Reactions of saccharides catalyzed by molybdate ions XXXIII.* Use of α[U-^{14}C]glucan for preparation of ^{14}C-labelled saccharides

[a]V. BÍLIK, [a]P. BIELY, and [b]J. KOLINA

[a]*Institute of Chemistry, Centre for Chemical Research, Slovak Academy of Sciences, CS-842 38 Bratislava*

[b]*Institute for Research, Production, and Use of Radioisotopes, CS-102 27 Prague*

Received 4 March 1983

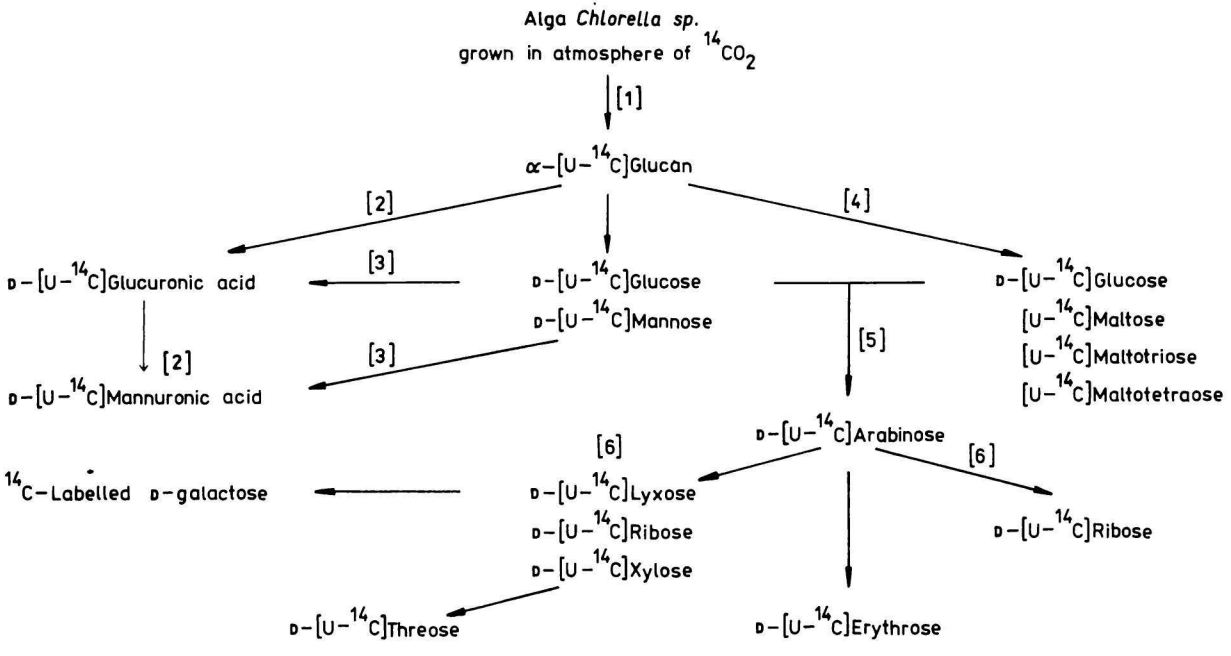
D-[U-^{14}C]Glucose obtained on acid hydrolysis of α-[U-^{14}C]glucan (2 M-HCl) is epimerized under a catalytic action of molybdate ions to D-[U-^{14}C]mannose isolated in 20 % iyeld. Oxidative degradation of 4-nitrophenylhydrazones of D-[U-^{14}C]arabinose and D-[U-^{14}C]xylose affords D-[U-^{14}C]erythrose and D-[U-^{14}C]threose, respectively, in 15 % yield when referred to the starting aldopentoses. Nitromethane synthesis with D-[U-^{14}C]lyxose followed by oxidative decomposition of the corresponding nitrohexitols gives ^{14}C-labelled D-galactose. Described is also the preparation of D-[U-^{14}C]arabinose from D-[U-^{14}C]glucose and the conversion of D-[U-^{14}C]arabinose to D-[U-^{14}C]xylose and D-[U-^{14}C]lyxose.

