

Recent Advances in Polyphenol Research

Volume 6

Edited by

Heidi Halbwirth

Karl Stich

Véronique Cheynier

Stéphane Quideau



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Recent Advances in Polyphenol Research

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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.

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Volume 6

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This sixth volume of *Recent Advances in Polyphenol Research* is dedicated to the memory of Ragai Ibrahim, Emeritus Professor of Biology at the University of Concordia, Montreal, Canada, who passed away on 19th November 2017, aged 88. Dr Ibrahim was an active member of Groupe Polyphénols since 1980, the organizer of the XIVth International Conference on Polyphenols in St Catharines, Ontario, Canada in 1988, and a role model for many scientists in the field, both within Groupe Polyphenols and beyond. He was internationally renowned for his cutting-edge research on the structure and biosynthetic pathways of flavonoids. His research group has been instrumental in the description of sulfated and prenylated flavonoid derivatives, the discovery of novel enzymes involved in their biosynthesis, and the study of their distribution and role in plants. His generous donation made possible the Ragai Ibrahim Prize, which has been since 2012, awarded every two years to an active graduate student or postdoctoral fellow who has co-authored during his/her doctoral studies a particularly relevant original scientific article.

Further, the editors also wish to memorialize in this volume the life and work of Takua Okuda, Professor of Pharmacognosy and Phytochemistry at the Okayama University, Japan, who sadly passed away on 31st December 2016, aged 89. Professor Okuda was a world-renowned expert in the structural characterization of bioactive plant polyphenols, in particular the most structurally complex polyphenols of the ellagitannin classes. The contributions of his research team over several decades have constituted major milestones in the acquisition of sound knowledge on these unique and fascinating natural products. Among his many awards and recognitions are the 2004 Tannin Award and the 2014 Groupe Polyphénols Medal.

Finally, the editors would like this volume to serve in remembrance of Werner Heller, who passed away on 18th March 2018, aged 72. Werner Heller was a key researcher in the plant biochemistry laboratory of Professor Grisebach at the University of Freiburg, Germany, and as such contributed significantly to the elucidation of many key reactions of the flavonoid pathway. He was internationally recognized for his series of reviews on the advances in research into flavonoid biosynthesis, which he wrote together with Gert Forkmann, and for his studies on the effects of UV-B radiation on secondary metabolites in plants.

In memoriam

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Preface

Polyphenols are secondary metabolites that are widely distributed in the plant kingdom and characterized by a large diversity of chemical structures. As supported by the international academic society Groupe Polyphénols, which organizes the biennial International Conference on Polyphenols (ICP), the term *polyphenol* should be exclusively used for plant secondary metabolites derived from the phenylpropanoid and/or polyketide pathway(s), featuring more than one phenolic ring and being devoid of any nitrogen-based functional group (www.groupepolyphenols.com/the-society/why-bother-with-polyphenols). Several thousand structures have been isolated and characterized from plants so far, ranging from quite simple phenolic molecules to highly polymerized compounds with molecular weights of more than 30 000 Da. As a result of the huge diversity of structures, polyphenols possess diverse physicochemical properties. Over the years, scientists from all over the world have been fascinated by these molecules, trying to shed light on their chemistry, properties and physiological relevance in plants, humans and ecosystems. In addition, there is increasing interest in the valorization of polyphenols obtained as natural by-products from, for example, the lignocellulose industry or agroindustrial waste streams for use as bioactive substances in dietary supplements and functional food, additives in food and cosmetic products to mediate antioxidant activity, natural coloration or flavours, and as raw materials for emerging products such as multifunctional polymer coatings or antibacterial packaging.

