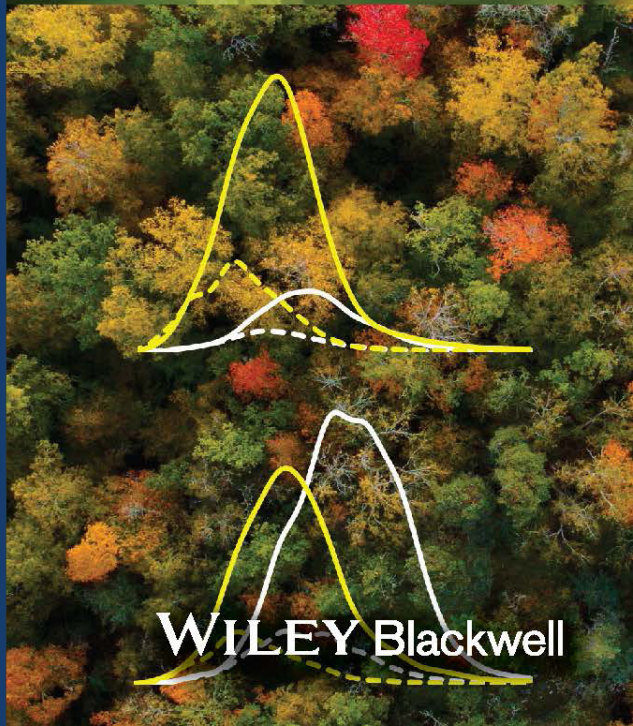


Recent Advances in Polyphenol Research

Volume 8

Edited by
Juha-Pekka Salminen
Kristiina Wähälä
Victor de Freitas
Stéphane Quideau



Recent Advances in Polyphenol Research

Recent Advances in Polyphenol Research

A series for researchers and graduate students whose work is related to plant phenolics and polyphenols, as well as for individuals representing governments and industries with interest in this field. Each volume in this biennial series focuses on several important research topics in plant phenols and polyphenols, including chemistry, biosynthesis, metabolic engineering, ecology, physiology, food, nutrition, and health.

Volume 8 Editors:

Juha-Pekka Salminen (University of Turku, Finland), Kristiina Wähälä (University of Helsinki, Finland), Victor de Freitas (University of Porto, Portugal), and Stéphane Quideau (University of Bordeaux, France)

Series Editor-in-Chief:

Stéphane Quideau (University of Bordeaux, France)

Series Editorial Board:

Oyvind Andersen (University of Bergen, Norway)
Denis Barron (Nestlé Research, Lausanne, Switzerland)
Luc Bidel (INRAE, Montpellier, France)
Véronique Cheynier (INRAE, Montpellier, France)
Catherine Chèze (University of Bordeaux, France)
Gilles Comte (University of Lyon, France)
Fouad Daayf (University of Manitoba, Winnipeg, Canada)
Olivier Dangles (University of Avignon, France)
Kevin Davies (Plant & Food Research, Palmerston North, New Zealand)
Maria Teresa Escribano-Bailon (University of Salamanca, Spain)
Sylvain Guyot (INRAE, Rennes, France)
Ann E. Hagerman (Miami University, Oxford, Ohio, USA)
Heidi Halbwirth (Vienna University of Technology, Austria)
Amy Howell (Rutgers University, Chatsworth, New Jersey, USA)
Victor de Freitas (University of Porto, Portugal)
Johanna Lampe (Fred Hutchinson Cancer Research Center, Seattle, Washington, USA)
Vincenzo Lattanzio (University of Foggia, Italy)
Stephan Martens (Fondazione Edmund Mach, IASMA, San Michele all'Adige, Italy)
Nuno Mateus (University of Porto, Portugal)
Fulvio Mattivi (University of Trento, Italy)
Jess Reed (University of Wisconsin-Madison, USA)
Annalisa Romani (University of Florence, Italy)
Erika Salas (Autonomous University of Chihuahua, Chihuahua, Mexico)
Juha-Pekka Salminen (University of Turku, Finland)
Pascale Sarni-Manchado (INRAE, Montpellier, France)
Celestino Santos-Buelga (University of Salamanca, Spain)
Kathy Schwinn (Plant & Food Research, Palmerston North, New Zealand)
Karl Stich (Vienna University of Technology, Austria)
David Vauzour (University of East Anglia, Norwich, UK)
Kristiina Wähälä (University of Helsinki, Finland)
Kumi Yoshida (Nagoya University, Japan)
Kazuhiko Fukushima (Nagoya University, Japan)

Recent Advances in Polyphenol Research

Volume 8

Edited by

Juha-Pekka Salminen

Professor, Natural Compound Chemistry

Department of Chemistry

University of Turku, Finland

Kristiina Wähälä

Professor, Organic Chemistry

Faculty of Medicine and Faculty of Science

University of Helsinki, Finland

Victor de Freitas

Professor, Food Chemistry

Chemistry and Biochemistry Department, Faculty of Sciences

University of Porto, Portugal

Stéphane Quideau

Professor, Organic and Bioorganic Chemistry

Institut des Sciences Moléculaires, CNRS-UMR 5255

University of Bordeaux, Talence, France

& Institut Universitaire de France, Paris, France

WILEY Blackwell

This edition first published 2023
© 2023 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 Juha-Pekka Salminen, Kristiina Wähälä, Victor de Freitas, and Stéphane Quideau to be identified as the authors of the editorial material in this work has been asserted in accordance with law.

Registered Offices

John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA

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

Trademarks: Wiley and the Wiley logo are trademarks or registered trademarks of John Wiley & Sons, Inc. and/or its affiliates in the United States and other countries and may not be used without written permission. All other trademarks are the property of their respective owners. John Wiley & Sons, Inc. is not associated with any product or vendor mentioned in this book.

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. 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. 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. 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 applied for

ISBN: 9781119844761

ISSN: 2474-7696

Cover Design: Wiley

Cover Images: The River Aura and the Turku Cathedral are the landmarks of the city of Turku, the venue for the XXX International Conference on Polyphenols, hosted by the Natural Chemistry Research Group at the University of Turku, Finland – The Onagraceae plant family produces the largest ellagitannins found to date in the plant kingdom: the Natural Chemistry Research Group found the undecameric ellagitannin first time in *Oenothera biennis* – The Island of Ruissalo in Turku harbors the most famous oak forest in Finland. Together with other deciduous tree species oaks produce a great color show with their autumn coloration. The two chromatograms on the top of the oak forest show how the group-specific LC-MS methodology developed by the Natural Chemistry Research group can visualize the chromatographic fingerprints of both terminal and extension units of procyanidin and prodelfphinidin units present in plant proanthocyanidins.

