

over pieces of copper wire heated to some 500° C.

The electromotive forces of the cells and the time passed since the last washing of the electrodes were measured. Cell (B) and (C) became constant in 20 hours since the last washing of the left electrodes had been effected.

The work has been completed at Laboratoriet for Fotokemi, Fotografi og Reproduktionsteknik, Technical University, Copenhagen. The authors thanks are due to dr. E. Güntelberg for information and help in the work.

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Paper Chromatography of Nucleoside Polyphosphates

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Paper chromatography according to the method of Hanes and Isherwood¹, namely descending chromatography with *n*-propanol-ammonia (*d* 0.880)-water (60:30:10) separates the 5'-mono-, di- and triphosphates of any one of the ribonucleosides adenosine, guanosine, inosine, cytidine and uridine from each other. On the other hand, difficulties are generally encountered on attempts to separate by this method mixtures which contain phosphates of the different nucleosides. Some time ago, the need arose in this laboratory for the resolution of mixtures containing different adenosine and inosine 5'-phosphates and a method for the separation of such mixtures by two-dimensional paper chromatography was devised². In the first direction, the chromatogram was developed with *n*-propanol-ammonia-water, separating the individual members in each series and in the second direction with a solvent system consisting of saturated ammonium sulphate solution-water-*iso*-propanol (79:19:2), which had been introduced³ for the separation of the pro-

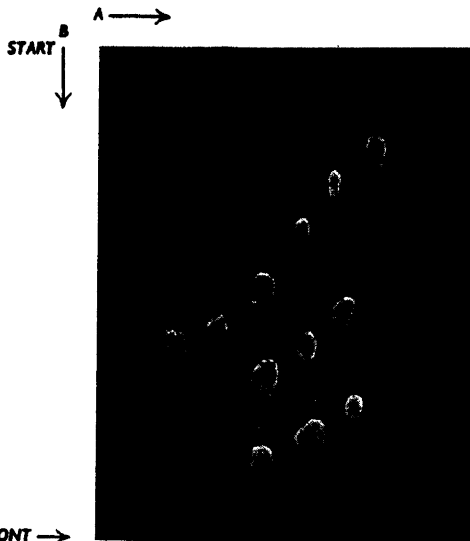


Fig. 1. Two-dimensional chromatogram (Whatman No. 1 paper) of a mixture of AMP, ADP, ATP, GMP, GDP, GTP, IMP, IDP, ITP, UMP, UDP and UTP, approximately 50 µg of each compound. (A) first solvent: *n*-propanol-ammonia-water (60:30:10), 60 hours. (B) second solvent: saturated ammonium sulphate solution-water-*iso*-propanol (79:19:2), 10 hours. (1) AMP; (2) ADP; (3) ATP; (4) GMP; (5) GDP; (6) GTP; (7) IMP; (8) IDP; (9) ITP; (10) UMP; (11) UDP; (12) UTP.

ducts of hydrolysis of nucleic acids. It was found that this solvent separates the two nucleoside series of phosphates from each other.

In the course of recent work on the isolation of the 5'-triphosphates of guanosine^{4,5} and uridine^{4,6} from muscle, the ammonium sulphate-water-*iso*propanol solvent system proved to be useful for the separation of the 5'-triphosphates of different nucleosides including those of guanosine, uridine, adenosine, inosine and xanthosine. We have therefore applied the previously described method of two-dimensional paper chromatography to the resolution of mixtures containing the 5'-mono-, di- and triphosphates of adenosine (AMP, ADP and ATP), guanosine (GMP, GDP and GTP), inosine (IMP, IDP and ITP) and uridine (UMP, UDP and UTP) and obtained adequate resolution of all the twelve components (Fig. 1). On the other hand,

the 5'-mono-, di- and triphosphates of cytidine (CMP, CDP and CTP) are not separated by this method from the corresponding 5'-phosphates of uridine.

Experimental. Materials. The adenosine and inosine polyphosphates⁷ and the cytidine, guanosine and uridine polyphosphates^{5,6} were prepared and purified as described previously, except for CMP and UMP which were obtained by courtesy of Prof. A. R. Todd, Cambridge. *Method* as described in previous communications for qualitative² purposes and for analysis⁸ on the eluted spots.

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Isotope Effect in the Hydrolysis of Grignard Compounds

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Hydrolysis of Grignard compounds of the type RMgX by water containing heavy hydrogen isotopes has been carried out, mostly for preparative purposes, by several investigators. In these reactions the water has usually been present in a deficient or approximately theoretical quantity.

In the present investigation Grignard compounds have been hydrolyzed by a large excess of tritiated water. Under such

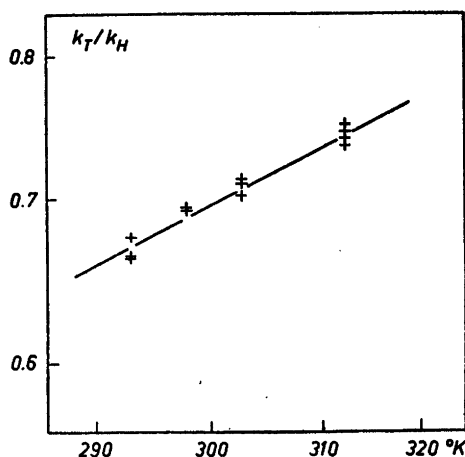
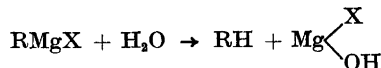


Fig. 71. The ratio k_T/k_H (logarithmic scale) for methane formation as a function of the absolute temperature (inverted scale.)

conditions, one might be able in a simple way to calculate the ratio k_T/k_H between the reaction rates of tritium and protium, respectively, and further, to draw conclusions about the reaction mechanism. Water has been present in an approximately ten-fold excess over the Grignard compound, and therefore the reaction in practice must be expected to take place according to



especially as no precipitates of basic magnesium salts are formed in the solvents used. Such precipitates include water and in this way withdraw it from the reaction with RMgX , and if the amount of water present is deficient both hydrogen atoms will react¹. The reaction may also be assumed to take place practically completely with regard to RMgX .

In the present experiments on the hydrolysis of methylmagnesium and phenylmagnesium iodide, the Grignard compound, dissolved in anisole, was added to a solution of water in pyridine by means of an injection syringe or by a dropping funnel. The anisole as well as the pyridine had been dried according to current methods and afterwards tested for moisture by the usual Zerewitinoff method. In the case of $\text{C}_6\text{H}_5\text{MgI}$ a fraction of the solvent was