

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. Burke¹ reported the reduction of pyrimidine nucleosides with sodium in liquid ammonia and ethanol. Cohn and Doherty² 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 reported³.

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 μ ⁴. 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 test⁵ 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 thymidine³. 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 lability 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 method⁵ 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 μ 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 sulphate¹. It has been synthesized by the condensation of a sulphur trioxide-pyridine complex with adenosine-5'-monophosphate² (AMP), and by the carbodiimide method³.

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: