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COMPARATIVE STUDIES OF MALTO- AND ISOMALTO-OLIGOSACCHARIDES AND THEIR CORRESPONDING ALDITOLS*†

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ABSTRACT

Gel-filtration chromatography (Bio-Gel P-4) has been used to separate series of homologous oligosaccharides and their corresponding alditols. The positions of the individual oligomers (d.p. 2–15), as expressed by their K_{av} values, have been used as a measure of their size in solution ("hydrodynamic volume"), using the malto-oligosaccharides as a common reference. In terms of the increase in the "hydrodynamic volume" generated by an α-D-(1 → 4)-linked glucose residue, we have obtained the following results: the conversion of a malto-oligosaccharide into the corresponding alditol leads to an increase by 0.8 of an α-D-(1 → 4)-linked glucose residue. An isomalto-oligosaccharide alditol is 0.5 of an α-D-(1 → 4)-linked glucose residue larger than the corresponding isomalto-oligosaccharide. Moreover, each additional α-D-(1 → 6)-linked glucose residue in an isomalto-oligosaccharide or its alditol seems to increase its "hydrodynamic volume" by 1.3 of an α-D-(1 → 4)-linked glucose residue. Viewed in terms of the addition of a D-glucitol group to an existing malto- or isomalto-oligosaccharide, the size in solution of the resultant alditol, for both series, increases by 1.8 of an α-D-(1 → 4)-linked glucose residue. Quantitative gel-filtration chromatography of reduced oligosaccharides is of importance in the structural analysis of "dextrins" and also permits the analysis of commercial, hydrogenated "glucose syrups".

INTRODUCTION

Quantitative gel-filtration chromatography[2] is a powerful method that permits the separation and determination of series of homologous oligosaccharides according to their molecular weight or d.p. (degree of polymerization). Although some isomeric oligosaccharides have quite different gel-filtration chromatographic properties[1,2], it is fortunate that the oligosaccharides in acid[3] and enzymic[4] hydrolyzates of starch can apparently be separated according to their molecular weights, although such

*Dedicated to Professor Dexter French on the occasion of his 60th birthday.
†Part II in the series: Gel Filtration Chromatography of Oligosaccharides. For Part I, see Ref. 1.

hydrolyzates contain several isomers[5]. In combination with the use of specific enzymes, we have favoured the use of gel-filtration chromatography in our studies on the structure of singly and multiply branched dextrins present in beer[6,7].

Structural analysis of branched dextrins and linear oligosaccharides containing mixed linkages frequently involves a parallel study of the reduced dextrin or oligosaccharide alditol. Analysis of the reaction products from partial acid-hydrolysis[8] or from the action of specific enzymes[9] may then provide important information that may not readily be obtained from the original branched dextrin or oligosaccharide. Paper chromatography appears to be a suitable method, which permits the separation and identification of oligosaccharide alditols. However, gel-filtration chromatography may also be a convenient and quantitative method for this purpose.

Accordingly, the present investigation was undertaken to study the gel-filtration chromatographic properties of series of homologous oligosaccharide alditols. We have examined the malto- and isomalto-oligosaccharide alditols and compared them to the parent malto- and isomalto-oligosaccharides (d.p. 2–15). In addition, as hydrogenated D-glucose syrups, such as Lycasin®, have become commercially available, an experimental sample of these starch-derived oligosaccharide alditols has been included in this study. A preliminary account of this study has been given elsewhere[10].

MATERIALS AND METHODS

Samples. — A. Malto-oligosaccharides, M_n-series. These were obtained by partial, acid hydrolysis of the α-D-(1 → 4)-linked glucan, amylose (Merck). To this hydrolyzate was added a purified specimen of maltopentaose; the increased amount of maltopentaose serves as a useful marker in the gel-filtration chromatographic pattern of this series of homologous, α-D-(1 → 4)-linked oligosaccharides composed of D-glucosyl residues. The weight-distribution pattern of the malto-oligosaccharides (maltose = M_2, maltotriose = M_3, ..., M_n) plus D-glucose (G) is shown in Fig. 1. Total carbohydrate "as glucose" was 22.4 mg/ml by the phenol–sulphuric acid method[11]. Reducing sugar "as glucose"[12,13] was 6.15 mg/ml, corresponding to 27.5% of the total carbohydrate compared to 26.4% obtained from the weight-distribution pattern.

