Original

Degradation of Lignin Substructure Models with Biphenyl Linkage by *Phanerochaete chrysosporium* Burds

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Abstracts.——A study was made for elucidating the degradption process of biphenyl structure by a white-rot fungus *Plhanerochaete chrysosporium* using two dehydrodimers (**II** and **V**) derived from pinoresinol monoethyl ether (**I**) and guaiacylglycerol-β-guaiacyl ether (**IV**). In both cases, alkyl-aryl (C_α–C_1) cleavage was found to be a major degradative reaction by the fungus.

Introduction

Guaiacyl type lignin substructure model compounds are largely condensed to give 5–5′ linked biphenyl dimers as a major product by white-rot fungi producing phenol-oxidizing enzymes[1,2] or by the enzymes[3]. Biphenyl structure is an important linkage unit in natural lignin macromolecule[4] and resistant to microbial attack[5]. Its degradation is presumed to be related closely to the aromatic ring cleavage, but the pathway or mechanism of the degradation by white-rot fungi has not been elucidated. In this point of view, we have investigated the degradation by a white-rot fungus *Phanerochaete chrysosporium* of two phenolic biphenyl models **II** and **V**, derived from pinoresinol monoethyl ether (**I**) and guaiacylglycerol-β-guaiacyl ether (**IV**), respectively. Alkyl-aryl (C_α–C_1) cleavage was found to be a major degradative reaction by the fungus, but no information was obtained for aromatic ring cleavage.

Materials and Methods

Fungus and Culture Conditions

Phanerochaete chrysosporium Burds. ME-446 was used. The fungus was grown in a nitrogen-limited (2.6 mmolar N), 1% glucose, dilute mineral salts medium (20 ml in a 300 ml Erlenmeyer flask) at 39°C without agitation[6,7]. Substrate (2.5 mg/culture) was added to 6-day-old cultures as *N*, *N*-dimethylformamide solution (50 μl).

Substrates

Compound **II** was obtained as a product of **I** by *P. chrysosporium* as previously

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described[2]. The structure was confirmed by ^{1}H- and ^{13}C-NMR spectroscopy[2].

Compound **V** was obtained as an oxidation product of guaiacylglycerol-β-guaiacyl ether (**IV**, *erythro/threo* mixture) with peroxidase and H_2O_2[8]. The structure was confirmed by ^{1}H- and ^{13}C-NMR specroscopy. ^{1}H-NMR (acetone-d_6) δ (ppm): 3.5–3.9 (4H, m, γ-CH_2—), 3.78, 3.83 and 3.85 (12H, s×3, —OCH_3), 4.2–4.4 (2H, m, β-CH—), 4.92 (2H, m, α-CH—), 6.75–7.25 (12H, m, Ar-H). ^{13}C-NMR (acetone-d_6) δ (ppm): 56.3 and 56.4 (—OCH_3), 61.9 and 62.0 (γ-C), 74.0 (α-C) 86.6 and 88.4 (β-C), 110.2–151.9 (21 signals, Ar-C).

Isolation and Identification of Degradation Products

Extraction of the cultures was conducted as previously described[2]. Products were isolated from the acetylated extracts by preparative TLC, and identified by ^{1}H-NMR and mass spectrometry. Analytical instruments were the same as previously described[2].

The uninoculated control culture did not catalyze the reaction described here.

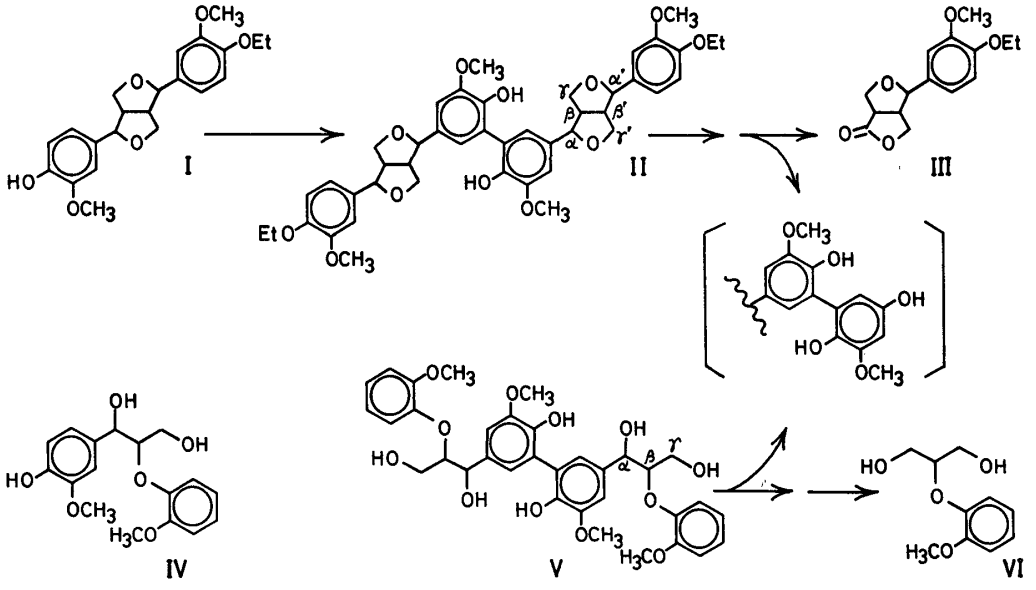

Fig. 1. Alkyl-aryl cleavage of lignin model compounds with biphenyl linkage by *Phanerochaete chrysosporium*.