D-[U-^{14}C]глюкоза, полученная при кислотном гидролизе α-[U-^{14}C]глюкана (2 M HCl), эпимеризуется под каталитическим действием молибдат-ионов на D-[U-^{14}C]маннозу, выделяемую с 20 % выходом. Окислительное разложение 4-нитрофенилгидразонов D-[U-^{14}C]арабинозы и D-[U-^{14}C]ксилозы ведет к D-[U-^{14}C]эритрозе и D-[U-^{14}C]треозе соответственно, получаемых с 15 % выходом из расчета на исходные альдопентозы. Нитрометановый синтез с D-[U-^{14}C]ликсозой с последующим окислительным разложением соответствующих нитрогекситолов приводит к меченной ^{14}C D-галактозе. Также описывается получение D-[U-^{14}C]арабинозы из D-[U-^{14}C]глюкозы и конверсия D-[U-^{14}C]арабинозы на D-[U-^{14}C]ксилозу и D-[U-^{14}C]-ликсозу.

* For Part XXXII see *Chem. Zvesti 37*, 791 (1983).

Transformations of saccharides in biological systems and in chemical reactions are easy to follow by means of ^{14}C-labelled saccharides. In our previous work we used as a starting material for the preparation of labelled saccharides an α-[U-^{14}C]glucan isolated from the alga *Chlorella sp.* grown in the atmosphere of $^{14}CO_2$ [1] (Scheme 1). The oxidation of dry α-[U-^{14}C]glucan by gaseous nitrogen dioxide followed by hydrolysis of the oxidized product by formic acid gives D-[U-^{14}C]glucuronic acid (yield = 40 %) [2] which on the molybdate-catalyzed epimerization affords an equilibrium mixture of D-[U-^{14}C]glucuronic acid and D-[U-^{14}C]mannuronic acid in the ratio 4:1 [2]. ^{14}C-Labelled aldohexuronic acids were also obtained by oxidation of *N*-arylglycosylamines of ^{14}C-labelled aldohexoses by nitrogen dioxide followed by hydrolytic removal of the aglycon [3]. Hydrolysis of α-[U-^{14}C]glucan with α-amylase immobilized on cellulose leads to [U-^{14}C]-labelled D-glucose, maltose, maltotriose, and maltotetraose [4]. In aqueous solution of molybdic acid D-[U-^{14}C]glucose is converted to an equilibrium mixture of the $C_{(2)}$-epimeric aldoses from which D-[U-^{14}C]mannose is isolated in 25 % yield [2]. Oxidative degradation of D-[U-^{14}C]glucose 4-nitrophenylhydrazone by hydrogen peroxide in the presence of molybdates gives D-[U-^{14}C]arabinose (yield = 50 %) [5]. Molybdate-catalyzed epimerization of D-[U-^{14}C]arabinose leads to an equilibrium mixture of the $C_{(2)}$-epimeric aldopentoses from which D-[U-^{14}C]ribose can be isolated in 25 % yield, or, under special reaction conditions, all four D-[U-^{14}C]aldopentoses (34 % yield of xylose, 29 % yield of arabinose, 24 % yield of lyxose, and 13 % yield of ribose) [6]. In the present work we describe a modified procedure for preparation of D-[U-^{14}C]glucose and D-[U-^{14}C]mannose from α-[U-^{14}C]glucan, further the methods for preparation of D-[U-^{14}C]erythrose and D-[U-^{14}C]threose from D-[U-^{14}C]arabinose and D-[U-^{14}C]xylose, respectively, and ^{14}C-labelled D-galactose from D-[U-^{14}C]lyxose.