B. Malto-oligosaccharide alditols, M_n-OH-series. These were obtained from the foregoing series of malto-oligosaccharides by reduction with sodium borohydride. The foregoing M_n-series (10 ml) was concentrated to 1 ml, and then sodium borohydride (50 mg) in water (1 ml) was added dropwise, essentially following the procedure outlined for the reduction of D-galactose to galactitol[14]. Finally the reduced malto-oligosaccharides were reconstituted to the original volume of 10 ml. Total carbohydrate "as glucose" was 16.0 mg/ml. Reducing sugar "as glucose" was 0.21 mg/ml, indicating that at least 96% of the available aldehyde groups has been converted into their corresponding alditols. The gel-filtration chromatographic

separation of the malto-oligosaccharide alditols (maltitol = M_2-OH, maltotriitol = M_3-OH, ..., M_n-OH) is shown in Fig. 2.

C. Isomalto-oligosaccharides, IM_n-series. These were prepared by partial, acid hydrolysis of the α-D-(1 → 6)-linked glucan, dextran (Pharmacia). The weight-distribution pattern shown in Fig. 4 represents isomaltopentaose (IM_5) and higher oligomers of the foregoing hydrolyzate to which isomaltotetraose (IM_4), isomaltotriose (IM_3), isomaltose (IM_2) (obtained by preparative, paper chromatography) and glucose (G) had been added. In this way, isomaltopentaose becomes a useful marker in the gel-filtration chromatographic pattern of this series of homologous, α-D-(1 → 6)-linked oligosaccharides composed of D-glucose. Total carbohydrate "as glucose" was 14.4 mg/ml. Reducing sugar "as glucose" was 4.33 mg/ml, corresponding to 30.1% of the total carbohydrate, as compared to 27.4% obtained from the weight-distribution pattern.

D. Isomalto-oligosaccharide alditols, IM_n-OH-series. A sample of the foregoing series of isomalto-oligosaccharides was sent to Mr. Steen Baggesen, Alfred Benzon Ltd., who kindly performed the reduction of these oligosaccharides to their corresponding alditols. The resultant syrup was diluted with water to 1.2 ml. Total carbohydrate "as glucose" was 2.44 mg/ml. Reducing sugar "as glucose" was 0.04 mg/ml, which indicates conversion of at least 96% of the available aldehyde groups. The gel-filtration chromatographic separation of the isomalto-oligosaccharide alditols (isomaltitol = IM_2-OH, isomaltotriitol = IM_3-OH, ..., IM_n-OH) is shown in Fig. 5.

E. Hydrogenated "glucose syrup". An experimental sample of a commercial, hydrogenated "glucose syrup" was chosen for this study, as it contained a wide range of oligosaccharide alditols derived from an acid-converted "glucose syrup". It was diluted with water to approximately 1% dry matter. This solution contained approximately 1.6 mg/ml of D-glucitol. Total carbohydrate "as glucose" was 6.70 mg/ml. Reducing sugar "as glucose" was nil. The gel-filtration chromatographic separation of this series of starch-derived oligosaccharide alditols (disaccharide alditols = 2, trisaccharide alditols = 3, and so on) is shown in Fig. 7.

Gel-filtration chromatography. — Bio-Gel P-4 (−400 mesh, control No. 41868) was obtained from Bio-Rad Laboratories. The Bio-Gel P-4 particles were further fractionated before use in order to obtain a narrower size-distribution. This was performed by air classification of the dry gel with a Multi-plex Zigzag Classifier (Alpine), in contrast to previous studies[1,7], where wet sieving was employed. Gel-filtration chromatography was performed as previously described[1] at 65°, with water as eluent. The parameters of the Bio-Gel P-4 column are given in Table I. The concentration of oligosaccharides in the eluate was determined by continuous recording of the absorbance at 420 nm, after colorimetric reaction with orcinol–sulphuric acid[2]. With respect to the oligosaccharide alditols in question, the colour response only reflects the intact D-glucose residues. D-Glucitol may be determined by using a refractive-index monitor (Laboratory Data Control, Model 1107L). Quantitation of the various oligosaccharides was obtained from their respective peak-areas assessed by computation by means of a System IV B Computing Integrator (Spectra-

