Reactions of saccharides catalyzed by molybdate ions. XXX.* Epimerization of D-[U-^{14}C]glucuronic acid

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Epimerization of D-[U-^{14}C]glucuronic acid catalyzed by molybdate ions offers an equilibrium mixture of D-[U-^{14}C]glucuronic and D-[U-^{14}C]mannuronic acid in the ratio 5 : 1 and small amounts of D-[U-^{14}C]lyxo-5-hexulosonic acid. D-[U-^{14}C]Glucuronic acid was obtained on hydrolysis of α-[U-^{14}C]glucan oxidized by nitrogen dioxide.

Эпимеризацией D-[U-^{14}C]глюкуроновой кислоты, катализированной ионами молибдата, образуется равновесная смесь D-[U-^{14}C]глюкуроновой и D-[U-^{14}C]манноновой кислот в отношении 5 : 1 и одновременно образуется малое количество D-[U-^{14}C]ликсо-5-гексулозоновой кислоты. D-[U-^{14}C]глюкуроновая кислота была приготовлена гидролизом α-[U-^{14}C]глюкана, окисленного двуокисью азота.

In alkaline aqueous solutions aldoses generally isomerize under formation of mixtures consisting of the starting aldose, its 2-epimer and the corresponding 2-ketose. Similar conditions (0.5 M-NaOH, 20°C, 24 h) lead to isomerization of D-glucuronic acid and from the reaction mixture besides D-glucuronic acid (39%), D-mannuronic acid (8.5%) and D-lyxo-5-hexulosonic acid (1.9%) were isolated [1]. Partially neutralized D-glucuronic acid (pH 7) undergoes more extensive isomerization at increased temperatures (100°C, 3 h) affording a mixture of D-glucuronic (36%), D-mannuronic (8%), D-lyxo-5-hexulosonic (35%), D-altruronic (5%), D-altruronic (1%), and L-ribo-5-hexulosonic acid (15%) [2]. In aqueous solutions containing certain metal ions (Mg, Zn, Ni, Al, Pb) decarboxylation of hexuronic acids takes place giving the corresponding aldopentoses [3]. In the presence of mineral acids and at increased temperatures (19% HCl, 145°C, 2 h) the decarboxylation proceeds quantitatively [4]. In mildly acid aqueous media containing molybdate ions aldohexoses, aldopentoses, and aldotetroses epimerize under formation of equilibrium mixtures of epimeric aldoses without formation of the corresponding ketoses [5]. The present study deals with epimerization of D-glucuronic acid in the presence of molybdic acid.

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The epimerization of D-glucuronic acid containing small amount of D-[U-^{14}C]-glucuronic acid as a radioactive tracer was carried out in 0.25% or 0.5% aqueous solution of molybdic acid (pH 1.6 ± 0.1) at 80°C. The content of D-glucuronic acid, D-mannuronic acid, D-lyxo-5-hexulosonic acid, and destruction products in the reaction mixtures was determined by measuring their radioactivity after separation of all products by paper chromatography (Table 1). Similar procedure was employed to follow the epimarization of D-[U-^{14}C]glucose performed for comparative purposes.

Table 1

Composition of the reaction mixture during epimerization of D-[U-^{14}C]glucuronic acid in aqueous solution of molybdic acid at 80°C

Reaction time h	Content of acid %						Destruction products %		Ratio D-GlcUA : D-ManUA	
	D-Glucuronic*		D-Mannuronic*		D-lyxo-5--Hexulosonic					
	a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
1	94	92	5	7	0	0	1	1	18.8	13.1
2	92	87	7	10	0	0	1	3	13.1	8.7
4	85	81	12	14	1	1	2	4	7.1	5.7
6	80	77	16	16	1	2	3	5	5.0	4.8
9	77	74	17	16	2	3	4	7	4.5	4.6
12	75	70	17	16	3	5	5	9	4.4	4.4

a) 0.25%; *b*) 0.5% aqueous solution of molybdic acid.
*Sum of free acid and its lactone.

