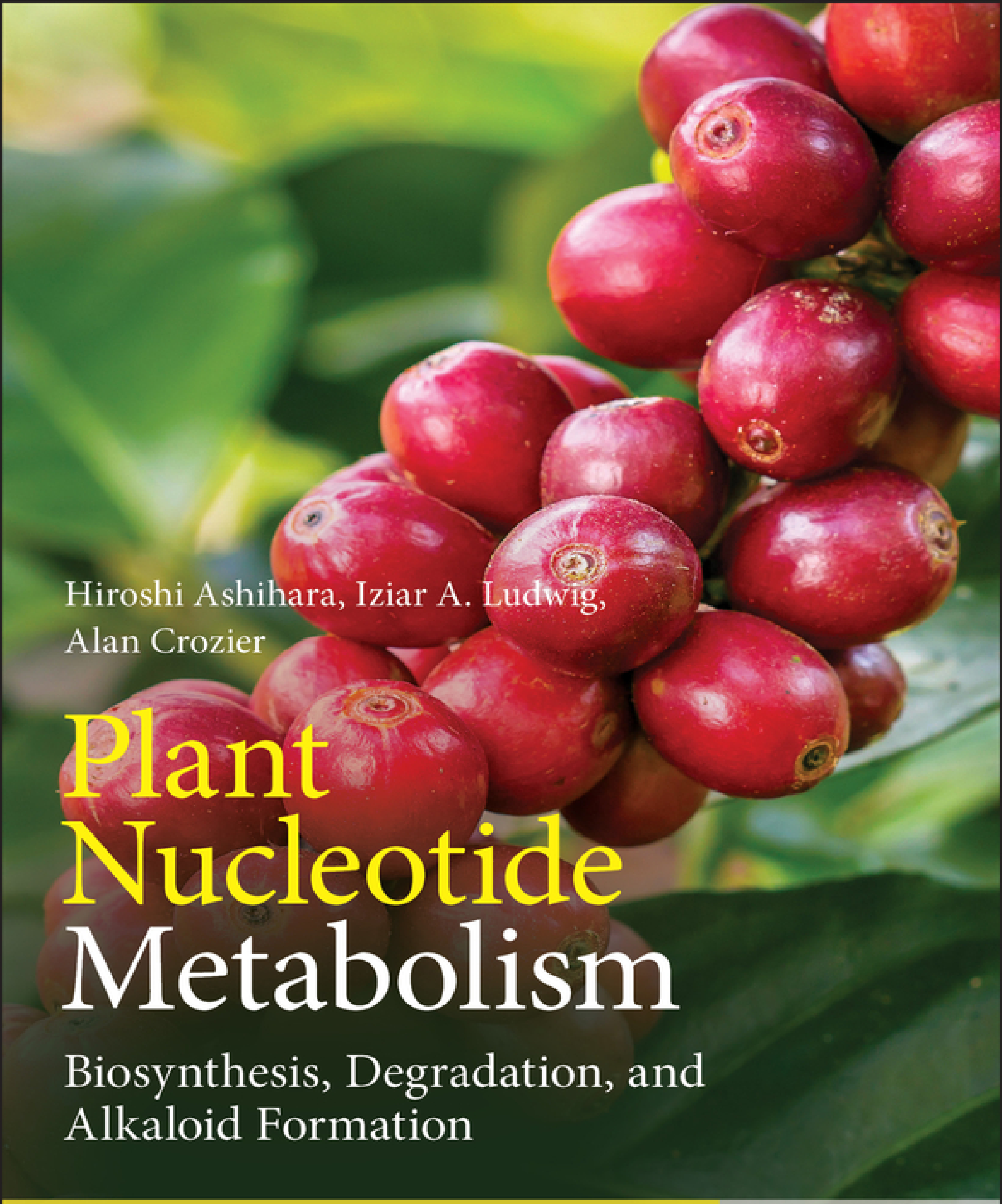

Hiroshi Ashihara, Iziar A. Ludwig,
Alan Crozier

Plant Nucleotide Metabolism

Biosynthesis, Degradation, and Alkaloid Formation

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Plant Nucleotide Metabolism – Biosynthesis, Degradation, and Alkaloid Formation

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Contents

Preface

Almost all organisms produce nucleobases, nucleosides and nucleotides of purines and pyrimidines. There have been a number of books on nucleotide metabolism in microorganisms and humans. However, this is the first to focus on plants which exhibit important differences to other organisms in key areas of nucleotide metabolism and function.