Based on our previous work it became generally known that epimerization of any aldose in mild acidic aqueous solution of molybdic acid (pH 2—3) at 70—100 °C for 1—8 h leads to an equilibrium mixture of the $C_{(2)}$-epimeric aldoses in which the aldose conformationally more stable predominates [7]. The epimerization of glucose or mannose (D- or L-series) gives the mixture of the aldoses in the ratio 3:1. The starting D-glucose is usually obtained on acid hydrolysis of α-glucans, most often in hydrochloric, sulfuric or oxalic acid [8]. In the present work the hydrolysis of α-[U-^{14}C]glucan was accomplished in 2 M-hydrochloric acid (90 °C, 21/2 h) giving D-[U-^{14}C]glucose in 90—95 % yield. Coupling of the α-glucan hydrolysis with D-glucose epimerization was found to be limited to some extent. From the examination of the effects of hydrochloric acid (at various concentration) and some lower aliphatic acids on the epimerization it follows: at concentrations higher than 0.1 mol dm^{-3}, hydrochloric acid decreases the ratio of D-mannose in the reaction mixture: at $c(HCl) = 2$ mol dm^{-3} (as well as at similar concentration of other mineral acids such as H_2SO_4, H_3PO_4, HNO_3) the epimerization is blocked


Alga Chlorella sp.
grown in atmosphere of $^{14}CO_2$
[1]
α-[U-^{14}C]Glucan
[2]
[3]
[4]
D-[U-^{14}C]Glucuronic acid
D-[U-^{14}C]Glucose
D-[U-^{14}C]Mannose
D-[U-^{14}C]Glucose
[U-^{14}C]Maltose
[U-^{14}C]Maltotriose
[U-^{14}C]Maltotetraose
[2]
[3]
[5]
D-[U-^{14}C]Mannuronic acid
D-[U-^{14}C]Arabinose
[6]
[6]
^{14}C-Labelled D-galactose
D-[U-^{14}C]Lyxose
D-[U-^{14}C]Ribose
D-[U-^{14}C]Xylose
D-[U-^{14}C]Ribose
D-[U-^{14}C]Threose
D-[U-^{14}C]Erythrose

Scheme 1

(Table 1). The ratio of epimeric aldoses is also influenced by 0.1 M-organic acids (n(D-glucose) : n(organic acid) : n(molybdic acid) = 100 : 10 : 1, 90 °C, 5 h) (Table 1). In the presence of lower fatty acids or dicarboxylic acids, with the exception of oxalic acid, the same ratio of D-glucose and D-mannose was obtained as in the presence of molybdic acid alone. The ratio of D-mannose was significantly lowered by α-hydroxycarboxyl acids. In the presence of oxalic acid and citric acid the epimerization does not proceed. Any decrease of D-mannose ratio in the reaction mixture can be ascribed to diminution of the concentration of catalytically effective complexes of aldoses with molybdate due to formation of more stable complexes of molybdate with α-hydroxycarbonyl acids and particularly with oxalic acid and citric acid. Complexes of molybdate with malic acid and oxalic acid were extensively studied [9]. The epimerization mixtures, depending on the acidity of the medium, also contain reversion products. Their amount is proportional to the acidity (Table 1). In the present procedure for the preparation of D-[U-^{14}C]glucose and D-[U-^{14}C]mannose the hydrolysis of α-[U-^{14}C]glucan is carried out in 2 M-HCl.

Table 1

Epimerization of D-glucose catalyzed by molybdate ions in solutions of organic acids and hydrochloric acid (θ = 90 °C, t = 5 h)

Acid (c/(mol dm^{-3}))	pH	D-Glucose	D-Mannose	Reversion products
		Yield/%		
Formic (0.1)	2.3	69	27	4
Acetic (0.1)	2.7	71	26	3
Monochloroacetic (0.1)	1.9	70	26	4
Trichloroacetic (0.1)	1.5	71	25	4
Malonic (0.1)	2.0	69	27	4
Succinic (0.1)	2.5	70	26	4
Maleic (0.1)	2.0	93	4	3
Tartaric (0.1)	1.9	90	5	5
Lactic (0.1)	2.0	91	6	3
Oxalic (0.1)	1.3	91	1	8
Citric (0.1)	2.0	95	2	3
HCl (0.05)	1.3	71	25	4
HCl (0.1)	1.0	70	25	5
HCl (0.5)	0.4	78	14	8
HCl (1.0)	1.0	83	7	10
Control*	2.6	72	26	2

* Epimerization with molybdic acid alone.

