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GEL CHROMATOGRAPHY OF (1→3), (1→4), AND MIXED-LINKAGE (1→3),(1→4)-β-D-GLUCO-OLIGOSACCHARIDES*

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ABSTRACT

Four series of D-gluco-oligosaccharides were chromatographed on Bio Gel P-2 with water and aqueous boric acid as eluents: β-(1→3), β-(1→4), a β-(1→4) series terminated on the reducing end by a β-(1→3), and a β-(1→4) series terminated on the non-reducing end by a β-(1→3) linkage. Each set gave a linear plot of $-\log K_{av}$ *vs* molecular weight. Although the elution volumes for each series followed a similar pattern, there were distinct differences between each group. A β-(1→3)-linked glucose residue had more effect on the elution volumes when on the reducing end of a β-(1→4)-chain than when on the non-reducing end. Borate decreased the elution volumes of the β-(1→3) series and the compounds of lower d.p. of the β-(1→4) series terminated on the reducing end by a β-(1→3)-linked glucose residue.

INTRODUCTION

Gel-filtration chromatography has proved to be very useful for separating oligosaccharides of different degrees of polymerization. The chromatography of glucose and various oligosaccharides[1,2], fructans[3] manno-oligosaccharides[4], and partial-hydrolysis products of amylose, gluco-oligosaccharides, pullulan, and starch[5] has been described in some detail. In addition, elution volumes and related constants have been reported for β-(1→3)-linked glucose oligosaccharides[6] and (1→4)-linked glucose oligosaccharides[6,7] on poly(acrylamide) gels. The elution volumes in water and in boric acid of β-linked glucose oligosaccharides joined by (1→3)-, (1→4)-, and mixed-linkage (1→3),(1→4)-β-D-glucosidic bonds are presented in this paper.

*In this paper, β-(1→3)-linked D-gluco-oligosaccharides are designated by an L followed by their degree of polymerization (d.p.), for example, L_2 = laminarabiose. β-(1→4)-linked oligosaccharide are designated by the letter C and their d.p., for example, cellobiose = C_2. The mixed-linkage compounds are designated by showing just the linkages. For example, *O*-β-laminarabiosyl-(1→4)-cellobiose would be indicated by $(1\rightarrow3)\text{-}(1\rightarrow4)_2$.

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EXPERIMENTAL

Gel filtration. — A column of Bio Gel P-2 (Bio-Rad Laboratories, Richmond California, Batch No. 11712) was prepared essentially as described in John *et al.*[1]. The exact details were presented previously[6]. A reference solution composed of glucose, sucrose, and raffinose was separated regularly to check the detector response. Dextran-40 was applied at intervals to determine the void volume.

Two column eluents were used, water and aqueous 0.1M boric acid. The water was deionized and degassed before use. When eluting with boric acid, each sample was made 0.2M in boric acid before applying to the column.

Elution volumes were calculated from the distance on the recorder paper between the point where the sample was applied and the peak of the elution profile as follows: The distance was divided by the recorder-paper speed (5 cm/h) and multiplied by the flow rate. The elution volumes were corrected for the dead volume in the system. Flow rates varied from 27.6 to 27.9 mL/h over the period covered by this work. The elution volumes for glucose when eluted with water varied from 74 to 79 mL. All elution volumes were normalized to the average for glucose (76.39 mL). The elution volumes for samples chromatographed in water represent the average of three or more determinations (except for one compound with two), which were typically obtained on different runs. All replicate values except three showed deviations from the mean of 0.61 mL or less. As the work progressed it was observed that the inclusion of an internal reference compound decreased the deviations. Thirteen replicates distributed over three compounds (C_2, C_3, and C_4) in which glucose was also present showed deviations from the mean of only ± 0.27 mL or less when normalized to the average value for glucose.

The elution volume of glucose in boric acid varied from 72 to 75 mL and again all elution volumes were normalized to the average for glucose (73.72 mL). All elution volumes in boric acid represent single determinations, except for laminarabiose and cellobiose.

The total bed-volume was 108.1 mL and the void volumes determined by using Dextran-40 were 25.97 mL in water and 28.60 mL in boric acid. K_{av} values were calculated from $K_{av} = (V_e - V_o)/(V_t - V_o)$, where V_e is the elution volume of the compound in question, V_o is the void volume, and V_t the total bed-volume.

