Note

Interaction of polysaccharides with iodine
Part II. The behavior of xylans in different salt solutions

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INTRODUCTION

In previous publications[1-4] the interaction of linear and branched polysaccharides in aqueous calcium chloride solutions with aqueous iodine–potassium iodide to give blue-colored complexes was described. The naturally occurring D-xylose-containing polysaccharides have various compositions and conformations according to their source and mode of isolation. Such polysaccharides were used in the present study of the effect of chemical structure on the reaction of the polymer with iodine in the presence of various salt solutions.

The polysaccharides selected are described in Table I and include an acid-hydrolyzed commerical xylan[5], bamboo[6], elm[7], and sapote gum[8], and a commercial xylan (probably from oat hulls). Of these polymers, only the xylan from elm has been extensively characterized by conventional chemical and physical techniques. This polymer is probably very similar to other hardwood xylans[9-11].

Two new polysaccharides, not differing appreciably in chain length from the original, were prepared from the elm xylan by altering the nature and the number of the branches attached to it. In one case, the 4-*O*-methyl-D-glucopyranosyluronic acid residues were reduced by a modification[7] of a diborane reduction-procedure[12] to anhydro-4-*O*-methyl-D-glucopyranosyl residues, with a conversion of over 90%. In the other preparation, advantage was taken[13,14] of the known alkali lability, at temperatures greater than 100°, of the glycosidic bond linking uronic acid residues to aglucones, and the 4-*O*-methylglucuronoxylan of elm was converted into a linear xylan substantially devoid of uronic acid branches[7].

A highly branched D-xylan was isolated[8,15] from sapote gum. This polysaccharide is a xylan in which there are no nonterminal D-xylopyranosyl residues that are not branch points; all of the components have a pyranose ring and the polysaccharide is, therefore, analogous in some respects to guaran, a hexose polysaccharide.

The structure of bamboo xylan has been investigated by Matsuzaki and coworkers[16]. As can be seen from Table I, the bamboo xylan employed in this investigation had the same proportion of uronic acid as elm xylan, and had a lower viscosity,

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TABLE I

THE COMPOSITION OF VARIOUS XYLOSE-RICH POLYSACCHARIDES

Composition, %	*Sapote gum xylan*	*Commercial xylan*	*Acid-hydrolyzed xylan*	*Bamboo xylan*	*Reduced elm xylan*	*Elm xylan*	*Degraded elm xylan*
Anhydro-D-xylose	45.3	73.6	99	78.8	88.7	86.7	98.1
Anhydro-L-arabinose	22.9	12.2	trace	6.9	0	0	0
Anhydro-4-*O*-methyl-D-glucuronic acid	16.1	3.9	0	13.4	1.1	13.3	1.8
Anhydro-4-*O*-methyl-D-glucose	0	0	0	0	10.1	0	0
Other components	11.3[a]	5.8[b]	0	0	0	0	0
Viscosity[c]	0.42	1.13	0.45	0.69	1.20	0.96	0.85

[a] Anhydro-D-glucuronic acid. [b] A mixture of anhydro-D-glucose, anhydro-D-galactose, and anhydro-D-mannose. [c] $dl.g^{-1}$.

but it had an appreciable content of L-arabinofuranosyl residues, making it more branched than the elm xylan.

Other polysaccharides were employed as controls in this investigation and include a xylan prepared by acid hydrolysis from a commercial xylan[5], and unmodified commercial xylan from Pfanstiehl Chemical Company, a commercial amylose preparation (Superlose), and a commercial amylopectin (Ramalin) obtained from Stein, Hall & Co., Inc.

RESULTS AND DISCUSSION

Introductory experiments. — Previous experiments have indicated that aqueous solutions of polysaccharides in solutions of many salts besides calcium chloride react with iodine to yield a blue product[1-4]. An initial, qualitative survey showed that solutions of commercial xylan in aqueous calcium chloride, calcium nitrate, calcium iodide, magnesium chloride, barium chloride, strontium chloride, aluminum nitrate, and zinc chloride and reacted with iodine to form blue complexes. The xylan did not react with iodine in solutions of aluminum chloride, mercuric chloride, lead chloride, cupric chloride, cadmium chloride, cobalt chloride, ferric chloride, potassium chloride, or sodium iodide. These results indicate that the nature of the cation is a predominating (but not the only) factor in complex formation.

