Alkali Metal Complexes of Carbohydrates. I. Interaction of Alkali Metal Salts with Carbohydrates in Alcoholic Media

JACOB A. RENDLEMAN, JR.

The Northern Regional Research Laboratory,[1] Peoria, Illinois 61604

Received September 20, 1965

The interaction of uni-univalent alkali metal salts with carbohydrates in anhydrous alcoholic media is shown to be rapidly reversible; isolable adducts, probably chelates, whose combining ratios often vary with salt concentration are produced. High salt concentration favors polycation adduct formation and increases carbohydrate solubility in alcohol. Stoichiometry for the reaction of potassium acetate with methyl β-D-glucopyranoside in homogeneous ethanolic solution was studied polarimetrically. Electrophoretic and ebulliometric studies showed little difference in complexing ability between sodium ion and potassium ion; lithium ion is relatively weak. There was no evidence that carbohydrates combine with free nonbasic univalent anions; however, the probability is great that anions are involved in the interaction of undissociated molecules (ion pairs) of salt with carbohydrates. Techniques for electrophoresis of alcoholic solutions on glass fiber paper were developed. Rates of carbohydrate migration are much faster in alcoholic than in aqueous salt solution. The order of decreasing ability to promote migration in methanolic salt solution is $Ca^{2+} > Na^+ > K^+ > NH_4^+ > Li^+$.

The formation of alkali metal complexes of polyhydroxy compounds in nonaqueous media was investigated to determine (1) the function of cation and anion in complex formation in solution, (2) complexing abilities of metal cations, (3) the extent of complexing in solution, and (4) the combining ratio of polyhydroxy compound to alkali metal. Electrophoresis, ebulliometry, and polarimetry have been helpful. Conductometric studies were initiated but discontinued when it was found that the presence of carbohydrates generally had little effect upon the electrical conductivity of dilute alcoholic solutions of alkali metal salts.

This paper describes the interaction of carbohydrates with uni-univalent salts whose anions are insufficiently basic to abstract hydroxylic protons. The highly basic hydroxide and cyanide ions will be treated in a separate paper. With the exception of a brief investigation by Watters, Hockett, and Hudson[2] on the formation of 1:1 potassium acetate–methyl glycoside adducts in ethanolic solution, there have been no comprehensive studies of alkali metal complexes of polyhydroxy compounds in nonaqueous media.

Experimental Section

Solvents.—Commercial absolute ethanol (0.04% water), reagent grade methanol (0.05% water), anhydrous ether, and ethyl acetate were used without further purification. *N*-Methyl-2-pyrrolidinone and 2-pyrrolidinone were purified by distillation.

Reactants.—All organic reactants were anhydrous and the purest available. Inorganic salts were reagent grade. Spray reagents for detecting carbohydrate spots in paper electrophoresis studies were (1) ammoniacal silver nitrate (for studies not involving halide salts), (2) permanganate–periodate,[3] and (3) *o*-aminobiphenyl.[4] Papers sprayed with either reagent 1 or 3 were first permitted to dry and then heated at approximately 100° to effect spot development.

Analyses. A. Elemental and Group Analysis.—Standard quantitative microanalytical methods were used to analyze for metal, nitrogen, alkoxyl (for ethanol of solvation), and acetyl (for acetate ion content). Halide ion was determined by silver nitrate titration. Anion content of adducts containing either formate, acetate, or propionate ion was occasionally determined by passing an aqueous solution of the adduct through an acidic cation-exchange column[5] and then titrating the resulting acidic solution with standard sodium hydroxide.

B. Polarographic Analysis.—Measurements on a solution of potassium acetate (0.001 *M*) in ethanol and on a solution of both potassium acetate (0.001 *M*) and methyl β-D-glucopyranoside (0.100 *M*) in ethanol were made at 25° with a dropping mercury electrode. A pool of mercury functioned as the anode. The dropping rate was 1 drop per 3 sec. Each solution was 0.16 *M* in tetramethylammonium chloride (recrystallized from ethanol and dried under vacuum) which served as the electrolyte support. Potential readings were reproducible to within ±0.01 v.

C. Ebulliometric Analysis.—All carbohydrates employed in the ebulliometric studies behave as monomers in solution. Alkali metal halides and acetates in boiling ethanol appear to exist largely as ion pairs, whereas in methanol they behave as if predominantly dissociated into free ions. For example, the apparent molecular weight of potassium iodide in ethanol, determined ebulliometrically, was 129 at 0.05 *M* salt concentration (actual molecular weight, 166). This apparent weight indicates a 29% dissociation of potassium iodide into free ions. In methanol, the apparent molecular weight was 94 at 0.05 *M* salt concentration, indicating 77% dissociation into free ions.

