hydrogen transfer at the positions C-1 and C-2 of the hexose. Extensive studies on the mechanism of transformation of saccharides in alkaline medium [5, 6] revealed that the transformation proceeds by an enediol mechanism, nevertheless, the results obtained by *Gleason* and *Barker* [7] indicate that the alkali-catalyzed epimerization of ribose to arabinose is an intramolecular process involving migration of hydrogen atom from C-1 to C-2 (D-ribose-2-^{3}H yields D-arabinose-1-^{3}H).

Under catalytic action of molybdenic acid aldotetroses [8], aldopentoses [9, 10], and aldohexoses [11—16] in aqueous solutions undergo epimerization with the formation of equilibrium mixtures of epimeric aldoses. No corresponding ketoses or significant amounts of destruction products are formed in the course of epimerization.

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Unsubstituted aldoses form with molybdate ions in acidic aqueous solutions complexes whose existence was proved by ionophoresis [17—19], polarimetry [20, 21], potentiometric titration [21], and optical rotation dispersion (circular dichroism) [22—25]. The efficiency of the epimerization reaction depends on the ability of saccharides to form complexes with molybdenic acid.

Epimerization of D-glucose-1-^{3}H or D-mannose-2-^{3}H catalyzed by molybdate ions leads to equilibrium mixtures containing radioactive D-glucose and D-mannose in a molar ratio approx. 7 3. The distribution of radioactivity in the individual aldose epimers corresponds roughly to their molar ratios (Table 1). It can be observed from the kinetics of the formation of the corresponding epimers that isotopic effect of tritium takes place to some extent. The relative isotopic effect of tritium in the case of D-mannose-2-^{3}H exhibits negative values while in the case of D-glucose-1-^{3}H epimerization the values of the relative isotopic effect are positive (Table 1). The tritium in the positions C-1 and C-2 was localized by chemical methods, measuring the radioactivity of appropriate degradation products. The radioactivity found in formaldehyde obtained after reduction of aldoses in the reaction mixture after epimerization of D-mannose-2-^{3}H and subsequent periodate oxidation proves that, in the course of epimerization, the displacement of tritium atom from C-1 to C-2 takes place.

Analogically, the radioactivity present in the aldonic acids obtained by oxidation of the epimerization product of D-glucose-1-^{3}H indicates the formation of C_2—^{3}H bond. The ratio of radioactivities of the individual aldoses in the reaction mixture is in accordance with the ratio of radioactivities found in aldonic acids and formaldehyde. Mass spectrum of penta-*O*-acetyl-β-D-glucopyranose prepared from D-glucose obtained by epimerization of D-mannose in heavy water is identical with that of the ame compound prepared from commercial D-glucose.

