Journal of Industrial Microbiology (1995) 15, 457–471

Review: Biocatalytic transformations of ferulic acid: an abundant aromatic natural product

JPN Rosazza, Z Huang, L Dostal, T Volm and B Rousseau

Division of Medicinal and Natural Products Chemistry, and The Center for Biocatalysis and Bioprocessing, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242-5000, USA

In this review we examine the fascinating array of microbial and enzymatic transformations of ferulic acid. Ferulic acid is an extremely abundant, preformed phenolic aromatic chemical found widely in nature. Ferulic acid is viewed as a commodity scale, renewable chemical feedstock for biocatalytic conversion to other useful aromatic chemicals. Most attention is focused on bioconversions of ferulic acid itself. Topics covered include cinnamoyl side-chain cleavage; nonoxidative decarboxylation; mechanistic details of styrene formation; purification and characterization of ferulic acid decarboxylase; conversion of ferulic acid to vanillin; *O*-demethylation; and reduction reactions. Biotransformations of vinylguaiacol are discussed, and selected biotransformations of vanillic acid including oxidative and nonoxidative decarboxylation are surveyed. Finally, enzymatic oxidative dimerization and polymerization reactions are reviewed.

Keywords: ferulic acid; biocatalysis; microbial and enzymatic biotransformations

Introduction

Biotechnology has been defined as *'The application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services'* [19]. Biocatalysis is a more focused term defining the use of enzymes or of living catalysts such as whole microbial cells for the production of useful chemicals. As in chemical processing, the biocatalyst initiates or accelerates a reaction between two or more substances while the catalyst itself remains unchanged and is recycled. Biocatalysis encompasses the field of microbial transformations of organic or inorganic compounds which result in a change in chemical structure. Useful biocatalytic reactions yield products (often called metabolites) which accumulate in the incubation medium. Biocatalytic processes are now used for the preparation of very valuable specialty chemicals for the pharmaceutical, agricultural, flavor and fragrance, nutritional and chemical industries. Such processes are also exploited for the preparation of commodity chemical substances on an enormous industrial scale.

In this review, we attempt to describe biocatalytic technologies and how they can be used with an extremely abundant, naturally occurring phenolic compound known as ferulic acid. We review the history, isolation and physical properties of ferulic acid and related phenolic cinnamic acids. The widespread occurrences and functions of ferulic acid in nature are described along with several emerging fields concerned with the antioxidant and putative chemopreventative properties of this family of compounds. The development of needed biocatalytic approaches toward the production of useful aromatic/phenolic chemicals from ferulic acid is also emphasized. The aim of producing valuable chemical products from ferulic acid is coupled with a description of what is known about biotransformation and biodegradation pathways of ferulic acid. Finally, we emphasize detailed efforts from our own laboratories devoted to unraveling mechanistic details associated with the biocatalysis of ferulic acid and its products.

Correspondence: JPN Rosazza, Division of Medicinal and Natural Products Chemistry, College of Pharmacy, University of Iowa, Iowa City, Iowa 52242-5000, USA
Received 3 July 1995; accepted 22 August 1995

Ferulic acid

Ferulic acid (**1**, in Figure 1), 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid is an extremely abundant and nearly ubiquitous phenolic cinnamic acid derivative. It was described in 1866 as a component of *Ferula foetida* Reg. (*Umbelliferae*) and various salts of the compound were prepared [129]. The name ferulic acid obviously derives from the botanical name of the plant from which it was obtained. In nature, ferulic acid is thought to arise biosynthetically in plants via cinnamic acid [54], which finds its origin in the shikimic acid pathway [35]. Since its discovery, numerous reports on the occurrence, chemistry, synthesis, spectral analysis, possible uses and biological properties have appeared in the literature. The structures of ferulic acid and related compounds have been confirmed by their synthesis, by ^{13}C-NMR spectral analysis [77,87] and by X-ray analysis [98]. Chemical and biological properties of ferulic acid were recently summarized in an exhaustive review by Graf [47].

The structure of ferulic acid (**1**) is shown in Figure 1 along with the structures of caffeic acid (**3a**) and isoferulic acid (**3b**) which also are abundant in nature. The unsaturated side-chain of ferulic acid can exist naturally in either *trans* or *cis* conformational structures such as **1** or **2**, where hydrogens at the adjacent 7- and 8-positions are either on opposite or the same sides of the double bond [2]. The double bond is subject to *trans-cis* isomerization [41]. Chlorogenic acid (**4**) is also shown in Figure 1 to illustrate

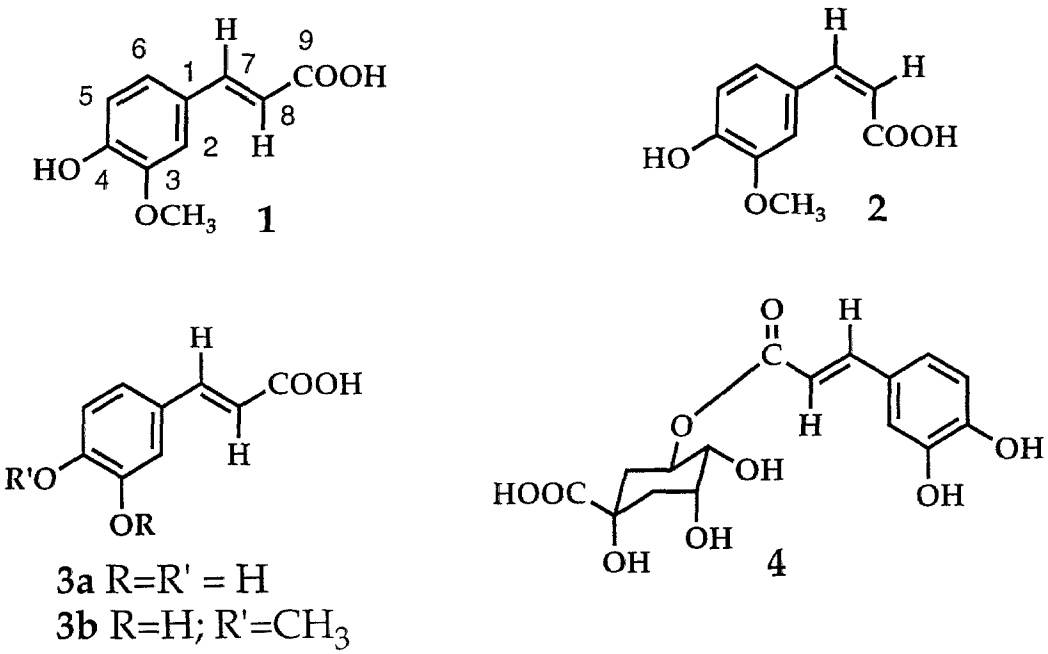

Figure 1 The structures of *trans*-ferulic acid (**1**), *cis*-ferulic acid (**2**), caffeic and isoferulic acids (**3a**, **3b**) and chlorogenic acid (**4**)

the fact that cinnamates may occur free in nature, they may be esterified as with **4**, or esterified to hydroxyl groups of polymeric backbones found within the plants that contain them.

In plants, ferulic acid is rarely found in free form [118]. It occurs widely linked to various carbohydrates as glycosidic conjugates, and it occurs as various esters and amides with a wide variety of natural products. As esters, ferulic acid is linked to polysaccharides where it may occur in monomeric or dimeric form [3,46,47]. It also occurs esterified with flavonoids and with various hydroxycarboxylic acids including malic, tartaric, tartronic, hydroxycitric, quinic, gluconic and hydroxy fatty acids [64,65]. Relatively nonpolar ferulate esters occur with long chain alcohols, and with various plant sterols [47]. Ferulic acid is also found esterified with a tetrahydroisoquinoline-monoterpene glucoside [72], a cyanogenetic glycoside [1,127] and an amino-hydroxycyclopentenone [36]. Levels of ferulic acid amides with putrescine, tyramine or tryptamine may be indicators of environmental stress in plants [90]. Amides of ferulic acid with amino acids or dipeptides are used as preservatives in baking [47]. Corn contains feruloyl tryptamide [37], and ferulic acid esters linked to a polysaccharide [102] and to dihydroxy β-sitosterol [106].

Ferulic acid occurs commonly in fruits, vegetables, grains, beans, leaves, seeds, nuts, grasses, flowers, and other types of vegetation [47,64]. It is abundant in some plants, varying from 1 g kg^{-1} in corn kernels [102,132] to 0.05 g kg^{-1} in refined wheat and 0.5 g kg^{-1} in whole wheat flour [47] to 1.5 g kg^{-1} in the spice turmeric [64]. Ferulic acid can be isolated in nearly 2% yield (wt/wt) from the hulls of corn kernels obtained from wet milling [10].

Ferulic acid and its derivatives are well recognized as members of a class of phenolic natural antioxidants [47], and many natural and presumed functions and potential uses of these compounds can be attributed to this property. The roles of ferulic acid in the plants that contain it and in human nutrition and well-being continue to be elaborated [47]. In plants, alleopathic regulation and protection mechanisms are related to ferulic acid content. Ferulic acid endows structural rigidity and strengthens cell wall architecture by cross-linking pentosan chains, arabinoxylans and hemicelluloses, rendering these components less susceptible to hydrolytic enzymes during germination [47,122]. Considerable evidence suggests that ferulic acid is involved in regulating plant growth. In roots, ferulic acid appears to inhibit growth of competing plants. Ferulic acid exhibits a detrimental effect on seedling growth, plant dry weight and leaf expansion [18,125]. High levels of ferulic acid appear to inhibit root growth and the development of root system architecture [16]. High levels of ferulic acid appear to be inversely related to mineral and water uptake [88], water utilization [18], hyphal elongation [130], and hydrolytic enzyme activities of germinating seeds [34]. Ferulic acid and its derivatives confer protection to plants against avian [52], insect [30,86,119], viral [90] and fungal [107] invasion. Increased levels of ferulylamides are found in plants acclimated to herbicide treatment and nutritional depletion [90]. The UV-absorbing properties of ferulic acid confer additional protection to plants.

Microbial transformations of ferulic acid

Microbial and/or enzymatic transformations of ferulic acid and related phenolic cinnamates have been examined from several interesting perspectives [111]. Ferulic acid and its microbial metabolites appear to play roles in plant germination and plant growth [126]. These compounds influence the makeup of microfloral consortia in soil by inhibiting the growth of certain microorganisms while stimulating others [116]. Styrene metabolites from hydroxycinnamates like ferulic acid confer pest-resistance in some plants [61]. Hydroxystyrenes and other aromatic microbial metabolites

of ferulic acid are also important flavor constituents in beer, wine, soy sauce, and fruit juices [12,26,117]. The rumen metabolism of ferulic acid in cows [101], and the possible involvement of antioxidants like ferulic acid and its metabolites in mammalian toxicology are of growing interest [114]. Ferulic acid also has been used as a 'lignin phenolic substrate to model microbial degradations of aromatic compounds from chemical and petroleum industrial wastes [51,67,78]. The great abundance of ferulic acid in nature has also prompted several investigations into the potential for using ferulic acid as a starting material for microbial or enzymatic synthesis of useful aromatic chemical compounds [33,69]. Conversions of ferulic acid to commercially valuable aromatic aldehydes like vanillin have been described [84,85].

Numerous studies on aerobic or anaerobic biotransformations of ferulic acid have been reported [5–8,25,38, 42,48,53,55,63,71,73,75,76,91,92,94,96,97,99,101,103,108, 115,117,120,123,124,126]. Major pathways of biotransformation by bacteria, fungi and yeasts are summarized in Figure 2, and in Table 1. The major pathways of metabolism can be summarized as: a) reductive, forming saturated side chains, aldehydes and alcohols such as **6**, **12** and **13**; b) cleavage of acetate from the unsaturated side-chain flowing through vanillin (**5**), a major branch point for the formation of other oxidative and reductive products; c) oxidation of vanillin to vanillic acid (**7**), a central metabolic product yielding guaiacol (**9**) by decarboxylation, protocatechuic acid (**10**) by *O*-dealkylation, and methoxyhydroquinone (**8**) by oxidative decarboxylation; d) other *O*-dealkylation reactions giving caffeic acid (**3a**) directly from ferulic acid; and e) as perhaps the oldest recorded reaction, nonoxidative decarboxylation to form styrenes such as vinylguaiacol (**14**). Further oxidative degradation of the aromatic ring of ferulic acid or its metabolites usually occurs through protocatechuic acid (**10**). Features of the major types of ferulic acid biotransformation reactions are summarized below.

