Margolis, R. U. (1967), *Biochim. Biophys. Acta 141*, 91.
Mathews, M. B., and Cifonelli, J. A. (1965), *J. Biol. Chem. 240*, 4140.
Narahashi, Y., and Yanagita, M. (1967), *J. Biochem. (Tokyo) 62*, 633.
Nemoto, T., and Yosizawa, Z. (1969), *Biochim. Biophys. Acta 192*, 37.
Pamer, T., Glass, G. B. J., and Horowitz, M. I. (1968), *Biochemistry 7*, 3821.
Rees, D. A. (1963), *Biochem. J. 88*, 343.
Ryan, L. C., Carubelli, R., Caputto, R., and Trucco, R. E. (1965), *Biochim. Biophys. Acta 101*, 252.
Singh, M., and Bachhawat, B. K. (1968), *J. Neurochem. 15*, 249.
Spencer, B. (1960), *Biochem. J. 75*, 435.
Suzuki, K. (1965), *J. Neurochem. 12*, 629.
Swann, D. A., and Balazs, E. A. (1966), *Biochim. Biophys. Acta 130*, 112.
Szabo, M. M., and Roboz-Einstein, E. (1962), *Arch. Biochem. Biophys. 98*, 406.
Whistler, R. L., and Durso, D. F. (1950), *J. Amer. Chem. Soc. 72*, 677.
Yamagata, T., Saito, H., Habuchi, O., and Suzuki, S. (1968), *J. Biol. Chem. 243*, 1523.

Synthesis of Cellulose Derivatives Containing the Dihydroxyboryl Group and a Study of Their Capacity to Form Specific Complexes with Sugars and Nucleic Acid Components*

H. L. Weith, J. L. Wiebers, and P. T. Gilham

ABSTRACT: Carboxymethylcellulose can be converted into *N*-(*m*-dihydroxyborylphenyl)carbamylmethylcellulose by reaction of its azide with an aqueous solution of *m*-aminobenzeneboronic acid, and aminoethylcellulose reacts with an aqueous solution of *N*-(*m*-dihydroxyborylphenyl)succinamic acid in the presence of *N*-cyclohexyl-*N'*-β-(4-methylmorpholinium)ethylcarbodiimide *p*-toluenesulfonate to yield *N*-[*N'*-(*m*-dihydroxyborylphenyl)succinamyl]aminoethylcellulose. These two cellulose derivatives have been shown to form specific complexes of variable stabilities with nucleic acid components, sugars, and other polyols. In chromatography on columns prepared from these celluloses the retention volume of a particular polyol depends on (i) the availability in the compound of a glycol group with the appropriate configuration and conformation, (ii) the pH of the elution solvent, (iii) the ionic strength and the nature of the cations in the elution solvent, and, in the case of nucleosides, (iv) the nature of the base attached to the glycol group. The application of these observations to studies on the fine structure of nucleic acids is indicated.

The unsubstituted 2',3'-diol groups which are located at the 3' terminals of RNA molecules and polyribonucleotides possess some unique properties and some of these have already been exploited in structural studies on nucleic acids (Gilham, 1970a). In a recent example of one of these studies a specific reaction exhibited by this diol group is used to effect the direct separation of ribonucleic acid fragments containing the group from those fragments in which one of the terminal hydroxyl groups is substituted with a phosphate group. In this procedure, the diol group of the polynucleotide is specifically oxidized with periodate and the resulting dialdehyde is selectively bound to aminoethylcellulose from which it can be subsequently recovered (Lee *et al.*, 1970). This oxidation reaction can also be used to bind RNA molecules and polyribonucleotides permanently to cellulose, and, in this case, the initial complex formed between the oxidized polynucleotide and the amino groups of aminoethylcellulose is stabilized by reduction with sodium borohydride (Gilham, 1970b).

Another specific property exhibited by the glycol groups of nucleic acid components is that of complex formation with the borate anion and the use of these complexes in the chromatographic and electrophoretic separation of these components has been reviewed (Khym, 1967). The basis of these separations rests on the change in physical characteristics exhibited by the diol in the presence of borate and, in particular, on the extra negative charge attained by the diol in its complexed form. Although these effects have been exploited successfully in the case of nucleosides and mononucleotides the extension of the method to the study of larger molecules, *e.g.*, polynucleotides, may not be too fruitful since the percentage change in physical properties and total negative charge would decrease with increase in chain length of the polymer. In initiating the present work it seemed that the study and exploitation of this type of complex formation with both small and large molecules containing the glycol group could be readily carried out by immobilizing the dihydroxyboryl group through its attachment to an insoluble polymer.

