



Hiroshi Ashihara, Iziar A. Ludwig,
Alan Crozier

Plant Nucleotide Metabolism

Biosynthesis, Degradation, and
Alkaloid Formation

WILEY

**Plant Nucleotide Metabolism – Biosynthesis,
Degradation, and Alkaloid Formation**

Plant Nucleotide Metabolism – Biosynthesis, Degradation, and Alkaloid Formation

Hiroshi Ashihara

Ochanomizu University
Bunkyo-ku, Tokyo, Japan

Izias A. Ludwig

University Rovira I
Virgili
Reus, Spain

Alan Crozier

University of California
Davis, CA, USA

University of Glasgow
Glasgow, UK

WILEY

This edition first published 2020
© 2020 John Wiley & Sons Ltd.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by law. Advice on how to obtain permission to reuse material from this title is available at <http://www.wiley.com/go/permissions>.

The right of Hiroshi Ashihara, Iziar A. Ludwig and Alan Crozier to be identified as the author(s) of this work has been asserted in accordance with law.

Registered Office(s)

John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial Office

9600 Garsington Road, Oxford, OX4 2DQ, UK

For details of our global editorial offices, customer services, and more information about Wiley products visit us at www.wiley.com.

Wiley also publishes its books in a variety of electronic formats and by print-on-demand. Some content that appears in standard print versions of this book may not be available in other formats.

Limit of Liability/Disclaimer of Warranty

While the publisher and authors have used their best efforts in preparing this work, they make no representations or warranties with respect to the accuracy or completeness of the contents of this work and specifically disclaim all warranties, including without limitation any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives, written sales materials or promotional statements for this work. The fact that an organization, website, or product is referred to in this work as a citation and/or potential source of further information does not mean that the publisher and authors endorse the information or services the organization, website, or product may provide or recommendations it may make. This work is sold with the understanding that the publisher is not engaged in rendering professional services. The advice and strategies contained herein may not be suitable for your situation. You should consult with a specialist where appropriate. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this work was written and when it is read. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

Library of Congress Cataloging-in-Publication Data

Name: Ashihara, Hiroshi, author.

Title: Plant nucleotide metabolism : biosynthesis, degradation, and alkaloid formation / Hiroshi Ashihara, Emeritus Professor, Ochanomizu University, Tokyo, Japan, Iziar A. Ludwig, University Rovira I, Virgili, Spain, Alan Crozier, University of California, Davis and University of Glasgow, UK.

Description: First edition. | Hoboken : Wiley, 2020. | Includes bibliographical references and index.

Identifiers: LCCN 2019047672 (print) | LCCN 2019047673 (ebook) | ISBN 9781119476122 (hardback) | ISBN 9781119476108 (adobe pdf) | ISBN 9781119476078 (epub)

Subjects: LCSH: Plants—Metabolism. | Nucleotides—Metabolism.

Classification: LCC QK881 .A74 2020 (print) | LCC QK881 (ebook) | DDC 572/.42—dc23

LC record available at <https://lcn.loc.gov/2019047672>

LC ebook record available at <https://lcn.loc.gov/2019047673>

Cover Design: Wiley

Cover Image: © Mazur Travel/Shutterstock

Set in 10/12pt WarnockPro by SPi Global, Chennai, India

Contents

Preface *xv*

Part I General Aspects of Nucleotide Metabolism 1

1	Structures of Nucleotide-Related Compounds	3
1.1	Introduction	3
1.2	Nomenclature and Abbreviations of Nucleotide-Related Compounds	3
1.3	Chemical Structures of Nucleotide-Related Compounds	5
1.3.1	Purines	5
1.3.1.1	Purine Bases	5
1.3.1.2	Purine Nucleosides	6
1.3.1.3	Purine Nucleotides	7
1.3.2	Pyrimidines	8
1.3.2.1	Pyrimidine Bases	9
1.3.2.2	Pyrimidine Nucleosides	9
1.3.2.3	Pyrimidine Nucleotides	10
1.3.3	Pyridines	11
1.4	Summary	11
	References	11
2	Occurrence of Nucleotides and Related Metabolites in Plants	13
2.1	Purines and Pyrimidines	13
2.1.1	Concentration of Purine and Pyrimidine Nucleotides	14
2.1.2	Concentration of Purine and Pyrimidine Bases and Nucleosides	16
2.2	Pyridine Nucleotides	17
2.2.1	Concentration of Pyridine Nucleotides	17
2.2.2	Concentration of Nicotinate and Nicotinamide	18
2.3	Concentration of Cytokinins	18
2.4	Alkaloids Derived from Nucleotides	18
2.5	Summary	19
	References	19
3	General Aspects of Nucleotide Biosynthesis and Interconversions	21
3.1	Introduction	21
3.2	<i>De Novo</i> Biosynthesis of Ribonucleoside Monophosphates	21

3.3	Interconversion of Nucleoside Monophosphates, Nucleoside Diphosphates, and Triphosphates	23
3.3.1	Nucleoside-Monophosphate Kinase	23
3.3.2	Specific Nucleoside-Monophosphate Kinases	24
3.4	Conversion of Nucleoside Diphosphates to Nucleoside Triphosphates	24
3.4.1	ATP Synthesis by Electron Transfer Systems	25
3.4.2	Substrate-Level ATP Synthesis	26
3.4.3	Nucleoside-Diphosphate Kinase	26
3.5	Biosynthesis of Deoxyribonucleotides	29
3.6	Nucleic Acid Biosynthesis	29
3.7	Supply of 5-Phosphoribosyl-1-Pyrophosphate	30
3.8	Supply of Amino Acids for Nucleotide Biosynthesis	33
3.9	Nitrogen Metabolism and Amino Acid Biosynthesis in Plants	33
3.10	Summary	34
	References	35