1. Elimination of two carbons from the cinnamoyl side-chain: Elimination of an acetate moiety from the unsaturated cinnamate side-chain is one of the most common pathways of ferulic acid metabolism in bacteria, yeasts and fungi. Different interpretations of the mechanism by which side-chain cleavage gives rise to biodegradation intermediates have been postulated, but relatively limited work has been done with the enzymes catalyzing this transformation reaction. A variety of pseudomonads catalyze the oxidation of **1** to vanillic (**7**) and protocatechuic (**10**) acids [113,124].

Toms and Woods [124] reported that *P. acidovorans* converted both *cis*- and *trans*-ferulic acid to vanillin (**5**) and

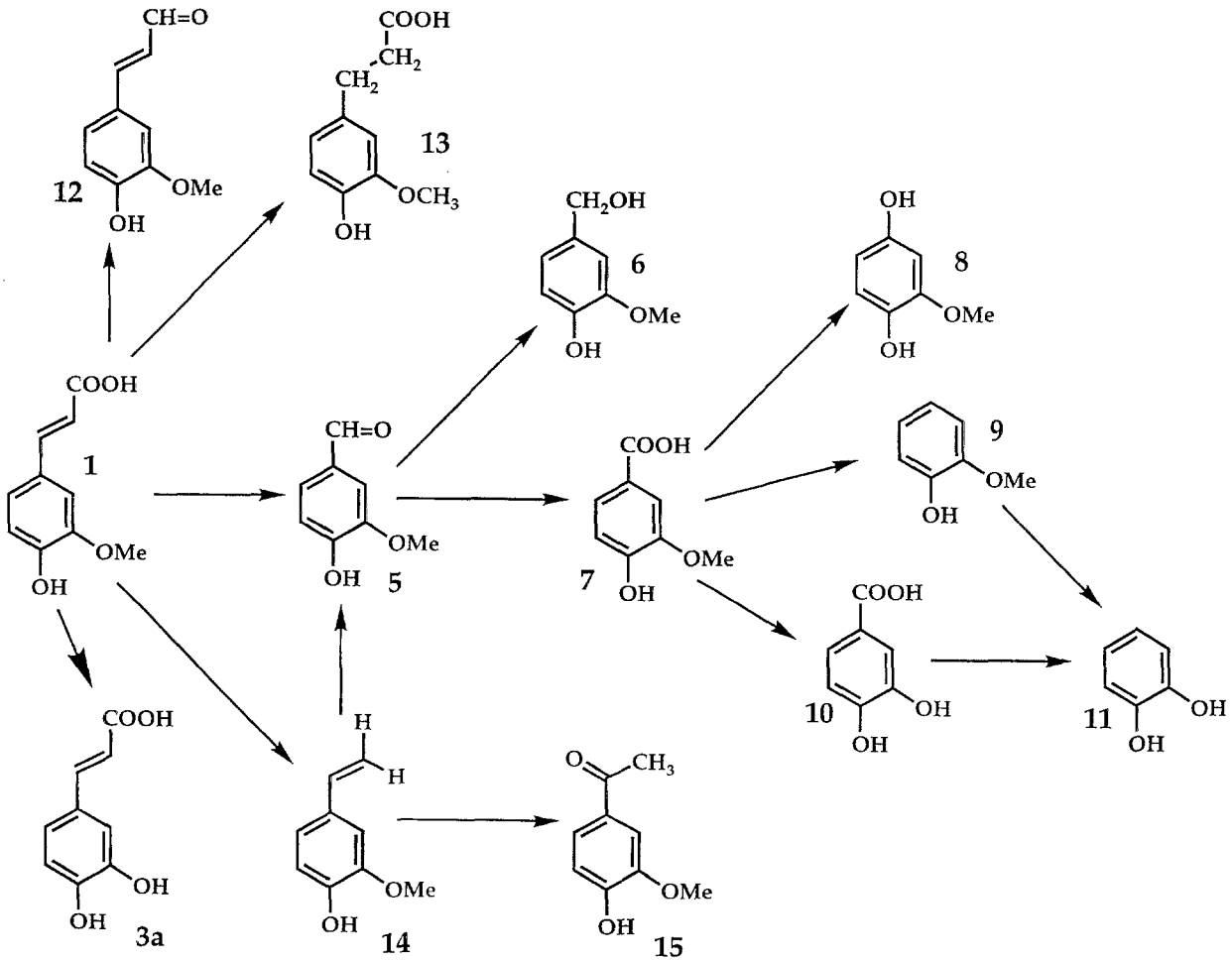

Figure 2 Major pathways of microbial transformation of ferulic acid

Table 1 A summary of ferulic acid metabolites formed by bacteria, fungi and yeasts

Organism	Compound no, shown in Figure 2:											Ring fission	Ref
	3a	5	6	7	8	9	10	11	12	13	14		
Aerobacter sp											+		[42]
Bacillus megaterium											+		[68,69]
B. pumulis (3)[a]											+		[12,33]
B. subtilis		+		+			+					+	[56]
B. subtilis											+		[68,69]
Corynebacterium glutamicum		+		+						+	+		[84,85]
Enterobacter DG-6	+						+					+	[48]
Erwinia uredovora											+	(caffeic acid)	[61]
Escherichia coli		+		+			+						[103]
Klebsiella oxytoca											+	(caffeic acid)	[61]
Margarinomyces mutabilis				+			+[b]					+[b]	[63]
M. heteromorph				+			+[b]					+[b]	[63]
Myobacterium sp											+		[66,69]
Nocardia sp											+		[68,69]
Pseudomonas acidovorans		+		+			+						[124]
P. cepacia										+	+	+	[8]
P. mira		+[b]		+									[75]
P. putida		+											[84]
P. putida Type A											+		[68,69]
P. sp TMY1009				+			+				+	+	[113]
Streptomyces rimosus											+		[68,69]
S. setonii		+		+			+						[120]
Wolinella succinogenes				+						+			[101]
Anaerobes from marsh sediment	+							+					[9]
Anaerobic microbial consortium										+			[96]
Methanogenic consortium	+											+	[51]
Rat gastrointestinal microflora										+	+		[114]
Unidentified bacterium				+									[73]
Aspergillus niger											+		[66,68,69]
A. niger		+											[84]
A. carneus											+		[12]
A. ochraceus (2)[a]											+		[12]
A. terreus (2)[a]											+		[12]
Candida intermedia											+		[12]
Corynespora cassiicola											+		[12]
Curvularia affinis											+		[12]
C. clavata											+		[12]
C. lunata (5)[a]											+		[12,68,69]
Fomes fomentarius		+[b]	+[b]						+[b]				[71]
Fusarium coerulum											+		[12]
F. dimerum											+		[12]
F. eumartii											+		[12]
F. moniliforme (2)[a]											+		[12]
F. oxysporum (5)[a]											+		[12]
F. roseum											+		[12]
F. solani (Mart) Sacc.		+		+			+				+	+[b]	[97]
F. solani (4)[a]											+		[12]
F. tritinctum											+		[12]
Hansenula anomala (2)[a]											+		[12,68,69]
H. beckii											+		[12]
H. capsulata											+		[12]
H. henricii											+		[12]
H. minuta											+		[12]
H. saturnus											+		[12]
Paecilomyces variotii		+	+	+		+		+			+	+	[108]
Penicillium frequentans											+		[68,69]
P. glaucans											+		[66]
P. rubrum	+						+					+	[123]
Pestalotia palmarum											+		[108]
Polyporus versicolor		+[b]	+[b]						+[b]				[71]
Pullularia pullulans			+[b]				+[b]					+	[63]
Rhizopus arrhizus											+		[68,69]
Rhodotorula rubra				+			+						[68,69]
R. sp											+		[126]
Saccharomyces cerevisiae (5)[a]										+	+		[12,68,69,89]
Sporotrichum pulverulentum					+[c]								[20]
Trametes sp			+	+									[99]

[a]Number of strains reported
[b]Proposed only
[c]From vanillic acid

subsequently to vanillic acid (**7**). The proposed pathway for acetate cleavage from ferulate in the side-chain shortening reaction involves water addition to the double bond to give **16**, subsequent oxidation of **16** to ketone **17** and removal of an acetate unit to give vanillin (**5**) (Figure 3).

Vanillin is further oxidized in a NAD^+-dependent reaction to form vanillic acid (**7**). In this work, vanillin and vanillic acid were established as metabolites by infrared spectroscopic comparisons of isolated metabolites to standard compounds. Evidence for the elimination of [2-^{14}C]-acetate from [2′-^{14}C]-*trans*-ferulic acid supported the proposed mechanism. However, attempts to synthesize the β-hydroxy intermediate (**16**) failed. Quinoid isomerization of the *p*-hydroxyl group on the aromatic ring causes elimination of the benzylic hydroxyl group of **16**. This work demonstrated that microorganisms are clearly capable of oxidizing *trans*-ferulic acid into smaller aromatic metabolites such as vanillic acid (**7**) common to basic bacterial metabolism.

Although detailed cell-free metabolism experiments were not generally conducted for most organisms listed in Table 1, it is clear that side-chain elimination of an acetate moiety occurs very commonly among bacterial species including *Bacillus* [56], *Corynebacterium* [84,85], *Escherichia* [103], *Pseudomonas* [75,124], *Streptomyces* [120] and other unidentified bacteria [73]; and among fungi and yeasts including species of *Aspergillus* [84], *Fomes* [71], *Fusarium* [97], *Polyporus* [71] and *Rhodotorula* [68,69].

The precise mechanism involved in the cleavage of two carbons from the ferulic acid cinnamoyl side-chain has not been fully established. Intermediates proposed during the cleavage reaction are unstable. Thus, it has not been possible to isolate or characterize them by classical chemical or biochemical means. With *Rhodotorula rubra*, we employed a combination of biochemical, mass spectral and NMR spectroscopic analytical approaches to probe the side-chain cleavage mechanism [69]. We first established that *R. rubra* catalyzed the conversion of **1** to **7** and later **9** by isolating these metabolites and characterizing them by mass spectrometry (MS) and nuclear magnetic resonance (NMR) analyses. Cell-free extracts were used to demonstrate that the side-chain cleavage reaction was CoA/ATP- and NAD^+-dependent. Similar results were previously reported with extracts of a *Polyporus* strain, and with *Streptomyces setonii* in ferulic acid transformations to vanillic acid [69,120]. These cofactor requirements suggested the involvement of a classical β-oxidation process similar to fatty acid oxidation, where steps include the hydration of **1** to **16**, oxidation to a ketone (**17**), and subsequent acetate cleavage (Figure 3).

We attempted to confirm this path by incubating *R. rubra* in buffers prepared with 20% $H_2{}^{18}O$ [69]. Based on available evidence, intermediates **18–21** (Figure 3) would all be labeled with ^{18}O. Three different ^{18}O-labeled species of vanillic acid **22**, **23**, **24** (Figure 3) and unlabeled **7** were obtained from this incubation and analyzed by MS and ^{13}C-NMR to measure both the extent of incorporation of labeled oxygen, and the precise location of oxygen in **7**. By MS, unlabeled vanillic acid (**7**, Figure 3) exhibits a molecular ion [M^+] of m/z 168. The intensity of the m/z 170 [M^+ +2] ion (36%) indicated an equal amount of species of **23** and **24** each of which contained 18% of ^{18}O. The intensity of the ion at m/z 172 [M^+ +4] is due to doubly labeled vanillic acid (**22**) containing 3.5% of ^{18}O. Since **21** contained approximately 20% ^{18}O, hydrolysis in 20% $H_2{}^{18}O$ buffer would theoretically afford doubly labeled **22** in 4% yield.