Before the epimerization the acid has to be removed by evaporation of the solution diluted by ethanol or the pH of the solution has to be increased by addition of about equimolar amount of ammonium acetate (±20 %). The epimerization of thus arranged solutions and subsequent paper chromatography afforded yields 20—22 % of D-[U-^{14}C]mannose, 60—65 % of D-[U-^{14}C]glucose, and 5—10 % of reversion products or limit dextrins.

Attempts to prepare D-[U-^{14}C]erythrose by hydrolysis of α-[U-^{14}C]glucan oxidized with lead tetraacetate or sodium periodate were unsuccessful. Erythrose liberated on hydrolysis of the starch dialdehyde is simultaneously decomposed to other products. The methods for preparation of aldotetroses are generally restricted because aldotetroses, in contrast to higher aldoses, readily undergo isomerization, dehydration and are especially easily oxidized to aldonic acids. A convenient route to aldotetroses is based on a controlled oxidation of the corresponding aldohexoses by lead tetraacetate using suitable mole ratios of aldohexose and the oxidation reagent [10]. Oxidative cleavage of the carbon chain of suitably substituted saccharide derivatives can be effected by sodium periodate. D-Erythrose is obtained in this way from 4,6-*O*-ethylidene-D-glucose [11, 12] and D-threose from 1,3-*O*-benzylidene-D-arabinitol [11]. These methods are not very convenient to be carried out on small scales due to the risk of overoxidation, and tedious preparation of the starting derivatives. In this work D-[U-^{14}C]erythrose and D-[U-^{14}C]threose were prepared by oxidative degradation of 4-nitrophenylhydrazones of D-[U-^{14}C]arabinose and D-[U-^{14}C]xylose, respectively, with hydrogen peroxide under catalytic action of molybdate ions. In the first step the aldopentoses are in a simple way converted to 4-nitrophenylhydrazones (80—90 % yield) which are without separation from the mixture oxidatively degraded to D-[U-^{14}C]aldotetroses which are isolated by paper chromatography in 15 % yield as referred to the starting D-[U-^{14}C]aldopentose. The oxidative degradation of 4-nitrophenylhydrazones of aldohexoses (D-[U-^{14}C]glucose 4-nitrophenylhydrazone to D-[U-^{14}C]arabinose [5] or D-galactose 4-nitrophenylhydrazone to D-lyxose [13]) affords aldopentoses in much higher yields (50 %). Under conditions of the oxidative degradation subsequent oxidation of liberated aldoses often takes place. From the above results it follows that the stable pyranoid structures of aldopentoses protect against the oxidation to aldonic acids more efficiently than the furanoid structures of aldotetroses. Other conclusion which may be drawn here is that it is very important for the reaction yields to maintain precise reaction conditions, mainly the duration of the oxidation.

Effective synthetic routes to galactose are based mainly on the lengthening of the carbon chain of lyxose. Cyanohydrine synthesis with D-[1-^{14}C]lyxose followed by hydrolysis and reduction of galactonic acid gives D-[2-^{14}C]galactose [14]. The same reaction with D-lyxose and ^{14}C-labelled hydrogen cyanide leads to D-[1-^{14}C]galactose [15]. A different approach is based on nitromethane synthesis with lyxose and

