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COMPLEXES OF CARBOHYDRATES WITH ALUMINATE ION. CHROMATOGRAPHY OF CARBOHYDRATES ON COLUMNS OF ANION-EXCHANGE RESIN (ALUMINATE FORM)*

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ABSTRACT

With water as sole eluant, the retention volumes for carbohydrates on an aluminate-resin column generally decrease in the order: ketoses > aldoses > alditols > methyl glycosides; retention increases with molecular size. Both aluminate ion and hydroxide ion contribute to the chromatographic properties of an aluminate resin. To avoid alkaline degradation and interconversion, which can occur extensively at 25°, chromatography of reducing sugars must be performed both rapidly and at low temperature. A mixture of D-glucose and D-fructose can be completely separated on a short aluminate column.

INTRODUCTION

Polyhydroxy compounds form complexes with aluminate ion in aqueous solution; however, little information on this subject has been reported. Although various investigators have used sodium aluminate solution to convert certain aldoses into the isomeric ketoses in high yield[1–3], no attempts were made to elucidate the mechanism responsible for the high yields. In this investigation, the effectiveness of aluminate ion for separating sugars by column chromatography with strongly basic anion-exchange resin has been evaluated. Subsequent papers in this Series on aluminate complexes will deal with the mechanism, scope, and certain applications of complexation of carbohydrates with aluminate ion.

RESULTS AND DISCUSSION

When the formate, acetate, or chloride form of a strongly basic, anion-exchange resin is stirred with 1.3M aqueous sodium aluminate ($NaAlO_2 \cdot 1.5H_2O$), the resin

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anions are partially replaced by aluminate and hydroxide ions. Although repeated treatment with sodium aluminate results in a resin of higher aluminate content and greater ability to retain a carbohydrate, the present chromatographic study was performed almost entirely with resin given only a single treatment. Data from chromatography of a number of saccharides at 25° on a column of this resin (ht. = 12 cm; vol. = 10.6 ml) are presented in Table I. A column of these dimensions

TABLE I

CHROMATOGRAPHY[a] WITH ALUMINATE RESIN OF NORMAL HYDROXIDE CONTENT AT 25°

Carbohydrate	*Wt. of sample (mg)*	*Flow rate (ml/min)*	$V_{initial}$ *(ml)*	V_{max} *(ml)*	V_{final} *(ml)*
Reducing saccharides					
Maltotetraose	25	2.5	800[b]	1,200[b]	2,200[b]
Maltotriose	25	2.5	375[b]	490[b]	900[b]
D-Fructose	30	2.5	302	545	830
Maltose	20	2.5	202	258	695
Cellobiose	10	2.5	137	181	355
Lactose	10	2.5	89	116	245
D-*manno*-Heptulose	30	2.5	84	136	245
D-*glycero*-D-*galacto*-Heptose	10	2.5	80	110	195
D-Glucose	10	2.5	68	91	173
	150	2.5	15	26	173
Nonreducing saccharides					
Cyclohexaamylose	20	2.5	470	720	1,500
Raffinose	10	2.5	31	56	155
Sucrose	10	2.5	27	45	125

[a]The resin bed (vol., 10.6 ml; ht., 12 cm) was prepared by treating 10.0 ml of formate resin once with 1.3M sodium aluminate solution. $V_{initial}$ = effluent volume at first appearance of carbohydrate; V_{max} = effluent volume at point of maximum concentration in effluent; V_{final} = retention volume = effluent volume at point of complete emergence of carbohydrate. Probable error in estimating volumes was ±5%. [b]Estimated values based upon data from older columns of lower retention ability. Maximum possible error in estimates is 20%.

can accommodate at least 150 mg of either D-glucose or D-fructose without the leading end of the solute band moving as fast as the solvent. Because $V_{initial}$ and V_{max} often decreased with increase in sample weight for quantities larger than 10 mg, comparison of retention properties of different carbohydrates should be made preferably with samples of equal size. The sample size necessary for adequate polarimetric detection depends upon the magnitude of the specific optical rotation and upon the retention characteristics of the carbohydrate. To facilitate detection of certain compounds (such as D-fructose, L-sorbose, lactulose, maltulose, and maltotetraose), relatively large weights are necessary.

