A novel C_α-C_β cleavage of a β-*O*-4 lignin model dimer with rearrangement of the β-aryl group by *Phanerochaete chrysosporium*

Toshiaki Umezawa and Takayoshi Higuchi

Research Section of Lignin Chemistry, Wood Research Institute, Kyoto University, Uji, Kyoto 611, Japan

Received 22 August 1985

Incubation of a β-*O*-4 lignin substructure model dimer, 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (I), with a culture of *Phanerochaete chrysosporium* yielded 2-guaiacoxyethanol (II) as one of the main catabolites. It was demonstrated by isotopic investigation with β-^{18}O- and γ-^{13}C-labeled substrates that the guaiacyl group at the β-position of (I) was rearranged to the adjacent γ-position in the formation of (II) by C_α-C_β cleavage.

Aromatic ring rearrangement *Lignin biodegradation* *β-O-4 substructure* *White-rot fungus*

1. INTRODUCTION

In the metabolism of β-*O*-4 (arylglycerol-β-aryl ether) lignin substructure models, the most frequent substructure of lignin [1], by white-rot fungi, many metabolites have been identified [2]. One of the major catabolites formed from the β-*O*-4 dimer by a white-rot fungus, *Phanerochaete chrysosporium*, 2-guaiacoxyethanol (II), was first identified by Enoki et al. [3]. We determined by use of γ-^{13}C- and β-^{18}O-labeled β-*O*-4 dimers as substrates that the guaiacyl group at the β-position underwent rearrangement to the γ-position in the formation of (II) by the fungus. The mechanism is distinct from that proposed by Enoki et al. [3].

2. MATERIALS AND METHODS

2.1. *Culture conditions*

Cultures (20 ml/300 ml Erlenmeyer flask) of *P. chrysosporium* Burds. (ME-446) were grown at 39°C without agitation in a nitrogen-limiting medium [4–6].

2.2. *Metabolism of lignin model compounds*

4-Ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (I) and its β-^{18}O and γ-^{13}C analogs [(I-β-^{18}O) and (I-γ-^{13}C), respectively] were metabolized by *P. chrysosporium* and the metabolites extracted and acetylated as in [5,6]. The acetylated metabolites were separated 3 times by TLC (Kieselgel 60, F_{254}, Merck, developing solvent: 1st, CH_2Cl_2/*n*-hexane = 2:1, v/v, once then CH_2Cl_2, 4 times; 2nd, CH_3OH/CH_2Cl_2 = 1:49, v/v, 4 times; 3rd, CH_2Cl_2/*n*-hexane = 2:1, v/v, once then CH_2Cl_2, 3 times) to give the acetate of 2-guaiacoxyethanol (II-Ac), which was identified by ^{1}H-NMR and MS.

2.3. *Preparation of compounds*

(I) and (I-β-^{18}O) were prepared as in [5,6]. (I-γ-^{13}C) was prepared from acetovanillone as follows: (1) ethyl iodide and K_2CO_3 in DMF, 50°C; (2) $CuBr_2$ in ethyl acetate, reflux; (3) guaiacol and K_2CO_3 in DMF, ambient temperature; (4) [^{13}C]paraformaldehyde (MSD, 99 atom% ^{13}C) and K_2CO_3 in DMSO, ambient temperature; (5) $NaBH_4$ in dioxane and methanol, 0°C. MS (acetate) *m/z* (%), 433(13.3), 432(0), 251(4.6), 250(6.4), 223(12.7), 210(22.8), 208(29.2), 207(28.8), 181(100). 2-Guaiacoxyethanol (II) and 2-[1-^{2}H$_2$]guaiacoxyethanol (II-2^{2}H) were prepared by reduction of ethyl-2-guaiacoxy acetate prepared

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

previously [7] with $LiAlH_4$ and $LiAl^2H_4$ (CEA, min. 99 atom% ^2H), respectively. 2-(*m*-Methoxyphenoxy)ethanol (III) and 2-(*p*-methoxyphenoxy)ethanol (IV) were prepared in the same way for the preparation of (II) from *m*-methoxyphenol and *p*-methoxyphenol instead of guaiacol. (II), (II-2^2H), (III) and (IV) were acetylated with Ac_2O/pyridine (1:1, v/v) to give (II-Ac), (II-2^2H-Ac), (III-Ac) and (IV-Ac), respectively. All the compounds prepared were identified by ^1H-NMR and MS. (II-Ac): MS *m/z* (%), 210(M^+, 3.7), 124(12.2), 109(18.0), 87(100). (II-2^2H-Ac): MS *m/z* (%), 212(10.7), 211(0.1), 210(0), 124(22.4), 109(29.8), 89(100). ^1H-NMR and mass spectra were taken with a Varian XL-200 FT-NMR spectrometer (solvent, $CDCl_3$; internal standard, TMS) and a Shimadzu-LKB 9000 gas chromatograph-mass spectrometer (EI-MS, 70 eV).