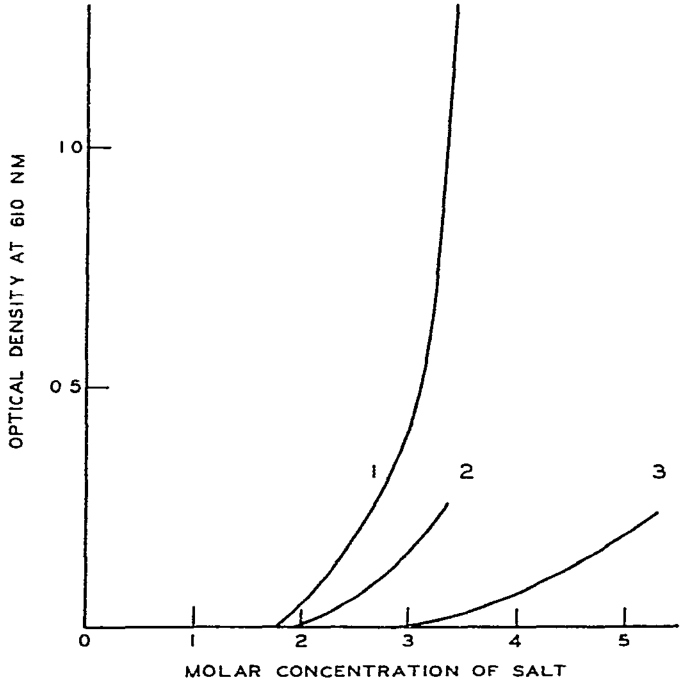

Fig. 1. Variation of optical density at 610 nm of colored iodine-complexes of commercial xylan (0 004%) in different concentrations of different aqueous divalent salt solutions with 0.05% I_2 and 0 21 KI. (1) Calcium chloride; (2) calcium nitrate; (3) magnesium chloride.

A quantitative measurement of the intensity of the color produced by the interaction of commercial xylan with iodine in various salt solutions such as calcium chloride, calcium nitrate, and magnesium chloride is given in Fig. 1. The results demonstrate that, at a given molar concentration, the extent of complex formation depends upon both the anion and cation components of the salt. The observed behavior of the calcium chloride, calcium nitrate, aluminum sulfate, and aluminum chloride solutions of the xylan may be rationalized as before by assuming that a particular conformation of the polysaccharide is necessary for reaction with iodine to occur. The maximum intensity of the reaction of xylan with iodine in Fig. 1 is limited by the solubility of the solvating salt in water, and no maximum intensity has yet been observed.

The data in Fig. 2 demonstrate that a linear relationship exists between the intensity of the color produced and the concentration of polysaccharide in solution in solutions of either calcium chloride or strontium chloride. In agreement with the previous data, the intensity of the reaction at a given concentration of polymer varies with the salt involved and was found always greater in calcium chloride solution than in other salt solutions.

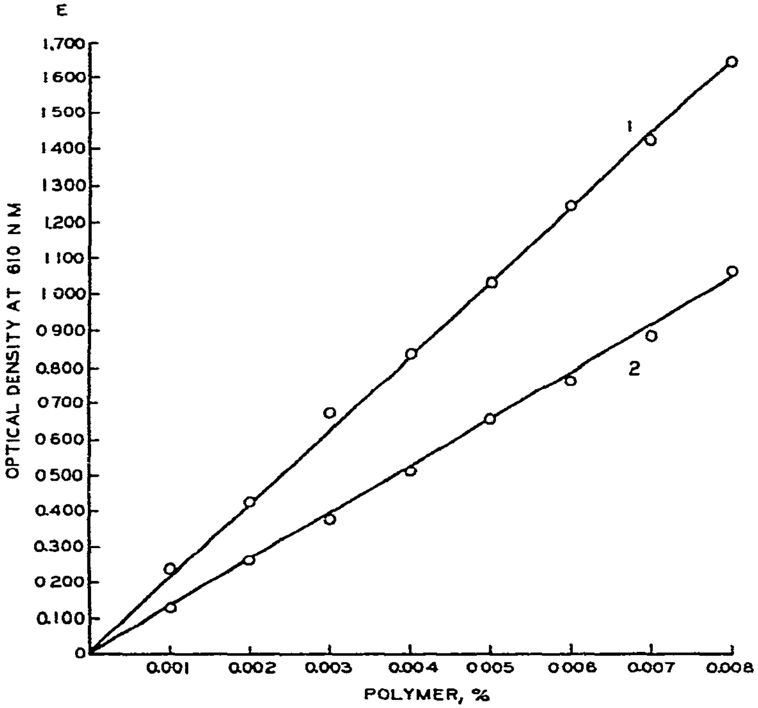

Fig. 2. Variation of optical density at 610 nm of colored iodine-complexes of elm xylan with increasing concentration of xylan, with 0.05% I_2 and 0.21 KI. (1) Aqueous calcium chloride solution (3.7M); (2) aqueous strontium chloride solution (3.7M).