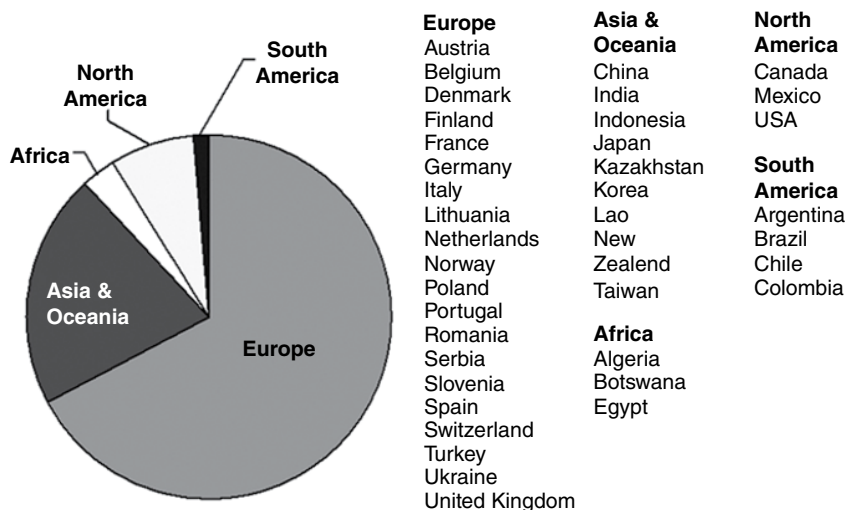
The book series *Recent Advances in Polyphenol Research* started in 2008 upon the occasion of the 24th ICP in Salamanca, Spain. The content of the first volume was mostly based on review articles written by plenary lecturers of the previous ICP, which had taken place in Winnipeg, Canada. Since then, this flagship publication of the Groupe Polyphénols has been released every two years to provide the reader with authoritative updates on various topics of polyphenol research written by ICP plenary lecturers and invited expert contributors.

This sixth volume of the series presents chapters representing a distillation of the topics covered during the 28th ICP, which was organized and hosted by the Technische Universität Wien in July 2016 in Vienna, Austria. This beautiful setting is represented on the cover by a photo of the dome of the stunning Art Nouveau church by Otto Wagner in Vienna. Participants were given a chance to visit this church in person during one of the social events organized during the conference.

Five main topics of the polyphenol sciences were selected for the scientific programme of this memorable ICP 2016 edition.

- **Chemistry and Physicochemistry**, covering structures, reactivity, organic synthesis, molecular modelling, fundamental aspects, chemical analysis, spectroscopy, molecular associations, and interactions of polyphenols.
- **Biosynthesis, Genetics and Metabolic Engineering**, covering molecular biology, genetics, enzymology, gene expression and regulation, trafficking, biotechnology, horticultural science, and molecular breeding related to polyphenols.
- **Roles in Plants and Ecosystems**, covering plant growth and development, biotic and abiotic stress, resistance, ecophysiology, sustainable development, valorization, plant environmental system, forest chemistry, and lignin and lignan.
- **Food, Nutrition and Health**, covering food ingredients, nutrient components, functional food, mode of action, bio-availability and metabolism, food processing, influence on food and beverage properties, cosmetics, antioxidant activity of polyphenols.
- **Applied Polyphenolics**, covering new findings on sources of isolated and standardized polyphenolic fractions and novel epigenetic polyphenol mechanisms, as well as industrial implementations of newly gleaned knowledge on polyphenols.

The 13 chapters of this volume highlight advances in our understanding of (i) polyphenol biosynthesis with a focus on (sub)cellular distribution and organization of the pathways, novel genes and transcription factors, (ii) bioactive and dietary compounds with a focus on health and taste, (iii) innovative sources of polyphenol compounds and their characterization and (iv) emerging products such as thermosetting polymers.

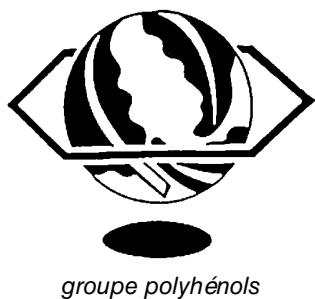


The conference was attended by 272 scientists from 40 countries, with 209 paper contributions, comprising 55 oral communications and 154 poster presentations.

The sixth volume of *Recent Advances in Polyphenol Research* contains chapters from 13 invited conference speakers and expert contributors. The support and assistance of the Groupe Polyphénols, the BachBERRY group, several Austrian academic associations and foundations, notably the Technische Universität Wien, the City of Vienna and

the Vienna Convention Bureau, and several private sponsors are gratefully acknowledged, as the great success of the 28th International Conference on Polyphenols would not have been possible without their contributions. As a final note, the editors would also like to deeply thank all of the plenary, communication and poster presenters for the quality of their contributions, from basic science to more applied fields, and all of the attendees.