Part II Purine Nucleotide Metabolism 39

4	Purine Nucleotide Biosynthesis De Novo	41
4.1	Introduction	41
4.2	Reactions and Enzymes	43
4.2.1	Synthesis of Phosphoribosylamine	44
4.2.2	Synthesis of Glycineamide Ribonucleotide	46
4.2.3	Synthesis of Formylglycineamide Ribonucleotide	46
4.2.4	Synthesis of Formylglycinamide Ribonucleotide	47
4.2.5	Synthesis of Aminoimidazole Ribonucleotide	47
4.2.6	Synthesis of Aminoimidazole Carboxylate Ribonucleotide	48
4.2.7	Synthesis of Aminoimidazole Succinocarboxamide Ribonucleotide	48
4.2.8	Synthesis of Aminoimidazole Carboxamide Ribonucleotide	49
4.2.9	Synthesis of IMP via Formamidoimidazole Carboxamide Ribonucleotide	49
4.2.10	Synthesis of AMP	50
4.2.11	Synthesis of GMP	51
4.3	Summary	52
	References	52
5	Salvage Pathways of Purine Nucleotide Biosynthesis	55
5.1	Introduction	55
5.2	Characteristics of Purine Salvage in Plants	56
5.3	Properties of Purine Phosphoribosyltransferases	59
5.3.1	Adenine Phosphoribosyltransferase	59
5.3.2	Hypoxanthine/Guanine Phosphoribosyltransferase	59
5.3.3	Xanthine Phosphoribosyltransferase	62
5.4	Properties of Nucleoside Kinases	62
5.4.1	Adenosine Kinase	62
5.4.2	Inosine/Guanosine Kinase	64
5.4.3	Deoxyribonucleoside Kinases	64

5.5	Properties of Nucleoside Phosphotransferase	65
5.6	Role of Purine Salvage in Plants	66
5.7	Summary	66
	References	66
6	Interconversion of Purine Nucleotides	71
6.1	Introduction	71
6.2	Deamination Reactions	71
6.2.1	Routes of Deamination of Adenine Ring	73
6.2.2	AMP Deaminase	73
6.2.3	Routes of Deamination of Guanine Ring	74
6.2.4	Guanosine Deaminase	75
6.3	Dephosphorylation Reactions	75
6.4	Glycosidic Bond Cleavage Reactions	76
6.4.1	Adenosine Nucleosidase	76
6.4.2	Inosine/Guanosine Nucleosidase	78
6.4.3	Non-specific Purine Nucleosidases	78
6.4.4	Recombinant Non-Specific Nucleosidases	78
6.5	<i>In Situ</i> Metabolism of ¹⁴ C-Labelled Purine Nucleotides	79
6.5.1	Metabolism of Adenine Nucleotides	79
6.5.2	Metabolism of Guanine Nucleotides	80
6.6	<i>In Situ</i> Metabolism of Purine Nucleosides and Bases	80
6.6.1	Metabolism of Adenine and Adenosine	82
6.6.2	Metabolism of Guanine and Guanosine	83
6.6.3	Metabolism of Hypoxanthine and Inosine	84
6.6.4	Metabolism of Xanthine and Xanthosine	84
6.6.5	Metabolism of Deoxyadenosine and Deoxyguanosine	85
6.7	Summary	88
	References	89
7	Degradation of Purine Nucleotides	95
7.1	Introduction	95
7.2	(S)-Allantoin Biosynthesis from Xanthine	97
7.2.1	Xanthine Dehydrogenase	99
7.2.2	Urate Oxidase	100
7.2.3	Allantoin Synthase	101
7.3	Catabolism of (S)-Allantoin	101
7.3.1	Allantoinase	103
7.3.2	Allantoate Amidohydrolase	104
7.3.3	(S)-Ureidoglycine Aminohydrolase	104
7.3.4	Allantoate Amidinohydrolase	105
7.3.5	Ureidoglycolate Amidohydrolase	105
7.3.6	(S)-Ureidoglycolate-urea Lyase	105
7.3.7	Urease	105
7.4	Purine Nucleotide Catabolism in Plants	106
7.5	Accumulation and Utilization of Ureides in Plants	107
7.5.1	Ureides in Plant Tissues and Xylem Sap	107

- 7.5.2 Role of Ureides in Nitrogen Storage and Transport 109
- 7.5.3 Role of Ureides in Germination and Development of Seeds 109
- 7.5.4 Ureide Formation in Nodules of Tropical Legumes 110
- 7.5.5 Other Role of Ureides in Plants 110
- 7.6 Summary 111
- References 111