All rights of the photos belong to Vesa Aaltonen (first photo), Marc Johnson and Kari Loikas (second photo), and Juha-Pekka Salminen (third photo). In addition, the cover includes the official logo of the ICP2020TURKU, designed by Juha Harju.

Set in 9.5/12.5pt STIXTwoText by Straive, Pondicherry, India

Dedications

In memoriam



Prof. Takashi Yoshida (born in 1939, deceased on 1 May 2021) was a Professor of Medicinal Plants Chemistry at Okayama University, Japan, from 1993 to 2005. I worked with him as a research associate in the same laboratory. He also worked at the College of Pharmacy, Matsuyama University, Japan, as a Professor of Pharmacognosy from 2006 to 2012 after his departure from Okayama University in 2005. His research interests included the isolation and structural determination of ellagitannins and related polyphenols, as well as terpenoids in medicinal plants and foods in Japan, China, South-East Asian, and South American countries and the examination of their physiological activities. His scientific activity is documented in 261 papers on the isolation and structure elucidation of tannins and terpenoids in many medicinal plants and foods, as well as their diverse pharmacological properties, such as anti-cancer, anti-*Helicobacter pylori*, antileishmanial, and anti-

methicillin-resistant *Staphylococcus aureus* activities. He received the Tannin Award at the Phytochemical Society of North America Annual Meeting in 2008 (Philadelphia, USA) and the Groupe Polyphenol Medal at the 8th Tannin Conference in 2014 (Nagoya, Japan) for his achievements in the field of polyphenolic natural products. He worked with many wonderful collaborators worldwide, including pharmacologists, biochemists, and microbiologists. He was respected and loved around the world for his caring friendship, and I have the deepest respect for him as a researcher and educator.

Professor Hideyuki Ito
Okayama Prefectural University, Japan

Prof. Hidetoshi Yamada (born in Ehime, Japan, on 4 August 1962, deceased on 23 November 2019) studied chemistry at Osaka City University, obtaining a bachelor's degree in 1985 and a master's degree in 1987. He was appointed Assistant Professor at Tokushima Bunri University, working with the late Professor Mugio Nishizawa. He received his PhD degree for the synthesis and structural revision of osladin, a sweet saponin. In 1997, he moved to Kwansei Gakuin University as Associate Professor and was promoted Full Professor in 2004. The key motif of his research was *curiosity*, centering particular attention on the conformations of carbohydrates, which were his familiar molecules from his early career. Interest in oligosaccharides led him to develop several viable glycosylation protocols, including thermal glycosylation, which allowed him to achieve the synthesis of L-rhamnose-based cyclodextrin. He and Mugio-sensei named the molecule "cycloawodorin," a facetious trivial name associated with Tokushima locality. Through these endeavors, he had a keen interest in the conformationally flipped glycosyl donors. An ingenious design of flipped donor by bridging allowed the β -selective glycosylation without aid of the neighboring-group participation and further led to the preparation of the smallest cyclodextrin. Motivated by the presence of inverted glucose motifs, he stumbled into the synthesis of ellagitannin-class polyphenols, featuring D-glucose as a core extensively esterified by digalloyl groups. Facing such a tremendous diversity, he was as ever positive to

make a slogan, “Let us synthesize these natural products all.” To tackle this formidable challenge, he focused on two key motifs embedded within these complex structures: (1) hexahydroxydiphenoyl (HHDP) diesters based on two gallic acids directly C—C linked together, and (2) C—O linked digallates for which two gallic acids are linked through an oxygen atom. For the former HHDP motif, he exploited intramolecular oxidative biaryl coupling between gallates appended on a glucose scaffold, where CuCl_2 and $n\text{-BuNH}_2$ were identified as effective reagents. For the latter O-linked digallate motif, *ortho*-quinone mono-acetal was used as a platform to undergo an *oxa*-Michael addition/elimination sequence. His chemical synthesis study opened a door to flexible, comprehensive access to the ellagitannin molecules as pure entities.



Prof. Toshiyuki Kan (born in Hokkaido, Japan, on 15 February 1964, deceased on 24 July 2021) studied chemistry at Hokkaido University, where he obtained his bachelor's degree (1986) and master's degree (1988). He received his PhD degree in 1993 under the guidance of Prof. Haruhisa Shirahama, working on the total synthesis of grayanotoxin III, a poisonous diterpene with a highly complex structure. After working as a researcher at Suntory Institute for Bioorganic Research with Prof. Yasufumi Ohfuné (1993–1996), he was appointed Assistant Professor at University

of Tokyo with Prof. Tohru Fukuyama in 1996 and was promoted as Associate Professor in 2003. In 2004, he moved to the University of Shizuoka as Full Professor. His research focus was complex natural product synthesis, as represented by grayanotoxin III (diterpenoid, skeletal complexity) and ecteinascidin 743 (alkaloid, skeletal/functional complexity). In addressing the synthesis of complex natural products, he was continuously exposed to care for the management of multiple functionalities, and he became able to come up with survival tactics or even strategies to achieve the total synthesis. Among others is the design of a new protecting group, “nosyl (Ns),” which served as bases for his alkaloid syntheses, and now one of the standard choices for protecting amino groups. Upon moving to his last destination, Shizuoka, one of the major tea and orange localities in Japan, he decided to study the synthesis and chemical biology of tea- and citrus-derived polyphenols, including chafuroside A and B (black tea), epigallocatechin gallate (EGCg), and nobiletin. Here the nosyl group was proven to work for protecting phenols, endorsing a theanine synthesis via otherwise difficult biomimetic oxidative dimerization. He designed molecular probes for catechins and nobiletin, fluorescein, and PET probes, serving for *in vivo* imaging and antibody generation as well. He further addressed the syntheses of sesamin and sesaminol by an organocatalytic process and biomimetic construction of the furofuran skeleton and of hybrid-type polyphenols, hedyotol A, princepin, and sophoraflavanone H by exploiting C—H insertion reactions. His study highlighted and gained insights into the traditional products indigenous to Shizuoka through chemical synthesis and chemical biology. He delivered a plenary lecture on “Total Synthesis of Hybrid Type Polyphenols” at the XXX International Conference on Polyphenols in Turku, Finland, in July 2021, which turned out to be his last lecture. The initial seven slides were a tribute to his friend, Hidetoshi Yamada. In many people's memory, they both would be remembered by their energy, courage, spirit in research, determination and leadership in society, and friendship and warm-hearted attitude toward everyone. They were intimate friends, sharing genuine scientific interests in complex natural product synthesis. Here they are (Hidetoshi on the left and Toshiyuki on the right) with their relaxed smiles like naughty kids, enjoying fine food and drinks!?! 合掌