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The Lignans

A Family of Biologically Active Polyphenolic Secondary Metabolites

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1.1 Introduction

Nature has long served as an important source of therapeutics, and lignans represent a large class of pharmacologically active compounds (Cunha et al. 2012). This family of molecules demonstrates a wide range of biological activities, which plants use as a front-line chemical defence against pathogens (Figure 1.1). Additionally, the anticancer, antimiotic, antiangiogenesis and antiviral properties possessed by lignans have made them appealing drug candidates, as well as starting points for drug discovery. Lignans currently employed for healthcare include (–)-podophyllotoxin (**1**), a treatment for warts, and its derivatives (–)-etoposide (**2**) and (–)-teniposide (**3**), two potent chemotherapeutic agents (Liu et al. 2007). Other members of this class with promising biological activities include (+)-gomisin J (**4**) and (+)-pinoresinol (**5**). Due to the established benefits of the lignans, both their biosynthesis and synthetic strategies to access them have been areas of extensive research.

In addition to their varied biological activities, lignans comprise a vast array of structurally distinct skeletons (Figure 1.2), including 6- and 8-membered carbocycles (**6**, **7**), linear dibenzylbutanes (**8**), and diversely oxidized tetrahydrofurans (**9–11**). Remarkably, their biosynthesis originates from a regio- and stereoselective, oxidative coupling of relatively simple monolignols (propenyl phenols) (**12**), to form the key 8–8 bond that serves to characterize all lignan natural products. Subsequent transformations, including cyclization and oxidation of the parent scaffold, convert the initially formed dimer to various family members, imparting unique functionalities. While this blueprint has served as a key source of inspiration for decades of biomimetic synthetic approaches to the lignans, issues of selectivity in the oxidative coupling have led researchers to alternative, target-oriented routes, which are often specific for an individual structural class. In this review, we summarize these recent efforts from 2009 to 2016, and provide an overview of contemporary research efforts interrogating the lignans. Previous

