Carbohydrate Research, 43 (1975) 215–224

STUDY OF THE INTERACTION OF POLYOLS WITH POLYMERS CONTAINING *N*-SUBSTITUTED [(4-BORONOPHENYL)METHYL]-AMMONIO GROUPS

ALEXANDER M. YURKEVICH, IVETTA I. KOLODKINA, ELENA A. IVANOVA, AND ELENA I. PICHUZHKINA

The All-Union Scientific Research Vitamin Institute, 117246 Moscow (U.S.S.R.)

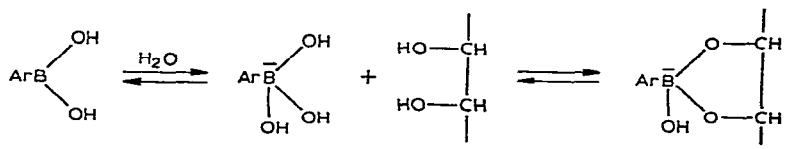
(Received February 6th, 1975; accepted for publication, April 23rd, 1975)

ABSTRACT

Polymers, based on dextran and cellulose, having 2-{[(4-boronophenyl)-methyl]-ethylammonio}ethyl and -diethylammonio}ethyl groups were prepared. It was shown that these polymers could be employed for absorption of *cis*-diol compounds. The polymers were found to be highly specific towards polyols, carbohydrates, nucleosides, and nucleotides over a wide range of pH. The polymer based on DEAE-Sephadex A-25 was used in separating nucleosides, and in fractionating mononucleotides and carbohydrates. The chromatographic behavior of carbohydrates is defined by their structure and conformation, which are also responsible for different stabilities of the boronic complexes generated.

INTRODUCTION

Most diols are known to afford cyclic complexes only with arylboronic* acid anions having coordination number 4.



In general, the larger the degree of dissociation of the arylboronic acid, the wider the range of pH in which the formation of complexes with *cis*-diols occurs. Thus, adenosine affords the most stable complexes with phenylboronic acid (pK_a 8.86) in the range of pH 7.5, (4-methylphenyl)boronic acid (pK_a 10.0) at pH 8.0, and (3-nitrophenyl)boronic acid (pK_a 7.30) at[1] pH 7.0. Similar results were obtained in the study of complexes of phenylboronic, (3-nitrophenyl)boronic, and (4-methoxyphenyl)boronic acids with carbohydrates[2].

**Editor's note.* The nomenclature employed in this article is based on that in *Chem. Abstr. Index Guide, Section IV*, 76 (1972) paragraph 239.

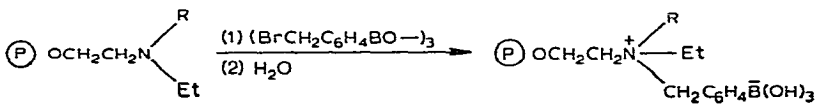
It is known that the degree of dissociation of arylboronic acids is defined by boron electrophilicity, and depends on the substituent on the aromatic ring[3,4]. However, even the replacement of hydrogen by the strongly electron-attracting nitro group in the *para* position of the benzene ring in an arylboronic acid provides no considerable increase in acid strength [pK_a of (4-nitrophenyl)boronic acid, 7.15][4]. Another method of stabilizing the arylboronic acid anion is the synthesis of bipolar derivatives, *e.g.*, with positive, quaternary ammonium groups. Thus, {4-[(hexamethylenetetramine)methyl]phenyl}boronic acid is one of the strongest among the known arylboronic acids (pK_a 7.04)[5].

In some studies, boron-containing polymers have been used in separating carbohydrates and nucleotides[6–9]. However, they always required a pH $\geqslant$7.0 for producing stable complexes of *cis*-diols with borono groups [$-B(OH)_2$] in the polymer. Naturally, a polymer having covalently bonded borono groups and a bipolar structure would (unlike the polymers just described) be interesting as regards expanding the stability region of boron complexes with *cis*-diols as compared with the known boron-containing polymers.

The present article is concerned with the synthesis of boronarylated polymers, based on dextran, having bipolar 2-{[(4-boronophenyl)methyl]-ethylammonio}ethyl and -diethylammonio}ethyl groups, and with a study of their properties as specific, complexing agents of polyols.

