

An improved paper chromatographic separation of four ribonucleosides, their 3'(2')-phosphates and 3'(2'),5'-diphosphates

While studying an enzymic hydrolysis of RNA, I tried to identify 5'-phosphoryl terminal, internal, and 3'-hydroxyl terminal nucleoside residues of the fragments (pXpYpY'p --- Z*). Upon alkaline hydrolysis¹ these residues can be liberated almost quantitatively as three groups of compounds, namely pXp, Yp (including Y'p ---), and Z, respectively, the separation of which is the present subject. The hydrolysed mixture was neutralized with Dowex 50-H⁺ and subjected to two-dimensional paper chromatography as described by FELIX *et al.*². This method had originally been devised for similar compounds of the deoxyribose type (T in place of U), and actually separates them well. However, no solvent system has ever been reported for separation of the corresponding ribose type compounds and separation was poor in the present case. The ribose type compounds, particularly the diphosphates, were closer to one another than the corresponding deoxyribose compounds in the first run in the ethanol-1 M ammonium acetate buffer, pH 7.5 (75:30). Furthermore, C and U, Cp and Up, and pCp and pUp, which were not clearly separated within each group of compounds segregated in the first run, again ran together in the second run in saturated ammonium sulfate-water-isopropanol (80:18:2).

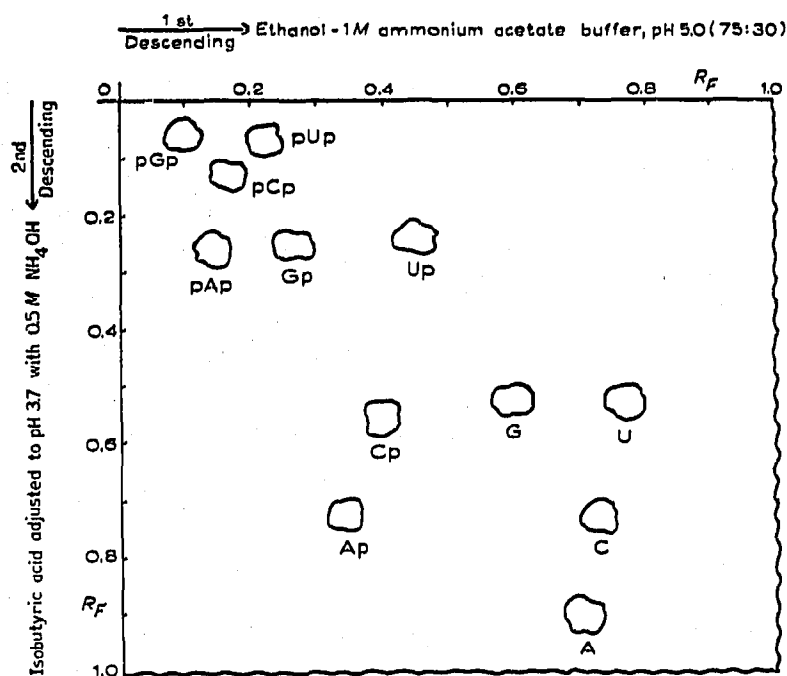


Fig. 1. Separation of ribonucleosides, ribonucleoside-3'(2')-phosphates, and ribonucleoside-3'(2'),5'-diphosphates.

This poor result prompted the development of a new and suitable solvent system. As shown in Fig. 1, ethanol-1 M ammonium acetate buffer, pH 5.0 (75:30), was used in the first direction. This is merely a slight modification of the solvent

* The abbreviations of the derivatives of nucleic acids are those used by *J. Biol. Chem.* When dealing with fragments of unknown composition, nucleosides are represented by capital letters X, Y, Y' and Z.

system used previously, the pH being changed from 7.5 to 5.0, but resulted in increased mobility, and so wider distribution, of all the components, particularly the diphosphates, while retaining the excellent group separation, except for pUp and Gp, which overlapped. Isobutyric acid adjusted to pH 3.7 with 0.5 M NH_4OH (ref. 3) was used in the second direction. This solvent separated C and U, Cp and Up, and pCp and pUp, respectively, with sufficient distance between them for clear distinction. pUp and Gp were also well separated. U and G, Up and Gp, and pUp and pGp, which almost co-chromatograph in this solvent, represent the extremes within each group of compounds in the first run.

Ultraviolet-absorbing spots were detected under an U.V. light, eluted, and determined spectrophotometrically. Commercial nucleosides and their 3'(2')-phosphates were used as standards. Diphosphates, which were not available, were identified from phosphorus/ribose/base ratio analyses as well as spectrophotometric data.

The solvent system suggested has proved useful in base-specificity work on ribonucleolytic enzymes in this laboratory.

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*Agricultural Chemistry Institute,
Kyushu University, Fukuoka (Japan)*

JUN-ICHIRO MUKAI

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The adsorption of metal ions from HF-HClO_4 mixtures on some ion exchange papers

Anion exchange from HF-HCl mixtures has found extensive analytical applications notably for those ions which complex strongly with HF such as Zr, Nb and Ta¹.

Only very few data on ion exchange from HF-HClO_4 can be found in the literature² and so far only the adsorption of Ta had been studied.

The present investigation is a comparison of HF-HCl and HF-HClO_4 for those ions which were shown by NELSON *et al.*¹ to adsorb from HF-HCl solutions. Both anion exchange papers (anionic resin paper SB-2 and Macherey, Nagel & Co. quaternary ammonium cellulose paper) and a cation exchange paper (sulphonic resin paper SA-2) were examined. The concentration of HCl or HClO_4 was maintained constant at 1N and the HF concentration was varied from 1 % to 10 %.

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