Part III Pyrimidine Nucleotide Metabolism 117

8 Pyrimidine Nucleotide Biosynthesis *De Novo* 119

- 8.1 Introduction 119
- 8.2 Reactions and Enzymes of the *De Novo* Biosynthesis 121
 - 8.2.1 Synthesis of Carbamoyl-phosphate 121
 - 8.2.2 Formation of Carbamoyl-aspartate 123
 - 8.2.3 Formation of Dihydroorotate from Carbamoyl-aspartate 123
 - 8.2.4 Formation of Orotate from Dihydroorotate 124
 - 8.2.5 Synthesis of UMP from Orotate 125
 - 8.2.6 Synthesis of CTP from UTP 126
- 8.3 Control Mechanism of *De Novo* Pyrimidine Ribonucleotide Biosynthesis 127
 - 8.3.1 Fine Control of the *De Novo* Pathway 127
 - 8.3.2 Coarse Control of the *De Novo* Pathway 129
- 8.4 Biosynthesis of Thymidine Nucleotide 129
 - 8.4.1 Formation of dUMP 129
 - 8.4.2 Conversion of UMP to dUMP via dUTP 130
 - 8.4.3 Conversion of dUMP to dTMP 130
 - 8.4.4 Thymidine Monophosphate Kinase 131
- 8.5 Summary 131
- References 131

9 Salvage Pathways of Pyrimidine Nucleotide Biosynthesis 137

- 9.1 Introduction 137
- 9.2 Characteristics of Pyrimidine Salvage in Plants 137
- 9.3 Enzymes of Pyrimidine Salvage 139
 - 9.3.1 Uracil Phosphoribosyl Transferase 140
 - 9.3.2 Uridine/Cytidine Kinase 142
 - 9.3.3 Thymidine Kinase 143
 - 9.3.4 Deoxyribonucleoside Kinase 144
 - 9.3.5 Nucleoside Phosphotransferase 144
- 9.4 Role of Pyrimidine Salvage in Plants 145
- 9.5 Summary 146
- References 146

10 Interconversion of Pyrimidine Nucleotides 149

- 10.1 Introduction 149
- 10.2 Deaminase Reactions 149
 - 10.2.1 Cytidine Deaminase 149
 - 10.2.2 Cytosine Deaminase 152
 - 10.2.3 Deoxycytidylate Deaminase 152
- 10.3 Nucleosidase and Phosphorylase Reactions 152
 - 10.3.1 Uridine Nucleosidase 152
 - 10.3.2 Thymidine Phosphorylase 153
- 10.4 *In Situ* Metabolism of ¹⁴C-Labelled Pyrimidines 153
 - 10.4.1 Metabolic Fate of Orotate 154
 - 10.4.2 Metabolic Fate of Uridine and Uracil 154
 - 10.4.3 Metabolic Fate of Cytidine and Cytosine 156
 - 10.4.4 Metabolic Fate of Deoxycytidine 157
 - 10.4.5 Metabolic Fate of Thymidine 158
- 10.5 Summary 159
- References 160

11 Degradation of Pyrimidine Nucleotides 165

- 11.1 Introduction 165
- 11.2 Enzymes Involved in the Degradation Routes of Pyrimidines 166
 - 11.2.1 Dihydropyrimidine Dehydrogenase 167
 - 11.2.2 Dihydropyrimidinase 167
 - 11.2.3 β -Ureidopropionase 168
- 11.3 The Metabolic Fate of Uracil and Thymine 168
- 11.4 Summary 169
- References 170

Part IV Physiological Aspects of Nucleotide Metabolism 173**12 Growth and Development 175**

- 12.1 Introduction 175
- 12.2 Embryo Maturation 175
- 12.3 Germination 180
 - 12.3.1 Purine Metabolism in Germination 180
 - 12.3.2 Pyrimidine Metabolism in Germination 183
- 12.4 Organogenesis 185
- 12.5 Breaking Bud Dormancy 186
- 12.6 Fruit Ripening 186
- 12.7 Storage Organ Development and Sprouting 186
- 12.8 Suspension-Cultured Cells 187
 - 12.8.1 Nucleotide Pools 187
 - 12.8.2 Nucleotide Biosynthesis 188
 - 12.8.3 Nucleotide Availability 188

- 12.9 Molecular Studies 189
- 12.10 Summary 189
- References 189

13 Environmental Factors and Nucleotide Metabolism 195

- 13.1 Introduction 195
- 13.2 Effect of Phosphate on Nucleotide Metabolism 195
- 13.3 Effect of Salts on Nucleotide Metabolism 199
- 13.4 Effect of Water Stress 202
- 13.5 Effect of Wound Stress 202
- 13.6 Effect of Iron Deficiency 205
- 13.7 Effect of Light 206
- 13.8 Summary 206
- References 206

Part V Purine Alkaloids 211

14 Occurrence of Purine Alkaloids 213

- 14.1 Introduction 213
- 14.2 Chemical Structure of Purine Alkaloids 213
- 14.3 Occurrence of Purine Alkaloids in Plants 215
 - 14.3.1 Purine Alkaloids in Tea and Related Species 215
 - 14.3.2 Purine Alkaloids in Coffee and Related Species 218
 - 14.3.3 Purine Alkaloids in Maté 220
 - 14.3.4 Purine Alkaloids in Cacao and Related Species 221
 - 14.3.5 Purine Alkaloids in Cola Species 223
 - 14.3.6 Purine Alkaloids in Guaraná and Related Species 223
 - 14.3.7 Purine Alkaloids in Citrus Species 224
 - 14.3.8 Purine Alkaloids in Other Plants 225
- 14.4 Summary 226
- References 226