Keisuke Suzuki
Professor Emeritus, Tokyo Institute of Technology, Japan

Contents

	Contributors	<i>xv</i>
	Preface	<i>xxi</i>
	Acknowledgments	<i>xxiii</i>
1	Lignins and Lignification: New Developments and Emerging Concepts	1
	<i>John Ralph, Hoon Kim, Fachuang Lu, Rebecca A. Smith, Steven D. Karlen, Nuoendagula, Koichi Yoshioka, Alexis Eugene, Sarah Liu, Canan Sener, Daisuke Ando, Mingjie Chen, Yanding Li, Leta L. Landucci, Sally A. Ralph, Vitaliy I. Timokhin, Wu Lan, Jorge Rencoret, and José C. del Río</i>	
1.1	Introduction	1
1.2	The Monolignol Pathway and Interacting Pathways – New Lignins	2
1.2.1	Truncated Monolignol Biosynthesis	3
1.2.1.1	CAD Deficiency	3
1.2.1.2	OMT Deficiency	11
1.2.2	Phenolics from Beyond the Monolignol Biosynthetic Pathway	11
1.2.3	Lignin Design, and the Concept of an Ideal Lignin	14
1.2.3.1	Zip-Lignins	15
1.2.3.2	The Concept of an “Ideal Lignin”	18
1.2.3.3	Introducing New Pathways into Lignification, New Units into Lignins	19
1.3	Lignin Conjugates, “Clip-Offs” – New Discoveries, and Enhancing Levels	20
1.3.1	Clip-Offs and Their Elevation	22
1.3.2	Exploring Monolignol Conjugates in Compositionally Extreme Lignins	23
1.4	Features of Lignification and the Possibility of New Polymerization Pathways	26
1.4.1	Features of Lignification	26
1.4.1.1	Lignification Reminders	26
1.4.1.2	Does Polymerization Have to Occur from the Phenolic End?	28
1.4.1.3	Do New Monomers Propound Possibilities for New Polymerization Mechanisms?	29
1.5	The Case for Model Studies and Synthesis	30
1.5.1	The Value of Proper Low-Molecular-Mass Model Compounds	31
1.5.2	Synthetic Lignin Polymers, Dehydrogenation Polymers (DHPs)	33
1.6	New or Improved Analytics	33
1.7	Conclusions and Opportunities	36
	Acknowledgments	37
	References	37

2 Synthesis of Epigallocatechin Gallate, Nobiletin, and Their Derivatives for Chemical-Biological Studies 51*Tomohiro Asakawa, Makoto Inai, and Toshiyuki Kan*

- 2.1 Synthetic Investigations of Catechin Derivatives 51
- 2.2 Synthesis and Application of Fluorescent Catechin Probes 54
- 2.3 Generation of Catechin Antibody 54
- 2.4 PET Imaging of Biodistribution of Catechin 55
- 2.5 Practical Synthesis of Nobiletin 56
- 2.6 Derivatization of Desmethyl Nobiletins 58
- 2.7 PET Imaging of Biodistribution of Nobiletin 59
- 2.8 Synthesis and Application of Fluorescent Nobiletin Probe 60
- 2.9 Conclusions 61
- References 61

3 Procyanidins in the Onset and Progression of Colorectal Cancer: Recent Advances and Open Questions 67*Wei Zhu, Gerardo G. Mackenzie, and Patricia I. Oteiza*

- 3.1 Introduction 67
- 3.2 Procyanidins: Chemistry and Metabolism 68
- 3.3 Procyanidins and CRC: Epidemiological Evidence 70
- 3.4 Procyanidins and CRC: Rodent Studies 74
- 3.5 Procyanidins and CRC: Mechanisms of Action 79
 - 3.5.1 Interactions with Membranes 79
 - 3.5.2 Inflammation and the NF- κ B Pathway 81
 - 3.5.2.1 Inflammation 81
 - 3.5.2.2 NF- κ B 82
 - 3.5.3 EGFR and IGF1R Pathways 83
- 3.6 Conclusions and Open Questions 84
 - Acknowledgments 85
 - Conflict of Interest Disclosure 85
 - References 85

4 The Potential of Low Molecular Weight (Poly)phenol Metabolites for Attenuating Neuroinflammation and Treatment of Neurodegenerative Diseases 95*Daniela Marques, Rafael Carecho, Diogo Carregosa, and Cláudia Nunes dos Santos*

- 4.1 Introduction: Neurodegenerative Disorders, Dietary (Poly)phenols and Neuroinflammation 95
- 4.2 (Poly)phenols: Metabolism and Distribution 96
- 4.3 (Poly)phenol Metabolites and Their Brain Permeability 119
- 4.4 LMW (Poly)phenol Metabolites as Effectors for Attenuating Neuroinflammation 121
- 4.5 Concluding Remarks 131
 - Acknowledgments 131
 - References 131

5	Deciphering Complex Natural Mixtures through Metabolome Mining of Mass Spectrometry Data: The Plant Specialized Metabolome as a Case Study	139
	<i>Justin J.J. van der Hooft, Madeleine Ernst, Daniel Papenberg, Kyo Bin Kang, Iris F. Kappers, Marnix H. Medema, Pieter C. Dorrestein, and Simon Rogers</i>	
5.1	Introduction	139
5.2	Materials and Methods	143
5.2.1	Case Studies	143
5.2.2	Metabolome Mining Tools	143
5.2.2.1	Molecular Networking	143
5.2.2.2	Substructure Discovery	146
5.2.3	Metabolome Annotation Tools	146
5.2.3.1	Elemental Formula Assignment – SIRIUS and ZODIAC	146
5.2.3.2	Candidate Structure Finding – Library Matching	147
5.2.3.3	Candidate Structure Finding – CSI:FingerID	148
5.2.3.4	Chemical Class Assignment – ClassyFire, NPClassifier, and CANOPUS	148
5.2.3.5	Combining All Structural Information – MolNetEnhancer	149
5.3	Results and Discussion	150
5.3.1	Rhamnaceae Case Study	150
5.3.2	Euphorbia Case Study	151
5.3.3	Pepper Case Study	154
5.3.4	Other Plant Metabolomics Studies	156
5.4	Current Limitations	156
5.5	Conclusions	157
5.6	Outlook	157
5.6.1	Extended Natural Product Candidate Structure Space	157
5.6.2	Improved Mass Spectral Similarity Scoring	158
5.6.3	Combined Genome and Metabolome Analyses	159
5.6.4	Linking Complementary Analytical Tools	160
5.6.5	Future Perspective: Chemically Informed Repository-Scale Analyses	160
	Acknowledgments	161
	References	162
6	Application of MS-Based Metabolomics to Investigate Biomarkers of Apple Consumption Resulting from Microbiota and Host Metabolism Interactions	169
	<i>Fulvio Mattivi and Maria M. Ulaszewska</i>	
6.1	Introduction	169
6.2	Materials and Methods	169
6.2.1	Acute Intake Study	169
6.2.2	Long-Term Intake Study	170
6.2.3	Metabolomic Analysis	170
6.2.3.1	Sample Extraction	170
6.2.3.2	Chromatography	171
6.2.3.3	Mass Spectrometry	171
6.2.4	Data Processing and Statistical Analysis	172
6.2.5	Metabolomic Data Sharing	172
6.3	Results and Discussion	173

