

## A General Method for Reduction of Pyrimidine Nucleosides and Nucleotides

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The reduction of the pyrimidine ring in nucleosides and nucleotides has recently attracted considerable attention. Burke<sup>1</sup> reported the reduction of pyrimidine nucleosides with sodium in liquid ammonia and ethanol. Cohn and Doherty<sup>2</sup> reduced catalytically pyrimidine nucleosides and nucleotides using a rhodium catalyst and simultaneously with this latter work the reduction of thymidine with sodium amalgam and the subsequent isolation of the sugar component was reported<sup>3</sup>.

This simple reduction method has now been extended to the following substances: deoxycytidine, deoxycytidylic acid, thymidylic acid, thymidine diphosphate, deoxyeytidine diphosphate, cytidylic acid, uridylic acid, cytidine and uridine. In all cases the reduction is carried out by shaking for a few hours at room temperature an aqueous solution of the substance with 3 % sodium amalgam. The complete reduction of the 4,5-ethylenic linkage in the pyrimidine ring was demonstrated by the disappearance of the UV absorption at 260 m $\mu$ <sup>4</sup>. With thymidylic acid and thymidine diphosphate the rate of reduction was slower. The present report concerns in particular the reduction of the deoxyribose nucleosides and nucleotides. The completeness of the reduction was also shown by the fact that the modified Dische diphenylamine test<sup>5</sup> after the reduction revealed the presence of between 90 and 100 % of the theoretical amount of 2-deoxy-D-ribose. Examination of the reduction products by paper chromatography, paper electrophoresis and ion exchange chromatography revealed the presence of several deoxyribosyl substances as was the case with thymidine<sup>3</sup>. This is due to the fission of the dihydropyrimidine ring under the prevailing alkaline condition. The reduction products are interesting. First some of them may be intermediates in the biosynthesis of nucleosides and nucleotides. Work to establish this is in progress. Secondly, for tracer work where the isolation of sugar and sugar phosphates is necessary, these reduction products are convenient because of the ability of the glycosidic

linkage towards acid, which enables isolation of the free sugar or the sugar phosphate. After short heating of reduction mixtures of thymidine, deoxycytidine, deoxycytidylic and thymidylic acid, respectively, with Amberlite IR-120 (H), 2-deoxy-D-ribose and 2-deoxy-D-ribose 5-phosphate were isolated in good yield by paper chromatography in propanol-ammonia (conc.), 6:4. Finally, subsequent to reduction, colour tests for the sugar component may be used for detecting and estimating pyrimidine nucleosides and nucleotides. Using the modified Dische diphenylamine method<sup>5</sup> for 2-deoxy-D-ribose, standard curves for the determination of thymidine, deoxycytidine, deoxycytidylic acid and thymidylic acid after reduction with 3 % sodium amalgam were made. Linear curves were obtained. It was shown that quantities of 50  $\mu$ g of nucleosides or nucleotides could be estimated. This method for deoxyribose pyrimidine nucleosides and nucleotides is particularly useful when the presence of other UV absorbing material, prevents the use of UV absorption spectroscopy.

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## The Synthesis and Characterization of Some AMP-Sulphates

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Adenosine-5'-phosphosulphate has been implicated as an intermediate in the enzymic activation of sulphate<sup>1</sup>. It has been synthesized by the condensation of a sulphur trioxide-pyridine complex with adenosine-5'-monophosphate<sup>2</sup> (AMP), and by the carbodiimide method<sup>3</sup>.

The carbodiimide method was used for the synthesis of some structurally related AMP-sulphates, containing sulphate linked to the hydroxyl groups of the ribose moiety. The following compounds were synthesized and characterized:

**AMP-monosulphates:** Adenosine-5'-phosphosulphate (APS). Inorganic sulphate was rapidly liberated from this compound by acid hydrolysis. It was somewhat more resistant to alkaline and CaO hydrolysis. It was degraded to adenosine, phosphate and sulphate by snake venom and prostatic phosphatases. It was an intermediate in the enzyme system for sulphate activation<sup>4</sup>. Adenosine-2'-sulphate-5'-phosphate and adenosine-3'-sulphate-5'-phosphate decomposed at a slower rate than APS during acid hydrolysis and were very slowly degraded by alkaline and CaO hydrolysis. Digestion with snake venom phosphatase produced adenosine, phosphate and sulphate. Prostatic phosphatase produced adenosine-sulphates + phosphate.