The relative magnitudes of V_{max} in Table I indicate that retention of a saccharide by aluminate resin is generally a function of molecular size. The larger the molecule, the greater the retention. Furthermore, reducing sugars are more strongly held than nonreducing sugars of similar molecular weight. Also, studies with D-

fructose, D-*manno*-heptulose, L-sorbose, lactulose, and maltulose indicate that aluminate resin generally retains ketoses more strongly than the corresponding isomeric aldoses (L-sorbose, lactulose, and maltulose are not included in Table I, because they were chromatographed on aluminate columns of different retention ability. Qualitatively, however, these three ketoses are more tightly bound than the corresponding aldoses.) The great difference in retention volume between D-glucose and D-fructose permits a facile separation of a mixture of these two sugars on relatively short columns (see Fig. 1).

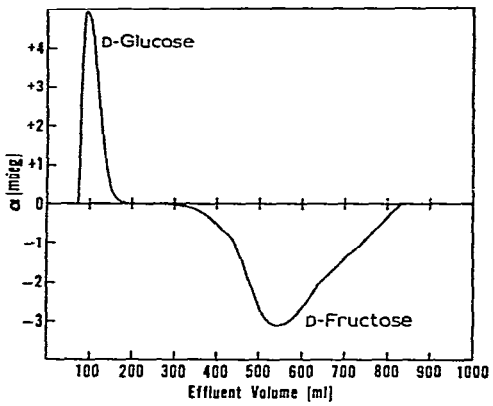

Fig. 1. Chromatography of mixture of D-glucose (10 mg) and D-fructose (30 mg) on aluminate resin of normal hydroxide content at moderate flow-rate (2.5 ml/min). Bed volume, 10.6 ml; column dimensions, 1 cm × 12 cm; detection by polarimetry in 0.4-dm cell; temperature, 24°.

In studies not mentioned in Table I, chromatography of methyl α-D-mannoside, methyl α- and β-D-glucopyranoside, D-glucitol, and erythritol have been shown to have retention volumes lower than that of D-glucose. The strongly acidic, anomeric hydroxyl group of reducing sugars apparently favors higher retention.

The retentivity of a resin in the aluminate form is generally much greater than that of the corresponding purely formate, acetate, or chloride form. However, the

TABLE II

CHROMATOGRAPHY[a] WITH FORMATE RESIN AT 25°

Carbohydrate	*Wt. of sample (mg)*	*Flow rate (ml/min)*	$V_{initial}$ *(ml)*	V_{max} *(ml)*	V_{final} *(ml)*
Maltotetraose	0.1	3	3.5 (±0.3)	6 (±1)	23 (±1)
Cyclohexaamylose	0.1	3	3.5 (±0.3)	5.5 (±0.5)	16 (±1)
D-Fructose	0.1	3	4.5 (±0.3)	6.5 (±0.5)	16 (±1)
	30.0	3	4.0 (±0.3)	6.5 (±0.5)	45 (±2)
D-Glucose	0.1	3	5.0 (±0.5)	6.5 (±0.5)	15 (±2)
	10.0	3	4.0 (±0.5)	7.0 (±0.5)	35 (±2)

[a]Bed vol., 10.0 ml. Bed ht., 12 cm. Volume symbols are described in footnote *a* of Table I.

ability to retain methyl glycosides of monosaccharides is low for all of the resins. The results of several runs with formate resin are given in Table II.

Contribution of hydroxide ion to retention ability of resin. — At 25° a 1.3M solution of $NaAlO_2 \cdot 1.5H_2O$ has a pH of 13.14, caused by the equilibrium $Al(OH)_4^- \rightleftarrows OH^- + Al(OH)_3(sol)$. Because the concentration of OH^- in such a solution is approximately 0.14M, there should be appreciable exchange between hydroxide ions in solution and anions of an anion-exchange resin that is in contact with the solution. Table III presents data from the chromatographic treatment of

