Pergamon

Tetrahedron 57 (2001) 7693–7699

TETRAHEDRON

Synthesis of substituted imidazoles and dimerization products using cells and laccase from *Trametes versicolor*

Annett Schäfer,[a,*] Michael Specht,[b] Annemarie Hetzheim,[c] Wittko Francke[b] and Frieder Schauer[a]

[a]*Institut für Mikrobiologie, Ernst-Moritz-Arndt-Universität, F.-L.-Jahn-Straße 15, D-17489 Greifswald, Germany*
[b]*Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany*
[c]*Institut für Chemie und Biochemie, Ernst-Moritz-Arndt-Universität, Soldmannstraße 16, D-17487 Greifswald, Germany*

Received 7 May 2001; accepted 19 July 2001

Abstract—Feeding *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides to cells of *Trametes versicolor* yielded two types of aromatic metabolites besides degradation products: (1) Novel heteroaromatic compounds, such as: *N*-[5-formyl-4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide, 4-phenyl-2-(propylamino)imidazole, *N*-(3-hydroxy-4-phenyl-2-propylimino-2,3-dihydroimidazol-1-yl)-acetamide, and *N*-(5-oxo-4-phenyl-2-propylimino-2,5-dihydroimidazol-1-yl)-acetamide; (2) Novel compounds arising from coupling of two ring-opened molecules of the substrate.

1. Introduction

Laccase, an extracellular enzyme from white rot fungi, has been implicated in lignin biodegradation, although the enzyme causes both depolymerization[1] and polymerization of lignin.[2,3] In addition, several white rot fungi, such as *Trametes versicolor*, transform pesticides and other anthropogenic pollutants through the action of their laccase.[4] Additionally, this enzyme is able to polymerize various halogen, alkyl, and alkoxy anilines,[5,6] phenols,[7] and biaryls.[8] Enzymatic copolymerization of pesticide-derived aromatic amines with phenolic humus monomers such as syringic, vanillic, protocatechuic, and ferulic acid resulted in the formation of several hybrid dimers, trimers, and tetramers.[9]

Hitherto laccase has not been widely used in organic synthesis[10,11] and little is known about the transformation of heteroaromatic compounds by *T. versicolor*.[12] The purpose of the present study was to investigate whether *T. versicolor* can transform *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides to yield novel substituted imidazoles or oligomerization products applicable for pharmacological purposes.

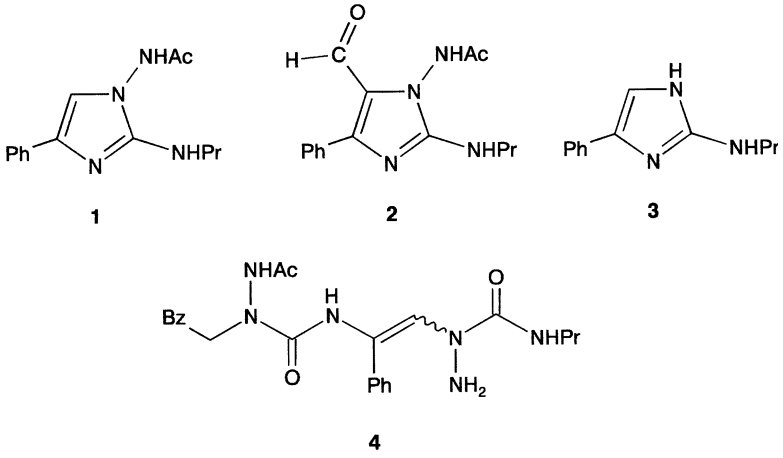

Figure 1. *N*-[4-Phenyl-2-(propylamino)imidazol-1-yl]-acetamide (substrate **1**) and products **2**–**4** formed during incubation with *T. versicolor*.

Keywords: imidazole; *Trametes versicolor*; transformation.
* Corresponding author. Tel.: +49-3834-864225; fax: +49-3834-864202; e-mail: annett-schaefer@gmx.de

0040–4020/01/$ - see front matter
PII: S0040-4020(01)00751-7

Table 1. Comparison of ^{1}H NMR spectra of substrate **1** (*N*-[4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide) with product **2** and **3**

1		**2**		**3**		Atom and structure
δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	
–		9.40	s	–		1H, CHO
7.64	d, ^3J=7.8	7.74	d, ^3J=7.3	7.88	d, ^3J=6.7	2H, H_o, C_6H_5
		7.45	m	7.46–7.31	m	3H, H_m, H_p, C_6H_5
7.29	dd, ^3J=7.8, ^3J=7.3					2H, H_m, C_6H_5
7.11	t, ^3J=7.3					1H, H_p, C_6H_5
7.08	s	–		7.46–7.31		1H, imidazole
5.86	t, ^3J=5.8	7.16	t, ^3J=5.8			1H, NH, *NH*–CH_2
3.19	m	3.29	m	3.11	m	2H, CH_2, *CH_2*–NH
1.97	s	1.99	s	–	–	3H, CH_3, *CH_3*–CONH
1.58	m	1.57	m	1.54	m	2H, CH_2, $NHCH_2$*CH_2*CH_3
0.91	t, ^3J=7.4	0.88	t, ^3J=7.4	0.91	t, ^3J=7.4	3H, CH_3, *CH_3*$(CH_2)_2$NH

2. Results and discussion

2.1. Transformation of *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides by *Trametes versicolor*

Several products generated from different *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides can be obtained from cultures of *T. versicolor* supplemented with these imidazole substrates.

