The Chemistry of Nucleosides and Nucleotides

A. M. MICHELSON

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PREFACE

". . . one can only hope that if we have not reached the last chapter in an interesting series of researches which was initiated by Miescher many years ago, we must be somewhat near the penultimate one."

W. D. HALLIBURTON

[*Annual Reports on the Progress of Chemistry*, **6**, 170, (1909)]

This book presents an attempt to describe the general chemistry of nucleosides and nucleotides and their major derivatives, the nucleotide coenzymes and nucleic acids, and also those biochemical aspects that are so intimately connected with the chemical approach that segregation becomes absurd. No description of carbohydrate or purine and pyrimidine chemistry *per se* has been essayed, since such topics are covered to a large extent by standard texts. Apart from giving, in the words of the publishers, " a bird's-eye-view of the subject of value to advanced students " it is hoped that this volume will be of some use to the numerous experimentalists whose efforts render the task of writing such an account both necessary and possible.

Paris
October 1962

A. M. MICHELSON

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CONTENTS

Chapter 1

INTRODUCTION

The biological significance of nucleosides, nucleotides and their derivatives is attested by the numerous publications now appearing. In large measure this renaissance is based on the emergence of a number of techniques which have facilitated the separation and identification of substances that were not amenable to treatment by the classical methods of organic chemistry, partly owing to the hybrid character of their properties and partly because of the relatively low stability of some of the linkages involved. A nucleotide is best defined as the phosphate ester of a sugar in glycosyl combination with a base molecule derived from purine or pyrimidine, that is, it is the phosphate ester of a nucleoside. In the naturally occurring compounds the nucleoside moiety is generally a β-D-ribofuranosyl or β-D-2-deoxyribofuranosyl derivative. Common usage embraces such substances as nicotinamide mononucleotide and riboflavin mononucleotide. The simplest units include adenosine-5′ phosphate, adenosine-5′ pyrophosphate and adenosine-5′ triphosphate as well as the mononucleotides obtained by degradative hydrolysis of nucleic acids. Others of a rather more complex nature are the diesterified pyrophosphate derivatives active as coenzymes, such as flavin adenine dinucleotide, coenzyme A, uridine diphosphate glucose and diphosphopyridine nucleotide, examples that also illustrate the somewhat haphazard nomenclature now prevalent. Finally, the polymeric nucleotide derivatives known as nucleic acids have molecular weights of up to several million, and exhibit biological properties close to life itself as components of chromosomes and viruses, and as agents in cell growth.

The earliest isolation of polynucleotide material reflects a connection with medical science that has grown significantly in recent times, many purine and pyrimidine derivatives being of considerable chemotherapeutic value. In 1869 Miescher[1] attempted the extraction of nuclear substances from pus cells (obtained from discarded surgical bandages) by digesting the cells with pepsin and dilute hydrochloric acid for prolonged periods, and then shaking the mixture with ether, when practically pure nuclear material settled at the bottom of the aqueous phase. From this he prepared a rather unusual acidic substance, *nuclein*, that contained a large amount of phosphorus, was insoluble in the usual organic solvents and in dilute acids, but was readily soluble

in dilute alkali. Although the scepticism of Hoppe-Seyler delayed publication for two years, until he and two of his research students could confirm and expand the work, further investigations by Miescher on his return to Basel soon showed that salmon sperm was a convenient source of high molecular weight nuclein and of a basic protein, which he named *protamine*, that could be extracted readily from the sperm with dilute acid. In later work by Altmann[2] (who introduced the term *nucleic acid* in 1889) and others, general methods were developed for the isolation of nucleic acids from yeast and a number of animal tissues, and in 1891 Kossel[3] described the hydrolysis of protein free nucleic acid. The existence of two main types of nucleic acid, now known as ribonucleic acid and deoxyribonucleic acid, was recognised at an early date in the pioneer work of Miescher and Kossel and later developed in the work of Hammarsten, Jones, Levene, and others. Historically, the subject has endured more disputes than usual, such as the controversy raised in 1899 by Bang's description of " guanylic acid " as a complex containing four guanine molecules, four phosphate groups joined together, three glycerols, and three pentoses; a controversy at times coloured with undue bitterness. Strong differences of opinion were expressed in the contest between a trinucleotide theory of nucleic acid advanced by Steudel and by Jones versus the tetranucleotide theory proposed by Levene, and an even more acrimonious discussion between Levene in the U.S.A., Gulland in Great Britain, and Bredereck in Germany ensued over an apocryphal guanine-uridylic acid.[4–11] The meretricious appeal of oversimplification has all too often succeeded in the absence of sound experimental evidence; in view of the nature of the problems involved and the techniques available, this is perhaps not too surprising.