TABLE III

CHROMATOGRAPHY[a] WITH HYDROXIDE RESIN AT 24°

Carbohydrate	*Wt. of sample (mg)*	*Flow rate (ml/min)*	$V_{initial}$ *(ml)*	V_{max} *(ml)*	V_{final} *(ml)*
With resin treated with 0.2M NaOH (bed vol., 10.5 ml; ht., 12 cm)					
Maltotetraose	8	3	310	525	745
Cyclohexaamylose	30	3	83	171	495
D-Fructose	30	3	39	54	145
	30	0.6	35	45	115
D-Glucose	10	3	37	51	95
	30	3	28	38	109
With resin treated with 0.65M NaOH (bed vol., 10.7 ml; ht., 12 cm)					
D-Fructose	10	3	114	145	265
D-Glucose	10	3	106	126	205

[a]The resin bed was prepared by treating 10.0 ml of formate resin once with sodium hydroxide solution of the desired concentration. Volume symbols are described in footnote *a* of Table I. Probable error in estimating volumes is ±5%.

several saccharides on columns of resin prepared by treating formate resin with 0.2M sodium hydroxide. It is obvious from these data that hydroxide ion contributes greatly toward the retentivity of an anion-exchange resin—either by converting the carbohydrate into an alcoholate ion that is tightly held by the resin, or through simple ion–dipole attraction between OH^- and carbohydrate hydroxyl groups—or both. The greater the hydroxide content of the resin, the greater is the contribution of hydroxide toward retention (see Table III for data on formate resin treated with 0.65M sodium hydroxide).

Although the effect of hydroxide ion on retention of D-fructose is only slightly greater (about 6–15%, based on V_{max}) than that on retention of D-glucose, the ratio of $V_{max\ (fructose)}$:$V_{max\ (glucose)}$ is about 6:1 for an aluminate column of normal hydroxide content (Table I). This high ratio indicates that aluminate ion affects the passage of D-fructose much more strongly than the passage of D-glucose. It is quite probable that strong aluminate–carbohydrate complexes are responsible for high retention volumes. Such complexes would not be expected to be of the ion-dipole–type, which is relatively weak in aqueous media[4].

Complex formation between carbohydrate and aluminate. — That complex formation between aluminate and carbohydrate can readily occur in aqueous solution has been demonstrated in this Laboratory by the ease with which a large number of such complexes have been isolated. Many aluminate complexes of alditols and reducing sugars are precipitated in high yield by the addition of ethanol to an aqueous, equimolar mixture of sodium aluminate and carbohydrate. For example, when 10 ml of ethanol is added to 1 ml of aqueous solution containing 0.6 mmole each of sodium aluminate and D-glucitol, the percentage of D-glucitol recovered in the form of an aluminate complex is 86%. Similarly, the complexes of D-fructose, D-glucose, and D-mannose are isolated in yields of 83, 85, and 77%, respectively. The amount of water in these reaction mixtures is sufficiently high to prevent coprecipitation of unreacted carbohydrate, sodium hydroxide–carbohydrate adduct, or sodium alcoholate. The molar ratio of carbohydrate:Na:Al in these complexes is very close to 1:1:1. All products were amorphous (nonbirefringent) powders.

Complexes of carbohydrates whose reactivity toward aluminate is exceptionally low cannot be isolated by the method just described. Among these weakly reactive compounds are sucrose, methyl α-D-glucopyranoside, and 1,6-anhydro-β-D-glucopyranose.

The method of isolation described here has been used to obtain rough estimates of relative reactivities for D-glucose, D-fructose, and D-glucitol. When a 1:1 mixture of D-glucose and D-fructose and a 1:1 mixture of D-glucose and D-glucitol are each treated with a small amount of sodium aluminate, the carbohydrates in each mixture compete for the aluminate. The extent to which aluminate is complexed with each carbohydrate depends upon the relative reactivities of the carbohydrates. Under the conditions of the experiment, each of these three carbohydrates forms an isolable 2:1 carbohydrate–sodium aluminate complex (to be discussed in a future paper). G.l.c. analyses of the isolated complexes revealed that D-fructose and D-glucitol are considerably more reactive than D-glucose. The competition study indicated that D-fructose is four times more reactive than D-glucose and that D-glucitol is three times more reactive than D-glucose.

