

Fig. 1. Electron-spin-resonance spectrum of Yonetani's (1960, 1961) preparation of cytochrome oxidase in the oxidized form. The concentration of enzyme was 0.8 mM (expressed as haem), and observation was in a circular tube of 3 mm. diameter at 77° K. The arrow shows the direction of increase of magnetic field.

mixed. The solution was frozen as quickly as possible after leaving the mixer and the time was estimated to be approx. 10 msec. (Bray, 1961). The finely divided ice crystals, suspended in heptane, contain only about half as much enzyme per unit volume as do the undiluted preparations of enzyme. A substantial portion of the total enzyme had been converted into the oxidized form in the 10 msec. or so between mixing and the completion of freezing. When the cytochrome oxidase solution was first equilibrated with CO and then mixed with O<sub>2</sub>, there was no signal due to oxidized copper.

#### DISCUSSION

Although the technique described by Bray (1961) represents a great improvement on the making of a mixture followed by freezing the tube containing it, the time of 10 msec. given by Bray for freezing

the droplets is still rather long in the study of a rapidly reacting enzyme such as cytochrome oxidase. The satisfactory signals which we have obtained, however, certainly show that a large proportion of the enzyme copper can become oxidized in that time, so that the participation of copper in the enzyme reaction is in no way excluded, as it would have been if the electron-spin-resonance signal had failed to appear. The protection given by carbon monoxide helps in drawing conclusions about the order in which the haem and copper react with oxygen. For reasons which are given in the Discussion section of the main paper, it seems unlikely that carbon monoxide itself combines with cuprous copper of the enzyme; if this is accepted it follows that cuprous copper does not react directly with oxygen but is oxidized indirectly through the intervention of haem iron, a conclusion which fits well with the observation that the kinetics of the reaction of oxygen with reduced cytochrome oxidase are the same when studied by the stopped-flow method and by the flow-flash methods at similar oxygen concentrations.

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## Studies on Dextran and Dextranases

### 3. STRUCTURES OF OLIGOSACCHARIDES FROM *LEUCONOSTOC MESPENTEROIDES* (BIRMINGHAM) DEXTRAN\*

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It has been shown that the adaptively produced dextranases of *Penicillium tilacinum* (I.M.I. 79197; NRRL 896) and of *P. funiculosum* (I.M.I. 79195; NRRL 1132) hydrolyse the virtually unbranched dextran of *Streptococcus bovis* to give mainly isomaltose and that the degree of hydrolysis of dextran possessing anomalous linkages depends on the percentage of such linkages in the dextran

(Bourne, Hutson & Weigel, 1962). This suggested that the dextranases could not hydrolyse the anomalous linkages of branched dextran. Isolation of the oligosaccharides which cannot be hydrolysed by the dextranases should aid the structural analysis of dextran and also elucidate further the mechanism of dextranase action. An analysis of the structures of 'branched' oligosaccharides produced by the action of the dextranase of *Lactobacillus bifidus* (Bailey & Clarke, 1959) on

\* Part 2: Bourne, Hutson & Weigel (1962).

*Leuconostoc mesenteroides* (Birmingham) dextran has already been made (Bailey, Hutson & Weigel, 1961). The smallest 'branched' oligosaccharides produced were 3<sup>3</sup>-glucosylisomaltotriose and 3<sup>2</sup>-glucosylisomaltotriose. We have now examined the oligosaccharides produced by the action of the dextranases from the two moulds on the same dextran.

### EXPERIMENTAL

**Dextranases.** The dextranase preparations of *P. lilacinum* (I.M.I. 79197; NRRL 896) (dextranase A) and *P. funiculosum* (I.M.I. 79195; NRRL 1132) (dextranase B) used in the present investigation were as described by Bourne *et al.* (1962).

**Dextran.** *L. mesenteroides* (Birmingham) dextran was from the same batch as was that used for structural studies and was shown to contain 12–15% of  $\alpha$ -(1→3)-linkages (Barker, Bourne, Bruce, Neely & Stacey, 1954).

**Dextranase digests.** Digests contained dextran (2%, w/v) and dextranase preparation (0.2%, w/v) in 0.1M-sodium citrate, pH 5.0. The digests were incubated under toluene for 16 hr. at 37°. The final number of reducing groups liberated was the same when the ratio of dextran to dextranase preparation was 10:1, 4:1 or 2:1 (by weight).

