

Degradation of β -1 Linked Dilignols by *Fusarium solani* M-13-1*

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Abstract—1,2-Diarylpropane-1,3-diol, which is one of major substructures of lignin, was found to be mainly degraded, yielding methoxybenzoquinone and 2-arylpropane-1,3-diol via alkyl-phenyl cleavage by *Fusarium solani* M-13-1. A part of the diarylpropane is degraded by α - β cleavage to give C6-C1 aldehyde and phenylglycol via an epoxide derivative.

1. Introduction

Lignin, which is a dehydrogenation polymer of *p*-hydroxycinnamyl alcohols, is composed of various microbiologically stable linkages such as β -O-4, β -5, β - β' , 5-5' and 4-O-5 substructures. To elucidate lignin biodegradation we have investigated degradation pathways for main dilignols as substructures of lignin molecule by a white-rot fungus, *Phanerochaete chrysosporium*, and *Fusarium solani* M-13-1.¹⁾ We expect that the degradation pathways for these dilignols are operative in the degradation of lignin polymer. In the following, the degradation of β -1 linked dilignol, 5–15% of which is contained in lignin, by *Fusarium solani* M-13-1 is presented.

2. Experimental

2.1 Microorganism

Fusarium solani M-13-1 which was isolated from soil by an enrichment technique²⁾ using coniferyl alcohol DHP as sole carbon source was used. Mycelia from the stock culture of the *Fusarium* were inoculated into the nutrient medium²⁾ (pH 6, 100 ml in a 500 ml flask) and shake-cultured for 2 days at 30°C. Mycelia were separated by filtration and washed with the inorganic medium²⁾, and used for degradation experiment of the dilignol.

2.2 Degradation of β -1 dilignols

β -1 Dilignol was dissolved in a small amount of N,N-dimethylformamide (DMF), and the solution was added into the sterilized inorganic medium. The medium was then inoculated with mycelia. Controls which contain only mycelia and/or

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substrate in the inorganic medium were prepared. Inoculated flasks were shaken at 30°C, together with the controls.

2.3 Analysis of metabolic products

Degradation of the substrates was monitored by UV spectroscopy and TLC analysis of the cultured filtrates. When compounds different from substrates were detected on a silica gel TLC plate under a short-wave UV light and with spray reagent (FeCl₃-potassium ferricyanide solution, 2,4-dinitrophenylhydrazine-HCl), mycelia were removed by filtration and washed with distilled water. The combined filtrate and washings were extracted three times with an equal volume of methylene chloride. The aqueous layer was then acidified with conc. HCl to pH 2 and extracted twice with an equal volume of ethyl acetate. Both organic layers were dried over anhydrous Na₂SO₄ and evaporated *in vacuo*. The residues were dissolved in methylene chloride and metabolic products were separated and purified by preparative TLC (Kieselgel 60 F₂₅₄, Merk). The products purified were identified by NMR and MS spectrometry. Authentic compounds were synthesized and used as reference for identification.

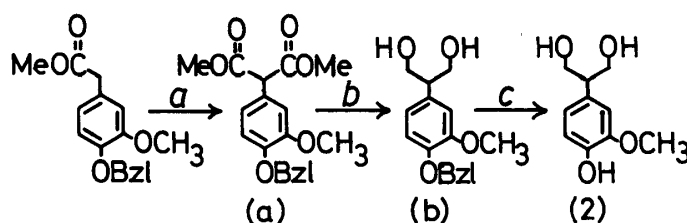
2.4 Preparation of compounds

1,2-Diguaiacylpropane-1,3-diol (1), and *1,2-disyringylpropane-1,3-diol (1')*

(1) was synthesized by the condensation of methylbenzylhomovanillate with benzylvanillin³⁾. 1,2-Disyringylpropane-1,3-diol (1') was also synthesized from syringyl derivatives by the same method.

