ION-EXCHANGE CHROMATOGRAPHY OF ARENEBORONIC ACID COMPLEXES OF NUCLEOSIDES AND MONONUCLEOTIDES

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The search for new methods of separating nucleotides has not lost its importance. Recently, methods of chromatography on Sephadexes [1], on activated carbons [2], on cation-exchange resins [3], and, in the case of nucleosides, on polyacrylamide gel [4] have been used ever more widely.

The method that we have found for separating nucleosides and nucleotides is based on the chromatography of their complexes with areneboronic acids which are obtained by the reaction of the 2',3'-diol system of ribofuranosides with boric acid derivatives. The nucleosides in the form of the areneboronate complexes acquire additional acid functions which facilitates their separation from derivatives substituted at the 2'- or 3'-hydroxyls.

The complexes with m-nitrobenzene- and benzeneboronic acids (NBBA and BBA) form rapidly when aqueous solutions of a nucleoside (or a 5'-mononucleotide) and a potassium areneboronate are mixed, being accompanied by a fall in the pH of the solutions.

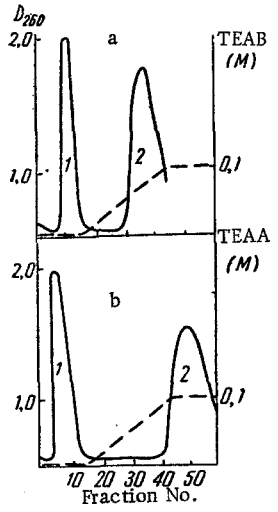

Fig. 1. Separation of a mixture of 2'-deoxyadenosine and adenosine by chromatography on "Dowex-1" resin (HCO_3^-) in the presence of benzeneboronic acid (a) and m-nitrobenzeneboronic acid (b). 1) 2'-Deoxyadenosine; 2) adenosine.

The maximum values of the depression of the pH are found for benzeneboronates of nucleosides at 7.0-8.0 and for benzeneboronates of nucleotides at 7.5-8.5.

Making use of the ΔpH values obtained, we have calculated the stability constants of the corresponding complexes (K_S) (Table 1) [5].

On analyzing the values of the stability constants for the complexes, it can be seen that they differ and change according to the nature of the aglycone and the nature of the substituent in the riboside moiety.

The results showing the existence in weakly alkaline aqueous media of stable anions of the complexes have been used in the chromatographic separation of the nucleosides and nucleotides.

The areneboronate complexes were obtained immediately before chromatography by mixing solutions of the nucleosides (or nucleotides) with a threefold molar excess of the areneboronic acid. They were chromatographed on Dowes 1 × 4 (HCO_3^- form) with a triethylammonium bicarbonate or acetate (TEAB, TEAA) gradient at pH 7.0-8.0, which corresponded to the pH region of the maximum stability of the complexes. A relatively small excess of

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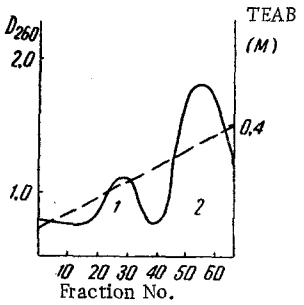


Fig. 2. Separation of a mixture of 5'- and 2'-AMP by chromatography on "Dowex-1" resin (HCO_3^-) in the presence of benzeneboronic acid. 1) 2'-AMP; 2) 5'-AMP.

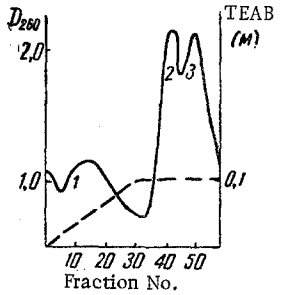
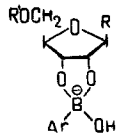


Fig. 3. Separation of a mixture of cytidine, adenosine, and uridine by chromatography on "Dowex-1" resin (HCO_3^-) in the presence of benzeneboronic acid. 1) Cytidine; 2) uridine; 3) adenosine.

