DOI: 10.1002/adsc.200700043

Laccase-Catalyzed Dimerization of Hydroxystilbenes

Chiara Ponzoni,[a] Elisa Beneventi,[a,b] Maria Rita Cramarossa,[a] Stefano Raimondi,[a] Giulia Trevisi,[a,b] Ugo Maria Pagnoni,[a] Sergio Riva,[b,*] and Luca Forti[a,*]

[a] Dipartimento di Chimica, Università di Modena e Reggio Emilia, Via Campi 183, 41100 Modena, Italy
Fax: (+39)-059-373-543; e-mail: forti.luca@unimore.it
[b] Istituto di Chimica del Riconoscimento Molecolare, CNR, Via Mario Bianco 9, 20131 Milano, Italy
Fax: (+39)-02-2890-1239; e-mail: sergio.riva@icrm.cnr.it

Received: January 23, 2007; Revised: March 30, 2007

Supporting information for this article is available on the WWW under http://asc.wiley-vch.de/home/.

Abstract: A series of hydroxystilbenes, analogues of the bioactive phytoalexin resveratrol, were synthesized and submitted to the catalytic action of a laccase from *Trametes pubescens* in a biphasic system made of ethyl acetate and acetate buffer. Oxidation took place at the 4′-hydroxy (4-hydroxy) position of the hydroxystilbenic moieties, followed by radical-radical coupling dimerization reactions. Most of the products were isolated in good yields and fully characterized. Depending on the substrates, three different dimeric products could be identified, the main products usually being 4-O-α-ß-5 (dihydrofuran-like) dimers.

Keywords: biotransformations; enzyme catalysis; laccase; oxidoreductases; radical reactions; resveratrol

Introduction

trans-Resveratrol (3,5,4′-trihydroxystilbene, **1**; Figure 1) is a stilbenic phytoalexin produced by plants, particularly by grapevines, pines and legumes, *via* a metabolic sequence induced in response to biotic or abiotic stress factors.[1] It has been proposed to be one of the components in red wines with beneficial effects to human cardiovascular health (French paradox).[2] In addition to resveratrol, its oligomers, the so-called "viniferins", have also been found in plants[3] as a result of infection or stress and are supposed to be formed by oxidative dimerization catalyzed by plant peroxidases and/or phenoloxidases.

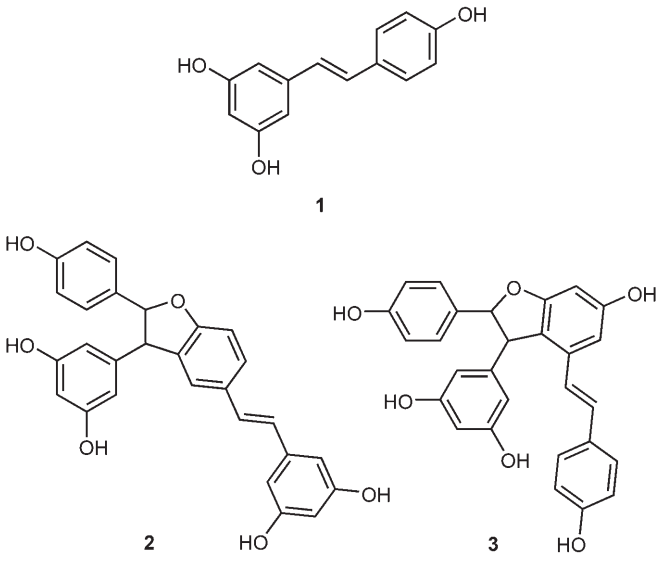


Figure 1.

Both monomeric and oligomeric stilbenes are reported to be potentially important cancer chemoprotective agents, being able to inhibit cellular events associated with carcinogenesis.[4] More recently it has been claimed that resveratrol might also have beneficial effects when used for treating obesity-related disorders and diseases of ageing.[5] Despite that, as many of these compounds are exclusively obtained by extraction from natural sources, the studies of their biological properties are limited by their very scarce availability.

Few synthetic approaches to the oxidative coupling of resveratrol have been reported. For instance, by treating **1** with 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) a major product was isolated in 18% yields and identified as *trans*-resveratrol dehydrodimer **2** (Figure 1).[6] On the other hand, the treatment of **1** with $FeCl_3$ in methanol failed to give **2**, affording the isomeric dimer ε-viniferin (**3**; Figure 1) as the sole product, although in low yield.[7] More recently, Sako described the non-enzymatic synthesis of **2** involving the treatment of **1** with AgOAc and other metallic oxidants.[8] Similarly, Takaya and co-workers described

the treatment of **1** with several oxidizing reagents and found that $Tl(NO_3)_3$ and $K_3[Fe(CN)_6]$ were the best oxidants to transform **1** into **3**, whereas $FeCl_3$ in acetone and MnO_2 in dichloromethane were the best catalysts to produce the dimer **2**.[9]

Different studies have shown that resveratrol is transformed *in vivo* (for instance by incubating **1** with *Botrytis cinerea* cultures) into various oxidized products (dimers), such as **2**.[10,11] On the other hand, to the best of our knowledge, there are only few reports on the *in vitro* biocatalyzed oxidation of **1**: two of them describe a horseradish peroxidase-hydrogen peroxide promoted dimerization of **1** to **2**,[12] one reports on the oxidative degradation of **1** by action of a soybean lipoxygenase (product structures not identified),[13] and a fourth one is on the formation of viniferin by action of a COX-1 peroxidase.[14] A purified laccase isolated from a *B. cinerea* strain was also used,[15] but this latter reaction was just run on an analytical scale, monitored by HPLC, and the formed product was identified as ε-viniferin (**3**) simply by comparison with the HPLC elution time of an authentic sample. Finally, the *B. cinerea* laccase-catalyzed dimerization of the resveratrol derivative pterostilbene (3,5-dimethoxy-4′-hydroxystilbene) was reported by Breuil[16] and co-workers, who described the synthesis and characterization of a pterostilbene *trans*-dehydrodimer.

Laccases are oxidoreductases (the so-called "blue oxidases"), widely distributed in fungi and in some bacteria and higher plants. The active site of these enzymes consists of a metallic cluster containing four copper atoms, all of them being involved in the redox process *via* a radical cyclic mechanism in which one oxygen molecule is reduced to give two water molecules, while four substrate molecules (usually phenols or aliphatic/aromatic amines) are oxidized to the corresponding reactive radicals.[17] Some of these enzymes have recently been cloned and overexpressed and are becoming commercially available.[18]

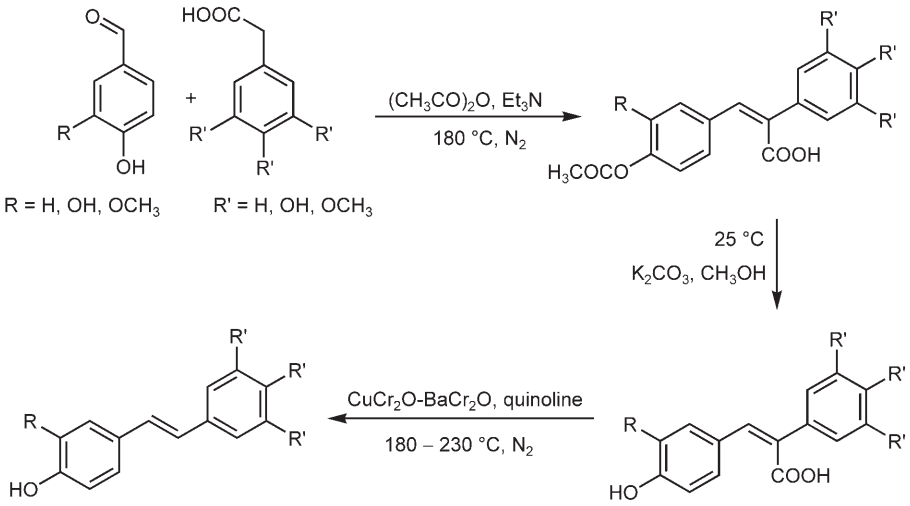
As stated before, phenols are among the most suitable laccases substrates.[17,19] The oxidations proceed *via* formation of radical cations and subsequent deprotonation of the phenolic hydroxy groups to give phenoxy radicals that can undergo a broad variety of coupling reactions.[20] The main drawback of these biotransformations is the extensive polymerization that may arise from the radical mechanism of the oxidative process, producing quite often a complex mixture of polyphenolic oligomers.[21]