DISCUSSION

The known methods of synthesis of polymers containing quaternary ammonium bases involve the reaction of amino-substituted polyhydroxy compounds with bifunctional reagents in aqueous alkaline solution[10]. However, this method could not be used in the case of tris[4-(bromomethyl)phenyl]boroxin, as it has highly active 4-(bromomethyl)phenyl groups that are readily hydrolyzed, even in neutral, aqueous solution. To avoid concurrent esterification of the 4-(bromomethyl)phenyl groups, the amino groups in Sephadexes and cellulose were alkylated with tris[4-(bromomethyl)phenyl]boroxin in *N,N*-dimethylformamide[11].



where (P) is the residue of Sephadex, hydroxypropylated Sephadex, or cellulose, and R = H, or Et.

O-(2-Diethylaminoethyl)Sephadex (DEAE-Sephadex A-25), hydroxypropylated Sephadex (DEAE-Sephadex LH-20), and *O*-[2-(ethylamino)ethyl]cellulose were used as matrices. In the last case, the reaction was conducted in the presence of the stoichiometric quantity of tributylamine[12]. [2-{[(4-Boronophenyl)methyl]diethylammonio}ethyl]Sephadex A-25 (DEBAE-Sephadex A-25) contained 1.52 mequiv.

of boron per g of the resin; DEBAE-Sephadex LH-20, 0.45 mequiv. of boron per g; and EBAE-cellulose (see Experimental section), 0.2 mequiv. of boron per g. On the basis of these data, the extent of modification of the amino groups was always 60–70%.

The specific, sorptive ability of the polymers towards the diol systems was investigated for adenosine and its phosphates under static conditions (see Tables I and II). With DEBAE-Sephadex A-25, adenosine was absorbed, even at pH 2.5; this effect could not have been due to complex-formation with the 2',3'-hydroxyl groups

TABLE I

CAPACITY OF DEBAE-SEPHADEX A-25 FOR ADENOSINE AND 5'-AMP

	Capacity (mmole.g^{-1})	
pH	*Adenosine*	*5'-AMP*
2.3	—	1.03
2.5	0.95	—
3.55	1.11	1.58
5.5	1.11	1.09
6.7	—	0.73
8.2	1.13	0.63

TABLE II

THE ABSORPTION OF ADENOSINE AND ITS 5'-PHOSPHATES ON DEBAE-SEPHADEX LH-20 AT pH 7.15

Compound	*Absorption*[a] (%)	*Compound*	*Absorption*[a] (%)
2'-Deoxyadenosine	0.0	5'-AMP	78.0
Adenosine	92.0	5'-ADP	67.5
Adenosine 5'-(hydrogen 4-morpholinylphosphonate)	100.0	5'-ATP	32.0

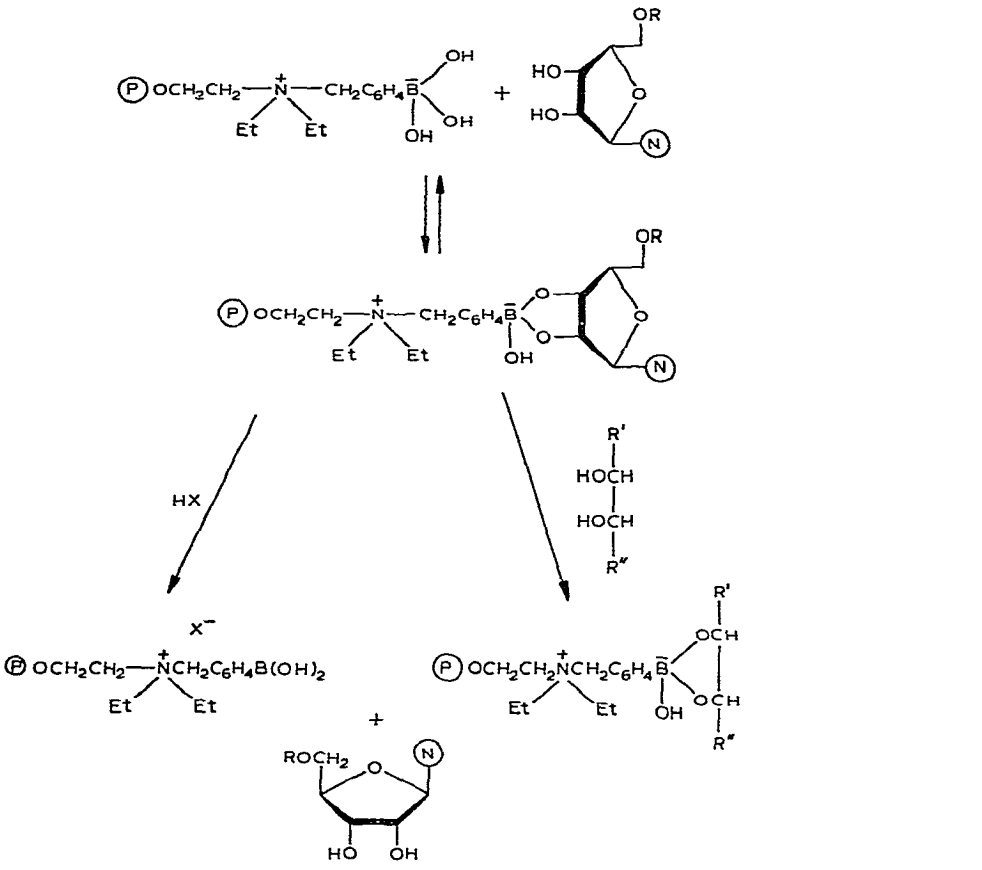
[a]The absorption of 0.45 mmole.g^{-1} was adopted as 100%; this corresponds to the boron content in the polymer (0.45 mequiv.g^{-1}).