- 6.3.1 Lessons from the Acute Study 173
 - 6.3.1.1 Two Nutrikinetics Patterns 173
 - 6.3.1.2 Dose–Response Relationship After Apple Juice Intake 174
 - 6.3.1.3 Inter-Individual Variability of Metabolic Response and Impact on Bioavailability 178
 - 6.3.1.4 Is Our Microbiota a Multiplier of Complexity? 182
 - 6.3.1.5 Microbial Catabolites of Flavanols are Persistent in the Body and can Reach High Concentrations 184
 - 6.3.1.6 Considerations of Bioequivalence Studies 185
- 6.3.2 Lessons from the Prolonged Exposure Study 187
 - 6.3.2.1 Renetta Apples and Cardiometabolic Biomarkers 187
 - 6.3.2.2 Investigating the Importance of Microbial Biodiversity of the Human Gut 188
- 6.4 Conclusion 190
 - Acknowledgments 190
 - Funding 190
 - References 191

7 **Non-Extractable Polyphenols Should be Systematically Included in Polyphenol Analysis** 193

Enrique Báez-García, Sonia G. Sáyago-Ayerdi, and Jara Pérez-Jiménez

- 7.1 Introduction: The Concept of Non-Extractable Polyphenols 193
- 7.2 Analysis of Non-Extractable Polyphenols 195
 - 7.2.1 Preparation of Solutions of Non-Extractable Polyphenols 195
 - 7.2.1.1 Alkaline and Acid Hydrolysis 197
 - 7.2.1.2 Enzymatic Hydrolysis 198
 - 7.2.1.3 Emerging Extraction Techniques for NEPP 202
 - 7.2.2 Analysis of the Profile of NEPP 204
 - 7.2.2.1 Spectrophotometric Methods for Analysis of NEPP 204
 - 7.2.2.2 Liquid Chromatography-Mass Spectrometry Analysis of NEPP 205
 - 7.2.3 Determination of the Content of Non-Extractable Polyphenols. Which Standard? 209
 - 7.2.4 Analysis of Dietary Fiber: Connection with Non-Extractable Polyphenols 210
- 7.3 Why Should Non-Extractable Polyphenols be Systematically Included in Polyphenol Analysis? 211
 - 7.3.1 Intake of NEPP in Different Populations 211
 - 7.3.2 Metabolism of NEPP 212
 - 7.3.3 Beneficial Effects Attributed to NEPP 213
- 7.4 Relevance of the Determination of Non-Extractable Polyphenols in Quality Control 216
 - 7.4.1 Comprehensive Characterization of Vegetal Materials 216
 - 7.4.2 Identification of New Botanical Sources with Potential Applications 217
 - 7.4.3 Comparison Between Varieties 219
 - 7.4.4 Evaluation of Processing Effects 219
- 7.5 Perspectives 223
 - References 225

8	Template-Mediated Engineering of Functional Metal–Phenolic Complex Coatings 239
	<i>Steve Spoljaric, J.J. Richardson, Yi Ju, and Frank Caruso</i>
8.1	Introduction 239
8.2	Template-Mediated Techniques to Deposit MPNs 248
8.3	MPN Film Properties 253
8.4	MPN Surface Interactions and Applications 254
8.5	Upscaling Considerations and Challenges 260
8.5.1	Reagent Considerations 261
8.5.2	Engineering Controls 261
8.5.3	Washing and Solvents 262
8.5.3.1	Dissolution of Reagents and Preparation of Buffers used in MPN Fabrication 262
8.5.3.2	Synthesis of Mesoporous Templates or Functionalized Polyphenols 262
8.5.3.3	Washing of MPN-Coated Templates and MPN Capsules 262
8.5.3.4	Dissolution of Particle Templates to Obtain MPN Capsules 263
8.5.4	Human Resources and Training 263
8.5.5	Environmental Health and Safety Considerations 264
8.6	Method Automation: Possibilities and Outlook 264
8.6.1	Automated Assembly Techniques 265
8.6.1.1	Robotic/Automated Immersive Assembly 265
8.6.1.2	Robotic/Automated Spray Assembly 266
8.6.1.3	Fluidic Assembly 267
8.6.1.4	Automated Washing and Filtration Techniques 269
8.7	Conclusions 269
	References 270
9	Highly Efficient Production of Dihydroflavonol 4-Reductases in Tobacco Cells and Refinement of the BuOH-HCl Enzymatic Assay 281
	<i>Lingping Zhu, Saku Mattila, Roosa Matomäki, Lorenzo Mollo, Sharmin Ahamed, Sara M. Abdou, Hany Bashandy, and Teemu H. Teeri</i>
9.1	Introduction 281
9.2	Results 283
9.2.1	Transient Expression from Hypertranslatable Vectors 283
9.2.2	BuOH-HCl Assay Revisited 284
9.2.3	Substrate Profiles of Different DFRs 291
9.3	Materials and Methods 291
9.3.1	Plant Material and Chemicals 291
9.3.2	Isolation of DFR Encoding Sequences and Plasmid Construction 293
9.3.3	Protein Extraction and Purification 293
9.3.4	BuOH-HCl Assay 295
9.3.5	HPLC 295
9.4	Discussion 295
	Acknowledgements 297
	References 297