As a part of our general interest in biocatalysis[22] and, more specifically, in the enzyme-catalyzed carbon-carbon bond formation,[23] we have started an investigation on the synthetic exploitation of laccases for the oxidative dimerization of phenolic derivatives such as tetrahydronaphthol,[24] estradiol,[25] and, more recently, totarol.[26] The preparative-scale oxidation of resveratrol was also one of our synthetic goals, and a couple of years ago we described the laccase-catalyzed dimerization of **1**: under the best reaction conditions, the dehydrodimer **2** was isolated in 31% yields; we have also shown that the laccase-mediated oxidation of **1** took place at the *para* 4′-OH.[27] In this paper we report an extension of this preliminary work, describing the laccase-mediated oxidation of a series of hydroxystilbenes in a biphasic system.

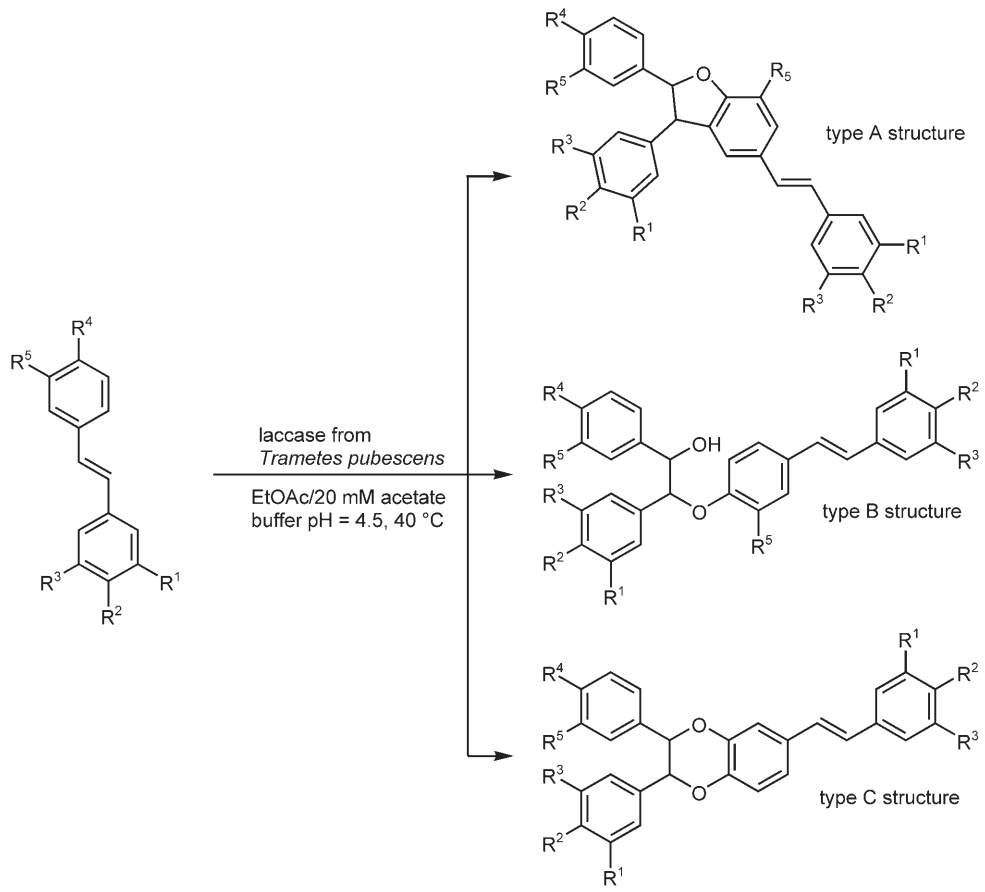
Results and Discussion

Synthesis of Substrates

As described in Scheme 1, the resveratol analogues were synthesized by Perkins condensation at 180 °C, starting from the suitably substituted hydroxybenzaldehydes and phenylacetic acids in the presence of



Scheme 1. Synthesis of the the hydroxystilbenes **4**–**13**.



Substrate	R^1	R^2	R^3	R^4	R^5	Products Type A	Type B	Type C
4	H	OH	H	OH	H	**4a**	**4b**	-
5	H	OCH_3	H	OH	H	**5a**	**5b**	-
6	H	OH	H	OH	OCH_3	**6a**	-	-
7	H	H	H	OH	H	**7a**	**7b**	-
8	OH	H	OH	OCH_3	H	-	-	-
9	OCH_3	H	OCH_3	OH	H	**9a**	**9b**	-
10	H	OH	H	OH	OH	**10a**	-	**10c**
11	OCH_3	OCH_3	H	OH	H	**11a**	**11b**	-
12	H	OCH_3	H	OH	OH	**12a**	-	**12c**
13	H	OCH_3	H	OH	OCH_3	**13a**	**13b**	-

Scheme 2. Structures of the substrates **4–13** and of the dimers isolated and characterized from the *T. pubescens* laccase-catalyzed oxydation of these hydroxystilbenes.

acetic anhydride and triethylamine. The resulting carboxylic acids were deprotected with K_2CO_3 in MeOH at room temperature and decarboxylated with $CuCr_2O$-$BaCr_2O$ and quinoline by refluxing the solution at 230 °C under an N_2 atmosphere.[28] In this way ten different hydroxystilbenes (compounds **4–13**, Scheme 2) were isolated and characterized (see Experimental Section and Supporting Information).

Laccase-Catalyzed Oxidative Dimerization of Compounds 1, 4–13

After some experimentation (reaction performed in monophasic organic solvents,[27] monophasic water/water miscible organic solvents,[26] biphasic systems made of water/water immiscible organic solvents,[24] use of immobilized enzyme[27]), it was found that the best reaction conditions were based on the use of a laccase from *Trametes pubescens* in a mildly shaken biphasic system made of an organic phase (AcOEt)

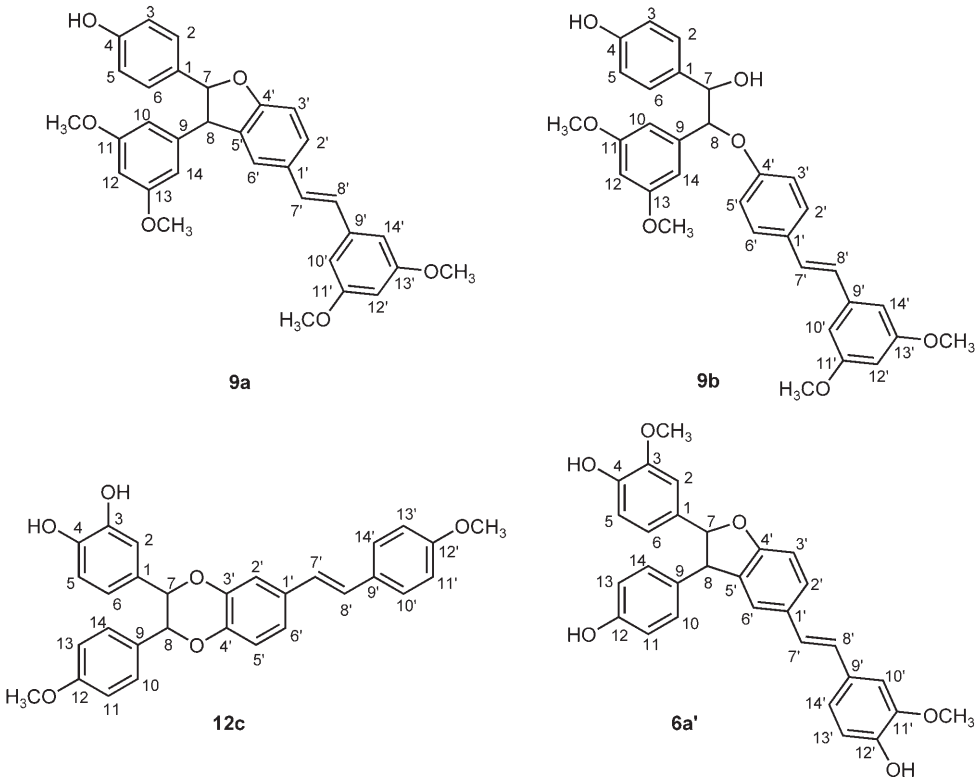


Figure 2.