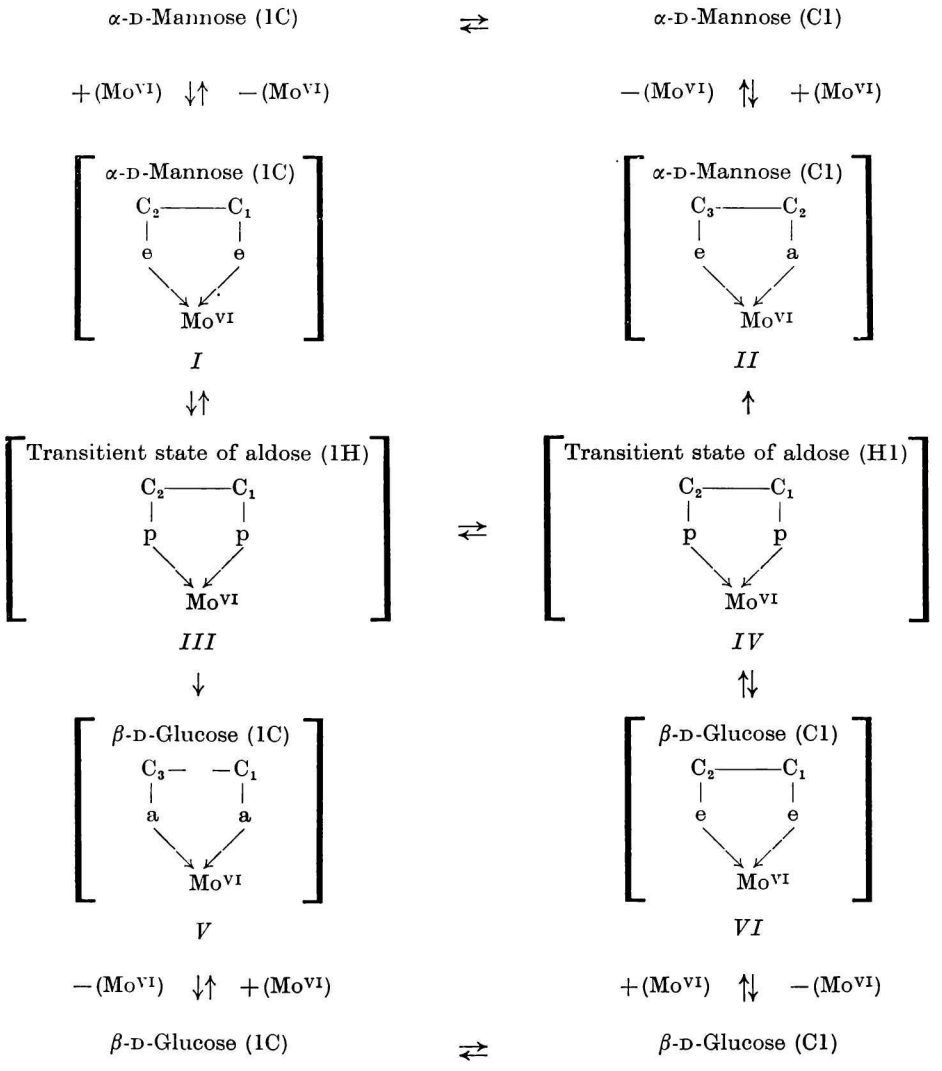
Table 1

Densitometric (*a*) and radiometric (*b*) determination of D-glucose and D-mannose in the epimerization mixtures as a function of time

Time	Epimeric aldose (%)		Δ
	a	*b*	
D-Mannose	D-Glucose		
15 min	30	21	−9
30 min	48	39	−9
45 min	58	49	−9
1 hr	65	54	−11
2 hrs	70	61	−9
4 hrs	71	64	−7
D-Glucose	D-Mannose		
15 min	6	18	+12
30 min	14	25	+11
45 min	29	28	−1
1 hr	26	27	+1
2 hrs	28	30	+2
4 hrs	29	26	−3

From the obtained results it follows that the epimerization reaction is an intramolecular process and therefore, it does not proceed by an enediol mechanism. This hypothesis is supported by following observations:

a) The water from the reaction mixture after the epimerization of tritium labelled D-glucose and/or D-mannose contains only unsignificant amount of radioactivity (about 3%).



Scheme 1

a — axial, e — equatorial, p — planar hydroxyl group.

b) The mass spectrum of peracetylated D-glucose obtained after epimerization of D-mannose in heavy water does not contain fragments indicating the presence of C—^{2}H bond.

c) The epimerization mixture does not contain fructose.

A mechanism involving the exange of hydrogen atoms at C-1 and C-2 in the course of epimerization can take place only when the hydrogen atoms linked to C-1 and C-2 are in the *trans*-axial configuration. Molybdate complexes of aldoses apparently enable this configuration by forming the complex with equatorial hydroxyl groups in the positions C-1 and C-2 (Scheme 1). The formed complex *I* or *IV* deforms part of the aldose molecule whereby the atoms C-1, C-2, and C-3, pyranoid oxygen atom and hydroxyl groups linked to carbons C-1 and C-2 (*III*, *IV*) lie in the plane (Scheme 2). The formation of a half-chair conformation is accompanied by a strong deformation of valence angles at carbon atoms C-1 and C-2 (originally in a tetrahedric arrangement), which causes rehybridization of electron orbitals in the mentioned carbon atoms. As a consequence, strong overlapping of the two parallel electron orbitals at the neighbouring atoms C-1 and C-2 takes place resulting in the formation of a transitory tricentric bond. In this transitient state the two hydrogen atoms are linked at the same time to both carbon atoms (Fig. 1). As a result of the migration of hydrogen atoms proceeds the change of configuration at carbon atoms C-1 and C-2. Transitient states of aldoses *III* and *IV* form a conformation equilibrium (Scheme 2) where, due to a greater conformational stability (equatorial $-CH_2OH$ group, equatorial $-OH$ group at C-4), conformation H1 is preferred (*IV*). The transitient states can reversibly undergo transformation into the original complexes