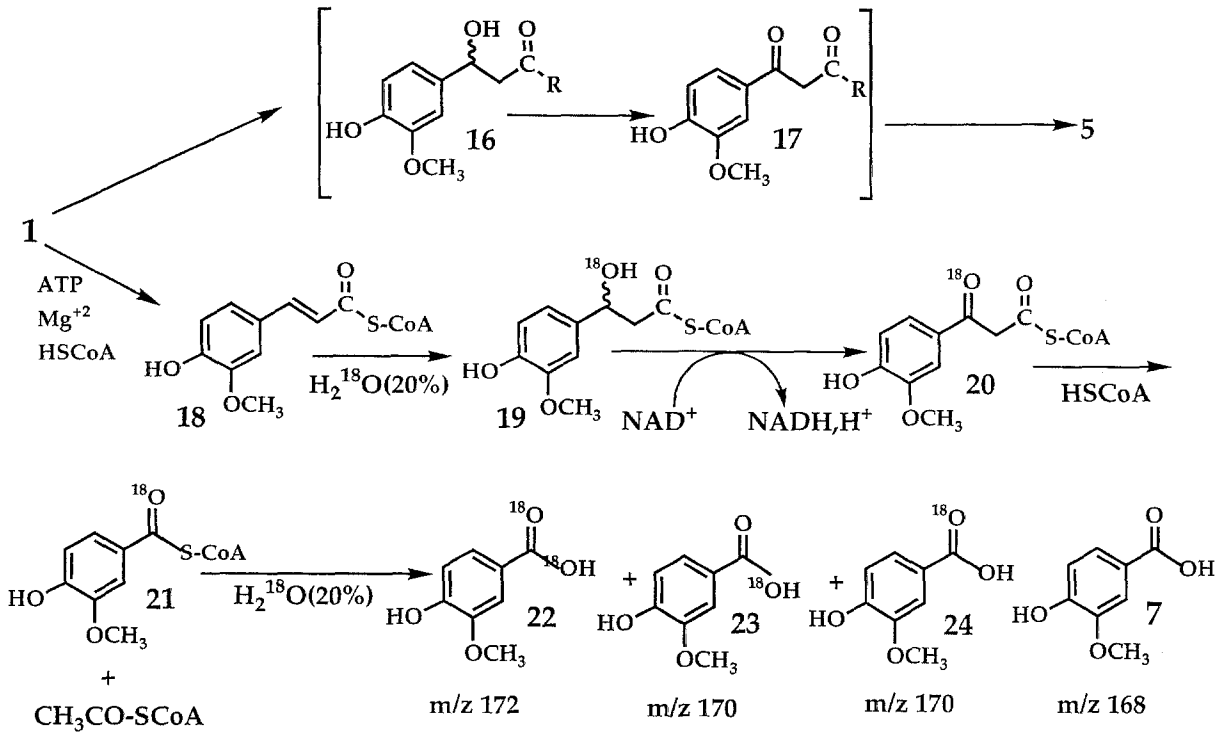

Figure 3 Details of the mechanism by which ferulic acid (**1**) is metabolized to labeled vanillic acids (**22**, **23**, and **24**) and unlabeled vanillic acid (**7**)

The incorporation of ^{18}O specifically into the carboxyl group was confirmed by ^{13}C-NMR where the upfield shift of 2.1 Hz (0.023 ppm) for the carboxyl carbon is caused by the presence of ^{18}O on the carboxyl-carbon atom [69].

*2. Nonoxidative decarboxylation of ferulic acid to 4-vinylguaiacol (4-hydroxy-3-methoxystyrene) (***14***):* Styrene formation is among the oldest reported biotransformations of cinnamic acid derivatives. In 1906, Herzog and Ripke [66] reported that species of *Penicillium glaucum* and *Aspergillus niger* converted cinnamic acid into styrene. Styrene is also produced from cinnamic acid by other molds [74] and yeast [27,93]. This is interesting because the decarboxylation of cinnamic acid apparently does not require an oxygenated aromatic ring, a common requirement for this biotransformation reaction in most microorganisms [12,42,68,69]. Table 1 reveals that styrene formation is perhaps the most common microbial transformation reaction observed with ferulic acid. The reaction generally occurs rapidly, and in high yield. Although not established with all microorganisms, the decarboxylation reaction appears to be nonoxidative in nature. Scheline observed that rat intestinal microflora convert ferulic acid into 4-vinylguaiacol, thus indicating the potential for this reaction to convert a common dietary chemical into a potentially toxic styrene intermediate in the gastrointestinal tract [114].

Bioconversion of ferulic acid to **14** is often accompanied by additional metabolites. For example, Nazareth and Mavinkurve reported the nonoxidative decarboxylation of *trans* ferulic acid to 4-vinylguaiacol by *Fusarium solani* (Mart.) Sacc. 4-Vinylguaiacol was thought to be further metabolized to vanillin, vanillic acid and protocatechuic acid, which undergoes *ortho*-ring cleavage to β-keto adipic acid [97]. Rahouti *et al* also suggested that *Paecilomyces* gave vinylguaiacol as a major metabolite that was converted further to vanillin [108]. However, the amounts of vanillin and vanillic acid observed were very small (probably less than 1% as determined by HPLC), thus casting doubt on this pathway. Samejima *et al* [113] found that *Pseudomonas* TMY1009 produced 4-vinylguaiacol from *trans*-ferulic acid via a proposed 7-hydroxy intermediate such as **16** (Figure 3, R = OH). Decarboxylation of caffeic acid (**3a**, Figure 1) to afford 3,4-dihydroxyphenylethane was thought to involve cinnamic acid double bond reduction and subsequent decarboxylation [134]. This reaction more likely involves initial formation of styrene first and subsequent reduction as described later for biotransformations of 4-vinylguaiacol.

3. Mechanism of styrene production of microorganisms: Surprisingly little work had been conducted until very recently on the mechanism of styrene formation from ferulic acid. Manitto *et al* reported on the stereochemistry of the decarboxylation reaction of 3,4-dimethoxycinnamic acid by cultures of *Saccharomyces cerevisiae* [89]. A deuterium atom on position 8 of the cinnamic acid (*H, Figure 4) was retained (by ^1H-NMR) in the resulting styrene product. These results were used to suggest that *in vivo* decarboxylation might occur by enzymatic *cis*-addition to the double bond followed by *trans*-decarboxylative elimination to maintain the observed stereochemistry of the retained deuterium atom.

Our screening studies [68] revealed several microorganisms capable of catalyzing the decarboxylation reaction in good yield. Among these, baker's yeast gave **14** and another metabolite identified as 4-hydroxy-3-methoxyphenylpropionic acid **13** (Figure 2). The side-chain reduction reaction did not occur under aerobic conditions. Reduction of the cinnamoyl side-chain has been observed with bacteria [8,96,101] and fungi [40,68]. An organism originally identified as *Pseudomonas fluorescens* UI-670 also converted **1** to **14** in excellent yield both under standard aerobic incubation conditions, and when the organism was incubated under argon. This organism has since been identified as a strain of *Bacillus pumilus* by fatty acid analysis and metabolic studies (unpublished results). We used this microorganism and *Saccharomyces cerevisiae* to probe the mechanism of decarboxylation further.

A nonoxidative mechanism was envisioned to involve initial enzymatic isomerization of **1** to quinoid intermediate **25** (Figure 4) [68]. If formed, the doubly vinylogous β-keto acid **25** would spontaneously decarboxylate to afford styrene. The mechanism would satisfy the requirement for a *para*-hydroxyl group in the decarboxylation process. Isomerization of **1** to **25** involves addition of a proton from water to the side-chain. Thus, we reasoned that evidence for the existence of **25** could be obtained by conducting the incubation reaction in deuterium oxide-containing media, and by isolating and characterizing the deuterium-labeled styrene **26** by mass spectrometry, and both deuterium and ^1H-NMR. The proposed mechanism is analogous in part to that proposed by Hopper and coworkers and Whited and Gibson for the hydroxylation of cresol by the enzyme cresol methylhydroxylase [133].

Conversions of **1** to **26** were obtained with both baker's yeast and *B. pumilus* UI-670 in media containing 0, 50, 75 or 100% D_2O. Incorporation of deuterium into all samples of **26** isolated from deuterium-containing medium was supported by the presence of a peak at 5.13 ppm in their ^2H-NMR spectra. The absence of any other deuterium peaks by ^2H-NMR indicated that deuterium incorporation was highly stereospecific. The deuterium incorporations were also confirmed by MS where the relative intensities of ions at m/z 150 (unlabeled vinylguaiacol **14**) and m/z 151 (**26**, Figure 4) were compared. All MS analyses indicated that **26** contained a single deuterium atom, the relative intensities of which were proportional to the amounts of D_2O used in incubation buffers. NMR and mass spectral results obtained for the decarboxylation of **1** by baker's yeast were identical to those obtained for *B. pumilus* [68]. These results provided confirmatory evidence for a novel microbial mechanism for the nonoxidative decarboxylation of naturally occurring 4-hydroxycinnamates.

4. Purification and characterization of ferulic acid decarboxylase: Ferulic acid decarboxylase was purified 2780-fold from cells of *B. pumilus* UI-670 by a combination of DEAE-cellulose, hydrophobic interaction chromatography, gel filtration, hydroxylapatite and mono-Q chromatographic steps [70]. The colorless enzyme is constitutive, stable in the cold and does not require exogen-

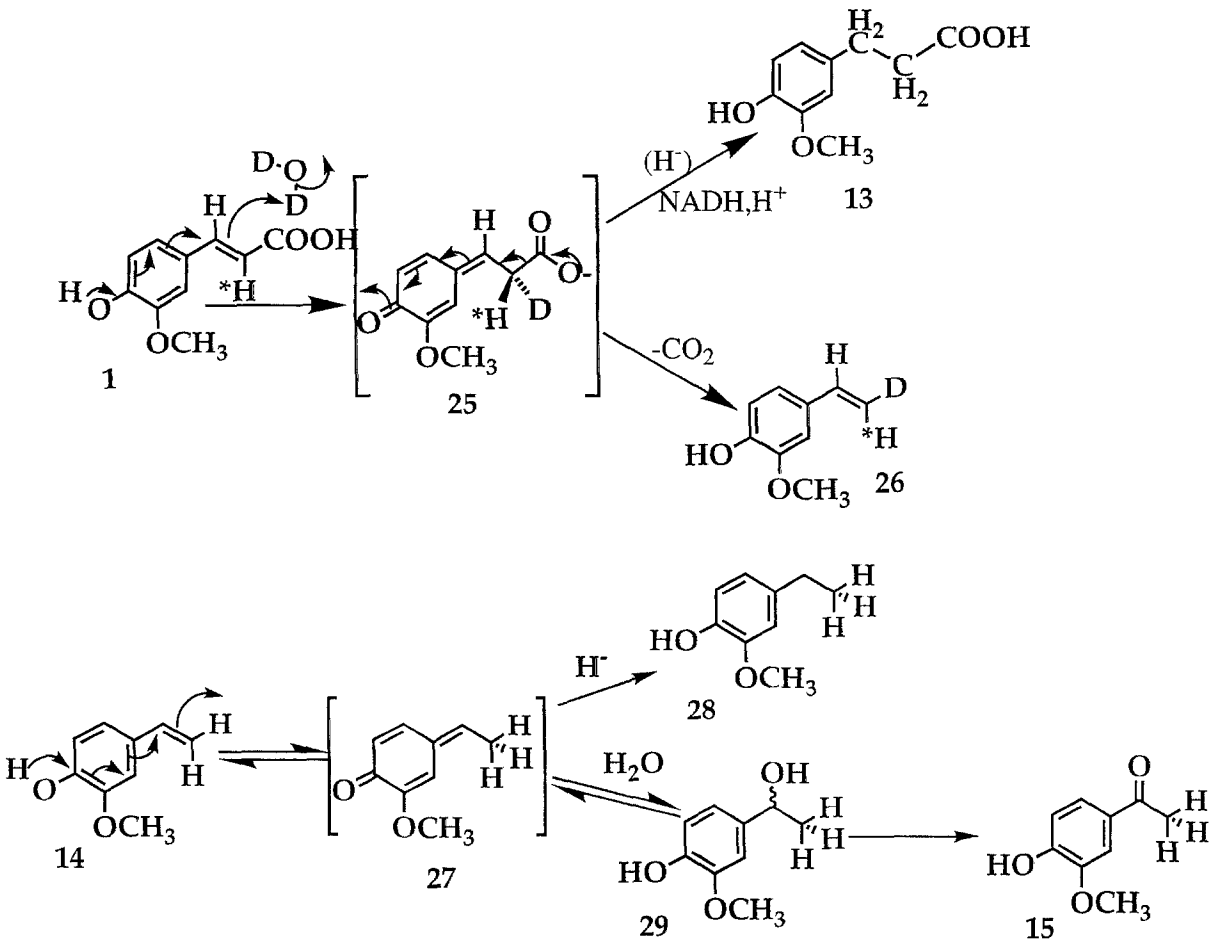

Figure 4 Mechanism of vinylguaiacol formation from ferulic acid in microorganisms, and further oxidation products of vinylguaiacol

