786. *Polysaccharides of Baker's Yeast. Part III.*[1] *The Presence of* 1 : 6-*Linkages in Yeast Glucan.*

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The glucan from baker's yeast has been examined by the technique of toluene-*p*-sulphonylation followed by the replacement of primary tosyl groups by iodine. It is concluded from the iodine content of the product that some 10—20% of the primary hydroxyl groups are involved in 1 : 6-linkages. These results support the evidence given in Part II of this series.[1]

The structure of the insoluble cell-wall polysaccharide from baker's yeast (yeast glucan) has been studied by methylation assay,[2-4] by periodate oxidation,[5] and more recently by partial acid hydrolysis and identification of the oligosaccharide fragments.[1] These investigations all lead to the conclusion that the predominant linkage in yeast glucan is β-1 : 3-glucosidic, but there is less certainty about the character of subsidiary linkages. Indeed methylation assay by earlier workers [2,3] appeared to show that no subsidiary linkages were present; on the other hand, Bell and Northcote, from the results of methylation assay and periodate oxidation, concluded that yeast glucan has a highly branched structure in which the branch linkage is 1 : 2-glucosidic [4] (see preceding paper). Peat, Whelan, and Edwards,[1] by examination of the products of partial acid hydrolysis, inferred a structure which is essentially unbranched and in which " blocks " of β-1 : 6-linked glucose units alternate with " blocks " of β-1 : 3-linked units. If it be assumed that the average chain is of such length that the formic acid derived, by periodate oxidation, from terminal groups may be neglected, then the 1 : 6-linkages constitute *ca.* 10% of the whole. In view of the dubiety involved in the methylation assay owing to incomplete methylation of the polysaccharide, and in the acid hydrolysis method owing to the possibility that some of the oligosaccharides isolated may be products of reversion synthesis, it was felt that independent methods for establishing the presence of other linkages were desirable in order to decide between the structures postulated. It is the purpose of this communication to describe the application of one such method to yeast glucan.

[1] Peat, Whelan, and Edwards, preceding paper.
[2] Zechmeister and Toth, *Biochem. Z.*, 1934, **270**, 309; 1936, **284**, 133.
[3] Hassid, Joslyn, and McCready, *J. Amer. Chem. Soc.*, 1941, **63**, 295.
[4] Bell and Northcote, *J.*, 1950, 1944.
[5] Barry and Dillon, *Proc. Roy. Irish Acad.*, 1943, **49**, *B*, 177.

Estimation of the number of primary hydroxyl groups in oligo- and poly-saccharides has been accomplished by the well-established method of Oldham and Rutherford.[6] This method is based on the facts that the primary hydroxyl group in a sugar unit is esterified by toluene-*p*-sulphonyl chloride much faster than are the secondary hydroxyl groups, and that the primary tosyl groups can be replaced quantitatively by iodine under conditions which leave secondary tosyl groups unaffected. Subsequent determination of the iodine and the sulphur content of the product can be used to estimate the number of esterified primary and secondary hydroxyl groups respectively. Provided that esterification of the primary hydroxyl groups in a saccharide is complete, the method provides a means of estimating the number of free primary hydroxyl groups in the original saccharide. The method has found extensive application in the polysaccharide field, to cellulose and its derivatives,[7] starch,[8] and other polysaccharides.[9] Application of this method to yeast glucan should indicate the number of free hydroxyl groups and hence, by difference, the number of primary hydroxyl groups involved in 1 : 6-linkages.

The method was initially tested on amylose since this is known to contain no appreciable proportion of 1 : 6-linkages. Amylose from potato starch was treated with toluene-*p*-sulphonyl chloride in pyridine for various times, and the esterified amylose was isolated and treated with sodium iodide in hexane-2 : 5-dione at 115—120°. The product, after isolation, was analysed for iodine and sulphur. The results indicated that a temperature of 35° gave a reasonable rate of esterification and that the primary hydroxyl groups were completely esterified after 24 hr. (Table 1). (Incidentally, this

TABLE 1. *Esterification of amylose by toluene*-p-*sulphonyl chloride at* 35°.

Time of esterification (hr.)	Ester groups per anhydroglucose unit		Time of esterification (hr.)	Ester groups per anhydroglucose unit	
	Primary	Secondary		Primary	Secondary
6	0·66	0·21	30	0·99	1·04
12	0·80	0·50	35	1·01	1·09
21	0·98	0·84	50	0·99	1·19
24	1·01	0·94			

experiment provides additional evidence of the absence of 1 : 6-links from amylose.) The fact that the apparent degree of primary esterification did not increase after this time, whereas the degree of secondary esterification did increase, confirmed the view that the replacement of tosyl groups by iodine is limited to the primary positions under the conditions employed. The reliability of the method being established, two samples of yeast glucan