Oligosaccharides. — β-(1→3)-Oligosaccharides were prepared by partial hydrolysis of laminaran in oxalic acid and separation of the products on carbon–Celite columns[8]. Six major peaks were obtained: D-glucose and L_2 through L_5. The L_2 through L_5 peaks were reseparated on carbon–Celite.

β-(1→4)-Oligosaccharides were prepared by partial hydrolysis of cellulose in fuming hydrochloric acid[8]. Again, the products were separated on carbon–Celite columns. The C_2 through C_6 peaks were reseparated on carbon–Celite.

β-(1→4)-Oligosaccharides terminated on the reducing end by a β-(1→3) linked glucose residue were prepared from an enzymic hydrolyzate of barley β-D-Glucan[9]. The hydrolyzate was separated on carbon–Celite.

TABLE 1

ELUTION VOLUMES

d.p.	H_2O					Boric acid				
	(1→3)	(1→4)	$(1\rightarrow4)_n$-(1→3)	(1→3)-$(1\rightarrow4)_n$	Glucan hydrolyzate	(1→3)	(1→4)	$(1\rightarrow4)_n$-(1→3)	(1→3)-$(1\rightarrow4)_n$	Glucan hydrolyzate
1					76.1					73.7
2	68.8	69.1			68.4	62.7	68.6			68.0
3	61.3	62.2	61.7	61.9	61.7	56.5	61.5	59.8	62.3	61.6
4	55.3	56.4	55.6	55.7	55.8	51.6	57.3	55.2	55.7	56.3
5	50.2	51.1	51.9	50.9[a]	51.3	48.7	52.1	51.3		52.0
6		46.9	47.5	47.0[a]	47.2		47.1	48.0		48.5
7					43.8					45.3
8					40.7					42.6
9					38.0					37.4
					29.7					29.4
	Glucose 76.4					73.7				
	Dextran-40 26.0					28.6				

[a]Even though these compounds showed only a single peak after carbon–Celite followed by Bio Gel P-2 separation, the results of experiments to sequence the linkages by alkaline degradation[6] indicate that these two compounds contain contaminants of d.p. 5 and d.p. 6, respectively[11].

β-(1→4)-Oligosaccharides terminated on the non-reducing end by a β-(1→3)-linked glucose residue were prepared by hydrolyzing barley β-D-glucan with an endo-(carboxymethyl)cellulase. The enzyme was prepared essentially as described by Moffa and Luchsinger[10]. The complete description of the preparation of the enzyme and the identification of the products is in preparation[11]. One portion of the hydrolyzate was subjected directly to gel filtration and is referred to as glucan hydrolyzate. A second portion was separated on carbon–Celite prior to gel filtration.

RESULTS AND DISCUSSION

The elution volumes for the four groups of oligosaccharides when subjected to gel filtration on Bio Gel P-2 are given in Table I. The $-\log K_{av}$ values for the compounds in Table I that were subjected to gel filtration in water are plotted against molecular weight in Fig. 1. The plots show a linear response of $-\log K_{av}$ to molecular weight through d.p. 7. There is a departure from linearity observed at d.p. 8 and 9. This departure may be caused by a greater interaction of the larger oligosaccharides with the gel matrix[7]. The plots for the (1→3) and (1→4) series originate at relatively similar $-\log K_{av}$ values and diverge as the d.p. values increase.