High-performance liquid chromatography (HPLC) analysis of the cell-free supernatant of cultures incubated with the substrate *N*-[4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide (substrate **1**, Fig. 1) revealed at least nine products. Sufficient amounts of these products for spectral characterization were formed over an incubation period of 24 h.

Two of the nine products were mixtures, which we were unable to resolve. Four of the seven separable products were extracted by ethyl acetate and identified as mandelic acid, oxophenyl acetic acid, benzyl alcohol, and benzoic acid by comparison of the spectroscopic data (HPLC/UV and gas chromatography–mass spectrometry GC–MS) with those of synthetic standards.

The separation of the remaining three products was performed by HPLC. Comparison of proton–proton spin systems and couplings of the ^{1}H NMR spectra of two of these compounds with those of substrate **1** showed structural similarities (Table 1) and led to the later identification of product **2** as *N*-[5-formyl-4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide and product **3** as 4-phenyl-2-(propylamino)imidazole (Fig. 1). These identifications are based on the following data: mass spectral analysis of compound **2** showed a molecular mass of 286. This mass, 28 units higher than substrate **1**, and the fragmentation of M^+-29 (M^+-CHO) in the mass spectrum of **2**, was attributed to an additional formyl group. This is confirmed by the presence of a proton showing a singlet at δ=9.40 ppm in the ^{1}H NMR spectrum of **2**, and a carbon signal at the low field value of δ=175.0 ppm in the ^{13}C NMR spectrum. Two-dimensional NMR experiments led to the assignment of the proton at δ=9.40 ppm to the carbon at δ=175.0 ppm, demonstrating the presence of an aldehyde structure. Finally, the singlet at δ=7.08 ppm, present in the ^{1}H NMR spectrum of substrate **1** and belonging to the C-5 position of the imidazole ring, is lacking in the spectrum of product **2**. Therefore, the additional formyl group was assigned to the C-5 position in compound **2**.

Table 2. ^{1}H NMR and ^{13}C NMR spectra of product **4**

^{1}H NMR, **4**			^{13}C NMR, **4**	
δ (ppm)	J (H)	Atom and structure	δ (ppm)	Atom and structure
7.64	t, ^3J=7.5	1H, H_p, C_6H_5–CO–CH_2–N	192.1	C=O, C_6H_5–*C*O–CH_2–N
7.62	d, ^3J=7.7	1H, H_o, C_6H_5–CO–CH_2–N	180.3	C=O
7.47	dd, ^3J=7.5, ^3J=7.7	1H, H_m, C_6H_5–CO–CH_2–N	169.6	C=O, CH_3–*C*ONH
7.42	d, ^3J=7.6	1H, H_o, C_6H_5	153.4	C=O bzw. C=NH
7.30	t, ^3J=7.2	1H, H_p, C_6H_5		CH_3–CH_2–*C*H_2–NH–*C*
7.26	t, ^3J=5.8	1H, NH,	134.0	C_p, C_6H_5–CO–CH_2–N
		CH_3–CH_2–CH_2–*NH*–C	133.3	C1, C_6H_5
7.19	dd, ^3J=7.6, ^3J=7.2	1H, H_m, C_6H_5	133.2	C1, C_6H_5–CO–CH_2–N
5.76	s	1H, CH	129.2	C_o, C_6H_5
3.52	s	2H, CH_2, C_6H_5–CO–*CH_2*–N	129.1	C_o, C_6H_5–CO–CH_2–N
3.27	m	2H, CH_2,	128.9	C_p, C_6H_5
		CH_3–CH_2–*CH_2*–NH–C	128.7	C_m, C_6H_5–CO–CH_2–N
1.68	s	3H, CH_3, *CH_3*–CONH	127.6	C_m, C_6H_5
1.55	m	2H, CH_2,	120.5	CH
		CH_3–*CH_2*–CH_2–NH–C	118.2	C-quaternary
0.86	t, ^3J=7.4	3H, CH_3,	69.8	CH_2, C_6H_5–CO–*CH_2*–N
		CH_3–CH_2–CH_2–NH–C	43.6	CH_2, CH_3–CH_2–*CH_2*–NH–C
			22.5	CH_2, CH_3–*CH_2*–CH_2–NH–C
			20.5	CH_3, *CH_3*–CONH
			11.1	CH_3, *CH_3*–CH_2–CH_2–NH–C

The molecular mass of compound **3** (201) is 57 units less than that of substrate **1** (258) indicating the loss of the acetylamino group. The absence of a fragment ion at *m/z* 43 in the mass spectrum of **3** indicates that an acetyl group is not present in this compound. Furthermore, all ^{1}H NMR signals of an acetylamino group (present in the substrate) are lacking in the ^{1}H NMR spectrum of product **3**, leading together with the remaining resonance signals to the structure of 4-phenyl-2-(propylamino)imidazole for this product.