The intensity of the color produced is dependent upon the nature and concentration of both the salt and the polysaccharide, and also as on the complicated equilibria existing between the complex and the various entities in solution. The existence of an intermediate complex, formed by the interaction of calcium ion and polysaccharide, suggested in the previous communication, and the observations made in this investigation support this proposal.

The intensity of the color produced by a given polysaccharide–salt system under controlled conditions is dependent upon the concentration of the potassium iodide in solution. This dependence is similar to that in formation of the starch–iodine complex, and is illustrated in Fig. 3 with reference to commercial xylan.

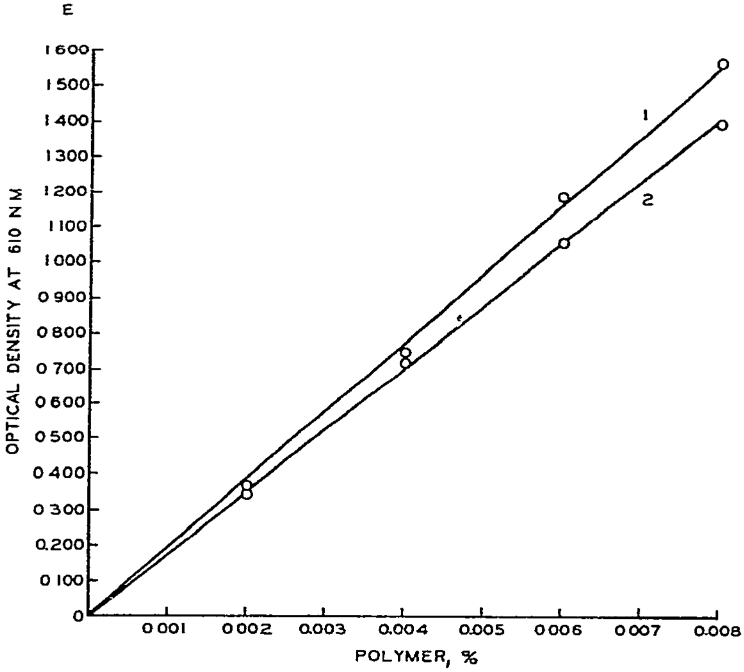

Fig. 3. Variation of optical density at 610 nm of colored iodine-complexes of commercial xylan with increasing concentration of xylan in 3.7M calcium chloride. (1) 0.05% I_2 and 0.21% KI; (2) 0.05% I_2 and 0.07% KI

The role of iodide ion in the amylose–iodine reaction has been the subject of much debate[17] and theories have been postulated showing that it may enhance the formation of a necessary iodide–iodine species[18,19] or that it may be necessary for the formation of a starch helix[20]. Since xylans in potassium iodide solution do not give a blue color with iodine, it is obvious that potassium iodide does not influence the conformation of xylan, but may enhance the formation of the iodide–potassium iodide species necessary for sorption or reaction with the polysaccharide, as has been suggested by others[17–19].

A comparison of the behavior of different xylans. — The change in absorption at 610 nm with changing composition of the polymer is plotted in Fig. 4 for each of the different xylans (Table I). The highly branched xylan from sapote gum did not react at all with iodine, and this behavior was similar to that of most other highly branched hexose polysaccharides, such as guaran, under these circumstances. The other, less-highly branched xylans, reacted with iodine to give the expected blue coloration. The elm xylan and the alkali-degraded elm xylan reacted almost identically, and gave at 610 nm the most intense absorptions observed. Since it had been expected that the unbranched, degraded xylan would have given a more intense reaction than that observed in Fig. 4, it must be assumed that the uronic acid groups of the undegraded elm xylan do not hinder formation of the complex. On the other hand, reduction of the uronic acid groups of this xylan to primary alcohol groups (producing a 4-*O*-methyl-D-gluco-D-xylan) decreased the extent to which the polysaccharide would react with iodine. The nature of the branch in this polysaccharide system has an important influence on the extent of reaction, and it can only be assumed at this point