containing the substrate and a water phase (acetate buffer, pH 4.5) containing the dissolved enzyme.

All the reactions, monitored by TLC and RP-HPLC, typically afforded at least four to five products. Most of them were isolated by flash chromatography and identified by EI-MS and extensive mono- and bi-dimensional NMR analysis. It is well-known that the laccase-catalyzed dimerization of phenolic compounds proceeds through the formation of a phenoxy radical. Due to the capability of electron-delocalized radicals to couple at various sites, different dimeric products are found, depending on the structural features of the starting substrates. As shown in Scheme 2, depending on the starting hydroxystilbenic substrate it was possible to identify three kinds of products: the substituted dihydrofuran dimers (type A structure, already isolated in the previously reported oxidation of resveratrol[27]), a group of "open" hydroxylated dimers (type B structure), and, in some cases, a group of dioxane-like dimers (type C structure).

As an example, the biocatalyzed oxidation of 3,5-dimethoxy-4′-hydroxystilbene (**9**) and the structural characterization of its dimers will be discussed in detail. The incubation of **9** (150 mg, dissolved in 7 mL AcOEt) with the *Trametes pubescens* laccase (10 U) furnished at least five products, as monitored by silica gel (eluent hexane/AcOEt, 7/3) or reverse phase TLC (eluent CH_3CN/H_2O, 7/3). The two most abundant products (**9a** and **9b**) were isolated by flash chromatography and characterized with HPLC-MS and mono- and bi-dimensional ^1H and ^{13}C NMR.

The mass analysis of a sample of the less polar product showed a quasi-molecular ion of 510 Da, consistent with the strucure of a dehydrodimer, while its ^1H NMR spectrum gave some additional information that allowed its identification with the structure **9a** of Figure 2.

Despite the fact that the product was a dimer, only one phenolic OH (a singlet at $\delta = 9.56$) and four aromatic AA′BB' protons (two doublets at $\delta = 7.22$ and 6.80, $J = 8.5$ Hz) were present, due to a single 4-hydroxybenzoyl moiety. Additional signals were due to two 3,5-dihydroxybenzoyl systems (a doublet – counting two protons – at $\delta = 6.41$ with $J = 2.0$ Hz, due to H-10 and H-14, a triplet at $\delta = 6.45$ with $J = 2.0$ Hz due to H-12, a triplet at $\delta = 6.38$ with $J = 1.6$ Hz due to H-12' and, finally, a doublet at $\delta = 6.74$ with $J = 1.6$ Hz due to H-10′ and H-14′), to two *trans* olefinic protons (two doublets at $\delta = 6.96$ and 7.23, with a large coupling constant of 16.9 Hz, H-7′ and H-8′) and to two aliphatic protons (doublets at $\delta = 4.62$ and 5.60, $J = 8.5$ Hz, H-8 and H-7). Further signals were due to aromatic protons H-2′ (doublet at $\delta = 7.46$, $J = 8.5$ Hz), H-3′ (doublet at $\delta = 6.93$, $J = 8.5$ Hz) and H-6′ (singlet at $\delta = 7.23$). As discussed in the introduction, the same product was previously isolated and characterized by Breuil et al.[16] However, this previous

report is lacking a detailed assignment of the carbon atoms as well as the geometrical arrangement with respect to the C-7/C-8 bond.

The correct structural assignements could be made on the basis of homonuclear bidimensional correlation (^{1}H,^{1}H-COSY), heteronuclear ^{1}H,^{13}C-HSCQ[29] and and long-range ^{1}H,^{13}C-HMBC[30] correlation experiments. The long-range correlation between the doublet at $\delta = 6.80$ and the aliphatic carbon at $\delta = 56.3$ (related to the doublet at $\delta = 5.60$) as well as between the doublet at $\delta = 7.22$ and the carbon at $\delta = 94.72$ (related to the doublet at $\delta = 4.62$) allowed us to establish that the 4-hydroxybenzoyl moiety was bonded to C-7 whereas one of the 3,5-dimethoxybenzoyl systems was linked to C-8. The low, but still detectable, observed correlation between H-7 ($\delta = 5.60$) and C-4′ ($\delta = 159.4$) suggested the presence of a fused oxygenated five-membered ring (and was in favor of a dihedral angle between H-7, C-7, the oxygen atom and C-4 close to 90°). All these data were consistent with the proposed "closed" dehydrodimer **9a**. Moreover, the value of 8.5 Hz for the coupling constant (^3J) between H-7 and H-8 suggested a predominant pseudo-*trans*-axial arrangement for these two aliphatic protons. This conformation was confirmed by NOESY experiments (400 ms mixing time):[31] the recorded spectrum clearly showed the absence of NOE in-phase cross-peaks between H-7 and H-8, and the presence of positive in phase cross-peaks between H-7 and H-10, H-14.

Finally, the enantiomeric composition [ratio of the (7*S*,8*S*)- and (7*R*,8*R*)- enantiomers] of the product was evaluted by HPLC analysis on a chiral Chiralcel OD column (retention time for the two enantiomers, 6.75 and 9.42 min, eluent petroleum ether:2-propanol, 65:35) and it was found that **9a** was a racemic mixture of the *trans*-diastereoisomer, thus showing that the enzyme-inducted radical reaction was non-stereoselective.

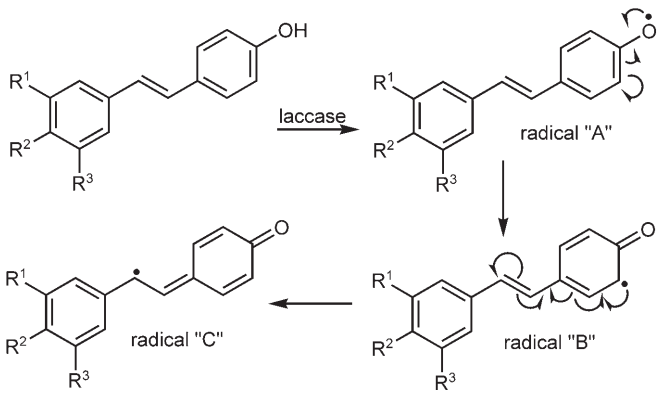
The mass spectrum of the more polar product had a quasi-molecular ion of 528 Da, that is 18 amu more than the value obtained with **9a**. Its ^{1}H NMR spectrum also showed significant differences with respect to the previous compound. The signals due to two protonic systems AA′BB′ were identified (AA′ at $\delta =$ 6.60, H-3 and H-5; BB′ at $\delta = 7.02$, H-2 and H-6 with $J = 8.5$ Hz. AA′ at $\delta = 6.90$ ppm, H-3′ and H-5′; BB′ at: $\delta = 7.42$, H-2′ and H-6′, $J = 8.7$ Hz) and an additional doublet at $\delta = 5.48$ was present and could be assigned to an alcoholic proton of an OH bonded to the C-7 (and, in turn, the signal due to the H-7 proton appeared as a double doublet). The heteronuclear longe-range ^{1}H,^{13}C-HMBC analysis showed that C-4′ was not correlated to H-7, thus excluding the presence of a furanic ring, but, instead, to H-8, thus showing the existence of a bond between C-8 and the oxygen linked to C-4′. The other signals confirmed the presence of two 3,5-dimethoxybenzoyl moieties, of one phenolic and of two *trans*-olefinic protons (for details see the Experimental Section and Supporting Information), thus allowing the identification of this compound with the "open" dimeric structure of **9b** shown in Figure 2.