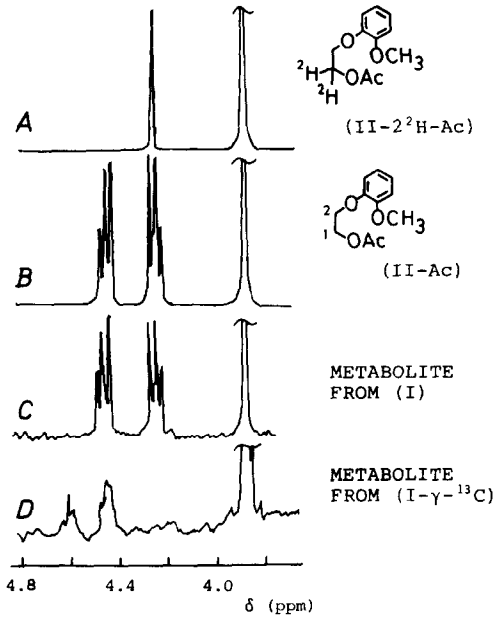

Fig.1. A part of the ^1H-NMR spectra of 2-guaiacoxyethanol (acetate). (A) Synthesized compound (II-2^2H-Ac), (B) synthesized compound (II-Ac), (C) metabolite from (I), (D) metabolite from (I-γ-^{13}C). In spectrum B, peaks at 3.86, 4.23 and 4.44 ppm were assigned to methoxyl group, protons at the 2-position and at the 1-position, respectively, since in A, the acetate of 2-[1-$^2H_2$]guaiacoxyethanol (II-2^2H-Ac), the peak at 4.44 ppm disappeared. Spectra B and C are identical, while the chemical shifts of the peaks of methoxyl groups of the *m*-isomer (III-Ac) and the *p*-isomer (IV-Ac) were 3.79 and 3.77 ppm, respectively (not shown). Decoupling measurement showed that in spectrum D, the peak at 4.23 ppm is split into 4.60 and 3.86 ppm by coupling with ^{13}C (coupling constant, 148 Hz). The peak at 4.44 ppm became a multiplet by coupling with ^{13}C (coupling constant, less than about 10 ppm). This result showed that the metabolite was labeled with ^{13}C at the 2-position, since $^1J_{C\text{-}H}$ and $^2J_{C\text{-}H}$ are usually >100 and <10 Hz, respectively.

3. RESULTS

After incubation of (I) with *P. chrysosporium* for 93 h, the acetate of 2-guaiacoxyethanol (II-Ac) was isolated from the acetate of the culture extracts and identified by comparison of the mass and ^1H-NMR spectra with those of (II-Ac), (III-Ac) and (IV-Ac) (fig.1). When 2 mg (I) was added to 20 ml culture, the yield of (II) was 30 μg, 3% (mol product formed/mol initial substrate $\times$ 100) based on the stable isotope dilution procedure using (II-2^2H) as an internal standard. Uninoculated control culture did not catalyze the conversion from (I) to (II). When (I-β-^{18}O) (61.7 atom% excess ^{18}O in the β-position) was incubated with the fungus, GC-MS analysis of the acetate of the metabolites [1% OV-1 on Chromosorb W (AW-DMCS), column temperature (150°C), glass column (2 m $\times$ 0.3 cm, i.d.), carrier gas (28 ml/min)] showed that ^{18}O was almost lost in the formation of 2-guaiacoxyethanol (table 1). In the incubation of (I-γ-^{13}C) as described above, MS analysis showed that the 2-guaiacoxyethanol formed re-

Table 1

Relative intensity of molecular ion region of the mass spectra of the acetates of 2-guaiacoxyethanol

m/z	Relative intensity (%)[d]		
	Metabolite 1[a]	Metabolite 2[b]	Unlabeled[c]
309	0	0	0
310	100	4.5	100
311	13.7	100	13.5
312	5.6	13.9	2.6

[a] Metabolite from (I-β-^{18}O)
[b] Metabolite from (I-γ-^{13}C)
[c] Unlabeled authentic compound (II-Ac)
[d] Background correction was not made

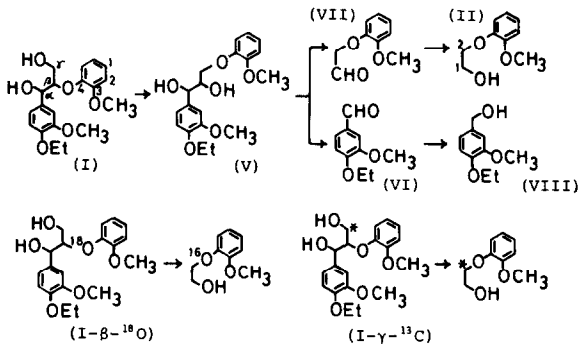

Fig.2. Proposed mechanism of formation for 2-guaiacoxyethanol (II) from 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (I) by *P. chrysosporium*. (V) and (VII): assumed compounds.

tained the ^{13}C atom (table 1), which was found to be at the 2-position of 2-guaiacoxyethanol by ^{1}H-NMR analysis (fig.1).

