STEREOSELECTIVITY IN THE ELUTION OF SUGARS FROM COLUMNS OF SEPHADEX G-15

NOELIE R. BERTONIERE, LAWRENCE F. MARTIN, AND STANLEY P. ROWLAND

Southern Regional Research Laboratory, Southern Marketing and Nutrition Research Division, Agricultural Research Service, U. S. Department of Agriculture, New Orleans, Louisiana 70119 (U. S. A.)

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ABSTRACT

The gel-permeation characteristics of selected sugars and sugar derivatives were determined on a column of Sephadex G-15*. The following observations were made: (*1*) the Sephadex G-15 column does not distinguish between enantiomeric saccharides, (*2*) a decrease in R_g value is observed on going from a methylene (deoxy) to a hydroxyl to a methoxyl group in monosaccharides, (*3*) methylation or reduction of a particular hydroxyl group affects the R_g values selectively, (*4*) the substituted (methyl or glucosyl) α anomer is retained on the column longer than the corresponding β anomer, and (*5*) sugars having either one or no axially attached hydroxyl group are eluted in the order (increasing R_g value): axially attached hydroxyl groups at C-4, at no carbon atom, at C-2, and at C-3.

INTRODUCTION

In preceding studies[1–5], the internal-pore structures of cotton celluloses and chemically modified celluloses have been characterized by inverse, gel-permeation chromatography. For this purpose, a series of mono-, di-, tri-, and tetra-saccharides were employed as the solutes, and the ball-milled or chopped fibrous celluloses constituted the "gel phase" in the column. Recent explorations have involved examination of the elution characteristics of a variety of types of solute. In the course of this study, it was observed that, at a given molecular weight, variations in the stereochemistry of mono- and di-saccharides resulted in significant differences in the elution volumes. Marsden[6], who employed columns of dextran gel crosslinked by divinyl sulfone instead of Sephadex crosslinked by means of epichlorhydrin, reported similar results for a variety of sugars. He ascribed the differences of elution volumes for isomeric sugars to their relative conformational stabilities; this may be a factor, but comparative measurements that we have made with other solutes[7] suggest that adsorption is a factor with the sugars and sugar derivatives now discussed. The results reported here are for studies conducted on columns of Sephadex G-15, as this material is substantially more stable than fibrous cotton cellulose in a column.

*Mention of a company or product by the U. S. Department of Agriculture does not imply approval or recommendation of the company or product to the exclusion of others that may also be suitable.

RESULTS AND DISCUSSION

Optically active adsorbents in chromatography have been shown to adsorb one enantiomer preferentially[8]. Sephadex G-15 is a highly crosslinked dextran, a polymer having a skeleton of α-D-(1→6) linked D-glucose residues. As both the dextran column and the compounds employed as test solutes are optically active, the elution characteristics of five enantiomeric pairs (four aldoses and one alditol) were examined. The results are given in Table I, from which it was concluded that the Sephadex G-15

TABLE I

EFFECT OF OPTICAL ISOMERISM ON THE RETENTIONS ON SEPHADEX G-15 OF ALDOSES AND AN ALDITOL RELATIVE TO THAT OF D-GLUCOSE

Compound	R_g	
	D	L
Xylose	1.047	1.047
Arabinose	1.022	1.024
Mannose	1.029	1.031
Glucose	1.000	1.000
Arabinitol	1.007	1.005

column does *not* distinguish between enantiomers. Such comparisons as those between D-mannose and L-rhamnose (6-deoxy-L-mannose) were therefore considered valid and appropriate.