A weighed sample of salt was introduced into an ebulliometer (±0.001° precision) containing a known weight of refluxing alcohol. A weighed sample of carbohydrate was then added and the change in boiling point, ΔT_s, was measured with ±2% precision. The procedure was then repeated without the introduction of salt and the change in boiling point, ΔT_0, recorded. The difference $\Delta T_0 - \Delta T_s$ was assumed to be proportional to the number of carbohydrate particles consumed through complexing with metal cations. Thus, the percentage of carbohydrate complexed equals $(\Delta T_0 - \Delta T_s)/\Delta T_0 \times 100$. This equation can be used only for solutions of relatively low salt concentration (around 0.2 *M* or less). At high salt concentration (for example, 1 *M*), not only may all the carbohydrate be in complex form, but more than one cation may be attached to each molecule of carbohydrate. In the latter, the boiling point of a salt solution is actually decreased by the addition of carbohydrate, and a determination of the approximate number of cations attached to the carbohydrate may be accomplished through knowledge of the individual colligative properties of both salt and carbohydrate solutions.

D. Electrophoresis.—The electrophoretic behavior of various carbohydrates in methanolic and ethanolic solutions of alkali and alkaline earth metal salts was examined (Tables I and II). Glass fiber paper was used as the support and chrysene as the reference compound. Both rate and direction of electroosmotic movement, as well as rate of carbohydrate migration, are a function of salt concentration. In the absence of salt, no carbohydrate migration occurs. Adsorption of carbohydrates on glass fiber paper does not occur to any significant extent, and is therefore not a factor affecting migration rates.

Small circles, 4 mm in diameter, were cut from a 57 × 13 cm strip of glass fiber paper at equidistant intervals along a straight line drawn widthwise across the exact center of the strip. The strip was placed on a 38 × 14 cm glass plate, such that 8.5 cm of

---

[1] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[2] A. J. Watters, R. C. Hockett, and C. S. Hudson, *J. Am. Chem. Soc.*, **56**, 2199 (1934).

[3] R. U. Lemieux and H. F. Brown, *Anal. Chem.*, **26**, 920 (1954).

[4] T. E. Timell, C. P. J. Glaudemans, and A. L. Currie, *ibid.* **28**, 1916 (1956).

[5] C. H. VanEtten and M. B. Wiele, *ibid.*, **25**, 1109 (1953).

TABLE I

ELECTROPHORETIC BEHAVIOR OF CARBOHYDRATES IN METHANOLIC SOLUTIONS OF VARIOUS SALTS[a]

	No salt		KOAc				NaOAc				NaI			
Compound	°C	Rate, mm/hr	°C	Salt concn, M	Rate, mm/hr	M_R	°C	Salt concn, M	Rate, mm/hr	M_R	°C	Salt concn, M	Rate, mm/hr	M_R
Chrysen (nonmigrating)	27	−6.0	42	0.3	+2.3		37	0.3	−3.0		38	0.03	−11.8	
	43	−12.3	48	0.3	+2.8						38	0.15	+1.2	
D-Ribose	27	−0.0	42	0.3	−7.3	1.00	37	0.3	−9.4	1.00	38	0.03	−6.6	1.00
											38	0.15	−15.2	1.00
											40	0.30	−15.8	1.00
	43	−0.0	48	0.3	−10.2	1.00	38	0.3	−9.3	1.00	47	0.15	−15.7	1.00
D-Arabinose			48	0.3	−9.1	0.90	38	0.3	−7.8	0.83	47	0.15	−11.6	0.74
D-Lyxose			48	0.3	−7.4	0.73	38	0.3	−6.0	0.65	47	0.15	−11.0	0.70
D-Xylose			42	0.3	−4.5	0.60	38	0.3	−4.7	0.50	38	0.03	−4.0	0.61
			48	0.3	−6.0	0.58								
D-Fructose			42	0.3	−10.2	1.00	37	0.3	−7.9	0.84	47	0.15	−13.4	0.85
D-Mannose														
D-Galactose														
α-D-Glucose			42	0.3	−4.8	0.64	38	0.3	−4.3	0.46	47	0.15	−10.1	0.64
L-Rhamnose														
Maltose			42	0.3	−4.8	0.64								
Sucrose			42	0.3	−4.1	0.58								
Raffinose							37	0.3	−4.3	0.46				
Melezitose							37	0.3	−3.4	0.36				
1,6-Anhydro-β-D-glucopyranose							37	0.3	−12.3	1.31				
Methyl α-D-mannopyranoside			42	0.3	−6.7	0.94								
Methyl α-D-glucopyranoside			42	0.3	−4.5	0.63								
Methyl β-D-glucopyranoside			42	0.3	−4.4	0.62								
D-Glucitol			42	0.3	−5.2	0.69								
Erythritol			42	0.3	−4.0	0.56								

[a] Rates listed for chrysene (nonmigrating reference compound) correspond to the rates of electroosmotic flow. Rates for carbohydrates are migration rates (migration rate = rate of total movement from initial spotting position less rate of electroosmotic flow).