**Paper chromatography and ionophoresis.** The solvents used for paper chromatography were: (a) the upper layer of ethyl acetate–water–pyridine (2:2:1, by vol.) (Jermyn & Isherwood, 1949); (b) ethyl acetate–water–pyridine–acetone (Malpress & Hytten, 1958). Ionophoresis was carried out at about 50 v/cm. in molybdate solution, pH 5.5 (Bourne, Hutson & Weigel, 1961), and in borate solution, pH 10 (Foster, 1953). Some of the  $M_s$  (mobility with respect to sorbitol) values reported here differ slightly from those reported previously. This is due to the application of smaller quantities, thus allowing a more accurate determination of the rates of migration. In all cases comparison was made with known compounds.

The reagents used for the detection of compounds were: (a) silver nitrate in acetone–ethanolic sodium hydroxide (Trevelyan, Procter & Harrison, 1950); (b) aniline hydrogen phthalate (Partridge, 1949); (c) *p*-anisidine–HCl (Hough, Jones & Wadman, 1950); (d) aniline–diphenylamine–phosphoric acid (Schwimmer & Bevenue, 1956); (e) triphenyltetrazolium chloride (Feingold, Avigad & Hestrin, 1956).

**Borohydride reduction.** Aqueous solutions of the sugars (about 1%, w/v) were mixed with an equal volume of potassium borohydride (tetrahydroborate) solution (1%, w/v) and allowed to stand overnight. The solutions were then de-ionized by treatment with Amberlite IR-120 (H<sup>+</sup> form) followed by repeated distillation with anhydrous methanol. The sugar alcohols were always purified by paper chromatography.

**Degree of polymerization of oligosaccharides.** The degree of polymerization of the oligosaccharides was determined by the following methods: (a) measurement of  $R_M$  values,  $\log[1/(R_G - 1)]$ , where  $R_G$  is the distance travelled compared with glucose; (b) comparison of  $M_s$  values (Bourne *et al.* 1961) of the reduced oligosaccharides with those of the reduced oligosaccharides of the isomaltose series; (c) determination of reducing power relative to glucose (Shaffer & Hartmann, 1921), with corrections for carbohydrate

content; the carbohydrate content was determined by acid hydrolysis and measurement of the reducing sugar produced (Shaffer & Hartmann, 1921), by applying the correction of Pirt & Whelan (1951) for the degradation of glucose by acid; (d) comparison of anthrone values before and after reduction with potassium borohydride (Peat, Whelan & Roberts, 1956).

### RESULTS

*Oligosaccharides produced by the action of dextranases A and B on Leuconostoc mesenteroides (Birmingham) dextran*

Digests containing *L. mesenteroides* (Birmingham) dextran were separately incubated with dextranases A and B. Paper chromatography in solvent (a) of the digest with dextranase A revealed the presence of components with  $R_G$  values identical with those of glucose, isomaltose, isomaltotriose and oligosaccharides which had slightly higher  $R_G$  values than isomalto-pentaose, -hexaose, -heptaose and -octaose. The digest with dextranase B contained, in addition to the above, a component which had a slightly higher  $R_G$  than that of isomaltotetraose. Unresolvable material with  $R_G$  smaller than that of isomalto-octaose was present in both digests. As glucose, isomaltose and isomaltotriose are the only products when the dextran-

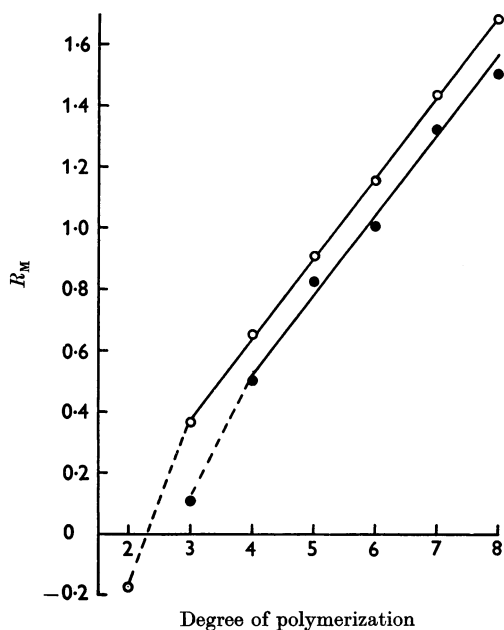


Fig. 1.  $R_M$  values (see the text) in ethyl acetate–water–pyridine (2:2:1, by vol.). O, Oligosaccharides of the isomaltose series; ●, 'branched' oligosaccharides produced by the action of dextranases A and or B on *L. mesenteroides* (Birmingham) dextran. 3<sup>1</sup>- (or 3<sup>2</sup>-)Glucosylisomaltose (Bailey *et al.* 1961) has been included for comparison.