TABLE 1. Stability Constants (K_S) of the Areneboronate Complexes of Nucleosides and Nucleotides

Ar	R	R'	log K_S at pH				
			7,0	7,5	8,0	8,9	9,5
C_6H_5	Adenine	H	—	3,11	—	3,02	2,92
$M—NO_2C_6H_4$		"	2,73	—	2,65	2,46	—
	Cytosine	"	—	2,98	3,06	3,04	3,01
	Uracil	"	—	3,15	—	2,61	2,12
	Hypoxanthine	"	—	3,06	—	2,27	1,81
C_6H_5	Adenine	PO_3H_2	—	—	2,75	2,59	2,37
	Cytosine	"	—	—	3,69	2,72	2,51
	Guanine	"	—	—	2,61	2,11	1,41
	Hypoxanthine	"	—	—	2,54	2,03	1,77

areneboronic acid and the use of the volatile TEAB, in contrast to known methods using elution with solutions of borax [6], simplifies the subsequent isolation of the substances.



Initially, the chromatographic separation of a mixture of 2'-deoxyadenosine and adenosine was carried out in the presence of benzeneboronic and m-nitrobenzeneboronic acids (Fig. 1). The replacement of benzeneboronic acid by the stronger m-nitrobenzeneboronic acid, forming a fairly stable complex with adenosine even at pH 6.5, permitted the complete separation of the mixture of nucleosides at pH 6.8. Apparently, depending on the nature of the substituent in the aromatic ring of the areneboronic acid it is possible to change the pH range in chromatography by this method fairly widely.

The use of benzeneboronate complexes for the separation of mixtures of 5'-, 2'-, and 3'-mononucleotides was demonstrated for the case of adenine derivatives. While under the standard conditions of separation according to Cohn the 5'-AMP is eluted first, in agreement with its higher pK_a value [7], in the presence of benzeneboronic acid the order of desorption of the nucleotides changed (Fig. 2). However, in this case satisfactory results were obtained only under the conditions of the previous saturation of the ion-exchange resin with a benzeneboronate. By subsequently chromatographing the mixture of adenosine phosphates without benzeneboronic acid and in the presence of it, each of the isomeric mononucleotides was isolated in the pure state.

By this method of ion-exchange chromatography, cytidine was separated completely from a mixture of adenosine and cytidine (Fig. 3). Although these nucleosides form complexes with benzeneboronic acid, the complex of cytidine, the N^4 amino group of which is the most basic, probably has the structure of a bipolar ion under the conditions of chromatography and is practically sorbed on the resin.

EXPERIMENTAL

Paper chromatography was carried out with Leningrad paper No. 2 and the following systems of solvents: 1) ethanol-ammonium acetate saturated with borax (5:2); 2) propan-2-ol–water–saturated ammonium sulfate solution (2:19:79); 3) propan-2-ol–conc. hydrochloric acid–water; and 4) butan-1-ol saturated with water. The qualitative determinations were carried out on the Brumberg UB-1 ultrachemiscope, and the quantitative determinations on an SF-4 spectrometer; the depressions of the pH values of the solutions were determined on a LPU-0,1 potentiometer. Samples from the Reanal firm (Hungarian People's Republic) dried in vacuum to constant weight were used to prepare the nucleoside and nucleotide samples. The benzeneboronic acid was synthesized by a published method [8] and was determined by the qualitative reaction with diphenylcarbazone [9]. The separations were carried out on a column (1 × 8 cm) containing Dowex 1 × 4 resin, 200-400 mesh (HCO_3^-). The rate of elution of the substances was 1 ml/min. The volume of the fractions was 6 ml. To collect and record the fractions a Radirak device with a Uvicord attachment ("LKB," Sweden) was used.

CHROMATOGRAPHIC SEPARATIONS

Adenosine and 2'-Deoxyadenosine. A. With Benzeneboronic acid. To a solution of 2.5 mg of adenosine and 2.5 mg of 2'-deoxyadenosine in 10 ml of water containing a threefold molar excess of benzeneboronic acid was added a 3% aqueous solution of ammonia to pH 8.0, and it was transferred to a column. The 2'-deoxyadenosine was eluted with 0.002 M TEAB, pH 8.0, R_f 0.6 (system 4). Then elution was carried out with a TEAB gradient (100 ml of 0.002 M to 100 ml of 0.1 M solution). The adenosine was eluted with 0.08 M TEAB, R_f 0.74 (system 4) (see Fig. 1a).