ously added cofactors or metal ions for full activity. It exhibits optima at pH 7.3 and 27° C in 20 mM phosphate buffer, and has a K_m of 7.9 mM for ferulic acid. Gel filtration indicated an apparent molecular mass of 40.4 (±6%) kDa whereas Na dodecylsulfate-polyacrylamide gel electrophoresis showed a molecular mass of 20.4 kDa. Thus, it appears that ferulate decarboxylase is a homodimer in solution. The enzyme catalyzed decarboxylations of *p*-coumaric acid, but not 2- or 3-hydroxycinnamic acids, thus indicating the requirement for a 4-hydroxyl group in the decarboxylation reaction. Ferulate decarboxylase contains no apparent flavin component. Further, the reaction was not enhanced by additions of FMN, FAD, NAD^+, $NADP^+$, NADH H^+, or NADPH H^+, and it was not coupled to typical electron acceptors. It was inhibited by mercuric and cupric ion, 4-chloromercuribenzoic acid, *N*-ethylmaleimide and iodoacetamide suggesting that sulfhydryl groups are required for catalysis. Lack of inhibition by diethyl pyrocarbonate suggests that histidine is not involved in the decarboxylation reaction.

The N-terminal sequence of 39 amino acids was determined to be:

5 10
NH_2-Met-Asp-Gln-Phe-Val-Gly-Leu-His-Met-Leu-Tyr-

15 20
Thr-Tyr-Glu-Asn-Lys-Trp-Glu-tyr-Glu-Ile-Tyr-Ile-

25 30 35
Lys-Asn-Asp-His-Thr-Ile-Asp-Tyr-Arg-Ile-His-Ser-

39
Gly-Met-Val-Gly-.

Some of the properties of our purified enzyme were similar to those described earlier for a partially purified *p*-coumarate decarboxylase from *Cladosporium phlei* [58], and, interestingly, for a vanillate hydroxylase (decarboxylating) enzyme from *Sporotrichum pulverulentum* and a vanillyl-alcohol oxidase from *Penicillium simplicissimum* [23]. *p*-Cresol-methylhydroxylase, which is mechanistically similar to our ferulate decarboxylase, is classically a ferrohemochromagen oxidoreductase [133]. This purified yellow enzyme contains a covalently bound flavin with absorption maxima at 454 and 344 nm in alkaline solution [133].

In January 1995, Degrassi *et al* reported the purification of ferulate and *p*-coumarate decarboxylase from *B. pumilus* [33]. The enzyme was induced in this culture, was slightly higher in molecular mass (23 kDa by Na dodecyl sulfate PAGE, 45 kDa by size exclusion), showed different pH (pH 5.5), buffer and temperature (37° C) optima and

kinetic properties (K_m 1.03 mM for ferulic acid). A 17-amino acid N-terminal sequence was identical to our ferulate decarboxylase for amino acids 1–9, but differed for amino acids 10 (Ile), 14 (Gln) and 16 (Gly). Interestingly, the decarboxylase reaction is inhibited when our enzyme is incubated in Tris buffer, the buffer used in the first isolation steps of purification of ferulate and *p*-coumarate decarboxylase from *B. pumilus* [33].

Ferulate decarboxylases can be useful biocatalysts for the production of styrenes that in themselves have value as starting materials for the preparation of aromatic feedstock chemicals. The styrenes themselves are useful as prepolymers, and are readily converted into other products by microorganisms.

5. Conversions of ferulic acid to vanillin: As a noteworthy goal, the conversion of ferulic acid to vanillin is of considerable interest. Some 12000 tons of synthetic vanillin are produced for flavoring purposes each year [57]. A number of bioconversions of ferulate to vanillin have been reported, but none of these accomplish the side-chain cleavage reaction (Figure 2 and Table 1) to yield the aldehyde (**5**) in good yield. Ishikawa *et al* [71] found that white rot fungi *Polyporus versicolor* and *Fomes fomentarius* could degrade ferulic acid to vanillin (**5**), which could be reversibly reduced to vanillyl alcohol (**6**) or oxidized to vanillic acid (**7**). Ötük found that *E. coli* metabolized ferulic acid to vanillin, then vanillic acid and protocatechuic acid [103]. Labuda *et al* [85] found that *Cornybacterium glutamicum* produced a mixture of vanillin (10–15% yield) and vanillic acid from ferulic acid. Iwahara *et al* [73] found an unidentified bacterial strain that converted ferulic acid to vanillic acid. Labuda *et al* [84] found that the conversion of ferulic acid to vanillin by *C. glutamicium*, *Aspergillus niger*, and *Pseudomonas putida* could be increased 10 to 100-fold by the addition of dithiothreitol in the medium. Addition of the thiol presumably precludes further reduction of the aldehyde functional group of vanillin by an aldehyde oxidoreductase.

6. O-Demethylation of ferulic acid to caffeic acid: The demethylation of ferulic acid to caffeic acid (**3a**) is a common biotransformation reaction. Tillet and Walker [123] reported a *Penicillium rubrum* strain that *O*-demethylated ferulic acid to caffeic acid (**3a**, Figure 2). The caffeic acid thus formed is metabolized by conversion to protocatechuic acid, followed by further aromatic ring cleavage. Angeles de la Torre and Gomez-Alarcon found that anaerobic microorganisms from marsh sediment converted ferulic acid to caffeic acid [9]. Grbic-Galic found that the facultative anaerobe *Enterobacter* DG-6 was capable of *O*-demethylating ferulic acid to caffeic acid (**3a**, Figure 2) under either anaerobic or aerobic conditions. Anaerobically, caffeic acid was metabolized to cinnamic acid by ring dehydroxylation. Cinnamic acid was then reduced to phenylpropionic acid which was subsequently metabolized to phenylacetic acid. Aerobically, caffeic acid was oxidized to protocatechuic acid [48]. The mechanism of ferulate demethylation was proposed to involve a *trans*-methylation reaction resulting in the production of both **3a** and methanol [50]. Grbic-Galic and Young described a methanogenic consortium that metabolized ferulic acid to caffeic acid, and then to CH_4 and CO_2 [51]. Scheline found that rat intestinal microflora could convert ferulic acid to *m*-hydroxyphenyl propionic acid, dihydrocaffeic acid and 4-ethylcatechol [114].

7. Reduction of ferulic acid to dihydroferulic acid (DFA): Side-chain reduction of ferulic acid to dihydroferulic acid (**13**, Figure 2) has been observed frequently. The reduction is catalyzed by anaerobic microbial consortia [9,96], methanogenic consortia [51], *Pseudomonas cepacia* [8] and *Wolinella succinogens* [101] grown anaerobically, and by cultures of *Cornyebacterium glutamicum* [85]. The side-chain reduction reaction also occurs in cultures of rat intestinal microflora [114] to give products including *m*-hydroxy-phenylpropionic acid, hydrocaffeic acid and 4-ethyl catechol [114]. Additional biotransformation reactions including dehydroxylation, demethylation, side-chain cleavage and further ring degradation often accompany the side-chain reduction reaction. Interestingly, the cinnamoyl side-chain reduction reaction of **1** to **13** was observed in our cultures of *Saccharomyces cerevisiae* [68]. With this yeast, **13** was obtained only when cells were incubated in an argon atmosphere. Under aerobic conditions ferulic acid was transformed to **14** (Figure 4). We suggested that the side-chain reduction reaction likely occurred by hydride attack of a quinoid intermediate (**25**) to afford **13** by an isomerization mechanism analogous to decarboxylations of ferulic acid and vanillic acid.

8. Biotransformations of vinylguaiacol: Since 4-hydroxy-3-methoxystyrene (**14**) is a valuable bioconversion product from the extremely abundant ferulic acid, we examined microorganisms for their abilities to catalyze further transformations of **14**. Metabolic transformation studies of styrene by microorganisms have revealed several common pathways. Styrene is metabolized via styrene *cis*-glycol to 3-vinylcatechol and 2-vinylmuconate, demonstrating double bond and aromatic ring oxygenation prior to ring fission [131]. Metabolism of styrene via styrene oxide gives phenylacetaldehyde and phenylacetic acid [59,60,81]. With anaerobic consortia, after water addition to the double bond, styrene is oxidized through the phenylacetate route to yield a variety of products including ethylbenzene, phenylethanol, 1-phenylethanone, phenylacetaldehyde, phenylacetic acid, benzyl alcohol, benzaldehyde and benzoic acid [49]. Styrene transformations have also been reported to occur with *Pseudomonas putida* [17] and the black yeast *Exophala jeanselmei* [31].

Of some fifty microorganisms screened for their abilities to transform vinylguaiacol (**14**) we discovered a *Nocardia* strain that efficiently converted the substrate to products. When incubations were conducted under an argon atmosphere with resting cells of *Nocardia* spp, **14** was transformed to 4-ethyl-2-methoxyphenol (**28**, Figure 4) in 35% yield, 1-(4′-hydroxy-3′-methoxyphenyl)-ethanol (**29**) in 43% yield, and 1-(4′-hydroxy-3′-methoxyphenyl)-ethanone (**15**) in 2% yield (unpublished results). $H_2{}^{18}O$ was used as before in mechanistic studies to demonstrate (by MS analysis) that bioconversion of **14** to **29** involved the addition of water to the vinyl group of vinylguaiacol. We

speculated that a tautomerized intermediate such as **27** was involved in the transformation reaction. Deuterium oxide (D_2O)-containing media were used to demonstrate that the reaction sequence shown from **14** to **27** and **27** to **29** (Figure 4) were essentially reversible reactions. Recoveries of all products and determinations of deuterium incorporations by MS and deuterium NMR revealed that recovered **14** contained deuterium at the terminal olefin position; that **28** contained deuterium at both positions on the reduced ethyl side chain; and that both **29** and **15** contained deuterium in the terminal methyl position (unpublished results).

9. Selected biotransformations of vanillic acid: Although the biotransformation of ferulic acid is the major theme of this review, a few interesting biotransformations are described for vanillic acid (**7**), a major ferulic acid metabolite. Three of these are summarized in Figure 5. *O*-Demethylation of **7** affords protocatechuic acid (**10**) [124]. With *P. acidovorans*, *O*-demethylation requires NADH, H^+ along with formaldehyde, Fe^{2+} and reduced glutathione [124]. Conversion of vanillic acid to protocatechuic acid followed by ring-degradation is a common biodegradative pathway in microorganisms [21,22,43,56,75,79,97,113, 124]. Vanillic acid may be degraded to quinone and acetophenone [75], and protocatechuic acid is degraded to 2-pyrone-4,6-dicarboxylic acid which is further metabolized to pyruvate and oxaloacetate [113] or β-ketoadipic acid [97] after *ortho*-ring fission.

Pometto, Sutherland and Crawford found that *Streptomyces setonii* converted ferulic acid to vanillin, then vanillic acid. Vanillic acid was subsequently oxidized to protocatechuic acid and catechol with ultimate cleavage of the catechol aromatic ring to *cis,cis*-muconic acid [105]. Unspecified degradation products were obtained when protocatechuic acid was cleaved by a dioxygenase from *Bacillus subtilis* [56]. Quantitative nonoxidative decarboxylation of vanillate (10 mM) to guaiacol occurred with succinate-grown cells of *Bacillus megaterium* over a 9-h period [32]. Thus, guaiacol is a major metabolic product obtainable from ferulic acid.