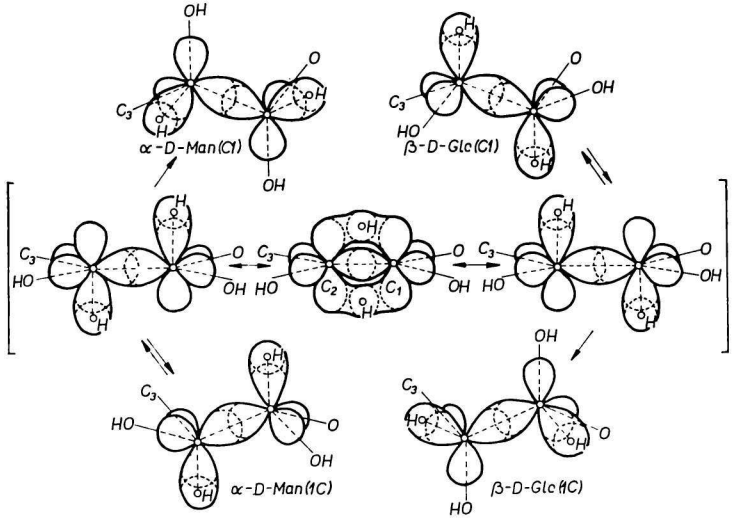
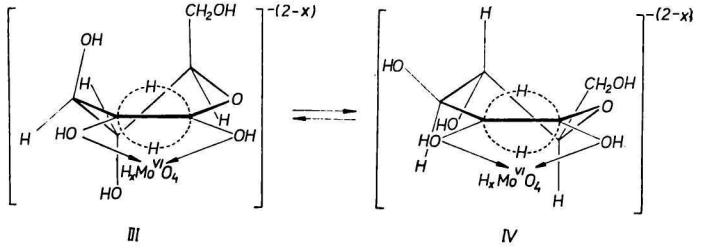


Fig. 1. Model of molecular orbitals of aldohexoses in the transitient state involving migration of hydrogen atoms.

I or *IV* or irreversibly transform into complexes *II* and *V* The last complexes do not fulfil the basic condition for the mentioned reversible process (necessity of equatorial hydroxyl groups at C-1 and C-2).



Scheme 2

Preferential occurrence of glucose in the equilibrium mixtures of epimeric aldoses (both from glucose and mannose) is determined by its greater conformational stability. From thermodynamic point of view, in an epimeric mixture of aldoses the one possessing less elements of conformational instability is more preferred [16].

Experimental

The used radioactive D-glucose-1-^{3}H and D-mannose-2-^{3}H were from the Radiochemical Centre, Amersham (England). The ratio of aldose epimers in the epimerization mixtures was determined after their separation by paper chromatography on Whatman No. 1 paper. The descending chromatography was carried out using the solvent system *n*-butanol—ethanol—water (5 : 1 : 4, v/v) during 120 hrs at room temperature. The following methods were used for quantitative determinations of aldoses:

a) densitometric measurement of sugar spots on paper detected by freshly prepared solution of anilinium hydrogen phthalate using densitometer ERI-10 (Zeiss, Jena);

b) determination of radioactivity on paper chromatograms measuring 1 cm paper strips in a liquid scintillation counter (Packard 3330, USA) with 5 ml of toluene scintillation mixture. The scintillation mixture contained 5 g of 2-phenyl-5-(4-diphenyl)-1,3,4-oxadiazole (PBD) and 0.3 g of 1,4-bis-2-(4-methyl-5-phenoxazolyl)benzene (dimethyl POPOP) per 1 l of toluene. The radioactivity of aldose solutions was measured using 10 ml of water-miscible scintillation mixture "Monophase" (Packard Instrument Co., USA) per 0.2 ml of the sample. No corrections for self-absorption and quenching were made. The mass spectra of investigated compounds were measured with HCh 1306 (USSR) mass spectrometer at the ionization potential of 70 eV and at 40°C in a direct inlet evaporation chamber.