The book covers the metabolism of purine, pyrimidine and pyridine nucleotides and nucleotide alkaloids in higher plants and points out differences from that occurring in other organisms. Likewise, differences in the salvage pathways and diversity of interconversions in plants, fungi and bacteria are highlighted. Various physiological aspects of these processes are covered along with their involvement in the control of plant growth and development. Among the topics covered are the purine alkaloids caffeine, theobromine and their metabolites which, in species including coffee, tea and cocoa, accumulate in quantity. There is also discussion of the function of purine alkaloids and the potential allelopathic role of caffeine. Studies, some making use of genetically-modified plants, have indicated that caffeine can play a role in a variety of plant defence strategies. Other investigations have provided evidence that trigonelline, found principally in coffee and legumes, has a role in resistance to salt stress and can act as a natural pesticide to reduce insect infestations. Finally, the book explores the absorption, metabolism and potential impact on health of dietary caffeine, theobromine and trigonelline

This is the only book on plant nucleotide metabolism. It provides comprehensive information on nucleotide structures and metabolic pathways and is a unique resource on a diversity of topics and as such is essential reading for students, researchers, and lecturers in plant biochemistry, physiology, chemistry, agricultural sciences, nutrition and the associated applied fields of research.

We owe special thanks Professors Tatsuhito Fujimura, Claudio Stasolla and Takao Yokota for their help with some of the figures and advice on genes encoding key enzymes and chemical structures for the book.

Hiroshi Ashihara
Iziar A. Ludwig
Alan Crozier

Part I

General Aspects of Nucleotide Metabolism

1

Structures of Nucleotide-Related Compounds

1.1 Introduction

The chemistry of purine, pyrimidine, and pyridine nucleobases, nucleosides, and nucleotides constitute one of the oldest topics in biochemistry. In this chapter, the nomenclature and structures of nucleotides are briefly described.

1.2 Nomenclature and Abbreviations of Nucleotide-Related Compounds

The nucleotide nomenclature and abbreviations employed in the text are those used by Henderson and Paterson (1973) in their textbook '*Nucleotide Metabolism – An Introduction*'. The terms 'nucleoside' and 'nucleotide' in the strictest sense refer, respectively, to *N*-glycosides and phosphorylated *N*-glycosides derived from nucleic acids. However, they are now used in a wider context. *N*-Ribosides, such as nicotinamide mononucleotide (NMN), are called nucleotides only by extension and analogy, and nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are referred to as dinucleotides. Flavin mononucleotide (FMN) is a step further removed, as it contains ribitol, a pentose alcohol formed by the reduction of ribose, instead of ribose, while flavin adenine dinucleotide (FAD) similarly extends the meaning of dinucleotide. *N*-Glycosides such as orotidine 5′-monophosphate (OMP) and adenylosuccinate (SAMP) are called nucleotides through their close relationship to the 'true' nucleotides. The terms ribonucleoside and ribonucleotide are used in preference to riboside and ribotides. The IUPAC-IUB Combined Commission on Biochemical Nomenclature has abbreviations and symbols for nucleotides and related compounds. However, as argued by Henderson and Paterson (1973), while they are appropriate for polynucleotides, the distinction between bases and nucleosides is not always immediately obvious, and this has limited their use. The abbreviations used here are more intuitive and better suited to the portrayal of reaction schemes in which the addition or removal of substituent groups occurs.

In Table 1.1, the abbreviations for the major nucleotides, ribo- and deoxyribonucleotides and nucleobases are presented. For readers convenience, the styles used both in this book (style #1) and those recommended by IUPAC (style #2) are shown.

Plant Nucleotide Metabolism – Biosynthesis, Degradation, and Alkaloid Formation, First Edition.
Hiroshi Ashihara, Iziar A. Ludwig and Alan Crozier.

Table 1.1 Nomenclature and abbreviations of purine and pyridine ribo- and deoxyribonucleotides and related compounds.