We explored the mechanism of nonoxidative decarboxylation of vanillic acid by the same approach used in probing the mechanism of ferulic acid decarboxylation [69]. We proposed that **7** could isomerize to **30** (Figure 5) during the course of the reaction. Tautomerization of **7** to **30** involves addition to the aromatic ring of a proton from water which should be retained in guaiacol (**31**) following decarboxylation. The tautomerization is enzyme-mediated; the decarboxylation reaction is a chemical event because **30** is a vinylogous β-ketoacid that would spontaneously decarboxylate once formed. Mass spectral analysis of **31** indicated that a single deuterium atom was incorporated into guaiacol (**31**) obtained from incubation mixtures containing 50% or 100% deuterium oxide. Proton NMR spectroscopy also indicated that the proton at position 4 of **31** had been replaced by deuterium. Thus, the mechanism for guaiacol formation is similar to that identified for ferulic acid decarboxylation in that both systems apparently involve quinoid and vinylogous β-keto acid intermediates prone to decarboxylation.

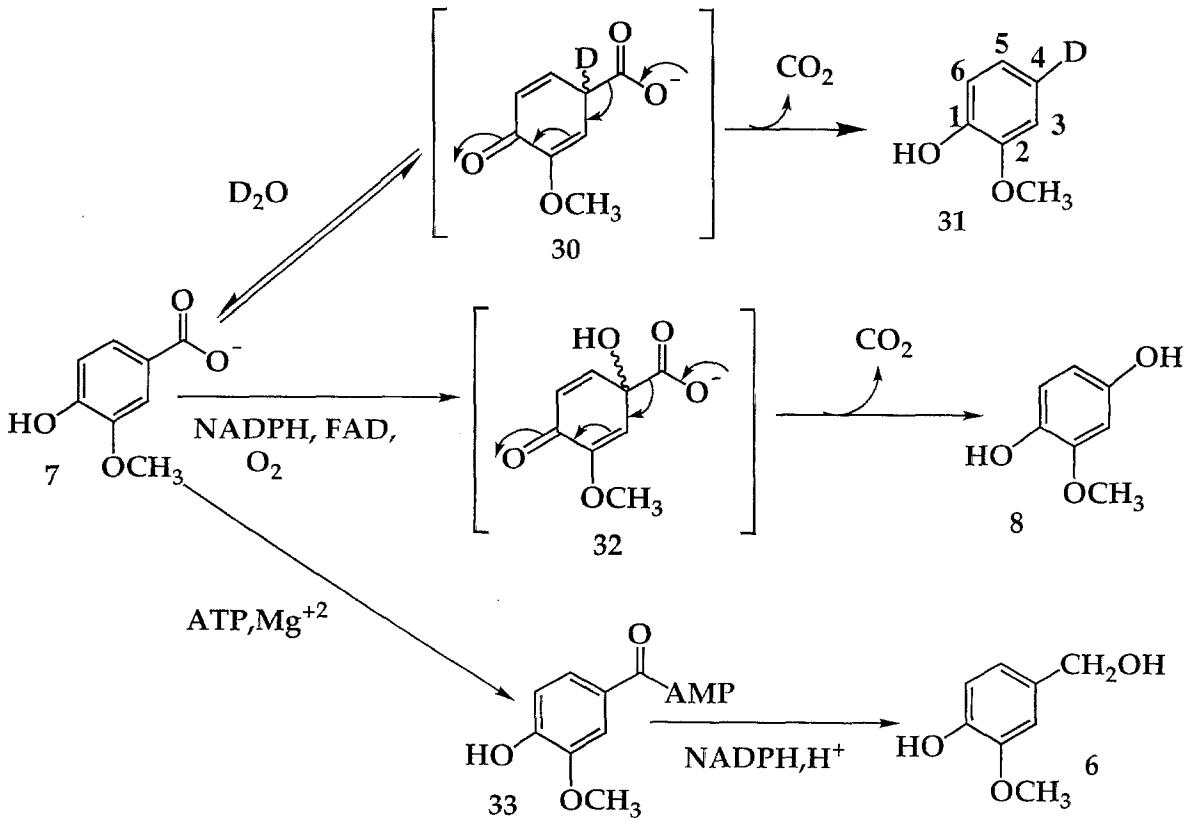

Figure 5 Selected oxidative and nonoxidative transformations of vanillic acid by microorganisms

Oxidative decarboxylation of vanillic acid to methoxyhydroquinone (**8**) (Figure 2) is also a common pathway for biotransformation in soft-rot, brown-rot and white-rot fungi [108]. Mycelial extracts prepared by sonication of cells of *Chaetomium*, *Humicola*, *Petriellidium*, *Daedalea*, *Lenzites*, *Fomes*, *Polyporus*, *Poria* and *Pycnoporus* contained high levels of an NADPH-dependent vanillate hydroxylase leading to the formation of **8**. Reaction progress was measured by the release of $^{14}CO_2$ from labeled vanillic acid. Vanillate decarboxylase from *Sporotrichum pulverulentum* requires both NADPH and molecular oxygen [20]. Vanillate hydroxylase from *S. pulverulentum* had a molecular weight of about 65 000 Da, had highest activity with both FAD and NADPH, required a *para*-hydroxyl group for decarboxylation, and worked best for vanillate, protocatechuate, *p*-hydroxybenzoate and 3,4-dihydroxybenzoate [23]. We suggest here that the oxidative decarboxylation mechanism leading to methoxyhydroquinone (**8**) involves a hydroxylated quinoid intermediate (**32**) that spontaneously decarboxylates to **8**.

Carboxylic acid reductases are a relatively unexplored group of enzymes. In many fungi and bacteria, carboxylic acid substrates like vanillic acid are activated by conversion to a carbonyl-AMP intermediate (Figure 5, **33**) followed by subsequent reduction to the benzylic alcohols [28]. The reaction is widespread in the microbial world, and we have observed its range to include aliphatic as well as aromatic acid substrates [28]. Facile reduction of carboxylic acids to their respective alcohols bears all of the interesting possibilities of regio- and enantioselectivities with multifunctional substrates. Furthermore, this reaction is biotechnologically interesting because the carboxylic acid substrates are water soluble.

10. Oxidations of ferulates by peroxidases: A number of investigations have focused attention on enzymatic oxidations of ferulic acid or its derivatives. Many of these studies serve as a basis for modeling the formation of compounds in nature known as lignans or neolignans. The availability of enormous quantities of ferulic acid suggests its potential for use in the generation of oxygenated and biodegradable polymers by enzyme catalysis. The presence of the phenolic functional group obviously directs attention to the involvement of free radical oxidations for ferulic acid and its derivatives. Generation of phenolic radicals can theoretically lead to a plethora of dimeric, oligomeric or polymeric products. The aromatic ring and the conjugated double bond of the side-chain presents additional sites where electrons generated enzymatically or chemically can be delocalized. Examples of the formation of 'dimers' of ferulates formed naturally or 'in vitro' are common. Less well defined are the structures of polymers formed from ferulic acid and its relatives.

Most attention has been directed towards the action of peroxidases as biocatalysts. Chemical oxidants such as ferric chloride or ammonium persulfate, and photochemical oxidations have been employed to transform ferulates and to mimic the catalytic activities of peroxidases in nature.

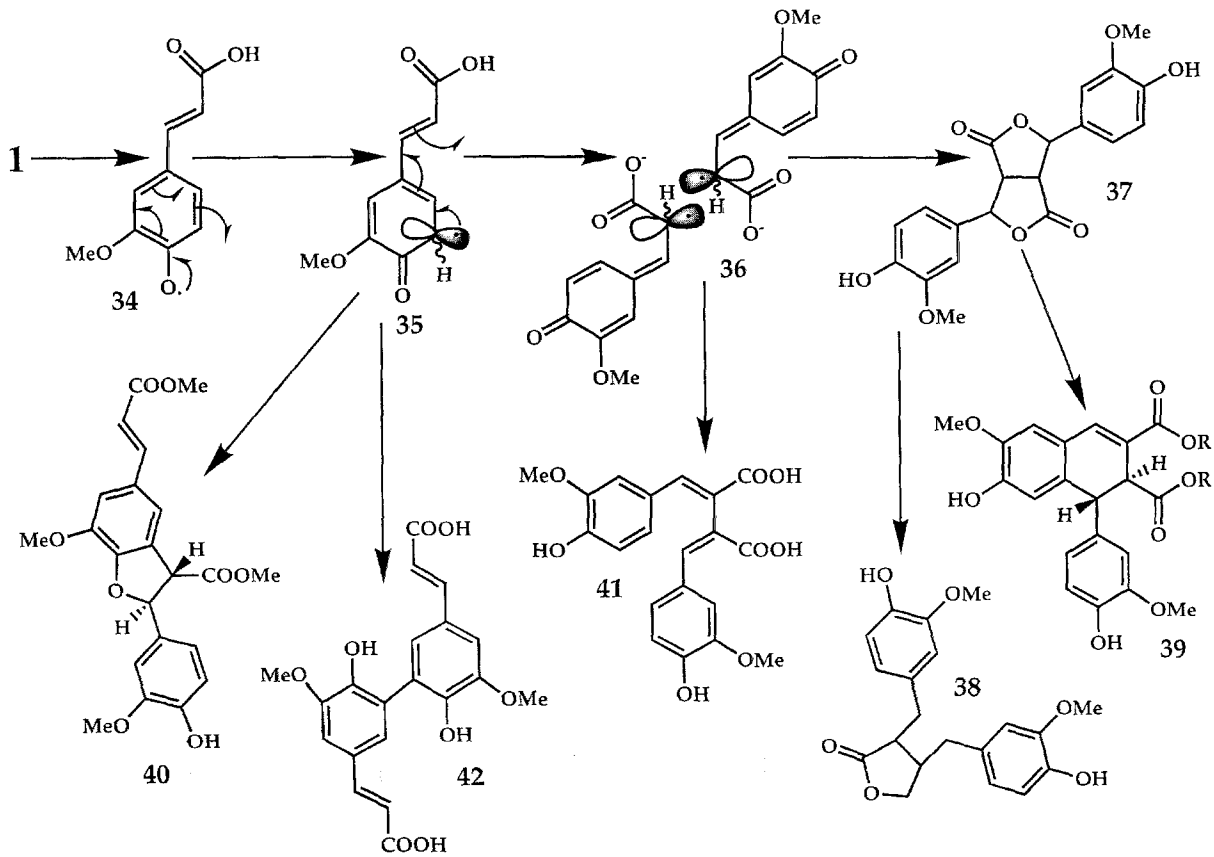

Figure 6 Products formed during enzymatic and chemical free radical oxidations of ferulic acid and derivatives