TABLE I

PARAMETERS OF THE BIO-GEL P-4 COLUMN

Column packing	*Column parameters*					
	Height (mm)	V_t *(ml)*	V_0 *(ml)*	*Flow-rate (ml/h)*	*Temperature (°C)*	
Bio-Gel P-4[a]	1614	101.99	28.11	10.16[b]	65.0	
Components in external standard	*Elution parameters*					
	V_e *(ml)*	K_{av}	W *(ml)*	N	*HETP (mm)*	R_s
D-Glucose	84.88	0.768	2.71	15696	0.103	1.54
Maltose	80.70	0.712	2.71	14188	0.114	
Dextran T-150	28.11	0	2.68	1760	0.917	

[a]Bio-Gel P-4, (−400 mesh, Control No. 41868), further fractionated by air classification before use (see text). [b]The flow-rate, as well as the elution volumes, (V_e), including V_0, are expressed corresponding to the volume of water at 65°. Other symbols (V_t, V_0, V_e, K_{av}, W, N, HETP, and R_s) are as previously described (Ref. 1).

Physics). This instrument also records the retention time for the peak concentration of the individual oligosaccharides, which facilitates and increases the precision of the determination of V_e values and V_0, and accordingly of $K_{av} = (V_e - V_0)/(V_t - V_0)$. The K_{av} value appears to be a convenient index that specifies the position of a given component in the elution profile. Moreover, for series of homologous oligosaccharides, a straight-line relationship of $-\log K_{av}$ versus d.p. (degree of polymerization) is obtained, as previously described[1].

RESULTS

General. — The malto-oligosaccharides have been used as a common reference in our previous study of the gel-filtration chromatographic behaviour of various α-D-(1 → 4)- and/or α-D-(1 → 6)-linked oligosaccharides composed of D-glucose. This also applies to the present study, which relates the malto- and isomalto-oligosaccharide alditols to their parent oligosaccharides. To ensure that the separation characteristics of the column did not change with time from one separation to the next, the malto-oligosaccharides were analysed at the beginning and at the end of the present series of experiments.

The separation of the M_n-series is shown in Fig. 1. The elution parameters for the individual malto-oligosaccharides are given in Table II, as well as the results of linear-regression analysis of $-\log K_{av}$ versus d.p. as expressed by the slope, the intersection with the ordinate, and the correlation coefficient of the straight line.

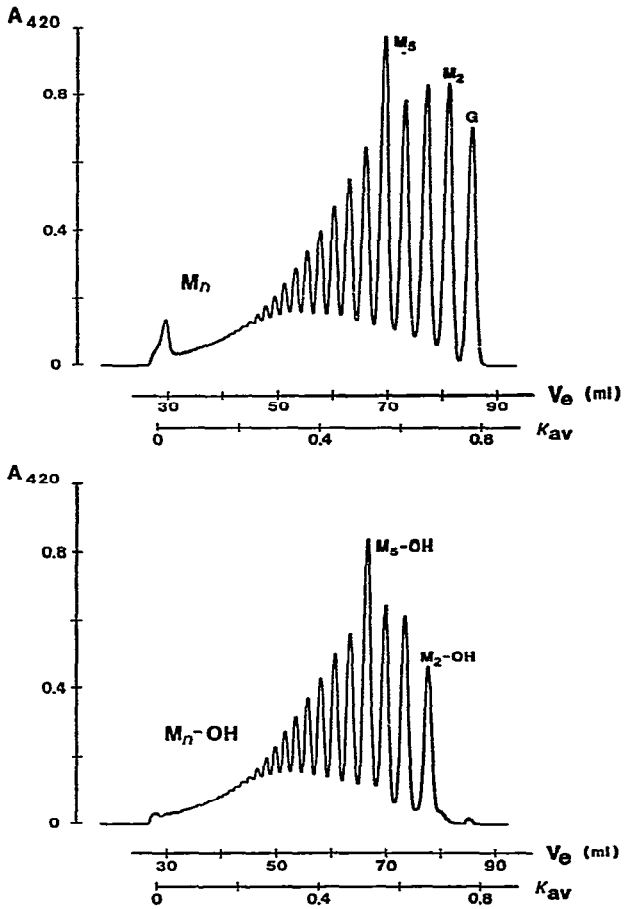

Fig. 1(upper). Elution profile of the malto-oligosaccharides, M_n-series. The sample was obtained by partial, acid hydrolysis of the α-D-(1 → 4)-linked glucan, amylose. The peaks indicated are: glucose (G), maltose (M_2), and maltopentaose (M_5). Sample volume: 16 μl. Conditions of separation: see Table I and text.