Table 1. *Ionophoresis of reduced oligosaccharides of the isomaltose series and reduced 'branched' oligosaccharides produced by dextranases A and B*

Experimental details are given in the text.  $M_s$ , mobility with respect to sorbitol in molybdate solution (Bourne *et al.* 1961).

Degree of polymerization of reduced oligosaccharides of isomaltose series	$M_s$	Reduced 'branched' oligosaccharides produced by dextranases A and B	$M_s$	
			A	B
2	0.78	—	—	—
3	0.62	—	—	—
4	0.53	Tetrasaccharide alcohol	—	0.52
5	0.44	Pentasaccharide alcohol	0.45	0.44
6	0.39	Hexasaccharide alcohol	0.39	0.39
7	0.35	Heptasaccharide alcohol	0.35	0.35
8	0.33	Octasaccharide alcohol	0.33	0.33

ases act on the virtually unbranched dextran of *S. bovis* (Bourne *et al.* 1962), the additional oligosaccharides produced from *L. mesenteroides* (Birmingham) dextran probably contain its  $\alpha$ -(1 $\rightarrow$ 3)-linkages.

In Fig. 1 the  $R_M$  values of these oligosaccharides are plotted against their expected degree of polymerization and compared with those of the oligosaccharides of the isomaltose series. A linear relationship was observed in both series, as was noted for the latter by Turvey & Whelan (1957). 3<sup>1</sup>- (or 3<sup>2</sup>-)Glucosylisomaltose and isomaltose do not obey this relationship. This indicates that in the solvent used the tetrasaccharide must be regarded as the lowest member of the former polymeric series whereas that of the latter is isomaltotriose.

The oligosaccharides containing one glucose unit joined through a (1 $\rightarrow$ 3)-linkage to a glucose unit of isomaltotriose or isomaltotetraose have slightly higher  $R_G$  values than those of isomaltotetraose and isomaltopentaose respectively. As the two plots of  $R_M$  against degree of polymerization in Fig. 1 are parallel it is reasonable to assume that the oligosaccharides produced by the dextranases are members of a homologous series of 'branched' oligosaccharides of which a 3-glucosylisomaltotriose is the lowest member.

The 'branched' oligosaccharides were eluted from paper chromatograms, reduced with potassium borohydride and subjected to ionophoresis in molybdate solution. The  $M_s$  values (Table 1) were identical with those of the reduced oligosaccharides of the isomaltose series of the same expected degree of polymerization.

The products of the action of the dextranases on the dextran were eluted quantitatively from paper chromatograms and their relative concentrations determined by the anthrone method (Yemm & Willis, 1954). The results are shown in Table 2, the assignation of degree of polymerization being based on chromatography and ionophoresis.

Table 2. *Relative weight yields of oligosaccharides produced by the action of dextranase A and B respectively on Leuconostoc mesenteroides (Birmingham) dextran*

Experimental details are given in the text. The relative weight yield is expressed relative to isomaltose (= 100).

Compound	Relative weight yield	
	With dextranase A	With dextranase B
Glucose	21	17
Isomaltose	100	100
Isomaltotriose	71	56
Tetrasaccharide	—	8
Pentasaccharide	9	6
Hexasaccharide	21	16
Heptasaccharide	29	22
Octasaccharide and unresolvable material	107	58

#### *Structural examination of oligosaccharides produced by dextranase A*

The oligosaccharides (series A) produced by the action of dextranase A on *L. mesenteroides* (Birmingham) dextran (10 g.) were fractionated on a charcoal-Celite column (Whistler & Durso, 1950) and further purified by paper chromatography. The yields, carbohydrate contents, reducing powers, optical rotations and periodate consumptions of the isolated oligosaccharides are shown in Table 3. The properties of the oligosaccharides are consistent with their molecular sizes already assigned.

*Pentasaccharide A.* Pentasaccharide A could be detected on paper chromatograms with spray reagents (a), (b), (c), (d) (green spot, specific for 6-O-substituted glucose) and (e). During ionophoresis in borate solution, pH 10, it migrated with the same  $M_G$  (mobility with respect to glucose) as isomaltopentaose (0.60).

