

COMMUNICATIONS TO THE EDITORS

*A Comparison of Synthetic Dextran with a Natural Product by Enzymic Methods*

Recently a dextran ( $\alpha$ -1,6 glucan) was synthesized by the cationic polymerization of 1,6-anhydro-2,3,4 tri-*O*-benzyl- $\beta$ -D-glucopyranose.<sup>4,5</sup> While characterized as predominantly a linear glucan, the question of minor deviations in structure arises. It is known that among the natural dextrans, i.e., those produced by *Leuconostoc* and related organisms, branching does occur and this branching may be quite extensive.<sup>3</sup> Are there structural deviations in the synthetic dextran? This report deals with a comparison of the synthetic dextran with a natural product, based on the use of enzymes; and was done at the suggestion of Dr. Schuerch.

The natural dextran, produced by *Leuconostoc mesenteroides* NRRL B512F, was received from Commercial Solvents Corp. as clinical dextran Lot 89001-D. It is reported to contain about 5% branch points,<sup>6</sup> and to have a molecular weight of 72,500. The synthetic dextran, produced via Lewis acid-catalyzed polymerization of the tribenzyl ether of levoglucosan followed by debenylation, was supplied by Dr. Schuerch. According to him, it has a molecular weight of 13,000 and an  $[\alpha]^{19} = +200^\circ$ .

Dextran (50 mg.) was incubated with an excess of the random-acting dextranase (*Penicillium funiculosum* QM 474) for 16 hr. (40°C.; pH 5.1). The hydrolyzate was concentrated to about 1 ml., streaked across 3 MM paper and the chromatogram developed with isopropanol-acetic acid-water (54:8:18). The components were located, eluted with water, and concentrated to a small volume. The amount of carbohydrate was determined by the phenol-sulfuric acid method (Table I).

TABLE I  
Hydrolysis Products of Dextrans Acted on by Dextranase

Component*	Yield, as per cent of recovered products	
	Dextran, natural	Dextran, synthetic
$R_G < 0.20$	25.6	7.7
$R_G 0.26 =$ isomaltotriose	5.6	7.7
$R_G 0.52 =$ isomaltose	57.4	72.8
$R_G 1.0 =$ glucose	11.4	11.7

\*  $R_G =$  distance moved by component/distance moved by glucose.

The hydrolysis had not gone to completion, since some trimer remained. There was no isomaltotetraose.

The  $R_G < 0.20$  fraction is important since it is made up of products that retain the branches present in the initial material. These products remain because the branch prevents hydrolysis by the random-acting  $\alpha$ -1,6 glucanase. The results show that the synthetic dextran has much less branching than the natural product. An estimate of the amount of branching can be made from the data of Bourne et al.<sup>2</sup>; based on the fact that the resistant fraction has an average DP of about 5, i.e., one branch in four linkages. Since the  $R_G < 0.20$  fraction from the natural dextran amounts to 25% (Table I), the original material must have had one branch in 16 or about 6%. Applying the same

reasoning to the *synthetic* dextran, one arrives at a frequency of 2% of linkages other than  $\alpha$ -1,6 (see below).

The products found in the  $R_G < 0.20$  fraction of *synthetic* dextran are different from those produced from native dextran. Three-day development of chromatograms showed five components in the native, but only three in the synthetic. All are different from each other and from the oligosaccharides of the isomaltose series (IM2, IM3, etc.).

Oligosaccharides from	$R_G$ values
Dextran, native, $R_G < 0.20$	0.006; 0.018; 0.025; 0.075; 0.155
Dextran, synthetic $R_G < 0.20$	0.021; 0.048; 0.11
Isomaltose series	0.06 (IM5); 0.12 (IM4); 0.26 (IM3); 0.52 (IM2)

The branched products from native dextran have been shown to be linear  $\alpha$ -1,6 oligoglucans, bearing a single glucose attached through an  $\alpha$ -1,3 linkage to one of the nonterminal units of the chain.<sup>2,6</sup> The  $R_G < 0.20$  products from synthetic dextran are branched in some other fashion. In order to investigate this branching further, these products were subjected to the action of various glycosidases. All were hydrolyzed by glucamylase, and by  $\alpha$ -glucosidase; none by  $\beta$ -glucosidase (almond). No products other than glucose were found. The  $R_G < 0.20$  products of native and of synthetic dextrans behaved in similar fashion.

The enzymic analyses confirm the basic similarity of the synthetic and natural dextrans. Both glucans were hydrolyzed at the same initial rate by dextranase and produced the same major products. Even the appearance and disappearance of tetramer and trimer followed the same time sequence.

The two glucans differ in minor ways. Glucamylase (an enzyme which successively removes glucose units from the nonreducing end of chains) acts twice as rapidly on the synthetic dextran as on the natural (31% vs. 16% hydrolysis in 24 hr.).

An attempt was made to obtain further information about the branched products ( $R_G < 0.20$ ) of the synthetic dextran by means of the Smith degradation technique.<sup>1</sup> The material (5.7 mg.) was treated successively with  $\text{NaIO}_4$ ,  $\text{KBH}_4$ , and 0.1N HCl; deionized, dried, silylated, and chromatographed on the Pye instrument using a column of 3% SE52 on Chromosorb W. No erythritol or glucosylglycerol was detected under conditions permitting the detection of 5  $\mu\text{g}$ . of authentic sample. These results indicate the absence of (a) internal glucofuranose units linked 1,6 and (b) internal glucopyranose units linked 1,4.

The synthetic dextran, then, contains a small amount of a linkage of unknown nature. The data indicate that the linkages are all  $\alpha$ -, and that the glucose units are in the pyranose configuration.

If there are single glucose branches, as in natural dextran, then these must be linked  $\alpha$ -1,4 or  $\alpha$ -1,2 to the main chain. However, it should be pointed out that the dextranase-resistant fragments could also result from (a) substitution by benzyl groups, resulting from incomplete debenzoylation, or (b) alternation of a "foreign"  $\alpha$ -linkage with  $\alpha$ -1,6 linkages in *straight* chain oligomers. The complete susceptibility of the residues to  $\alpha$ -glucosidases, and the failure to detect *substituted* glucose among the products, seem to rule out (a). It appears likely that all glucoses are in the pyranose form, and  $\alpha$ -linked. The minor linkage either remains within the straight chain or forms a branch from it.

#### References

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*f. 1.3 is correct  
James  
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Synth

Prob:

dextran with a few  $\alpha$ 1,4 linked  
glucose side chains - as the  $\alpha$ 1,4  
would be more susc to glucosylase  
than  $\alpha$ 1,3 or  $\alpha$ 1,2