of the nucleoside, as 2'-deoxyadenosine was not absorbed by the polymer under such conditions. It is interesting that the polymer capacity for adenosine is almost constant over a wide range of pH (3.0–8.0). For adenosine 5'-phosphate (5'-AMP), unlike adenosine, the nucleotide association increases in the range of pH 3.2–3.85, probably due to the total effect of the polymer as anionite and complexing agent. The capacity decreases with further increase of pH, and its minimum is at pH 7.4–8.2. Such a decrease in the absorption may be due to an electrostatic repulsion of the boron complex (anion) and the adenosine 5'-phosphate dianion (pK_a 6.57) which lessens the effect of complex formation.

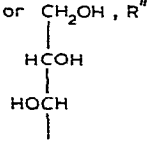
On DEBAE-Sephadex LH-20, a decrease of absorption in the series:

adenosine>5′-AMP>5′-ADP>5′-ATP may be due to the higher electrostatic repulsion, with an increasing general negative charge in the ligand molecule.

A column of DEBAE-Sephadex A-25 was used for chromatographic separation of a mixture of nucleosides and nucleotides[13]. In separating adenosine and 2′-deoxyadenosine, the latter was not absorbed by the column, unlike adenosine and other ribonucleosides eluting only at pH 6.1. It should be noted that, unlike the reports of Gilham *et al.*[7] and Reske and Schott[9], ribonucleosides were not desorbed from DEBAE-Sephadex in the weakly alkaline zone by buffers of high ionic strength. Probably, this could also be due to the higher concentration of the arylboronate anions in the polymer investigated.



where R = H or —PO(OH)$_2$, R′ = H or CH_2OH, R″ = H or CH_2OH, X = Cl or AcO,



(N) = residue of a heterocyclic base, and (P) = Sephadex or hydroxypropylated Sephadex.

Two methods are used in desorbing nucleosides from DEBAE-Sephadex. The first is by treatment with buffers (pH <7.0); here, the quaternary amino groups of the polymer coordinate with an appropriate counter-ion, leading to an equilibrium splitting of the boronium complex at the expense of suppressing the dissociation of the complex boronic acid. The second method is by ligand substitution, using other diols (such as D-glucitol or ethylene glycol) as eluting agents. The latter process requires no acid solutions, and provides a practically constant pH. However, the ribonucleosides were not separated in all cases; this may be explained by the fact that the stability constants in the neutral and weakly alkaline media are practically the same for all nucleosides, and they are defined by the total structure of the five-membered, tetrahedral complexes of β-D-ribofuranosides[1] (see Table III).