15 Biosynthesis of Purine Alkaloids 231

- 15.1 Introduction 231
- 15.2 A Brief History of Caffeine Biosynthesis Research 231
- 15.3 Caffeine Biosynthesis Pathway 234
 - 15.3.1 *N*-Methyltransferase Nomenclature 236
 - 15.3.2 Formation of 7-Methylxanthine from Xanthosine 236
 - 15.3.3 7-Methylxanthosine Synthase 237
 - 15.3.4 *N*-Methylnucleosidase 240
 - 15.3.5 Formation of Caffeine from 7-Methylxanthine 241
 - 15.3.6 Caffeine Synthase 241
 - 15.3.7 Theobromine Synthase 244
- 15.4 Genes and Proteins of Caffeine Synthase Family 245
- 15.5 Xanthosine Biosynthesis from Purine Nucleotides 249
 - 15.5.1 *De Novo* Purine Route 250

15.5.2	Adenosine Monophosphate Route	251
15.5.3	S-Adenosyl-L-methionine Cycle Route	251
15.5.4	Nicotinamide Adenine Diphosphate Catabolism Route	252
15.5.5	Guanosine Monophosphate Route	253
15.6	Summary	253
	References	253
16	Physiological and Ecological Aspects of Purine Alkaloid Biosynthesis	259
16.1	Introduction	259
16.2	Physiology of Caffeine Biosynthesis	259
16.2.1	Purine Alkaloid Biosynthesis in Different Species	261
16.2.2	<i>Camellia</i>	261
16.2.3	<i>Coffea</i>	264
16.2.4	<i>Theobroma</i>	264
16.2.5	Maté	266
16.2.6	Guaraná	267
16.2.7	<i>Citrus</i>	268
16.3	Subcellular Localization of Caffeine Biosynthesis	268
16.3.1	Caffeine Synthase	268
16.3.2	The <i>De Novo</i> Route Enzymes	269
16.3.3	The AMP Route Enzymes	270
16.3.4	The SAM Route Enzymes	270
16.3.5	Subcellular Localization and Transport of Intermediates	270
16.4	Regulation of Caffeine Biosynthesis	270
16.5	Ecological Roles of Caffeine	271
16.5.1	Allelopathic Function Theory	271
16.5.2	Effect of Caffeine on Plant Growth	272
16.5.3	Allelopathy in Natural Ecosystems	273
16.5.4	Chemical Defence Theory	274
16.6	Summary	274
	References	275
17	Metabolism of Purine Alkaloids and Biotechnology	281
17.1	Introduction	281
17.2	Metabolism of Purine Alkaloids	281
17.2.1	Methylurate Biosynthesis	281
17.2.2	The Major Pathway of Caffeine Degradation	282
17.2.3	Purine Catabolic Pathways in Alkaloid Plants	284
17.3	Diversity of Purine Alkaloid Metabolism in Plants	285
17.3.1	<i>Coffea</i> Species	285
17.3.2	<i>Camellia</i> Species	286
17.3.3	Maté Species	290
17.3.4	Cacao Species	290
17.3.5	Other Plant Species	290
17.3.6	Bacteria	291
17.4	Biotechnology of Purine Alkaloids	293

- 17.4.1 Decaffeinated Coffee Plants 293
- 17.4.2 Decaffeinated Tea Plants 294
- 17.5 Caffeine-Producing Transgenic Plants 295
 - 17.5.1 Antiherbivore Activity 295
 - 17.5.2 Antipathogen Activity 296
- 17.6 Summary 298
- References 298

Part VI Pyridine Nucleotide Metabolism 301

- 18 Pyridine (Nicotinamide Adenine) Nucleotide Biosynthesis *De Novo* 303**
 - 18.1 Introduction 303
 - 18.2 Two Distinct Pathways of *De Novo* Nicotinate Mononucleotide Biosynthesis 303
 - 18.3 The Outline of the *De Novo* Pathway of NAD Biosynthesis in Plants 304
 - 18.4 Enzymes Involved in *De Novo* NAD Synthesis in Plants 307
 - 18.4.1 L-Aspartate Oxidase and Quinolinate Synthase 308
 - 18.4.2 Quinolinate Phosphoribosyltransferase 309
 - 18.4.3 Nicotinate Mononucleotide Adenylyltransferase 309
 - 18.4.4 NAD Synthetase 310
 - 18.4.5 NAD Kinase 310
 - 18.5 Summary 310
 - References 310
- 19 Pyridine Nucleotide Cycle 315**
 - 19.1 Introduction 315
 - 19.2 Pyridine Nucleotide Cycle 315
 - 19.2.1 Major Pyridine Nucleotide Cycles in Plants 317
 - 19.2.2 Alternative Pyridine Nucleotide Cycles in Plants 318
 - 19.2.3 Rate-Limiting Step of the Pyridine Cycle 319
 - 19.3 Catabolism of NAD 320
 - 19.3.1 Reactions from NAD to Nicotinate 320
 - 19.3.2 Degradation of Pyrimidine Ring 320
 - 19.3.3 Nicotinate Conversion to Nicotinate-*N*-Glucoside and *N*-Methylnicotinate 321
 - 19.4 Enzymes Involved in NAD Catabolism 321
 - 19.4.1 Direct NAD Cleavage Enzymes 321
 - 19.4.2 NAD Pyrophosphatase 321
 - 19.4.3 5'-Nucleotidase and Nicotinamide Riboside Nucleosidase 322
 - 19.4.4 Nicotinamidase and Nicotinamide Riboside Deaminase 322
 - 19.5 Salvage of Nicotinamide and Nicotinate 323
 - 19.5.1 Nicotinate Phosphoribosyltransferase 323
 - 19.5.2 Nicotinate Riboside Kinase 324
 - 19.6 Summary 325
 - References 325