Pentasaccharide A (1 mg.) was heated for 4 hr. at 100° in 1.5N-hydrochloric acid (2 ml.). Chromatography of the de-ionized hydrolysate showed a single component identical with glucose. A partial hydrolysate, obtained by heating for 4 hr. at 100° in aq. 1% (w/v) oxalic acid was shown by paper chromatography in solvents (a) and (b) to contain glucose and isomaltotetraose as the main products. Isomaltose and isomaltotriose were present as traces. After elution from paper chromatograms the isomaltotetraose was further identified by incubation with dextranase A, which resulted in rapid hydrolysis with isomaltose as the main product (Bourne *et al.* 1962).

Pentasaccharide A alcohol (Table 1) was partially hydrolysed by heating for 4 hr. at 100° in aq. 1% oxalic acid. The de-ionized hydrolysate was fractionated by paper chromatography in solvent (a) into six fractions in addition to unchanged material (Table 4). Each fraction was subjected to ionophoresis in molybdate solution, pH 5.5, when fractions 3, 4, 6 and 7 were each resolved into two components. The non-migrating fraction 3 (i) was, after further reduction with potassium borohydride, mobile during ionophoresis

in molybdate solution. Fraction 5 was likewise reduced. Ionophoresis in molybdate solution resolved this reduction product into two components, fractions 5 (i) and 5 (ii).

*Hexasaccharide A.* Hexasaccharide A could be detected with the same spray reagents as pentasaccharide A. Paper chromatography of a complete hydrolysate showed a single component identical with glucose, whereas a partial hydrolysate showed glucose and isomaltopentaose as the main products.

Hexasaccharide A alcohol (Table 1) was partially hydrolysed and the products were fractionated by paper chromatography. In addition to isomaltopentaol and its expected hydrolysis products, components were found which had the same  $R_G$  values as those of the partial hydrolysate of pentasaccharide A alcohol shown in Table 4. The fractions which corresponded to fractions 1, 3 and 5 ( $R_G$  values 0.17, 0.29 and 0.44 respectively) were subjected to ionophoresis in molybdate solution. Those corresponding to fractions 1 and 3 were each resolved into an immobile (corresponding to 'branched' pentasaccharide and fraction 3a respectively) and a mobile ( $M_s$  0.45 and 0.50 re-

Table 3. *Some properties of oligosaccharides produced by dextranase A*

Experimental details are given in the text. The calculated periodate consumption is for oligosaccharides containing one (1→3)-'branch'-link/molecule.

	Yield (mg.)	[ $\alpha$ ] <sub>D</sub> in water	Carbohydrate content (%)	Reducing power (% of theoretical)	Periodate consumption (mol.prop.)	
					Found	Calc.
Pentasaccharide A	100	178°	98.0	90.1	9.3	9
Hexasaccharide A	340	186	94.2	105.6	11.1	11
Heptasaccharide A	570	190	95.4	97.7	13.2	13
Octasaccharide A	560	194	96.6	95.6	14.3	15

Table 4. *Fractionation of products from partial hydrolysis of pentasaccharide A alcohol*

Experimental details are given in the text.

Paper chromatography in solvent (a)		Ionophoresis in molybdate solution			
Fraction no.	$R_G$	Before further reduction		After further reduction	
		$M_s$	Identity	$M_s$	Identity
1	0.17	0.45	Pentasaccharide A alcohol	—	—
2	0.21	0.50	(VIII)	—	—
3	0.29	(i) 0	(IX) or (XI)	0.50	(X) or (XII)
		(ii) 0.50	(X)	—	—
4	0.34	(i) 0	(XV)	—	—
		(ii) 0.65	(XIV)	—	—
5	0.44	0	(XVI) and (XVIII)	(i) 0	(XXIV)
				(ii) 0.65	(XVII)
6	0.60	(i) 0	(XX)	—	—
		(ii) 0.78	(XIX)	—	—
7	1.0	(i) 0	(XXII)	—	—
		(ii) 1.0	(XXIII)	—	—

spectively) component. That with  $R_G$  0.44 (fraction 5) remained immobile during ionophoresis.

**Heptasaccharide A.** Heptasaccharide A could be detected with the same spray reagents as pentasaccharide A. Complete hydrolysis was shown, by paper chromatography, to give only glucose. The partial hydrolysate contained glucose and isomaltohexaose as the main products.

Heptasaccharide A alcohol (Table 1) was partially hydrolysed and the hydrolysate fractionated as described for pentasaccharide A alcohol. In addition to isomaltohexaitol and its expected hydrolysis products, components were found that had the same  $R_G$  values as those of the partial hydrolysate of pentasaccharide A alcohol shown in Table 4. Ionophoresis in molybdate solution resolved the fraction corresponding to fraction 1 ( $R_G$  0.17) into an immobile ('branched' pentasaccharide) and a mobile component with  $M_n$  0.45 (reduced 'branched' pentasaccharide). The fractions corresponding to fractions 3 ( $R_G$  0.29) and 5 ( $R_G$  0.44) did not migrate during ionophoresis in molybdate solution.