Molybdate catalyzed epimerization of D-glucuronic acid gives an equilibrium mixture of D-glucuronic and D-mannuronic acid in the ratio 5 : 1. Raise in the concentration of molybdic acid as well as extention of the reaction time lead to increase in the content of D-lyxo-5-hexulosonic acid and destruction products (Table 1). During epimerization of D-glucose, carried out under the same conditions, the equilibrium of D-glucose and D-mannose in the ratio 3 : 1 is established in a much shorter time (15—30 min). D-Fructose was not detected in the mixtures even after prolonged reaction time. The equilibrium between the pyranose form of D-glucuronic acid and its 3,6-lactone existing in aqueous solutions [6] is probably the main reason of the different behaviour of D-glucuronic acid during the epimerization regarding the epimerization rate and products in the reaction mixture, in comparison with D-glucose. Certain analogy of this situation can be found in the case of molybdate catalyzed epimerization of 3-deoxy-D-*ara*-

bino-hexose and 3-deoxy-D-ribo-hexose which leads exlusively to 3-deoxy-D-*erythro*-hexulose [7]. Because the epimerization mixtures of D-glucuronic acid contain besides the epimeric uronic acid, 2-ketouronic acid, and destruction products also the uronic acid lactones (Fig. 1), the isolation of individual components is rather difficult. Moreover, isolation of D-[U-^{14}C]mann-

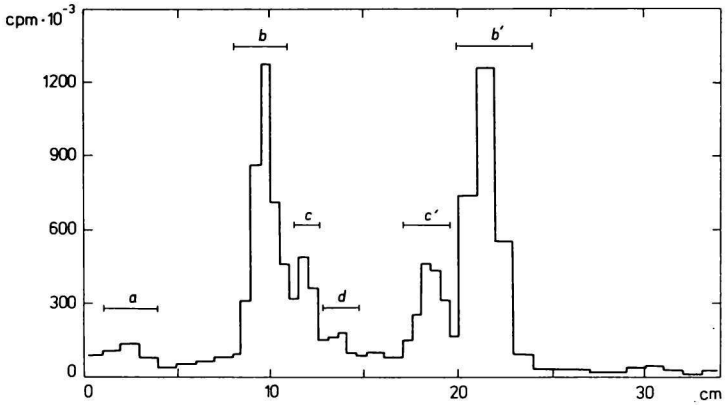

Fig. 1. Distribution of radioactivity on paper chromatogram (solvent *A*) of the epimerization mixture of D-[U-^{14}C]glucuronic acid.
a) Destruction products; *b*) D-glucuronic acid; *b'*) D-glucuronic acid lactone; *c*) D-mannuronic acid; *c'*) D-mannuronic acid lactone; *d*) D-lyxo-5-hexulosonic acid.

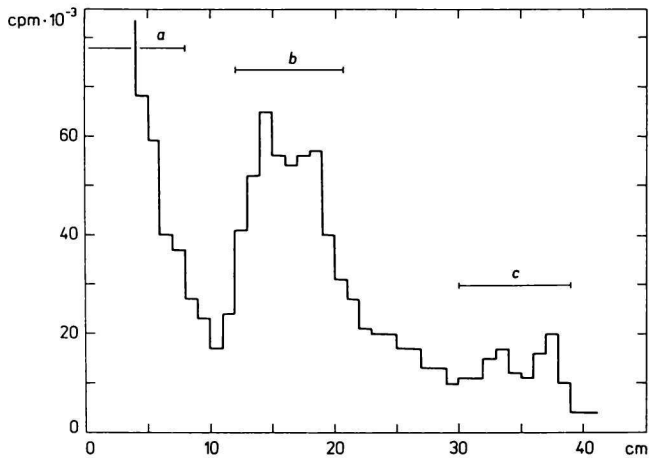

Fig. 2. Distribution of radioactivity on paper chromatogram of the hydrolysate of α-[U-^{14}C]glucan oxidized with nitrogen dioxide.
a) Polysaccharides, D-glucose, and D-glucuronic acid; *b*) D-glucuronic acid 3,6-lactone; *c*) formyl esters of D-glucose and D-glucuronic acid.

uronic acid by paper chromatography and rechromatography is accompained by irreversible losses of the compound due to decomposition. On the contrary, D-[U-^{14}C]mannose can be recovered from the epimerization mixtures of D-[U-^{14}C]glucose and purified by paper chromatography without significant losses (in 25% yield).