Although inosinic acid was discovered by Liebig[12] in 1847 (the structure was not fully determined till some 90 years later) it was not until the isolation of muscle adenylic acid in 1927 by Embden and Zimmerman[13] and of adenosine triphosphate two years later by Lohmann[14] and by Fiske and Subbarow,[15] that a second major branch of nucleotide derivatives began to be explored. This group of compounds, loosely classified as nucleotide coenzymes, has grown considerably in the past decade, while the central significance of adenosine-5′ triphosphate in a multitude of biochemical reactions continues to be demonstrated, undiminished by the recognition of di- and triphosphates of the other major nucleosides. Methods of isolation, characterisation, and synthesis have now become routine, a marked contrast to the situation less than fifteen years ago when the chemical synthesis of adenosine triphosphate was regarded as a somewhat ludicrous and futile ambition.

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Chapter 2

CHEMISTRY OF NUCLEOSIDES*

The name *nucleoside*, introduced by Levene and Jacobs in 1909, was originally applied to the purine–carbohydrate derivatives isolated from alkaline hydrolysates of yeast ribonucleic acid.[1]

Isolation

Although nucleosides can be obtained either enzymically or by chemical means from any type of naturally occurring nucleotide material, the main sources of these glycosyls are the nucleic acids isolated from various tissues and organisms. Degradation of ribonucleic acid to a mixture of the component nucleosides can be accomplished in a number of ways, among which may be mentioned hydrolysis with dilute ammonia at elevated temperatures,[2] treatment with aqueous pyridine under reflux for several days,[3] and hydrolysis catalysed by various metal ions.[4] In a recently described method,[5] the ribonucleic acid (or mononucleotide) is refluxed in aqueous formamide for several hours at pH 4. Separation and isolation of the nucleosides has been greatly improved by the application of ion exchange methods.[6]

Chemical methods for the hydrolysis of deoxyribonucleic acids to deoxynucleosides have been unsuccessful for a variety of reasons; a rather laborious enzymic digestion with deoxyribonuclease, diesterase, and monophosphatase must therefore be used.[7] Again, the earlier isolation procedures have been considerably simplified by the application of ion exchange chromatography.[8]

A number of nucleosides, including some with antibiotic activity, occur naturally as such and can be extracted directly without previous hydrolytic procedures.

Structure

The major nucleosides obtained from ribonucleic acids are the purine derivatives, adenosine and guanosine, and the ribosyl pyrimidines, cytidine and uridine. In addition, certain ribonucleic acids contain a relatively high proportion of an isomer of uridine, pseudouridine.

* The older numbering system is used for pyrimidine derivatives, in conformity with purines. There seems little point in excepting the latter class from standard rules, but not the former.

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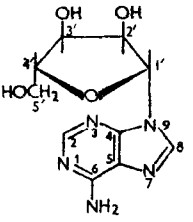
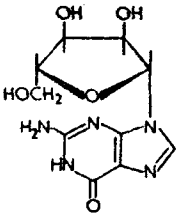
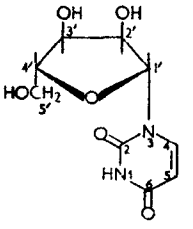
Various ribonucleosides derived from methylated purines and pyrimidines, including thymine, 5-methylcytosine, 1-methyladenine, 2-methyladenine, 6-methylaminopurine, 6-dimethylaminopurine, 1-methylguanine, 6-hydroxy-2-methylaminopurine, and 6-hydroxy-2-dimethylaminopurine (the so-called minor bases), occur in small amounts.[9] Nucleosides substituted at the sugar 2′ hydroxyl group have also been isolated.[10, 11] They are probably 2′-*O*-methyl derivatives.