**Octasaccharide A.** Octasaccharide A could be detected with the same spray reagents as pentasaccharide A. The complete hydrolysate contained only glucose whereas the partial hydrolysate contained glucose, isomaltohexaose, possibly isomaltoheptaose, and a component with  $R_G$  identical with that of heptasaccharide A.

Octasaccharide A alcohol (Table 1) was partially hydrolysed and fractionated as described for pentasaccharide A alcohol. In addition to the main products which had  $R_G$  values corresponding to those of the 'branched' heptasaccharide A, possibly isomaltoheptaose, isomaltohexaose, 'branched' hexasaccharide A, isomaltopentaose and glucose, components were found which had the same  $R_G$  values as those of the partial hydrolysate of pentasaccharide A alcohol (Table 4). Ionophoresis in molybdate solution of the fractions with  $R_G$  values 0.17, 0.29 and 0.44 gave the same results as the corresponding fractions of the partial hydrolysate of heptasaccharide A alcohol.

#### *Structural examination of oligosaccharides produced by dextranase B*

The tetra-, penta- and hexa-saccharides (series B) produced by the action of dextranase B on *L. mesenteroides* (Birmingham) dextran (40 g.) were fractionated and purified as described for those of series A. Determinations of the degree of polymerization by the method of Peat *et al.* (1956) gave values of 4.2, 4.9 and 6.0 respectively. The preliminary assignation of molecular size (Fig. 1 and Table 1) was thus correct.

**Tetrasaccharide B.** The methods used were as for pentasaccharide A. Tetrasaccharide B could be

detected on paper chromatograms with spray reagents (a), (b), (c), (d) (green spot) and (e). During ionophoresis in borate solution, pH 10, it migrated with the same  $M_n$  as that of isomaltotetraose (0.65). Complete hydrolysis gave a single reducing component that was chromatographically identical with glucose, whereas a partial hydrolysate contained components corresponding to glucose and isomaltotriose, as the main products, and isomaltose and nigerose.

The component corresponding to isomaltotriose was isolated by paper chromatography. When benzoylated (Turvey & Whelan, 1957) it gave a crystalline product, which had m.p. 223–225° (Found: C, 69.2; H, 4.6.  $C_{95}H_{76}O_{27}$  requires C, 69.2; H, 4.6%). Admixture with authentic undeca-*O*-benzoyl- $\beta$ -isomaltotriose (m.p. 225–227°) caused no depression in melting point.

The disaccharide corresponding to nigerose maintained its identity during ionophoresis in borate solution, pH 10. When reduced with potassium borohydride it did not migrate during ionophoresis in molybdate solution, pH 5.5. This shows conclusively the presence of a (1→3)-glucosidic linkage (Bourne *et al.* 1961). The disaccharide was not hydrolysed by  $\beta$ -glucosidase, unlike laminaribiose, thus confirming the presence of an  $\alpha$ -linkage.

Tetrasaccharide B consumed 7.1 mol.prop. of periodate. After destruction of the excess of periodate, the oxidation product was reduced with potassium borohydride and hydrolysed with hydrochloric acid (Bailey *et al.* 1961). The hydrolysate was fractionated by paper chromatography in solvent (a) when a component corresponding to glucose was revealed. This component was determined by the anthrone method (Yemm & Willis, 1954). The yield of glucose from tetrasaccharide B corresponded to 28% of the glucose present in a tetrasaccharide containing glucose only.

Tetrasaccharide B alcohol was partially hydrolysed by heating for 4 hr. at 100° in aq. 1% oxalic acid. By the methods described by Bailey *et al.* (1961) it was shown that the hydrolysate contained 6- $\alpha$ -isomaltosylsorbitol, 3<sup>2</sup>-glucosylisomaltose, isomaltose, 6- $\alpha$ -D-glucopyranosylsorbitol, nigerose, glucose and sorbitol. 6-Nigerosylsorbitol and 3<sup>1</sup>-glucosylisomaltose were absent from the hydrolysate.

**Pentasaccharide B and hexasaccharide B.** The  $R_G$  values and the compositions of the complete and partial hydrolysates were identical with those of pentasaccharide A and hexasaccharide A respectively. The partial hydrolysates of pentasaccharide B alcohol and hexasaccharide B alcohol (Table 1) contained the same components as those of the alcohols of pentasaccharide A and hexasaccharide A respectively.