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Solms and Deuel (1957) have prepared an insoluble polymer containing the dihydroxyboryl group with a view to using the material for the selective binding of polyhydroxy compounds. The material was prepared by polymerizing *m*-phenylenediamine and formaldehyde in the presence of *m*-aminobenzeneboronic acid and, although some preliminary studies on the separation of mixtures of sugars were performed, no details of the experiments have been published. Letsinger and Hamilton (1959) have described the preparation of insoluble popcorn-type polymers containing the dihydroxyboryl group. These were prepared by the copolymerization of a derivative of *p*-vinylbenzeneboronic acid, styrene, and diallyl maleate. The interaction of polyhydroxy compounds with such polymers has apparently not been examined. For the present work, in anticipation of using complex formation for the study of larger biologically important molecules, where restricted diffusion of the molecules to and from the incorporated dihydroxyboryl groups might become a problem, it was decided that cellulose powder should be used as the insoluble matrix.

Synthesis of Cellulose Derivatives. The commercially available derivatives of cellulose, carboxymethylcellulose, and aminoethylcellulose were used for the preparation of the dihydroxyboryl derivatives (Figure 1). Carboxymethylcellulose was converted into its hydrazide and then to the azide using modifications of the methods described by Micheel and Ewers (1949). The capacity of this azide to react with aromatic amines in aqueous solution was first tested with aniline. Spectrophotometric analysis of the product showed that the amine was covalently bound to the extent of 0.3 mmole per g of dry cellulose, a yield which corresponded to the substitution of half of the carboxyl groups originally present in the cellulose. The reaction was repeated with *m*-aminobenzeneboronic acid (I) resulting in the formation of the derivative, *N*-(*m*-dihydroxyborylphenyl)carbamylmethylcellulose (II) which contained about 0.2 mmole of dihydroxyboryl group per g of dry cellulose as determined spectrophotometrically and by direct boron analysis. In aqueous suspensions of pH values above 5, this cellulose derivative is anionic in character due to the fact that some of the original carboxyl groups remain unsubstituted, and, since it was expected that, in some circumstances, the study of the interactions of this polymer with certain polyhydroxy compounds might be compromised by the presence of negative charges, an alternative route to the preparation of dihydroxyboryl-substituted celluloses was investigated.

m-Aminobenzeneboronic acid was converted into *N*-(*m*-dihydroxyborylphenyl)succinamic acid (III) by reaction with succinic anhydride, and the condensation of this acid in aqueous solution with aminoethylcellulose was studied using *N*-cyclohexyl-*N'*-β-(4-methylmorpholinium)ethylcarbodiimide *p*-toluenesulfonate as the activating agent. This carbodiimide was originally prepared by Sheehan and Hlavka (1956) as a water-soluble condensing reagent for use in their studies on peptide synthesis. The solubility properties of the reagent together with those of the hydration product which is formed during the reaction were found to facilitate the isolation of the peptide derivative after the condensation step. For the present work it is important to note that, in aqueous solution, the reagent undergoes acid-catalyzed hydration to the corresponding urea. However, it has been shown that, if, in the activation of carboxylic acids and monoalkyl phosphates, the pH of the aqueous reaction mixture is maintained at about 6, a reasonable balance between the rate of destruction of the reagent and the rate of the activation of the acid and the subsequent condensation reaction can be achieved (Naylor and Gilham, 1966). Thus, the exposure of aminoethylcellulose to an aqueous solution of III and the water-soluble carbodiimide at pH 6 gave *N*-[*N'*-(*m*-dihydroxyborylphenyl)succinamyl]aminoethylcellulose (IV). The product contained 0.6 mmole of the dihydroxyboryl group per dry g of cellulose as determined spectrophotometrically and confirmed by boron analysis, and this yield corresponds to a 60% substitution of the amino groups originally present in the cellulose. Thus, in suspensions of neutral pH this cellulose derivative should exhibit the properties expected of a polycation.