TABLE II

ELECTROPHORETIC BEHAVIOR OF CARBOHYDRATES IN ETHANOLIC SOLUTIONS OF ALKALI METAL SALTS[a]

	No salt		KOAc				NaI				LiCl			
Compound	°C	Rate, mm/hr	°C	Salt concn, M	Rate, mm/hr	M_R	°C	Salt concn, M	Rate, mm/hr	M_R	°C	Salt concn, M	Rate, mm/hr	M_R
Chrysene (nonmigrating)	33	−7.1	33	0.3	+0.83		33	0.15	+5.5		33	0.3	+1.11	
							34	0.3	+5.2					
D-Ribose	33	0.0	33	0.3	−2.6	1.00	33	0.15	−9.1	1.00	33	0.3	−1.0	1.00
							34	0.3	−9.8	1.00				
D-Arabinose			33	0.3	−2.4	0.95	33	0.15	−7.3	0.80	33	0.3	−0.3	0.3
D-Lyxose			33	0.3	−2.3	0.91	33	0.15	−6.8	0.75	33	0.3	0	0
D-Xylose			33	0.3	−2.0	0.78	34	0.3	−7.7	0.79	33	0.3	0	0
α-D-Glucose			31	0.3	−2.1	0.74	34	0.3	−8.0	0.82				
Erythritol			33	0.3	−1.3	0.51								
2,3,4,6-Tetra-O-methyl-D-glucose							33	0.15	−3.1	0.34	33	0.3	−0.3	0.3

[a] See footnote for Table I.

the paper hung free at each end. By means of a pipet, the paper was rapidly and evenly moistened with alcoholic salt solution of the desired concentration. One of the circle cutouts was impregnated with alcoholic chrysene solution and inserted into one of the holes in the paper strip. Each of the remaining cutouts was impregnated with an alcoholic carbohydrate solution (0.05–0.08 M, solubility permitting) and placed in a separate hole in the strip. A second glass plate, identical with the first, was immediatedly placed on top of the strip to form a "sandwich," which was then sealed together lengthwise along the edge with masking tape to prevent evaporation of solvent. Spring clamps, one near each corner, held the sandwich tightly together. The combination was placed on a level metal support so that the protruding ends of the paper strip hung free and could be immersed in alcoholic salt solutions in glass tanks equipped with platinum wire electrodes. The entire apparatus was finally wrapped with a polyethylene sheet.

After a 30-min period to allow equilibrium to be reached (no spot movement occurred during this period), a potential of 16.7 v/in. was applied across the paper for periods varying from 5 to 46 hr. The paper strip was then removed and hung vertically for 30 min to dry. At 30-sec intervals, for 10 min after drying commenced, the strip was inverted to prevent gravitational movement of the spots The dried strip was then examined under ultraviolet light to determine the position of the chrysene spot (not visible while wet). Positions of carbohydrate spots were disclosed by means of the proper spray reagent.

E. Polarimetry.—Optical rotations of ethanolic solutions of methyl β-D-glucopyranoside and potassium acetate were measured on a polarimeter (±0.02° precision) in 4-dm tubes at 20°

NaBr				NaCl				$CaCl_2$				$MgCl_2 \cdot 6H_2O$			
°C	Salt concn, M	Rate, mm/hr	M_R	°C	Salt concn, M	Rate, mm/hr	M_R	°C	Salt concn, M	Rate, mm/hr	M_R	°C	Salt concn, M	Rate, mm/hr	M_R
38	0.15	−1.5		34	0.03	−12		39	0.15	+12.2		44	0.15	+13	
				34	0.15	−1									
38	0.15	−12.7	1.00	34	0.03	−6.6	1.00	39	0.15	−25.4	1.00	44	0.15	−4.1	1.00
				34	0.15	−10.3	1.00								
				34	0.15	−7.3	0.71	39	0.15	−16.8	0.66	44	0.15	−1.7	0.41
				34	0.15	−6.5	0.63	39	0.15	−21.0	0.83	44	0.15	−1.2	0.29
38	0.15	−7.2	0.57	34	0.03	−3.8	0.58	39	0.15	−14.0	0.55	44	0.15	−0.8	0.2
				34	0.15	−5.7	0.55								
38	0.15	−9.9	0.78					39	0.15	−18.4	0.72	44	0.15	−2.9	0.71
38	0.15	−9.1	0.72												
38	0.15	−8.2	0.65	34	0.15	−5.6	0.54	39	0.15	−10.2	0.40	44	0.15	−1.7	0.41
38	0.15	−6.5	0.51												
				34	0.15	−6.2	0.60								

A − or + before a rate value indicates that the movement is toward the cathode or anode, respectively. Temperatures listed are the maximum reached by the "sandwich" during electrophoresis. Voltage potential, 16.7 v/in.; M_R, rate of migration relative to D-ribose.

(Table III). The glucoside–salt solutions containing from 0.3 to 0.8 mole fraction of glucoside were metastable; adduct precipitated from these mixtures after varying lengths of time, the tendency to precipitate being greatest for the 1:1 mixture (0.5 mole fraction). Precipitates from solutions of mole fractions 0.5 and 0.8 were isolated, washed with a 3:1 ether–ethanol mixture, dried overnight under vacuum, and analyzed for potassium content.