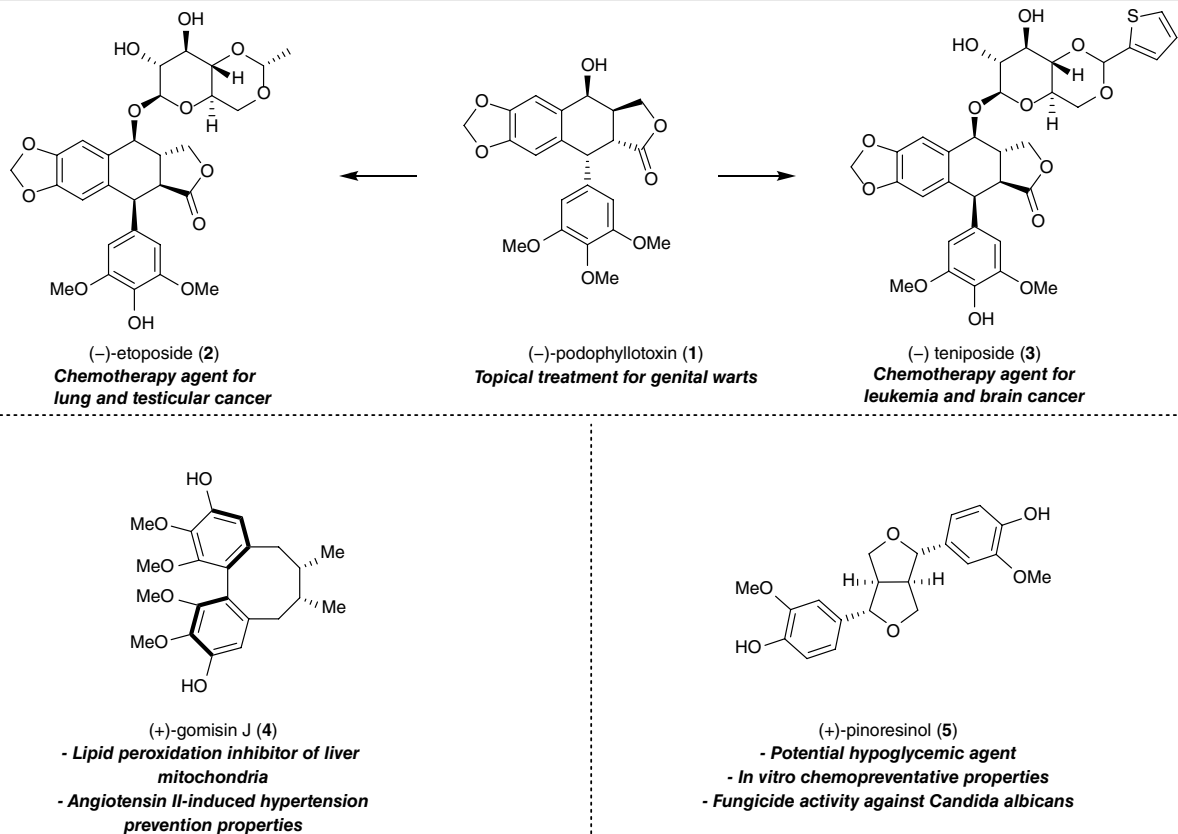


Figure 1.1 Selected biologically active lignan natural products.