Fig. 2(lower). Elution profile of the malto-oligosaccharide alditols, M_n-OH-series. The sample was obtained from the sample in Fig. 1 by reduction with sodium borohydride. The peaks indicated are: maltitol (M_2-OH) and maltopentaitol (M_5-OH). Sample volume: 16 μl.

D-Glucose was not included as it does not contain the characteristic α-D-(1 → 4-) linkage of the M_n-series.

Similarly, the separation of the M_n-OH-series is shown in Fig. 2, and the $-\log K_{av}$ values for maltitol to maltopentadecaitol are given in Table III. As D-glucitol does not give any colour reaction with orcinol–sulphuric acid, the position of D-glucitol in the elution profile is not visible in Fig. 2. The tailing to the right of the maltitol peak probably arises from unreacted maltose, and the small peak to the very right is unreacted D-glucose. By means of a refractive-index monitor, we have been able to identify an additional component having the same position ($V_e \sim 82.0$

TABLE II

ELUTION PARAMETERS FOR THE MALTO-OLIGOSACCHARIDES AND LINEAR-REGRESSION ANALYSIS[a] OF $-\log K_{av}$ VERSUS D.P.

Component	V_e *(ml)*	K_{av}	$-log\ K_{av}$
(G)	(84.81)	(0.768)	(0.115)
M_2	80.55	0.710	0.149
M_3	76.63	0.657	0.183
M_4	72.71	0.604	0.219
M_5	68.98	0.553	0.257
M_6	65.60	0.508	0.295
M_7	62.58	0.467	0.331
M_8	59.82	0.429	0.367
M_9	57.25	0.394	0.404
M_{10}	54.93	0.363	0.440
M_{11}	52.79	0.334	0.476
M_{12}	50.82	0.307	0.512
M_{13}	49.01	0.283	0.548
M_{14}	47.37	0.261	0.584
M_{15}	45.91	0.241	0.618

[a]Linear-regression analysis: d.p.-range 2–15, slope 0.0363, intersection 0.0758, and correlation coefficient 1.0000.

TABLE III

VALUES OF $-\log K_{av}$ FOR THE M_n-OH-SERIES, THE IM_n-SERIES, THE IM_n-OH-SERIES, AND THE HYDROGENATED GLUCOSE SYRUP, AND LINEAR-REGRESSION ANALYSIS[a] OF $-\log K_{av}$ VERSUS D.P.

D.p.	M_n-*OH* $-log\ K_{av}$	IM_n $-log\ K_{av}$	IM_n-*OH* $-log\ K_{av}$	*Hydr. G-syrup* $-log\ K_{av}$
(1)	(0.115)	(0.114)	—	—
2	0.177	0.164	0.184	0.175
3	0.216	0.214	0.233	0.214
4	0.251	0.263	0.282	0.250
5	0.288	0.312	0.330	0.287
6	0.325	0.360	0.378	0.323
7	0.360	0.407	0.425	0.359
8	0.397	0.454	0.471	0.395
9	0.433	0.501	0.518	0.432
10	0.469	0.547	0.565	0.467
11	0.504	0.593	0.610	0.502
12	0.539	0.639	0.655	0.538
13	0.575	0.683	0.701	0.573
14	0.610	0.728	0.747	0.608
15	0.645	0.773	0.792	0.642
[a]Linear-regression analysis:				
D.p. range	2–15	2–15	2–15	2–15
Slope	0.0359	0.0468	0.0467	0.0359
Intersection	0.1081	0.0768	0.0956	0.1068
Correlation coefficient	1.0000	0.9999	0.9999	0.9999

ml) in the elution profile as an authentic sample of D-glucitol, having $-\log K_{av} = 0.138$.

Comparison of the M_n-OH-series to the M_n-series. — Inspection of the elution profiles and the data in Tables II and III for the malto-oligosaccharides and the reduced malto-oligosaccharides reveals that not only do the peak heights differ (as expected), but the position of malto-oligosaccharide alditols is shifted towards higher molecular weight in a manner not expected from the minute increase in molecular weight arising from hydrogenation of the malto-oligosaccharides. We shall deal with these two aspects separately.

First of all, the data agreed well with the anticipated colour-response for an oligosaccharide alditol (such as M_n-OH) being $(n - 1)/n$ times that of the corresponding oligosaccharide, for example M_n, where n is the number of monosaccharide residues (d.p.).

