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Note

Novel application of sugar–borate complexation for separation of ribo-, 2'-deoxyribo-, and arabinonucleosides on cation-exchange resin

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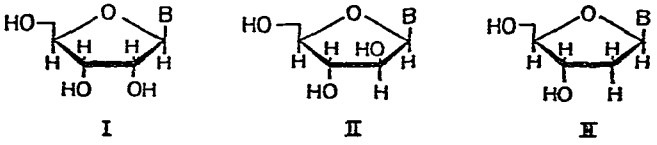
Conversion of ribonucleosides to anions by borate complexation provides an additional parameter for separation on anion-exchange resins. The principle was exploited in the early 1950s by Jaenicke and von Dahl[1] and by Khym[2] and Cohn[3] to separate sugars and nucleosides by anion exchange on Dowex 1 borate columns. The discovery of anion-exclusion chromatography by Singhal[4] led investigators to apply borate complexation for the separation of nucleosides on cation exchangers. Khym[5] has demonstrated group separation of ribonucleotides, ribonucleosides, and purine and pyrimidine bases by anion-exclusion chromatography on Bio-Gel P-2 in the presence of borate ions. Pal *et al.*[6] separated ribonucleosides from deoxyribonucleosides by chromatography on Aminex A-6 (a cation-exchange resin) in the presence of borate.

I wish to report now that the same principle may be utilized to separate ribonucleosides (I), arabinonucleosides (II), and deoxyribonucleosides (III).



EXPERIMENTAL

Materials and equipment

Ribonucleosides and deoxyribonucleosides were obtained from Schwarz/Mann (Orangeburg, N.Y., U.S.A.). 1-β-D-Arabinofuranosyluracil (ara-Urd), 1-β-D-arabinofuranosylcytosine (ara-Cyd) hydrochloride, and 1-β-D-deoxyribofuranosyluracil were obtained from Calbiochem (La Jolla, Calif., U.S.A.). 9-β-D-Arabinosyladenine (ara-Ado) was procured from Sigma (St. Louis, Mo., U.S.A.). The nucleosides were used without any further purification. All other chemicals were reagent grade. The cation-exchange resin, Aminex A-6 ($17 \pm 2.5\,\mu m$), was purchased from Bio-Rad Labs. (Richmond, Calif., U.S.A.) and was cleaned as described previously[4]. A jacketed glass

column (Cat. No. LC-6M-23, Laboratory Data Control., Riviera Beach, Fla., U.S.A.) was used for the A-6 resin. These columns have minimum dead volume at the ends and are connected with narrow-bore PTFE tubing (I.D. 0.3 mm) so as to minimize zone-spreading. An off-column septum injector (LDC Cat. No. 164A11) was used for injecting samples of 2 to 6 μl into the column with a Hamilton syringe fitted with a hypodermic needle. The column effluent was continuously monitored and recorded by essentially the same procedure as described in detail by Uziel *et al.*[7].

Solvent and samples

Borate buffer (0.1 *M*, pH 7.4) was prepared by adjusting the solution of boric acid in distilled water to pH 7.4 with ammonia. Samples were prepared by dissolving the nucleosides in water, except ara-Ado, which was dissolved in 0.1 *M* HCl (about 100 A_{260} units/ml). A sample mixture was obtained by mixing equal volumes of solutions of different nucleosides. One A_{260} unit is defined as the amount of material in 1 ml of a solution that has an absorbance of 1.0 when it is measured with a 1.0-cm optical path at 260 nm.

RESULTS AND DISCUSSION

Separation of the three groups of nucleosides: (1) Urd, ara-Urd, and dUrd; (2) Cyd, ara-Cyd, and dCyd; (3) Ado, ara-Ado, and dAdo are shown in Figs. 1–3,

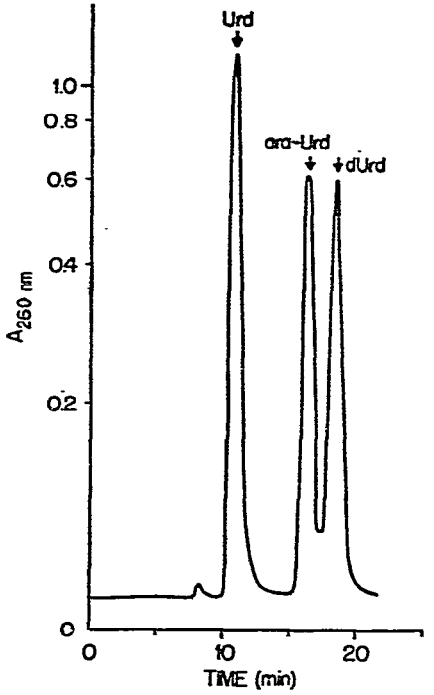


Fig. 1. Separation of a mixture of Urd, ara-Urd, dUrd. A 6-μl sample was injected into an Aminex A-6 column (20 × 0.63 cm; maintained at 50°) and eluted with 0.1 *M* boric acid adjusted to pH 7.4 with 1 *M* ammonia at 0.31 ml/min (50 p.s.i. pressure).