The extremely small retention volume of D-glucitol on an aluminate column (see Table IV) shows that D-glucitol does not complex significantly with the aluminate ion on an aluminate resin. This is in strong contrast with the behavior of D-glucitol toward aluminate ion in sodium aluminate solution. A possible explanation for the absence of complexation on the resin is that, in order for a strong D-glucitol–aluminate interaction to occur, the alditol must be able to form a quadridentate chelate in which the alditol is "wrapped around" the aluminum atom. For steric reasons, envelopment of the aluminum atoms may not be possible on a resin surface. With D-fructose, however, envelopment of the D-glucitol type is apparently unnecessary for strong complexation on the resin.

Chromatography of carbohydrates on aluminate-resin columns of low hydroxide content. — The hydroxide content of a sodium aluminate solution can be decreased almost tenfold by careful addition of cation-exchange resin (H^+ form). In this manner,

the pH of 1.3M sodium aluminate is lowered from an initial 13.14 to 12.22 without significant precipitation of aluminum hydroxide. The solution of pH 12.22 is less stable than the original of pH 13.14; precipitation of aluminum hydroxide begins to occur in the former solution in 30–45 min, whereas in the latter, no precipitate forms for several hours or more.

When 1.3M sodium aluminate of decreased hydroxide content (pH 12.22) is used to prepare an aluminate resin, the proportion of hydroxide ion in the resin prepared from this solution should be lower than that in aluminate resin prepared from 1.3M sodium aluminate of normal hydroxide content (pH 13.14). The lower proportion of OH^- is evidenced by the greater stability of reducing sugars on columns of this resin (discussed later). Generally, a lower hydroxide content results in a lower retention-volume for carbohydrates that do not complex strongly with aluminate ion. (Compare the retention volumes for D-glucose and sucrose in Table I with those in Table IV. Neither of these sugars complexes strongly with aluminate ion in solution.)

Table IV presents chromatographic data for a large number of carbohydrates on columns of aluminate resin of low hydroxide content at 24–26° and low-to-moderate flow rate. Chromatographic peaks for certain carbohydrates such as D-allose, L-rhamnose, and D-xylose are nearly symmetrical, whereas with many other carbohydrates such as lactose, D-lyxose, and D-ribose, there is considerable peak-tailing. We can only conjecture as to some of the causes of peak dissymmetry. Sample size, as mentioned earlier, often has an important influence on $V_{initial}$ and V_{max}. For example, $V_{initial}$ and V_{max} for lactulose at 100 mg are much lower than the corresponding values at 30 mg. This decrease in retention with increase in sample weight is not unexpected, because of the relatively large ratio of the number of carbohydrate

TABLE IV

CHROMATOGRAPHY[a] WITH ALUMINATE RESIN OF LOW HYDROXIDE CONTENT

Carbohydrate	*Wt. of sample (mg)*	*Flow rate (ml/min)*	*t (°)*	$V_{initial}$ *(ml)*	V_{max} *(ml)*	V_{final} *(ml)*
Monosaccharides						
D-Fructose	30	2	25	310	640	820
L-Sorbose	50	3	24	135	197	500 (±50)
D-Ribose	100	3	24	47	81	425
D-Allose	10	3	24	103	150	196
D-Mannose	10	3	24	87	112	154
D-Xylose	10	3	24	66	105	148
D-Lyxose	30	3	24	52	77	143
L-Rhamnose	20	3	25	43	59	122
D-Glucose	10	0.4	25	47	57	112
	10	2	24	62	74	118
	10	3	24	58	77	117
L-Arabinose	10	3	24	49	75	117
D-Galactose	10	3	24	49	69	113

Table continued on p. 161.