Part VII Pyridine Alkaloids 329**20 Occurrence and Biosynthesis of Pyridine Alkaloids 331**

- 20.1 Introduction 331
- 20.2 Occurrence of Pyridine Alkaloids 333
 - 20.2.1 Trigonelline in Plants 333
 - 20.2.2 Other Pyridine Alkaloids in Plants 334
- 20.3 Biosynthesis of Pyridine Alkaloids 335
 - 20.3.1 Trigonelline Biosynthesis 335
 - 20.3.2 Nicotinate *N*-Glucoside Biosynthesis 336
 - 20.3.3 The Diversity of Biosynthetic Reactions 337
 - 20.3.3.1 Ferns 338
 - 20.3.3.2 Gymnosperms 338
 - 20.3.3.3 Angiosperms 339
 - 20.3.3.4 Nicotinate Conjugate Formation 340
 - 20.3.4 Biosynthesis of Ricinine 341
 - 20.3.5 Biosynthesis of Nicotine (Pyridine Ring) 343
- 20.4 Summary 345
- References 345

21 Physiological Aspect and Biotechnology of Trigonelline 351

- 21.1 Introduction 351
- 21.2 Physiological Aspect of Trigonelline Biosynthesis 351
 - 21.2.1 Coffee 351
 - 21.2.2 Leguminous Plants 354
- 21.3 Physiological Aspects of Nicotinate *N*-Glucoside Biosynthesis 356
- 21.4 The Role of Trigonelline in Plants 356
 - 21.4.1 Role of Trigonelline as a Nutrient Source 357
 - 21.4.2 Role of Trigonelline as a Compatible Solute 357
 - 21.4.3 Trigonelline and Nyctinasty 358
 - 21.4.4 Cell Cycle Regulation 358
 - 21.4.5 Detoxification of Nicotinate 359
 - 21.4.6 Signal Transduction 360
 - 21.4.7 Role of Host Selection by Herbivores 360
- 21.5 Biotechnology of Trigonelline 360
- 21.6 Summary 362
- References 363

Part VIII Other Nucleotide-Related Metabolites 367**22 Sugar Nucleotides 369**

- 22.1 Introduction 369
- 22.2 The Sugar Nucleotide Moiety 370
- 22.3 Enzymes of Sugar Nucleotide Biosynthesis 371
 - 22.3.1 UDP-Glucose Pyrophosphorylase 371
 - 22.3.2 UDP-Sugar Pyrophosphorylase 374

- 22.3.3 Sucrose Synthase 376
 - 22.4 Localization of UDP-Glucose-Producing Enzymes 377
 - 22.5 UDP-Glucose-Interconversion 377
 - 22.6 Other Metabolites 379
 - 22.6.1 Cyclic Nucleotides 379
 - 22.6.2 Diadenosine Tetraphosphate 381
 - 22.6.3 Purine Alkaloid Glucosides 382
 - 22.7 Summary 382
 - References 382
- 23 Cytokinins 387**
- 23.1 Introduction 387
 - 23.2 Adenosine Phosphate-Isopentenyl Formation 388
 - 23.3 *trans*-Zeatin Phosphate Synthesis 389
 - 23.4 Formation of Cytokinin Bases 389
 - 23.5 Effect of Nucleotide Enzymes in Cytokinins 390
 - 23.5.1 Cytokinin Inactivation by Adenine Phosphoribosyltransferase 390
 - 23.5.2 Homeostasis of Cytokinin by Adenosine Kinase 392
 - 23.5.3 Endodormancy of Potato and Purine Nucleoside Phosphorylase 392
 - 23.6 New Purine-Related Plant Growth Regulators 392
 - 23.7 Summary 393
 - References 394

Part IX Dietary Plant Alkaloids, Their Bioavailability, and Potential Impact on Human Health 397

- 24 Bioavailability and Potential Impact on Human Health of Caffeine, Theobromine, and Trigonelline 399**
- 24.1 Caffeine 399
 - 24.1.1 Dietary Caffeine 399
 - 24.1.2 Bioavailability and Bioactivity of Caffeine 400
 - 24.2 Theobromine 404
 - 24.2.1 Interactions with Flavan-3-ols 404
 - 24.2.2 Toxicity of Theobromine 406
 - 24.3 Trigonelline 406
 - 24.3.1 Dietary Trigonelline 406
 - 24.3.2 Bioavailability and Bioactivity of Trigonelline 407
 - 24.4 Summary 409
 - References 409

Index 415

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 Tatsuhiro 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
Izias 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.

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 (uridylylate), and NMP for any ribonucleoside monophosphate. Similarly, dAMP stands for deoxyadenosine monophosphate (deoxyadenylate), dUMP for deoxyuridine monophosphate (deoxyuridylylate), and dNMP for any deoxyribonucleoside monophosphate. Thymidine monophosphate (thymidylylate) 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

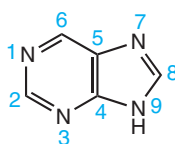
Studies on purines and pyrimidines began in 1776 when the Swedish pharmacist Carl Wilhelm Scheele isolated uric acid from bladder stones. In 1846, Unger isolated guanine from the guano of Peruvian sea birds. At the end of the nineteenth century, several purines (adenine, xanthine, and hypoxanthine) and 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 Verkhatsky (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

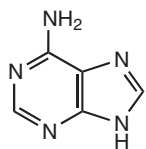
A purine is a heterocyclic compound that consists of a pyrimidine ring fused to an imidazole ring. The word, 'purine' ('Purum' + 'Uricum') was coined by Emil Fischer (1884).

