TREHALOSE AS A METABOLIC PRODUCT OF D-XYLOSE IN *Rhodotorula gracilis*

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Received March 17th, 1971

External D-xylose is utilized by the lipid-forming yeast *Rhodotorula gracilis* to form carbon dioxide as the final product. It appears that prior to final oxidation D-xylose is converted to threhalose which may be isolated from cells incubated with D-xylose in substantial amounts. Its identity was demonstrated by the determination of the melting point, the IR spectrum and gas chromatography.

The lipid-forming yeast *Rhodotorula gracilis* is known to possess an exclusively aerobic metabolism[1,2] and to transport monosaccharides into cells against a concentration gradient[3]. Both D-glucose and D-xylose are metabolized by the yeast but the rate of glucose utilization is such that practically no free glucose can be detected in the cells while D-xylose is accumulated. Upon simultaneous addition of the two sugars glucose is utilized first, up to complete exhaustion from the medium before xylose conversion sets in[4].

While the details of glucose and xylose metabolism are to be published subsequently we wish to report here on the appearance of a labelled compound different from D-xylose which accumulates in *Rhodotorula gracilis* on incubation with [^{14}C]-D-xylose. The melting point, the IR spectrum and the gas chromatographic behaviour of the isolated substance demonstrate its identity with trehalose.

Since this disaccharide cannot be formed directly from D-xylose a conversion of D-xylose to glucose or one of its phosphorylated derivatives prior to formation of trehalose must be postulated. Experiments on the elucidation of xylose metabolism leading up to trehalose in *Rhodotorula gracilis* are now under way.

Rhodotorula gracilis Fres/Harrison was grown as described before[3] for 24 h whereafter the cells were washed thoroughly with water and aerated overnight at room temperature. A suspension (10–15 mg dry weight/ml) was then incubated at 30°C for 8–10 h in 0·15M-KH_2PO_4 in the presence of 5–6 mM D-xylose uniformly labelled with ^{14}C (0·7 μCi/mmol). After incubation, the cells were washed in ice-cold distilled water and extracted for 20 min with boiling distilled water. D-Xylose was estimated both in the medium and in the hot-water extract according to Meijbaum[5]. Radioactivity of parallel samples was counted in a 2π methane-flow Frieseke-Hoepf-

ner counter. The activity found in the cell extract exceeded several times the amount corresponding to free D-xylose and its source was then investigated.

The cell extract was concentrated at 50°C at reduced pressure to a thick sirup, applied to Whatman No 1 paper and developed in acetic acid–butanol–water (1 : 4 : 5) for 24 – 30 h. Detection with Bonner's reagent for α-diol groups revealed three spots, two of them radioactive. The one with lower radioactivity had the same R_F as D-xylose, the one with higher radioactivity corresponded to a disaccharide with glucose moieties. Detection with benzidine showed only two spots, one of them radioactive, and due to D-xylose. The radioactive disaccharide was isolated by preparative paper chromatography and the water eluate evaporated at reduced pressure at 50°C. The remaining sirup crystallized from aqueous ethanol. The substance was then examined by gas chromatography.

Acetylation was done in parallel in the isolated substance and in authentic trehalose. Each of the compounds was dissolved in hot pyridine, acetic anhydride was added and the reaction mixture left to stand for 50 h at room temperature. Distilled water was then added in excess and the acetylated derivative was extracted with chloroform. After washing the extract with water, hydrochloric acid, potassium bicarbonate and water, the solution was evaporated at reduced pressure to dryness and crystallized from hot ethanol, the m. p. of both was 98° – 101°C, the mixed melting point showed no depression. The IR spectra were identical. The optical rotation of the acetylated isolated compound was +153·3°C which is within the range of values reported in the literature for octaacetyl trehalose. The gas chromatography was done on a Packard chromatograph with flame-ionization detection. The column used was filled with Gas Chrom P (60 – 80 mesh), the liquid phase was 3% SE-30 silicone oil (Applied Science Laboratories Inc.). The analyses were done under a programmed heating regime from 125 to 250°C at a rate of 3·5°C/ min. The carrier gas was nitrogen flowing at 60 ml/min. Before analysis, the compounds were converted to trimethylsilyl ethers[6].

Thanks are due to Dr J. Pacák, Department of Organic Chemistry, Charles University, Prague, for invaluable advice.

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Translated by A. Kotyk.