Results and Discussion

In the previous research we found that pinoresinol monoethyl ether (**I**), a β-β' linked lignin substructure model, was largely dimerized at free *ortho*-position of the phenolic hydroxyl group, probably by the action of fungal phenol-oxidizing enzymes[2]. In the present study, the fate of this dehydrodimer **II** was examined in cultures of *P. chrysosporium*. The biphenyl dimer **II** was rather stable, but slowly degraded to give 6-oxo-2-(4-ethoxy-3-methoxyphenyl)-3, 7-dioxabicyclo [3, 3, 0] octane (**III**) in 5 days.

About 75% of II was recovered. The structure of **III** was identified from its ^{1}H-NMR and mass spectra referring to those of a related analog[2]. ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.46 (3H, t, J=7 Hz, $-CH_3$), 3.13 (1H, m, β'-CH$-$), 3.45 (1H, br. dt, J=4.0, 8.8 Hz, β-CH$-$), 3.89 (3H, s, $-OCH_3$) 4.11 (2H, q, J=7Hz, $-OCH_2CH_3$), 4.19 (1H, dd, J=9.3, 4.0 Hz) and 4.37 (1H, approx. t, J=9 Hz) (γ-CH_2-), 4.33 (1H, dd, J=9.7, 2.1 Hz) and 4.51 (1H, dd, J=9.7, 6.8 Hz) (γ'-CH_2-), 4.63 (1H, d, J=6.7 Hz, α'-CH$-$), 6.85–6.93 (3H, m, Ar-H). MS *m/z* (%): 278 (M^+, 100), 249 (12), 247 (9), 233 (14.1), 186 (13), 180 (21.7), 165 (33.7), 163 (18.5), 152 (57.6), 151 (41.3), 149 (19.6), 137 (42.4), 135 (15.2). The results indicated that this biphenyl dimer **II** was degraded mainly via alkyl-aryl (C_α–C_1) cleavage, after oxidation of the benzylic position (C_α), as has been found with syringaresinol having an additional methoxyl group at *ortho* to the phenolic hydroxyl group[2]. Direct C_α–C_β side chain cleavage was not found. This was also consistent with the previous results[2].

On the other hand, another type of biphenyl model **V** prepared from the β-ether dimer **IV** was more actively degraded by the fungus, and disappeared completely in 5 days. Only one product was isolated and identified as glycerol-2-guaiacyl ether (**IV**) from its ^{1}H-NMR and mass spectra referring to those of a related analog[9]. ^{1}H-NMR (diacetate, $CDCl_3$) δ (ppm): 2.07 (6H, s, $-OCOCH_3$), 3.84 (3H, s, $-OCH_3$), 4.34 (4H, d, J=5 Hz, $-CH_2-$), 4.54 (1H, quintet, J=5 Hz, $-CH-$), 6.83–7.07 (4H, m, Ar-H). MS (diacetate) *m/z* (%): 228 (M^+, 2.3), 159 (48.5), 124 (25.8), 109 (13), 99 (6.1), 43 (100). Other compounds did not allow the structural elucidation because of their small quantities. These results indicated that **V** was also degraded via alkyl-aryl cleavage, followed by reduction of glyceraldehyde-2-guaiacyl ether as speculative intermediate to give **VI**. The aldehyde intermediate has been isolated as a product of **IV** with peroxidase and H_2O_2[8]. The accumulation of **VI** might be ascribed to its relative stability in cultures of the fungus. Other various reactions including the C_α–C_β side chain cleavage were also considered to be operative in the degradation of **V** by *P. chrysosporium*.

In both cases, counterpart products released by the cleavage reaction were not characterized, but hydroquinone (see Fig. 1) or the corresponding *p*-quinone derivatives were assumed as possible intermediates. Apparently, these compounds are unstable and presumed to be metabolized or polymerized faster than **III** and **VI** in the culture.

From these experiments, information concerning the aromatic ring cleavage by *P. chrysosporium* was not obtained. Considering the results with vanillic acid[10], however, hydroquinone derivatives described above would undergo ring cleavage reaction. The formation of 5-carboxyvanillic acid from dehydrodivanillic acid by *P. chrysosporium* was reported, but the pathway remained unclear[11]. Hence, some

attempts were made using such simple biphenyl compounds, but ring cleavage products could not be isolated from the complex mixture of the products.

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