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Note

A method for preparing laminaribiose by using yeast endo-(1→3)-β-D-glucanase

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Yeast endo-(1→3)-β-D-glucanases hydrolyse susceptible substrates (*e.g.*, laminarin) in a random fashion, giving, as intermediates, a mixture of laminaribiose, laminaritriose, and higher laminaridextrins, in addition to D-glucose[1,2]. If the enzymic reaction goes to completion, laminaribiose and D-glucose are the products[2]. Laminaribiose is not available from commercial sources; it can be prepared by partial hydrolysis of laminarin[3], although the method is tedious and gives low yields. However, the chemical method is useful for preparing oligosaccharides up to d.p. 7.

We now report on the use of an endo-(1→3)-β-D-glucanase isolated from the yeast *Kluyveromyces phaseolosporus* for the hydrolysis of either laminarin or yeast (1→3)-β-D-glucan into laminaribiose and D-glucose. The yield of the former was ~100-fold higher than that obtained by chemical hydrolysis (Table I) and is therefore

TABLE I

PRODUCTS OF ENZYMIC AND CHEMICAL HYDROLYSIS OF LAMINARIN AND YEAST CELL-WALL (1→3)-β-D-GLUCAN (50-mg SAMPLES)

	Laminarin		*Yeast (1→3)-β-D-glucan*	
	Laminaribiose (mg)	*D-Glucose (mg)*	*Laminaribiose (mg)*	*D-Glucose (mg)*
Enzymic hydrolysis	23.6	10.2	10.1	4.1
Chemical hydrolysis[2]	0.2	n.d.[a]	n.d.	n.d.

[a]Not determined.

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a more convenient method for preparing laminaribiose. The inconvenience of preparing the endo-(1→3)-β-D-glucanase is partly compensated by the fact that the enzyme can be recovered (up to 85%) and re-used.

Laminaritriose can also be prepared by our method, by appropriate interruption of the enzymic hydrolysis. However, the steady-state concentration of the trisaccharide is relatively low, and it is difficult to obtain good yields. Better yields may be expected by using an endo-(1→3)-β-D-glucanase from the yeast *Hanseniospora valbyensis*[3].

EXPERIMENTAL

Endo-(1→3)-β-D-glucanase I prepared[2] from *K. phaseolosporus* UCD-FS & T 50–80, had a specific activity of 2.6 units/mg of protein. The laminarin (Nutritional Biochemicals Corp.) had[4] d.p. 45. Yeast (1→3)-β-D-glucan was prepared from purified cell-walls[5] of *Saccharomyces cerevisiae*, except that the last remnants of (1→6)-β-D-glucan were removed as previously described[2].

Preparative p.c. was performed on Whatman No. 3MM paper with ethyl acetate–pyridine–water[6] (8:2:1) and detection with alkaline silver nitrate[7]. The appropriate areas of the chromatograms were excised, and the sugars eluted with distilled water–ethanol (5:2). The yields of D-glucose and laminaribiose, together with those obtained by chemical hydrolysis, are summarised in Table I. Partial hydrolysis of laminarin[2], enzyme assays[8], definition of enzyme units[4], and quantification[9,10] of reducing sugars followed literature procedures.

Enzymic degradations. — Standard reaction mixtures (4 ml) contained 15 units of endo-(1→3)-β-D-glucanase activity, and laminarin or yeast (1→3)-β-D-glucan (50 mg), buffered at pH 6.0 with 100 μl of 2M sodium acetate. Sodium azide (to 0.02%) was added to prevent bacterial growth. The reaction was monitored by determining the increase in reducing sugars in samples (50 μl) taken at 1-h intervals. After 5 h, no further increase in reducing power occurred. Before the mixtures of laminaribiose and D-glucose were subjected to preparative p.c., the enzyme was partially recovered (~85%) by using[2] DEAE-Sephadex A50 batch-wise.

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