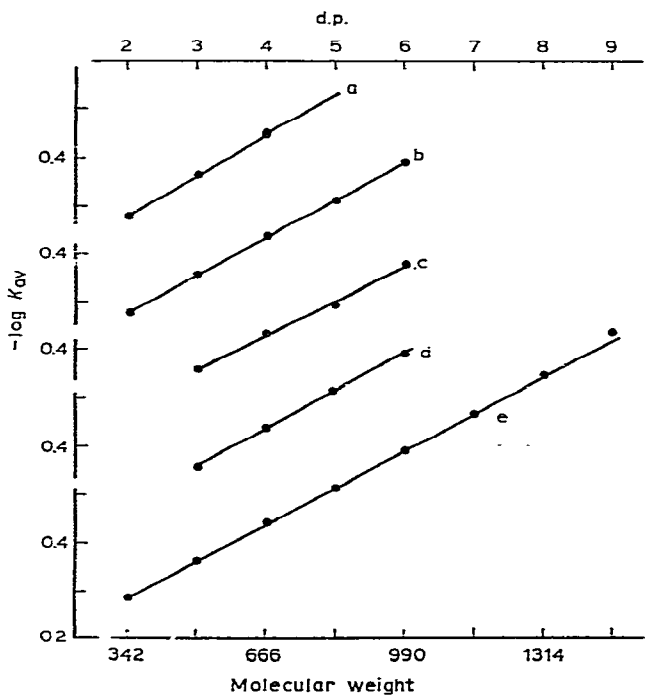

Fig. 1. Plots of $-\log K_{av}$ *vs* molecular weight of oligosaccharides separated in water: (*a*) (1→3) series, (*b*) (1→4) series, (*c*) $(1\rightarrow4)_n$-(1→3) series, (*d*) (1→3)-$(1\rightarrow4)_n$ series, and (*e*) Barley glucan hydrolyzed by endo(carboxymethyl)cellulase (glucan hydrolyzate).

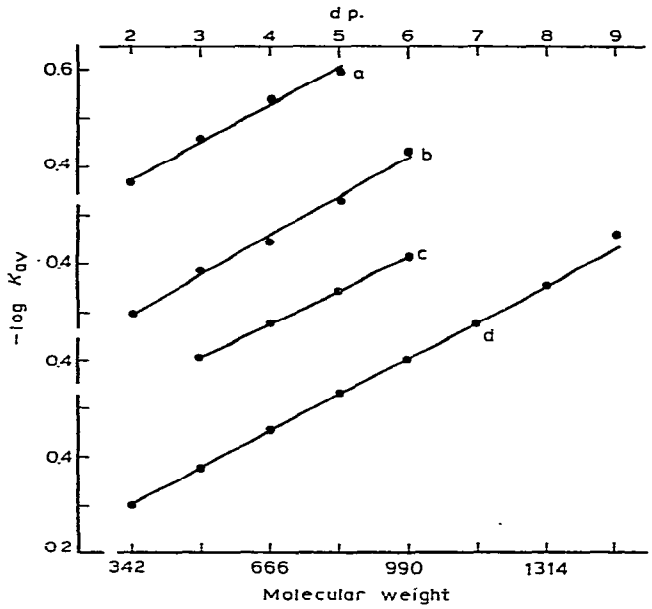

Fig. 2. Plots of $-\log K_{av}$ vs molecular weight of oligosaccharides separated in boric acid. (*a*) (1→3) series, (*b*) (1→4) series, (*c*) $(1\rightarrow4)_n$-(1→3), and (*d*) barley glucan hydrolyzed by endo-*O*-(carboxymethyl)cellulase (glucan hydrolyzate).

The $-\log K_{av}$ values for four of the series of compounds, when subjected to gel chromatography with borate solution, are plotted against molecular weight in Fig. 2. Again, there is a linear response of $-\log K_{av}$ to molecular weight for each series, with a deviation from linearity at d.p. 8 and 9.

Chromatography in borate was used because it is known that borate complexes (1→3)-linked oligosaccharides. Because of the manufacturers claim, it was assumed that the column is stable in boric acid. Even so, its elution volume suggests that there may be some interaction between boric acid and the column. The elution volume for boric acid chromatographed with water as a solvent is 87 mL, whereas it might have been expected to be essentially excluded, as is the case with several other inorganic compounds[6]. Chromatography in borate had little effect on the elution volumes of the β-(1→4), the $(1\rightarrow3)$-$(1\rightarrow4)_n$, and the compounds in the glucan hydrolyzate (Table I). Contrarily, there was a distinct decrease in the elution volumes of the β-(1→3) series, indicating that they were complexing with borate and thereby behaving as larger molecules. There also is an effect on the $(1\rightarrow4)_n$-(1→3) series which will be considered later.

The chromatographic results for the β-(1→3) and β-(1→4) series of compounds in water were compared with data on such compounds from other laboratories. The elution volumes showed very similar patterns, and a detailed account of the similarities and differences seems unnecessary.