For compound **4**, the NMR experiments revealed several systems of resonance signals (Table 2), pointing to two different phenyl groups, divers aliphatic groups (a propylamino and a methylene group) and an acetyl amino group. Mass spectral analysis with electron impact ionization (70 eV, direct inlet- and coupled GC–MS, with and without prior derivatization) led to inconsistent mass spectra, not suitable for structure elucidation. The mass spectrum obtained by use of the FAB technique showed no molecular ion peak, but significant peaks were detected at *m/z* 43 (acetyl group), 77 (phenyl group) and 105 (benzoyl group). The existence of a benzoyl substructure, also shown by the detection of benzoyl formic acid in further degradation of compound **4**, demonstrates that the imidazole ring is cleaved during metabolism. This together with further NMR experiments (^{1}H NMR, ^{13}C NMR, DEPT, and the two-dimensional NMR experiments HMBC, HMQC and NOESY, not shown), corroborate a structure formed by coupling of two ring-opened molecules of substrate **1**. Fig. 2 shows the proposed formation pathway of product **4**, assigned as 2-{{2-{[2-acetyl-1-(benzoylmethyl)hydrazino]carbonyl-amino}-2-phenylvinyl}}-4-propylsemicarbazide in accordance with all obtained spectral data. The *E*,*Z*-isomerism of product **4** were not analyzed.

Thus, after transformation of *N*-[4-phenyl-2-(propylamino)-imidazol-1-yl]-acetamide by *T. versicolor*, seven products were identified; the well known substances mandelic acid, oxophenyl acetic acid, benzoic acid, and benzyl alcohol, and the, up to now, synthetically not available compounds *N*-[5-formyl-4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide **2**, 4-phenyl-2-(propylamino)imidazole **3**, and the coupling product **4**. Two additional products, assigned as **5** and **6**, were detected by HPLC/UV analysis, which were not separable by the methods used. Sufficient amounts of these compounds for characterization were obtained in the following incubation experiments with a crude preparation of laccase.

HPLC/UV analysis of the cell-free supernatant of the culture incubated with the substrate *N*-(2-hexylamino-4-phenylimidazol-1-yl)-acetamide revealed also nine products, in accordance with the metabolism outlined above. As well as in the incubation experiments with substrate **1**, mandelic acid, oxophenyl acetic acid, benzoic acid and benzyl alcohol were formed. Additional analogous products were *N*-(5-formyl-2-hexylamino-4-phenylimidazol-1-yl)-acetamide, 2-hexylamino-4-phenylimidazole, a coupling product like **4**, now with a hexylamino group instead of a propylamino group, and, again, two other unseparable products (data not shown).

2.2. Transformation of *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides by crude preparation of laccase

In cultures supplemented with *N*-[4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide, *T. versicolor* secreted extracellular laccase with activity at a level of 400 nmol mL^{-1} min^{-1}. Under these conditions, *T. versicolor* also produced manganese peroxidase with an activity of smaller amounts (140 nmol mL^{-1} min^{-1}). No lignin peroxidase was detected. In order to distinguish between laccase- and manganese peroxidase-catalyzed reactions, crude preparations of the enzymes were tested for their ability to transform substrate **1**. After incubation with crude preparation of manganese peroxidase (activity 1000 nmol mL^{-1} min^{-1}), no decrease of substrate **1** and no products derived from it were found by HPLC/UV analysis.

In contrast, a crude preparation of laccase with an activity at a level of 800 nmol mL^{-1} min^{-1} transformed the *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides.

HPLC/UV analysis after 5 h of incubation with substrate **1** revealed only product **4** (Fig. 1) and the two products **5** and **6** already found in the transformation by the fungus. These three products were formed in higher yields and in shorter time by transformation with crude preparation of laccase than with cultures (whole cells) of *T. versicolor*.

The mixture of the two products **5** and **6**, not separable by the HPLC conditions used, was analyzed by GC/MS. The GC/MS analysis revealed two separated peaks with molecular masses of 274 and 272, respectively. The mass differences to substrate **1** (16 for compound **5** in accordance with a hydroxyl group) and the fragmentations in both mass spectra, combined with the known reactions induced by laccase (formation of radicals,[6,13–18] and reported reactions of oxygen with these radicals[19]) led to the suggested mechanism, shown in Fig. 3, for the formation of products **5** and **6**. The first step is the homolytic abstraction of hydrogen from the amino group of the substrate, as usual for formation of radicals by laccase.[6,18] Steric hindrance of the substituents suppresses the oligomerization of the radicals and the reactive centers add oxygen.[19] From this addition of oxygen at the two isomeric radical positions 3-N and 5-C and further conversion by the elimination of water yields the products *N*-(3-hydroxy-4-phenyl-2-propylimino-2,3-dihydroimidazol-1-yl)-acetamide **5** and the *N*-(5-oxo-4-phenyl-2-propylimino-2,5-dihydroimidazol-1-yl)-acetamide **6**.

