

PREPARATION OF 6-DEOXY-D- $[U-^{14}C]$ GLUCOSE

V. SIGLEROVÁ, K. VEREŠ

Institute of Nuclear Biology and Radiochemistry,
Czechoslovak Academy of Sciences, Vídeňská 1083,
142 20 Prague 4, Czechoslovakia

SUMMARY

A synthesis of 6-deoxy-D- $[U-^{14}C]$ glucose is described. The compound, having a specific radioactivity of 912.6 MBq/μmol, was obtained in a 11.8 % overall radiochemical yield from the starting D- $[U-^{14}C]$ glucose.

Key words: Carbon-14, 6-deoxy-D- $[U-^{14}C]$ glucose

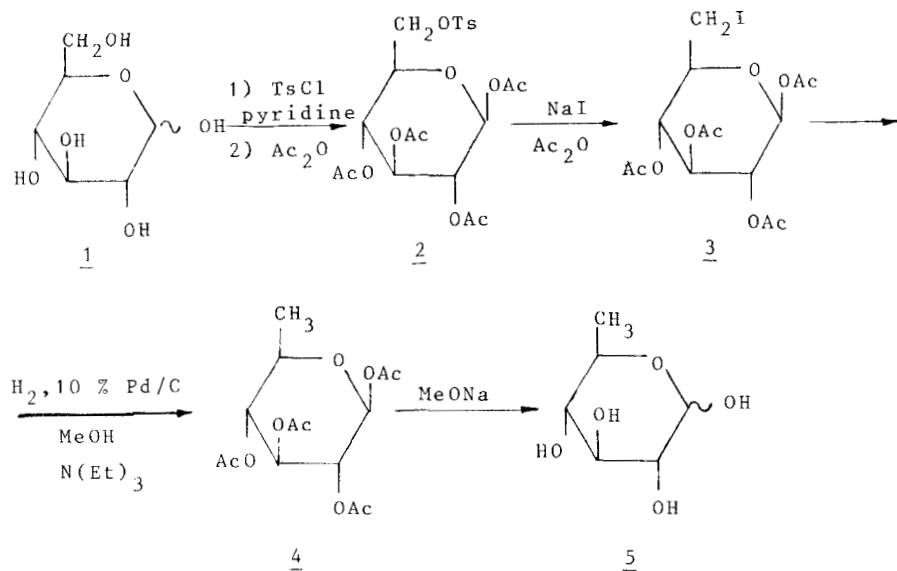
INTRODUCTION

Transport studies of non-metabolizable sugars in the yeasts *Rhodotorula glutinis* and *Saccharomyces cerevisiae* required the synthesis of commercially unavailable sugar derivatives labelled with hydrogen and carbon radionuclides. This work describes the preparation of 6-deoxy-D-glucose uniformly labelled with ^{14}C .

The starting compound in the synthesis of 6-deoxy-D- $[U-^{14}C]$ glucose was D-glucose uniformly labelled with ^{14}C . The synthesis was a modification of the procedure used by Hardegger and Montavon (1) for the preparation of unlabelled 1,2,3,4-tetra-O-acetyl derivatives of 6-deoxy-D-glucose. The

procedure has been successfully used for the synthesis of 6-deoxy-D- $[\delta\text{-}^3\text{H}_1]$ glucose (2).

Tosylation of D-glucose 1 in pyridine and subsequent acetylation (Scheme I) yields 1,2,3,4-tetra-O-acetyl-6-O-toluene-sulfonyl- β -D-glucopyranose 2. In keeping with the data of Staněk and Tajmr (3) we obtained product 2 in a 25 % yield; in addition the reaction gives rise to α -anomer, 2,6-ditosyl derivative and pentaacetyl-D-glucose (3,4). A large excess of tosyl chloride results in the formation of the 2,6-ditosyl derivative (4) but, as verified in model experiments, a small excess of tosyl chloride (1.46 equiv.) enhances the overall yield of 6-deoxy-D- $[\delta\text{-}^{14}\text{C}]$ -glucose 5.



Scheme I

We attempted to directly remove the 6-tosyloxy group and obtain the deoxy derivative 4 by treatment with zinc in the presence of sodium iodide in 1,2-dimethoxyethane (5). Under the conditions used (10 h, 80 °C), however, no reductive detosylation of compound 2 took place.

Tosyl derivatives are readily converted to deoxyiodo derivatives through the action of sodium iodide; in sugar derivatives the reaction can be performed in acetone (6). We found that the reaction of the tetraacetyl derivative 2 with sodium iodide in acetone under reflux does not occur while in methyl ethyl ketone it is very slow. For this reason we performed the reaction in acetic anhydride according to Hardegger and Montavon (1) and obtained iodo derivative 3 in a 65 % yield.

Hydrogenolysis of ¹⁴C-deoxyiodo derivative 3 in methanol in the presence of 10 % palladium on charcoal and triethylamine gives acetylated 6-deoxy derivative 4 in a high yield.