All the other substrates furnished the same kind of products, with the exception of the 4′-methoxy derivative **8** which, as expected, was recovered unreacted. Additional dimeric structures could be identified from the oxidative reactions of the substrates carrying a cathecolic group (**10** and **12**) or two *para*-phenolic moieties (**6**). For instance, the laccase oxidation of **12** afforded two main products. The ^{1}H NMR spectrum of the more polar compound allowed its identification with **12a**, possessing the typical dihydrofuran "type A" dimeric structure. Conversely, the usual careful mono- and bi-dimensional NMR analysis of the less polar compound characterized it as the dioxan-like dimer **12c** with a "type C" structure (Figure 2, see Experimental Section and Supporting Information for details). Finally, a particular case was represented by the smoothly reacting substrate **6**, which gave **6a** ("type A" dimer) as the major product and **6a′** (Figure 2) as the minor one (see below for a comment on the mechanism explaining its formation).

The overall results are collected in Table 1 and can be summarized as follows: a) The products derived from substrates with only one reactive phenolic OH group (**1**, **4**, **5**, **7, 9, 11** and **13**) were of two types: *trans*-dihydrofurans dimers (type A structure) and

Table 1. Reaction times and products isolated from the laccase-catalyzed oxidation of the hydroxystilbenes **1, 4–13**.

Substrate (mg)	Reaction Time [h]	Products (mg)
1 (100)	24	**1a** (33)
		1b (5)
4 (100)	70	**4a** (19)
		4b (8)
5 (90)	120	**5a** (31)
		5b (11)
6 (200)	70	**6a** (16)
		6a′ (37)
7 (50)	120	**7a** (10)
		7b (5)
8 (65)	120	-
9 (150)	120	**9a** (88)
		9b (9)
10 (65)	24	**10a** (6)
		10c (12)
11 (150)	52	**11a** (27)
		11b (8)
12 (65)	96	**12a** (9)
		12c (9)
13 (150)	48	**13a** (44)
		13b (12)



Scheme 3.

open dimers (type B structure). b) Substrates carrying an *orthio*-diphenolic group (**10** and **12**) afforded the *trans*-dihydrofurans dimers (type A structure) and the *trans*-dioxane dimers (type C structure). c) The substrate carrying two reactive *para*-phenolic groups (**6**) gave two different *trans*-dihydrofurans dimers (**6a** and **6a′**). d) The *meta*-diphenolic groups remained unreactive under our reaction conditions. Accordingly, compound **8** did not give any product.

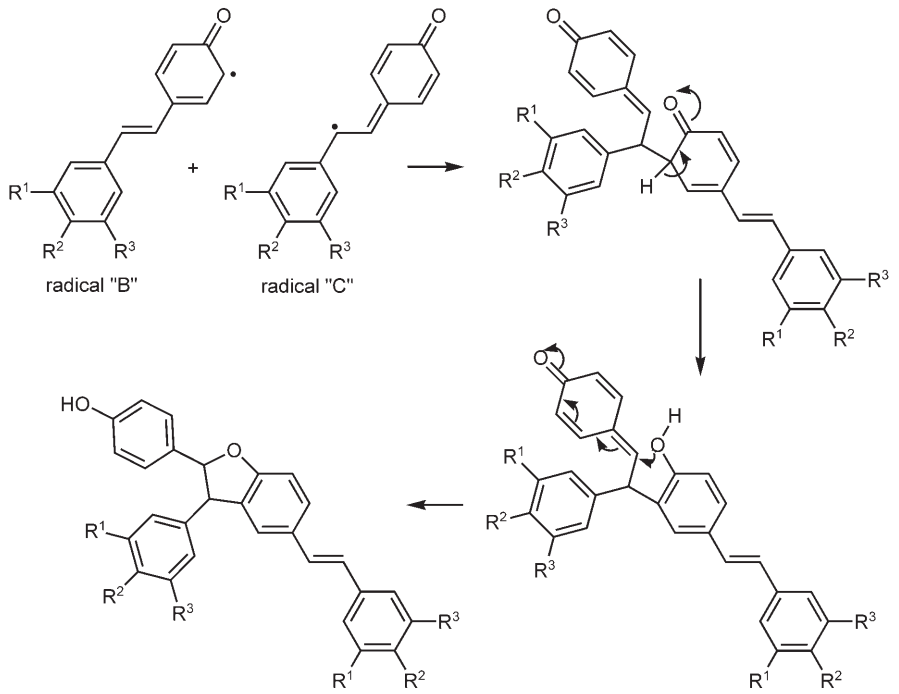
On the basis of the results obtained, a mechanism for the synthesis of the different dimeric derivatives can be proposed. The dimeric structures indicate that the oxidation reactions took place at the 4′-OH (4-OH) position of the hydroxystilbenic moieties. In turn, the formed phenoxy radicals could delocalize themselves as reported in Scheme 3.

Successively, the coupling of one radical "B" and one radical "C", followed by tautomeric rearrangment and intramolecular nucleophilic attack to the intermediate quinone, gave the dihydrofuran dimers **Xa** (Scheme 4). A particular case was represented by the reaction of the smoothly reacting substrate **6**, which could be oxidized at the two reactive phenolic OH and, accordingly, two dimers (**6a** and **6a′**) could be formed with the same mechanism.

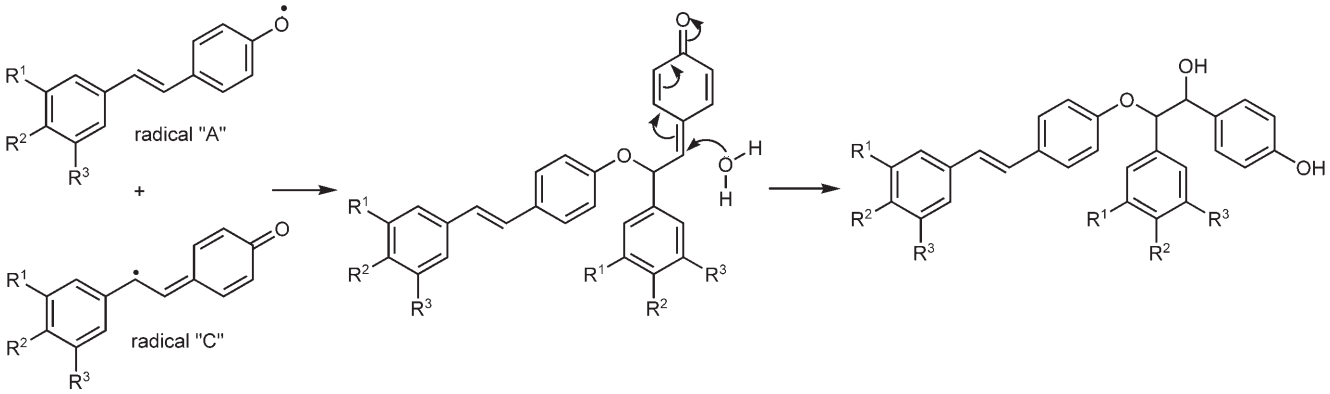
In turn, the formation of the "open" hydroxylated dimers (type "B"structure) can be easily explained by the coupling of one radical "A" with one radical "C", followed by addition of a water molecule to the intermediate quinones (Scheme 5).