2.3. Comparison of reaction by crude preparation of laccase and transformation by culture of *T. versicolor*

In agitated cultures, *T. versicolor* transformed *N*-[4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide **1** to at least nine products. The new compounds **2**–**6** were the predominant products after 24 h. Extended incubation (48 h) led to a decrease of products **2**–**6** and to a simultaneous enhancement of the transformation products mandelic acid, oxophenyl acetic acid, benzoic acid, and benzyl alcohol. The products **4**–**6** were formed in higher yields and in shorter time (5 h) by transformation with a crude preparation of laccase. The other products, which derive from extended

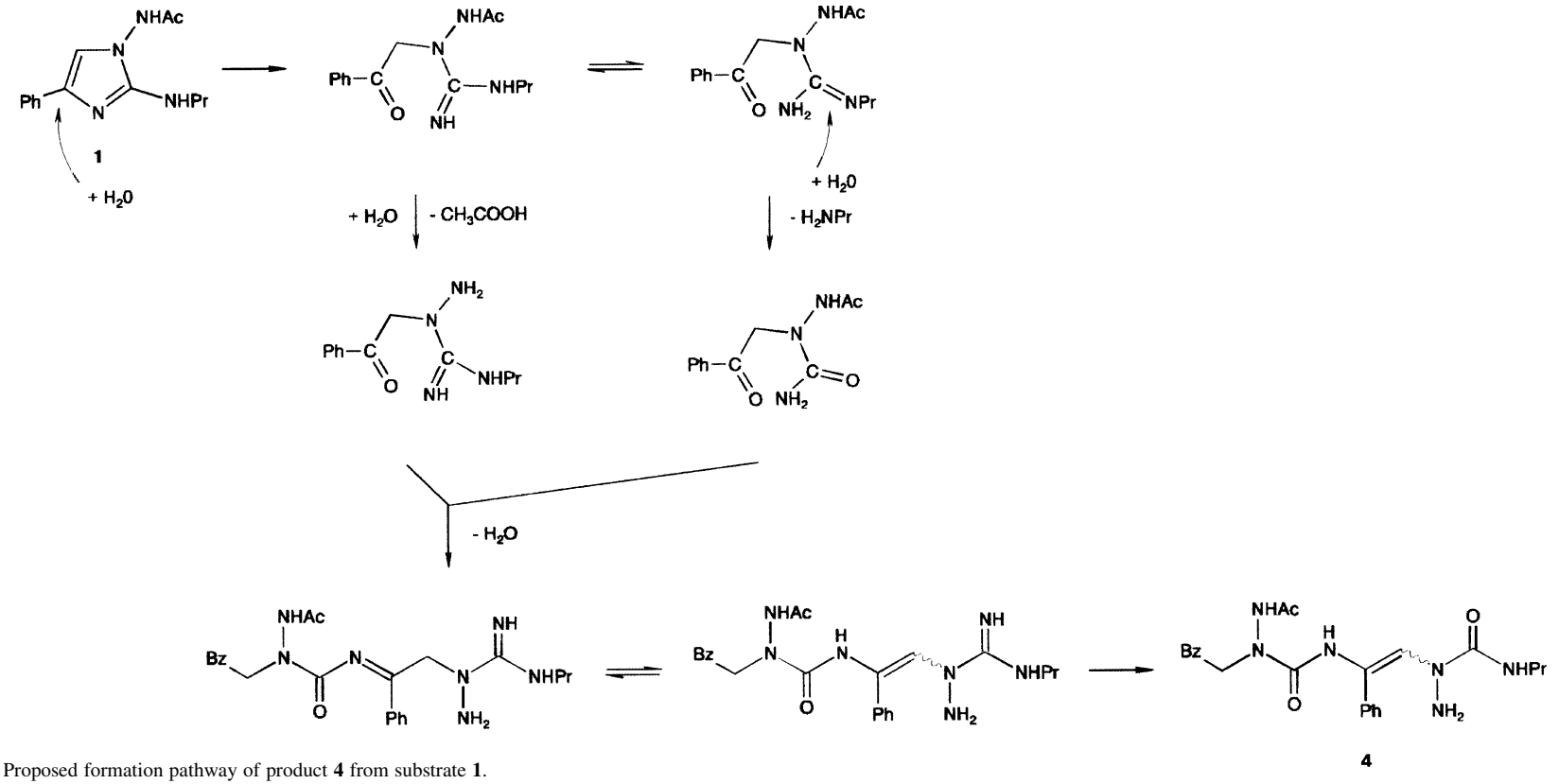

Figure 2. Proposed formation pathway of product **4** from substrate **1**.