TABLE IV (*continued*)

Carbohydrate	*Wt. of sample (mg)*	*Flow rate (ml/min)*	*t (°)*	$V_{initial}$ *(ml)*	V_{max} *(ml)*	V_{final} *(ml)*
Reducing oligosaccharides						
Maltulose	50	3	24	210 (±20)	425	800 (±100)
Maltose	10	3	24	210	315	450
Lactulose	30	3	26	161	230	375
	100	3	26	82	128	365
Cellobiose	20	3	24	103	139	260
Lactose	20	3	24	76	94	185
Nonreducing oligosaccharides						
Raffinose	10	3	24	21	45	93
	3	3	24	20	46	85
Sucrose	10	3	24	20	37	74
α,α-Trehalose	10	3	24	6	11	74
Glycosides						
Phenyl β-D-glucopyranoside	10	3	24	39	75	150
Methyl β-maltoside	14	3	24	8	16	106
Methyl α-D-mannoside	3	3	24	6	9	27 (±3)
Methyl α-D-glucopyranoside	3	3	24	4	8	28
	0.6	0.2	26	4	7	24
Methyl β-D-glucopyranoside	3	3	24	6	9	23
Methyl α-D-galactopyranoside	3	3	24	4	7	20
Alditols						
D-Glucitol	10	3	24	12	23	~39
Erythritol	10	3	24	10	14	~29

[a]The resin bed (vol., 10.6 ml; ht., 12 cm) was prepared by treating 10.0 ml of formate resin once with 1.3M sodium aluminate solution of decreased alkalinity (pH 12.2). Volume symbols are described in footnote *a* of Table I. Probable error in estimating volumes is ±5%, unless indicated otherwise.

molecules to available aluminate-ion and/or hydroxide-ion binding sites. In other words, there can be an overloading effect.

Interconversion and degradation of reducing sugars (discussed in the next section) are factors that may affect peak shape, because the monitoring system is sensitive to the different specific rotations of the species being eluted. The peak tailing that is so pronounced with such aldoses as D-ribose and lactose is possibly caused largely by aldose–ketose interconversion. The different forms may not only have different specific rotations but also different retention properties. Use of higher flow-rates can minimize interconversion and degradation and, in some instances, produce a more symmetrical peak. To illustrate, the shape of a peak for D-fructose is nearly symmetrical at high flow rates at 24° (see Fig. 2), whereas at much lower flow-rates the shape is considerably distorted (see Fig. 1).

The flow rate of 3 ml/min commonly used in these studies does not seem to be so rapid as to prevent establishment of equilibrium between free carbohydrate and bound carbohydrate. Evidence that the establishment of equilibrium is not an

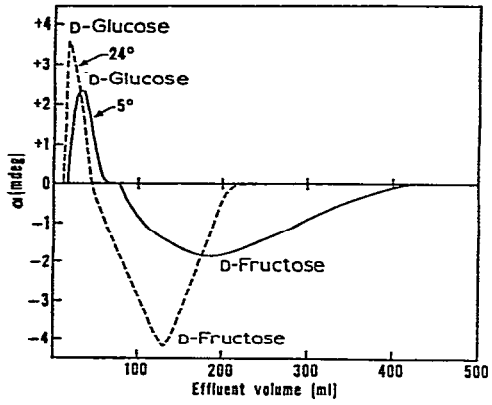

Fig. 2. Chromatography of mixtures of D-glucose and D-fructose at high flow-rate on aluminate resin of low hydroxide content. Bed volume, 3.3 ml; column dimensions, 0.6 cm × 13 cm; detection by polarimetry in 0.4-dm cells. *At 24°:* 3.3 mg of D-glucose and 10 mg of D-fructose; flow rate, 14 ml/min. *At 5°:* 6.7 mg of D-glucose and 15 mg of D-fructose; flow rate, 9 ml/min.

important factor in the studies with aluminate resin is provided by data on methyl α-D-glucopyranoside (see Table IV). The peak shape for this glycoside at a flow rate of 3 ml/min does not differ appreciably from the shape at 0.2 ml/min. Furthermore, the peak for D-fructose is more symmetrical at 14 ml/min (Fig. 2) than at 2.5 ml/min (Fig. 1). The reverse would be expected if an equilibrium factor were involved.

Fig. 2 shows that mixtures of D-glucose and D-fructose are separable at very high flow-rates. Separation at 5° is better than at 24° because the retention ability of the resin is greater at the lower temperature. For adequate separation of a mixture of two carbohydrates, V_{final} for the faster component should not be significantly larger than $V_{initial}$ for the slower component.