Finally, the coupling of the same radicals ("A" and "C") originated by the catechol substrates **10** and **12**, followed by intramolecular nucleophilic attack to the intermediate quinones can explain the production of the dioxane-like dimers (type "C" structure, Scheme 6).

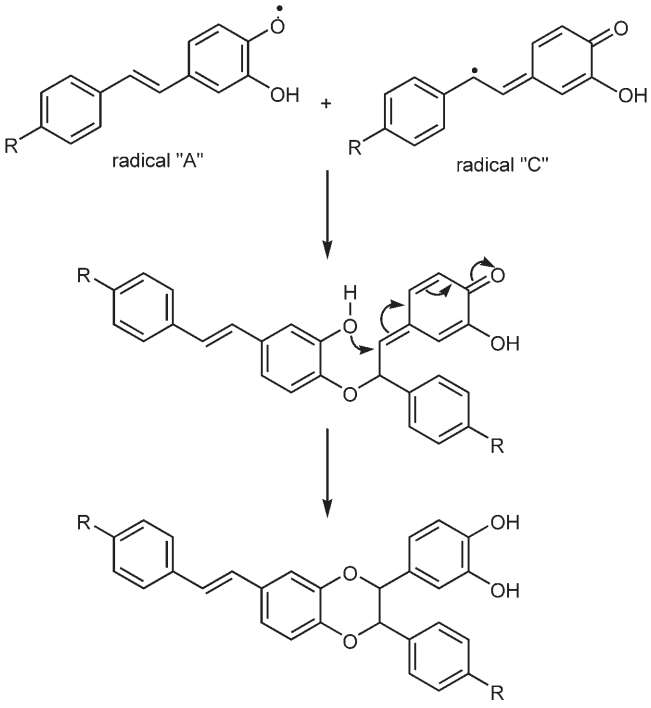
In order to verify the proposed mechanisms, that is to confirm that the dimer formation proceeded *via* radical-radical coupling and not *via* radical-to-double bond addition, a series of laccase-mediated oxidations of mixtures of two substrates were performed. In particular, the reaction between equimolar amounts of the reactive resveratrol (**1**) and of the unreactive hydroxystilbene **8** afforded exclusively the dimeric derivatives of resveratrol **1a** and **1b**. Moreover, in the laccase-mediated oxidation of the quite reactive substrate **12** in the presence of equimolar amount of the less reactive **7**, only the dimeric derivatives of the



Scheme 4.



Scheme 5.



Scheme 6.

more reactive substrate **12a** and **12c** could be isolated. The results of these two experiments clearly supported our hypothesis that the reaction proceeded through radical-radical coupling and not through the radical addition to the double bond of a non-radical substrate. These results were in accordance with most of the literature reports[8,9] and not with the hypothesis suggested by Penning and co-workers, who proposed a mechanism in which the resveratrol dimerization occurs *via* radical attack to a second molecule of resveratrol.[14]

Conclusions

Exploiting laccase catalysis, dimerization of various hydroxystilbenes could be observed. It has been shown that these biocatalyzed reactions, which represent an example of carbon-carbon bond formation under mild conditions, proceeded through radical-radical coupling, and the principal reaction products (that is the ß-O-4, the 4-O-α-ß-5, and the 3-O-α-ß-O-4 dimer, Figure 3) were obtained in different amounts depending on the structure of the starting substrates.

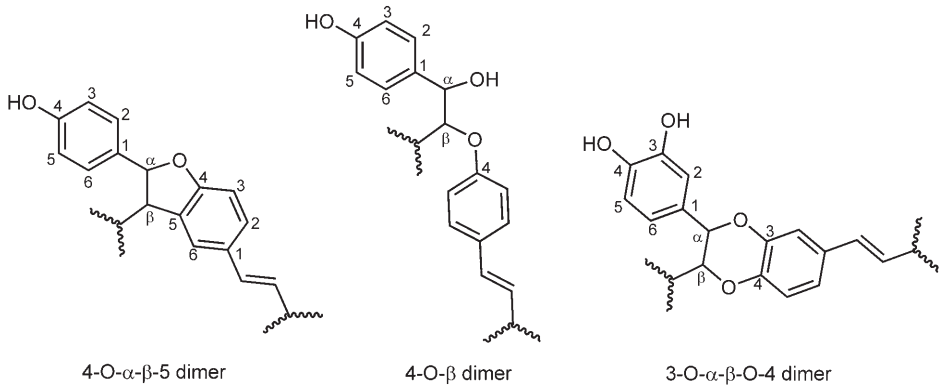


Figure 3.

To the best of our knowledge, up to now this is the most comprehensive report on the production and oxidation of hydroxystilbenes, analogues of the phytoalexin resveratrol, even considering previous protocols exploiting chemical catalysts.

As oligomeric hydroxystilbene derivatives have been reported to be potentially important cancer chemoprotective agents,[32] the products obtained in this work will be tested for their antiproliferative activity.[4c] The results will be reported in due course.

Experimental Section

Materials and Methods

TLC: precoated silica gel 60 F254 plates (Merck). Flash chromatography: silica gel 60 (70–230 mesh, Merck). Mass spectra were acquired with a combined HPLC (HP-1050) particle beam MS-engine (HP-5989). NMR spectra were recorded in DMSO-d_6 solutions at 400 MHz (Bruker FT-NMR AVANCE400) unless otherwise stated and the chemical shifts reported are in the δ (ppm) scale relative to tetramethylsilane as external standard.

The laccase from *Trametes pubescens* was provided by Prof. Haltrich (BOKU University, Wien, Austria). The enzyme was isolated as previously described,[33] and its activity evaluated by monitoring the oxidation of ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] at 436 nm. An enzymatic solution (10 μL) was added to a 1 mL cuvette containing 20 mM acetate buffer pH 3.5 (890 μL) and ABTS (100 μL of a 10 mM solution of ABTS in H_2O). One enzyme unit is defined as the amount of laccase that oxidizes 1 μmol of ABTS per minute under these conditions (ε ABTS 29.3 $mM^{-1} cm^{-1}$).

Similar results were obtained with the laccase from *Trametes versicolor* commercialized by Fluka.

General Procedure for the Synthesis of the Substrates 4–13

Synthesis of 3,5-dimethoxy-4′-hydroxystilbene (9)*; a) Perkin reaction:* Under nitrogen, 2 g of 3,5-dimethoxybenzaldehyde, 2.59 g of 4-hydroxyphenylacetic acid (1,2 equivs.), 5 mL of triethylamine and 5 mL of acetic anhydride, were heated at 180 °C for 10 h. The reaction was monitored with TLC (hexane/AcOEt/MeOH, 4/6/0.5). The reaction mixture was cooled at room temperature, diluted with 50 mL H_2O and 50 mL AcOEt and washed with 5% HCl. The aqueous layer was extracted with AcOEt (3×50 mL). The organic layer was washed with brine (50 mL), dried over $MgSO_4$ and concentrated. The intermediate product obtained was identified by GC-MS.

b) Deacetylation and decarboxylation: The product was then dissolved in 75 mL MeOH and treated with K_2CO_3 (10 g) at room temperature for 3 h. The deacetylation reaction was monitored by TLC (hexane/AcOEt/MeOH, 4/6/0.5). The reaction mixture was diluted with 5% $NaHCO_3$ and extracted with AcOEt. The aqueous phase was acidified with HCl and extracted with AcOEt. The organic phase was dried over Na_2SO_4 and concentrated.