Secondly, when the $-\log K_{av}$ values for the M_n-series and M_n-OH-series are depicted in a plot versus d.p., as shown in Fig. 3, it is apparent that the malto-oligosaccharide alditols also give a straight-line relationship, as has previously been reported[1] for series of homologous oligosaccharides. The two straight lines in Fig. 3 have almost the same slopes, and there appears to be an almost constant increment from the M_n-series to the M_n-OH-series of $\Delta -\log K_{av} \sim 0.029$. Compared with the average increment in $-\log K_{av}$ value of ~ 0.036 in going from one member in the M_n-series to the next higher member in this series of α-D-(1 → 4)-linked oligosaccharides composed of D-glucose, this means that the malto-oligosaccharide alditols are positioned in the elution profile as if they were displaced to the left by 0.80 of a D-glucose residue as compared with the corresponding malto-oligosaccharide.

Comparison of the IM_n-OH-series with the IM_n-series. — The separations obtained for the isomalto-oligosaccharides and their corresponding alditols are

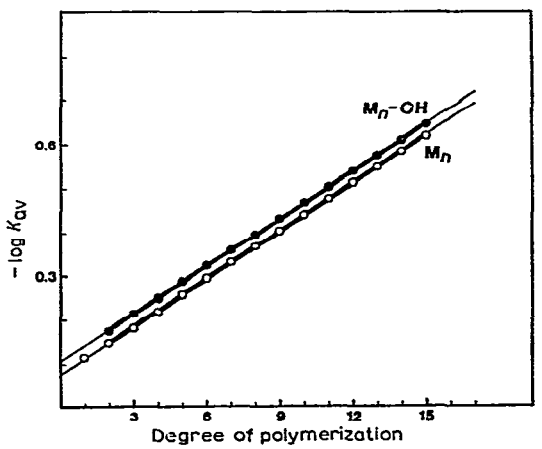

Fig. 3. Values of $-\log K_{av}$ versus d.p. for the M_n- and M_n-OH-series. The plots give values of $-\log K_{av}$ versus d.p. for the malto-oligosaccharides and their corresponding alditols, and the two straight lines were obtained by linear-regression analysis (d.p. 2–15).

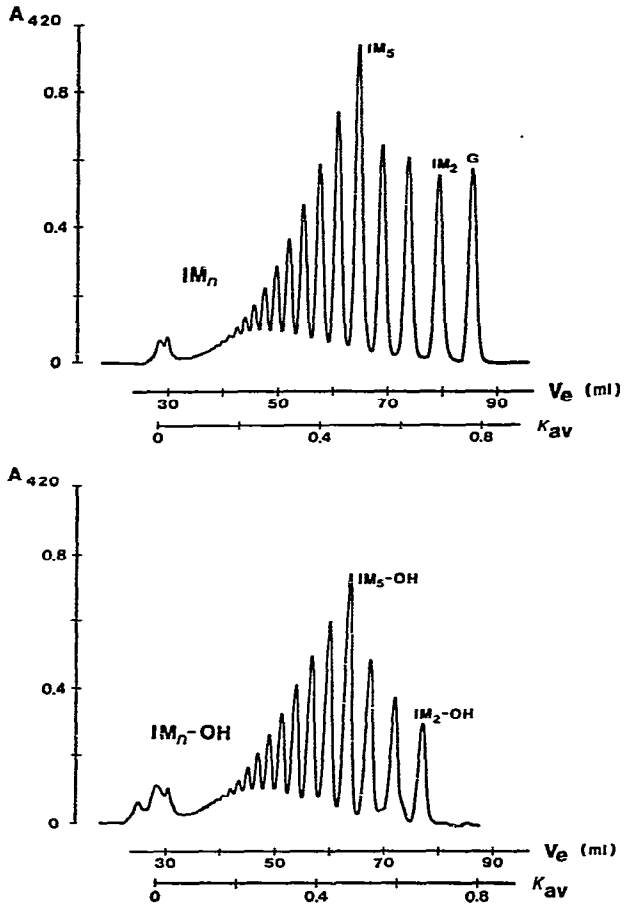

Fig. 4.(upper). Elution profile of the isomalto-oligosaccharides, IM_n-series. The sample was obtained by partial, acid hydrolysis of the α-D-(1 → 6)-linked glucan, dextran. The peaks indicated are: glucose (G), isomaltose (IM_2), and isomaltopentaose (IM_5). Sample volume: 18 μl.