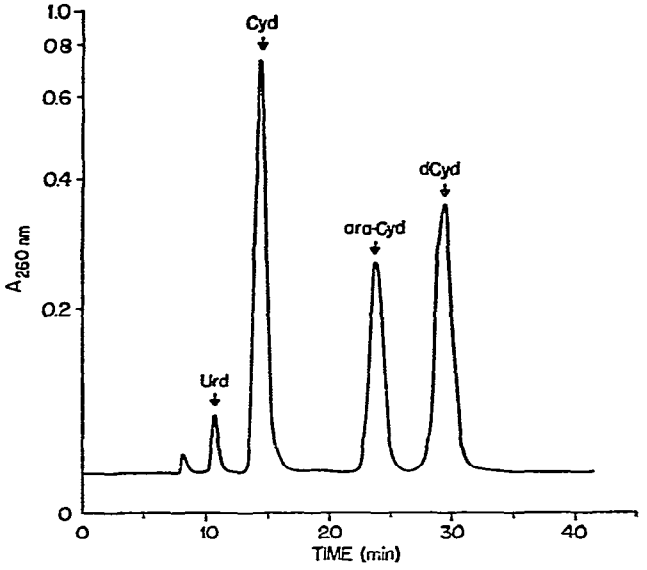


Fig. 2. Separation of a mixture of Cyd, ara-Cyd, dCyd, and Urd (impurity in the Cyd sample). Other conditions same as in Fig. 1.

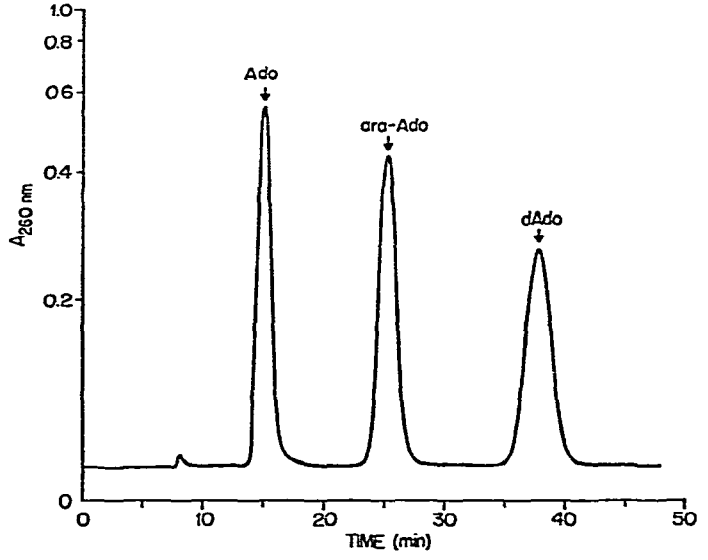


Fig. 3. Separation of a mixture of Ado, ara-Ado, dAdo. Other conditions same as in Fig. 1.

respectively. There is a slight overlap between the ara-Urd and dUrd peaks. Separation of the group of nucleosides, Guo, ara-Guo, and dGuo was not studied because ara-Guo was not available. In all three cases, ribonucleosides were eluted first, followed by arabinonucleosides and deoxyribonucleosides. This is in accord with our concept of stability and formation of borate complexes. In ribonucleosides (I) the 2′- and 3′-

hydroxyl groups are in the *cis* position, which facilitates the formation of a borate complex, whereas in arabinonucleosides (II) the 2'- and 3'-hydroxyl groups are in the *trans* position and complex less well with borate. The 2'-deoxyribonucleosides (III) lack vicinal hydroxyl groups and hence do not form borate complexes at all. The anions are excluded according to the amount of negative charge carried by them as a result of borate complexation. The order of elution is ribonucleosides, arabinonucleosides, and deoxyribonucleosides.

The chromatographic system will be useful in studies on the incorporation of arabinonucleosides in nucleic acids[8] and in the analysis of nucleotide pools from tumors being treated with ara-Cyd. It is not capable of separating all the ribonucleosides, 2'-deoxyribonucleosides, and arabinonucleosides in one step, but a sequential combination of this system with another chromatographic system such as the one reported by Uziel *et al.*[7] may be exploited for positive identification of a nucleoside. A fraction from the borate column containing the nucleoside of interest can be injected directly into the formate column, since the nucleosides are eluted in small volumes, and the weak buffer (0.1 *M* ammonium borate) is effectively neutralized by the strong formate buffer (0.4 *M* ammonium formate, pH 4.7). Using this technique, we have identified 5-chlorodeoxyuridine in the liver DNA hydrolysate obtained from mice kept on 5-chlorouracil saturated water, although the isolated DNA was contaminated with a small amount of RNA[9]. The advantage of this chromatographic system over conventional ones using anion exchange is quite obvious. Here the column is always ready for re-use, whereas in the latter system the column has to be regenerated after each use with the initial buffer solution.

ACKNOWLEDGEMENT

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