Ribonucleotides		Ribonucleosides	#1	#2	Nucleobases	#1	#2
Adenosine-5′-monophosphate	AMP	Adenosine	AR	Ado	Adenine	A	Ade
Guanosine-5′-monophosphate	GMP	Guanosine	GR	Guo	Guanine	G	Gua
Inosine-5′-monophosphate	IMP	Inosine	IR	Ino	Hypoxanthine	H	Hyp
Xanthosine-5′-monophosphate	XMP	Xanthosine	XR	Xao	Xanthine	X	Xan
Uridine-5′-monophosphate	UMP	Uridine	UR	Urd	Uracil	U	Ura
Cytidine-5′-monophosphate	CMP	Cytidine	CR	Cyd	Cytosine	C	Cyt
Orotidine-5′-monophosphate	OMP	Orotidine	OR	Ord	Orotic acid	O	Oro

Deoxyribonucleotides		Deoxyribonucleosides	#1	#2	Nucleobases	#1	#2
Deoxyadenosine-5′-monophosphate	dAMP	Deoxyadenosine	AdR	dAdo	Adenine	A	Ade
Deoxyguanosine-5′-monophosphate	dGMP	Deoxyguanosine	GdR	dGuo	Guanine	G	Gua
Deoxyuridine-5′-monophosphate	dUMP	Deoxyuridine	UdR	dUrd	Uracil	U	Ura
Deoxycytidine-5′-monophosphate	dCMP	Deoxycytidine	CdR	dCyd	Cytosine	C	Cyt
Thymidine-5′-monophosphate	dTMP	Thymidine	TdR	dThd	Thymine	T	Thy

Two types of symbols are used for nucleoside and nucleobases. Style #1: recommended in *Nucleotide Metabolism* (Henderson and Paterson 1973). Style #2: recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (1970). In this book, style #1 is adopted.

Abbreviations for ribonucleosides and 2-deoxyribonucleosides are derived from those used for the bases plus those for the ribosyl or 2′-deoxyribosyl groups. Thus AR stands for adenosine and AdR is the abbreviation for deoxyadenosine. In the case of inosine, (hypoxanthine + ribose) HR may be possible, but IR is often used. The latter is used in this text.

For nucleotides, the traditional abbreviations based on the term ‘nucleoside monophosphate’ are used. Thus AMP stands for adenosine monophosphate (adenylate), UMP for uridine monophosphate (uridylate), and NMP for any ribonucleoside monophosphate. Similarly, dAMP stands for deoxyadenosine monophosphate (deoxyadenylate), dUMP for deoxyuridine monophosphate (deoxyuridylate), and dNMP for any deoxyribonucleoside monophosphate. Thymidine monophosphate (thymidylate) often does not have the ‘deoxy’ prefix in its name, because thymidine is thymine deoxyriboside. However, the symbol including a ‘d’ is commonly used in biochemistry textbooks, so dTMP is adopted in this article.

1.3 Chemical Structures of Nucleotide-Related Compounds

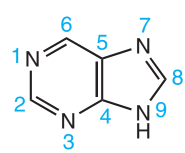
Studies on purines and pyrimidines began in 1776 when the Swedish pharmacist Carl Wilhelm Scheele isolated uric acid from bladder stones. In 1846, Unger isolated guanine from the guano of Peruvian sea birds. At the end of the nineteenth century, several purines (adenine, xanthine, and hypoxanthine) and pyrimidines (thymine, cytosine, and uracil) were discovered by the German biochemist, Albrecht Kossel who believed they constituted the main part of cell nuclei. In 1874 Friedrich Miescher isolated nuclear material rich in phosphorus which he called ‘nuclein’. In the same period, Emil Fischer (1884) elucidated the structures of caffeine and related compounds which he confirmed by chemical synthesis. Further information can be found in a historical survey by Burnstock and Verkhratsky (2012). The pyridine nucleotide, NAD was discovered by the British biochemists Arthur Harden and William John Young in the early twentieth century (Harden and Young 1906).

1.3.1 Purines

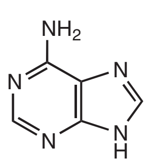
A purine is a heterocyclic compound that consists of a pyrimidine ring fused to an imidazole ring. The word, ‘purine’ (‘Purum’ + ‘Uricum’) was coined by Emil Fischer (1884).