Fig. 5(lower). Elution profile of the isomalto-oligosaccharide alditols, IM_n-OH-series. The sample was obtained by reduction of the samples described in Fig. 4. The peaks indicated are: isomaltitol (IM_2-OH) and isomaltopentaitol (IM_5-OH). Sample volume: 90 μl.

shown in Fig. 4 and Fig. 5. The $-\log K_{av}$ values for the individual components in these two series are listed in Table III. The linear-regression analysis shows that the isomalto-oligosaccharide alditols also give a straight line in a plot of $-\log K_{av}$ versus d.p. From Table III and as depicted in Fig. 6, it is seen that the two straight lines for the IM_n- and the IM_n-OH-series are parallel. This means that there is an almost constant increment in the $-\log K_{av}$ value from one member in the IM_n-series to the next higher member of Δ $-\log K_{av}$ ~0.047, and similarly for the IM_n-OH-series, Δ $-\log K_{av}$ ~0.047. However, the $-\log K_{av}$ value for any given isomalto-oligosaccharide alditol is larger than the $-\log K_{av}$ value for its parent isomalto-oligosaccharide, corresponding to Δ $-\log K_{av}$ ~0.018.

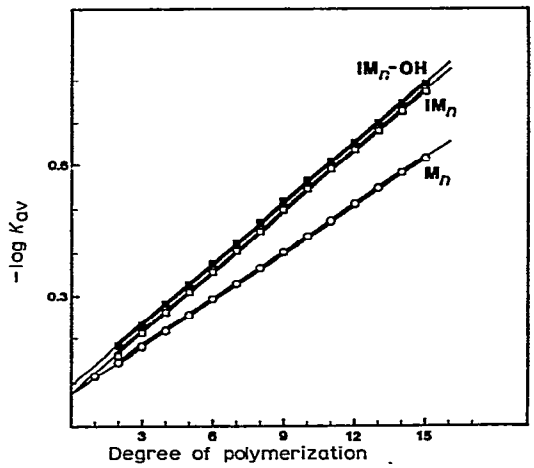

Fig. 6. Values of $-\log K_{av}$ versus d.p. for the IM_n- and IM_n-OH-series. The plots give values of $-\log K_{av}$ versus d.p. for the isomalto-oligosaccharides and their corresponding alditols, and the two straight lines were obtained by linear-regression analysis (d.p. 2–15). The straight line for the malto-oligosaccharides from Fig. 3 is included for comparison.

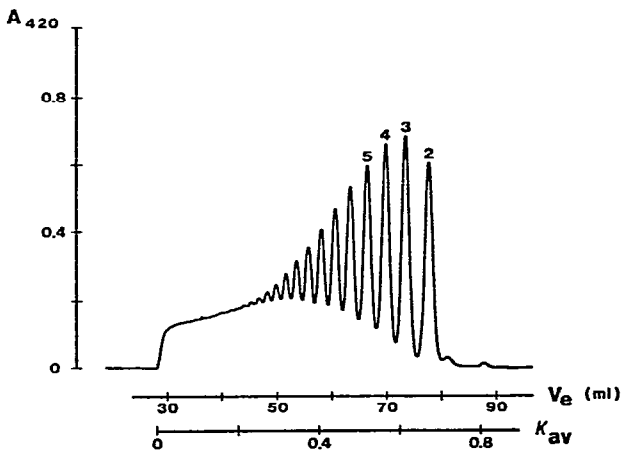

Fig. 7. Elution profile of a hydrogenated "glucose syrup". The sample is an experimental one, the parent glucose syrup being a partial, acid-hydrolyzate of starch. The peaks indicated are: disaccharide alditols (2), trisaccharide alditols (3), tetrasaccharide alditols (4), and pentasaccharide alditols (5). The sample volume was 45 μl.

Gel-filtration chromatography of a hydrogenated "glucose syrup". — The oligosaccharides present in an acid-converted "glucose syrup" consist of several isomers composed of D-glucose residues, linked either α-D-(1 → 4) only (malto-oligosaccharides), or containing one or more α-D-(1 → 6) linkages in addition to the α-D-(1 → 4) linkages. Accordingly, a hydrogenated "glucose syrup" contains several isomeric oligosaccharide alditols. The separation of one such hydrogenated "glucose syrup" is shown in Fig. 7, and the $-\log K_{av}$ values for the individual peaks are given in Table III.

