Carbohydrate Research, 93 (1981) 300–303
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

A method for preparing lower [U-^{14}C]-labelled (1→4)-*β*-D-xylo-oligosaccharides

ZDENĚK KRÁTKÝ, PETER BIELY, AND MÁRIA VRŠANSKÁ
Institute of Chemistry, Slovak Academy of Sciences, Bratislava (Czechoslovakia)

(Received October 7th, 1980; accepted for publication, December 18th, 1980)

Linear (1→4)-*β*-D-xylo-oligosaccharides (xylobiose, xylotriose, *etc.*) are commonly prepared from plant (1→4)-*β*-D-xylans by partial hydrolysis with acid[1–3] or enzymic hydrolysis[4,5], the latter method affording relatively high yields[6]. Synthetic routes are available for xylobiose[7–9]. Only enzymic hydrolysis seems to be convenient for the preparation of [U-^{14}C]-labelled xylo-oligosaccharides, but it requires the isolation of [U-^{14}C]xylans from plants grown in an atmosphere of $^{14}CO_2$. [U-^{14}C]-labelled (1→4)-*β*-D-xylo-oligosaccharides are important for elucidating the mechanism of action of *β*-D-xylanases that exhibit catalytic activities other than simple hydrolysis of glycosidic bonds[10–12].

Lower [U-^{14}C]-labelled xylo-oligosaccharides can be prepared easily from phenyl *β*-D-[U-^{14}C]xylopyranoside in a reaction catalysed by extracellular *β*-D-xylanase of the yeast *Cryptococcus albidus*. The enzyme degrades aryl *β*-D-xylopyranosides by a complex reaction-pathway involving a series of transglycosylation reactions followed by hydrolysis of larger intermediates to give lower xylo-oligosaccharides[13]. We now report that the oligosaccharides obtained in this way are (1→4)-*β*-D-linked and that a crude preparation of the enzyme may be used.

Incubation of phenyl *β*-D-xylopyranoside with purified *β*-D-xylanase of *C. albidus* yielded xylobiose, xylotriose, xylotetraose, and traces of xylopentaose. The yields were proportional to the concentration of the substrate, which, however, cannot be increased over 200mM, due to its limited solubility in water. At the highest concentration of phenyl *β*-D-[U-^{14}C]xylopyranoside examined (150mM), the oligosaccharides were formed as shown in Table I. The concentrations of [U-^{14}C]xylotriose and [U-^{14}C]xylotetraose reached a maximum after 2-h incubation and these oligosaccharides could be isolated in yields of 15 and 4%, respectively. Longer incubation increased the concentrations of [U-^{14}C]xylobiose and D-[U-^{14}C]xylose, but decreased that of the higher oligosaccharides, which are relatively good substrates for the enzyme. [U-^{14}C]Xylobiose could be obtained in a yield of ~50% by allowing the reaction to reach equilibrium.

TABLE I

FORMATION OF [U-^{14}C]XYLO-OLIGOSACCHARIDES AND D-[U-^{14}C]XYLOSE FROM 150mM PHENYL β-D-[U-^{14}C]XYLOPYRANOSIDE DURING INCUBATION WITH PURIFIED β-D-XYLANASE OF *Cryptococcus albidus*

Time (h)	*Radioactivity (%)*			
	Xylose	*Xylobiose*	*Xylotriose*	*Xylotetraose*
0.5	2.7	8.9	5.7	2.2
1	2.7	14.5	10.1	3.3
2	4.4	28.9	15.2	4.1
3	5.6	38.9	13.8	3.9
4	7.8	38.4	12.5	3.4
5	9.4	47.2	8.5	2.1

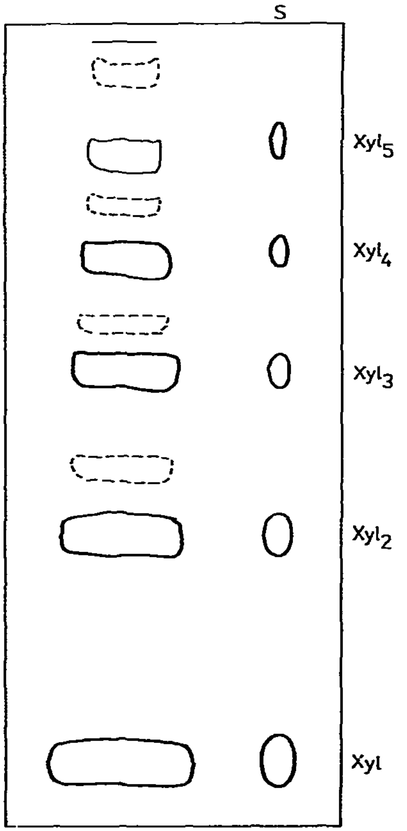

Fig. 1. Paper chromatography of reducing saccharides formed from phenyl β-D-xylopyranoside under the action of *C. albidus* β-D-xylanase: S, (1→4)-β-D-linked standards; detection with aniline hydrogenphthalate.