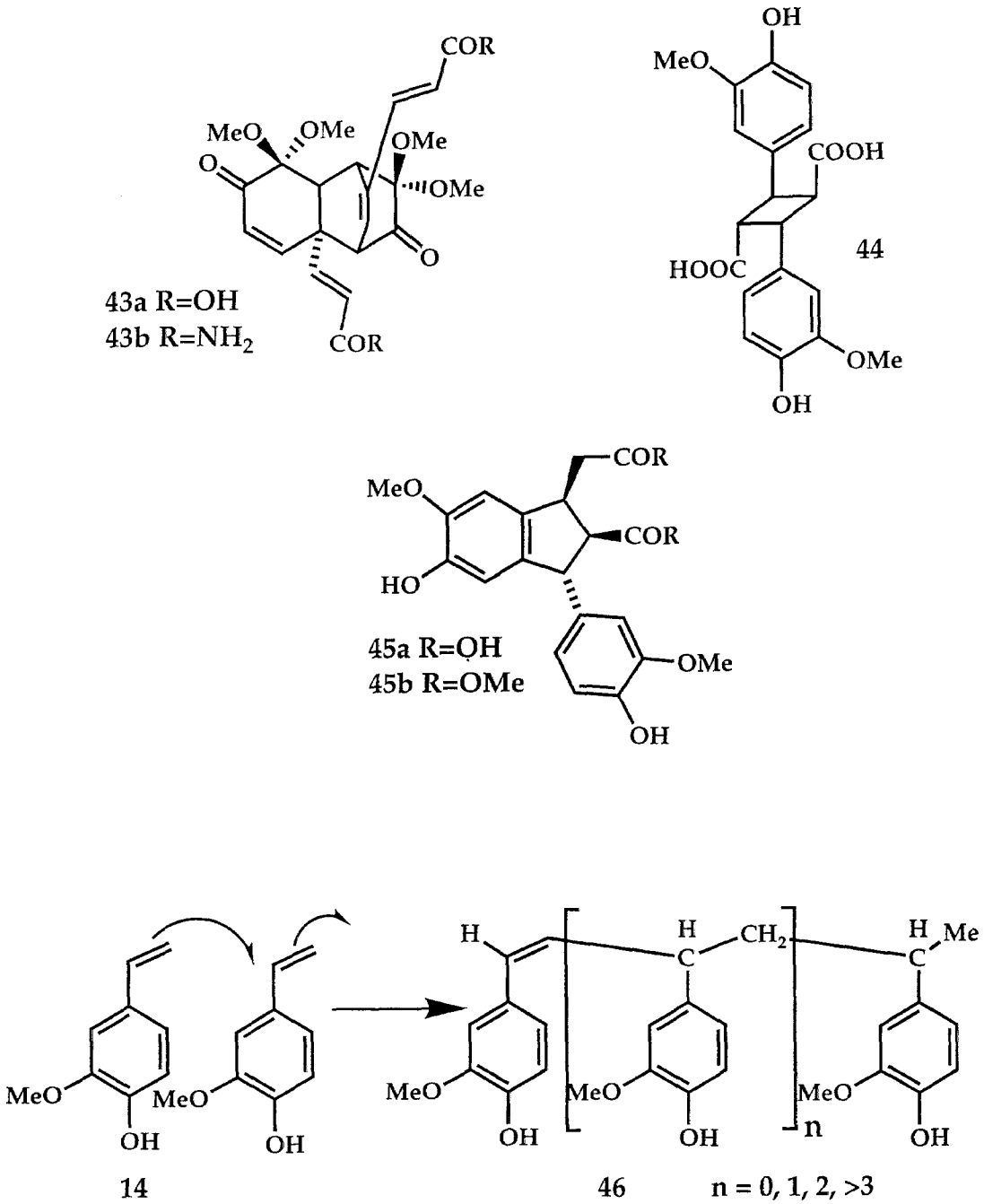

Figure 7 Structures of aryl indanes and some higher polymers of ferulic acid

Such work has a significant history. In 1944, Cartwright and Haworth studied the oxidation of ferulic acid with ferric chloride or ammonium persulfate [24]. These one-electron oxidants afforded the dilactone **37** as the major product (20% yield). Takei *et al* [121] demonstrated that **37** could be transformed into a matairesinol-like compound **38** by hydrogenolysis over palladium on carbon. Acidic treatment of **37** transformed the compound into the dihydronaphthalene derivatives **39** [121]. Thus, a pathway was established for the formation of **37** as an initial coupling product that serves as an intermediate for the formation of other lignan products. The initial steps (Figure 6) likely result from formation of phenolic radical (**34**) that is delocalized to the aromatic ring (**35**) and subsequently into the side-chain as in **36**. Pairing of radicals in **36** followed by Michael-type addition ring closures gives **37**.

Oxidation of ferulic acid with horseradish peroxidase (HRP) gives complex mixtures of various products, many of which have not been characterized [15]. Ferulate methyl ester is transformed into the phenyl-coumaran type compound **40** in 25% yield with HRP [14,29]. This compound is also formed by oxidation with iodosylbenzene in the presence of tetraphenylporphyrinatomanganese (III) acetate (22%) [14,29] or with silver (I) oxide (44%) [11]. Ferulic acid has been used as a model substrate in studies with other peroxidases [45,128,135]. Different peroxidases appear to catalyze dimerization reactions in different ways. Caffeate peroxidase is reported to convert ferulic acid into

$\beta\beta'$ linked-type dimers such as **41** [45], while peanut peroxidase converts **1** into biphenyl type compounds like **42** [128]. Both compounds are formed by the initial oxidation of the phenolic functional group of **1** followed by electron delocalization to the ring as in **35** to form **42**, or to the unsaturated side-chain (**36**) to form **41**.

Electrochemical oxidations of ferulic acid afforded the asatone type dimer **43a** and the dilactone **37** as a minor product with yields depending on oxidation conditions [100]. Ferulamide is transformed only into the asatone type dimer **43b** in 56% yield electrochemically. Photochemical oxidation of ferulic acid by irradiation under 'daylight' fluorescent tubes affords low yields (*ca* 2%) of the truxilic acid-like compound **44** [44]. Photoxidation of its methyl ester with visible light in the presence of methylene blue as a photosensitizer afforded dihydronaphthalene **39** and phenyl coumaran **40** (less than 10% each) [83]. Ferulic acid and its methyl ester are also dimerized by standing in trifluoroacetic acid into aryl indane compounds **43a** and **43b** in 80% yield [4] (Figure 7). Similar products have not yet been reported by enzyme oxidation.

Higher polymers of ferulic acid were obtained by polymerization with horseradish peroxidase under weakly basic conditions (pH = 8) [112] or chemically with thionyl chloride [39] or oxalyl chloride [104]. Although the structures of the polymers remain undefined, these synthetic polymerized lignins demonstrated some level of anti-HIV and anti-tumor activities [13,82,95]. In studies on lignification in the cell wall to determine the nature of lignin-hydroxycinnamic acids interactions, some codimers from methyl 5-*O*-(E)-feruloyl-α-L-arabinofuranoside and conyferyl alcohol were synthesized [109,110].

Studies on the oxidative coupling of 4-hydroxy-3-methoxystyrene (**14**) obtainable enzymatically from ferulic acid have not been reported. However, the tendency for **14** to polymerize was confirmed by Kodaira *et al* who identified oligomers like **46** when the phenolic styrene was held at room temperature under an inert atmosphere in the dark [62,80]. A polymeric form of **14** was obtained by bulk polymerization in the presence of the chemical oxidant 2,2′-azobisisobutyronitrile as an initiator. The polymer was decomposed by microorganisms in soil, and four chemical degradation products were identified. Vanillic acid is formed, and ring cleavage of vanillic acid gives the monomethyl ester of β-carboxymuconic acid. These studies indicate that potentially useful and biodegradable polymers of phenolic styrenes can be generated readily by chemical and, yet to be demonstrated, enzymatic catalysis.

Conclusions

This review has focused attention on microbial and enzymatic transformations of ferulic acid. Related 'lignin' monomeric compounds *p*-coumaric acid and caffeic acid are also abundant in nature. Together, these phenylpropanoids are attractive preformed aromatic substrates of great value as precursors for other useful chemical products. The field of biocatalysis with these cinnamic acid derivatives is well established, and it is, in fact, quite old. Nevertheless, the exploitation of biocatalytic methods for the conversion of these abundant feedstock chemicals into other useful products remains in its infancy. Many biotransforming enzymes remain to be isolated and characterized. As described in this review, ferulic acid has been transformed into useful phenolic aromatic chemical compounds of value as flavors (vanillin), leuko dyes for use in diagnostics (guaiacol), biodegradable polymer feedstock chemicals (vinylguaiacol), and dimeric products of potential value as drugs. Much work remains to be done. Preliminary investigations on mechanistic aspects of some enzymatic reactions have been reported, especially those dealing with conversions of ferulic acid to vanillic acid and vinyl guaiacol, and on the decarboxylation of vanillic acid into guaiacol. However, the catalytic mechanisms by which ferulate decarboxylase and vanillate decarboxylase function without apparent cofactor needs remain unknown. Although preliminary N-terminal sequences of some enzymes have been determined, none of the enzymes involved in reported biotransformations have yet been cloned and overexpressed. These approaches should yield an abundance of fruitful information about enzyme mechanism, and should avail sufficient amounts of enzymes for practical bioreactor applications.

References

1 Abbott TP, LK Nakamura, TC Nelsen, HJ Gasdorf, GA Bennett and R Kleiman. 1990. Microorganisms for degrading simmondsin and related cyanogenic toxins in jojoba. Appl Microbiol Biotechnol 34: 270–273.
2 Agarwal UP and RH Atalla. 1990. Formation and identification of cis/trans ferulic acid in photoyellowed white spruce mechanical pulp. J Wood Chem Technol 10: 169–190.
3 Akin DE, RD Hartley, LL Rigsby and WH Morrison III. 1992. Phenolic acids released from bermudagrass (*Cynodon dactylon*) by sequential sodium hydroxide treatment in relation to biodegradation of cell types. J Sci Food Agric 58: 207–214.
4 Al-Farhan E, PM Keehn and R Stevenson. 1992. Dimerization of 3,4-disubstituted cinnamic acids and esters. Synthesis 959–961.
5 Ander P, I Stoytschev and K-E Eriksson. 1987. Microbial demethoxylation/demethylation of lignin and lignin model compounds. In: Lignin Enzymic and Microbial Degradation. pp 58–62. Symposium International, Paris, Institut National de la Recherche Agronomique, 145 rue de l'Université, 75007 Paris.
6 Ander P, I Stoytschev and K-E Eriksson. 1988. Cleavage and metabolism of methoxyl groups from vanillic and ferulic acids by brown-rot and soft-rot fungi. Cellulose Chem Technol 22: 255–266.
7 Andreoni F, S Bernasconi, P Bestetti and M Villa. 1991. Metabolism of lignin-related compounds by *Rhodococcus rhodochrous*: bioconversion of anisoin. Appl Microbiol Biotechnol 36: 410–415.
8 Andreoni V, E Galli and G Galliani. 1984. Metabolism of ferulic acid by a facultatively anaerobic strain of *Pseudomonas cepacia*. Syst Appl Microbiol 5: 299–254.
9 Angeles de la Torre M and G Gomez-Alarcon. 1991. Ferulic acid anaerobic degradation by marsh sediment microorganisms. Microbios 68: 119–127.
10 Antrim RL and DW Harris. 1977. Method for treatment of corn hulls. United States Patent number 4038481.
11 Antus S, A Gottsegen, P Kolonits and H Wagner. 1989. Total synthesis of two naturally occurring neolignans of potential biological activity. Liebigs Ann Chem 593–594.
12 Arfmann HA and WR Abraham. 1989. Microbial formation of substituted styrenes. Z Naturforschung Section C, J Biosciences 44: 765–770.
13 Asanoma M, K Takahashi, M. Miyabe, K Yamamoto, N Yoshimi, H Mori and Y Kawazoe. 1994. Inhibitory effect of topical application of polymerized ferulic acid, a synthetic lignin, on tumor promotion in mouse skin two-stage tumorigenesis. Carcinogenesis 15: 2069–2071.
14 Bassoli A, G Di Gregorio, B Rindone, S Tollari, F Chioccara and M Salmona. 1988. Peroxidase-, mixed-function oxidase- and metal-