Effect of multiple treatment of formate resin with sodium aluminate. — Treating formate resin twice with 1.3M sodium aluminate solution of low hydroxide content results in an aluminate resin whose retentivity is often much greater than that of a resin treated only once. For example, $V_{initial}$, V_{max}, and V_{final} (in ml) for D-glucose (10 mg) and lactose (20 mg) on a double-treatment column were 120, 133, 245 and 152, 201, and 355, respectively. On a single-treatment column of the same dimensions, the respective values were 58, 77, 117 and 76, 94, and 185. D-Fructose exemplifies a carbohydrate whose retention is less affected by a double treatment ($V_{initial}$, V_{max}, V_{final} = 467, 661, 955 and 310, 640, 820 for a 30-mg sample on a double- and single-treatment column, respectively).

Stability of aluminate ion and reducing sugars on aluminate resin. — The passage of a large volume of water alone through an aluminate column has very little, if any, effect on the retentivity of the resin, provided that the water is free of carbon dioxide. The retention volume of D-glucose was not noticeably altered by passing 4,500 ml of freshly boiled, distilled water through an aluminate column of the kind described in Table I (resin volume, 10.6 ml). Nor does age appear to affect retention. When not in

use, aluminate columns remain unchanged for at least 7 days. Longer periods were not investigated.

Certain reducing sugars may alter significantly the retentivity of an aluminate resin. At 25°, D-glucose and D-mannose have little or no effect, whereas D-fructose causes considerable change. Passing 0.3 g of D-fructose through 10.6 ml of aluminate resin (normal hydroxide content) at such a flow rate that elution was complete in 3 h resulted in a 20% decrease in retentivity. This decrease seems to be the result of decomposition of aluminate ion. Fine particles of aluminum oxide were found mixed with the resin after various reducing sugars had been chromatographed. Although no full explanation for the decomposition can yet be offered, it is probable that loss of aluminate ion is caused by alkaline degradation of reducing sugars to acidic substances.

Alkaline degradation and interconversion of reducing sugars may occur extensively at 25° on aluminate columns. Therefore, low-hydroxide resin is preferred for chromatographic separations of reducing sugars. Rapid chromatography at 3–5° practically eliminates degradation and interconversion. Table V shows the effect of elution time, temperature, and hydroxide content on the recovery of D-fructose. For comparison, the table also includes data on hydroxide-resin columns. The resins were prepared according to the methods described in Tables I, III, and IV.

TABLE V

EFFECT OF ELUTION TIME AND TEMPERATURE ON RECOVERY OF D-FRUCTOSE[a]

Column	*Bed vol. (ml)*	*Bed ht. (cm)*	*t (°)*	*Time for complete elution (h)*	*Fructose-recovered (%)*
Aluminate (normal hydroxide content)	10.6	12	25	5.2	56
	5.3	6	24	3.9	74
	5.3	6	24	2.3	84
	5.3	6	3	6.6	91
Aluminate (low hydroxide content)	10.6	12	25	6.8	66
	3.3	13	24	0.44	95
	3.3	13	24	0.32	97
	3.3	13	3	0.72	100
Hydroxide (0.2M NaOH treatment)	10.5	12	24	0.8	89
Hydroxide (0.65M NaOH treatment)	10.7	12	25	1.5	69

[a]Amount of D-fructose sample = 30.0 mg.

Table VI presents data on the stability of D-glucose held stationary at 3 and 23° on a column of aluminate resin of low hydroxide content. D-Glucose is converted largely into D-fructose. At 55° on the same column, D-glucose apparently undergoes

rapid degradation; after a reaction period of 4 h, the eluate contained only a trace of D-fructose and no D-glucose. On similar columns of low hydroxide content, D-mannose is converted into D-fructose in 9% yield in 4 h at 23°; lactose is converted into lactulose in 41% yield in 4 h at 25°; and lactulose is consumed (by conversion and/or degradation) to the extent of 41% in 24 h at 25°.