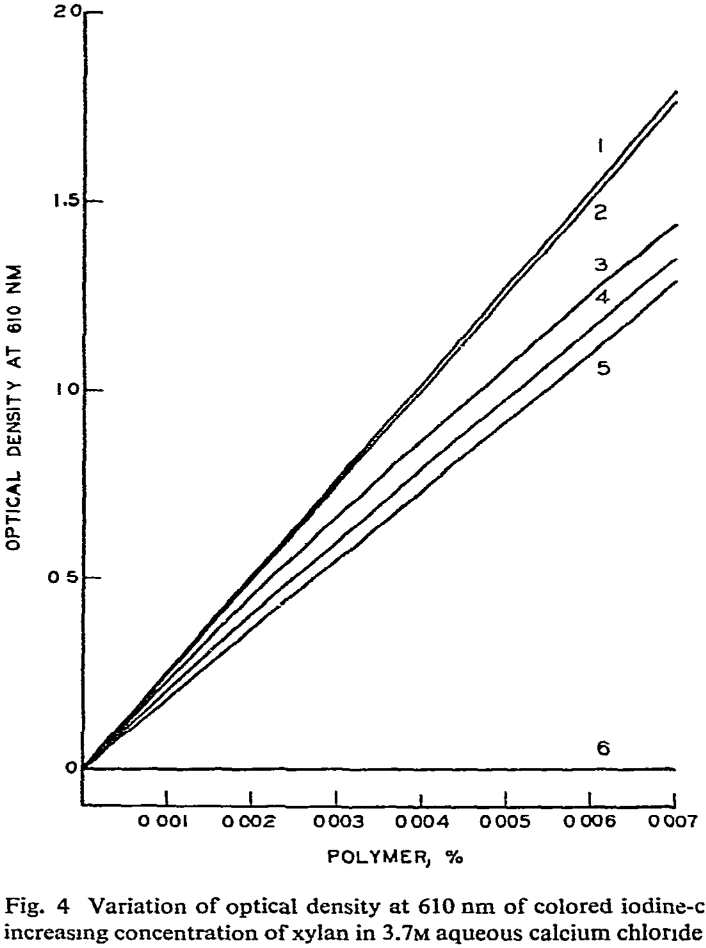

Fig. 4 Variation of optical density at 610 nm of colored iodine-complexes of different xylans with increasing concentration of xylan in 3.7M aqueous calcium chloride solution and 0.05% I_2 and 0 21% KI. (1) Elm xylan; (2) alkali-degraded elm xylan; (3) reduced elm xylan; (4) commercial xylan; (5) bamboo xylan; and (6) xylan isolated from sapote gum.

that the formation of the calcium salt of the uronic acid in some way counteracts the inhibitory action of the branch.

Potentiometric titration has been used as a sensitive tool for determining the degree of reaction with iodine in the case of amylose–iodine and amylopectin–iodine interactions[21]. The difference in oxidation potential of the sample and the control can be used to calculate an "iodine-binding capacity" that is characteristic of a given sample. The results of the potentiometric titration of the various xylans in calcium chloride are recorded in Table II. They show that the reduced elm xylan, even though it reacts with iodine under these circumstances to form a blue complex, does not react in such a manner as to change the oxidation potential of the system from that of the control. Thus, the behavior of this polymer is similar to the behavior of D-gluco-D-mannan, "amyloid", and polyvinyl alcohol, and it does not yield an "iodine-binding capacity" as a result of the titration. The alkali-degraded xylan, composed primarily of a linear, unbranched sequence of D-xylopyranosyl residues, does react with iodine, however, to produce a greater change in oxidation potential (and hence in "iodine-binding capacity") than the unmodified elm xylan control. Comparison of the behavior of these three closely related xylans demonstrates that the "iodine-binding capacities" are dependent upon the number and type of short terminal-branches that exist in the polymer.

TABLE II

A COMPARISON OF THE IODINE CONTENT OF VARIOUS "XYLAN"–IODINE COMPLEXES

Polysaccharide	*Iodine, %, found by:*		*Calcium content of precipitated complex* (%)
	Potentiometric titration[a]	*Analysis of precipitated complex*	
Acid-hydrolyzed xylan	6	21.9	
Commerical xylan	10	16.6	6.8
Bamboo xylan	4	14.5	
Elm xylan	7	19.3	
Diborane-reduced elm xylan	0	17.9	
Alkali-degraded elm xylan	12	18.5	18.2
Amylose	15.5[b]	23.5	11.5
Amylopectin	7.6[c]		

[a] Determined in aqueous calcium chloride solution. [b] 17.8% in water. [c] 9.6% in water.