Deacetylation of 4 in methanol in the presence of sodium methanolate afforded almost quantitatively 6-deoxy-D-[U-¹⁴C]glucose 5. The radioactive synthesis was carried out without isolating individual intermediates.

6-Deoxy-D-[U-¹⁴C]glucose was prepared from D-[U-¹⁴C]glucose in an overall radiochemical yield of 11.8 % with a specific radioactivity of 912,6 MBq/mmol. The radiochemical purity of the product (99 %) was determined by checking the radioactivity of the TLC-plates. In addition, no radiochemical impurities were detected by autoradiography.

EXPERIMENTAL

D-[U-¹⁴C]Glucose, specific radioactivity 10 285 MBq/mmol, was purchased from the Institute for Research, Production and Application of Radioisotopes, Prague, Czechoslovakia.

Samples of radioactive compounds were measured on a liquid scintillation counter Beckman LS 9800.

Thin-layer chromatography was carried out on precoated silica gel plates (60 F₂₅₄, 0.2 mm thickness, Merck,

Darmstadt), spots of sugar derivatives were detected by UV and an aniline-diphenylamine spray (7). The reactions with labelled compounds were monitored by TLC using previously prepared intermediates as reference.

TLC was carried in the following solvent systems:

a) hexane-ethyl acetate (3:2), b) acetonitrile-water (85:15), and c) acetone-chloroform-water (85:10:5).

The radiochemical purity of radioactive compounds was determined by measuring the radioactivity of the TLC-plates on a Berthold LB 2832 Thin-layer Scanner II and by autoradiography on X-ray films.

Mass of radioactive 6-deoxy-D-glucose was determined spectrophotometrically (8).

1,2,3,4-Tetra-O-acetyl-6-O-p-toluenesulphonyl- β -D-[U-¹⁴C]glucopyranose 2 (8)

A solution of D-[U-¹⁴C]glucose (76.6 MBq) in 50 % ethanol was evaporated to dryness and the residue was dried over phosphorus pentoxide. To the solid residue was added 12 mg (0.067 mmol) anhydrous D-glucose, 0.5 ml absolute pyridine and the reaction mixture was stirred until most of the D-glucose was dissolved. p-Toluene-sulphonyl chloride (20.6 mg; 0.108 mmol) was added at 0 °C under stirring and the reaction mixture was left to stand for 24 h at room temperature. Acetic anhydride (0.1 ml) was added at 0 °C and the reaction mixture left to stand for 48 h at room temperature. Thereafter the mixture was evaporated to dryness and the product was used for the next step without purification.

1,2,3,4-Tetra-O-acetyl-6-deoxy-6-iodo- β -D-[U-¹⁴C]glucopyranose 3

Crude product 2 was dissolved in 1 ml acetic anhydride, 22.2 mg (0.148 mmol) anhydrous sodium iodide was added

and the reaction mixture was evaporated to dryness. Then 1 ml water was added to the brown evaporation residue and the product was extracted to ethyl acetate. The ethyl acetate extract was washed with a solution of sodium bicarbonate, then with sodium sulphite and water. After removal of the solvent the crude product was dried over phosphorus pentoxide. The yield was 27.9 mg of product 3.

1,2,3,4-Tetra-O-acetyl-6-deoxy-β-D-[U-¹⁴C]glucopyranose 4

Crude product 3 was dissolved in 3 ml absolute methanol, 20 μl triethylamine and 28.3 mg 10 % Pd/C were added and the reaction mixture was hydrogenated for 2 h at a pressure of 82.6 kPa. The catalyst was removed by centrifugation and the reaction mixture was evaporated to dryness. Crude product 4 was dissolved in ethyl acetate and washed with water. The solvent was evaporated and the product was dried over phosphorus pentoxide.

6-Deoxy-D-[U-¹⁴C]glucose 5

Crude product 4 was dissolved in 1 ml absolute methanol, and 0.1 ml of 0.1 N sodium methanolate was added to the reaction mixture and this was left to stand for 1 h at room temperature. The deacetylation was monitored by TLC in system b; the R_f of D-glucose was 0.16, R_f of 6-deoxy-D-glucose was 0.33. Measurement of radioactivity of the TLC plate showed that the reaction mixture contained 22 % of product 5 (6-deoxy-D-[U-¹⁴C]glucose) and 73 % of the starting D-[U-¹⁴C]glucose 1. The reaction mixture was evaporated to dryness and purified by preparative thin-layer chromatography in system b. Elution with water yielded 9,06 MBq (11.8 % - overall radiochemical yield) of 6-deoxy-D-[U-¹⁴C]glucose 5, with a specific radioactivity of 912,6 MBq/μmol. The radiochemical purity was determined by TLC in systems b and c.

31.7 MBq (41.4 %) of the starting D- $\text{U-}^{14}\text{C}$ /glucose was then recovered back from the reaction mixture.

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