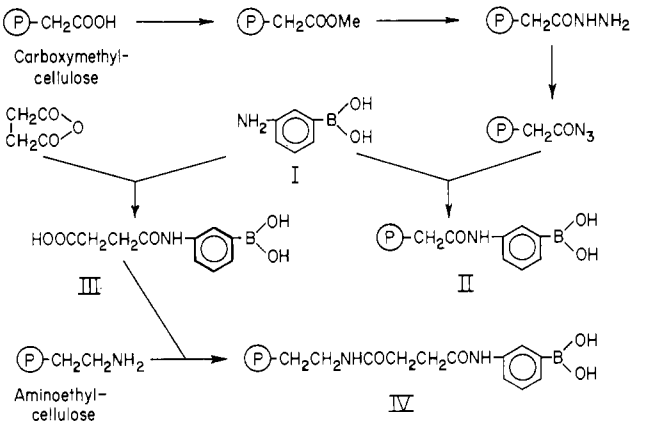


FIGURE 1: Reaction scheme for the preparation of cellulose derivatives.

Dihydroxyboryl groups may also be readily bound to preformed cross-linked polyacrylamide beads. The hydrazide derivative of polyacrylamide prepared according to the methods of Inman and Dintzis (1969) can be converted into its azide and reacted with *m*-aminobenzeneboronic acid as in the preparation of the cellulose derivative, II. In this way, the polyacrylamide originally containing 1 mmole of hydrazide group per dry g gave a product containing 0.3 mmole of dihydroxyboryl group per dry g.

Complex Formation with Nucleosides. In order to determine the binding properties of the incorporated dihydroxyboryl groups in each cellulose, initial studies have been made on their interaction with simple polyhydroxy compounds such as nucleosides and sugars. The elution pattern for the eight ribo- and deoxyribonucleosides obtained at pH 7.5 with a column of the substituted cellulose II is shown in Figure 2. It will be noted that each of the ribonucleosides are retained to a considerable degree when compared with their deoxyribo-counterparts. With preliminary experiments of this type it became apparent that there are four main factors which determine the position of elution of a particular nucleoside: (i) the presence of a *cis*-glycol system in the sugar moiety, (ii) the pH of the elution solvent, (iii) the ionic strength of the solvent, and (iv) the nature of the nucleoside base. It could also be shown that pH and ionic strength of the solvent were operative in determining the position of elution only if the nucleoside contained the ribose moiety. For example, at pH values higher than 7.5 the ribonucleosides are bound increasingly more firmly whereas the retention volumes of the de-

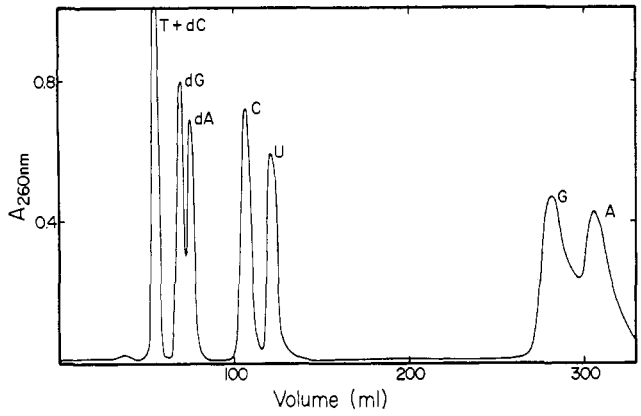
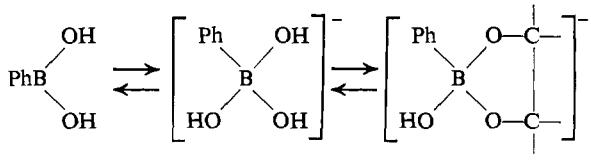


FIGURE 2: Elution pattern obtained from the chromatography of a mixture of thymidine (T), deoxycytidine (dC), deoxyguanosine (dG), cytidine (C), uridine (U), guanosine (G), and adenosine (A) on a column of *N*-(*m*-dihydroxyborylphenyl)carbamylmethylcellulose. The column had dimensions 55 × 1 cm and the elution was effected with 0.1 M sodium phosphate buffer, pH 7.5 at 20° and at a flow rate of about 10 ml/hr.

oxyribonucleosides are essentially unchanged. A comparison of the binding of nucleosides to a column of cellulose derivative II in the presence of 0.1 M and 0.2 M sodium phosphate solution, pH 7.5 has also been made and the retention volumes are listed in Table I. It is clear that the retention volumes for the ribonucleosides were increased by the presence of the higher salt concentration while the retention volumes for the deoxyribose derivatives were again unaffected.