1.3.1.1 Purine Bases

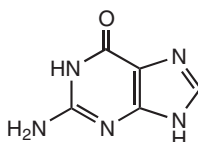
As shown in structure **1** the atoms of the purine ring are numbered in an anticlockwise manner. In plants, there are several naturally occurring purine bases. They include adenine (**2**) and guanine (**3**), which are constituents of nucleic acids, and hypoxanthine (**4**), xanthine (**5**), and uric 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).



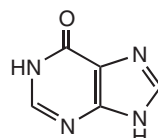
Purine: numbering system (1)



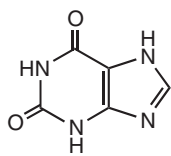
Adenine (2)



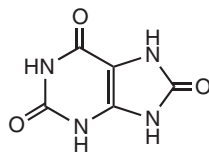
Guanine (3)



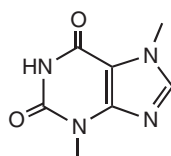
Hypoxanthine (4)



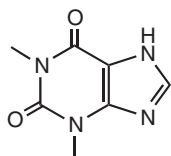
Xanthine (5)



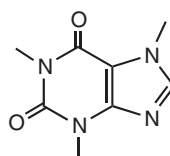
Uric acid (6)



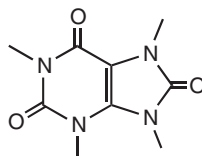
Theobromine (7)



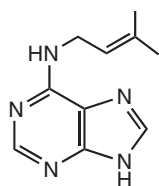
Theophylline (8)



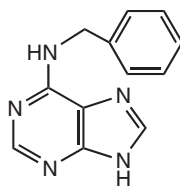
Caffeine (9)



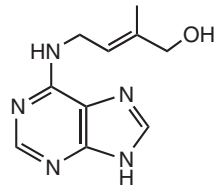
Theacrine (10)



Isopentenyladenine (11)

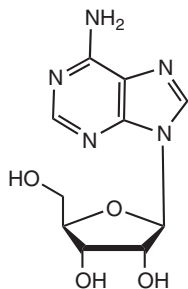


Benzyladenine (12)

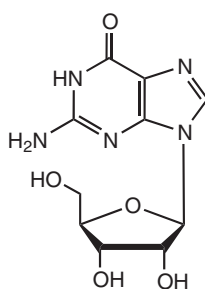
*trans*-Zeatin (13)

1.3.1.2 Purine Nucleosides

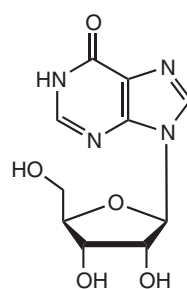
A nucleoside consists of a nucleobase and a five-carbon sugar (ribose or deoxyribose). Adenosine (14), guanosine (15), inosine (16), and xanthosine (17) are catabolites of purine ribonucleotides and RNA while deoxyadenosine (18) and deoxyguanosine (19) are catabolites of DNA. Cytokinins also occur as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



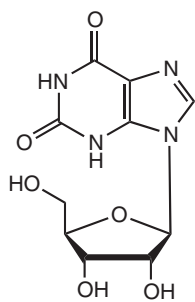
Adenosine (14)



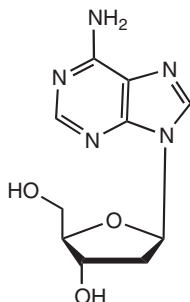
Guanosine (15)



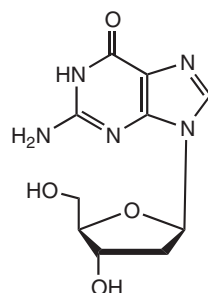
Inosine (16)



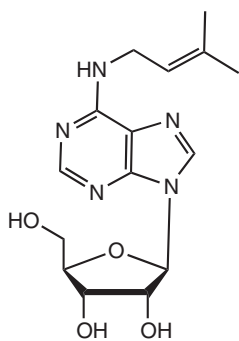
Xanthosine (17)



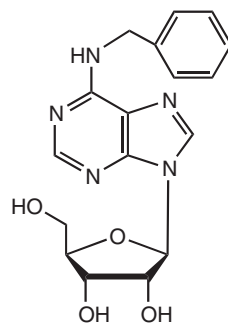
Deoxyadenosine (18)



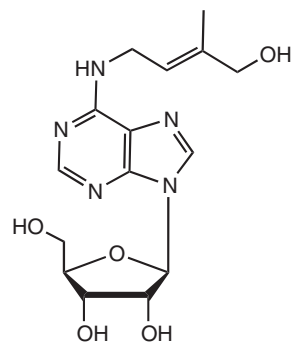
Deoxyguanosine (19)



Isopentenyladenine riboside (20)

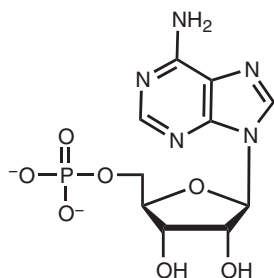


Benzyladenine riboside (21)

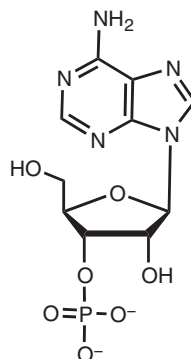
*trans*-Zeatin riboside (22)

1.3.1.3 Purine Nucleotides

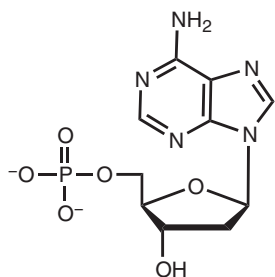
A nucleotide is composed of a purine base, a sugar moiety (ribose or deoxyribose) and at least one phosphate group. The phosphate group is attached to either the C3' or C5' position of the sugar. Nucleoside-5'-phosphates (5'-nucleotides) are the main purine nucleotides in plants as well as other organisms. 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).