TABLE III

CHROMATOGRAPHIC SEPARATION OF NUCLEOSIDES AND NUCLEOTIDES ON DEBAE-SEPHADEX

Compound	*Absorption conditions*	*Elution conditions*
Mixture of the ribonucleosides: adenosine, cytidine, guanosine, and uridine	1mM TEAB, pH 8.0	0.12M acetate buffer, pH 6.1[a] 1.5M ethylene glycol in 0.01M TEAB[b]
	2mM Tris buffer, pH 8.45	M D-glucitol in 0.2M Tris buffer, pH 8.35
2′(3′)-AMP	1mM bicarbonate buffer, pH 8.0 1mM acetate buffer, pH 6.55	0.24M bicarbonate buffer, pH 8.0[c] 0.16M acetate buffer, pH 6.55[d]
5′-AMP	1mM bicarbonate buffer, pH 8.0 1mM acetate buffer, pH 6.55	0.2M acetate buffer, pH 5.0[c] 0.21M acetate buffer, pH 5.0[d]

[a]The elution was performed consecutively with a gradient of TEAB concentrations; reservoir, 25 ml of 0.5M TEAB, pH 7.5; mixer, 25 ml of 1mM TEAB, pH 7.2, by water, 1mM ammonium acetate, pH 6.5; in the gradient of acetate buffer concentrations: reservoir, 150 ml of 0.3M NH_4OAc, pH 6.1; mixer, 150 ml of 0.1M NH_4OAc, pH 6.1. [b]The elution was conducted with a gradient of ethylene glycol concentrations: reservoir, 200 ml of 3.0M ethylene glycol; mixer, 200 ml of 0.05M ethylene glycol. [c]The elution was performed consecutively with a gradient of ammonium hydrogen carbonate concentrations: reservoir, 25 ml of 0.5M BA, pH 8.0; mixer, 25 ml of 0.1 BA, pH 8.0, 1mM acetate buffer, pH 5.65; in the gradient of acetate buffer concentrations: reservoir, 100 ml of 0.5M NH_4OAc, pH 4.0; the mixer, 100 ml of 0.01M NH_4OAc, pH 5.65. [d]The elution was performed consecutively with a gradient of acetate buffer concentrations: reservoir, 50 ml of 0.6M NaOAc, pH 6.55; the mixer, 50 ml of 1mM NaOAc, pH 6.55, 6mM acetate buffer, pH 5.0, in the gradient of acetate buffer concentrations: reservoir, 50 ml of 0.3M NaOAc, pH 5.0; the mixer, 50 ml of 6mM NaOAc, pH 5.0.

In separating isomeric mononucleotides, adenosine 2′(3′)-monophosphates were removed from adenosine 5′-monophosphate, although 2′(3′)-AMP was absorbed by the polymer as anionite. This nucleotide may readily be removed from the polymer with triethylammonium hydrogen carbonate (TEAB) in weakly alkaline medium, whereas the strongly sorbed 5′-AMP could be desorbed only at pH 5.0.

A more interesting example of the use of a boron-containing polymer was found on studying the chromatographic behavior of carbohydrates on DEBAE-Sephadex

A-25. The differences revealed were certainly due to the conformational specificities of carbohydrates, and they provide excellent possibilities for separation thereof. In general, an increase in the number of *cis*-hydroxyl groups of carbohydrates gives more strongly retained complexes. Here, an equilibrium among conformational, tautomeric, and anomeric forms of carbohydrates may be altered when one of the forms gives a stronger complex (than the others) with the boronic acid derivative. For example, certain sugars (specifically, those having the higher mutarotation constants, such as 2-ketoses) may more readily give the acyclic or furanose forms, which are more favorable for complex-formation[14].

Several aldoses were chromatographed on DEBAE-Sephadex A-25; D-glucose, D-mannose, D-arabinose, and D-ribose; the ketose D-fructose; the disaccharide lactose; the sugar alcohol D-glucitol, and D-arabinono- and D-glucono-lactones. Some of the results are given in Table IV, from which it may be seen that, in the neutral medium, D-glucitol and D-fructose are strongly retained by the polymer, and may

TABLE IV

THE CHROMATOGRAPHY OF CERTAIN CARBOHYDRATES ON A COLUMN OF DEBAE-SEPHADEX AT VARIOUS pH VALUES

Compound	*0.01M Tris buffer, pH 8.4*		*0.01M Acetate buffer, pH 6.5*		*0.01M Acetate buffer, pH 5.0*	
	v_e[a]	v_r[b]	v_e	v_r	v_e	v_r
Lactose	—	—	1.5[c]	2.8[c]	—	—
			2.0	40.0	—	—
D-Glucose	no elution[d]		3.0[c]	40.0[c]	—	—
D-Ribose	2.0	25.5	14.0[c]	41.5[c]	—	—
D-Mannose	8.5	55.0	—	—	—	—
	—	80.0	—	—	—	—
D-Arabinose	2.0	28.0[e]	—	—	—	—
D-Glucitol	—	—	no elution[f]		16.6	69.0
D-Fructose	—	—	no elution[c,f]		10.6	58.0