Epimerization of labelled aldoses

50 mg of D-glucose-1-^{3}H or D-mannose-2-^{3}H (specific activities 1—2 μCi per mg) and 2—2.5 mg of molybdenic acid were dissolved in 1 ml of water and heated on water bath at 80—82°C. At appropriate time intervals two parallel 5 μl samples were with-

drawn and applied on chromatographic paper for separation and quantitative densitometric and radiometric determination of individual aldoses in the reaction mixture.

Determination of radioactivity in the aqueous solvent after epimerization of aldoses labelled with tritium

After 4 hrs epimerization the reaction mixture was diluted with 4 ml of water and distilled under atmospheric pressure until its volume was about 2 ml. The radioactivity in an aliquot portion of the distillate was measured and calculated for the whole volume of the reaction mixture. It has been found that the radioactivity of the aqueous solvent after epimerization represented only about 3% of the total radioactivity of the reaction mixture.

Determination of tritium at C-1

After 4 hrs epimerization, 0.5 ml of the reaction mixture, containing 25 mg of aldohexoses, was diluted to 50 ml with water, 100 mg of $NaBH_4$ were added and the mixture was left at room temperature for 8 hrs. The reduction was terminated by the addition of 0.2 ml of glacial acetic acid.

After standing for 15 hrs 50 ml of 0.1 M-$NaIO_4$ were added and the oxidation was carried out for 24 hrs at room temperature. Then 50 ml of dimedone solution were added (3 g of dimedone dissolved in 10 ml of ethanol and diluted with 15 ml of a mixture of 0.1 M citric acid and 0.2 M-NaH_2PO_4 and the volume adjusted with water to 500 ml) and the mixture was left to stand for 24 hrs. The precipitated condensation product of dimedone with formaldehyde was filtered, dried in the air, dissolved in the mixture of ethyl acetate—methanol and the radioactivity of the solution was determined. The radioactivity of the condensation product of formaldehyde with dimedone represented $65 \pm 7\%$ of the original radioactivity present in D-mannose-2-^{3}H or D-glucose-1-^{3}H.

Determination of tritium at C-2

After 4 hrs epimerization, the solution containing 25 mg of aldohexoses was concentrated under reduced pressure at 30°C to approx. 0.2 ml. To the residue after evaporation 0.1 ml of water and 0.2 ml of bromine was added. The reaction mixture was left to stand for 48 hrs at room temperature. The bromine was then evaporated under reduced pressure and the traces of bromine were removed by a subsequent evaporation with the following solvents: dioxan, ethanol, water, 5 ml of each in the given order under reduced pressure at 30°C. The evaporation procedure was repeated three times. After this procedure the residue (0.2 ml), checked by means of the paper chromatography for the absence of aldoses, was dissolved in water and its radioactivity was determined. The radioactivity of the obtained aldonic acids represented $35 \pm 7\%$ of the original radioactivity of the epimerization mixture before the oxidation.

Epimerization of D-*mannose in deuterium oxide*

3 g of D-mannose was dissolved in 3 ml of heavy water and the solution was evaporated to a sirup under reduced pressure. The procedure of evaporation was repeated once more. Similarly, 3—4 mg of molybdenic acid were dissolved in 1 ml of heavy water and evaporated to dryness. The residue was dissolved in 5 ml of heavy water (isotopic purity 99%) and added to the sirup of D-mannose. The mixture was heated at 80°C for 3 hrs.

The reaction mixture was then concentrated under reduced pressure and the residual sirup dissolved in a mixture of ethanol and methanol. By crystallization from this mixture crystalline D-glucose was obtained. By acetylation of obtained D-glucose with acetic anhydride in the presence of sodium acetate crystalline penta-*O*-acetyl-β-D-glucopyranose was obtained giving mass spectrum identical with that of penta-*O*-acetyl-β-D-glucose prepared from commercial D-glucose.

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