The gel-permeation mechanism is complicated by sorption of solutes to the dextran gel[7]. The effects of methylation, and of replacement by hydrogen (reduction), of specific hydroxyl groups were explored in this regard. However, such alterations in the structure of a sugar result in changes in size and shape, as well as in changes in hydrogen-bonding (sorption) capacity. Replacement of a hydroxyl group by a methoxyl group should significantly lessen the sorption; however, this methoxyl group simultaneously increases the size of the molecule. Both effects will lower the observed R_g values, and if simultaneously operative, determination of the individual contributions to the overall effect will then not be possible. Replacement of a specific hydroxyl group by hydrogen (to form a deoxy sugar), or removal of the hydroxymethyl substituent at C-5 to form an aldopentopyranose, eliminates hydrogen bonding completely at that site, while simultaneously decreasing the molecular size. These changes will have *opposite* effects on the R_g values. The results obtained with several sugars, some methylated and some reduced at specific hydroxyl groups, and some lacking a hydroxymethyl group at C-5 (aldopentopyranoses), are summarized in Table II.

The deoxy sugars and the aldopentopyranoses, for which decreased sorption and smaller size will have opposite effects in gel permeation, are retained to a greater extent than the corresponding aldohexose. Thus, elimination of hydrogen bonding

TABLE II

EFFECT OF STRUCTURAL MODIFICATIONS OF MONOSACCHARIDES ON THEIR RELATIVE RETENTIONS ON SEPHADEX G-15

Monosaccharide	*Corresponding aldopentopyranose*[a]	*Substituent at indicated carbon atom*		
		H[b]	*OH*	*OMe*
		Carbon-2		
Ribose		1.086	1.063	
Glucose		1.042	1.000	0.974
Galactose		1.029	0.990	
		Carbon-3		
Glucose			1.000	0.977
		Carbon-6		
Mannose	1.062	1.040[c]	1.029	
Glucose	1.047		1.000	0.957
Galactose	1.024	0.997[c]	0.990	

[a]Hydroxymethyl substituent on C-5 of the aldohexose replaced by H. [b]Deoxy sugars. [c]Measurement made with the L monosaccharide.

at only one site is insufficient to overcome the accompanying changes in size and shape. The smaller size of hydrogen than that of a *C*-methyl group on C-5 is reflected by the higher R_g values obtained with the aldopentopyranoses relative to those of the 6-deoxy-aldohexoses.

The expected decreases in R_g values relative to those of unmethylated sugar are observed with the methylated D-glucoses. The effect of methylation at specific hydroxyl groups is approximately additive for polymethylated D-glucoses. Using values of $1-R_g$, calculations for the 2,3-di- and 2,3,4,6-tetra-*O*-methyl derivatives are given as examples in Table III. In the first example, the sum of the effects caused

TABLE III

ADDITIVITY OF THE EFFECTS OF METHYLATION OF SPECIFIC HYDROXYL GROUPS ON ELUTION FROM SEPHADEX G-15

Methyl α-D-glucopyranoside, methylated at the hydroxyl group on carbon atoms		$1-R_g$	*D-Glucose, methylated at the hydroxyl group on carbon atoms*	$1-R_g$
2 and 3		0.080	2	0.026
None		0.029	3	0.023
	Difference	0.051	Sum	0.049
2, 3, 4, and 6		0.125	2, 3, 4, and 6	0.088
None		0.029	None	0.000
	Difference	0.096	Sum or difference	0.088

by individually methylating the hydroxyl groups at C-2 and C-3 of D-glucose is compared with the effect of 2,3-di-*O*-methylation of methyl α-D-glucopyranoside. The second example compares the effects of methylation of the 2-, 3-, 4-, and 6-hydroxyl groups of methyl α-D-glucopyranoside and the corresponding tetra-*O*-methylation of D-glucose.