subsequent oxidative decomposition of 1-deoxy-1-nitrogalactitol [16]. This principle was applied for the preparation of ^{14}C-labelled galactose with a slight modification. The method starts with the epimerization of D-[U-^{14}C]xylose giving a mixture of D-[U-^{14}C]xylose and D-[U-^{14}C]lyxose which is subjected to nitromethane synthesis and subsequent oxidative decomposition. Under the given conditions of the oxidative decomposition, nitrohexitols formed from D-[U-^{14}C]xylose are quantitatively decomposed to the starting pentose, but the nitrohexitols formed from lyxose are converted to aldohexoses. For these reasons the paper chromatography of the mixture affords besides D-[2,3,4,5,6-^{14}C]galactose 25 %, D-[U-^{14}C]-xylose 60—70 % with small amount of D-[U-^{14}C]lyxose. The aldopentoses can be used in further cycle of the preparation of ^{14}C-labelled D-galactose.

Experimental

Water-soluble α-[U-^{14}C]glucan (7.4×10^3 MBq/mmol of anhydroglucose) isolated from the alga *Chlorella sp.* and D-[U-^{14}C]glucose (6.6×10^3 MBq/mmol) were from the Institute for Research, Production, and Use of Radioisotopes (Prague), and α-glucan (oyster glycogen) was from J. T. Baker Chemical Co. (USA). D-[U-^{14}C]Arabinose was prepared from D-[U-^{14}C]glucose by oxidative degradation of its 4-nitrophenylhydrazone [5]. D-[U-^{14}C]Xylose was obtained by the molybdate-catalyzed epimerization of D-[U-^{14}C]arabinose [6]. Reaction mixtures were analyzed and the products isolated by chromatography on Whatman No. 1 paper in the solvent systems V(1-butanol): :V(ethanol): V(water) = 5:1:4 (A), V(ethyl acetate): V(pyridine): V(water) = 8:2:1 (B), and V(ethyl acetate): V(acetic acid): V(4 % aqueous solution of boric acid) = 9:1:1 (C). Proportion of saccharides in the reaction mixtures was determined on the basis of radioactivity distribution on paper chromatograms found by measuring radioactivity of 1 cm wide strips with a Tri-Carb Scintillation Spectrometer Packard 3330 (USA) using a toluene scintillation fluid (Tesla, Přemyšlení, Czechoslovakia).

Epimerization of D-*glucose in the presence of organic acids and hydrochloric acid*

0.2 M-solution of an organic acid (1 cm^3) was mixed with 1 cm^3 of aqueous solution of 2 M-D-glucose containing molybdic acid (18 g of D-glucose and 0.2 g of $H_2MoO_4 \cdot 2H_2O$ in 50 cm^3 of water). Epimerization solutions of D-glucose containing various concentration of hydrochloric acid were prepared in a similar way. After addition of D-[U-^{14}C]glucose (40 kBq) the mixtures were heated at 90 °C for 5 h. Aliquots of the mixtures were then chromatographed in solvent system A and radiometrically analyzed for the proportion of saccharides (Table 1).

D-[U-^{14}C]*Glucose and* D-[U-^{14}C]*mannose from* α-[U-^{14}C]*glucan*

Procedure A

A mixture of α-glucan (10 mg of oyster glycogen), α-[U-^{14}C]glucan (130 kBq), and 2 M-hydrochloric acid (1 cm^3) was heated at 90 °C for 21/2 h. The hydrolyzate was diluted with 96 % ethanol (3 cm^3), concentrated at a reduced pressure and 20 °C to one third of its volume and, after addition of new ethanol portion (3 cm^3), concentrated to 0.5—1.0 cm^3. This residue was mixed with 1 % aqueous solution of molybdic acid (1 cm^3), heated at 90 °C for 1 h and then chromatographed on one sheet of Whatman No. 1 paper in solvent system *A* (mobility of D-mannose relative to that of D-glucose was 1.3, mobility of dextrins and reversion products 0.0—0.3). Distribution of radioactivity on chromatograms was established and used for localization of radioactive products and determination of their proportions.

Procedure B

The hydrolyzate (see Procedure *A*) was mixed with 4 M-ammonium acetate (0.5 cm^3), 2 % aqueous solution of molybdic acid (0.5 cm^3), heated at 90 °C for 1 h and analyzed as described above.