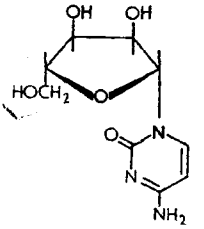
Adenosine



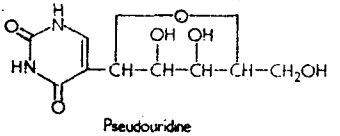
Guanosine



Uridine



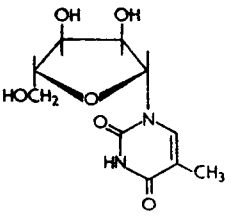
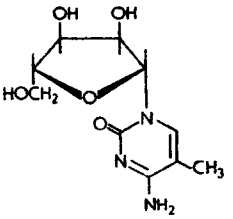
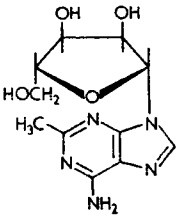
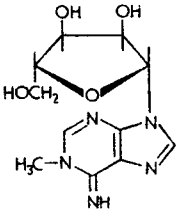
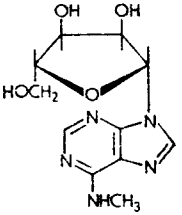
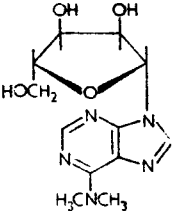
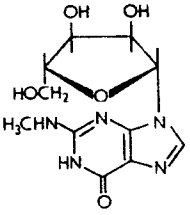
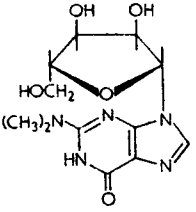
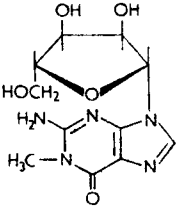
Cytidine



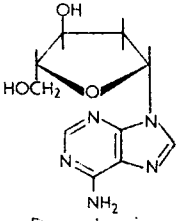
Pseudouridine

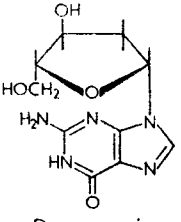
Major Ribonucleosides

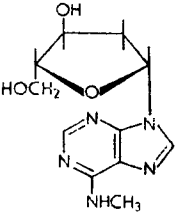
Deoxyribonucleic acids contain deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine as major nucleosides except in *Escherichia coli* T *even* numbered bacteriophage deoxynucleic acids where the cytosine is entirely replaced by 5-hydroxymethylcytosine[12] (or the 5-glucoside of 5-hydroxymethylcytosine). Deoxyribosyl-5-methylcytosine occurs in some deoxynucleic acids, particularly those from wheat germ,[13, 14] and small amounts of deoxyribosyl-6-methylaminopurine have been identified in deoxyribonucleic acids from various bacteria and bacteriophage.[15]

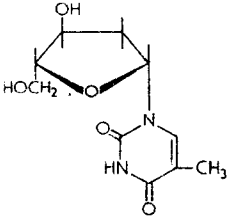










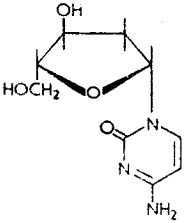
Minor ribonucleosides

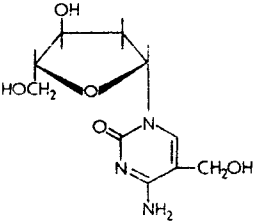

Deoxyadenosine

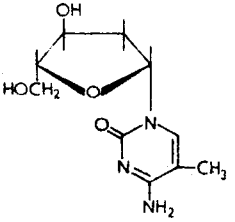

Deoxyguanosine




Thymidine


Deoxycytidine


5—Hydroxymethyldeoxycytidine



Deoxyribonucleosides

Deoxyuridine has been isolated from deoxynucleic acids of rather doubtful history[16] but is probably an artefact arising from deamination of cytosine residues.*

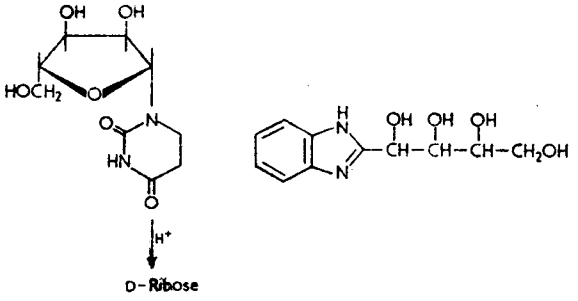
Complete knowledge of the structure of nucleosides involves the determination of (*i*) the nature of the base, (*ii*) the nature of the sugar, (*iii*) the mode of union and position of attachment of the sugar to base, (*iv*) the lactol structure of the sugar, and (*v*) the configuration at the sugar-base linkage.

Since the bases of the "classical" ribonucleosides—adenosine, guanosine, cytidine, and uridine—are readily obtained by acidic hydrolysis, they were early identified as adenine, guanine, cytosine, and uracil respectively.[17] In like manner, the bases present in deoxynucleosides and in the "newer" ribonucleosides (except pseudouridine) were isolated and identified after liberation by acidic hydrolysis. Comparison and identification with known purines and pyrimidines can now be made with extremely small amounts of material, using ultraviolet absorption spectra at several pH values, paper chromatography, paper electrophoresis, and ion exchange chromatography, techniques which have greatly facilitated the recognition and isolation of purine and pyrimidine derivatives that are present in nucleic acids in minor proportions.