[a]The value of v_e is the eluate volume (ml), namely, the volume of eluate collected from the moment of addition of the sample at the top of the column to the moment of appearance of the maximum of an elution peak. [b]The value of v_r is the retention volume (ml), namely, the volume of eluate collected from the moment of addition of the sample at the top of the column to the moment of decrease of the elution peak to the minimum. [c]These results were obtained with a double quantity of the polymer. [d]Desorption with 0.1M buffer, pH 6.0, $v = 32$ ml. [e]Elution with 0.1M buffer, pH 8.4. [f]The desorption was carried out in a gradient of buffer concentrations: reservoir, 90 ml of 0.2M NaOAc, pH 6.0; mixer 90 ml of 0.01M NaOAc, pH 6.0. The compound was eluted with 0.08M NaOAc, pH 6.0.

readily be separated from aldoses, as D-glucitol has a chain conformation that may produce five- and six-membered complexes. Such complexes are more stable than others, because of the presence of free 1,2- or 1,3-hydroxyl groups. In addition, D-fructose may also give such complexes of its acyclic form. It is interesting that, even at pH 5.0, D-glucitol and D-fructose may yield complexes with the borono groups of

the polymer; this consitutes evidence that the complexed boronium anion stabilized by the bipolar polymeric structure may exist in weakly acid medium as well. On the other hand, lactose (having only a pair of hydroxyl groups for generation of complex) is only weakly retained by the polymer.

In separating aldoses (D-glucose and D-ribose) in the neutral medium, it was noted that their retention volumes are almost the same. However, a negligible increase of the output volume of D-ribose may be due to the 1,3-diaxial orientation of its hydroxyl groups, which may to some extent provide a six-membered complex. It was also found that, at the higher pH, the behavior of D-glucose in regard to complex-formation differs from that of the other aldoses investigated. Thus, at pH 8.4, D-glucose is strongly absorbed on DEBAE-Sephadex A-25, and could only be eluted with 0.1M buffer of pH 6.0; no conversion of D-glucose into D-fructose was observed. This difference may be attributable to the pyranose–furanose interconversion of D-glucose in alkaline media in the presence of boric and phenylboronic acids[15]. In this instance, arylboronates may additionally involve the 5- and 6-hydroxyl groups for α and β anomers whose stabilities are close to those of boronium complexes of alditols and D-fructose. Alternatively, formation of the most stable, six-membered complexes *via* the hydroxyl groups on C-3 and C-5 may be assumed. Such behavior was earlier found for D-glucose in the chromatography of sugars with solvents containing basic organoboronates. Among all of the monosaccharides studied, only D-glucose had a considerably lower lability, and its R_F value was found to be close to those of alditols[16]. This was manifested by the ^{11}B n.m.r. and p.m.r. data[17]. The complex-formation of D-glucose is enhanced owing to participation of the furanose forms on increase of the pH from 7.0 to 12.0. Unlike D-glucose, the other aldoses investigated (in the most stable conformation of their pyranose forms) have a higher probability of reacting with the borono groups of the polymer; thus, no conversion may ever occur, or it may proceed to a lesser extent. The "abnormal" behavior of D-glucose may be used in separating it from other aldoses.

The effect of the ionic strength of the buffer upon the elution was investigated for D-arabinose. Its retention volume in elution with 0.1M solution is almost one-third of that for 0.01M buffer. Formation of the complexes of aldonolactones with the borono polymer discloses new possibilities for separation, and for proving their structures. In aqueous solutions of aldonic acids, the equilibrium is known to shift towards formation of the γ-lactones. Actually, it was found that D-arabinono-1,4-lactone, having no *cis*-hydroxyl groups, is practically unretained by the polymer, whereas D-glucono-1,4-lactone affords stable complexes with borono groups of the polymer, and could only be desorbed with 0.1M buffer at pH 5.0. The acyclic forms of the acids present in solution behave similarly (see Fig. 1).

Use of volatile buffers (ammonium acetate and TEAB) produces no essential change in the elution, but facilitates isolation of the compounds. For the compounds investigated, the yields from the column were always within 80–100%.