**AMP-disulphates:** The disulphates were probably a mixture of three different compounds, which were not further separated. Two of the compounds conceivably contained one phosphosulphate and one ribose-sulphate linkage. The third compound contained both sulphates attached to ribose.

**AMP-trisulphate:** Adenosine-2',3'-disulphate-5'-phosphosulphate was degraded to adenosine-2',3'-disulphate-5'-phosphate and sulphate during acid, alkaline and CaO hydrolysis. Further decomposition occurred at a slower rate. Snake venom phosphatase produced adenosine-2',3'-disulphate-5'-phosphate + sulphate. Prostatic phosphatase produced adenosine-2,3'-disulphate, phosphate and sulphate.

The factors influencing the yield of the different AMP-sulphates were studied. APS appeared as the primary product. By increasing the amount of sulphuric acid in the reaction mixture and prolonging the reaction time a total yield of AMP-sulphates amounting to 90—100% (calc. on AMP) could be obtained. It was possible to obtain a 40—50% conversion of AMP to APS without more than small amounts of other AMP-sulphates.

The different AMP-sulphates could be separated from each other by column electrophoresis on a gram scale.

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4. We wish to thank Dr. P. W. Robbins for testing the enzymic activity of our APS.

## Complex Formation of Nucleoside Triphosphates with Metal Ions\*

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Transphosphorylation reactions with nucleoside triphosphates as the phosphate donor require metals as cofactors, especially  $Mg^{2+}$ ,  $Mn^{2+}$  or  $Ca^{2+}$ . It has therefore been suggested that metal nucleotide complexes are the actual coenzymes. In the present work the dissociation constants of several nucleoside triphosphates of  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Co^{2+}$  and  $Mn^{2+}$  have been determined. An ion exchange method according to the principle reported by Schubert has been adopted. An anion exchange resin (Dowex-1) in the chloride form has been used. The dissociation constants of mononucleotides with different metals have been determined under the following conditions. A total volume of 20 ml contained (concentrations):  $0.5 \times 10^{-4}$  M nucleotide, 0.1 M NaCl, 0.005 M tris(hydroxymethyl)aminomethane buffer, pH 8.2, and usually 50 mg resin. Equilibration was obtained without and with addition of metal salts to the mixture by shaking for 3 h at 23 °C. The concentration of nucleotide not bound to the resin was determined by reading the extinction at appropriate wavelengths in ultraviolet region on a Beckman spectrophotometer. From these data the dissociation constants  $k_c$  for ATP with metals have been calculated.

$$k_c \text{ Ca} > k_c \text{ Mg} > k_c \text{ Co} > k_c \text{ Mn} \\ 1.7 \times 10^{-4} \quad 0.9 \times 10^{-4} \quad 0.23 \times 10^{-4} \quad 0.17 \times 10^{-4}$$

Dissociation constants for different metal complexes with ADP and AMP show the same sequence as reported for ATP. However, as an approximation complexes with ADP are 10 times more dissociated and complexes with AMP are 100 times more dissociated than the corresponding ATP metal complexes. This can be illustrated in the case of complexes with Mg

$$\text{ATP} < \text{ADP} < \text{AMP} \\ 0.9 \times 10^{-4} \quad 6.4 \times 10^{-4} \quad 1.1 \times 10^{-2}$$

The dissociation constants for CTP, GTP, UTP and ITP with the different metals show, with a few exceptions, the same sequence as reported for ATP. The values approximately correspond to those given for ATP metal complexes.

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