TABLE VI

STABILITY OF D-GLUCOSE HELD STATIONARY ON ALUMINATE RESIN OF LOW HYDROXIDE CONTENT[a]

t (°)	*Sample amount (mg)*	*Time on resin (h)*	*Eluate analysis*		
			A	*B*	*100−(A+B)*
			D-*Glucose* (%)	D-*Fructose* (%)	
23	30	1	88	9	3
23	30	2	84	15	1
23	30	4	70	25	5
3	30	66		16	
3	30	168	62	29	9

[a]The resin was prepared according to the method described in Table IV. Each D-glucose sample was applied as a 0.5-ml solution to a column of resin (13 cm × 0.6 cm; vol., 3.3 ml) and pressed in with an additional 5 ml of water. After a chosen period of time, the column was quickly cooled to 3°; elution was then effected rapidly with 500 ml of water and the eluate analyzed for both D-glucose and D-fructose.

EXPERIMENTAL

Materials. — All carbohydrates were the purest available and were used without further purification. The sodium aluminate ($NaAlO_2 \cdot 1.5H_2O$; mol. wt. taken as 109) was commercial-grade of high purity. To facilitate dissolution in water, the sodium aluminate was thoroughly ground in a mortar. Concentrated solutions of aluminate are metastable and should be used within several h after preparation.

Analyses of effluents. — D-Fructose and lactulose were determined quantitatively in the presence of D-glucose by a modified anthrone method[5]. To determine D-glucose, the effluent was first evaporated to dryness in a rotary evaporator (bath temperature, 50°). The residue was then dissolved in water to make the concentration of D-glucose at least 50 mg per 100 ml of solution. A portion (1–2 ml) of this solution was used for analysis by means of D-glucose oxidase[6].

pH Measurements. — The pH values were measured with a Corning combination electrode designed for the full pH range of 0 to 14.

Chromatographic detection. — Generally, column effleunts were monitored by an automatic Bendix polarimeter equipped with a 0.4-dm flow-through cell. Where a flow-through system was impractical (such as, at 3–5°), 2-ml fractions were collected for polarimetry. Specific rotations of alditols are too small for polarimetric monitoring; hence, 3-ml fractions were collected and treated individually with 0.75 ml of periodic

acid–silver nitrate reagent; the rate of formation of precipitate permitted an estimate of the relative concentration of alditol in each fraction.

Reagents. — Reagents for determining D-glucose and D-fructose were as described in references already cited for analyses of these two sugars. Periodic acid–silver nitrate reagent was prepared as follows: 2.5 ml of conc. nitric acid was added dropwise with stirring to a solution of H_5IO_6 (1.0 g) in 100 ml of water; to this was added a solution of silver nitrate (0.2 g) in 3 ml of water.

Preparation of resins for chromatography. — Aluminate and hydroxide resins employed in experiments that provided data for the Tables and Figures were prepared by appropriate treatment of Bio-Rad AG 1-X8 (200–400 mesh) strongly basic anion-exchange resin (formate form; 3.2 meq. per gram, dry weight). Exactly 10.0 ml of wet resin (formate form) was stirred for 5 min in 15 ml of sodium aluminate or sodium hydroxide solution. After filtration with a fritted-disc funnel, the resin was washed with several portions of distilled water (freshly boiled to eliminate dissolved carbon dioxide, which may decompose aluminate ion). In the determination of retention volumes, column packings were discarded when their retentivity decreased to 95% of their original values.

A. *Aluminate resin of normal hydroxide content.* A measured volume of formate resin was treated once with 1.3M sodium aluminate. The relative proportions of aluminate, hydroxide, and formate ion in the product were not determined.

B. *Aluminate resin of low hydroxide content.* With constant stirring of 25 ml of 1.3M sodium aluminate, increments of cation-exchange resin (H^+ form) were added until the pH had fallen from 13.14 to 12.22. A faint turbidity in the mixture, caused by precipitation of aluminum hydroxide, indicated the end point. Immediately, the faintly turbid solution was separated from the resin by decantation and centrifuged to remove the small amount of aluminum hydroxide. The clear supernatant is stable for at least 30 min. Formate resin (10.0 ml) was stirred with 15 ml of the supernatant in order to prepare aluminate resin of low hydroxide content. The relative proportions of aluminate, hydroxide, and formate ion in the product were not determined.