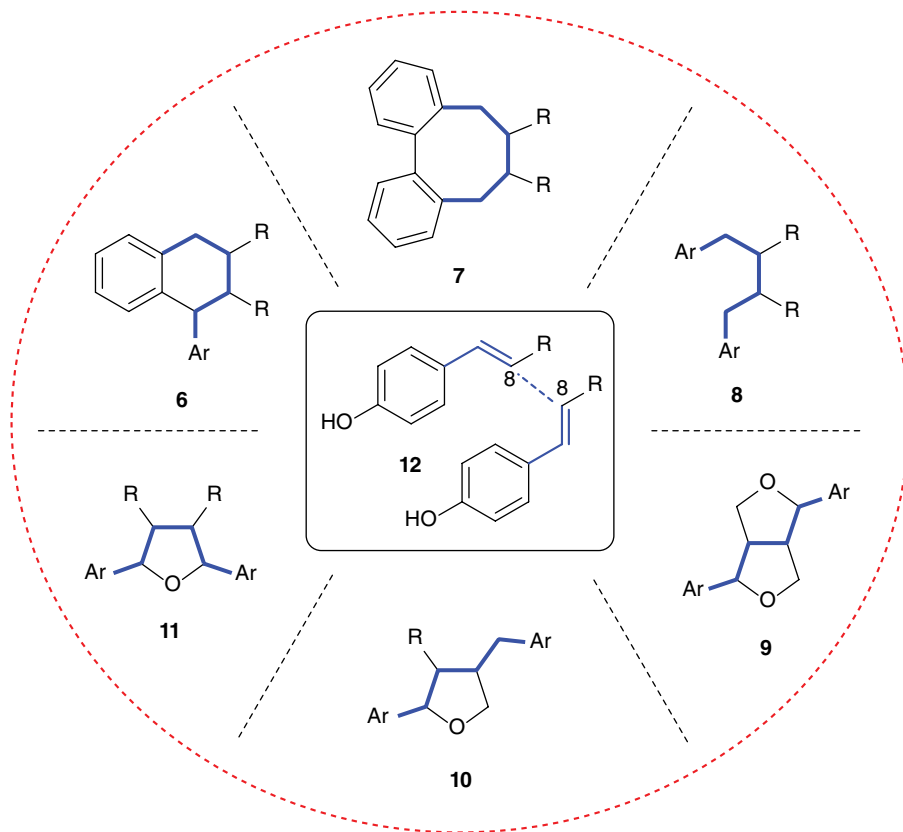
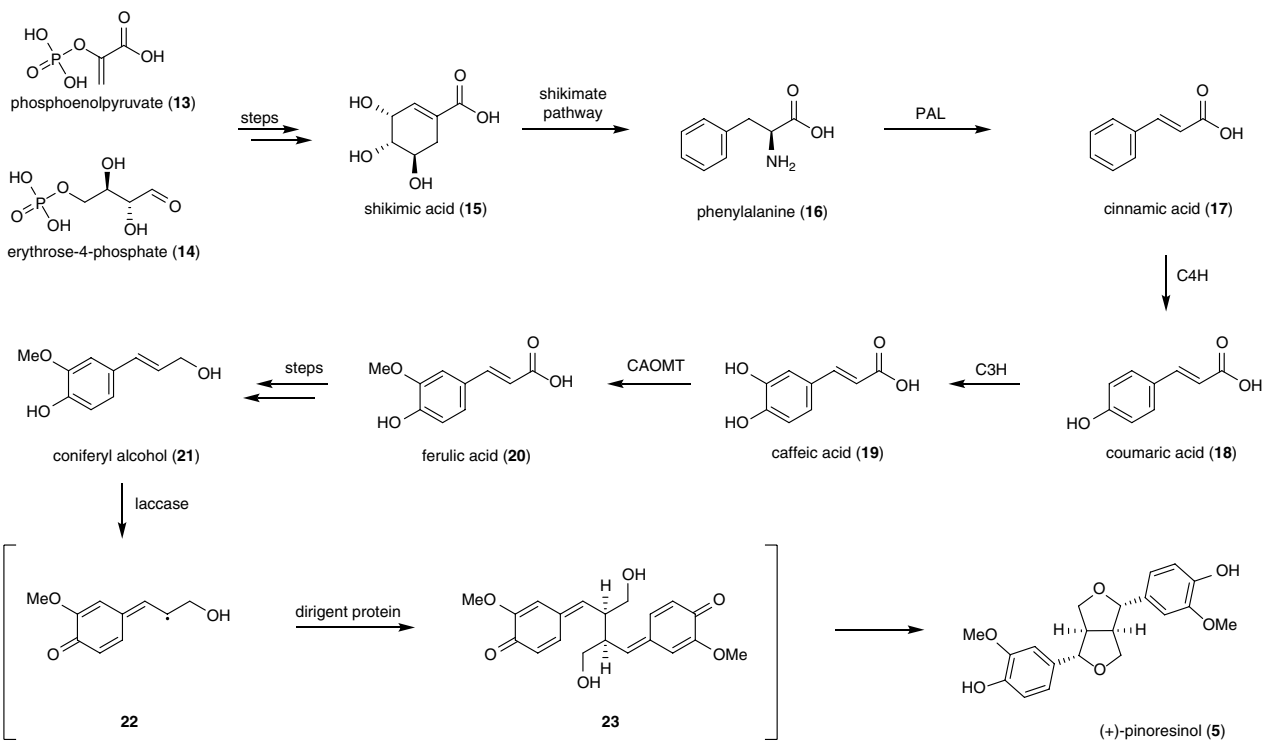


Figure 1.2 Structural classes of lignans.

reviews on this subject cover 2000–2004 (Saleem et al. 2005), 2005–2008 (Pan et al. 2009), and 2009–2015 (Teponno et al. 2016).

1.2 Biosynthesis of Lignans

Due to their biological activity and fundamental importance to plant biology, significant efforts have been made to elucidate lignan biosynthesis (Suzuki and Umezawa 2007; Umezawa 2009; Petersen et al. 2010). Lignans originate from cinnamic acids, which are themselves biosynthesized from phenylalanine (Scheme 1.1). The shikimate pathway, which produces several aromatic amino acids including phenylalanine (**16**), is preceded by the synthesis of shikimic acid (**15**) from phosphoenolpyruvate (**13**) and erythrose-4-phosphate (**14**). The conversion of phenylalanine to cinnamic acid (**17**) is carried out by phenylalanine ammonia-lyase (PAL). Substitution of the aromatic ring is performed by cinnamate hydroxylases (C4H and C3H), to access coumaric acid (**18**) and caffeic acid (**19**). The methyl ether found in ferulic acid (**20**) is installed by caffeic acid *O*-methyltransferase (CAOMT). Several additional steps convert the carboxylic acid to the primary alcohol, affording coniferyl alcohol (**21**). This propenyl phenol undergoes