D-[U-^{14}C]Glucuronic acid was prepared by hydrolysis of α-[U-^{14}C]glucan oxidized by nitrogen dioxide. From the hydrolysate that portion of D-[U-^{14}C]glucuronic acid was isolated by paper chromatography which is present in the form of its 3,6-lactone (Fig. 2). This way of preparation of [U-^{14}C]-labelled D-glucuronic acid is very simple and offers a product of high purity already after one rechromatography. Attempts to prepare D-mannuronic acid employing selective oxidation of the primary hydroxyl group of D-mannose by nitrogen dioxide under simultaneous protection of the second active oxidation site using D-mannose phenylhydrazone or 4-nitrophenylhydrazone, were unsuccessful. The oxidation of D-mannose phenylhydrazones led in all cases to a mixture of nonreducing saccharides (aldonic and aldaric acid and their lactones) containing only trace amounts of D-mannuronic acid.

Experimental

A water-soluble glycogen-type α-[U-^{14}C]glucan isolated from an alga *Chlorella sp.* was from the Institute for Development, Production and Use of Radioisotopes (ÚVVVR), Prague, oyster glycogen from J. T. Baker Chemical Co., USA, liquid nitrogen dioxide from Syntézia, Pardubice. Paper chromatography of saccharides was done in the following solvent systems: solvent *A* [1, 2], ethyl acetate—acetic acid—water (3 : 1 : 1), relative mobilities: D-glucuronic acid lactone 1.00, D-mannuronic acid lactone 0.83, D-lyxo-5-hexulosonic acid 0.63, D-mannuronic acid 0.52, D-glucuronic acid 0.42; solvent *B*, 1-butanol—ethanol—water (5 : 1 : 4), (D-glucuronic acid lactone 1.00, D-mannuronic acid lactone 0.75, D-mannuronic acid 0.50, D-glucuronic acid 0.35); solvent *C*, ethyl acetate—1-butanol—water (6 : 2 : 1), (D-glucose 1.00, D-mannose 1.48, D-fructose 1.54). Radioactivity of saccharides was measured with a scintillation spectrometer Packard, type 3330 (USA) using a toluene scintillation liquid (Tesla, Přemyšlení, Czechoslovakia).

Epimerization of D-*glucuronic acid*

A mixture of D-glucuronic acid (25 mg), D-[U-^{14}C]glucuronic acid (25 mg, 5 μCi) and 1 ml of 0.25% or 0.5% aqueous solution of molybdic acid was incubated at 80°C for 12 h. At time intervals aliquots of the reaction mixtures were taken and chromatographed in solvent *A*. The content of reaction products was determined on the basis of radioactivity distribution on paper chromatograms (Table 1).

Isolation of D-*mannuronic acid*

D-Glucuronic acid (5 g) was dissolved in 0.25% aqueous solution of molybdic acid (100 ml) and heated at 80°C for 6 h. The reaction mixture was then evaporated, the distillation residue dissolved in methanol (30 ml) and chromatographed on Whatman No. 1 paper (15 sheets) in solvent *A*. D-Mannuronic acid (100 mg) having $[\alpha]_D^{23} = +93°$ (*c* 2, water, 24 h) was isolated after twofold rechromatography. Similarly was obtained D-lyxo-5-hexulosonic acid. Ref. [8] gives for D-mannuronic acid $[\alpha]_D^{25} = +71 \rightarrow +92°$ (*c* 1, water, 20 min).