In conclusion, it should be noted that all results obtained on the chromatography of nucleotides and carbohydrates are in good agreement with the stability

constants (K_c) earlier observed for complexes of various polyols with phenylboronic acid[1,3]; this also indicates the high specificity of the bipolar polymers employed.

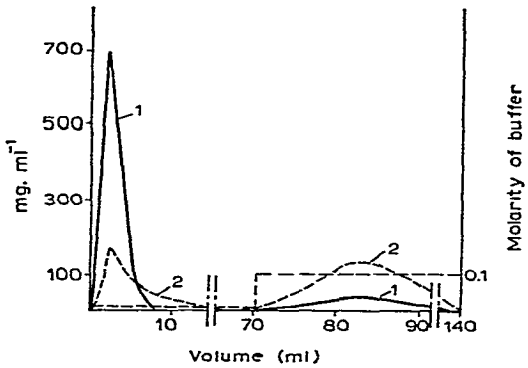


Fig. 1. The chromatography of D-glucono- and D-arabinono-lactones on DEBAE-Sephadex. (1, D-arabinonic acid; 2, D-gluconic acid.)

EXPERIMENTAL

Materials and general techniques. — The paper used for chromatography was FN 11 paper, and the solvent systems were: (1) 1-butanol saturated with water, (2) 43:7:1 2-propanol–concentrated hydrochloric acid–water, (3) 5:2 ethanol–0.5M ammonium acetate saturated with borax, (4) 40:6:1 saturated ammonium sulfate solution–M sodium acetate–2-propanol, (5) 4:1:5 1-butanol–acetic acid–water. Quantitative spectrophotometric measurements of the eluted solutions of carbohydrates were performed with L-cysteine–sulfuric acid[18], and with periodate–chromotropic acid[19]. The separations were conducted on columns of DEBAE-Sephadex A-25 (11×0.4 cm for carbohydrates, and 8×0.7 cm for nucleotides). The respective rates of elution of nucleotides and carbohydrates were 1 $ml.min^{-1}$ and 10–15 $ml.h^{-1}$. The samples investigated were 1 mg of a nucleotide, and 5 mg of a carbohydrate. The absorption and elution conditions for nucleosides and nucleotides are shown in Table III, and the eluate and retention volumes of carbohydrates are listed in Table IV.

DEBAE-Sephadex A-25. — DEAE-Sephadex A-25 (free form; 1.5 g) was added to a solution of tris[4-(bromomethyl)phenyl]boroxin (3.0 g) in *N,N*-dimethylformamide (15 ml), and the mixture was stirred in a flask under a stream of argon for 3 h at 60° and then for 12 h at 18–20°. The polymer was filtered off, and successively washed with *N,N*-dimethylformamide, ethanol, water, 0.1M sodium hydroxide, and water. The polymer (free from the monomeric [4-(bromomethyl)phenyl]boronic acid) was successively washed with M potassium chloride, water, ethyl alcohol, and ether, and dried in a vacuum desiccator. Found: B, 1.64%; capacity, 1.52 $mequiv.g^{-1}$.

DEBAE-Sephadex LH-20. — DEAE-Sephadex LH-20 (free form[20]; 0.6 g) was added to a solution of tris[4-(bromomethyl)phenyl]boroxin (0.39 g) in *N,N*-dimethyl-

formamide (3 ml). The reaction was conducted under a stream of argon for 4.5 h at 60°, and then for 12 h at 18–20°. The polymer was treated as described in the previous experiment. Found: B, 0.48%; capacity, 0.45 mequiv. g^{-1}.

EBAE-cellulose. — Tributylamine (0.4 ml) and tris[4-(bromomethyl)phenyl]-boroxin (0.5 g) were added to a suspension of *O*-(2-aminoethyl)cellulose (2.0 g) in *N,N*-dimethylformamide (9 ml). The mixture was stirred under a stream of argon for 12 h at 100°; then, treatment similar to that described for the Sephadexes was performed. Found: B, 0.23%; capacity, 0.2 mequiv. g^{-1}.

Determination of absorption of nucleotides on DEBAE-Sephadexes. — (*a*) The ligand solution (1–2 ml), having a 2:3 ratio of mequiv. of B per mmole of nucleoside, was added to DEBAE-Sephadex A-25 (10 mg) swollen in 0.2 ml of 0.01M buffer of appropriate pH (TEAB or sodium acetate in acetic acid), or in hydrochloric acid. The mixture was stirred for 1–2 h and filtered. The capacity of the polymer was determined by measurement of the optical absorbance of the filtrate at 260 nm (see Table I).