TABLE III

EFFECT OF POTASSIUM ACETATE ON THE OPTICAL ROTATION OF METHYL β-D-GLUCOPYRANOSIDE IN ETHANOL[a]

Concn of glucoside, moles/l.	α_1	α_2	$\Delta\alpha$
0.030	−0.87	−0.72	0.15
0.060	−1.71	−1.50	0.21
0.090	−2.54	−2.25	0.29
0.120	−3.37	−2.99	0.38
0.150	−4.17	−3.78	0.39
0.180	−4.85	−4.53	0.32
0.210	−5.56	−5.28	0.28
0.240	−6.25	−6.06	0.19
0.270	−6.90	−6.79	0.11
0.300		−7.54	

[a] α_1 and α_2 represent degrees of rotation for solutions with and without potassium acetate, respectively. Combined concentration = 0.300 M. Sodium light (5893 A) was employed.

Anal. Calcd for a 1:1 glucoside–potassium acetate adduct (assuming no ethanol of solvation): K, 13.4. Found for an adduct from 0.5 mole fraction mixture: K, 13.4. Found for an adduct from 0.8 mole fraction mixture: K, 13.3.

Preparation of Carbohydrate–Salt Adducts.—Brief preparative procedures are given in Table IV. Volumes of solvent, not listed, vary according to the solubility of reactants and product. Care was taken not to create a condition whereby free carbohydrate or salt would coprecipitate with the adduct. Because of the generally low solubility of adducts in alcoholic media and because combining ratios are often a function of salt concentration, no attempts were made to purify the adducts by recrystallization. All ratios in Table IV were calculated on the basis of either a metal or an anion analysis. Small deviations from whole numbers in these ratios are probably due largely to impurities (carbohydrate or salt) occluded to the precipitated complex. Alcohol of solvation not removed in the drying process is generally very small and should have only a slight effect on calculated ratios.

Results and Discussion

In alcoholic media, polyhydroxy compounds react with alkali metal salts of the uni-univalent type to give crystalline (birefringent) adducts whose bonding can be attributed to weak ion–dipole forces of attraction between metal ion and two or more hydroxyl groups of each donor moiety (reactions 1 and 2). The ability

$$n\text{ROH} + \text{M}^+ \rightleftharpoons \text{M}^+ \cdot n\text{ROH} \qquad (1)$$

$$n = 1 \text{ or } 2;\ \text{ROH} = \text{carbohydrate}$$

$$\text{ROH} + n\text{M}^+ \rightleftharpoons \text{ROH} \cdot n\text{M}^+ \qquad (2)$$

$$n > 1$$

of kojic acid to form an adduct with potassium acetate indicates that carbonyl groups can also participate as donors. The weak nature of the linkage and the rapid reversibility of the reaction in solution are indicated not only by the mass action effect on extent of complexing, as shown by ebulliometric studies (Table V),

TABLE IV
SALT ADDUCTS ISOLATED FROM NONAQUEOUS MEDIA

Adduct	Combining ratio,[a] ligand:salt	Analysis: % metal	Analysis: % anion	Analysis: % alcohol of solvation	Method of preparation[b]	Molar ratio of polyhydroxy compound to salt in reaction mixture
α-D-Glucose–potassium acetate	2.3:1	...	11.5	...	I	4:1
	1.0:1	14.7	...	...	III	1:2
α-D-Glucose–sodium bromide	2.0:1	...	17.6	1.3	II	1:10
α-D-Glucose–sodium formate	2.3:1	4.77	10.4	...	III	4:1
α-D-Glucose–sodium iodide	2.2:1	...	23.4	...	II	1:5
Kojic acid–potassium acetate	1.0:1	16.1	22.0	...	IV	2:1
D-Mannose–potassium acetate	0.9:1	...	22.3	3.9	II	1:5
D-Mannose–sodium iodide	1.0:1	...	38.7	...	II	1:5
Methyl β-D-glucopyranoside–potassium acetate	1.0:1	13.4	...	...	II	1:1
Sucrose–potassium acetate	2.2:1	...	6.84	...	III	2:1
Sucrose–potassium acetate	1:1.9	...	21.1	...	III	1:20
Sucrose–sodium propionate	2.2:1	2.72	9.82	...	III	2:1

[a] Alcohol of solvation is generally very small and was assumed to be negligible in calculating combining ratios. [b] I: A solution of salt in ethanol was added to a solution of carbohydrate in a mixture of 2-pyrrolidinone, ethanol, and ethyl acetate. II: Salt was dissolved in a solution of carbohydrate in ethanol; addition of ether was, in most instances, necessary to effect precipitation. III: Ether (or ethyl acetate) was added to a solution of carbohydrate and salt in a mixture of ethanol and 2-pyrrolidinone or *N*-methyl-2-pyrrolidinone. IV: Ether was added to a solution of polyhydroxy compound and salt in methanol. Precipitates were washed with ethanol-ether or ethanol-ethyl acetate and then dried overnight under vacuum at 25°. Gas chromatographic analysis of adducts prepared from α-D-glucose showed that the sugar moiety was largely (>90%) in the α form. All adducts were birefringent.