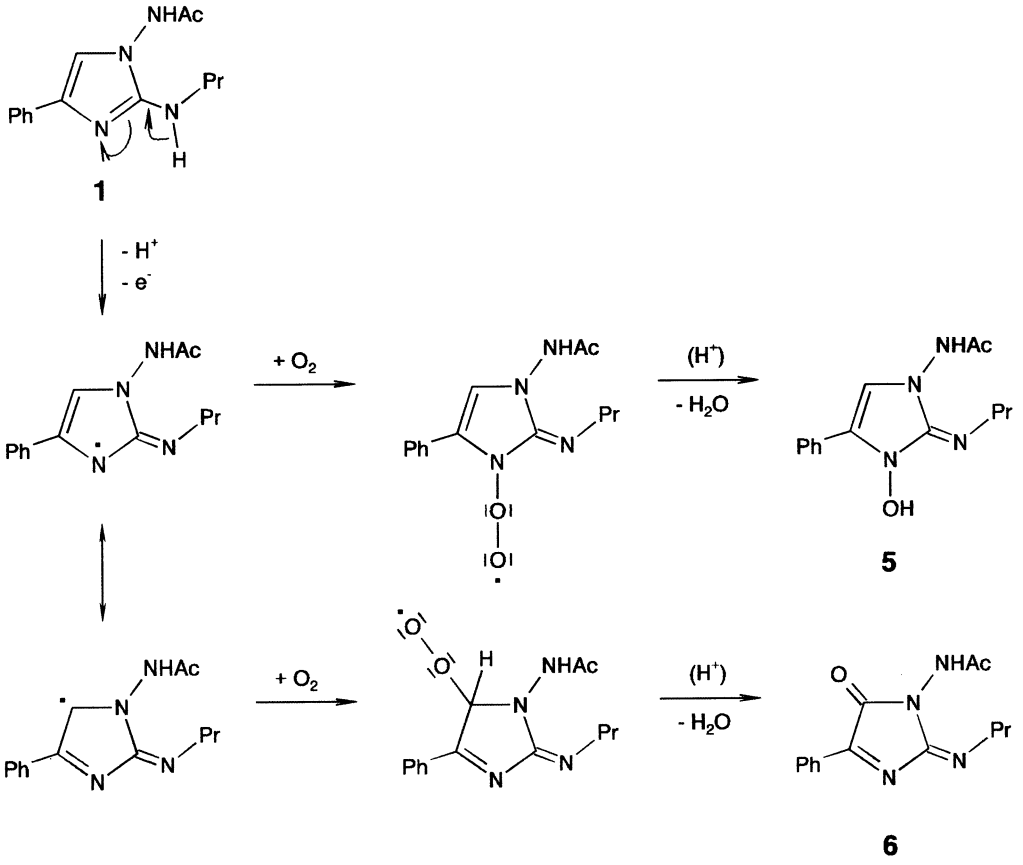

Figure 3. Proposed laccase-catalyzed formation of radicals and products **5** and **6** from substrate **1**.

degradation of the metabolites, could not be detected under these conditions. After 24 h, there was no evidence for further transformation of product **4** and only a small decrease of compounds **5** and **6**. These results demonstrate that the formation of compounds **4**–**6** are laccase-catalyzed reactions and that the extracellular enzyme laccase is not responsible for the transformation of substrate **1** to mandelic acid, oxophenyl acetic acid, benzoic acid, benzyl alcohol, as well as to the products **2** and **3**. It is likely, that intracellular enzymes catalyze these transformations.

The transformation of *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides by whole cells of *T. versicolor* and by a crude preparation of laccase to novel substituted imidazoles and a novel coupling product 2-{{2-{[2-acetyl-1-(benzoylmethyl)hydrazino]carbonyl-amino}-2-phenylvinyl}}-4-propylsemicarbazide, provides an attractive and practicable synthetic route to a variety of new structures that could possess pharmacological activity.

3. Experimental

3.1. Fungal strain

Trametes versicolor SBUG-M 1050 was isolated from the wood of a deciduous tree in the north of Germany. The white rot fungus is deposited at the strain collection of the Department of Biology of the University of Greifswald (SBUG).

3.2. Culture media and conditions

3.2.1. Transformation of *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides using whole cells of *Trametes versicolor* SBUG-M 1050. *T. versicolor* was initially cultivated on malt agar plates that were incubated for 7 days at 30°C and then maintained at 4°C. Broth cultures were prepared by inoculating a nitrogen-rich medium[8] with two or three 1 cm^2 agar culture fragments. Incubation was performed under static conditions at 30°C for 7 days. A uniform inoculum was obtained by homogenization of this culture with an Ultra-Turrax homogenizer T25 (IKA Labortechnik, Staufen, Germany) at 17000 rpm. For the production of transformation products, 75 mL medium inoculated with 7.5 mL of the homogenized pre-culture was incubated with 30 mg of *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamide in 300 mL Erlenmeyer flasks for 24 h. Cultures were agitated in a water bath (GFL model 1092, Burgwedel, Germany) at 30°C and 160 rpm.

3.3. Transformation of *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides using crude preparation of laccase

Crude preparation of laccase was prepared as follows: *T. versicolor* was cultivated as described with the substrate **1** substituted by 3,4-dimethoxybenzyl alcohol (10 mM), a known inducer of laccase. The culture medium was filtered through a glass fiber filter in a Buchner funnel to separate the medium from whole cells. The cell-free culture medium was incubated with Q-Sepharose for 1 h and the adsorbed

enzymes were extracted from the Q-Sepharose with 20 mM histidine buffer (pH 6.3). This enzyme extract was then concentrated by ultrafiltration (Centriprep, 10 kDa, Amicon GmbH, Witten, Germany) and desalted with a Sephadex G-25 Superfine column (Pharmacia, Freiberg, Germany).