catalysed oxidation of phenylpropenoidic compounds. Gazz Chim Ital 118: 763–768.
15 Baumgartner J. 1973. Beitrag zur oxidation pflanzlicher phenole insbesondere der ferulasäure. Thesis Nr 4923 Diss ETH Zürich.
16 Ben-Hayyim G, J-P Damon, J Martin-Tanguy and D Tepfer. 1994. Changing root system architecture through inhibition of putrescine and feruloyl putrescine accumulation. FEBS Lett 342: 145–148.
17 Bestettyi G, E Galli, C Benigni, F Orsini and F Pelizzoni. 1989. Biotransformation of styrenes by a *Pseudomonas putida*. Appl Microbiol Biotechnol 25: 252–256.
18 Blum U, BR Dalton and JR Shann. 1985. Effects of various mixtures of ferulic acid and some of its microbial products on cucumber leaf expansion and dry matter in nutrient culture. J Chem Ecol 11: 619–641.
19 Bull A, G Holt and M Lilly. 1982. Biotechnology. International Trends and Perspectives. Organization for Economic Co-Operation and Development, Paris.
20 Buswell JA, P Ander, B Pettersson and K-E Eriksson. 1979. Oxidative decarboxylation of vanillic acid by *Sporotrichum pulverulentum*. FEBS Lett 103: 98–101.
21 Buswell JA and K-E Eriksson. 1979. Aromatic ring cleavage by the white-rot fungus *Sporotrichum pulverulentum*. FEBS Lett 104: 2589–2600.
22 Buswell JA, K-E Eriksson, JK Gupta, SG Hamp and I Nordh. 1982. Vanillic acid metabolism by selected soft-rot, brown-rot and white-rot fungi. Arch Microbiol 131: 366–374.
23 Buswell JA, K-E Eriksson and B Pettersson. 1981. Purification and partial characterization of vanillate hydroxylase (decarboxylating) from *Sporotrichum pulverulentum*. J Chromatogr 215: 99–108.
24 Cartwright NJ and RD Haworth. 1944. The constituents of natural phenolic resins. Part XIX. The oxidation of ferulic acid. J Chem Soc 535–537.
25 Casey J and R Dobb. 1992. Microbial routes to aromatic aldehydes. Enzyme Microb Technol 14: 739–747.
26 Chatonnet P, D Dubourdieu, JN Boidron and M Pons. 1992. The origin of ethylphenols in wines. J Sci Food Agric 60: 165–178.
27 Chen SL and HJ Peppler. 1956. Conversion of cinnamaldehyde to styrene by a yeast mutant. J Biol Chem 221: 101–106.
28 Chen Y and JPN Rosazza. 1994. Microbial transformation of ibuprofen by a *Nocardia* species. Appl Environ Microbiol 60: 1292–1296.
29 Chioccara F, S Poli, B Rindone, T Pilati, G Brunow, P Pietikäinen and H Setälä. 1993. Regio- and Diastereo-selective synthesis of dimeric lignans using oxidative coupling. Acta Chem Scand 47: 610–616.
30 Classen D, JT Arnason, JA Serratos, JDH Lambert, C Nozzolillo and BJR Philogène. 1990. Correlation of phenolic acid content of maize to resistance to *Sitophilus zeamais*, the maize weevil, in CIMMYT'S collections. J Chem Ecol 16: 251–315.
31 Cox HHJ, JHM Houtman, HJ Doddema and W Harder. 1993. Enrichment of fungi and degradation of styrene in biofilters. Appl Microbiol Biotechnol 39: 372–376.
32 Crawford RL and PP Olson. 1978. Microbial catabolism of vanillate: decarboxylation to guaiacol. Appl Environ Microbiol 36: 539–543.
33 Degrassi G, PP DeLaureto and CV Bruschi. 1995. Purification and characterization of ferulate and *p*-coumarate decarboxylase from *B. pumilus*. Appl Environ Microbiol 61: 326–332.
34 Devi S and MNV Prasad. 1992. Effect of ferulic acid on growth and hydrolytic enzyme activities of germinating maize seeds. J Chem Ecol 18: 1981–1990.
35 Dewick PM. 1989. The biosynthesis of shikimate metabolites. Natural Prod Rept 6: 263–290.
36 Ebenezer WJ. 1991. Colabomycin co-metabolites. Synthesis of 2880-II, a metabolite related to ferulic acid. Synth Commun 21: 351–358.
37 Ehmann A. 1974. *N*-(*p*-Coumaryl)-tryptamine and *N*-ferulyl-tryptamine in kernels of *Zea mays*. Phytochemistry 13: 1979–1983.
38 Elder DJE, P Morgan and DJ Kelly. 1992. Evidence for two differentially regulated phenylpropenoyl-coenzyme A synthase activities in *Rhodopseudomonas palustris*. FEMS Microbiol Lett 98: 255–260.
39 Elias HG and JA Placios. 1985. Poly(ferulic acid) by thionyl chloride activated polycondensation. Makromol Chem 186: 1027–1045.
40 Enoki A, Y Yajima and MH Gold. 1981. Olefin saturation and acid reduction of 3,4-dimethoxycinnamic acid and derivatives by *Phanaerochaete chrysosporium*. Phytochemistry 20: 1543–1546.
41 Fenton TW, MM Mueller and DR Clandinin. 1978. Isomerization of some cinnamic acid derivatives. J Chromatogr 152: 517–522.
42 Finkle BJ, JC Lewis, JW Corse and RE Lundin. 1962. Enzyme reactions with phenolic compounds: formation of hydroxystyrenes through the decarboxylation of 4-hydroxycinnamic acids by *Aerobacter*. J Biol Chem 237: 2926–2931.
43 Fonseca A and JP Sampaio. 1992. *Rhodosporidium lusitaniae* sp nov, a novel homothallic basidiomycetous yeast species from Portugal that degrades phenolic compounds. Syst Appl Microbiol 15: 47–51.
44 Ford CW and RD Hartley. 1989. GC/MS characterization of cyclodimers from *p*-coumaric and ferulic acids by photodimerization—a possible factor influencing cell wall biodegradability. J Sci Food Agric 46: 251–260.
45 Frías I, JM Siverio, C González, JM Trujillo and JA Pérez. 1991. Purification of a new peroxidase catalyzing the formation of lignan-type compounds. Biochem J 273: 109–113.
46 Friend J. 1981. Plant phenolics, lignification and plant disease. In: Progress in Phytochemistry, vol 7 (Reinhold L, JB Harborne and T Swain, eds), pp 197–261, Pergamon Press, Oxford, UK.
47 Graf E. 1992. Antioxidant potential of ferulic acid. Free Radical Biol Med 3: 435–448.
48 Grbic-Galic D. 1985. Fermentative and oxidative transformation of ferulate by a facultatively anaerobic bacterium isolated from sewage sludge. Appl Environ Microbiol 50: 1052–2057.
49 Grbic-Galic D, N Churchman-Eisel and I Mrakovic. 1990. Transformation of styrene by anaerobic consortia. J Appl Bacteriol 69: 247–260.
50 Grbic-Galic D and L La Pat-Polasko. 1985. *Entero cloacae* DG-6: a strain that transforms methoxylated aromatics under aerobic and anaerobic conditions. Curr Microbiol 12: 321–324.
51 Grbic-Galic D and LY Young. 1985. Methane fermentation of ferulate and benzoate: anaerobic degradation pathways. Appl Environ Microbiol 50: 292–297.
52 Greig-Smith PW and MF Wilson. 1989. Benzene compounds useful for avian control. UK Patent 88-29167.9.
53 Gross B, M Asther, G Corrieu and P Brunerie. 1991. Production de vanilline par bioconversion de precurseurs benzeniques. European Patent number 91401031.9.
54 Gross GG. 1985. Biosynthesis and metabolism of phenolic acids. In: Biosynthesis and Biodegradation of Wood Components (Higuchi T, ed), pp 229–271, Academic Press, New York.
55 Gupta JK, P Sharma, HW Kern and H Sahm. 1990. Degradation of synthetic lignins and some lignin monomers by the yeast *Rhodotorula glutinis*. World J Microbiol Biotechnol 6: 53–58.
56 Gurujeyalakshmi G and A Mahadevan. 1987. Dissimilation of ferulic acid by *Bacillus subtilis*. Curr Microbiol 16: 69–73.
57 Hagedorn S and B Kaphammer. 1994. Microbial biocatalysis in the generation of flavor and fragrance chemicals. Ann Rev Microbiol 48: 773–800.
58 Hartada T and Y Mino. 1976. Some properties of *p*-coumarate decarboxylase from *Cladosporium phlei*. Can J Microbiol 22: 1258–1262.
59 Hartmans S, JP Smits, MJ Van der Werf, F Volkering and JAM DeBont. 1989. Metabolism of styrene oxide and 2-phenyl-ethanol in styrene-degrading *Xanthobacter* strain 124X. Appl Environ Microbiol 55: 2850–2855.
60 Hartmans S, MJ Van der Werf and JAM DeBont. 1990. Degradation of styrene involving a novel flavin adenine dinucleotide-dependent styrene monooxygenase. Appl Environ Microbiol 56: 1347–1351.
61 Hashikado Y, M Urashima, T Yoshida and J Mizutani. 1993. Decarboxylative conversion of hydroxycinnamic acids by *Klebsiella oxytoca* and *Erwinia uredovora*, epiphytic bacteria of *Polymnia sonchifolia* leaf, possibly associated with formation of microflora on the damaged leaves. Biosci Biotech Biochem 57: 215–219.
62 Hatakeyama H, E Hayashi and T Haraguchi. 1977. Biodegradation of poly(3-methoxy-4-hydroxy styrene). Polymer 18: 759–763.
63 Henderson MEK. 1961. The metabolism of aromatic compounds related to lignin by some Hyphomycetes and yeast-like fungi of soil. J Gen Microbiol 26: 155–165.
64 Herrmann K. 1989. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. CRC Crit Rev Food Sci Nutr 28: 315–347.
65 Herrmann K. 1990. Significance of hydroxycinnamic compounds in food. I. Antioxidant activity—effects on the use, digestibility, and