1.3.1.1 Purine Bases

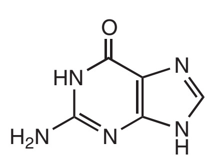
As shown in structure **1** the atoms of the purine ring are numbered in an anticlockwise manner. In plants, there are several naturally occurring purine bases. They include adenine (**2**) and guanine (**3**), which are constituents of nucleic acids, and hypoxanthine (**4**), xanthine (**5**), and uric acid (**6**), which are produced as catabolites of adenine and guanine. Purine alkaloids, such as theobromine (3,7-dimethylxanthine) (**7**), theophylline (1,3-dimethylxanthine) (**8**), caffeine (1,3,7-trimethylxanthine) (**9**), and theacrine (1,3,7,9-tetramethyluric acid) (**10**) are derived from purine nucleotides, as are the major cytokinin plant hormones isopentenyladenine (**11**), benzyladenine (**12**), and *trans*-zeatin (**13**) (Ashihara et al. 2013).



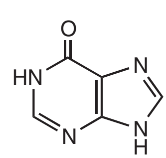
Purine: numbering system (**1**)



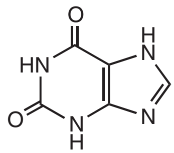
Adenine (**2**)



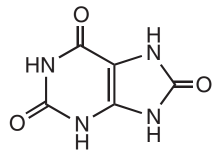
Guanine (**3**)



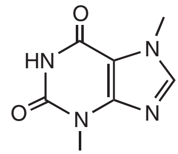
Hypoxanthine (**4**)



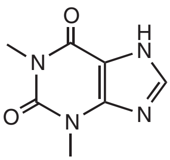
Xanthine (**5**)



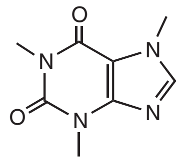
Uric acid (**6**)



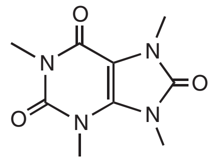
Theobromine (**7**)



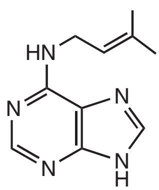
Theophylline (**8**)



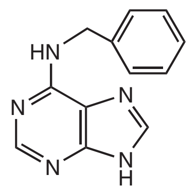
Caffeine (**9**)



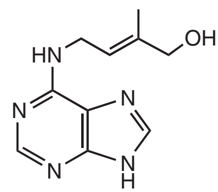
Theacrine (**10**)



Isopentenyladenine (**11**)



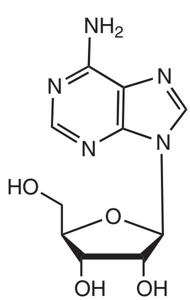
Benzyladenine (**12**)



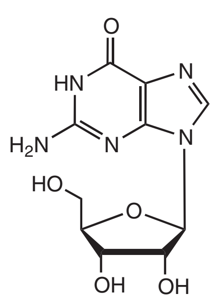
trans-Zeatin (**13**)

1.3.1.2 Purine Nucleosides

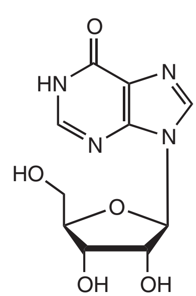
A nucleoside consists of a nucleobase and a five-carbon sugar (ribose or deoxyribose). Adenosine (**14**), guanosine (**15**), inosine (**16**), and xanthosine (**17**) are catabolites of purine ribonucleotides and RNA while deoxyadenosine (**18**) and deoxyguanosine (**19**) are catabolites of DNA. Cytokinins also occur as ribosides, namely, isopentenyladenine riboside (**20**), benzyladenine riboside (**21**), and *trans*-zeatin riboside (**22**) (Ashihara et al. 2013).



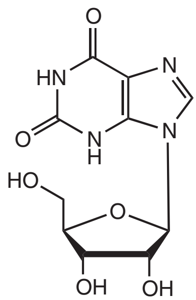
Adenosine (**14**)



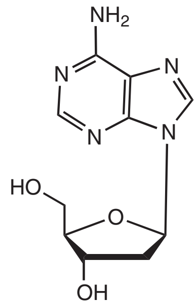
Guanosine (**15**)



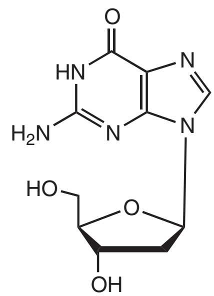
Inosine (**16**)



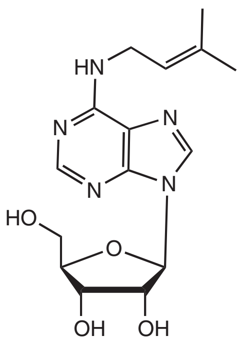
Xanthosine (**17**)