The crude preparation of laccase (activity 800 nmol mL^{-1} min^{-1}) was diluted 1:4 with 20 mM sodium acetate buffer, pH 5. 6 mg *N*-(2-alkylamino-4-phenylimidazol-1-yl)-acetamide was added to 6 mL of this solution, and the reaction mixture was incubated for 5 h at 30°C with agitation at 100 rpm.

3.4. Analytical HPLC

For routine analysis, culture samples were centrifuged to remove cells and analyzed by a HPLC system (Hewlett-Packard GmbH, Bad Homburg, Germany) consisting of an HP 1050 Series Pump, and HP 1040 M Series I Diode-Array-Detector, and an HP HPLC Chem Station. An endcapped, 5-μm, LiChroCart 125-4 RP 18 column (Merck, Darmstadt, Germany) was used at a flow rate of 1 mL/min. A solvent system consisting of methanol (eluent A) and phosphate buffer pH 4.8 (eluent B), starting from an initial ratio of 20% A and 80% B and reaching 100% methanol within 14 min, was used.

3.5. Synthesis

N-(2-alkylamino-4-phenylimidazol-1-yl)-acetamides were prepared according to literature procedures.[20]

3.6. Isolation: general procedure for isolation of transformation products

An RP18 silicagel column (polypropylene 3 mL, 200 mg absorbent material, Baker, Gross-Gerau, Germany) was charged with 10 mL cell-free culture media. The substrate and several products were eluted together with 1 mL of methanol.

The aqueous filtrate was extracted three times with an equal volume of ethyl acetate, first at pH 6 and then at pH 2. The unified ethyl acetate phases, which contained the products mandelic acid, oxophenyl acetic acid, benzoic acid, and benzyl alcohol, were dried over anhydrous sodium sulfate and then evaporated with a vacuum rotator at 40°C. All samples obtained were redissolved in 1 mL methanol.

The separation of products **2**–**6** from the methanolic fraction was performed on a HPLC module system (Merck, Darmstadt, Germany) equipped with a Model L 6200A Intelligent Pump, a Rheodyne 7161 injection valve with a 20-μL loop, a Model L-4250 absorbance detector operating at 220 nm. High purity of products was achieved on an endcapped, 5-μm, LiChroCart 125-4 RP 18 column (Merck, Darmstadt, Germany) at a flow rate of 1 mL/min. For sufficient separation the solvent system, consisting of methanol (eluent A) and phosphate buffer pH 4.8 (eluent B) in a ratio of 45% A and 55% B, was held constant for 5 min, then changed to a ratio of 50% A and 50% B within 30 s and held constant for another 4.5 min. Then the ratio was changed within 30 s to 60% A and 40% B, held for 2.5 min and finally changed again within 30 s to 100% A for additional 2 min. The products **2**–**4** were obtained in high purity, compounds **5** and **6** as an inseparable mixture.

3.7. Characterization

Analysis of purified products by gas chromatography/mass spectrometry was carried out on a gas chromatograph GC 8000 linked to a mass selective detector MD 800 (Fisons Instruments, Mainz, Germany) operating at 70 eV and fitted with a 30-m BPX5 ms column (0.21 mm; 0.25 μm film, SGE, Weiterstadt, Germany). Other mass spectral determinations were made by electron impact analysis at 70 eV with sample introduction via a direct insertion probe. Molecular weights were confirmed by fast atom bombardment (+FAB) analysis on a M-40 mass spectrometer (AMD Intectra) using 3-nitrobenzyl alcohol as a matrix.

The ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Bruker (Karlsruhe, Germany) ARX 300 instrument at 300 MHz or DRX 500 at 500 MHz in DMSO-d_6. Tetramethylsilane was used as an internal standard.

The four products separated by ethyl acetate extraction were identified as mandelic acid, oxophenylacetic acid, benzoic acid, and benzyl alcohol by comparison of the HPLC/UV and GC–MS data with those of synthetic standards.

3.7.1. *N*-[5-Formyl-4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide 2. This compound was received as a transformation product of *N*-[4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide by *T. versicolor*. Isolation from culture medium was achieved by the use of RP18-silicagel column. Separation was carried out as described before: yield 4 mg (13%). 300 MHz ^1H NMR (DMSO-d_6) δ: 9.40 (s, 1H, CHO), 7.74 (d, ^3J=7.3 Hz, 2H, H_o, C_6H_5), 7.45 (m, 3H, H_m, H_p, C_6H_5), 7.16 (t, ^3J=5.8 Hz, 1H, NH, *NH*–CH_2), 3.29 (m, 2H, CH_2, *CH_2*–NH), 1.99 (s, 3H, CH_3, *CH_3*–CONH), 1.57 (m, 2H, CH_2, $NHCH_2$*CH_2*CH_3), 0.88 (t, ^3J= 7.4 Hz, 3H, CH_3, *CH_3*$(CH_2)_2NH$); ^{13}C NMR (DMSO-d_6): 175.0 (C=O, CHO), 169.3 (C=O, NH–*C*OCH_3), 152.9, 151.1 (C-4, C-2, imidazole), 132.7 (C-1, C_6H_5), 128.9 (C_p, C_6H_5), 128.5 (C_o, C_6H_5), 128.4 (C_m, C_6H_5), 122.1 (C-5, imidazole), 43.4 (CH_2, CH_3CH_2*CH_2*NH), 22.5 (CH_2, CH_3*CH_2CH_2*NH), 20.7 (CH_3, *CH_3*–CONH), 11.1 (CH_3, *CH_3*$(CH_2)_2NH$); MS (GC/MS): M^+=286, 257 (M^+-29, COH), 243 (M^+-43, $COCH_3$), 228 (M^+-58, $HNCOCH_3$), 200 (M^+-86, $HNCOCH_3/C_2H_4$), 186, 158, 131, 104, 103, 77, 43