Methylation or reduction of particular hydroxyl groups affects the R_g values selectively (see Table II). Retention is decreased about twice as much for the 6-*O*-methyl (1.000 − 0.957 = 0.043) derivative as for either the 2-*O*- (0.026) or 3-*O*-methyl (0.023) derivative. These results indicate that the methylation of the 6-hydroxyl group (which has free rotation) may increase the effective size of the molecule more than do methoxyl groups on C-2 or C-3 of the ring. Alternatively, etherification of the 6-hydroxyl group may cause a larger decrease of R_g, because this hydroxyl group is approximately twice as effective as the 2- or 3-hydroxyl groups in hydrogen-bonding sorption. Substitution at the 6-hydroxyl group by a D-glucosyl group also results in a greater diminution of R_g than similar substitution at O-4 (as in the disaccharides listed in Table IV). It is possible that size and sorption factors contribute to

TABLE IV

EFFECT OF SITE OF LINKAGE OF SELECTED DISACCHARIDES OF D-GLUCOSE ON THEIR RELATIVE ELUTION FROM SEPHADEX G-15

Disaccharide	*Linkage*	R_g
Maltose	α-D-(1→4)	0.922
Isomaltose	α-D-(1→6)	0.889
Cellobiose	β-D-(1→4)	0.907
Gentiobiose	β-D-(1→6)	0.870

these results. In contrast to the results of methyl substitution, replacement of the 6-hydroxyl group by hydrogen increases the R_g value, and similar replacement of the 2-hydroxyl group (on the ring) causes a greater increase than that at the primary position. These increases are consistent with the smaller sizes of the deoxy sugars relative to those of their parent compounds. The relative effect, being less for replacement by hydrogen at the primary than at the secondary position, is in opposition to the relative effect of methylation of the corresponding hydroxyl groups.

Relative elutions are selectively affected, not only by the site of attachment but also by the orientation of the hydroxyl group bearing the substituent. The relative elution volumes of glycosides and disaccharides differing only in the configuration at the anomeric carbon atom are listed in Table V. For all such compounds studied, the substituted α-D anomer is retained to a greater extent than the corresponding β-D anomer. The α and β anomers respectively have an axially and an equatorially oriented hydroxyl group at C-1 in their favored chair conformation. The substituted β anomer seems to be effectively larger than the corresponding α anomer, regardless of the nature of the substituent at O-1.

TABLE V

EFFECT OF CONFIGURATION AT THE SUBSTITUTED ANOMERIC CARBON ATOM ON THE RELATIVE RETENTIONS OF METHYL GLYCOSIDES AND DISACCHARIDES ON SEPHADEX G-15

Compound	R_g
Methyl α-D-xylopyranoside	1.021
Methyl β-D-xylopyranoside	1.005
Methyl α-D-glucopyranoside	0.971
Methyl β-D-glucopyranoside	0.954
4-*O*-α-D-Glucopyranosyl-D-glucopyranose	0.922
4-*O*-β-D-Glucopyranosyl-D-glucopyranose	0.907
6-*O*-α-D-Glucopyranosyl-D-glucopyranose	0.889
6-*O*-β-D-Glucopyranosyl-D-glucopyranose	0.870

The effect of variations in the configuration at specific carbon atoms was explored by comparing the gel-permeation characteristics of several aldopentoses, aldo- and keto-hexoses, and disaccharides. The configuration at the anomeric carbon atom significantly affects the relative elution volumes of methyl glycosides (see Table V). Unlike the conformationally stable D-glucopyranosides, however, aqueous solutions of free sugars are mixtures. The elution volumes of equilibrated free sugars are, therefore, actually those of equilibrium mixtures, as their maxima are determined from a superposition of weighted curves. Whether or not interaction with the column material alters the equilibrium composition of the solution is not yet known. It is assumed here that an approximately constant contribution from the anomeric hydroxyl group occurs within a series. It is further assumed that the sugars exist in solution mainly in the favored chair conformation of their pyranose forms[9]. The results are given in Table VI.

Sugars having either one or no hydroxyl group axially attached are eluted in a specific order that is consistent throughout all four series, namely, increasing R_g values in the order: hydroxyl group axial at C-4, at no position, at C-2, and at C-3. These systematic differences due to variations in the stereochemistry of saccharides of the same molecular weight reflect subtle differences in their sizes or their hydrogen-bonding capacities, or both.