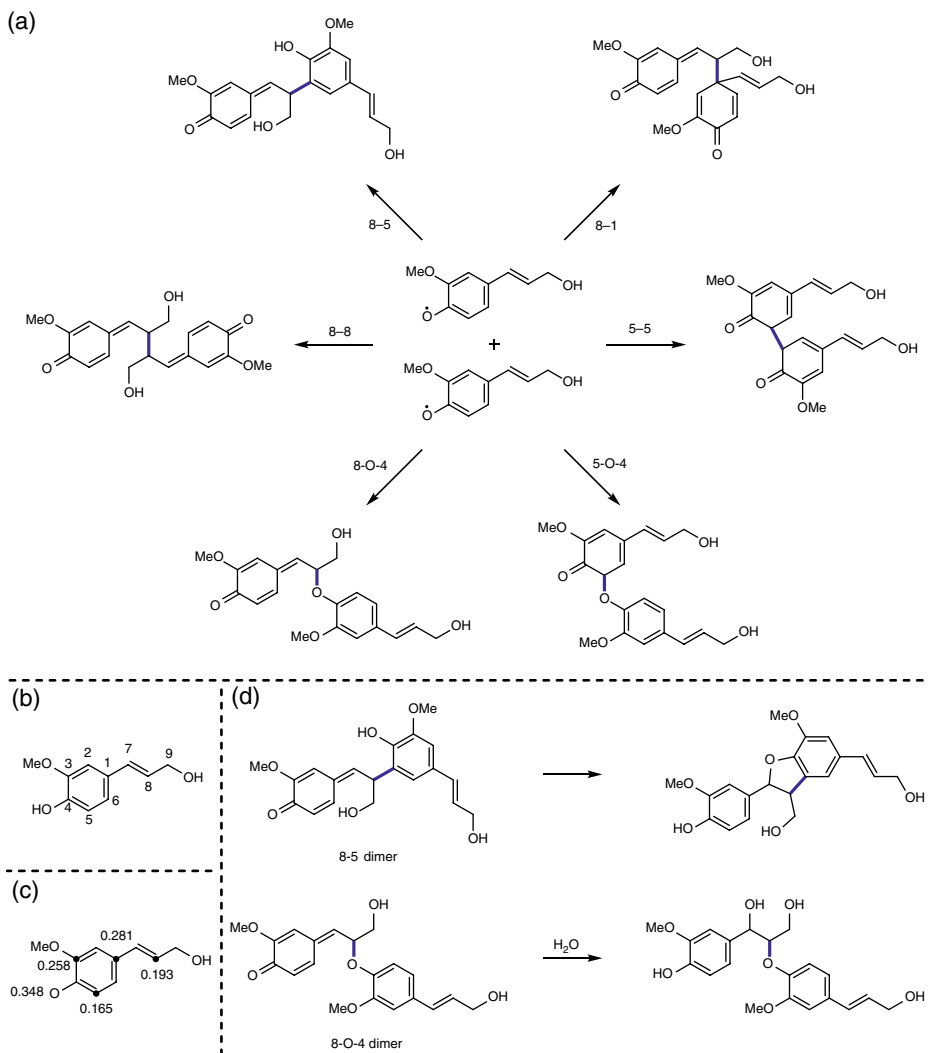
Scheme 1.1 Biosynthesis of (+)-pinoresinol.

an oxidative coupling, the first step in the biosynthesis of pinoresinol (**5**). The oxidative coupling has been extensively investigated (Hapiot et al. 1994; Gavin and Huai-Bing 1997; Halls et al. 2004; Pickel et al. 2010), and involves a unique mechanism, starting with a one-electron oxidation of the phenol, believed to be carried out by a laccase. Two phenoxyl radicals (**22**) are then proposed to combine in the presence of a dirigent protein to form a bis-*para*-quinone methide (**23**), which undergoes subsequent cyclization to provide the furofuran **5**.