3.7.2. 4-Phenyl-2-(propylamino)imidazole 3. This compound was found as a transformation product of *N*-[4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide by *T. versicolor*. Isolation from culture medium was carried out by the use of RP18-silicagel column and preparative HPLC as described before: yield 4 mg (13%). 300 MHz ^1H NMR (DMSO-d_6) δ: 7.88 (d, ^3J=6.7 Hz, 2H, H_o, C_6H_5), 7.46–7.31 (m, 3H, H_m, H_p, C_6H_5, 1H, imidazole), 3.11 (m, 2H, CH_2, *CH_2*–NH), 1.54 (m, 2H, CH_2, $NHCH_2$*CH_2*CH_3), 0.91 (t, ^3J=7.4 Hz, 3H, CH_3, *CH_3*$(CH_2)_2NH$); MS (GC/MS): M^+=210, 173 (M^+-28, C_2H_4), 160, 104, 103, 77.

3.7.3. 2-{{2-{[2-Acetyl-1-(benzoylmethyl)hydrazino]carbonyl-amino}-2-phenylvinyl}}-4-propylsemicarbazide 4. This compound was detected after transformation of *N*-[4-phenyl-2-(propylamino)imidazol-1-yl]-acetamide by whole cells of *T. versicolor* and by crude preparation of laccase. For structure elucidation, compound **4** was produced in reaction mixture with crude preparation of laccase, because of the shorter reaction time and a higher accumulation of the product. Isolation from the reaction mixture was carried out by use of a RP18-silicagel column and the product was purified with HPLC as described before: yield 2.8 mg (47%). 500 MHz ^{1}H NMR (DMSO-d_6) δ: 7.64 (t, ^3J=7.5 Hz, 1H, H_p, C_6H_5–CO–CH_2–N), 7.62 (d, ^3J=7.7 Hz, 1H, H_o, C_6H_5–CO–CH_2–N), 7.47 (dd, ^3J=7.5 Hz, ^3J=7.7 Hz, 1H, H_m, C_6H_5–CO–CH_2–N), 7.42 (d, ^3J=7.6 Hz, 1H, H_o, C_6H_5), 7.30 (t, ^3J=7.2 Hz, 1H, H_p, C_6H_5), 7.26 (t, ^3J=5.8 Hz, 1H, NH, CH_3–CH_2–CH_2–*NH*–C), 7.19 (dd, ^3J=7.6 Hz, ^3J=7.2 Hz, 1H, H_m, C_6H_5), 5.76 (s, 1H, CH), 3.52 (s, 2H, CH_2, C_6H_5–CO–*CH*$_2$–N), 3.27 (m, 2H, CH_2, CH_3–CH_2–*CH*$_2$–NH–C), 1.68 (s, 3H, CH_3, *CH*$_3$–CONH), 1.55 (m, 2H, CH_2, CH_3–*CH*$_2$–CH_2–NH–C), 0.86 (t, ^3J=7.4 Hz, 3H, CH_3, *CH*$_3$–CH_2–CH_2–NH–C); ^{13}C NMR (DMSO-d_6): 192.1 (C=O, C_6H_5–*C*O–CH_2–N), 180.3 (C=O), 169.6 (C=O, CH_3–*C*ONH), 153.4 (C=O bzw. C=NH, CH_3–CH_2–*C*H_2–NH–*C*), 134.0 (C_p, C_6H_5–CO–CH_2–N), 133.3 (C1, C_6H_5), 133.2 (C1, C_6H_5–CO–CH_2–N), 129.2 (C_o, C_6H_5), 129.1 (C_o, C_6H_5–CO–CH_2–N), 128.9 (C_p, C_6H_5), 128.7 (C_m, C_6H_5–CO–CH_2–N), 127.6 (C_m, C_6H_5), 120.5 (CH), 118.2 (C-quaternary), 69.8 (CH_2, C_6H_5–CO–*C*H_2–N), 43.6 (CH_2, CH_3–CH_2–*C*H_2–NH–C), 22.5 (CH_2, CH_3–*C*H_2–CH_2–NH–C), 20.5 (CH_3, *C*H_3–CONH), 11.1 (CH_3, *C*H_3–CH_2–CH_2–NH–C); MS (FAB): 105 (H_5C_6CO), 77, 43.