The effect of acyclic as compared with cyclic structure on the gel-permeation characteristics of polyhydroxy compounds was examined by comparison of the behavior of two readily available hexitols with that of *myo*-inositol. The results are given in Table VII. Unlike the monosaccharides investigated, the configurational differences between the acyclic hexitols, D-glucitol and D-mannitol, is not reflected in their relative elution-volumes. The hexitols had an R_g value higher than that of *myo*-inositol. The observed difference ($0.980-0.963=0.017$) is less than that between the extreme values for the aldopentoses ($1.065-1.024=0.041$) and aldohexoses ($1.029-0.990=0.029$). It is probable that the nine inositols would give a similar range in R_g values, possibly overlaping those of the (acyclic) hexitols. Therefore, no

conclusion concerning the relative elution volumes of the cyclic and acyclic compounds can be drawn at this time.

TABLE VI

EFFECT OF CONFORMATION OF SOME MONO- AND DI-SACCHARIDES ON THE RELATIVE RETENTIONS ON SEPHADEX G-15

Saccharide	*Assumed conformation*[a]	*Axial OH on carbon atom*	R_g
Pentoses (mol.wt. 150)			
D-Ribose	CA	3	1.065
D-Lyxose	CA	2	1.062
D-Xylose	CA	none	1.047
D-Arabinose	CE	4	1.024
Hexoses (mol.wt. 180)			
A. Aldoses			
D-Mannose	CA	2	1.029
D-Glucose (reference)	CA	none	1.000
D-Galactose	CA	4	0.990
B. Ketoses			
D-Psicose	CA	3	1.040
D-Sorbose	CA	none	1.026
D-Fructose	CE	4	1.018
Disaccharides (mol.wt. 342)			
4-*O*-β-D-Glucopyranosyl-D-glucopyranose	CA, CA	none	0.907
4-*O*-β-D-Galactopyranosyl-D-glucopyranose	CA, CA	4[b]	0.895
6-*O*-α-D-Glucopyranosyl-D-glucopyranose	CA, CA	none	0.889
6-*O*-α-D-Galactopyranosyl-D-glucopyranose	CA, CA	4[b]	0.877

[a]CA = Reeves[9] *C1*(D); CE = Reeves *1C*(D). [b]Axial hydroxyl group in the (nonreducing) D-galactopyranosyl group.

TABLE VII

EFFECT OF ACYCLIC AND CYCLIC STRUCTURE OF POLYHYDRIC ALCOHOLS ON THEIR RELATIVE RETENTION ON SEPHADEX G-15

Polyhydric alcohol	R_g
Acyclic	
D-Glucitol	0.979
D-Mannitol	0.980
Cyclic	
myo-Inositol	0.963

EXPERIMENTAL

Materials. — Sephadex G-15 in spherical-bead form was used as supplied by Pharmacia Fine Chemicals, Inc. Most of the sugars and sugar derivatives were obtained from commercial sources. The various methyl ethers of D-glucose were

prepared essentially according to published procedures[10–14], the technique described by Gillis[15] usually being used instead of the classical methylation procedures. The methylation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside is described as an example.

Preparation of methyl 4,6-O-benzylidene-2,3-di-O-methyl-α-D-glucopyranoside. — To 56.4 g (0.2 mole) of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside in methyl sulfoxide (~200 ml) was added 50 g of powdered sodium hydroxide; then, 228 g (1.6 moles) of methyl iodide was added slowly, with stirring. Cooling was required, in order to control the exothermic reaction that ensued. The reaction mixture, which solidified after being stirred for ~3 h at room temperature, was kept overnight. A large excess of distilled water was then added to the solid mass, the slurry was stirred for ~1 h, and the crystalline product was collected by suction filtration, washed with water, and dried. The methylation was essentially quantitative. Recrystallization from benzene–hexane gave 44 g (71%) of pure methyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-α-D-glucopyranoside, m.p. 119–121° (lit.[14] m.p. 122–123°).