Quinoline (15 mL, 11 equivs.) was then added to the crude residue together with 5000 mg of copper chromite. The reaction flask was heated for 7 h at 240 °C under nitrogen. The mixture was then cooled and filtered over celite, which was washed with 2×20 mL of AcOEt. The organic layer was washed with 2×20 mL 5% HCl, dried over $NaSO_4$ and concentrated. The brown oil obtained was purified by chromatography on silica gel (hexane/AcOEt, 6.5/3.5) to give 3,5-dimetoxy-4′-hydroxy-stilbene (**9**) as a white solid; yield: 44%. ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta = 3.78$ (s, 6H, $2OCH_3$), 6.38 (dd, 1H, $J = 2.22$ Hz, H-4), 6.73 (d, 2H, $J = 2.22$ Hz, H-2,6), 6.78 (d, 2H, $J = 8.61$ Hz, H-3′,5′), 6.86 and 6.96 (AB system, 2H, $J = 16.42$ Hz, CH=CH), 7.43 (d, 2H, $J = 8.61$ Hz, H-2′,6′), 9.56 (s, 1H, OH).

All the other substrates, with the exception of **8**, were synthesized following the same procedure.[28]

Synthesis of *trans*-3,5-Dihydroxy-4′-methoxystilbene (8)[28c]

a) 3,5-Diisopropoxybenzaldehyde: Isopropyl bromide (3.1 mL, 4.5 equivs.) and then anhydrous potassium carbonate (3.3 g, 3.3 equivs.) were added at room temperature to 1 g of 3,5-dihydroxybenzaldehyde dissolved in 10 mL DMF. After 24 h the reaction mixture was quenched with H_2O (30 mL), the organic layer was extracted with AcOEt (3×30 mL), dried over $MgSO_4$, filtrated and concentrated. The resulting dark red oil was purified by chromatography on silica gel (hexane/AcOEt 9:1) to give a pale yellow oil.

b) Perkin reaction and decarboxylation: The Perkin reaction between 3,5-diisopropoxybenzaldehyde and 4-methoxyphenylacetic acid and the subsequent decarboxylation were performed as reported in the previous section to give a mixture of *cis*- and *trans*-3,5-diisopropoxy-4-methoxystilbene).

c) Isomerization: Iodine (230 mg) was added to the above product dissolved in 25 mL AcOEt. The reaction was stirred at room temperature for 90 min, after which the solution was washed thoroughly with saturated aqueous sodium metabisulfite to destroy the excess of iodine. The yellow solution was washed with H_2O, dried ($MgSO_4$), and concentrated under vacuum furnishing the *trans*-stilbene derivative as a white solid.

d) Isopropyl ether cleavage: BCl_3 (11 mL of a 1M solution in CH_2Cl_2) was added at −78 °C to the *trans*-3,5-diisopropoxy-4-methoxystilbene (700 mg) dissolved in 15 mL of CH_2Cl_2. The mixture was maintained for 1.5 h at −60 to −30 °C, then for 45 min at 0 °C. Following the addition of the borane, a deep yellow precipitate appeared and remained during all the reaction. AcOEt (20 mL) was added at 0 °C to dissolve the precipitate, followed by 15 mL of 2M NaOH. The aqueous layer was extracted with AcOEt (3×20 mL), the organic layer was dried over $MgSO_4$ and concentrated. The oil obtained was purified by chromatography on silica gel (hexane/AcOEt, 8/2, then 6/4, then 5/5) to give the *trans*-3,5-dihydroxy-4-methoxystilbene (**8**) as a white powder; yield: 19%. ^{1}H NMR (DMSO-d_6, 200 MHz): $\delta =$ 3.76 (s, 3H, OCH_3), 6.12 (t, 1H, $J = 2.10$ Hz, H-4), 6.39 (d, 2H, $J = 2.10$ Hz, H-2,6), 6.92 (d, 2H, $J = 8.76$ Hz, H-3′,5′), 6.87 and 6.98 (AB system, 2H, $J = 16.56$ Hz, CH=CH), 7.51 (d, 2H, $J = 8.76$ Hz, H-2′,6′), 9.20 (s, 2H, 2OH).

Laccase-Catalyzed Dimerization of the Hydroxystilbenes; General Procedure

One of the hydroxystilbenic substrates (100 mg) was dissolved in 7 mL AcOEt, while the laccase (10 U) was dissolved in 7 mL of 20 mM acetate buffer, pH 4.5. The biphasic system was shaken at room temperature and monitored by TLC. When the TLC spots indicated that the products were prevalent with respect to the initial substrate, the reaction was quenched by phase separation followed by AcOEt extraction of the water solution, and the organic solvent was evaporated. The crude residue was purified by flash chromatography.

6a: Substrate: 200 mg; reaction time: 70 h; TLC (PetEt:AcOEt, 4:6): $R_f = 0.16$; TLC_{rp} ($CH_3CN:H_2O$, 1:1): $R_f = 0.21$; flash chromatography: PetEt:AcOEt, 6:4/5.5:4.5/5:5/3:7/2:8; yield: 37 mg (19%). ^{1}H NMR (DMSO-d_6): $\delta =$ 3.74 (s, 3H), 3.87 (s, 3H), 4.62 (d, $J = 9.28$ Hz, 1H), 5.41 (d, $J = 9.28$ Hz, 1H), 6.68 (bs, 1H), 6.73 (d, $J = 8.64$ Hz, 2H), 6.74 (d, $J = 8.50$ Hz, 2H), 6.75 (bs, 2H), 6.93 (s, 2H), 6.95 (bs, 1H), 7.02 (d, $J = 8.50$ Hz, 2H), 7.12 (bs, 1H), 7.35 (d, $J = 8.64$ Hz, 2H), 9.10 (s, 1H), 9.39 (s, 1H), 9.50 (s, 1H); ^{13}C NMR (DMSO-d_6): $\delta = 55.7$, 56.1, 56.2, 93.7, 110.4, 111.3, 115.3, 115.7, 116.0 (4C), 117.9, 125.9, 126.5, 127.9 (2C), 128.9, 129.7 (2C), 130.7, 131.6, 132.2, 133.1, 144.5, 147.3 (2C), 148.1, 156.9, 157.3.

6a′: Substrate: 200 mg; reaction time: 70 h; TLC (PetEt:AcOEt, 4:6): $R_f = 0.21$; TLC_{rp} ($CH_3CN:H_2O$, 1:1): $R_f = 0.15$; flash chromatography: PetEt:AcOEt, 6:4/5.5:4.5/5:5/3:7/2:8; yield: 16 mg (8%). ^{1}H NMR (DMSO-d_6): $\delta =$ 3.74 (s, 3H), 3.79 (s, 3H), 4.62 (d, $J = 9.19$ Hz, 1H), 5.43 (d, $J = 9.19$ Hz, 1H), 6.72 (d, $J = 8.12$ Hz, 1H), 6.75 (s, 1H), 6.75 (bs, 1H), 6.75 (d, $J = 8.61$ Hz, 2H), 6.88 and 7.00 (AB system, $J = 16.70$ Hz, 2H), 6.89 (d, $J = 8.70$ Hz, 1H), 6.92 (dd, $J = 1.89$, 8.12 Hz, 1H), 6.96 (bs, 1H), 7.03 (d, $J = 8.61$ Hz, 2H), 7.10 (bs, 1H), 7.14 (d, $J = 1.89$ Hz, 1H), 7.38 (dd, $J = 1.45$, 8.70 Hz, 2H), 9.01 (s, 1H), 9.07 (s, 1H), 9.37 (s, 1H); ^{13}C NMR (DMSO-d_6): $\delta = 55.4$, 56.0, 56.1, 93.5, 109.8, 110.1, 111.1 (2C), 115.7, 115.9, 116.1, 119.8, 120.1, 122.6, 125.8, 126.5, 127.3, 129.5, 129.7 (2C), 132.5, 146.7, 147.2, 148.1, 148.2, 157.0, 158.9.