## DISCUSSION

The assignment of the structures of the oligosaccharides produced by the action of dextranases A and B on *L. mesenteroides* (Birmingham) dextran is based on the facts that: (a) in the solvents used the reducing sugars have the same  $R_G$  values as their reduction products; (b) oligosaccharides of the isomaltose series have slightly lower  $R_G$  values than their isomers containing (1→3)-linkages; (c) glucose and oligosaccharides of glucose do not migrate during ionophoresis in molybdate solution and can thus be separated from their reduction products, provided the latter are not 3-substituted sorbitols; (d) 3-substituted sorbitols do not migrate during ionophoresis in molybdate solution; (e) isomeric oligosaccharide alcohols containing the same substituted polyol component, e.g. 6- $\alpha$ -isomaltosylsorbitol and 6- $\alpha$ -nigerosylsorbitol, have identical  $M_s$  values during ionophoresis in molybdate solution.

The evidence presented shows that the oligosaccharides investigated in detail are tetra-, penta-, hexa- and hepta-saccharides in which one glucose unit is joined through a (1→3)-linkage to a glucose unit, other than the reducing one, of isomaltotriose, -tetraose, -pentaose and -hexaose respectively. It was also shown that pentasaccharide A and hexasaccharide A are identical with pentasaccharide B and hexasaccharide B respectively. As the (1→3)- as well as the (1→6)-glucosidic linkages in this dextran are of the  $\alpha$ -type it is reasonable to assume that the (1→3)-glucosidic linkages in the oligosaccharides also are  $\alpha$ -(1→3)-linkages. This is confirmed by the characterization of nigerose as one of the products of the hydrolysis of tetrasaccharide B and its reduction product.

Tetrasaccharide B was shown conclusively to be 3<sup>3</sup>- $\alpha$ -glucosylisomaltotriose (I) (Fig. 3). The products expected from the 3<sup>2</sup>-isomer were absent from the hydrolysate of tetrasaccharide B alcohol.

There are three possible structures, (II)–(IV) (Fig. 2), for a pentasaccharide containing one glucose unit joined through an  $\alpha$ -(1→3)-linkage to a glucose unit, other than the reducing one, of isomaltotetraose. The structure was elucidated further by a partial hydrolysis of the reduction product (V), (VI) or (VII).

The products that would be expected from all three structures (Fig. 2), namely 6-isomaltotri-  
sorbitol (VIII) (fraction 2), 6-isomaltosylsorbitol (XIV) (fraction 4ii), isomaltotriose (XV) (fraction 4i), 6-glucosylsorbitol (XIX) (fraction 6ii), isomaltose (XX) (fraction 6i), glucose (XXII) (fraction 7i) and sorbitol (XXIII) (fraction 7ii), were identified by paper chromatography and ionophoresis. Under the conditions used it was not possible to detect nigerose (XXI), which also would be expected from all three structures.

Structure (IV) is eliminated for the pentasaccharide since (XIII) and (XVII) have been shown to be absent from the partial hydrolysate of the pentasaccharide alcohol.