- 10 A Long and Winding Road: The Evolution of Transcriptional Regulation of Polyphenol Biosynthesis 301**
Cathie Martin, Jie Li, and Nick W. Albert
- 10.1 Introduction 301
- 10.2 The Importance of R2R3Myb Transcription Factors (TFs) in the Regulation of Phenylpropanoid Metabolism in Plants 303
- 10.2.1 R2R3Myb TFs Regulate Specialized Branches of Polyphenol Metabolism 305
- 10.2.2 R2R3Myb Transcriptional Repressors Controlling Phenylpropanoid Metabolism 306
- 10.2.3 Stand-Alone R2R3Myb Transcriptional Activators 307
- 10.2.4 R2R3Myb TFs Working in MBW Complexes to Regulate Phenylpropanoid Metabolism 308
- 10.3 The Role of bHLH Proteins in the Regulation of Phenylpropanoid Metabolism 310
- 10.3.1 Roles of bHLH-1 and bHLH-2 Clades in Regulating Anthocyanin Biosynthesis 312
- 10.3.2 Roles of bHLH-1 and bHLH-2 Clades in the Regulation of Proanthocyanidin Biosynthesis 313
- 10.4 The Role of the WDR in the MBW Complex in the Regulation of Polyphenol Metabolism 316
- 10.5 Additional Factors Regulating Transcriptional Control of the MBW Complex 317
- 10.6 Conclusions 318
Acknowledgments 318
References 318
- 11 Analysis of Proanthocyanidins in Food Ingredients by the 4-(Dimethylamino) cinnamaldehyde Reaction 325**
Daniel Esquivel-Alvarado, Emilia Alfaro-Viquez, Andrew Birmingham, Abigail Kramschuster, Christian G. Krueger, and Jess D. Reed
- 11.1 Introduction 325
- 11.2 Background on the 4-(Dimethylamino)cinnamaldehyde (DMAC) Reaction with PACs 326
- 11.3 Mechanism of the Acid-Catalyzed DMAC Reaction with PACs 327
- 11.4 Absorption and Emission Spectra of the DMAC Reaction Products 334
- 11.5 Standards for the DMAC Reaction and Accuracy of the Method 337
- 11.6 Interaction of PAC-DMAC Reaction Products with Extra-Intestinal Pathogenic *Escherichia coli* 340
- 11.7 Conclusion 341
References 342
- 12 Reactions of Ellagitannins Related to Their Metabolism in Higher Plants 347**
Takashi Tanaka
- 12.1 Introduction 347
- 12.2 Structural Variety of Ellagitannin Acyl Groups 348

12.3	Reactions of the DHHDP Group	349
12.4	Decomposition of 1,4-DHHDP- α -D-glucose	353
12.5	Amariin as a Precursor of Geraniin	353
12.6	Triterpenoid HHDP Esters in <i>Castanopsis sieboldii</i>	355
12.7	Highly Oxidized Ellagitannins in <i>Carpinus japonica</i>	356
12.8	Similarity of Catechin Oxidation to Oxidation of Methyl Gallate	357
12.9	Production Mechanism of DHHDP and HHDP	358
12.10	Oxidative Degradation of Ellagitannins	359
12.10.1	Degradation of Pedunculagins in the Leaves of Common Camellia Species	360
12.10.2	Degradation of Vescalagin in the Leaves of Japanese Blue Oak	360
12.10.3	Degradation of Vescalagin with Wood-Decaying Fungi	361
12.11	Conclusions	362
	References	362

Index	369
--------------	-----

Contributors

Sara M. Abdou

Department of Agricultural Sciences,
Viikki Plant Science Centre, University of
Helsinki, Helsinki, Finland

Sharmin Ahamed

Department of Agricultural Sciences,
Viikki Plant Science Centre, University of
Helsinki, Helsinki, Finland

Nick W. Albert

Plant & Food Research, Palmerston North,
New Zealand

Emilia Alfaro-Viquez

Reed Research Group, Department
of Animal and Dairy Sciences, University
of Wisconsin-Madison, Madison,
WI, USA

Daisuke Ando

Great Lakes Bioenergy Research
Center, The Wisconsin Energy
Institute, University of Wisconsin,
Madison, WI, USA

Current address: Institute of Wood
Technology, Akita Prefectural University,
Noshiro, Japan

Tomohiro Asakawa

Department of Fisheries-Food Science,
Tokai University, Shizuoka, Japan

Enrique Báez-García

Institute of Food Science, Technology
and Nutrition, Spanish Research Council
(ICTAN-CSIC), Madrid, Spain

Tecnológico Nacional de Mexico/Instituto
Tecnológico de Tepic, Tepic, Nayarit, Mexico

Hany Bashandy

Department of Agricultural Sciences,
Viikki Plant Science Centre, University of
Helsinki, Helsinki, Finland

Department of Genetics, Cairo University,
Giza, Egypt

Andrew Birmingham

Complete Phytochemical Solutions LLC,
Cambridge, WI, USA

Rafael Carecho

NOVA Medical School, Faculdade de
Ciências Médicas, Universidade NOVA de
Lisboa, Lisboa, Portugal

Diogo Carregosa

NOVA Medical School, Faculdade de
Ciências Médicas, Universidade NOVA de
Lisboa, Lisboa, Portugal

Frank Caruso

Department of Chemical Engineering, The
University of Melbourne, Parkville, VIC,
Australia

Mingjie Chen

Great Lakes Bioenergy Research Center, The Wisconsin Energy Institute, University of Wisconsin, Madison, WI, USA
Current address: Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China

José C. del Río

Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Seville, Spain

Pieter C. Dorrestein

Collaborative Mass Spectrometry Innovation Center, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, USA

Cláudia Nunes dos Santos

NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal

Madeleine Ernst

Section for Clinical Mass Spectrometry, Department of Congenital Disorders, Danish Center for Neonatal Screening, Statens Serum Institut, Copenhagen, Denmark

Daniel Esquivel-Alvarado

Reed Research Group, Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI, USA

Alexis Eugene

Great Lakes Bioenergy Research Center, The Wisconsin Energy Institute, University of Wisconsin, Madison, WI, USA

Makoto Inai

School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Yi Ju

Department of Chemical Engineering, The University of Melbourne, Parkville, VIC, Australia

Toshiyuki Kan

School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Kyo Bin Kang

College of Pharmacy, Sookmyung Women's University, Seoul, Korea

Iris F. Kappers

Laboratory of Plant Physiology, Plant Sciences Group, Wageningen University and Research Wageningen, the Netherlands

Steven D. Karlen

Great Lakes Bioenergy Research Center, The Wisconsin Energy Institute, University of Wisconsin, Madison, WI, USA

Hoon Kim

Great Lakes Bioenergy Research Center, The Wisconsin Energy Institute, University of Wisconsin, Madison, WI, USA

Abigail Kramschuster

Complete Phytochemical Solutions LLC, Cambridge, WI, USA

Christian G. Krueger

Reed Research Group, Department of Animal and Dairy Sciences, University of Wisconsin-Madison, Madison, WI, USA
Complete Phytochemical Solutions LLC, Cambridge, WI, USA

Wu Lan

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute, University
of Wisconsin, Madison, WI, USA

Current address: State Key Laboratory
of Pulp and Paper Engineering, South
China University of Technology,
Guangzhou, China

Leta L. Landucci

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute,
University of Wisconsin, Madison,
WI, USA

Jie Li

Department of Biochemistry and
Metabolism, John Innes Centre,
Norwich Research Park,
Colney, Norwich, UK

Yanding Li

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute,
University of Wisconsin, Madison,
WI, USA

Current address: BeiGene, Zhongguancun
Life Science Park, Changping District,
Beijing, China

Sarah Liu

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute,
University of Wisconsin, Madison,
WI, USA