TABLE 2. *Esterification of yeast glucan by toluene*-p-*sulphonyl chloride.*

Time of esterification (hr.)	Ester groups per anhydroglucose unit		Time of esterification (hr.)	Ester groups per anhydroglucose unit	
	Primary	Secondary		Primary	Secondary
	At 65°, Specimen I			At 85°, Specimen I	
21	0·45	0·29	32	0·81	1·26
30	0·83	0·61	46	0·82	1·38
46	0·80	0·85	64	0·81	1·56
60	0·84	1·06			
72	0·84	1·14		At 80°, Specimen II	
84	0·82	1·18	24	0·82	1·07
			29	0·81	1·16

were examined by the same procedure. Esterification of yeast glucan proceeded more slowly than for amylose at 35° and the temperature was raised to 65° before a comparable rate was achieved (Table 2). Cellulose is also known to require a higher temperature in

[6] Oldham and Rutherford, *J. Amer. Chem. Soc.*, 1932, **54**, 366.
[7] Heuser, Heath, and Shockley, *J. Amer. Chem. Soc.*, 1950, **72**, 670 and references cited therein.
[8] Hess and Eveking, *Ber.*, 1934, **67**, 1908.
[9] Carson and Maclay, *J. Amer. Chem. Soc.*, 1948, **70**, 2220; Low and White, *ibid.*, 1943, **65**, 2430.

order to give a satisfactory rate of esterification; Honeyman [10] obtained complete esterification of primary hydroxyl groups in cellulose only after 16 hr. at 100°. At 65°, esterification of primary hydroxyl groups in yeast glucan reached a limiting value of 0·82 mol. per $C_6H_{10}O_5$ unit after 30 hr. and thereafter remained constant within the limits of experimental error, while esterification of secondary hydroxyl groups increased steadily. Raising the temperature to 85° did not increase this limit for specimen I, and the same result was observed with specimen II.

The margin of possible error in this method is dependent on a number of factors not all of which can be checked. For instance, the figures quoted are not corrected for the observation that the polyglucose content of the glucan was only 92·4%. The estimated content of 1 : 6-linkages could be 7—8% too high if all the non-hydrolysable material present in the original glucan persists in the acylated-iodinated product. No dubiety attaches however, to the conclusion that 10—20% of the glucosidic linkages in yeast glucan are 1 : 6-links, a conclusion which reinforces the earlier evidence.[1]

Experimental

Analytical Methods.—Iodine was determined by the bromine oxidation method.[11] For the determination of sulphur, the polysaccharide (100 mg.) was digested in a boiling-tube with perchloric acid (*d* 1·54; 4 ml.) under gentle reflux until frothing had ceased. Hydrogen peroxide (100-vol.; 0·1 ml.) was then added and the digestion continued, with addition of further portions of hydrogen peroxide, until the solution became colourless. After cooling, the contents of the tube were washed into a beaker, neutralised with 3N-sodium hydroxide, diluted to 100 ml., and made just acid with 3N-hydrochloric acid. The sulphate in this solution was estimated gravimetrically as barium sulphate by the usual procedure.

Polysaccharides.—Amylose was prepared from potato starch by the method of Hobson *et al.*[12] but, before use, was " activated " by precipitation from aqueous solution with pyridine, washing well with pyridine, and drying in a vacuum over phosphoric oxide. Specimen I of yeast glucan was that used by Peat *et al.*; [1] specimen II was prepared by the method of Bell and Northcote [4] but contained glycogen, as judged by iodine-staining. This specimen (25 g.) was therefore suspended in water (1 l.), and salivary amylase (50% saliva in water, centrifuged to remove mucins; 50 ml.) was added. Incubation at 35° for 24 hr. gave a product (23 g.), after filtration and washing, which was no longer stained by iodine. This gave glucose only on complete acid hydrolysis and had a polyglucose content of 92·4%.

Method.—The dried polysaccharide (0·5 g.) was suspended in dry pyridine (30 ml.) and toluene-*p*-sulphonyl chloride (6 g.) was added. The mixture was heated in a water bath and stirred under anhydrous conditions for the appropriate time (see Tables) and was then cooled to 0°. Excess of reagent was destroyed by adding a few ml. of water, the mixture was poured into iced water (500 ml.) and stirred for several hours, and the precipitated polysaccharide collected by filtration. The precipitate, after being washed with water, methanol, and ether and dried over phosphoric oxide, was heated at 115—120° for 7 hr. with an equal weight of anhydrous sodium iodide in dry hexane-2 : 5-dione (50 ml. per g.). The solvent was distilled off at 80—90° under reduced pressure and the residue was shaken with acetone (50 ml.) before being poured into iced water (1 l.). The precipitate was collected on a filter and washed with methanol, 0·1N-sodium thiosulphate, water, methanol, and finally with ether. After drying at 60° over phosphoric oxide, the product was analysed for sulphur and iodine. Extension of the time of heating with sodium iodide from 7 to 12 hr. indicated that iodination is complete in 7 hr.

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[10] Honeyman, *J.*, 1947, 168.
[11] E. P. Clark, " Semimicro Quantitative Organic Analysis," Academic Press Inc., New York, 1943, p. 62.
[12] Hobson, Pirt, Whelan, and Peat, *J.*, 1951, 801.