The Al content of aluminate resin, prepared by stirring formate resin once with sodium aluminate solution, was 1.8% by weight (based upon ash analysis performed upon aluminate resin that had been vacuum-dried to constant weight at 23°), indicating a 21% displacement of formate ions by aluminate ions. A second treatment with sodium aluminate solution increased the Al content to 3.0%.

C. *Hydroxide resins.* Formate resin was treated once with 0.2M sodium hydroxide to afford a resin of moderate hydroxide content; 0.65M sodium hydroxide was used to prepare a resin of higher hydroxide content. The relative proportions of hydroxide and formate ion in the product were not determined.

Chromatographic apparatus. — The column was a simple, heavy-walled glass tube equipped with a Teflon stopcock (to regulate the flow rate) and a small plug of glass wool to support the resin bed. For polarimetric monitoring, the effluent was passed first through a flow-through polarimetric cell before being collected.

General chromatographic procedure. — A sample of carbohydrate, applied to the

top of the resin bed as an aqueous solution (0.2–0.5 ml), was pressed into the column with 5–10 ml of water. Water was the only eluant. Hydrostatic pressure from a 2-ft column of water above the resin bed permitted an elution rate of 3 ml/min or less. Higher rates necessitated greater pressure, which was provided by compressed nitrogen. The effluent was generally collected in graduated cylinders. Chromatography was performed at room temperature, which varied by no more than $\pm 0.5°$. Low-temperature experiments were conducted in a cold room at 3–5°.

Precipitation of sodium aluminate–carbohydrate complex from aqueous solution. — A. Competition between two carbohydrates for aluminate ion. Ethanol (15 ml) was added to a stirred solution of sodium aluminate (0.0327 g; 0.3 mmole), D-glucitol (0.1100 g; 0.6 mmole), and D-glucose (0.1080 g; 0.6 mmole) in 1 ml of distilled water. The precipitated, solid complex was washed with ethanol and dried at room temperature under vacuum; yield, 0.1042 g. The molar ratio of D-glucitol/D-glucose was 3.1 (by g.l.c.). This procedure was also applied to the study of competition for aluminate between D-glucose and D-fructose.

G.l.c. analysis of an isolated complex for the purpose of determining carbohydrate was performed as follows. A sample of complex (20.0 mg) was dissolved in 5 ml of distilled water containing 10.0 mg of methyl α-D-mannoside as internal standard. Carbon dioxide was bubbled rapidly through the solution for 2 min to decompose the complex. After centrifugation to remove aluminum hydroxide (~0.2 ml), a 1.5-ml portion of the supernatant was evaporated to dryness in a 50-ml round-bottomed flask on a rotary evaporator (bath temperature, 50°). Dry pyridine (1 ml) was added, followed by appropriate amounts of silylating reagents to form the trimethylsilyl ethers. G.l.c. of the ethers was performed on an F & M Series 700 instrument equipped with a flame-ionization detector. The temperature was programmed at 2°/min from 140° to 188°. The 8 ft × 1/8 in. stainless-steel column was packed with Gas Chrom Q support (80–100 mesh) with 3% JXR as the liquid phase.

B. Preparation of 1:1 complexes of D-glucitol, D-glucose, D-fructose, and D-mannose with sodium aluminate. Abs. ethanol (10 ml) was added with stirring to a solution of carbohydrate (0.6 mmole) in 1.0 ml of 0.6M sodium aluminate. The precipitated complex was separated by centrifugation, washed twice with ethanol, and dried at room temperature under vacuum. The carbohydrate content of the complex was determined by g.l.c. Sodium and aluminum were determined by atomic absorption spectrometry of samples dissolved in dilute nitric acid.

Quantitative analyses showed the following ratios of carbohydrate:Na:Al for the 1:1 aluminate complexes: D-glucitol, 1.00:0.97:0.91; D-glucose, 1.00:0.98:0.89; D-fructose, 1.00:0.98:0.89; and D-mannose, 1.00:1.16:0.95.

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