As previously shown[1] for the starch-derived oligosaccharides having $-\log K_{av}$ values close to those for the exclusively α-D-(1 → 4)-linked malto-oligosaccharides, the starch-derived oligosaccharide alditols also have $-\log K_{av}$ values close to those for the reduced malto-oligosaccharides. This means that the oligosaccharide alditols in commercial, hydrogenated glucose syrups, for example Lycasin®, may be analysed by gel-filtration chromatography. It should, however, be borne in mind that the oligosaccharide alditols in these hydrogenated "glucose syrups" are displaced by approximately 0.8 of an α-D-(1 → 4)-linked glucose residue as compared with the oligosaccharides present in traditional glucose syrups.

DISCUSSION

The present study concerns the gel-filtration chromatographic properties of oligosaccharide alditols composed of D-glucose, linked α-D-(1 → 4) and/or α-D-(1 → 6). The study encompassed 14 malto-oligosaccharide alditols (maltitol–maltopentadecaitol) and 14 isomalto-oligosaccharide alditols (isomaltitol–isomaltopentadecaitol), as well as starch-derived oligosaccharide alditols present in a hydrogenated "glucose syrup". The position in the elution profile of these oligosaccharide alditols has been determined and expressed by their K_{av} value or, rather, $-\log K_{av}$ value.

It follows that, for a series of homologous oligosaccharide alditols, a plot of $-\log K_{av}$ versus d.p. gives a straight line. Comparison of isomeric oligosaccharide alditols shows that these are not necessarily positioned at the same place in the elution profile and the observed $-\log K_{av}$ values may be related to their structure.

Correlation between the structure of the oligosaccharide alditols and their $-\log K_{av}$ *values.* — For a given series of homologous oligosaccharide alditols, there is an almost constant increase in the $-\log K_{av}$ value for the reduced oligosaccharide as compared with the corresponding oligosaccharide. This increase seems to depend on the structure of the parent oligosaccharide. With respect to the malto-oligosaccharides, containing exclusively α-D-(1 → 4)-linked glucose residues, it amounts to $\Delta -\log K_{av} \sim 0.029$, whereas for the isomalto-oligosaccharides [exclusively α-D-(1 → 6)-linked glucose residues], it appears to be less, corresponding to $\Delta -\log K_{av} \sim 0.018$. Although we did anticipate a possible effect on the $-\log K_{av}$ value with respect to the carbon atom (for instance, C-4 or C-6) through which the D-glucitol group was linked to the remainder of the molecule, the foregoing difference did surprise us at first. The following may offer an explanation, which implies that the difference is less than the face values indicate.

The linear relationship of the $-\log K_{av}$ values versus d.p. for the M_n- and the IM_n-series indicates that $\Delta -\log K_{av} = 0.036$ per α-D-(1 → 4)-linked glucose residue and $\Delta -\log K_{av} = 0.047$ per α-D-(1 → 6)-linked glucose residue. As the transformation of, for example, M_n to M_n-OH ($\Delta -\log K_{av} = 0.029$) may be viewed as a removal of an α-D-(1 → 4)-linked glucose residue ($\Delta -\log K_{av} = -0.036$) and the subsequent addition of a D-glucitol group, the contribution of the latter should be $\Delta -\log K_{av} = 0.065$ to meet the experimental data. Similarly, the transformation of IM_n to IM_n-OH

($\Delta -\log K_{av} = 0.018$) may be viewed as the removal of an α-D-(1 → 6)-linked glucose residue ($\Delta -\log K_{av} = -0.047$) and, accordingly, the contribution of the addition of the D-glucitol group needs to be $\Delta -\log K_{av} = 0.065$.

Comments on the size or "hydrodynamic volume" of oligosaccharides in solution. — If we assume that the position in the elution profile of a given oligosaccharide alditol reflects its size in solution, gel-filtration chromatography offers a means of determining the size or "hydrodynamic volume" of oligosaccharide alditols, at least relative to other oligosaccharide alditols or oligosaccharides. We wish to emphasize that all separations have been performed in water at 65°, and that the size of the molecules in solution determined in this way refers to these conditions, and does not necessarily imply that the same relations hold at, for instance, room temperature or in buffered solutions. The "hydrodynamic volume" so determined may also differ from the size that might be derived from X-ray crystallographic data.