Fachuang Lu

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute,
University of Wisconsin, Madison,
WI, USA

Gerardo G. Mackenzie

Department of Nutrition, University of
California-Davis, Davis, CA, USA

Daniela Marques

NOVA Medical School, Faculdade de
Ciências Médicas, Universidade NOVA de
Lisboa, Lisboa, Portugal

Cathie Martin

Department of Biochemistry and
Metabolism, John Innes Centre, Norwich
Research Park, Colney, Norwich, UK

Roosa Matomäki

Department of Agricultural Sciences,
Viikki Plant Science Centre, University of
Helsinki, Helsinki, Finland

Saku Mattila

Department of Agricultural Sciences,
Viikki Plant Science Centre, University of
Helsinki, Helsinki, Finland

Fulvio Mattivi

Department of Cellular, Computational
and Integrative Biology (CIBIO),
University of Trento, Trento, Italy

Department of Food Quality and Nutrition,
Fondazione Edmund Mach, Research
and Innovation Centre, San Michele
all'Adige, Italy

Marnix H. Medema

Bioinformatics Group, Plant
Sciences Group, Wageningen
University and Research,
Wageningen, the Netherlands

Lorenzo Mollo

Department of Agricultural Sciences,
Viikki Plant Science Centre, University of
Helsinki, Helsinki, Finland

Department of Environmental and Life
Sciences, Laboratory of Plant and Algae
Physiology, Università politecnica delle
Marche, Ancona, Italy

Nuoendagula

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute,
University of Wisconsin, Madison,
WI, USA

Patricia I. Oteiza

Department of Nutrition, University of
California-Davis, Davis, CA, USA

Daniel Papenberg

Bioinformatics Group, Plant Sciences
Group, Wageningen University and
Research, Wageningen, the Netherlands

Laboratory of Plant Physiology,
Plant Sciences Group, Wageningen
University and Research, Wageningen,
the Netherlands

Jara Pérez-Jiménez

Institute of Food Science, Technology
and Nutrition, Spanish Research Council
(ICTAN-CSIC), Madrid, Spain

John Ralph

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute, University
of Wisconsin, Madison, WI, USA

Department of Biochemistry, University of
Wisconsin, Madison, WI, USA

Sally A. Ralph

The US Forest Products Laboratory, One
Gifford Pinchot Drive, Madison, WI, USA

Jess D. Reed

Reed Research Group, Department of
Animal and Dairy Sciences, University of
Wisconsin-Madison, Madison, WI, USA

Complete Phytochemical Solutions LLC,
Cambridge, WI, USA

Jorge Rencoret

Instituto de Recursos Naturales y
Agrobiología de Sevilla (IRNAS), CSIC,
Seville, Spain

J.J. Richardson

Department of Chemical Engineering,
The University of Melbourne,
Parkville, VIC, Australia

Simon Rogers

School of Computing Science, University
of Glasgow, Glasgow, UK

Sonia G. Sáyago-Ayerdi

Tecnológico Nacional de Mexico/
Instituto Tecnológico de Tepic, Tepic,
Nayarit, Mexico

Canan Sener

Great Lakes Bioenergy Research
Center, The Wisconsin Energy
Institute, University of Wisconsin,
Madison, WI, USA

Rebecca A. Smith

Great Lakes Bioenergy Research
Center, The Wisconsin Energy
Institute, University of Wisconsin,
Madison, WI, USA

Steve Spoljaric

Department of Chemical Engineering, The
University of Melbourne, Parkville, VIC,
Australia

Takashi Tanaka

Graduate School of Biomedical Sciences,
Nagasaki University, Nagasaki, Japan

Teemu H. Teeri

Department of Agricultural Sciences,
Viikki Plant Science Centre,
University of Helsinki, Helsinki, Finland

Vitaliy I. Timokhin

Great Lakes Bioenergy Research
Center, The Wisconsin Energy
Institute, University of Wisconsin,
Madison, WI, USA

Maria M. Ulaszewska

Department of Food Quality and Nutrition,
Fondazione Edmund Mach, Research
and Innovation Centre, San Michele
all'Adige, Italy

PROMEFA Facility, San Raffaele Scientific
Institute, Center for Omics Sciences,
Milan, Italy

Justin J.J. van der Hooft

Bioinformatics Group, Plant Sciences
Group, Wageningen University and
Research, Wageningen, the Netherlands

Koichi Yoshioka

Great Lakes Bioenergy Research Center,
The Wisconsin Energy Institute, University
of Wisconsin, Madison, WI, USA

Wei Zhu

Department of Nutrition, University of
California-Davis, Davis, CA, USA

Lingping Zhu

Department of Agricultural Sciences,
Viikki Plant Science Centre, University of
Helsinki, Helsinki, Finland

Preface

Every 2 years, Groupe Polyphénols (GP) hosts the International Conference on Polyphenols (ICP). The anniversary XXX ICP was planned to be held in 2020 in Turku, Finland. Unfortunately, the COVID pandemic forced GP to postpone the conference by one year, although everything was already set and organized for the conference in 2020. After one year of pandemic, GP decided that the ICP2020TURKU should not be postponed further and it was successfully organized as a fully virtual conference from 13 to 15 July, 2021. This was the first ever virtual ICP hosted by GP since its foundation in 1972. Groupe Polyphénols is the world's premier society of scientists in the fields of polyphenol chemistry, synthesis, bioactivity, nutrition, industrial applications, and ecology. Luckily, the great success of this virtual ICP encouraged GP to plan for new types of scientific activities for its members also in between the biannual ICPs. Since the ICP2020TURKU, GP has already organized the first Webinar in Polyphenols Research that will gather polyphenol scientists virtually three to four times a year to attend presentations of both established and young scientists. This approach thus also continues one of the main aims of the ICP2020TURKU by giving good opportunities to young scientists to present their recent research findings on polyphenols.

The city of Turku is a city full of history. It was the first capital of Finland, before Helsinki, and it had the first Finnish-speaking university in Finland. The main organizers of the ICP2020TURKU, the Natural Chemistry Research Group, had planned to organize the ICP at the main campus of the University of Turku, close to the River Aura and the Turku Cathedral (see the front cover). Other history-oriented activities such as the gala dinner in the medieval Turku Castle were also planned and booked. However, now it remains for all the ICP participants to visit Turku on a later notice, once the COVID pandemic allows.

The XXX ICP was attended by 250 registrants from 36 countries, with 105 invited and contributed presentations. This great number of presentations was achieved by parallel sessions that maximized the opportunities given to young scientists to present their work. This eighth edition of *Recent Advances in Polyphenol Research* has 12 chapters that represent the work of the invited speakers at the XXX ICP and reflect the depth of science in this important field of natural product chemistry. The conference included 19 sessions on structure, reactivity, and synthesis; bioactivity and bioavailability; metabolomics, targeted analysis, and big data; quality control and standardization; biogenesis and functions in plants and ecosystems; and biomaterials and applied sciences.