9a: Substrate: 150 mg; reaction time: 120 d; TLC (PetEt:AcOEt, 7:3): $R_f = 0.35$; TLC_{rp} ($CH_3CN:H_2O$, 7:3): $R_f = 0.20$; flash chromatography: PetEt:AcOEt, 7:3/6:4; yield 88 mg (59%). ^{1}H NMR (DMSO-d_6): $\delta = 3.71$ (s, 6H), 3.75 (s, 6H), 4.62 (d, $J = 8.49$ Hz, 1H), 5.60 (d, $J = 8.49$ Hz, 1H), 6.38 (t, $J = 1.64$ Hz, 1H), 6.41 (d, $J = 2.03$ Hz, 2H), 6.45 (t, $J = 2.03$ Hz, 1H), 6.74 (d, $J = 1.64$ Hz, 2H), 6.80 (d, $J = 8.49$ Hz, 2H), 6.93 (d, $J = 8.54$ Hz, 1H), 6.96 and 7.23 (AB system, $J = 16.95$ Hz, 2H), 7.22 (d, $J = 8.49$ Hz, 2H), 7.23 (bs, 1H), 7.46 (d, $J = 8.54$ Hz, 1H), 9.56 (s, 1H); ^{13}C NMR (DMSO-d_6): $\delta = 55.6$ (2C), 56.3, 92.6, 99.0, 100.0, 104.6 (2C), 106.8 (2C), 109.9, 115.8 (2C), 123.4, 126.2, 128.1, 128.3 (2C), 129.3, 130.5, 130.8, 131.9, 140.0, 144.1, 158.1, 159.4, 161.1 (2C), 161.2 (2C).

9b: Substrate: 150 mg; reaction time: 120 h; TLC (PetEt:AcOEt, 7:3): $R_f = 0.21$; TLC_{rp} ($CH_3CN:H_2O$, 7:3): $R_f = 0.39$; flash chromatography: PetEt:AcOEt, 7:3/6:4; yield: 9 mg (6%). ^{1}H NMR (DMSO-d_6): $\delta = 3.60$ (s, 6H), 3.76 (s, 6H), 4.74 (dd, $J = 4.51$, 6.26 Hz, 1H), 5.21 (d, $J = 6.26$ Hz, 1H), 5.48 (d, $J = 4.51$ Hz, 1H), 6.27 (t, $J = 2.03$ Hz, 1H), 6.30 (d, $J = 2.03$ Hz, 2H), 6.37 (t, $J = 2.02$ Hz, 1H), 6.60 (d, $J = 8.46$ Hz, 2H), 6.70 (d, $J = 2.02$ Hz, 2H), 6.90 (d, $J = 8.70$ Hz, 2H), 6.96 and 7.14 (AB system, $J = 16.31$ Hz, 2H), 7.02 (d, $J = 8.46$ Hz, 2H), 7.42 (d, $J = 8.70$ Hz, 2H), 9.20 (s, 1H); ^{13}C NMR (DMSO-d_6): $\delta = 55.4$ (2C), 55.6, 76.5, 84.4, 99.4, 100.0, 104.6 (2C), 106.1 (2C), 114.7 (2C), 116.5, 126.7, 128.1, 128.9 (2C), 129.0, 130.0, 132.3, 139.9, 141.4, 158.3, 160.2 (2C), 161.1 (2C).

12a: Substrate: 65 mg; reaction time: 96 h; TLC (PetEt:AcOEt, 6:4): $R_f = 0.12$; TLC_{rp} ($CH_3CN:H_2O$, 7:3): $R_f = 0.45$; flash chromatography: PetEt:AcOEt, 6:4; yield: 9 mg (14%). ^{1}H NMR (DMSO-d_6): $\delta = 3.74$ (s, 3H), 3.75 (s, 3H), 5.07 (d, $J = 8.70$ Hz, 1H), 5.35 (d, $J = 8.70$ Hz, 1H), 6.58 (bs, 1H), 6.63 (dd, $J = 2.05$, 8.02 Hz, 1H), 6.71 (d, $J = 8.02$ Hz, 1H), 6.78 (d, $J = 2.05$ Hz, 1H), 6.83 and 6.93 (AB system, $J = 16.21$ Hz, 2H), 6.89 (d, $J = 8.70$ Hz, 2H), 6.91 (bs, 1H), 6.92 (d, $J = 8.70$ Hz, 2H), 7.14 (d, $J = 8.70$ Hz, 2H), 7.45 (d, $J = 8.70$ Hz, 2H), 8.37 (s, 1H), 8.97 (s, 1H), 9.40 (s, 1H); ^{13}C NMR (DMSO-d_6): $\delta = 55.5$, 55.6, 56.3, 93.3, 113.8, 114.2, 114.4, 114.5 (2C), 114.7 (2C), 115.8, 118.2, 125.6, 126.9, 127.8 (2C), 129.6 (2C), 130.5, 131.3, 131.8, 133.0, 133.9, 141.8, 145.7, 146.0, 146.8, 158.8, 159.0.

12c: Substrate: 65 mg; reaction time: 96 h; TLC (PetEt:AcOEt, 6:4): $R_f = 0.22$; TLC_{rp} ($CH_3CN:H_2O$, 7:3): $R_f = 0.26$; flash chromatography: PetEt:AcOEt, 6:4; yield: 9 mg (14%). ^{1}H NMR (DMSO-d_6): $\delta = 3.72$ (s, 3H), 3.78 (s, 3H), 5.02 (d, $J = 7.98$ Hz, 1H), 5.07 (d, $J = 7.98$ Hz, 1H), 6.49 (d, $J = 7.99$ Hz, 1H), 6.59 (d, $J = 7.99$ Hz, 1H), 6.65 (bs, 1H), 6.83 (d, $J = 8.62$ Hz, 2H), 6.93 (d, $J = 8.37$ Hz, 2H), 6.95 (d, $J = 8.42$ Hz, 1H), 7.00 and 7.07 (AB system, $J = 17.15$ Hz, 2H), 7.10 (d, $J = 8.42$ Hz, 1H), 7.18 (d, $J = 8.62$ Hz, 2H), 7.20 (bs, 1H), 7.50 (d, $J = 8.37$ Hz, 2H), 8.83 (s, 1H), 8.94 (s, 1H); ^{13}C NMR (DMSO-d_6): $\delta = 55.5$, 55.6, 78.5, 79.7, 113.9 (2C), 114.6 (3C), 115.7, 115.9, 117.4, 119.8, 120.0, 126.2, 126.9, 127.8, 128.0 (2C), 129.2, 129.8 (2C), 130.4, 131.5, 143.9, 144.4, 145.3, 145.9, 159.2, 159.6.

Supporting Information

More detailed characterization data for products **4–13** are available in the Supporting Information.

Acknowledgements

We gratefully thank Prof. Dietmar Haltrich (BOKU University, Wien, Austria) for a generous gift of Trametes pubescens laccase.

References

[1] J. Burns, T. Yokota, H. Ashihara, M. E. J. Lean, A. Crozier, *J. Agric. Food Chem.* **2002**, *50*, 3337–3340.
[2] E. N. Frankel, A. L. Waterhouse, J. E. Kinsella, *Lancet* **1993**, *341*, 1103–1104.
[3] R. H. Cichewicz, S. A. Kouzi, in: *Studies in natural products chemistry*, Vol. 26: *Bioactive natural products (Part G)*, (Ed.: Atta-ur-Rahman), Elsevier, Dordrecht, **2002**, pp 507–579.
[4] a) N. Latruffe, D. Delmas, B. Jannin, M. Cherkaoui Malki, P. Passilly-Degrace, J. P. Berlot, *Int. J. Mol. Med.*

2002, *10*, 755–760; b) K. Roemer, M. Mahyar-Roemer, *Drugs Today* **2002**, *38*, 571–580; c) L. A. Stivala, M. Savio, F. Carafoli, P. Perucca, L. Bianchi, G. Maga, L. Forti, U. M. Pagnoni, A. Albini, E. Prosperi, V. Vannini, *J. Biol. Chem.* **2001**, *276*, 22586–22594; d) P. Waffo-Téguo, M. E. Hawthorne, M. Cuendet, J. M. Mérillon, A. D. Kinghorne, J. M Pezzuto, R. G. Mehta, *Nutrition and Cancer*, **2001**, *40*, 173–179; e) M. Ohyama, T. Tanaka, T. Ito, M. Iinuma, K. F. Bastow, K.-H. Lee, *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3057–3060; f) M. S Jang, E. N. Cai, G. O. Udeani, K. V. Slowing, C. F. Thomas, C. W. W. Beecher, H. H. S. Fong, N. R. Farnsworth, A. D. Kinghorn, R. G. Mehta, R. C. Moon, J. M. Pezzuto, *Science* **1997**, *275*, 218–220.