B. With m-Nitrobenzeneboronic Acid. The areneboronate complex was obtained in a similar manner. The solution was transferred to a column previously washed with a 0.002 M solution of TEAA having pH 6.8 and was eluted successively with 0.002 M TEAA and a gradient of TEAA (100 ml of 0.002 M to 100 ml of 0.1 M solution) and with 0.1 M TEAA having pH 6.8. The 2'-deoxyadenosine was eluted by the 0.002 M TEAA and the adenosine by the 0.1 M TEAA (Fig. 1b).

Separation of Isomeric Mononucleotides. 1. Without Benzeneboronic Acid. A solution of 5 mg of 5'-AMP and 5 mg of 2'(3')-AMP in 20 ml of water with pH 8.8 was transferred to a column. The substances were eluted by a TEAB gradient (100 ml of 0.15 M to 100 ml of 0.4 M solution). A mixture of 5'- and 2'-AMP's was eluted by the 0.2 M TEAB, R_f 0.0, 0.12 (system 1). $R_{5'\text{-AMP}}$ 1.0, 0.92 (system 2). The 3'-AMP was eluted by the 0.3 M TEAB, R_f 0.12 (system 1), $R_{5'\text{-AMP}}$ 0.65 (system 2).

2. With Benzeneboronic Acid. The solution containing the 2'- and 5'-AMP's from experiment 1 was evaporated in vacuum to 10 ml and was mixed with a threefold excess of benzeneboronic acid, and at pH 8.2 it was transferred to a column through which 15 ml of a 0.1 M solution of benzeneboronic acid with pH 8.2 had previously been passed. The excess of benzeneboronic acid was washed out with 0.1 M TEAB. The 2'-AMP was eluted by 0.22 M TEAB, $R_{5'\text{-AMP}}$ 3.0 (system 1), $R_{5'\text{-AMP}}$ 0.92 (system 2). The 5'-AMP was eluted with 0.35 M TEAB, R_f 0.0 (system 1), $R_{5'\text{-AMP}}$ 1.0 (system 2) (see Fig. 2).

Separation of Nucleosides. A solution with pH 7.0 containing 2 mg of cytidine, 2 mg of adenosine, 2 mg of uridine, and a threefold excess of benzeneboronic acid was transferred to a column. The cytidine was eluted with 0.05 M TEAB, R_f 0.45 (system 3). A mixture of adenosine and uridine was eluted with 0.1 M TEAB, R_f 0.38, 0.67 (system 3) (see Fig. 3).

CONCLUSIONS

A method for the chromatographic separation of a number of nucleosides and mononucleotides has been proposed which is based on the formation of anionic complexes with m-nitrobenzeneboronic and benzeneboronic acids.

LITERATURE CITED

1. R. Braun, Biochim. Biophys. Acta, 142, 267 (1967); S. Zadrazie, Z. Sormove, and F. Sorm, Coll. Czech. Chem. Comm., 26, 2643 (1961).
2. V. Sanno, Chem. Pharm. Bull., 9, 657 (1961); M. Nonjo, V. Furukawa, and K. Kobayashi, Chem. Pharm. Bull., 14, 1061 (1966).

3. British Patent No. 959,326 (1964); Chem. Abstr., 61, 9716 (1964); I. S. Kulaev and V. N. Belozerskii, Biokhim., 26, 188 (1961).
4. M. Carrara and G. Bernardi, Biochim. Biophys. Acta, 155, 1 (1968).
5. J. P. Lorand and J. O. Edwards, J. Org. Chem., 24, 769 (1959).
6. J. X. Kxym, and W. E. Cohn, Biochim. Biophys. Acta, 15, 139 (1954).
7. W. E. Cohn, J. Am. Chem. Soc., 72, 1471 (1950).
8. Collection: Organic Syntheses [Russian translation], No. 2, p. 5, Moscow (1961).
9. F. J. Welcher, Org. Anal. Reagents, 3, 456 (1957).