We owe a special thanks to Tina Ahonen from the Aboa Congress and Event Services for her professional and excellent help in the organization of the conference. The great

execution of the virtual ICP would not have been possible without the help of professionals of RajuLive Ltd. that made sure that all tiny details of the virtual conference, including the presentation recordings were of prime quality. The members and students of the Natural Chemistry Research Groups deserve our sincere thanks for their huge efforts in making the intermission activities in the form of entertaining videos that were also uploaded on the social media and for helping with the practical organization on site. Finally, we thank all the participants, who took active part in the conference sessions, and initiated a lot of scientific discussion in the conference chat, both after and between the presentations. You created a warm atmosphere for the conference and made it a really enjoyable event and a great learning experience during these otherwise difficult COVID times. We think that the ICP2020TURKU will always be remembered as a special conference, but luckily only for very good and positive reasons.

*Juha-Pekka Salminen
Kristiina Wähälä
Victor de Freitas
Stéphane Quideau*

Acknowledgments

The editors wish to thank all members of the “Groupe Polyphénols” Board Committee (2018–2021) for their guidance and assistance throughout this project.

Dr. Denis Barron

Dr. Luc Bidel

Dr. Catherine Chèze

Dr. Peter Constabel

Prof. Olivier Dangles

Dr. Kevin Davies

Prof. M Teresa Escribano Bailon

Prof. Victor de Freitas

Prof. Kazuhiko Fukushima

Dr. David Gang

Dr. Sylvain Guyot

Prof. Ann E. Hagerman

Dr. Irene Mueller-Harvey

Prof. Stéphane Quideau

Prof. Jess Reed

Dr. Erika Salas

Prof. Juha-Pekka Salminen

Prof. Kristiina Wähälä

1

Lignins and Lignification

New Developments and Emerging Concepts

John Ralph^{1,2}, Hoon Kim¹, Fachuang Lu¹, Rebecca A. Smith¹, Steven D. Karlen¹, Nuoendagula¹, Koichi Yoshioka¹, Alexis Eugene¹, Sarah Liu¹, Canan Sener¹, Daisuke Ando^{1,*}, Mingjie Chen^{1,**}, Yanding Li^{1,***}, Leta L. Landucci¹, Sally A. Ralph³, Vitaliy I. Timokhin¹, Wu Lan^{1,****}, Jorge Rencoret⁴, and José C. del Río⁴

¹Great Lakes Bioenergy Research Center, The Wisconsin Energy Institute, University of Wisconsin, Madison, WI, USA

²Department of Biochemistry, University of Wisconsin, Madison, WI, USA

³The US Forest Products Laboratory, One Gifford Pinchot Drive, Madison, WI, USA

⁴Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), CSIC, Seville, Spain

*Current address: Institute of Wood Technology, Akita Prefectural University, Noshiro, Japan

**Current address: Institute of Microbiology, Guangdong Academy of Sciences, Guangzhou, China

***Current address: BeiGene, Zhongguancun Life Science Park, Changping District, Beijing, China

****Current address: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China

1.1 Introduction

In the previous volume in this series, Volume 7, Chapter 7 highlighted recent discoveries relating to the interactions between monolignol pathways with flavonoid and stilbenoid pathways producing monomers for lignification (del Río et al. 2021). The notion that lignification, the process of polymerization from monomers to the lignin polymer, may tolerate or even favor the use of monomers beyond the canonical monolignols (*p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) is becoming mainstream even if there might not be universal agreement about what exactly constitutes lignification. Advances continue as ever more is revealed about the way cell wall polymers in various tissues in “natural plants” are derived and how the perturbations of genes, now in various interacting pathways, can affect lignification and the consequent composition and structure of the polymer. Although “structure” has little meaning for a polymer lacking defined repeating units of any length and possessing an overwhelming stereochemical complexity (Ralph et al. 2008), this newly revealed complexity to the composition and structure of lignin polymers, and the blurring of the definition of lignification, may seem alarming. It is worth emphasizing, however, that the process of lignification itself is a delightfully simple

one involving a single, purely chemical mechanism and, accordingly, lignification is a much simpler process than the ones involved in the formation of hemicellulosic polysaccharides, for example.

Findings expanding the definition of lignin continue, even as the ramifications of the COVID-19 pandemic have impeded research progress in general since 2020. To document this rapidly advancing field, we cover some new findings and a few of the emerging notions on lignification. So much is happening in this field that we cannot comprehensively cover even merely the work from our own labs; this chapter is best regarded as an update in which we concentrate on some of the research on a common theme that interests us. We also cover areas that often must be jettisoned from research papers, weaving in concepts that are in principle well-known but occasionally need to be reemphasized because they have particular importance and/or may be distinctive to lignification and unfamiliar to researchers new to the field. Along with the evaluation and contemplation of “new” pathways and mechanisms, we also include minor subsections on the value and use of lignin models to understand reaction pathways, and the continued importance of developing diagnostic analytics to provide unambiguous new insight.

The chapter has been laid out in seven sections but keeping the ideas discretely under those headings has not been fully realized. Just as an example, we decided that observations on the use of monolignol conjugates in lignification needed their own section, yet much of the material could easily fit under sections preceding it. Similarly, model, synthetic, and analytical work often accompanies any discovery, but we have chosen to split one aspect out to provide some recognition for such crucial research components. Finally, there are some concepts that we wanted to convey here that simply do not fit well under the chosen headings. We trust that this will nevertheless be a readable and useful contribution despite these limitations.

1.2 The Monolignol Pathway and Interacting Pathways – New Lignins

The monolignol biosynthetic pathway produces the three canonical monolignols for lignification, *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, differing in their degrees of methoxylation *ortho* to the phenol (Freudenberg and Neish 1968; Sarkanen and Ludwig 1971). Some of the enzymes are quite specific, whereas others are more general in nature. As summarized in Figure 1.1, and as has been reviewed (Boerjan et al. 2003; Ralph et al. 2004b; Shi et al. 2010; Weng and Chapple 2010), the major flux through the pathway was simplified from the full metabolic grid originally considered (Dixon et al. 2001; Higuchi 2006; Matsui et al. 2000) as favored substrates and pathways through the grid have been elucidated (Humphreys et al. 1999a, 1999b; Li et al. 2000; Osakabe et al. 1999), and new steps and their enzymes continue to be discovered, as reviewed (Bonawitz and Chapple 2010; Mottiar et al. 2016; Ralph et al. 2019; Vanholme et al. 2010a, 2019a). Figure 1.1 attempts to capture the modern notion of broader lignification, integrating in phenolic components beyond the strict monolignol pathway. Perturbing the various genes

along the pathway from phenylalanine to the monolignols, or even from shikimic acid and further back, not only provides a rich source of insight into the pathway processes but is also capable of producing some striking lignins. With reference to the line in Michael Chrichton's original Jurassic Park book (Chrichton 1990) and in the movie that "Life will find a way," plants do not simply give up and die because they find themselves unable to synthesize a monolignol. Although the very notion was once considered heresy, plants can survive, even from "instantaneous" perturbations from which they do not have the luxury of evolving, by producing a functional polymer from other available phenolic components. This is most readily evident in plants utilizing "products of truncated monolignol biosynthesis," a term we might have first introduced in print in 2003 (Boerjan et al. 2003), to produce the lignin polymer, sometimes quite successfully, but it is also clear that nature itself has, over time, explored options well beyond just utilizing pathway intermediates and still has plenty of surprises for us. The following recent examples are illustrative but are neither unique nor exhaustive.