Several dirigent proteins have been isolated, including those that are selective for either enantiomer of pinoresinol. They display a unique ability to control the regio- and stereoselectivity of phenoxyl C–C coupling, despite not having any oxidative activity themselves. This has led to a biosynthetic proposal that requires an exogenous oxidant, followed by diffusion of the phenoxyl radicals into the dirigent protein's active site. In their absence, the oxidative coupling of coniferyl alcohol leads to a complex mixture (Scheme 1.2), from which pinoresinol is isolated in only trace quantities. The first crystal structure of such proteins was obtained from a pea plant, *Pisum sativum* (Figure 1.3), affording (+)-pinoresinol (Kim et al. 2015). While it was not co-crystallized with the substrate, several aspects of the protein are consistent with the proposed biosynthesis. A trimer structure was determined, which was observed to have six conserved residues in the proposed active site with other proteins that produce (+)-pinoresinol. These include arginine and aspartic acid residues that are on opposite sides of the pocket but are sufficiently close to co-ordinate to the phenolic and primary hydroxylic oxygens of the oxidized substrate. However, since several loops surrounding the potential binding cavity were not resolved in the structure, alternative modes of substrate binding and coupling could not be confirmed.

While the exact mechanistic steps involved in the dimerization have not been conclusively determined, it is now accepted that the dirigent protein is critical for controlling selectivity during the oxidative coupling. This is readily apparent from numerous studies on the free radical coupling of monolignols (Table 1.1). In the presence of various oxidants, coniferyl alcohol rarely forms pinoresinol but instead affords dimers arising from radical coupling at carbon 8 with carbon 5 and oxygen 4 (Scheme 1.2a and b), along with extensive polymerization and decomposition. Attempts at directly mimicking the biosynthetic pathway by employing laccases (Wan et al. 2007; Lu and Miyakoshi 2012) (Table 1.1, entries 1–4) and peroxidases (Chioccare et al. 1993; Mitsuhashi et al. 2008; Matsutomo et al. 2013) (entries 5–7) afford mixtures that vary significantly depending on the specific enzyme used, as well as the method of isolation and purification of the oxidase. Due to the sensitivity of the enzymes, temperature and pH play a large role in the product distribution. More traditional synthetic oxidants, such as peroxides (Dellagreca et al. 2008) (entry 8) and metal salts (Brežný and Alföldi 1982; Vermes et al. 1991; Kasahara et al. 2006; Lancefield and Westwood 2015) (entries 9–12), have been utilized and suffer from similar challenges with regioselectivity and decomposition.

These issues of selectivity result from delocalization of the phenoxyl radical, which places partial spin density at carbons 1, 3, 5, 8 and oxygen 4 (Scheme 1.2c) (Sangha et al. 2012). Although the calculated spin density at carbons 1 and 3 is higher than at other carbons, steric factors and the inability to restore aromaticity make coupling at these positions unlikely. Calculated enthalpic values show that 8–O–4, 8–8, and 8–5 dimers are 5–20 kcal mol⁻¹ more stable than the 5–O–4, 5–5, and 8–1 dimers. The 8–5 and 8–O–4 linkages allow for rearomatization by nucleophilic attack of the *para*-quinone methide (Scheme 1.2d). Intramolecular cyclization by the phenol in the 8–5 dimer and an external nucleophilic attack on the 8–O–4 dimer provide the core structures of the



Scheme 1.2 (a) Main coupling pathways for oxidative coupling of coniferyl alcohol. (b) Atom labelling of coniferyl alcohol. (c) Calculated spin density for atoms contributing most to coniferyl radical. (d) Conversion of radical-coupled products to neolignans.

neolignan class of molecules. The 8–O–4 linkage is the most thermodynamically favourable, which is consistent with experimental studies. Additionally, this coupling is the predominant interunit linkage observed in lignin, the plant polymer synthesized from the oxidation of monolignols. The ability of plants to form other linkages in both the polymer and the lignans is thus likely to result from factors controlling the orientation of the radicals during coupling. Without the dirigent protein to position the phenoxyl radicals appropriately, controlling selectivity remains a significant challenge.

The C–C linkage adjoining two units of coniferyl alcohol is conserved in all the lignan natural products, with subsequent transformations of this core structure leading to downstream derivatives (Scheme 1.3). These steps have been carefully studied for the