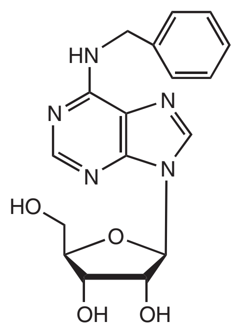
Deoxyadenosine (**18**)



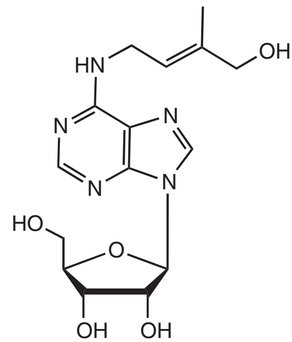
Deoxyguanosine (**19**)



Isopentenyladenine riboside (**20**)



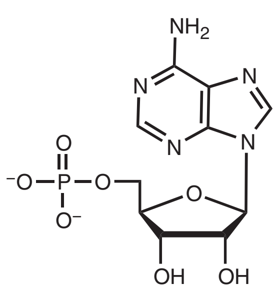
Benzyladenine riboside (**21**)



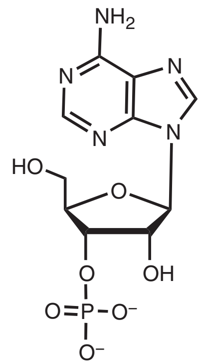
trans-Zeatin riboside (**22**)

1.3.1.3 Purine Nucleotides

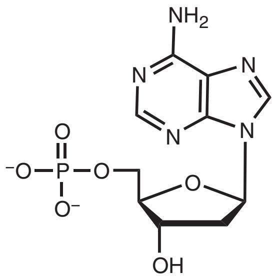
A nucleotide is composed of a purine base, a sugar moiety (ribose or deoxyribose) and at least one phosphate group. The phosphate group is attached to either the C3′or C5′ position of the sugar. Nucleoside-5′-phosphates (5′-nucleotides) are the main purine nucleotides in plants as well as other organisms. Small nucleoside-3′-monophosphate (3′-nucleotides) pools are mainly produced as catabolites of nucleic acids. Examples of different forms of nucleotides are adenosine-5′-monophosphate (5′-AMP, usually abbreviated as AMP) (**23**), adenosine-3′-monophosphate (3′-AMP) (**24**), deoxyadenosine-5′-monophosphate (dAMP) (**25**), and isopentenyladenine ribotide (**26**).



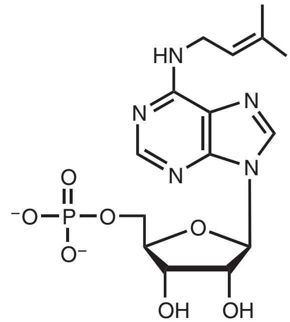
Adenosine-5′-monophosphate (**23**)



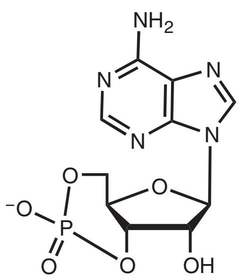
Adenosine-3′-monophosphate (**24**)



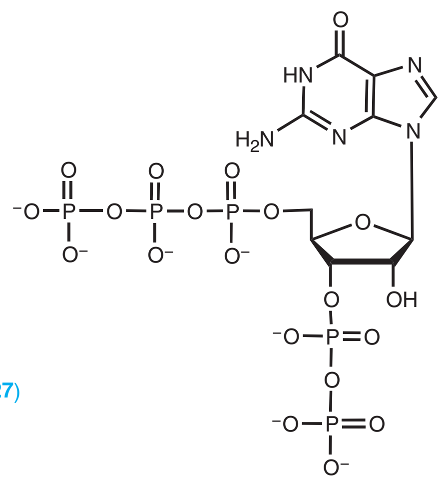
Deoyxadenosine-5′-monophosphate (**25**)



Isopentenyladenine ribotide (**26**)



Cyclic adenosine-3′,5′-monophosphate (**27**)

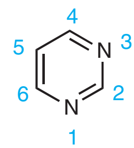


Guanosine pentaphosphate (**28**)