The oligosaccharides can also be prepared by using a crude enzyme, *i.e.*, the concentrated and dialysed, xylan-spent, culture fluid of *C. albidus*. From a reaction mixture containing unlabelled phenyl β-D-xylopyranoside, xylobiose and xylotriose were isolated by preparative p.c. The crystalline disaccharide was characterised (m.p., optical rotation, mass spectrometry of its hexa-*O*-methyl derivative) as 4-*O*-β-D-xylopyranosyl-D-xylose. The crystalline trisaccharide was characterised (m.p., optical rotation) as (1→4)-β-D-xylotriose. Xylotetraose and xylopentaose, which were formed only in small amounts, had chromatographic mobilities identical with those of authentic (1→4)-β-D-linked markers[6]. A linear relationship between the chromatographic-mobility parameter $\log(R_{\mathrm{Xyl}}/1 - R_{\mathrm{Xyl}})$ and the d.p. of all the major products confirmed that the transfer reactions catalysed by *C. albidus* β-D-xylanase led to a homologous series of (1→4)-β-D-linked products. However, the enzyme is not specific for (1→4)-β transfer. On chromatograms loaded with a large amount of material, traces of other reducing saccharides were detected (Fig. 1). The nature of these products was not investigated, but they did not interfere with the isolation of the lower (1→4)-β-D-linked products.

EXPERIMENTAL

The cultivation of the yeast *C. albidus* on wood xylans, the isolation of extracellular β-D-xylanase, and the definition of the enzyme unit have been described[14]. Crude enzyme was obtained by concentration (40°, *in vacuo*) of the xylan-spent culture fluid to 10% of the original volume and subsequent dialysis against distilled water containing 0.01% of sodium azide.

The preparation of phenyl β-D-[U-^{14}C]xylopyranoside (2.1 Ci/mol) and the quantitative analysis of the reaction products have been described[13]. Preparative p.c. was performed on Whatman No. 3MM paper (washed with distilled water) with ethyl acetate–acetic acid–water (18:7:8).

Preparation and characterisation of xylobiose and xylotriose. — Phenyl β-D-xylopyranoside (400 mg) was incubated with crude β-D-xylanase (30 U) in distilled water (10 ml) containing 0.01% of sodium azide at 30°. After 24 h, the mixture was concentrated *in vacuo*, and fractionated by preparative p.c. to give xylobiose and xylotriose, which were crystallised from methanol. Xylobiose had m.p. 182.5–187°, $[\alpha]_D^{20}$ −26° (*c* 0.5, water; 1 h); lit.[1] m.p. 186–187°, $[\alpha]_D^{25}$ −25.5° (*c* 1, water; 1 h). Xylotriose had m.p. 215–218°, $[\alpha]_D^{20}$ −48.5° (*c* 0.55, water; 1 h); lit.[5] m.p. 215–216°, $[\alpha]_D$ −48.1°.

Crystalline xylobiose (5 mg) was methylated[15], and the α,β-mixture was purified by elution from a column of silica gel (Merck) with chloroform–acetone (10:1). Its mass spectrum was consistent with the hexa-*O*-methyl derivative of 4-*O*-β-D-xylopyranosyl-D-xylose[16].

Preparation of [U-^{14}C]xylo-oligosaccharides. — A solution of phenyl β-D-[U-^{14}C]xylopyranoside (1.15 mg, 6.6 μCi) in water (50 μl) was incubated with purified β-D-xylanase (0.15 U) for 2 h. The mixture was fractionated on Schleicher

Schüll 2043b paper. After detection of guide strips and localisation of radioactive saccharides by autoradiography, [U-^{14}C]xylobiose, [U-^{14}C]xylotriose, and [U-^{14}C]-xylotetraose were eluted with water in yields of 35, 10, and 2%, respectively.

ACKNOWLEDGMENTS

We thank Dr. P. Kováč for advice in the methylation of xylobiose, and Dr. V. Mihálov for measuring the mass spectrum.

REFERENCES

1 R. L. Whistler and C. C. Tu, *J. Am. Chem. Soc.*, 73 (1951) 1389–1390.
2 R. L. Whistler and C. C. Tu, *J. Am. Chem. Soc.*, 74 (1952) 3609–3612.
3 C. T. Bishop, *Can. J. Chem.*, 33 (1955) 1073–1078.
4 R. L. Whistler and E. Masah, *J. Am. Chem. Soc.*, 77 (1955) 1241–1243.
5 T. E. Timell, *Sven. Papperstidn.*, 65 (1962) 435–447.
6 I. Kusakabe, T. Yasui, and T. Kobayashi, *Nippon Nogei Kagaku Kaishi*, 49 (1975) 383.
7 D. V. Myhre and F. Smith, *J. Org. Chem.*, 26 (1961) 4609–4612.
8 G. O. Aspinall and K. M. Ross, *J. Chem. Soc.*, (1961) 3674–3677.
9 P. Kováč, *Chem. Zvesti*, 33 (1979) 365–370.
10 H. Björndal, K. E. Eriksson, P. J. Garegg, B. Lindberg, and B. Swan, *Acta Chem. Scand.*, 19 (1965) 2309–2315.
11 R. F. H. Dekker and G. N. Richards, *Carbohydr. Res.*, 39 (1975) 97–114.
12 J. A. Gascoigne and M. M. Gascoigne, *J. Gen. Microbiol.*, 22 (1960) 242–248.
13 P. Biely, M. Vršanská, and Z. Krátký, *Eur. J. Biochem.*, in press.
14 P. Biely, M. Vršanská, and Z. Krátký, *Eur. J. Biochem.*, 108 (1980) 313–321.
15 P. Kováč and D. Anderle, in K. Blau and G. King (Eds.), *Handbook of Derivatives for Chromatography*, Heyden, London, 1977, pp. 209–210.
16 V. Kováčik, V. Mihálov, and P. Kováč, *Carbohydr. Res.*, 88 (1981) 189–201.