Chromatographic Separations of Cytosine Containing Compounds

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During work on the biological formation of deoxyribosidic compounds it became important to separate deoxyribosides and deoxyribotides from the corresponding ribosides and ribotides. The present note describes the separation of different cytosine containing compounds by a simple procedure involving chromatography on Dowex 50 (H+-form, 200–400 mesh). Fig. 1 shows a complete separation of cytidine-5′-phosphate, deoxycytidine-5′-phosphate, cytidine, deoxycytidine and cytosine on such a column. Cytidine-2′ (and -3′)-phosphate moved as the deoxyribotide. The recovery of all substances was 95–100 %. The compounds may be recovered quantitatively by simple evaporation *in vacuo* of the solvent.

The compounds could also be separated by paper chromatography with a borate containing solvent (20 ml of 5 M ammonium acetate, pH 9.5, 80 ml of saturated sodium tetraborate, 220 ml ethanol and 0.5 ml of 0.5 M versene). This solvent is a modification of the one originally described by Plesner [1]. The following R_F values were obtained: Cytidine-5′-phosphate = 0.06; deoxycytidine-5′-phosphate = 0.12; cytidine = 0.27; cytosine = 0.68; and deoxycytidine = 0.76. The R_F values changed considerably in different experiments but the relative positions of the substances remained the same. The same solvent could also be used for a similar separation of uracil or thymine containing compounds.

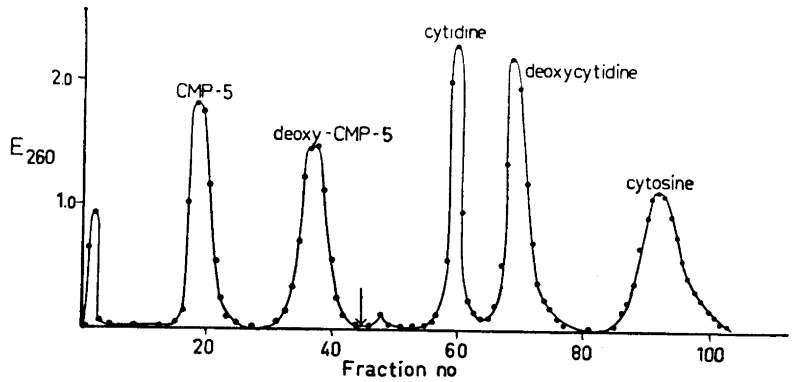

Fig. 1. Separation of 4—5 μmoles each of cytidine-5′-phosphate, deoxycytidine-5′-phosphate, cytidine, deoxycytidine, and cytosine on Dowex 50 (H+-form, length 10 cm, diam. 0.9 cm). Fraction size: 4—5 ml per 30 min. The chromatogram was first developed with 0.2 N acetic acid; at the arrow elution was started with N HCl. The first peak represents uracil containing compounds present as impurities in the commercial cytosine derivatives.

These methods have been used for the preparation of deoxycytidine and deoxycytidine-5′-phosphate from *in vitro* experiments [2].

1. Plesner, P. *Acta Chem. Scand.* **9** (1955) 197.
2. Reichard, P. *Biochim. et Biophys. Acta* **27** (1958) 434.

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