These effects offer some support for the contention that the binding of the ribonucleosides is, at least in part, due to complex formation with the covalently bound dihydroxyboryl groups. The effect of change in pH of the eluting solvent can be understood in terms of the change in the overall concentration of the form of the dihydroxyboryl group which is active in complex formation. Studies on benzeneboronic acid have indicated that the benzeneboronate anion has a tetrahedral structure (McDaniel and Brown, 1955; Torssell, 1957; Lorand and Edwards, 1959) and the p*K* for this ionization has



been reported as 8.86 (Branch *et al.*, 1934). Thus it is to be expected that the concentration of similar anions deriving from the cellulose-bound dihydroxyboryl groups would be dependent on the pH of the surrounding solvent. The observed increase in the binding of ribonucleosides with an increase in solvent pH would be consistent with the concept that the complexes formed also have the tetrahedral configuration and that their concentration is dependent on the concentration of benzeneboronate anions.

The change in retention volumes caused by a change in salt concentration can be accounted for by the same assumption that the tetrahedral boronate anion is the active species in complex formation. Under the conditions used for the chromatography of the nucleosides, the substituted cellulose II

TABLE I: Retention Volumes of Nucleosides on Columns of Dihydroxyboryl-Substituted Celluloses.

Nucleoside	*N*-(*m*-Dihydroxyborylphenyl)carbamylmethylcellulose[a]		*N*-[*N'*-(*m*-Dihydroxyborylphenyl)-succinamyl]aminoethylcellulose[a]	
	0.1 M Sodium Phosphate, pH 7.5, ml	0.2 M Sodium Phosphate, pH 7.5, ml	0.1 M Sodium Phosphate, pH 7.0, ml	0.2 M Sodium Phosphate, pH 7.0, ml
Thymidine, Deoxycytidine	55	55	55	54
Deoxyguanosine	69	68	72	69
Deoxyadenosine	75	76	76	73
Cytidine	106	115	152	135
Uridine	122	133	165	148
Guanosine	282	317	543	469
Adenosine	308	377	630	560

[a] The columns had the dimensions 55 × 1 cm and each separation was carried out at 20° and at a flow rate of 10 ml/hr. The retention volumes of each nucleoside were taken as the volumes of buffer required to elute the nucleoside and were measured from the point of addition of the sample at the top of the column to the point of its peak concentration eluting from the bottom.

would have contained a considerable number of negative charges. These would have arisen both from the remaining carboxyl groups which had not been derivatized and from the benzeneboronate groups themselves. It may be postulated that field effects due to the proximity of neighboring negatively charged groups would tend to raise the p*K* of the ionization of the cellulose-bound dihydroxyboryl groups above that to be expected for the ionization of corresponding unbound dihydroxyboryl derivatives. With an increase in the ionic strength of the solvent, however, these field effects should be diminished, resulting in a decrease in the p*K* of the ionization of the dihydroxyboryl group and the subsequent increase in the extent of complex formation with the polyol, with this increase manifesting itself in an increased retention volume. These effects are analogous to those observed with suspensions of carboxymethylcellulose where a decrease in the p*K* value of the carboxyl group is brought about by changing from a solvent of low ionic strength to one of high ionic strength (Morris and Morris, 1963).

Although there appears to be a small amount of interaction between the deoxyribonucleosides and the substituted cellulose, this effect is not thought to result from complex formation with the dihydroxyboryl groups. The interaction probably arises from other binding forces such as H bonding and these forces are presumably analogous to those discussed by Tomlinson and Tener (1963) in their studies on the interaction of nucleosides with other cellulose derivatives. It will be noted that the deoxyribonucleosides are bound to cellulose

derivative II to different extents as indicated by the large differences in the retention volumes of the pyrimidine and purine compounds. These effects are apparently operative also with the ribonucleosides but, in these cases, the differences in retention volumes are greatly enhanced because of the concomitant boronate complex formation.