(*b*) The ligand solution (3 ml), having a 5:7 ratio of mequiv. of B per mmole of ligand, was added to DEBAE-Sephadex LH-20 (100 mg) swollen in 1 ml of 1mM TEAB, pH 7.15. The mixture was stirred for 2–3 h, and filtered, and the polymer was washed with the same buffer until minimal optical absorbance of the washings was achieved.

Chromatography of D-*arabinono- and* D-*glucono-lactones.* — A solution o D-arabinono-1,4-lactone (5 mg) in 0.1 ml of 0.01M sodium acetate–acetic acid buffer (pH 5.0) was placed on the column with 100 mg of the polymer in the same buffer. Elution with the same buffer afforded a peak with 3.49 mg of D-arabinonolactone (70% of the original sample), $v_e = 2.0$ ml, $v_r = 7.0$ ml. Elution with 0.1M acetate buffer, pH 5.0, gave the peak for D-arabinonic acid (25–30%) (13 ml).

The same absorption conditions were used for D-gluconolactone. Two peaks were eluted in the way just described. Yield of the first fraction, 18% of the original quantity (D-glucono-1,5-lactone), $v_e = 2.3$ ml, $v_r = 13$ ml. The initial compound (~75%) was eluted with 0.1M acetate buffer (pH 5.0) (see Fig. 1).

REFERENCES

1 E. A. IVANOVA, I. I. KOLODKINA, AND A. M. YURKEVICH, *Zh. Obshch. Khim.*, 41 (1971) 455–459.
2 S. A. BARKER, A. K. CHOPRA, B. W. HATT, AND P. J. SOMERS, *Carbohydr. Res.*, 26 (1973) 33–40.
3 J. P. LORAND AND J. O. EDWARDS, *J. Org. Chem.*, 24 (1959) 769–774.
4 K. TORSELL, J. H. MCLENDON, AND G. F. SOMERS, *Acta Chem. Scand.*, 12 (1958) 1373–1385.
5 E. I. PICHUZHKINA, I. I. KOLODKINA, AND A. M. YURKEVICH, *Zh. Obshch. Khim.*, 43 (1973) 2275–2277.
6 J. SOLMS AND H. DEUEL, *Chimia*, 11 (1957) 311.
7 H. L. WEITH, J. L. WIEBERS, AND P. T. GILHAM, *Biochemistry*, 9 (1970) 4396–4401.
8 S. A. BARKER, B. W. HATT, P. J. SOMERS, AND R.R. WOODBURY, *Carbohydr. Res.*, 26 (1973) 55–64.
9 K. RESKE AND H. SCHOTT, *Angew. Chem. Int. Ed. Engl.*, 12 (1973) 417–418.
10 Brit. Pat. 936,039 (1963).
11 I. I. KOLODKINA, E. I. PICHUZHKINA, E. A. IVANOVA, AND A. M. YURKEVICH, Avtorskoe svidet., No. 406,841 (1973); *Byul. Izobret. i Tovarnykh Znakov*, No. 46 (1973).
12 H. Z. SOMMER, H. I. LIPP, AND L. L. JACKSON, *J. Org. Chem.*, 36 (1971) 824–828.

13 E. A. IVANOVA, I. I. KOLODKINA, AND A. M. YURKEVICH, *Zh. Obshch. Khim.*, 44 (1974) 430–434.
14 J. M. GORNER, *J. Inorg. Nucl. Chem.*, 32 (1970) 3545–3548; P. J. ANTIKAINEN, *Suom. Kemistilehti B*, 31 (1958) 255–260.
15 J. BÖESEKEN, *Advan. Carbohydr. Chem.*, 4 (1949) 189–210.
16 H. CARMINATTI, S. PASSERON, M. DANKERT, AND E. RECONDO, *J. Chromatogr.*, 18 (1965) 342–348.
17 G. R. KENNEDY AND M. J. HOW, *Carbohydr. Res.*, 28 (1973) 13–19.
18 Z. DISCHE, *J. Biol. Chem.*, 181 (1949) 379–392.
19 N. SPENCER, *J. Chromatogr.*, 30 (1967) 566–571.
20 E. A. PETERSON AND H. A. SOBER, *Biochem. Prepn.*, 8 (1961) 39–42.