Epimerization test

Epimerization of the isolated chromatographically homogeneous D-mannuronic acid under the conditions described for epimerization of D-glucuronic acid gave a reaction mixture containing D-glucuronic acid, D-lyxo-5-hexulosonic acid and starting D-mannuronic acid.

Preparation of D-*[U-*^{14}*C]glucuronic acid*

A water-soluble α-[U-^{14}C]glucan (10 μCi) was diluted with unlabelled α-glucan (50 mg of oyster glycogen), dried over P_2O_5 (24 h), placed into a desiccator with atmosphere of nitrogen oxides (2—3 ml of liquid nitrogen dioxide) and left to stand at room temperature for 4—5 days. After removal of residues of nitrogen oxides *in vacuo* on a rotatory evaporator, 85% aqueous solution of formic acid (1 ml) was added and the mixture heated at 90°C for 12 h. The mixture was then evaporated to dryness, dissolved in water (1 ml), heated at 90°C for 1 h and evaporated again. The whole procedure was done in one test tube provided with a glass-ground stopper. Finally, the distillation residue was dissolved in water (1 ml), heated at 90°C for 2 h and, after cooling, chromatographed on Whatman No. 1 paper in solvent *B* for 17—19 h at room temperature. After detection of a guiding strip (Fig. 2) the corresponding zones of the paper were eluted: *a*) polysaccharides, D-glucose, and D-glucuronic acid; *b*) D-glucuronic acid lactone; *c*) formyl esters of D-glucose and D-glucuronic acid. Isolated D-[U-^{14}C]glucuronic acid (as its lactone) contained 38—43% of the original radioactivity of the starting α-[U-^{14}C]glucan.

Oxidation of phenylhydrazones of D-*mannose*

Crystalline D-mannose phenylhydrazone (3 g) or D-mannose 4-nitrophenylhydrazone (3 g) was kept in the atmosphere of nitrogen oxides for 1 or 3 days. After removal of nitrogen oxides *in vacuo* (rotatory evaporator), the oxidized product was mixed with water (30 ml), ethanol (3 ml), and benzaldehyde (3 ml) and heated at 80°C for 3 h. After cooling the mixture was filtered and the filtrate extracted with ethyl acetate (3 × 10 ml). The aqueous phase was analyzed by paper chromatography. Saccharides were detected with anilinium hydrogen phthalate reagent followed by periodate oxidation. The chromatographic examination of one-day oxidation mixture (treated with benzaldehyde) showed the presence of D-mannose as the main product, small amounts of nonreducing saccharides, and

only traces of D-mannuronic acid. A three-day oxidation mixture contained nonreducing saccharides as the main fraction, small amounts of D-mannose, and traces of D-mannuronic acid.

Epimerization of D-[U-^{14}C]glucose

A solution of D-glucose (50 mg) supplied with D-[U-^{14}C]glucose (10 μCi) in 0.25% aqueous solution of molybdic acid (1 ml) was heated at 80°C. At time intervals aliquots were taken and chromatographed on Whatman No. 1 paper in solvent *C* to determine radiometrically the reaction products. It was found that the epimerization equilibrium between D-[U-^{14}C]glucose and D-[U-^{14}C]mannose (63 : 27) was established within 15—30 min. D-[U-^{14}C]Fructose was not detected in the reaction mixture even after 6 h epimerization.

Isolation of D-[U-^{14}C]mannose

A solution of D-glucose (10 mg) and D-[U-^{14}C]glucose (10 μCi) in 0.25% aqueous solution of molybdic acid (1 ml) was heated at 80°C for 30 min. The reaction mixture was then chromatographed on Whatman No. 1 paper in solvent *C* at room temperature for 60—70 h. From the paper chromatographically homogeneous D-[U-^{14}C]mannose (in 25% yield) and D-[U-^{14}C]glucose were isolated.

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