Retention volumes of nucleosides on columns of cellulose derivative IV are also listed in Table I. This cellulose contains a greater number of dihydroxyboryl groups and the retention volumes measured at pH 7.5 were excessively large. At a lower pH (7.0), however, the patterns of the retention volumes resemble those obtained with cellulose derivative II. Also, the effects due to changes in pH and the nature of the base in the nucleoside are similar and, here again, the deoxyribonucleosides are apparently unaffected by the presence of the dihydroxyboryl groups. However, with cellulose derivative IV the effect resulting from the change in ionic strength is reversed. In this case, an increase in salt concentration in the solvent causes a reduction in the retention volumes of the ribonucleosides, and this observation can be explained by invoking field effects as before. Cellulose IV contains an excess of positive charges at the pH at which these experiments are run and, in the presence of a solvent of low ionic strength, these charges would be expected to cause the pK of the boronate ionization to be reduced below its normal value. As before, the field effect should be minimized by increasing the ionic strength of the solvent and, in this case, the pK of the ionization should rise with a resultant reduction in the number of boronate anions and the subsequent reduction in retention volumes.

Table II lists the retention volumes for nucleosides on columns of cellulose II under different solvent conditions and, in this table, the effects described above are more dramatically illustrated. There is a large change in the binding of ribonucleosides at pH 7.5 caused by changing the sodium acetate concentration in the solvent from 0 to 1 M and a large increase in the elution volume of uridine caused by increasing the pH of the solvent by one unit. At pH 8.5 the purine ribonucleosides are bound so strongly that they cannot be eluted from the column within reasonable volumes. Again, the retention volumes of the deoxyribonucleosides are practically insensitive to both changes in pH and salt concentration in the solvent.

Complex Formation with Sugars. In the analysis of the binding of various sugars and other polyols it has become apparent that the most important factor is the availability in the polyol of a glycol group containing the required configuration and conformation. The relative degree of binding of polyols follows a pattern similar to that which can be derived from previous studies on the interaction of such compounds with boric acid and its anion. For example, it has been concluded that the enhancement of the conductivity of an aqueous mixture of a polyol and boric acid is probably due to the presence in the polyol of a glycol group with a coplanar oxygen–carbon–carbon–oxygen arrangement together with a cis configuration of the two hydroxyl groups (Böeseken, 1949). For the simple polyols the conductivity increments increase in the order: erythritol, adonitol, arabinitol, xylitol, mannitol, dulcitol, and sorbitol (Steinberg, 1964). It will be noted in Table II that this order is also indicated in the degree to which these polyols bind to cellulose II as expressed by their retention volumes measured at pH 7.5. Thus, it seems that the relative retention volumes of polyhydroxy compounds are determined purely by their content of *cis*-glycol groups possessing the coplanar conformation. The retention volumes of other polyols shown in Table II are also consistent with this view. For example, sucrose and the two inositols which are incapable of locating any two of their hydroxyls in this configuration pass through the column with little or no interaction. Finally, in the case of sugars and other polyols, the secondary binding forces such as those exhibited by the nucleosides are apparently either nonexistent or comparatively insignificant.

TABLE II: Retention Volumes of Nucleosides and Sugars on Columns of *N*-(*m*-Dihydroxyborylphenyl)carbamylmethylcellulose.[a]

Polyol	Volume (ml)		
	0.05 M *N*-Methylmorpholinium-Cl, pH 7.5	0.05 M Morpholinium-Cl, pH 8.5	1 M NaOAc–0.05 M *N*-Methylmorpholinium-Cl, pH 7.5
Thymidine	55	54	56
Deoxycytidine	58		56
Deoxyguanosine	72	65	72
Deoxyadenosine	74		74
Cytidine	78		137
Uridine	82	219	152
Guanosine	145		365
Adenosine	150		400
Erythritol	51		51
Adonitol	51		56
L-Arabinitol	56		76
Xylitol	62		93
D-Mannitol	66		109
Dulcitol	68		111
Sorbitol	92		182
myo-Inositol	51	56	56
(+)-Inositol	52		52
Sucrose	51		52
D-Glucose	52		52
D-Ribose	58		85
D-Fructose	72		128

[a] The columns had the dimensions 55 × 1 cm and each separation was carried out at 20° and at a flow rate of 10 ml/hr. The retention volumes of each polyol were taken as the volumes of buffer required to elute the polyol and were measured from the point of addition of the sample at the top of the column to the point of its peak concentration eluting from the bottom.