For most of the other compounds, the methylated products did not crystallize upon addition of distilled water to the reaction mixture; therefore, these reaction mixtures were extracted with chloroform or ethyl ether, and the extracts were processed in the usual way.

Methods. — The procedure described by Flodin and Kupke[16] was used for packing the gel-permeation columns. An extension tube, equal in length and diameter to the columns, was employed. The swollen gel was allowed to settle slowly from an agitated suspension. The columns were of Chromatronix, precision bore (2.54 cm × 45–50 cm between the top and bottom bed-supports). Solutes were introduced individually, as 4% solutions, through a 0.1-ml sample-loop, thereby minimizing the dead volume. Elution was conducted with water at a linear flow-rate estimated at ~8.5 cm/h. The eluate was monitored continuously by means of a Waters Associates R-4, recording, differential refractometer. Volumes were determined gravimetrically by collecting the eluate in tared test-tubes, and summing the weights of fractions and proportional parts of fractions between injection and the peak of the recorded elution-curve for each solute. D-Glucose was included as one of every set of six or seven test solutes as the reference standard. Nine measurements of the elution volume of glucose on one Sephadex column averaged 158.02 ml with a standard deviation of 0.16 ml. Data are expressed as R_g values, *i.e.*, as the ratios of the total elution volumes of the solutes to that of glucose in the same test. The values are accurate to within $\sim \pm 0.003$.

REFERENCES

1 L. F. MARTIN AND S. P. ROWLAND, *J. Chromatogr.*, 28 (1967) 139.
2 L. F. MARTIN, F. A. BLOUIN, N. R. BERTONIERE, AND S. P. ROWLAND, *Tappi*, 52 (1969) 708.
3 L. F. MARTIN, N. R. BERTONIERE, F. A. BLOUIN, M. A. BRANNAN, AND S. P. ROWLAND, *Textile Res. J.*, 40 (1970) 8.
4 F. A. BLOUIN, L. F. MARTIN, AND S. P. ROWLAND, *Textile Res. J.*, 40 (1970) 809.

5(a) F. A. BLOUIN, L. F. MARTIN, AND S. P. ROWLAND, *Textile Res. J.*, 40 (1971) 959; (b) L. F. MARTIN, F. A. BLOUIN, AND S. P. ROWLAND, *Separation Sci.*, 6 (1971) 287.
6 N. V. B. MARSDEN, *Ann. N. Y. Acad. Sci.*, 125 (1965) 428.
7 L. F. MARTIN, N. R. BERTONIERE, AND S. P. ROWLAND, *J. Chromatogr.*, submitted.
8 E. L. ELIEL, *Stereochemistry of Carbon Compounds*, McGraw–Hill, New York, 1966, p. 61.
9 R. E. REEVES, *J. Amer. Chem. Soc.*, 72 (1950) 1499; H. S. ISBELL AND R. S. TIPSON, *Science*, 130 (1959) 793.
10 J. E. HODGES AND C. E. RIST, *J. Amer. Chem. Soc.*, 74 (1952) 1498.
11 J. C. IRVINE AND J. P. SCOTT, *J. Chem. Soc.*, 103 (1913) 564.
12 L. HOUGH, J. K. N. JONES, AND M. S. MAGSON, *J. Chem. Soc.*, (1952) 1525.
13 J. C. IRVINE AND J. P. SCOTT, *J. Chem. Soc.*, 103 (1913) 575.
14 W. N. HAWORTH, *J. Chem. Soc.*, 107 (1915) 8.
15 R. G. GILLIS, *Tetrahedron Lett.*, (1968) 1413.
16 P. FLODIN AND D. W. KUPKE, *Biochim. Biophys. Acta*, 21 (1956) 368.