microbial spoilage in food. Chem Mikrobiol Technol Lebensm 12: 137–144.
66 Herzog RO and O Ripke. 1908. Notiz uber die Umwandlung von Zimtsaure in Styrol durch Schimmelpilze. Z Physiol Chem 57: 43–45.
67 Hinteregger C, R Leitner, M Loidl, A Ferschl and F Streichsbier. 1992. Degradation of phenol and phenolic compounds by *Pseudomonas putida* EKII. Appl Microbiol Biotechnol 37: 252–259.
68 Huang Z, L Dostal and JPN Rosazza. 1993. Microbial transformations of ferulic acid by *Saccharomyces cereviseae* and *Pseudomonas fluorescens*. Appl Environ Microbiol 59: 2244–2250.
69 Huang Z, L Dostal and JPN Rosazza. 1994. Mechanisms of ferulic acid conversions to vanillic acid and guaiacol by *Rhodotorula rubra*. J Biol Chem 268: 23594–23598.
70 Huang Z, L Dostal and JPN Rosazza. 1994. Purification of a ferulic acid decarboxylase from *Pseudomonas fluorescens*. J Bacteriol 176: 5912–5918.
71 Ishikawa H, WJ Schubert and FF Nord. 1963. Investigations on lignins and lignification. XXVIII. The degradation by *Polyporus versicolor* and *Fomes fomentarius* of aromatic compounds structurally related to softwood lignin. Arch Biochem Biophys 100: 140–149.
72 Itoh A, T Tanahashi and N Nagakura. 1992. 6′-*O*-Feruloyl- and 6′-*O*-Sinapoyl-demethylalangisides, tetrahydroisoquinoline-mono-terpene glucosides from *Alangium platanifolium*. Phytochemistry 31: 1037–1040.
73 Iwahara S, Y Yagi and M Tabuchi. 1981. Microbial degradation of ferulic acid. Nippon Nogeikagaku Kaishi 55: 1089–1092.
74 Jaminet F. 1950. Hydrocarbonic fermentation in a tolu sirup mixture. J Pharm Belg (NS) 5: 191–201.
75 Jurkova M and M Wurst. 1993. Biodegradation of aromatic carboxylic acids by *Pseudomonas mira*. FEMS Microbiol Lett 111: 245–250.
76 Katayama Y and T Fukuzumi. 1978. Bacterial degradation of dimers structurally related to lignin. II. Initial intermediate products from dehydroconiferyl alcohol by *Pseudomonas putida*. Mokuzai Gakkaishi 24: 643–649.
77 Kelley CJ, RC Harruff and M Carmack. 1976. The polyphenolic acids of *Lithospermum ruderale*. II. Carbon-13 nuclear magnetic resonance of Lithospermic and Rosmarinic acids. J Org Chem 41: 449–455.
78 Khanna P, B Rajkumar and N Jothikumar. 1992. Anoxygenic degradation of aromatic substances by *Rhodopseudomonas palustris*. Curr Microbiol 25: 63–67.
79 Kirk TK and LF Lorenz. 1973. Methoxyhydroquinone, an intermediate of vanillate catabolism by *Polyporus dichrous*. Appl Microbiol 26: 173–175.
80 Kodaira K, Y Onishi and K Ito. 1980. An oligomerization of 2-methoxy-3-vinylphenol. Makromol Chem Commun 1: 427–431.
81 Korn M, R Wodarz, W Schjoknecht, H Weichardt and E Bayer. 1984. Styrene metabolism in man—Gas-chromatographic separation and mandelic-acid enantiomers in the urine of exposed persons. Arch Toxicol 55: 59–63.
82 Kunisada T, H Sakagami, M Takeda, T Naoe, Y Kawazoe, H Ushijima, WEG Muller and T Kitamura. 1992. Effect of lignins on HIV-induced cytopathogenicity and myeloperoxidase activity in human myelogenous leukemic cell lines. Anticancer Res 12: 2225–2228.
83 Kuo YH, PC Kuo and ST Lin. 1983. The sensitized photooxidation of methyl (E)-ferulate. Proc Natl Sci Counc, Repub China, Part B 7: 28–34.
84 Labuda IM, SK Goers and KA Keon. 1992. Bioconversion process for the production of vanillin. US Patent 5128253
85 Labuda IM, KA Keon and SK Goers. 1993. Microbial bioconversion process for the production of vanillin. In: Progress in Flavor Precursor Studies. Analysis-Generation-Biotechnology, Proceedings of the International Conference (Schreier P and P Winterhalter, eds), pp 477–482, Allured Publishing Corporation, 362 S Schmale Road, Carol Stream, IL 60188.
86 Lattanzio V, V Linsalata, G Maruca, R Marullo, BB Singh and P Perrino. 1988. Preexisting phenols and resistance to aphid in cowpea. Genet Agrar 42: 461–462.
87 Lüdemann HD and H Nimz. 1974. ^{13}C-Kernresonanzspektren von Ligninen, 1. Die Makromolekulare Chem. 175: 2393–2407.
88 Lyu S-W and U Blum. 1990. Effects of ferulic acid, an allelopathic compound, on net P, K, and water uptake by cucumber seedlings in a split-root system. J Chem Ecol 16: 2429–2439.
89 Manitto P, P Gramatica and BM Ranzi. 1975. Stereochemistry of the decarboxylation of phenolic cinnamic acids by *Saccharomyces cerevisiae*. J Chem Soc, Chem Comm 1975: 442–443.
90 Martin-Tanguy J. 1985. The occurrence and possible function of hydroxylcinnamoyl acid amides in plants. Plant Growth Regul 3: 381–399.
91 Middelhoven WJ. 1993. Catabolism of benzene compounds by ascomycetous and basidiomycetous yeasts and yeastlike fungi. Antonie van Leeuwenhoek 63: 125–144.
92 Middelhoven WJ, M Koorevaar and GW Schuur. 1992. Degradation of benzene compounds by yeasts in acidic soils. Plant and Soil 145: 37–43.
93 Middelhoven WJ and MD Sollewijn Gelpke. 1995. Partial conversion of cinnamic acid into styrene by growing cultures and cell-free extracts of the yeast *Cryptococcus elinovii*. Antonie van Leeuwenhoek 67: 217–219.
94 Muller HW, W Trosch and KD Kulbe. 1988. Effect of phenolic compounds on cellulose degradation on some white rot basidiomycetes. FEMS Microbiol Lett 49: 87–93.
95 Nakashima H, T Murakami, N Yamamoto, T Naoe, Y Kawazoe, K Konno and H Sakagami. 1992. Lignified materials as medicinal resources. V. Anti-HIV (human immunodeficiency virus) activity of some synthetic lignins. Chem Pharm Bull 40: 2102–2105.
96 Nali M, B Rindone, S Tollari, V Andreoni and V Treccani. 1985. Anaerobic microbial conversion of three hydroxycinnamic acids. Experientia 41: 1351–1353.
97 Nazareth S and S Mavinkurve. 1986. Degradation of ferulic acid via 4-vinylguaiacol by *Fusarium solani* (Mart) Sacc. Can J Microbiol 32: 494–497.
98 Nethaji M, V Pattabhi and GR Desiraju. 1988. Structure of 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid (ferulic acid). Acta Crystallogr, Sect C: Cryst Struct Commun C44: 275–277.
99 Nishida A and T Fukuzumi. 1978. Formation of coniferyl alcohol from ferulic acid by the white rot fungus *Trametes*. Phytochemistry 17: 417–419.
100 Nishiyama A, H Eto, Y Tereda, M Iguchi and S Yamamura. 1983. Anodic oxidation of 4-hydroxycinnamic acids and related phenols. Chem Pharm Bull 31: 2845–2852.
101 Ohmiya K, M Takeuchi, W Chen, S Shimizu and H Kawakami. 1986. Anaerobic reduction of ferulic acid to dihydroferulic acid by *Wolinella succinogenes* from cow rumen. Appl Microbiol Biotechnol 23: 274–279.
102 Ohta T, S Yamasaki, Y Egashira and H Sanada. 1994. Antioxidative activity of corn bran hemicellulose fragments. J Agric Food Chem 42: 653–656.
103 Ötük G.1985. Degradation of ferulic acid by *Escherichia coli*. J Ferment Technol 63: 501–506.
104 Palacios JA and C Perez. 1990. Poly(ferulic acid) by oxalyl chloride activated polycondensation. New Polymeric Mater 2: 167–174.
105 Pometto AL III, JB Sutherland and DL Crawford. 1981. *Streptomyces setonii*: catabolism of vanillic acid via guaiacol and catechol. Can J Microbiol 27: 636–638.
106 Pratt DE. 1992. Natural antioxidants from plant material. In: Phenolic Compounds in Food and Their Effects on Health. II. Antioxidants and Cancer Prevention. ACS Symposium Series 507 (Huang M-T, C-T Ho and CY Lee, eds), Ch 5, pp 54–71, American Chemical Society, Washington, DC.
107 Putman LJ, PE Laks and MS Pruner. 1989. Chemical constituents of black locust bark and their biocidal activity. Holzforschung 43: 219–224.
108 Rahouti M, F Seigle-Murandi, R Steiman and K-E Eriksson. 1989. Metabolism of ferulic acid by *Paecilomyces variotii* and *Pestalotia palmarum*. Appl Environ Microbiol 55: 2391–2398.
109 Ralph J, RF Helm, S Quideau and RD Hatfield. 1992. Lignin-feruloyl ester cross-links in grasses. Part 1. Incorporation of feruloyl esters into coniferyl alcohol dehydrogenation polymers. J Chem Soc, Perkin Trans I: 2961–2969.
110 Ralph J, RH Helm and S Quideau. 1992. Lignin-feruloyl ester cross-links in grasses. Part 2. Model compound syntheses. J Chem Soc, Perkin Trans I: 2971–2980.
111 Renard G, J-C Guilleux, C Bore, V Malta-Valette and DA Lerner. 1992. Synthesis of L-phenylalanine analogs by *Rhodotorula glutinis*. Bioconversion of cinnamic acids derivatives. Biotechnol Lett 14: 673–678.
112 Sakagami H, T Oh-Hara, K Kohda and Y Kawazoe. 1991. Lignified

materials as a potential medicinal resource. IV. Dehydrogenation polymers of some phenylpropenoids and their capacity to stimulate polymorphonuclear cell iodination. Chem Pharm Bull 39: 950–955.
113 Samejima M, N Tatarazako, T Arakawa, Y Saburi and T Yoshimoto. 1987. Metabolism of 3,4-dimethoxycinnamic acid and ferulic acid by mutant strains derived from *Pseudomonas* sp TMY1009. Mokuzai Gakkaishi 33: 728–734.
114 Scheline RR. 1968. Metabolism of phenolic acids by the rat intestinal microflora. Acta Pharmacol Toxicol 26: 189–205.
115 Seigle-Murandi F, R Steiman, M Rahouti, JL Benoit-Guyod and KEL Eriksson. 1990. Metabolism of ferulic and syringic acids by micromycetes. Microbiologica 13: 191–200.
116 Shafer SR and U Blum. 1991. Influence of phenolic acids on microbial populations in the rhizosphere of cucumber. J Chem Ecol 17: 369–389.
117 Stead D. 1993. The effect of hydroxycinnamic acids on the growth of wine-spoilage lactic acid bacteria. J Appl Bacteriol 75: 135–141.
118 Strack D. 1990. Metabolism of hydroxycinnamic acid conjugates. Bull Liaison—Groupe Polyphenols (15) 1990: 55–64.
119 Suga T, S Ohta, K Munesada, N Ide, M Kurokawa, M Shimizu and E Ohta. 1993. Endogenous pine wood nematicidal substances in pines. *Pinus massoniana*, *P. strobus* and *P. palustris*. Phytochemistry 33: 1395–1401.
120 Sutherland JB, DL Crawford and AL Pometto III. 1983. Metabolism of cinnamic, *p*-coumaric and ferulic acids by *Streptomyces setonii*. Can J Microbiol 29: 1253–1257.
121 Takei Y, K Mori and M Matsui. 1973. Synthesis of DL-Matairesinol dimethyl ether, dehydrodimethylconidendrin and dehydrodimethylretodendrin from ferulic acid. Agr Biol Chem 37: 637–641.
122 Tan K-S, T Hoson, Y Masuda and S Kamisaka. 1992. Effect of ferulic and *p*-coumaric acids on *Oryza* coleoptile growth and the mechanical properties of cell walls. J Plant Physiol 140: 460–465.
123 Tillet R and JRL Walker. 1990. Metabolism of ferulic acid by a *Penicillium* sp. Arch Microbiol 154: 206–208.
124 Toms A and JM Wood. 1970. The degradation of trans-ferulic acid by *Pseudomonas acidovorans*. Biochemistry 9: 337–343.
125 Toro GIR, GR Leather and FA Einhellig. 1988. Effects of three phenolic compounds on *Lemna gibba* G3. J Chem Ecol 14: 845–853.
126 Turner JA and EL Rice. 1975. Microbial decomposition of ferulic acid in soil. J Chem Ecol 1: 41–58.
127 Van Boven M, S Toppet, MM Cokelaere and P Daenens. 1994. Isolation and structural identification of a new simmondsin ferulate from jojoba meal. J Agric Food Chem 42: 1118–1121.
128 Van Huystee RB and X Zheng. 1993. Cationic peanut peroxidase and the oxidation of ferulic acid. Phytochemistry 34: 933–939.
129 Von Hlasiwetz H and L Barth. 1866. Ueber einige Harze [Zersetzungsproducte derselben durch schmelzendes Kali]. Liebig's Annalen der Chemie 138: 61–76.
130 Wacker TL, GR Safir and CT Stephens. 1990. Effects of ferulic acid on *Glomus fasciculatum* and associated effects on phosphorus uptake and growth of asparagus. J Chem Ecol 16: 901–909.
131 Warhusrt AM, KF Clark, RA Hill, RA Holt and CA Fewson. 1994. Metabolism of styrene by *Rhodococcus rhodochrous* NCIMB 13259. Appl Environ Microbiol 60: 1137–1145.
132 Watson SA. 1987. Structure and composition. In: Corn: Chemistry and Technology (Watson SA and PE Ramsted, eds), pp 53–82, American Association of Cereal Chemists, St Paul, MN.
133 Whited GM and DT Gibson. 1991. Separation and partial characterization of the enzyme of the toluene-4-monooxygenase catabolic pathway in *Pseudomonas mendocina* KR1. J Bacteriol 173: 2517–2519.
134 Whiting GC and JG Carr. 1959. Metabolism of cinnamic acid and hydroxy-cinnamic acids by *Lactobacillus pastorianus* var *quinicus*. Nature 184: 1427–1428.
135 Zimmerlin A, P Wojtaszek and GP Bolwell. 1994. Synthesis of dehydrogenation polymers of ferulic acid with high specificity by a purified cell-wall peroxidase from french bean (*Phaseolus Vulgaris* L.). Biochem J 299: 747–753.