Conclusion. The two cellulose derivatives containing the dihydroxyboryl group were prepared initially for the study of nucleic acid primary structure. The information that has been obtained with regard to the interaction of nucleosides and polyols with these celluloses has permitted the extension

of the technique to the study of oligo- and polynucleotides (Rosenberg *et al.*, 1970). It has been found that an oligo- or polynucleotide can be selectively retained by columns of these celluloses and that its retention volumes are dependent on (i) the availability in the polynucleotide of a glycol group with the appropriate configuration (*e.g.*, an unsubstituted 2′,3′-diol group at its 3′ terminus), (ii) the nature of the nucleotide bases within the immediate vicinity of this glycol group, (iii) the chain length and the total charge on the polynucleotide, (iv) the pH of the elution solvent, and (v) the ionic strength and the nature of the cations in the elution solvent. In addition, polynucleotides which contain terminal phosphate groups may be chemically modified such that they are selectively retained by these columns. This can be achieved by the incorporation of sorbitol groups at the terminal phosphate of the polynucleotide using methods reported previously (Naylor and Gilham, 1966). These experiments and the application of the results to sequence analysis of ribonucleic acid will be discussed in a forthcoming publication.

Experimental Section

m-Aminobenzeneboronic Acid. The hemisulfate salt of *m*-aminobenzeneboronic acid (Seaman and Johnson, 1931) was purchased from Aldrich Chemical Co., Milwaukee, Wis., and converted into the free base. An aqueous solution (40 ml) containing 3 g of the salt was brought to pH 7 with sodium hydroxide solution and then concentrated to dryness *in vacuo*. The residue was extracted with dioxane, the extracts were filtered and evaporated to dryness. The product (2.7 g), after recrystallization from water, had mp 164–165° (on an aluminum block). In 0.2 M ammonia the substance had λ_{max} 280 nm, ϵ_{max} 1230, and in 1 M sodium phosphate, pH 7, λ_{max} 293 nm, ϵ_{max} 1560.

N-(m-Dihydroxyborylphenyl)succinamic Acid. m-Aminobenzeneboronic acid (1.37 g) and succinic anhydride (1.1 g) were dissolved in dry pyridine (5 ml) and the mixture was allowed to stand for 12 hr. Water (5 ml) was added and, after 1 hr, the solution was evaporated to dryness *in vacuo*. The product was dissolved in water (10 ml) and again the solvent was removed *in vacuo*. Finally, the product was dissolved in water (10 ml) and the pH of the solution was adjusted to 1 with concentrated hydrochloric acid. The mixture was cooled in ice and the crystalline precipitate removed by filtration and washed with cold water (2 × 5 ml). The dried product (1.98 g, 84%) was recrystallized from hot water to yield crystals which had mp 173–174° (on an aluminum block), λ_{max} 243 nm, and ϵ_{max} 10,800. *Anal.* Calcd for $C_{10}H_{12}BO_5N$: C, 50.71; H, 5.07; B, 4.56; N, 5.91. Found C, 50.89; H, 5.04; B, 4.51; N, 6.11.

Carboxymethylcellulose Hydrazide. Carboxymethylcellulose (Whatman CM 23, 0.6 mequiv/g) was converted into its hydrazide by procedures similar to those used by Micheel and Ewers (1949). The sodium form of the carboxymethylcellulose (50 g) was washed extensively with 0.1 N hydrochloric acid and then with water to remove the acid. The product was dried *in vacuo* and suspended in anhydrous methanol (400 ml), and a solution of about 5 g of diazomethane in ether (200 ml) was added. The mixture was occasionally agitated and after about 2 hr the product was collected by filtration and washed with methanol. The product was added to a solution of hydrazine (100 ml) in methanol (300 ml) and the mixture was kept at 37° for 3 days with occasional shaking. The product was collected by filtration, washed with methanol and then extensively with water, and finally dried *in vacuo*.