[5] J. A. Baur, K. J. Pearson, N. L. Price, H. A. Jamieson, C. Lerin, A. Karla, V. V. Prabhu, J. S. Allard, G. Lopez-Lluch, K. Lewis, P. J. Pistell, S. Poosala, K. G. Becker, O. Boss, D. Gwinn, M. Wang, S. Ramaswamy, K. W. Fishbein, R. G. Spencer, E. G. Lakatta, D. Le Couteur, R. J. Shaw, P. Navas, P. Puigserver, D. K. Ingram, R. De Cabo, D. A. Sinclair, *Nature* **2006**, *444*, 337–342.

[6] M. F. Wang, Y. Jin, C.-T. Ho, *J. Agric. Food Chem.* **1999**, *47*, 3974–3977.

[7] a) K.-S. Huang, M. Lin, L.-N. Yu, M. Kong, *Tetrahedron* **2000**, *56*, 1321–1329; b) K.-S. Huang, M. Lin, Y. H. Wang, *Chinese Chem. Lett.* **1999**, *10*, 817–820.

[8] M. Sako, H. Hosokawa, T. Ito, M. Inuma, *J. Org. Chem.* **2004** , 69, 2598–2600.

[9] Y. Takaya, K. Terashima, J. Ito, Y.-H. He, M. Tateoka, N. Yamaguchi, M. Niwa, *Tetrahedron* **2005**, *61*, 10285–10290.

[10] R. H. Cichewicz, S. A. Kouzi, M. T. Hamann, *J. Nat. Prod.* **2000**, *63*, 29–33.

[11] a) R. Pezet, V. Pont, K. Hoang-Van, *Physiol. Mol. Plant. Pathol.* **1991**, *39*, 441–450; b) A. C. Breuil, M. Adrian, N. Pirio, P. Meunier, R. Bessis, P. Jeandet, *Tetrahedron Lett.* **1998**, 39, 537–540.

[12] a) P. Langcake, R. J. Pryce, *Chem.Commun.* **1977**, 208–210; b) Y. Hirano, R. Kondo, K. Sakai, *J. Wood Sci.* **2002**, *48*, 64–68.

[13] M. D. Pinto, J. A. Garcia-Barrado, P. Macias, *J. Agric. Food Chem.* **2003**, *51*, 1653–1657.

[14] L. M. Szewczuk, S. H. Lee, I. A. Blair, T. Penning, *J. Nat. Prod.* **2005**, *68*, 36–42.

[15] R. Pezet, *FEMS Microbiol. Lett.* **1998**, *167*, 203–208.

[16] A.-C. Breuil, P. Jeandet, M. Adrian, F. Chopin, N. Pirio, P. Meunier, R. Bessis, *Phytopathology* **1999**, *89*, 298–302.

[17] a) S. Riva, *Trends Biotechnol.* **2006**, *24*, 219–226; b) F. Xu, *J. Biol. Chem.* **1997**, *272*, 924–928.

[18] See, for instance, R. M. Berka, P. Schneider, E. J. Golightly, S. H. Brown, M. Madden, K. M. Brown, T. Halkier, K. Mondorf, F. Xu, *Appl. Env. Microbiol.* **1997**, *63*, 3151–3157. Laccases are presently marketed by Fluka and by Novozymes.

[19] A. M. Mayer, R. Staples, *Phytochemistry* **2002**, *60*, 551–565.

[20] a) T. H. J. Niedermeyer, A. Mikolasch, M. Lalk, *J. Org. Chem.* **2005**, *70,* 2002–2008; b) S. Ciecholewski, E. Hammer, K. Manda, G. Bose, V. T. H. Nguyen, P. Langer, F. Schauer, *Tetrahedron* **2005**, 61, 4615–4619; c) H. Agematu, T. Tsuchida, K. Kominato, N. Shibamoto, T. Yoshioka. H. Nishida, R. Okamoto, *J. Antibiot.* **1992**, *46*, 141–148; d) G. Lugaro, G. Carrea, P. Cremonesi, M. M. Casellato, E. Antonini, *Arch. Biochem. Biophys.* **1973**, *159,* 1–6; e) S. Tranchimand, T. Tron, C. Gaudin, G. Iacazio, *J. Mol. Cat. B: Enzymatic* **2006**, *42,* 27–31.

[21] N. Aktas, H. Ciçek, A. T. Ünal, G. Kibarer, N. Kolankaya, A. Tanyolic, *Bioresource Technol.* **2001**, *80*, 29–36.

[22] a) S. Riva, *J. Mol. Cat. B: Enzymatic*, **2002**, *19–20*, 43–54; b) G. Carrea, S. Riva, *Angew. Chem. Int. Ed.* **2000**, *39*, 2226–2254.

[23] G. Roda, S. Riva, B. Danieli, H. Griengl, U. Rinner, *Tetrahedron* **2002**, *58*, 2979–2983, and references cited therein.

[24] A. Intra, S. Nicotra, S. Riva, B. Danieli, *Adv. Synth. Catal.* **2005**, *347*, 973–977.

[25] A. Intra, S. Nicotra, G. Ottolina, S. Riva, B. Danieli, *Tetrahedron: Asymmetry*, **2004**, *15*, 2927–2931.

[26] S. Ncanana, L. Baratto, L. Roncaglia, S. Riva, S. G. Burton, *Adv. Synth. Catal.* **2007**, *349*, 1507–1513.

[27] S. Nicotra, M. R. Cramarossa, A. Mucci, U. M. Pagnoni, S. Riva, L. Forti, *Tetrahedron* **2004**, *60*, 595–600.

[28] a) K. Felfoldi, M. Sutyinszky, N. Nagy, I. Palinko, *Synth. Commun.*, **2000**, *30*, 1543–1553; b) K. Gaukroger, J. A. Hadfield, L. A. Hepworth, N. J. Lawrence, A. T. McGown, *J. Org. Chem.* **2001**, *66*, 8135–8138; c) G. Solladié, Y. Pasturel-Jacopé, J. Maignan, *Tetrahedron* **2003**, *59*, 3315–3321.

[29] G. Bodenhausen, D. J. Ruben, *J. Chem. Phys. Lett.* **1980**, *69*,185–189.

[30] A. Box, M. F. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093–2094.

[31] D. Neuhaus, M. P. Williamson, *The Nuclear Overhauser Effect*, in: *Structural and Conformational Analysis*, VCH, New York, **1989**.

[32] E.-K. Seo, H. Chai, H. L. Constant, T. Santisuk, V. Reutrakul, C. W. W. Beecher, N. R. Farnsworth, G. A. Cordell, J. M. Pezzuto, A. D. Kinghorn, *J. Org. Chem.* **1999**, *64*, 6976–6983.

[33] C. Leitner, J. Hess, C. Galhaup, R. Ludwig, B. Nidetzky, K. Kulbe, D. Haltrich, *Appl. Biochem. Biotechnol.* **2002**, *98*, 497–507.