Of the original radioactivity of α-[U-^{14}C]glucan, in both cases 20—22 % was found in the zone of D-[U-^{14}C]mannose, 60—65 % in the zone of D-[U-^{14}C]glucose, and 5—10 % in the zone of reversion products. Further purification of D-[U-^{14}C]mannose or D-[U-^{14}C]glucose was achieved by paper chromatography in solvent system *B*.

D-[U-^{14}C]*Erythrose and* D-[U-^{14}C]*threose*

A mixture of D-arabinose or D-xylose (10 mg) and the corresponding D-[U-^{14}C]aldopentose (130 kBq) in 2 % methanolic solution of 4-nitrophenylhydrazine (1.5 cm^3) was heated at 60 °C for 4 h. After addition of 2.6 % aqueous solution of ammonia (0.5 cm^3), 0.5 % aqueous solution of sodium molybdate (0.5 cm^3) and 30 % aqueous solution of hydrogen peroxide (0.5 cm^3), the mixture was left to stand for half an hour at room temperature. After new addition of hydrogen peroxide (1 cm^3) and standing for 21/2 h, the mixture was filtered and chromatographed on one sheet of Whatman No. 3 paper in solvent system *A*. The chromatographic mobilities of compounds referred to that of arabinose are: erythrose 1.9, D-arabinose 4-nitrophenylhydrazone 3.4, and 4-nitrophenylhydrazine 4.6. The mobilities referred to that of xylose are: threose 1.5, D-xylose 4-nitrophenylhydrazone 2.6 and 4-nitrophenylhydrazine 3.5. Aldonic acids or their ammonium salts move only little beyond the starting line.

The above way of preparation of D-[U-^{14}C]erythrose led to the following distribution of radioactivity on paper chromatogram: 10—14 % of total counts in the zone of D-[U-^{14}C]-erythrose, 40—45 % in the zone of D-[U-^{14}C]arabinose, and 37—47 % in the zone of aldonic acids. Analogous data in the case of D-[U-^{14}C]threose preparation are: 10—15 % of total radioactivity in the zone of D-[U-^{14}C]threose, 30—35 % in the zone of the starting D-[U-^{14}C]xylose, and 43—53 % in the zone of aldonic acids.

Separated D-[U-^{14}C]erythrose or D-[U-^{14}C]threose are stored on dry paper chromatograms in air-tight dark vessels. From the paper they are eluted just before use. The two aldotetroses are well separated one from another by chromatography in solvent system *C*.

D-[2,3,4,5,6-^{14}C]*Galactose*

A mixture of D-xylose (25 mg), D-[U-^{14}C]xylose (130 kBq) in 0.1 % aqueous solution of molybdic acid (2 cm^3) was heated at 90 °C for 2 h. After evaporation *in vacuo* the dry residue was dissolved in methanolic solution of sodium methanolate (0.5 cm^3, 0.3 g of sodium in 10 cm^3 of methanol), mixed with nitromethane (0.2 cm^3), left to stand at room temperature for 20 h and, after addition of 30 % aqueous solution of hydrogen peroxide (1 cm^3), for another 24 h. The solution was then deionized on a column (1 cm × 20 cm) of the mixed ion exchanger Bio-Deminrolit (Permutit) and the eluate (60—80 cm^3) concentrated and chromatographed in solvent system *A*. Of the original radioactivity of the starting D-[U-^{14}C]xylose 25 % was found in the zone of ^{14}C-Labelled D-galactose and 65—70 % in the zone of D-[U-^{14}C]aldopentoses. ^{14}C-Labelled D-galactose was further purified by chromatography in solvent system *B*. Simultaneously recovered D-[U-^{14}C]aldopentoses were used in further cycles of the preparation of ^{14}C-labelled D-galactose.

Acknowledgements. The authors thank A. Puškáčová for technical assistance.

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Translated by P. Biely