We have used the malto-oligosaccharides as the common reference for the various oligosaccharides included in this study. Based on the $-\log K_{av}$ values for the various oligosaccharide alditols, we have calculated their size relative to the malto-oligosaccharides. With respect to oligosaccharide alditols, for which the observed $-\log K_{av}$ values fall within those for maltose and maltopentadecaose, we have used the observed $-\log K_{av}$ value for the oligosaccharide alditols in question as well as for the malto-oligosaccharides. Whenever the observed $-\log K_{av}$ values exceeded that for M_{15} (this applies to M_{15}-OH, IM_{12}–IM_{15}, and IM_{12}-OH–IM_{15}-OH) the relative size has been determined by using the straight-line relationship of $-\log K_{av}$ versus d.p. for these series as well as for the M_n-series. The values for the relative size of the various oligosaccharide alditols are given in Table IV.

TABLE IV

SIZE OF OLIGOSACCHARIDES AND THEIR ALDITOLS: THE SIZE, OR "HYDRODYNAMIC VOLUME", OF THESE COMPONENTS AS DETERMINED BY GEL-FILTRATION CHROMATOGRAPHY ON BIO-GEL P-4 IN WATER AT 65°, RELATIVE TO THE MALTO-OLIGOSACCHARIDES

D.p.	*Hydrogenated glucose syrup*	M_n	M_n-*OH*	IM_n	IM_n-*OH*
1					
2	2.77	2.00	2.83	2.44	3.05
3	3.85	3.00	3.91	3.86	4.37
4	4.81	4.00	4.85	5.16	5.68
5	5.79	5.00	5.83	6.48	6.96
6	6.79	6.00	6.82	7.80	8.28
7	7.78	7.00	7.81	9.09	9.58
8	8.76	8.00	8.80	10.40	10.87
9	9.76	9.00	9.80	11.68	12.16
10	10.74	10.00	10.79	12.97	13.46
11	11.71	11.00	11.76	14.26	14.77
12	12.70	12.00	12.74	15.55	15.99
13	13.70	13.00	13.75	16.75	17.24
14	14.69	14.00	14.76	18.04	18.52
15	15.63	15.00	15.72	19.32	19.80

In our previous study[1], we determined the relative size of cyclohexa- and cycloheptaamyloses and 38 structurally distinct, linear, α-D-(1 → 4), α-D-(1 → 6)-linked oligosaccharides (d.p. 3–27) similarly composed of D-glucose, as well as the 14 α-D-(1 → 6)-linked isomalto-oligosaccharides, also included in this study. We used the malto-oligosaccharides (maltose–maltopentadecaose) as the common reference. The relative sizes of the isomalto-oligosaccharides given in Table IV agree well with the values previously reported. This may reflect the essential similarity of the experimental conditions (the gel being prepared from the same batch of Bio-Gel P-4, and so on), and yet the two sets of experiments were separated in time by more than two years. Based on the assumption that the position in the elution profile reflects the size in solution of a given oligosaccharide or oligosaccharide alditol, we have, as part of the present study, listed the "hydrodynamic volume" of 28 oligosaccharide alditols relative to the malto-oligosaccharides. As an example, maltoheptaitol is 0.8 of an α-D-(1 → 4)-linked glucose residue larger than maltoheptaose. With respect to the isomalto-oligosaccharides, each additional α-D-(1 → 6)-linked glucose residue seems to increase the "hydrodynamic volume" by 1.3 of an α-D-(1 → 4)-linked glucose residue, whereas reduction to the corresponding isomalto-oligosaccharide alditol leads to a further increase by 0.5 of an α-D-(1 → 4)-linked glucose residue. As may be seen from Table IV (for instance, M_7-OH − M_6 = 7.81 − 6.00 = 1.81 and IM_7-OH − IM_6 = 9.58 − 7.80 = 1.78), the hypothetical addition of a D-glucitol group to an oligosaccharide increases the size by 1.8 of an α-D-(1 → 4)-linked glucose residue, apparently regardless of the linkage point (C-4 or C-6) of the D-glucitol group. It may be relevant to mention that D-glucitol itself occupies a position in the elution profile between those of D-glucose and maltose, its relative size being 1.68.

In order to relate the relative sizes observed to the structure of the oligosaccharides and oligosaccharide alditols, model building has been attempted and this seems to indicate compatibility. Computerized conformational-statistics have not been performed, but would certainly be of interest in the present context.

Gel-filtration chromatography is frequently defined as a method of separating molecules according to their size in solution. If so, it offers a means of determining the relative sizes of oligosaccharides and oligosaccharide alditols (Table IV). Independent, experimental values may be obtained by measuring other hydrodynamic properties, for instance, as indicated for the cello-oligosaccharide alditols[15] by determination of diffusion rates, friction coefficients, and Einstein–Stokes hydrodynamic radii.

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