The sugar moiety of adenosine and guanosine remained unidentified for many years until in 1911 Levene and Jacobs isolated it in crystalline form, and succeeded in characterising it as D-ribose, hitherto unknown.[18] Uridine and cytidine proved more difficult to investigate as the hydrolytic conditions necessary to rupture the glycosyl linkage are sufficiently vigorous to degrade the liberated sugar. However, preliminary hydrogenation gave a glycosyl-4,5-dihydropyrimidine that could be hydrolysed with dilute acid to yield D-ribose; in addition, simultaneous hydrolysis and oxidation of uridine with hydrobromic acid and bromine gave D-ribonic acid.[19] Confirmation of the presence of D-ribose in these four nucleosides was obtained by Gulland and co-workers,[20] who converted the sugar components into D-ribobenzimidazole, though this evidence was somewhat ambiguous owing to epimerisation reactions. As yet, no sugar other than D-ribose has been observed in a nucleoside shown to be derived from ribonucleic acid.

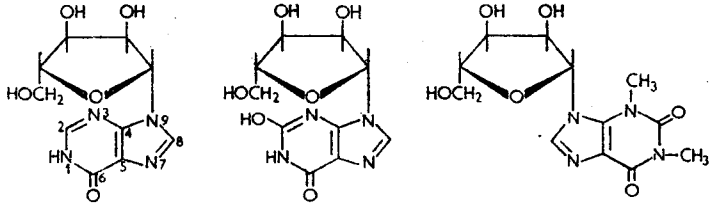
Considerable difficulty was encountered in determining the structure of the sugar component in the deoxynucleosides, as application of the hydrolytic conditions used on the purine ribonucleosides invariably yielded levulinic acid, and for some time the sugar was considered to be a hexose. However, Levene and his co-workers eventually isolated a crystalline deoxypentose by extremely mild acidic hydrolysis of deoxyguanosine.[21] The sugar did not form an osazone, showed many of the

* More recently it has been found that thymine is replaced by uracil in DNA from the *Bacillus subtilis* bacteriophage SP2; in another bacteriophage with the same host 5-hydroxymethyluracil is present instead of thymine.[369]



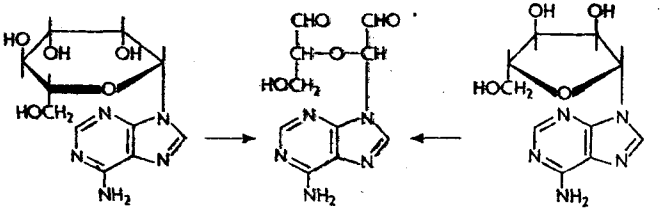
properties characteristic of 2-deoxysugars, and was identical with synthetic L-2-deoxyribose except that the specific rotation, while of the same numerical value, was opposite in sign.[22] More recently the crystalline benzyl mercaptal of D-2-deoxyribose has been isolated directly from deoxyribonucleic acid by mercaptanolysis,[23] and the sugar of the purine deoxynucleosides in a number of deoxynucleic acids (isolated from different sources) has been shown to be chromatographically identical with 2-deoxyribose.[24] Reduction of thymidine and deoxycytidine with sodium and ethanol, or with sodium amalgam in water, followed by mild acidic hydrolysis also yields D-2-deoxyribose,[25–27] while D-2-deoxyribose benzylphenylhydrazone has been prepared from deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine.[28] In the absence of contrary evidence it is generally assumed that the sugar in deoxynucleic acids is uniformly D-2-deoxyribose.

The rapid acidic hydrolysis of purine nucleosides suggested that the sugar was linked to the base as a ring *N*-glycosyl rather than via a C—C linkage, the amino groups of adenosine and guanosine being excluded since both substances could be deaminated (to inosine and xanthosine respectively) without loss of the sugar residue. Since xanthosine could be methylated to give ribosyl theophylline, positions 1 and 3 were eliminated leaving only N7 or N9 as the point of attachment.[29]