When the concentration of iodine in solutions from the potentiometric determinations is increased, the iodine complex precipitates and it can be isolated by conventional techniques. After storage in vacuum in a desiccator on a porous plate until no water or free iodine is detected, the complexes from the xylans become dark-blue films that can be stored in the open in the laboratory for prolonged periods of time without obvious signs of decomposition. Analysis of these complexes (Table II) shows the presence of more iodine than can be accounted for by potentiometric titration. Some of the difference might be due to the greater proportion of iodine present in solution at the time of precipitation, and some is probably due to the presence in the complexes of iodine able to cause coloration but not able to affect the potentiometric titration. Although appreciable calcium ion was present in the isolated iodine complexes, as determined by flame spectrometry, X-ray diffraction techniques showed no crystalline component to be present. The lack of suitable manipulating and purifying techniques made it impossible to determine whether or not the calcium ion is actively associated with the complexes.

An attempt was made to determine whether or not the iodine in the complexes was firmly bound to the polymer in a definable ratio, as is the case with starch. The variation of optical density of the colored complex of the polymer at 610 nm was plotted against the relative percentage of iodine at different total (xylan+iodine) compositions. With starch, the maximum optical density always occurred at a constant composition, indicating a favored stoichiometry. Although the alkali-degraded elm xylan is a linear polymer, thus resembling starch, the data in Table III show that this xylan does not interact with iodine in a favored stoichiometric ratio. Instead, it behaves in a manner typical of the branched xylan.

TABLE III

THE COMPOSITION OF "XYLAN" AND IODINE AT WHICH MAXIMUM EXTINCTION COEFFICIENT OCCURS AT DIFFERENT TOTAL COMPOSITONS OF "XYLAN" AND IODINE

Percentage (polymer and iodine) of solution	*Relative percentage of iodine at which max extinction occurs*				
	Commercial xylan	*Bamboo xylan*	*Acid-hydrolyzed commercial xylan*	*Elm xylan*	*Reduced xylan*
0.01	60			70	
0.02		70	60	60	65
0.03	50	60	50		55
0.04		50			

On the basis of these results, it may be postulated that the stability of the iodine–xylan complexes is not due to bonding of the iodine to the polymer, since the existence of the color does not coincide with changes in oxidation potential ("iodine-binding capacity") of the polysaccharide–salt–iodine system. The important influence of structural arrangement of the components attached to the chain of D-xylopyranosyl residues suggests that a controlling factor in the formation and stability of the blue coloration with iodine is the stability of the intermediate complex that is able to react with iodine.

EXPERIMENTAL

Preparation of samples. — The amylose and amylopectin used were "Superlose" and "Ramalin", respectively (Stein, Hall & Co., Inc.). A commercial oat-hull xylan (Pfanstiehl Chemical Company) was employed as a control.

The highly branched sapote xylan used was isolated by the procedure already described[8]. The elmwood xylan was originally isolated for a study of the effect of alkali at elevated temperatures on polysaccharides[7]. A portion of the isolated polymer was acetylated and then reduced to a 4-*O*-methyl-D-gluco-D-xylan by a modification of the diborane technique described by Smith and Stephen[12] (made necessary in order to prevent reduction of a few of the *O*-acetyl groups of the polymer to *O*-ethyl groups). A xylan devoid of most of the uronic acid branches was also prepared from the unmodified elm xylan by heating it in 1.55M sodium hydroxide for 2 h at 140°. The analyses of these polysaccharides are given[7] in Table I.

Reagents and analytical solutions. — The various salt solutions were prepared from analytical-grade reagents and distilled water. The stock solutions of calcium chloride used for spectrophotometric and potentiometric experiments were prepared by making up 412.1 g of analytical-grade reagent in 1 liter of water to give a specific gravity of 1.3. Strontium chloride solutions of similar molality were also prepared. The stock solutions of iodine were prepared by dissolving 2.000 g of iodine and 8.3 g of potassium iodide in 100 ml of water, and dilutions were made to the concentrations required with stock solutions of calcium chloride or other salts. The stock solutions of polysaccharides were prepared by dissolving 100 to 500 mg of polymer in 5 ml of M sodium hydroxide, neutralizing with hydrochloric acid and adding the stock solution of salt to a volume of 100 ml. These solutions, like those previously described[4], were slightly viscous and did not precipitate or decompose on standing.

Hydrolysis and quantitative analysis of the polysaccharides was effected by the chromatographic method of Saeman, *et al.*[22], and the uronic acid contents were determined estimated by the technique of Whistler, *et al.*[23]. Methoxyl analyses were performed by conventional procedures[24] and iodine complexes were isolated and analyzed by the procedures described previously. The potentiometric and spectrophotometric determinations were identical with those used in the previous report[4].

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