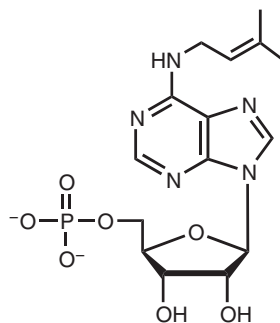
Adenosine-5'-monophosphate (23)



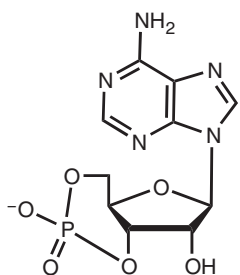
Adenosine-3'-monophosphate (24)



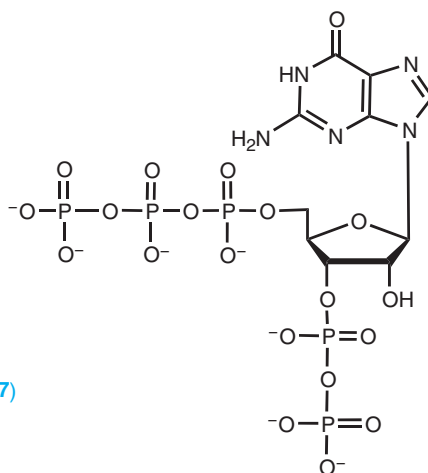
Deoxyadenosine-5'-monophosphate (25)



Isopentenyladenine ribotide (26)



Cyclic adenosine-3',5'-monophosphate (27)

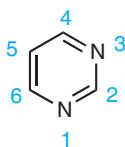


Guanosine pentaphosphate (28)

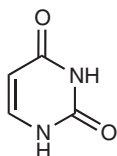
The cyclic monophosphates, cyclic adenosine-3',5'-monophosphate (cAMP) (27) and cyclic guanosine-3',5'-monophosphate (cGMP) occur in plants. These cyclic nucleotides are derived from ATP and GTP and act as second messengers. In addition, some unusual nucleotides, such as guanosine tetraphosphate (ppGpp), guanosine pentaphosphate (pppGpp) (28), diadenosine triphosphate (Ap₃A), and diadenosine tetraphosphate (Ap₄A), 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

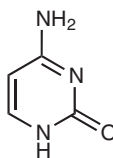
Pyrimidine (29) is an aromatic heterocyclic organic compound similar to pyridine. The systematic study of pyrimidines was carried out and named 'pyrimidin' by a German chemist, Adolf Pinner (1885).



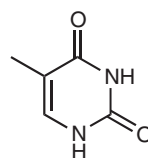
Pyrimidine: numbering system (29)



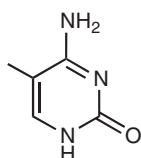
Uracil (30)



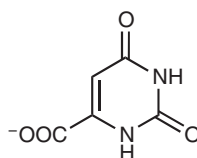
Cytosine (31)



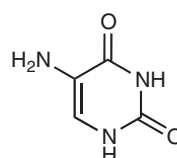
Thymine (32)



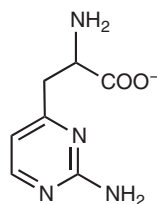
5-Methylcytosine (33)



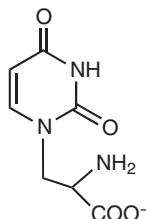
Orotate (34)



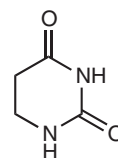
5-Aminouracil (35)



Lathyrine (36)



Willardine (37)



Dihydrouracil (38)

1.3.2.1 Pyrimidine Bases

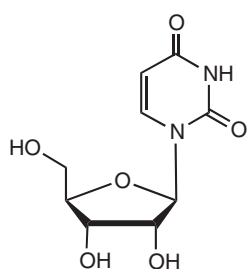
Uracil (30), cytosine (31), and thymine (32) are the common nucleobases of nucleic acids. DNA and RNA also contain other bases that have been modified after formation of the nucleic acid chain. In the case of DNA, the most common modified base is 5-methylcytosine (33).

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).

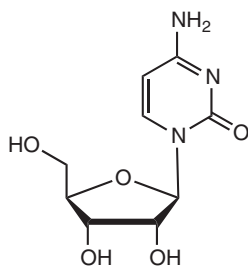
1.3.2.2 Pyrimidine Nucleosides

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

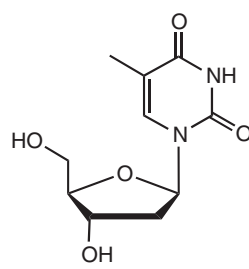
are many modified bases in RNA, including those contained in the nucleosides, pseudouridine (5-ribosyluracil) (42), and dihydrouridine (43). Pseudouridine is an isomer of the nucleoside uridine (39) in which the uracil is attached via a carbon–carbon linkage instead of a nitrogen–carbon glycosidic bond. It is the most prevalent of the over 100 different modified nucleosides found in RNA.