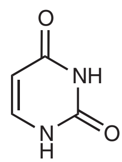
The cyclic monophosphates, cyclic adenosine-3′,5′-monophosphate (cAMP) (**27**) and cyclic guanosine-3′,5′-monophosphate (cGMP) occur in plants. These cyclic nucleotides are derived from ATP and GTP and act as second messengers. In addition, some unusual nucleotides, such as guanosine tetraphosphate (ppGpp), guanosine pentaphosphate (pppGpp) (**28**), diadenosine triphosphate (Ap_3A), and diadenosine tetraphosphate (Ap_4A), known as alarmones, which act as intracellular signal molecules, are produced in response to harsh environmental conditions (Boniecka et al. 2017; Pietrowska-Borek et al. 2011). Possible roles of these unusual nucleotides in plants are outlined in Part VIII.

1.3.2 Pyrimidines

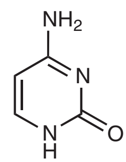
Pyrimidine (**29**) is an aromatic heterocyclic organic compound similar to pyridine. The systematic study of pyrimidines was carried out and named ‘pyrimidin’ by a German chemist, Adolf Pinner (1885).



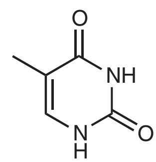
Pyrimidine: numbering system (**29**)



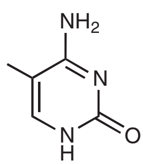
Uracil (**30**)



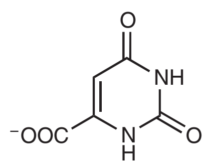
Cytosine (**31**)



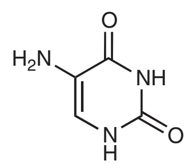
Thymine (**32**)



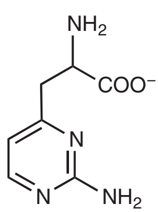
5-Methylcytosine (**33**)



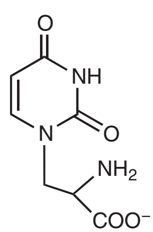
Orotate (**34**)



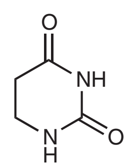
5-Aminouracil (**35**)



Lathyrine (**36**)



Willardine (**37**)



Dihydrouracil (**38**)

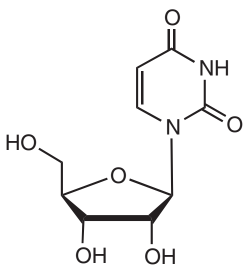
1.3.2.1 Pyrimidine Bases

Uracil (**30**), cytosine (**31**), and thymine (**32**) are the common nucleobases of nucleic acids. DNA and RNA also contain other bases that have been modified after formation of the nucleic acid chain. In the case of DNA, the most common modified base is 5-methylcytosine (**33**).

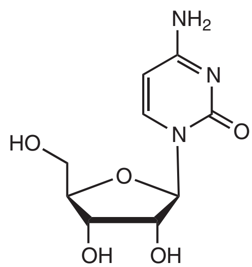
Orotate (pyrimidine carboxylic acid) (**34**) is an intermediate of the *de novo* pyrimidine biosynthesis. A number of secondary products, such as 5-aminouracil (**35**), lathyrine (**36**), and willardine (**37**) occur in plants (see Part VIII). Dihydrouracil (**38**) is an intermediate of uracil catabolism (see Part III).

1.3.2.2 Pyrimidine Nucleosides

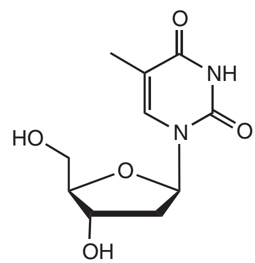
A pyrimidine nucleoside consists of a pyrimidine base and a five-carbon sugar, either ribose or deoxyribose. Uridine (**39**) and cytidine (**40**) are produced as catabolites of pyrimidine ribonucleotides and RNA. Thymidine (**41**) is a catabolite of DNA. There are many modified bases in RNA, including those contained in the nucleosides, pseudouridine (5-ribosyluracil) (**42**), and dihydrouridine (**43**). Pseudouridine is an isomer of the nucleoside uridine (**39**) in which the uracil is attached via a carbon–carbon linkage instead of a nitrogen–carbon glycosidic bond. It is the most prevalent of the over 100 different modified nucleosides found in RNA.