TABLE V
APPARENT PERCENTAGE OF CARBOHYDRATE COMPLEXED WITH ALKALI METAL SALTS IN ALCOHOLIC SOLUTION AT REFLUX TEMPERATURE[a]

Carbohydrate	Salt	Molality of solution: Carbohydrate	Molality of solution: Salt	% carbohydrate complexed: EtOH	% carbohydrate complexed: MeOH
1,6-Anhydro-β-D-glucopyranose	KI	0.0639	0.0639	27	
1,3:4,6-Dimethylene-D-mannitol	KI	0.0639	0.0639	1	
Erythritol	KI	0.0639	0.0639	8	
D-Glucitol	KI	0.0797	0.0338	6	
	KI	0.0797	0.0507	14	
	KI	0.0639	0.0639	21	
	KI	0.0639	0.1278	36	
D-Glucose	KI	0.0797	0.0507	14	
	KI	0.0639	0.0639	25	9
	KI	0.0639	0.1278	34	
	KI	0.0639	0.1489	42	
D-Mannose	KI	0.0639	0.0639	21	
Methyl α-D-glucopyranoside	KI	0.0639	0.0639	16	
	KOAc	0.0639	0.0639	19	
	NaOAc	0.0639	0.0639	15	
	LiCl	0.0639	0.0639	0	
Methyl β-D-glucopyranoside	KI	0.0639	0.0639	17	2
	KI	0.0467	0.176		19
	KOAc	0.0639	0.0639	21	
L-Rhamnose	KI	0.0639	0.0639	13	
D-Ribose	KI	0.0639	0.0639	21	
Sucrose	KI	0.0467	0.0467		5
2,3,4,6-Tetra-*O*-methyl-D-glucose	KI	0.0639	0.0639	0	

[a] From ebulliometric measurements at atmospheric pressure.

but also by the fact that the half-wave potential for potassium ion in ethanol ($E_{1/2}$ at 25°, −1.83 v) was virtually unaffected by the presence of a large excess of methyl β-D-glucopyranoside ($E_{1/2}$ at 25° for K^+ in the presence of the glycoside was −1.85 v). The heights and shapes of the two polarographic curves were the same.

In Table IV are listed various alkali metal salt adducts isolated from alcoholic media. Only sodium bromide·2α-D-glucose,[6] sodium iodide·2α-D-glucose,[7] and potassium acetate·methyl β-D-glucopyranoside[2] have been previously reported in the literature.

Generally the maximum number of carbohydrate ligands per metal cation in an isolated adduct is two. In some, a maximum of one is found, as in potassium acetate·methyl β-D-glucopyranoside. When carbohydrate-to-metal combining ratios greater 1:1 than are possible, the adducts with ratios greater than 1:1 are best isolated from solutions with concentration of carbohydrate higher than that of salt. Increasing the concentration and proportion of salt can lead to lower ratios. The production of 2:1 and 1:2 sucrose–potas-

---

[6] M. Hönig and M. Rosenfeld, *Chem. Ber.*, **10**, 871 (1877).

[7] J. A. Wülfing, German Patent 196,605 (March 16, 1907).

sium acetate adducts exemplifies this concentration effect. There are two possible explanations for the variability of combining ratio: (1) The coordination number of the cation may be sufficiently large to accommodate more than one carbohydrate molecule and (2) the presence of a large number of hydroxyl (donor) groups in a single carbohydrate molecule may allow, under the proper concentration conditions, more than one cation to become attached to that molecule.

High salt concentrations can increase the solubility of a carbohydrate in alcohol. For example, at 25°, the solubility of sucrose in absolute ethanol is less than 0.1 g per 100 ml of solvent. If a 1 *M* solution of potassium acetate in ethanol is used as solvent, the solubility is increased to approximately 3 g per 100 ml. At least two possible mechanisms can be postulated to explain such phenomena, and both are based upon the formation of *polycation adducts* (adducts possessing more than one cation per carbohydrate molecule). First, intermolecular hydrogen bonding between carbohydrate molecules would be less extensive, the greater the degree of formation of polycation adduct. A decrease in hydrogen bonding could increase the carbohydrate solubility. Secondly, the ions in a polycation adduct, solvated by alcohol, would impart a more solventlike character to the carbohydrate and thereby increase its solubility.

Alkali metal halides form adducts of 2:1 carbohydrate-to-salt ratio with greater facility than do acetates, as evidenced by the ability of α-D-glucose and sucrose to form such complexes in the presence of excess halide (Table IV). In the presence of a similar amount of metal acetate, these same sugars can give preponderantly 1:1 adduct. The nature of the anion in a carbohydrate–salt complex therefore appears to have an influence upon the ease with which an alkali metal ion can accommodate a second carbohydrate ligand.

The preparation of complexes in alcoholic media is often hampered by low carbohydrate solubility. Solubility can be increased and the preparation of complexes facilitated by employing either 2-pyrrolidinone or *N*-methyl-2-pyrrolidinone as a cosolvent. These solvents are not recommended for use in the preparation of metal halide adducts because of their tendency to complex with the adducts, possibly with the halide ion itself. This tendency is not apparent with adducts of alkali metal formate, acetate, and propionate.