3.7.4. *N*-(3-Hydroxy-4-phenyl-2-propylimino-2,3-dihydroimidazol-1-yl)-acetamide 5 and *N*-(5-oxo-4-phenyl-2-propylimino-2,5-dihydroimidazol-1-yl)-acetamide 6. These products were initially detected by HPLC/UV in culture medium of whole cells of *T. versicolor* and in the reaction mixture using the crude preparation of laccase. The products formed by transformation with the crude preparation of laccase were used for spectral analysis, because of the shorter reaction time and the higher yield of products. Isolation from the reaction mixture was carried out by the use of RP18-silicagel column and the products were received as mixture by preparative HPLC as described before: yield 3.4 mg (57%).

MS (GC/MS compound **5**): M^+=274, 246 (M^+−28, C_2H_4) 232 (M^+−42, H_2CCO), 216 (M^+−58, $HNCOCH_3$), 204 (M^+−70, H_2CCO/C_2H_4), 203 (M^+−71, $COCH_3/C_2H_4$), 190 (M^+−84, H_2CCO/C_3H_6), 188 (M^+−86, $HNCOCH_3/C_2H_4$), 173, 158, 131, 127, 106, 104, 91, 77, 43.

MS (GC/MS compound **6**): M^+=272, 230 (M^+−42, H_2CCO), 214 (M^+−58, $HNCOCH_3$), 201 (M^+−71, $COCH_3/C_2H_4$), 187 (M^+−85, $COCH_3/C_3H_6$), 174, 160, 104, 103, 77, 43.

Acknowledgements

We thank Ute Sack for providing the crude preparation of manganese peroxidase, M. Kindermann, S. Siegert, B. Witt for performing NMR spectra, and R. Jack for help in preparing the manuscript.

References

1. Yaropolov, A. I.; Skorobogat'ko, O. V.; Vartanov, S. S.; Varfolomeyev, S. D. *Appl. Biochem. Biotechnol.* **1994**, *49*, 257–280.
2. Kawai, S.; Umezawa, T.; Higuchi, T. *Arch. Biochem. Biophys.* **1988**, *262*, 99–110.
3. Bourbonnais, R.; Paice, M. G.; Reid, I. D.; Lanthier, P.; Yaguchi, M. *Appl. Environ. Microbiol.* **1995**, *61*, 1876–1880.
4. Collins, P. J.; Koterman, M. J. J.; Field, J. A.; Dobson, A. D. W. *Appl. Environ. Microbiol.* **1996**, *62*, 4563–4567.
5. Hoff, T.; Liu, S. Y.; Bollag, J. M. *Appl. Environ. Microbiol.* **1985**, *49*, 1040–1045.
6. Simmons, K. E.; Minard, R. D.; Bollag, J. M. *Environ. Sci. Technol.* **1987**, *21*, 999–1003.
7. Leonowicz, A.; Edgehill, R. U.; Bollag, J. M. *Arch. Microbiol.* **1984**, *137*, 89–96.
8. Jonas, U.; Hammer, E.; Schauer, F.; Bollag, J. M. *Biodegradation* **1998**, *8*, 321–328.
9. Bollag, J. M.; Minard, R. D.; Liu, S. Y. *Environ. Sci. Technol.* **1983**, *17*, 72–80.
10. Potthast, A.; Rosenau, T.; Chen, C. L.; Gratzl, J. S. *J. Org. Chem.* **1995**, *60*, 4320–4321.
11. Agematu, H.; Tsuchida, T.; Kominato, K.; Shibamoto, N.; Yoshioka, T.; Nishida, H.; Okamoto, R. *J. Antibiot.* **1993**, *46*, 141–148.
12. Heinfling, A.; Bergbauer, M.; Szewzyk, U. *Appl. Microbiol. Biotechnol.* **1997**, *48*, 261–266.
13. Bollag, J. M. Enzymes Catalyzing Oxidative Coupling Reactions of Pollutants. *Metal Ions in Biological Systems*; Sigel, H., Sigel, A., Eds.; Marcel Dekker: New York, 1992; Vol. 28, pp. 205–217.
14. Bollag, J. M.; Myers, C.; Pal, S.; Huang, P. M. The Role of Abiotic and Biotic Catalysts in the Transformation of Phenolic Compounds. In *Environmental Impacts of Soil Component Interactions*; Huang, P. M., Berthelin, J., Bollag, J. M., McGill, W. B., Page, A. L., Eds.; Lewis Publishers: Chelsea, MI, 1995; pp. 297–308.
15. Tatsumi, K.; Freyer, A.; Minard, R. D.; Bollag, J. M. *Environ. Sci. Technol.* **1994**, *28*, 210–215.
16. Youn, H. D.; Hah, Y. C.; Kang, S. O. *FEMS Microbiol. Lett.* **1995**, *132*, 183–188.
17. Youn, H. D.; Kim, K. J.; Maeng, J. S.; Han, Y. H.; Jeong, I. B.; Jeong, G.; Kang, S. O.; Hah, Y. C. *Microbiology* **1995**, *141*, 393–398.
18. Dec, J.; Bollag, J. M. *Environ. Sci. Technol.* **1995**, *29*, 657–663.
19. Kawai, S.; Umezawa, T.; Shimada, M.; Higuchi, T. *FEBS Lett.* **1988**, *236*, 309–311.
20. Hetzheim, A.; Peters, O.; Beyer, H. *Chem. Ber.* **1967**, *100*, 3418–3426.