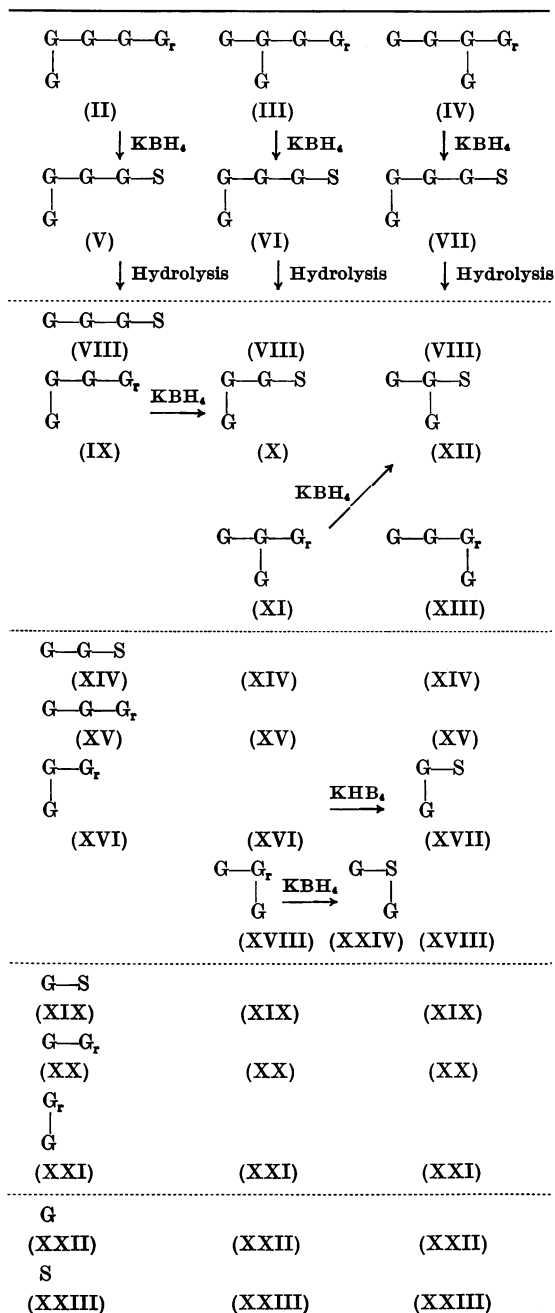


Fig. 2. Products of reduction-hydrolysis of pentasaccharides (II), (III) and (IV). —,  $\alpha$ -(1→6)-Link; |,  $\alpha$ -(1→3)-link;  $G_r$ , reducing glucose unit; G, glucosyl unit; S, sorbitol.

Reduction of the branched trisaccharide fragments (fraction 5) followed by ionophoresis in molybdate solution revealed the presence in the hydrolysate of (XVI) and (XVIII). As structure (IV) has been eliminated the presence of 3<sup>1</sup>-glucosylisomaltose (XVIII) in the hydrolysate shows conclusively that the pentasaccharide contains structure (III). 3<sup>3</sup>-Glucosylisomaltotriose (IX) is the only fragment derivable solely from structure (II) for the pentasaccharide. However, the method used did not distinguish between this and 3<sup>2</sup>-glucosylisomaltotriose (XI) [from (III)]. Thus the pentasaccharides A and B are 3<sup>3</sup>-glucosylisomaltotetraose (III), alone or in admixture with 3<sup>4</sup>-glucosylisomaltotetraose (II).

Hexasaccharides A and B on partial hydrolysis gave glucose and isomaltopentaose. Their reduction products likewise gave isomaltopentaitol, a 'branched' pentasaccharide, pentasaccharide A alcohol and the same hydrolysis products as the pentasaccharide A alcohol. Thus it is concluded that each hexasaccharide is 3<sup>3</sup>-glucosylisomaltopentaose (XXVI), alone or in admixture with 3<sup>4</sup>-glucosylisomaltopentaose or both.

The results obtained with heptasaccharide A show that it contained one glucose unit joined through a (1→3)-linkage to a glucose unit, other than the reducing one, of isomaltohexaose. Partial hydrolysis of heptasaccharide A alcohol produced in addition to isomaltohexaitol and its expected hydrolysis products components which had the same  $R_G$  values as those of the partial hydrolysate of pentasaccharide A alcohol. The structure of the heptasaccharide could thus have been similar to those of the penta- and hexa-saccharides. However, when the components with  $R_G$  values corre-

sponding to 'branched' tri- and tetra-saccharides or their reduction products were subjected to ionophoresis in molybdate solution fragments with structures (X) or (XVII) could not be detected. Hence the component that had the same  $R_G$  and  $M_g$  values as pentasaccharide A alcohol must have structure (V). Thus it is concluded that heptasaccharide A is 3<sup>4</sup>-glucosylisomaltohexaose (XXVII), alone or in admixture with isomers in which the branching occurs further towards the non-reducing end of the isomaltohexaose unit.

Octasaccharide A consumed 14.3 mol.prop. of periodate. The theoretical consumption of periodate of an octasaccharide containing one glucose unit joined through a (1→3)-linkage to isomaltoheptaose (XXVIII) is 15 mol.prop., whereas that containing two glucose units joined through (1→3)-linkages to isomaltohexaose (XXIX) is 13 mol.prop. It is thus likely that octasaccharide A is a mixture of compounds with structures similar to (XXVIII) and (XXIX). This is supported by the fact that the main products of the partial hydrolysis of octasaccharide A were glucose, isomaltohexaose, possibly isomaltoheptaose and a component with  $R_G$  identical with that of heptasaccharide A. The results of the partial hydrolysis of octasaccharide A alcohol show that the octasaccharide contains a 3<sup>4</sup>-glucosylisomaltotetraose unit as does heptasaccharide A. It is thus concluded that octasaccharide A is a mixture of 3<sup>4</sup>-glucosylisomaltoheptaose (XXVIII), possibly isomers in which the branching occurs further towards the non-reducing end of the isomaltoheptaose unit, and octasaccharides with structures similar to that of (XXIX).