Ebulliometric Studies.—Ebulliometric measurements provided information concerning the extent to which carbohydrates complex with alkali metal salts in boiling alcoholic media. The percentages of carbohydrate complexed were calculated with the reasonable assumption that there is negligible formation of polycation adduct (reaction 2, where $n > 1$) in dilute salt solution. Furthermore, I assumed that the degree of dissociation of uncomplexed metal salt into free ions does not differ significantly from that of complexed salt. Although this assumption may not be strictly valid, it was necessary to facilitate the calculation of approximate values for the extent of complexing. There is at this time no obvious way to distinguish quantitatively between reactions 1 and 3. Reaction 4 may be disregarded because electrophoretic studies (described later) strongly suggest that it is almost, if not entirely, nonexistent.

$$n\mathrm{ROH} + \mathrm{M^+A^-} \rightleftharpoons n\mathrm{ROH}\cdot\mathrm{M^+A^-} \qquad (3)$$

$$n = 1 \text{ or } 2;\ \mathrm{A^-} = \text{anion}$$

$$n\mathrm{ROH} + \mathrm{A^-} \rightleftharpoons n\mathrm{ROH}\cdot\mathrm{A^-} \qquad (4)$$

Apparent percentages of carbohydrate complexed in alcoholic salt solution are given in col 5 and 6 of Table V. Differences of less than 5% between the value for one carbohydrate and that for another under identical concentration conditions are probably not meaningful because of the limited precision of ebulliometric analysis. Nevertheless, the data adequately show the ease with which sodium and potassium ions complex with carbohydrates possessing two or more hydroxyl groups in close proximity to one another. They also show that the extent of complexing in solution is a function of salt concentration and is probably greater in ethanol than in methanol. That carbohydrates need not be cyclic to possess high complexing ability is indicated by the close similarity in the extent to which D-glucitol and D-glucose react with potassium iodide in ethanol. Although the percentages in col 5 and 6 are probably closely related to complexing ability, stability constants have not been calculated from these data because of the general lack of knowledge concerning combining ratio in alcoholic solution. Only for methyl β-D-glucopyranoside in ethanolic potassium acetate solution is there reasonably reliable information on combining ratio (see Polarimetric Studies). Use of Table V to determine relative complexing abilities (rough approximations, at best) requires that only those results for solutions of identical salt and carbohydrate concentrations be compared.

The inability of methyl α-D-glucopyranoside to combine measurably with lithium chloride under the conditions employed indicates not only that $\mathrm{Li^+}$ has weak complexing ability compared with $\mathrm{Na^+}$ and $\mathrm{K^+}$, but also that free chloride ions have little, if any, tendency to complex. On the one hand, the weak ability of $\mathrm{Li^+}$ might be due to its enclosure by a tightly bound, difficultly penetrable or displaceable shell of solvent molecules. On the other hand, $\mathrm{Na^+}$ and $\mathrm{K^+}$, which are larger in radius than $\mathrm{Li^+}$, are less firmly solvated and undergo considerable complex formation. The low reactivity of 2,3,4,6-tetra-*O*-methyl-D-glucose toward potassium iodide and the extraordinary ease with which most polyhydroxy compounds react with salts of sodium and potassium, even at low carbohydrate concentration, strongly suggest that the main interaction of polyhydroxy compounds with alkali metal salts is the formation of chelates rather than simple complexes involving only one donor group per carbohydrate molecule.

The presence of polycation complexes in homogeneous solution at high salt concentration has been demonstrated ebulliometrically. The addition of sucrose (2.92 mmoles) to a 1.03 *m* solution of potassium acetate in ethanol (59.2 g) causes a large decrease in boiling point ($\Delta t = -0.091°$). Calculations indicate apparently 2.5 potassium ions attached to each carbohydrate molecule. This 2.5:1 ratio agrees roughly with the 1.9:1 ratio for a potassium acetate–sucrose adduct isolated at room temperature under similar concentration conditions.

Electrophoretic Studies.—The use of anhydrous alcoholic media appears to have been ignored by other workers employing electrophoresis. The author has found that electrophoretic phenomena in alcoholic media are similar to those in aqueous media. In the *absence* of a dissolved electrolyte, with either a cellulose or a glass fiber paper support, electroosmotic movement of either water or alcohol is in the direction of the cathode. Mechanisms that have already been postulated to explain the movement of water[8] are applicable to alcoholic systems.

In alcoholic solutions of alkali and alkaline earth metal salts, cations appear to be adsorbed by the support in preference to anions. Electroosmotic flow of alcohol can be either decelerated, stopped, or reversed in direction by means of a suitable salt concentration. A similar effect of electrolyte on the movement of water was reported by Elissafoff.[9]

In alcoholic media, there is no migration of carbohydrate in the absence of dissolved electrolyte. In the presence of uni-univalent salts of sodium and potassium, migration toward the cathode occurs, the velocity being slightly greater in methanol than in ethanol. The faster migration in methanol may be caused, at least partly, by the higher degree to which salts are dissociated into free ions in this medium. The influence of alkali metal halide on carbohydrate migration rate increases in the order: sodium chloride < sodium bromide < sodium iodide. This order is the same as that found by Izmaĭlov and co-workers[10] for the increasing degree of dissociation of sodium halides in methanol. At salt concentrations below 0.15 M, carbohydrate migration rates vary considerably. The lower the concentration, the lower is the rate. Above 0.15 M, variation of rate with concentration is small. This behavior at high concentration gives reproducible data. Relative rates of migration (M_R) vary only slightly, if at all, with salt concentration over the entire range studied (0.03–0.3 M), nor do moderate variations in carbohydrate concentration have significant effect upon rates of migration, provided the salt concentration is relatively high.

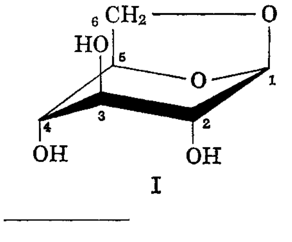
Some mixtures of carbohydrates are separated by electrophoresis in alcoholic media. For example, a mixture of D-ribose (M_R 1.00) and D-xylose (M_R 0.6) separates easily in methanolic 0.3 M potassium acetate solution with no apparent alteration of the individual migration rates. Where M_R values for different carbohydrates in alkali metal salt solutions are similar, a satisfactory separation might be possible if an alkaline earth metal salt is chosen as the electrolyte. M_R values in solutions of alkaline earth metal salts do not necessarily resemble or parallel M_R values for corresponding compounds in solutions of alkali metal salts (Table I).

Tables I and II contain the conditions and results of various electrophoreses with glass fiber paper and alcoholic solutions of alkali and alkaline earth metal salts. Comparing relative rates in methanol with those in ethanol shows that methanol is the better solvent in which to separate carbohydrate mixtures. The wider spread of M_R values in methanol, compared with ethanol, is probably caused by greater dissociation (instability) of complexes in methanol.

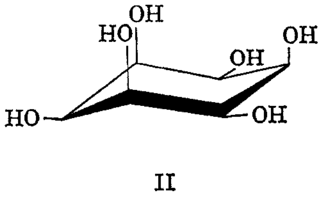
Because factors other than complex stability can influence migration rate, M_R values are not a reliable gauge of relative complex stability. However, electrophoretic studies provide information on relative abilities of alkali metal ions to form complexes with polyhydroxy compounds. The conclusions drawn from these data closely resemble those from ebulliometric data. Sodium and potassium ions possess similar complexing abilities since M_R values for carbohydrates in a sodium salt solution do not generally differ much from those in a potassium salt solution. Had there been any great difference in complexing ability between Na^+ and K^+, the less reactive, and therefore more selective, cation would probably have given a much wider range of M_R values than the more reactive cation. Unlike Na^+ and K^+, Li^+ causes relatively little carbohydrate migration. In ethanolic lithium chloride solution, some carbohydrates appear not to move at all. This immobility may well be due to low stability of lithium chelates.

The similarity between M_R values for a particular carbohydrate in methanolic solutions of sodium acetate, sodium chloride, sodium bromide, and sodium iodide indicates that free univalent anions (nonbasic) complex not at all or only very weakly in alcoholic solution. Mills[11] obtained electrophoretic evidence that sulfate ion complexes weakly with cyclitols and alditols in *aqueous* solution; however, he detected no complexing with acetate, nitrate, and perchlorate ions. In contrast, polarimetric studies of Wiklund[12] and of Ramaiah and Vishnu[13] strongly indicate that anions are involved in the formation of carbohydrate complexes in solution. In view of the electrophoretic results, this involvement may be interpreted as a formation of electrically neutral complexes. Such uncharged complexes could originate by either one of two possible processes, or both: (1) addition of an undissociated salt molecule to a carbohydrate (reaction 3) or (2) addition of a free ion (most likely a cation) to a carbohydrate to form a charged species that subsequently combines with an oppositely charged ion to give an electrically neutral adduct. Conceivably these neutral complexes could comprise a substantial part of all the complex species present in either aqueous or alcoholic media. Uncharged species cannot be detected electrophoretically, and their formation should not greatly influence relative migration rates.

There is no obvious correlation between electrophoretic behavior and carbohydrate structure, although the behavior of levoglucosan (1,6-anhydro-β-D-glucopyranose, I) is an exception. The relative migration rate of levoglucosan is extremely high; yet only two of



I



II

---

[8] H. R. Kruyt, "Colloids," John Wiley and Sons, Inc., New York, N. Y., 1930, pp 102–109.

[9] G. von Elissafoff, *Z. Physik. Chem.*, **79**, 385 (1912).

[10] N. A. Izmaĭlov, E. I. Vaĭl, and N. N. Salatnikov, *Uch. Zap. Khar'kovsk. Gos. Univ. Tr. Khim. Fak. i Nauchn.-Issled. Inst. Khim.*, **71**, No. 14, 29 (1956).

[11] J. A. Mills, *Biochem. Biophys. Res. Commun.*, **6**, 418 (1961/62).

[12] O. Wiklund, *Zucker*, **8**, 266 (1955).