The elution volumes (in water) of the lower-d.p. compounds of the $(1\rightarrow4)_n$-

TABLE II

STATISTICAL DATA FOR THE $(1\rightarrow4)_n$-(1→3) SERIES

d.p.	*3*	*4*	*5*	*6*
Elution volume (mL)	62.0	56.2	52.0	47.3
	61.3	55.8	51.9	46.8
	61.3	56.0	51.7	47.3
	62.1	54.4	52.0	48.7
			51.9	
			52.1	
$\bar{X}$	61.7	55.6	52.0	47.5
Standard deviation	0.3	0.8	0.2	0.8

TABLE III

SLOPES, INTERCEPTS, AND CORRELATION COEFFICIENTS FOR THE LINES PLOTTED IN FIGS. 1 AND 2

	H_2O			*Boric acid*		
	Intercept	*Slope* × 10^4	r	*Intercept*	*Slope* × 10^4	r
(1→3)	0.108	5.09	0.9999	0.209	4.80	0.9962
(1→4)	0.111	4.86	0.9998	0.123	5.03	0.9961
(1→3)-$(1\rightarrow4)_n$	0.120	4.79	0.9997			
$(1\rightarrow4)_n$-(1→3)	0.140	4.43	0.9979	0.191	4.26	0.9999
d.p. 3 and 5[a]	0.145	4.29				
d.p. 4 and 6[a]	0.156	4.29				

[a]Results obtained when the d.p. 3 and 5 compounds of the $(1\rightarrow4)_n$-(1→3) series are treated as one family and the d.p. 4 and 6 compounds as another family.

(1→3) series show a decrease in elution volume, and hence an increase in the relative sizes, as compared to the (1→4) series (Table I). On the other hand, the highest-d.p. compound of the $(1\rightarrow4)_n$-(1→3) series showed a decrease in the relative size as compared with the (1→4) series. The differences in the elution volumes are small and so a statistical analysis of the data was made (Table II). There is also an "alternating" scatter of the $-\log K_{av}$ values in the compounds of the series with a reducing terminal (1→3)-linked glucose that is not observed with the other compounds (Fig. 1a–e). It seems likely from these differences that the elution volumes are reflecting a structural response determined by whether the reducing-terminal (1→3)-linked glucose terminates an odd- or an even-numbered (1→4) chain. In addition, when the data are treated as two families, two lines are obtained that are 2.0 standard deviations apart (Table III). Because the differences are small and the d.p. 5 and 6 compounds may not be pure, it cannot be determined whether or not the $-\log K_{av}$ values for the d.p. 3 and 4 compounds of the (1→3)-$(1\rightarrow4)_n$ series are reflecting a similar structural effect caused by a (1→3) bond at the non-reducing end.

The slopes and intercepts (Table III) indicate that the β-(1→3) and β-(1→4) polymers started from similar "sized" molecules, and the β-(1→4) "fold" more compactly (or are more flexible) as the molecules grow. The $(1\rightarrow3)$-$(1\rightarrow4)_n$ compounds "start" perhaps a little larger and then generally resemble the β-(1→4) series as the molecules grow. The $(1\rightarrow4)_n$-(1→3) series started larger, but the presence of the reducing-terminal (1→3)-linked glucose residue apparently caused that series to form relatively more-compact molecules than the others as the d.p. increased. It is also apparent that the β-(1→3)-linked glucose on the reducing end of a (1→4) series shows greater ability to bind borate than when on the non-reducing end. The elution volume of (1→4)-(1→3) is 1.7 mL less than cellotriose, and $(1\rightarrow4)_2$-(1→3) is 2.1 mL less than cellotetraose (Table I). The effects of borate on the elution volumes disappeared as the d.p. increased. Contrarily, there appeared to be little or no complexing of borate with a (1→3)-linked glucose on the non-reducing end of the (1→4) series.

Although the effects of the structural differences on the elution volumes were small, the results support the concept that gels particularly designed for certain molecular sizes should prove to be useful tools for detecting structural changes. A practical advantage for such use is the simplicity of the procedures and the fact that difficultly prepared compounds may be readily recovered, often in a purer state than when applied to the gel.

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