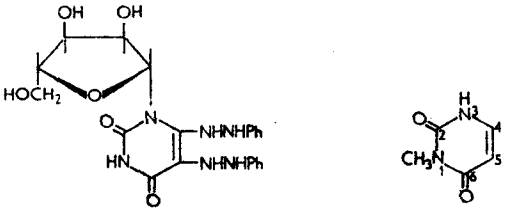


Whereas the ultraviolet absorption spectra of xanthosine are quite unlike those of 7-methylxanthine (or 1- or 3-methylxanthine) they

closely resemble spectra of the 9-methyl derivative. Similarly, ultraviolet absorption spectra of adenosine, inosine, and guanosine are very similar to those of 9-methyladenine, 9-methylhypoxanthine, and 9-methylguanine respectively, rather than the 7-methyl derivatives.[30–32] These results strongly indicated that the purine nucleosides are 9-ribosylpurines, a proof that is also valid for deoxyadenosine and deoxyguanosine since these substances show ultraviolet absorption spectra almost identical with those of adenosine and guanosine. Confirmation of the location of the sugar at position 9 in the purine nucleosides was provided by Todd and his co-workers through the unambiguous synthesis of 9-D-mannopyranosyladenine.[33] Periodate oxidation of this nucleoside gave a dialdehyde identical with that obtained from adenosine.



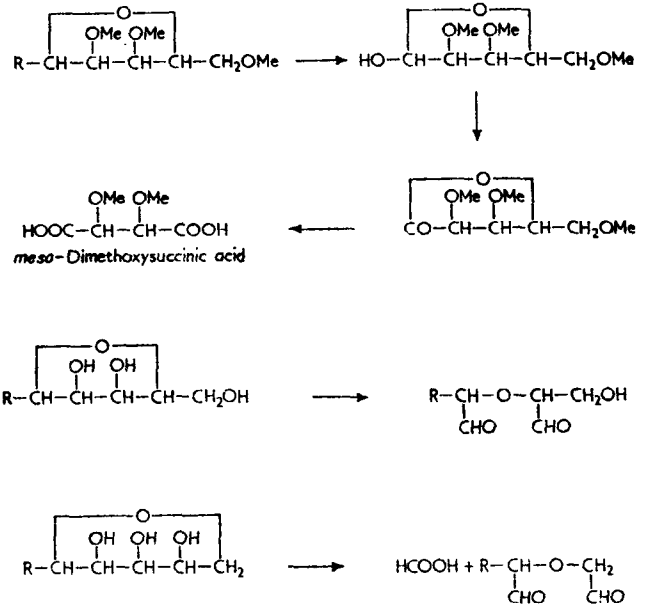
Since deamination of cytidine gives uridine, it is clear that the glycosyl linkage in the pyrimidine nucleosides does not involve C6, and bromination (or nitration) or uridine to the 5-bromo (or 5-nitro) derivative eliminates position 5. The action of phenylhydrazine on uridine previously treated with bromine gives 4,5-diphenylhydrazinouridine indicating the absence of substituents at position 4; the known instability of uracil O^2-glycosides towards dilute acids leaves only N1 and N3 as the point of glycosyl linkage.[34] Methylation of 2′,3′-di-*O*-acetyl-5′-*O*-trityluridine with diazomethane gave the *N*-methylated derivative which could be hydrolysed to 1-methyluracil, and therefore uridine and cytidine are 3-glycosyls.[35]



The isolation of 1-methylthymine from the hydrolysis products of methylated deoxyribonucleic acid indicates, but does not prove, that

thymidine is likewise a 3-glycosyl pyrimidine,[36] and the similarity in ultraviolet absorption spectra of cytidine and deoxycytidine provides evidence for the structure of the latter nucleoside. Confirmation of the structure of all these nucleosides is provided by the synthetic studies and interconversions described later. The structure of pseudouridine, a naturally occurring ribosyl pyrimidine containing a C—C glycosyl linkage, will be considered separately.

The furanose nature of the ribosyl residue in adenosine, guanosine, and uridine (and hence cytidine) was decisively demonstrated by Levene and Tipson.[37] Simultaneous methylation and deacetylation of tri-*O*-acetyladenosine gave a tri-*O*-methyl-*N*-methyladenosine that on acidic hydrolysis yielded 6-methylaminopurine and a trimethylribose, identified as 2,3,5-trimethyl-D-ribofuranose by oxidation first to trimethyl-γ-D-ribonolactone and then to *meso*-dimethoxysuccinic acid. The same oxidation products were obtained from methylated guanosine and methylated dihydrouridine. Subsequent synthesis of 2,3,5-trimethyl-D-ribofuranose gave final proof of the assigned structures.[38]



A more convenient proof of the ribofuranosyl structure of these nucleosides is provided by periodate titration.[39] A pentofuranosyl derivative consumes one molar equivalent of periodate on oxidation to the dialdehyde, as do the natural ribonucleosides; with pyranose isomers, two mols of periodate are consumed and one mol of formic acid

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