[13] N. A. Ramaiah and Vishnu, *Sharkara*, **2**, 3 (1959).

its three hydroxyl groups can possibly chelate with a metal ion. The *cis*-axial orientation of these two hydroxyls, located at C-2 and C-4, and the inflexibility of the carbohydrate ring appear to favor high complex stability and, consequently, a high M_R value. *cis*-Inositol (II), which has hydroxyl groups similarly positioned and oriented, is known to have exceptional mobility in *aqueous* solutions of alkali metal salts.[11]

Mills[11] has shown that carbohydrates such as aldoses and alditols have low migration rates in aqueous solutions of alkali metal salts. The order of decreasing effectiveness in promoting carbohydrate movement was $Na^+ > K^+ > Li^+ > NH_4^+$. Present studies in *alcoholic* media show an almost identical order, $Na^+ > K^+ > NH_4^+ > Li^+$, but the migration rates are greater in alcoholic than in aqueous media.

The ease with which carbohydrates complex with alkali metal cations in alcohol, relative to their ease of complexing in water, may be biologically significant. The environment in cell walls undoubtedly is largely nonaqueous, which condition favors the formation of complexes between polyhydroxy compounds and alkali metal cations.

Polarimetric Studies.—The effect of dissolved alkali metal salts upon the optical rotation of carbohydrates is greater in alcohol than in water, the influence increasing in the order water < methanol < ethanol. Wiklund[12] and Ramaiah and Vishnu[13] made use of Job's principle[14] and the property of optical rotation to show that the predominant combining ratio for adducts formed in homogeneous *aqueous* solution from the reaction of various alkali metal halides with either sucrose or D-fructose is 1:1. Wiklund also observed the formation of a 1:1 adduct from sodium acetate and sucrose. In the present study, methyl β-D-glucopyranoside and potassium acetate were chosen as reactants because of their relatively high solubilities in ethanol.

According to Job's principle, at a fixed combined molar concentration of salt and carbohydrate, the change in optical rotation effected by a salt is maximal when the ratio of total carbohydrate (free and complexed) to total salt in the solution equals the ratio of carbohydrate to salt in the adduct. Ethanolic solutions were thus prepared in which the mole fraction of salt varied from zero to one. The combined concentration of carbohydrate and salt was 0.3 *M* in each solution. The optical rotation was measured rapidly because the majority of the solutions were metastable. After the measurements, the solutions were shaken to induce precipitation. Precipitates from those solutions with carbohydrate-to-salt ratios of 1:1 and 4:1 were isolated and found to be 1:1 adducts. The polarimetric analyses, given in Table III, indicate that predominantly a 1:1 adduct is formed in homogeneous ethanolic solution, in complete harmony with the fact that only a 1:1 adduct was isolated under two widely differing sets of concentration conditions.

Unfortunately low carbohydrate solubility precludes a similar study of α-D-glucose and sucrose. Each of these compounds is capable of forming adducts of 1:1 and 2:1 carbohydrate-to-salt ratio.

Acknowledgment.—The author is indebted to Mr. J. E. Hodge for general supervision and to Mrs. Clara E. McGrew and Mrs. Bonita Heaton for the microanalyses.

[14] P. Job, *Ann. Chim.*, **9**, 113 (1928).

Alkali Metal Complexes of Carbohydrates. II. Interaction of Bases with Carbohydrates in Alcoholic Media[1]

JACOB A. RENDLEMAN, JR.

The Northern Regional Research Laboratory,[2] Peoria, Illinois 61604

Received September 20, 1965

Reaction between alkali metal hydroxides and carbohydrates in anhydrous alcoholic media at 25° produces isolable carbohydrate alcoholates, probably chelates. A minor fraction of the product appears to be a carbohydrate–alkali metal hydroxide adduct. Adducts are produced exclusively if the reaction medium contains 10% or more water. Some adducts can be thermally converted to alcoholates, provided the carbohydrate moiety does not simultaneously decompose. Under the proper conditions, most alcoholates combine with one or, in rare instances, two additional molecules of carbohydrate. Reactions that produce carbohydrate–metal hydroxide adducts show a stoichiometry of even greater variability. Combining ratio is a function of cation radius, hydroxide concentration, and carbohydrate geometry. Alkali metal cyanides react with carbohydrates in ethanolic media to give a mixture of carbohydrate alcoholate and carbohydrate–metal cyanide adduct. The alcoholate predominates. Sodium 1-butoxide, prepared from sodium hydroxide and 1-butanol by removing water of formation by azeotropic distillation, reacts with methyl α-D-glucopyranoside in refluxing 1-butanol to give the monosodium alcoholate.

The reaction of simple carbohydrates with alkali metal hydroxides and alkoxides in alcoholic and aqueous alcoholic media has been described often in the literature. In only a few instances has there been any degree of certainty regarding the composition of the isolated reaction product. Various investigators have described the products from reactions with hydroxides as either alcoholates (carbohydrate salts formed by the loss of one or more hydroxylic protons)[3] or carbohydrate–metal hydroxide adducts. With one possible exception, no one has ever offered sufficient evidence to permit the two types of product to be differentiated.

---

[1] Part I: J. A. Rendleman, Jr., *J. Org. Chem.*, **31**, 1839 (1966).

[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

[3] The term "alcoholate" is used rather than "alkoxide" to differentiate between the oxyanion of a carbohydrate and that of a monohydric alcohol. In no instance does "carbohydrate alcoholate" signify a carbohydrate solvated with alcohol.