An interesting feature is the production of 3<sup>3</sup>-glucosylisomaltotriose (I) by dextranase B but not by dextranase A. Dextranase B hydrolyses isomaltotriose appreciably faster than does dextranase A (Bourne *et al.* 1962). It is thus possible that dextranase B is capable of hydrolysing the main dextran chain closer to the non-reducing side of the branch point than dextranase A. Alternatively, dextranase B could be contaminated with an enzyme capable of hydrolysing terminal  $\alpha$ -(1→6)-glucosidic linkages at the non-reducing end of the chain. The presence, or absence, of such an enzyme must be investigated before a full assessment of the above results can be made.

Further, the 'branching' of the oligosaccharides consists of only one glucosyl unit, as with the oligosaccharides produced by the action of *L. bifidus* dextranase on the same dextran (Bailey *et al.* 1961).

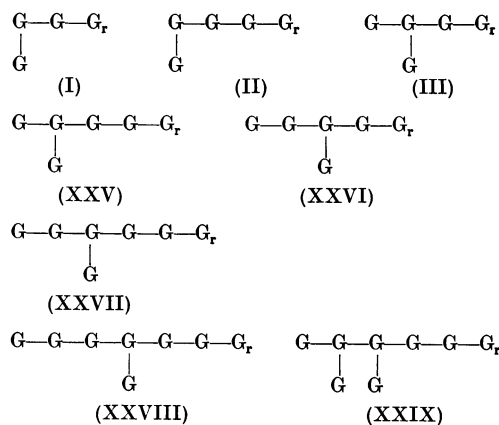


Fig. 3. Structures of oligosaccharides produced by the action of dextranases on *L. mesenteroides* (Birmingham) dextran. —,  $\alpha$ -(1→6)-Link; |,  $\alpha$ -(1→3)-link; G<sub>r</sub>, reducing glucose unit; G, glucosyl unit.

## SUMMARY

1. The dextranases of *Penicillium lilacinum* (I.M.I. 79197; NRRL 896) (dextranase A) and *P. funiculosum* (I.M.I. 79195; NRRL 1132) (dex-

tranase B) have been shown to hydrolyse *Leuconostoc mesenteroides* (Birmingham) dextran to complex mixtures of oligosaccharides.

2. Both dextranases produced glucose, isomaltose, isomaltotriose and 'branched' penta-, hexa-, hepta- and octa-saccharides. Dextranase B produced, in addition, a 'branched' tetrasaccharide. Unresolvable material of degree of polymerization greater than 8 was produced by both dextranases. The 'branched' oligosaccharides were shown to be members of an homologous series.

3. The tetrasaccharide produced by dextranase B was shown to be 3<sup>8</sup>- $\alpha$ -glucosylisomaltotriose.

4. The pentasaccharides produced by dextranases A and B were identical and shown to be a 3<sup>8</sup>-glucosylisomaltotetraose, alone or in admixture with 3<sup>4</sup>-glucosylisomaltotetraose. Likewise, the hexasaccharides were identical and shown to be 3<sup>8</sup>-glucosylisomaltopentaose, alone or in admixture with isomers in which the branching occurred further towards the non-reducing end of the isomaltopentaose unit.

5. The heptasaccharide and octasaccharide produced by dextranase A were shown to contain 3<sup>4</sup>-glucosylisomaltohexaose and 3<sup>4</sup>-glucosylisomaltoheptaose respectively. Both saccharides could have been in admixture with isomers in which the branching occurred further towards the non-reducing end of the isomalto-hexaose and -heptaose units respectively. The octasaccharide also contained a saccharide in which two glucose units are joined through (1 $\rightarrow$ 3)-linkages to isomaltohexaose.

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## The Effects of Antimicrobial Agents on Deoxyribonucleic Acid Polymerase

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The synthesis of DNA is carried out by an enzyme, deoxyribonucleic acid polymerase (deoxyribonucleic acid nucleotidyltransferase; deoxynucleoside triphosphate-deoxyribonucleic acid deoxynucleotidyltransferase, EC 2.7.7.7), which re-

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quires the presence of 4 mol.prop. of deoxyribonucleotide triphosphates and 1 mol.prop. of a DNA primer (Bessman, Lehman, Simms & Kornberg (1958). Kirk (1960) reported that the enzyme is inhibited by the antibacterial and antitumour agent, actinomycin D, at concentrations considerably in excess of those needed for marked effects on cell growth. This paper describes work in which