1.2.1 Truncated Monolignol Biosynthesis

We will update just two examples here, those of plants deficient in the last enzyme of the pathway, cinnamyl alcohol dehydrogenase (CAD), and in one of the two primary *O*-methyltransferases (OMT, i.e. CCoAOMT or COMT), Figures 1.1–1.3. The result in both cases is product monomers of incomplete monolignol biosynthesis, hydroxycinnamaldehydes in the former case, and the catecholic monomers caffeoyl alcohol or 5-hydroxyconiferyl alcohol in the latter. Both produce lignin polymers that function satisfactorily in the plant and, at least in the case of the catechols, are used as significant or even sole components to fabricate natural lignins in specialized tissues such as seedcoats.

1.2.1.1 CAD Deficiency

Lignins have long been known to contain low levels of hydroxycinnamaldehyde (and hydroxybenzaldehyde) units. The characteristic lignin stain, the Wiesner or phloroglucinol stain, is somewhat specific for hydroxycinnamaldehyde endgroups (Adler et al. 1948; Pomar et al. 2002); it has always been ironic that this common stain owes its utility to the incorporation of low levels of components that are often not even acknowledged as being involved in lignification. Cinnamaldehyde endgroups might be produced in the lignin in one of three ways. First, they could result from the oxidation of cinnamyl alcohol endgroups in lignin, themselves resulting from initial dimerization reactions of monolignols, particularly coniferyl alcohol. The conundrum here is that it is quite difficult to oxidize etherified hydroxycinnamyl alcohols, the decades that the lignin might remain in a tree notwithstanding. A second is for coniferaldehyde or sinapaldehyde to be produced by oxidizing the monolignols in the lignifying region of the cell wall by the action of H_2O_2 , for example – such oxidation with H_2O_2 can be demonstrated, and "always" accompanies synthetic lignin preparations in which monolignols are mixed with peroxidase and H_2O_2 (Kim et al. 2003; Zhao et al. 2013). These hydroxycinnamaldehyde monomers can then

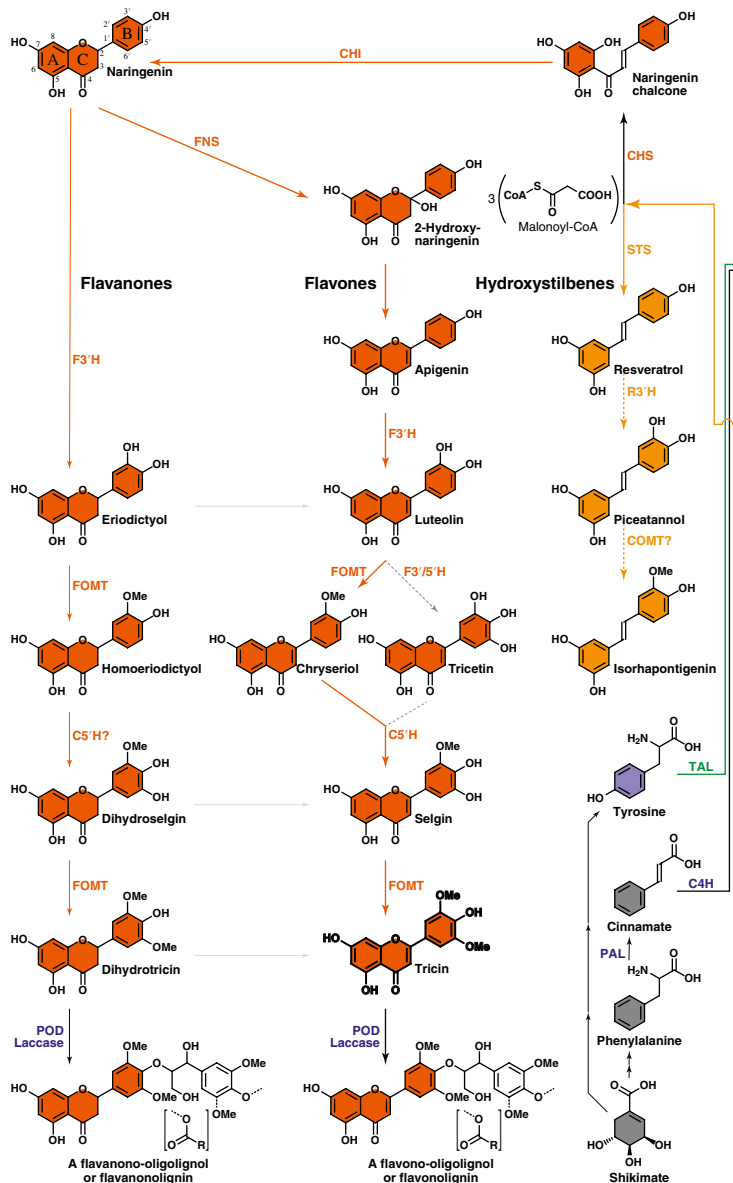


Figure 1.1 Biosynthetic pathways feeding into lignification. The primary biosynthetic pathway leading to the monolignols and the two related hydroxycinnamyl alcohols, bolded and highlighted in yellow (in the colored version of this figure), is to the right and is shown as a metabolic grid as in the old days (Dixon et al. 2001; Higuchi 2006; Matsui et al. 2000); unfortunately, since modifications to the pathway flux have been discovered, it is not possible to order the intermediates in a linear fashion vertically – conversions jump rows in a way that is simply not pleasing (and, were this a circuit-board, would represent bad design but seems unavoidable here). Where possible, the primary pathways are shown with black arrows, and the major pathways are slightly bolder, whereas minor pathways, or those that might not be completely demonstrated are in a light gray; dashed arrows represent pathways (and genes/enzymes) that are not yet known. Note that we do not try to show the pathway from *p*-hydroxybenzoate, via its CoA thioester