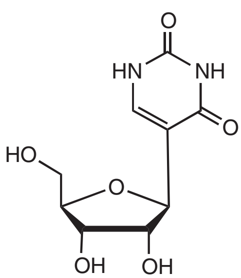
Uridine (**39**)



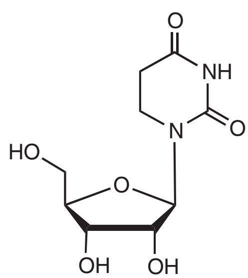
Cytidine (**40**)



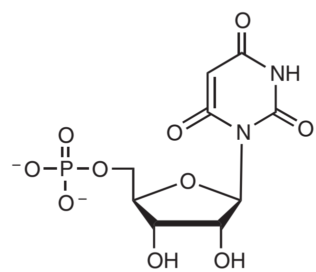
Thymidine (**41**)



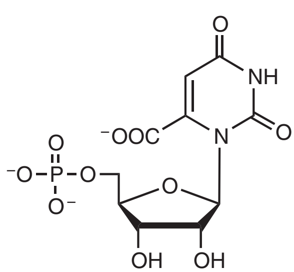
Pseudouridine (5-ribosyluracil) (**42**)



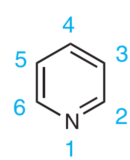
Dihydrouridine (**43**)



Uridine-5′-monophosphate (**44**)



Orotidine-5-monophosphate (**45**)



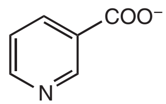
Pyridine numbering system (**46**)

1.3.2.3 Pyrimidine Nucleotides

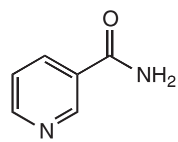
A pyrimidine nucleotide is composed of a pyrimidine base, a sugar (ribose or deoxyribose) and at least a phosphate group. Examples of different forms of pyrimidine nucleotide structures are uridine-5′-monophosphate (5′-UMP usually abbreviated as UMP) (**44**) and orotidine-5′-monophosphate (5′-OMP) (**45**), an intermediate of the *de novo* pyrimidine biosynthesis.

1.3.3 Pyridines

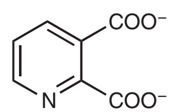
Pyridine (**46**) is a basic heterocyclic organic compound. It is structurally related to benzene, with one methine group replaced by a nitrogen atom, and was discovered in 1849 by a Scottish chemist, Thomas Anderson, as one of the constituents of bone oil. Pyridine-related compounds include the catabolites nicotinate (**47**) and nicotinamide (**48**), and quinolinate (**49**) and NMN (**50**), which are intermediates of the biosynthesis of NAD (**51**) and NADP. These compounds act as common coenzymes involved in many redox reactions, carrying electrons from one reaction to another in all living cells. Each coenzyme consists of pyridine purine nucleotides joined through their phosphate groups. NAD(P) exists in two forms: an oxidized and reduced form abbreviated as $NAD(P)^+$ and NAD(P)H respectively.



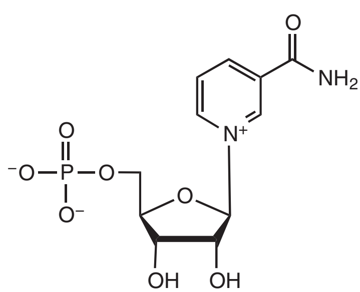
Nicotinate (**47**)



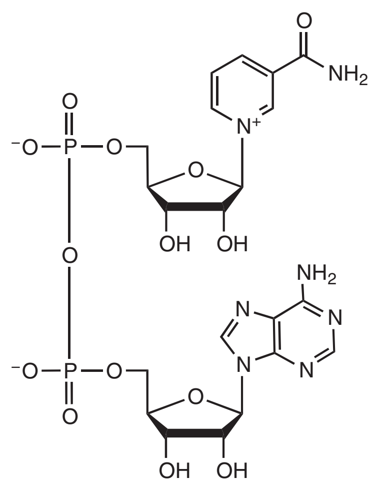
Nicotinamide (**48**)



Quinolinate (**49**)



Nicotinamide mononucleotide (**50**)



Nicotinamide adenine dinucleotide (**51**)

1.4 Summary

Nomenclature and abbreviations of nucleotide-related compounds and major chemical structures of purines, pyrimidine, and pyridine are described.

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