N-(m-Dihydroxyborylphenyl)carbamylmethylcellulose. Carboxymethylcellulose hydrazide (10 g) was suspended in 0.5 M hydrochloric acid (1000 ml) and stirred for 1 hr. The mixture was cooled to 0° and all subsequent steps in the preparation were carried out at this temperature. To the mixture was added, with stirring, 0.5 M sodium nitrite (100 ml) over a period of 15 min. The suspension was then stirred for a further 20 min and the product was collected by filtration on a Büchner funnel and washed extensively with water until the filtrate was neutral. While still at 0° the carboxymethylcellulose azide was transferred directly to the solutions containing the amine to be incorporated. In order to test the capacity of this azide to covalently bind aromatic amines, preliminary experiments were carried out with aniline. Aniline (930 mg, 10 mmoles) was dissolved in water (40 ml) and cooled to 0°. The azide from 10 g of carboxymethylcellulose hydrazide was added and the thick slurry was allowed to stand for 24 hr with occasional stirring. Concentrated ammonia (10 ml) was then added with stirring and the mixture was allowed to come to room temperature and allowed to stand for 1 hr with occasional stirring. The product was collected by filtration and washed with 0.2 M ammonia and then with water. Spectrophotometric analysis at 280 nm of the combined ammonia washings indicated that a total of 3.1 mmoles of aniline had been bound. The same procedures were carried out with an aqueous solution (40 ml) of *m*-aminobenzeneboronic acid (685 mg, 5 mmoles) in place of the aniline. Spectrophotometric analysis at 280 nm of the dilute ammonia washings of the product showed that 2.0 mmoles of the boronic acid had been bound to the 10 g of the derivatized carboxymethylcellulose. A small sample of the product, after drying *in vacuo* at room temperature, was found to contain 0.16% boron.

The *m*-dihydroxyborylphenyl group was also incorporated into polyacrylamide in a similar way using polyacrylamide hydrazide prepared by the method of Inman and Dintzis (1969) from Bio-Gel P60, 50–150 mesh (Bio-Rad Laboratories, Richmond, Calif.). The hydrazide prepared from 1 g of dry polyacrylamide and containing 1 mmole of hydrazide group per dry g of polymer was suspended in 0.3 M hydrochloric acid at 0°. The mixture was treated with 1 M sodium nitrite (10 ml) and occasionally stirred for 20 min at 0° and the product was then collected by filtration and washed extensively with water at 0°. The azide was added to a cold solution of *m*-aminobenzeneboronic acid which had been prepared by dissolving its hemisulfate salt (186 mg, 1 mmole) in 5 ml of water and adjusting the pH to 8.5 with sodium hydroxide. After 24 hr at 0° with occasional agitation the mixture was allowed to warm up to room temperature and treated with an equal volume of concentrated ammonia for 0.5 hr. The product was washed with 0.2 M ammonium hydroxide and spectrophotometric analysis of the washings as before showed that 0.29 mmole of the boronic acid had been incorporated.

N-[N′-(m-Dihydroxyborylphenyl)succinamyl]aminoethylcellulose. Aminoethylcellulose (1 g, Whatman AE11, 1.0 mequiv/g) was washed with 1 M sodium chloride and then with water and finally suspended in water (20 ml). *N-(m*-Dihydroxyborylphenyl)succinamic acid (237 mg) was added and the suspension was adjusted to pH 6.0 with 5 N sodium hydroxide solution. The mixture was cooled to 0° and *N*-cyclohexyl-

N'-β-(4-methylmorpholinium)ethylcarbodiimide *p*-toluenesulfonate (Aldrich Chemical Co., Milwaukee, Wis., 425 mg) was added and the suspension was stirred at 0° for 4 hr while the pH of the solution was maintained at 6.0 by the addition of 2 N hydrochloric acid. The reaction mixture was then allowed to come to room temperature and stirred for a further 4 hr after which time the pH was 6.2. The mixture was then placed in a small column containing a cotton plug and the cellulose was washed with 0.2 M ammonia (*ca.* 100 ml), then with 0.5 M sodium phosphate (pH 7), and finally with water. Spectrophotometric analysis at 260 nm of the combined ammonia washings (using ϵ_{260} 4800 for the substituted succinamic acid in dilute ammonia and ϵ_{260} 344 for the carbodiimide reagent) showed that 0.62 mmole of the boryl derivative had been incorporated onto the cellulose. This result was confirmed by boron analysis on a sample of the product dried *in vacuo* at room temperature. *Anal.* Found: B, 0.69%.