4. DISCUSSION

We propose a possible mechanism of formation for (II) from (I) by *P. chrysosporium* as shown in fig.2. First, the guaiacyl group was rearranged to the γ-position to give (V), which was subsequently cleaved between C_α and C_β to form 4-ethoxy-3-methoxybenzaldehyde (VI) and 2-guaiacoxyacetaldehyde (VII). Both (VI) and (VII) were reduced to 4-ethoxy-3-methoxybenzyl alcohol (VIII) and (II). This mechanism is quite distinct from that proposed for the formation of (II) by Enoki et al. [3]. We previously reported that C_α-C_β cleavage of a γ-benzyl ether analog of (V), 4-ethoxy-3-methoxyphenylglycerol-γ-benzyl ether was cleaved by the fungus to give (VIII) and benzyloxyethanol [4,8]. Intradiol cleavages of phenylglycol structures to give benzaldehydes by enzyme preparations from the fungus have also been reported [9,10]. The disappearance of ^{18}O in (II) in the degradation of (I-β-^{18}O) could be explained by exchange between the carbonyl oxygen of (VII) and that of H_2O as that between [*aldehyde*-^{18}O]benzyloxyacetaldehyde and H_2O under the same culture conditions [8]. Since a preliminary experiment showed that the ratio of yields, (II)/(VIII), was about 0.4, the C_α-C_β cleavage proposed here, which is distinct from the mechanism proposed by Tien and Kirk [9], may be major among C_α-C_β cleavage pathways to give (VIII).

As for the rearrangement of the guaiacyl group, 2 possible mechanisms are conceivable: activation of the guaiacyl aromatic ring and of the γ-hydroxyl group of (I). At present, the latter has not been substantiated by any experimental results, while the former, activation of the aromatic ring, was recently reported by Kersten et al. [11]. They showed, based on ESR analysis, that *P. chrysosporium* ligninase produced cation radicals from methoxylated benzenes. The rearrangement of the guaiacyl group might be explained as follows. The guaiacyl nucleus at the β-position of (I) is oxidized to a cation radical by the ligninase. The cation radical is attacked by the γ-hydroxyl group of (I), followed by leaving of the β-oxygen to give (V). Another major metabolite from (I), 4-ethoxy-3-methoxyphenylglycerol [2], could be formed by attack of H_2O on the cation radical instead of the γ-hydroxyl group. Recently, oxidative degradation of lignin models by *P. chrysosporium* enzyme, enzyme model systems and single electron transfer reagents were reported and discussed in relation to one-electron oxidation and C_α-C_β cleavage of β-1 and β-*O*-4 lignin models [12–15].

ACKNOWLEDGEMENTS

The authors are grateful to Dr M. Shimada, Wood Research Institute, Kyoto University, Japan, for his valuable discussions. This research was partly supported by a Grant-in-Aid for Scientific Research (nos 59760124, 60760130, 60440015) from the Ministry of Education of Japan.

REFERENCES

[1] Sakakibara, A. (1980) Wood Sci. Technol. 14, 89–100.
[2] Higuchi, T. (1985) in: Biosynthesis and Biodegradation of Wood Components (Higuchi, T. ed.) pp.557–578, Academic Press, Orlando, FL.
[3] Enoki, A., Goldsby, G.P. and Gold, M.H. (1980) Arch. Microbiol. 125, 227–232.
[4] Umezawa, T., Nakatsubo, F. and Higuchi, T. (1983) Agric. Biol. Chem. 47, 2677–2681.
[5] Umezawa, T. and Higuchi, T. (1985) FEMS Microbiol. Lett. 26, 123–126.

[6] Umezawa, T. and Higuchi, T. (1985) FEBS Lett. 182, 257–259.
[7] Nakatsubo, F., Sato, K. and Higuchi, T. (1975) Holzforschung 29, 165–168.
[8] Umezawa, T., Higuchi, T. and Nakatsubo, F. (1983) Agric. Biol. Chem. 47, 2945–2948.
[9] Tien, M. and Kirk, T.K. (1984) Proc. Natl. Acad. Sci. USA 81, 2280–2284.
[10] Gold, M.H., Kuwahara, M., Chiu, A.A. and Glenn, J.K. (1984) Arch. Biochem. Biophys. 234, 353–362.
[11] Kersten, P.J., Tien, M., Kalyanaraman, B. and Kirk, T.K. (1985) J. Biol. Chem. 260, 2609–2612.
[12] Schoemaker, H.E., Harvey, P.J., Bowen, R.M. and Palmer, J.M. (1985) FEBS Lett. 183, 7–12.
[13] Harvey, P.J., Schoemaker, H.E., Bowen, R.M. and Palmer, J.M. (1985) FEBS Lett. 183, 13–16.
[14] Habe, T., Shimada, M., Okamoto, T., Panijpan, B. and Higuchi, T. (1985) J. Chem. Soc. Chem. Commun. 1323–1324.
[15] Hammel, K.E., Tien, M., Kalyanaraman, B. and Kirk, T.K. (1985) J. Biol. Chem. 260, 8348–8353.