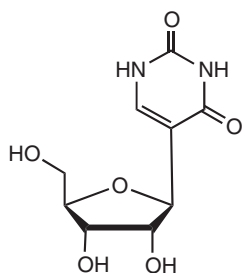
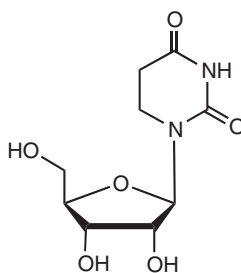
Uridine (39)



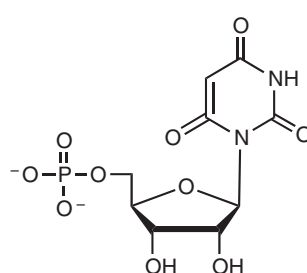
Cytidine (40)



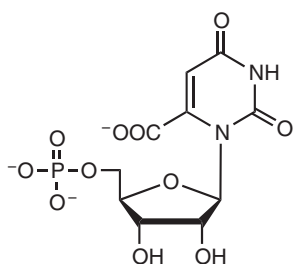
Thymidine (41)

Pseudouridine
(5-ribosyluracil) (42)

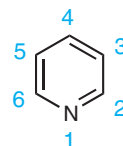
Dihydrouridine (43)



Uridine-5'-monophosphate (44)



Orotidine-5'-monophosphate (45)



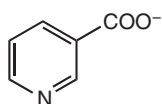
Pyridine numbering system (46)

1.3.2.3 Pyrimidine Nucleotides

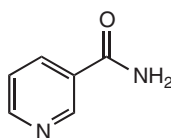
A pyrimidine nucleotide is composed of a pyrimidine base, a sugar (ribose or deoxyribose) and at least a phosphate group. Examples of different forms of pyrimidine nucleotide structures are uridine-5'-monophosphate (5'-UMP usually abbreviated as UMP) (44) and orotidine-5'-monophosphate (5'-OMP) (45),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

1.3.3 Pyridines

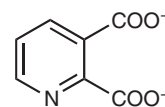
Pyridine (**46**) is a basic heterocyclic organic compound. It is structurally related to benzene, with one methine group replaced by a nitrogen atom, and was discovered in 1849 by a Scottish chemist, Thomas Anderson, as one of the constituents of bone oil. Pyridine-related compounds include the catabolites 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.



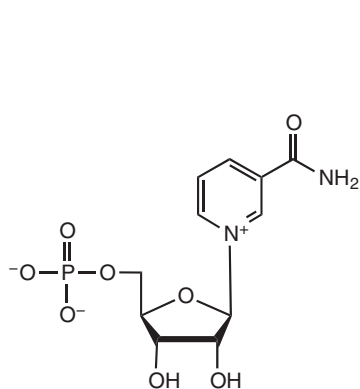
Nicotinate (**47**)



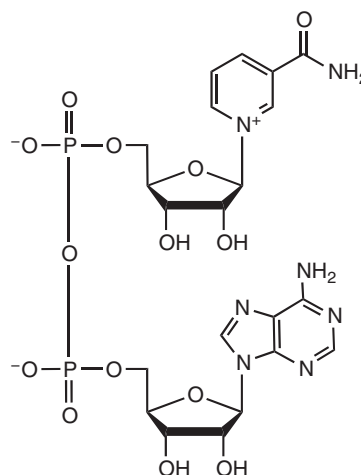
Nicotinamide (**48**)



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.

References

Ashihara, H., Yokota, T., and Crozier, A. (2013). Purine alkaloids, cytokinins, and purine-like neurotoxin alkaloids. In: *Natural Products: Phytochemistry, Botany and*

- Metabolism of Alkaloids, Phenolics and Terpenes* (eds. K.G. Ramawat and J.-M. Mérillon), 953–975. Berlin, Heidelberg: Springer.
- Boniecka, J., Prusińska, J., Dąbrowska, G.B., and Goc, A. (2017). Within and beyond the stringent response-RSH and (p)ppGpp in plants. *Planta* 246: 817–842.
- Burnstock, G. and Verkhatsky, A. (2012). Early history of purinergic signalling. In: *Purinergic Signalling and the Nervous System*, 7–66. Berlin, Heidelberg: Springer.
- Fischer, E. (1884). Ueber die Harnsäure. I. *Ber. Dtsch. Chem. Ges.* 17: 328–338.
- Harden, A. and Young, W.J. (1906). The alcoholic ferment of yeast-juice. Part II.—The coferment of yeast-juice. *Proc. R. Soc. London, Ser. B* 78: 369–375.
- Henderson, J.F. and Paterson, A.R.P. (1973). *Nucleotide Metabolism - an Introduction*. New York: Academic Press.
- IUPAC-IUB Commission on Biochemical Nomenclature (1970). Abbreviations and symbols for nucleic acids, polynucleotides and their constituents. Recommendations 1970. *J. Biol. Chem.* 245: 5171–5176.
- Pietrowska-Borek, M., Nuc, K., Zielezińska, M., and Guranowski, A. (2011). Diadenosine polyphosphates (Ap3A and Ap4A) behave as alarmones triggering the synthesis of enzymes of the phenylpropanoid pathway in *Arabidopsis thaliana*. *FEBS Open Bio* 1: 1–6.
- Pinner, A. (1885). Ueber die Einwirkung von Acetessigäther auf die Amidine Pyrimidin. *Ber. Dtsch. Chem. Ges.* A18: 759–760.