Retention Volumes. The conditions under which the column separations were carried out are indicated in Figure 2 and Tables I and II. The nucleosides were usually studied in 1-mg quantities and the sugars and other polyols in 10-mg quantities. The positions of elution of the nucleosides were detected spectrophotometrically and their identity confirmed by ultraviolet spectra, while the sugars were located by spotting the eluent on chromatography paper followed by spraying with a silver nitrate reagent (Trevelyan *et al.*, 1950).

References

Böeseken, J. (1949), *Advan. Carbohyd. Chem. 4*, 189.

Branch, G. E. K., Yabroff, D. L., and Bettman, B. (1934), *J. Amer. Chem. Soc. 56*, 937.

Gilham, P. T. (1970a), *Annu. Rev. Biochem. 39*, 227.

Gilham, P. T. (1970b), *Methods Enzymol. 12* (in press).

Inman, J. K., and Dintzis, H. M. (1969), *Biochemistry 8*, 4074.

Khym, J. X. (1967), *Methods Enzymol. 12*, 93.

Lee, J. C., Weith, H. L., and Gilham, P. T. (1970), *Biochemistry 9*, 113.

Letsinger, R. L., and Hamilton, S. B. (1959), *J. Amer. Chem. Soc. 81*, 3009.

Lorand, J. P., and Edwards, J. O. (1959), *J. Org. Chem. 24*, 769.

McDaniel, D. H., and Brown, H. C. (1955), *J. Amer. Chem. Soc. 77*, 3756.

Micheel, F., and Ewers, J. (1949), *Makromol. Chem. 3*, 200.

Morris, C. J. O. R., and Morris, P. (1963), Separation Methods in Biochemistry, New York, N. Y., Interscience, p 241.

Naylor, R., and Gilham, P. T. (1966), *Biochemistry 5*, 2722.

Rosenberg, M., Wiebers, J. L., and Gilham, P. T. (1970), 160th National Meeting of the American Chemical Society, Chicago, Ill.

Seaman, W., and Johnson, J. R. (1931), *J. Amer. Chem. Soc. 53*, 711.

Sheehan, J. C., and Hlavka, J. J. (1956), *J. Org. Chem. 21*, 439.

Solms, J., and Deuel, H. (1957), *Chimia 11*, 311.

Steinberg, H. (1964), Organoboron Chemistry, New York, N. Y., Interscience, p 661.

Tomlinson, R. V., and Tener, G. M. (1963), *Biochemistry 2*, 697.

Torssell, K. (1957), *Arkiv. Kemi 10*, 541.

Trevelyan, W. E., Procter, D. P., and Harrison, J. S. (1950), *Nature (London) 166*, 444.

Effect of Divalent Metal Ions on the Reversed-Phase Chromatographic Separation of Transfer Ribonucleic Acids*

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ABSTRACT: Divalent metal ions alter the elution sequence and position, multiplicity, and stability of *Escherichia coli* K-12 MO tRNAs during reversed-phase chromatography. The group IIA ions, Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+}, were all equivalent in effect, compared to reversed-phase chromatography runs with no divalent metal or with EDTA. They shifted the elution position to lower sodium chloride concentrations sharpened some tRNA peaks while broadening others and stabilized certain tRNAs.

Practical application can be made of these divalent metal ion effects for the purification of individual transfer ribonucleic acids.

Magnesium ion has pronounced effects on the reversed-phase chromatographic[1] separation of tRNAs (Kelmers *et al.*, 1965; Weiss and Kelmers, 1967; Weiss *et al.*, 1968). Numerous publications describe the dramatic effect of magnesium ion on many tRNA properties, including conversion between active and inactive forms (Lindahl *et al.*, 1966; Gartland and Sueoka, 1966), protection from nuclease degradation (Nishi-

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[1] Abbreviations used are: RPC, reversed-phase chromatography; A_{260} unit, quantity of tRNA having an absorbance of 1 in 1 ml at 260 mμ in a 1-cm cell; HEPES, *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid; BICINE, *N,N*-bis(2-hydroxyethyl)glycine.