2-Guaiacylpropane-1,3-diol (2)

(2) was synthesized from homovanillate derivative as shown in Fig. 1. To a stirred solution of lithium diisopropylamide (4 mmol) in 15 ml anhydrous THF, homovanillate derivative (858 mg, 3 mmol) in 7.5 ml anhydrous THF was added dropwise over a period of 20 min. below -70°C under nitrogen. The stirring was continued for additional 45 min. at the same temperature, and then dimethyl



a : (MeO)₂CO, LDA, THF, -78 °C

b : LAH, THF, 50 °C

c : 10% Pd/C, MeOH, H₂, r. t.

Fig. 1 Synthetic route for 2-guaiacylpropane-1,3-diol.

carbonate (4 mmol) in 5 ml anhydrous THF was added dropwise over a period of 15 min. After stirring for 30 min. the reaction mixture was neutralized with dry ice, and then partitioned between ethyl acetate and brine. The ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in 20% MeOH in methylene chloride and methylated with a limited amount of diazomethane to derive methyl ester of the product. The methylated product (a) was purified by silica gel TLC (solvent, ethyl acetate/*n*-hexane, 1:4). Yield of the compound a, 453.9 mg (61%).

To a stirred solution of lithium aluminum hydride (870 mg, 23 mmol) suspended in 20 ml anhydrous THF, the compound a (1.57 g, 4.6 mmol) in anhydrous THF (20 ml) was added dropwise over a period of 30 min. at 50°C under nitrogen. The stirring was continued for additional 30 min. and then excess hydride was decomposed by addition of a mixture of THF and H_2O (9:1) at 0°C. The reaction mixture was partitioned between ethyl acetate and brine, and the ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to result in compound b.

The compound b (crude oil) was dissolved in MeOH (10 ml) and 500 mg 10% palladium/carbon was added under stirring. After 60 min. stirring under nitrogen, the catalyst was filtered. The filtrate was evaporated *in vacuo* to give crystals of the target compound (2) which was recrystallized from methylene chloride. Yield, 684 mg (75.4%).

2,2'-Dihydroxy-3,3'-dimethoxy-5,5'-di(1,3-dihydroxy-2-propyl)-biphenyl (3)

The compound, which is 5,5'-condensate of 2-guaiacylpropane-1, 3-diol, was synthesized from 2-guaiacylpropane-1,3-diol with peroxidase/ H_2O_2 . To a stirred solution of 2-guaiacylpropane-1,3-diol and horse radish peroxidase (1 mg) in 5 ml water, H_2O_2 (equivalent amount) was added and stirred for 60 min. The reaction mixture was partitioned between ethyl acetate and brine, and the organic layer was dried over anhydrous Na_2SO_4 . Both layers were evaporated *in vacuo* separately. The residue of the aqueous layer was dissolved in acetone, and the solution was evaporated to give a crude product (21.1 mg). The product was acetylated overnight with acetic anhydride/pyridine at room temperature. The acetylated product was purified by silica gel TLC (solvent, ethyl acetate/*n*-hexane, 3:2) to afford the pure compound (3.6 mg).

NMR (CDCl_3) δ (ppm): 2.06 (12H,s, alc. acetate), 2.09 (6H,s, arom. acetate), 3.31 (2H,t, $J=6$, —CH), 3.88 (6H,s, OCH_3), 4.36 (8H, d, $J=6$, $\text{CH}-(\text{CH}_2\text{OAc})_2$), 6.74, 6.87 (4H, two d, $J=2$, arom.). MS m/z : 604 ($M+42$), 562, 544, 502, 460, 443, 442, 400, 382, 369, 349, 340, 327, 322, 309, 307, 297, 295, 289, 283, 279, 277, 267, 265, 263, 60, 43 (base ion).

3. Results

When *F. solani* M-13-1 was incubated in the medium containing compound (1), UV absorption of the culture filtrate at 279 nm decreased gradually and followed by a large shoulder at 300–340 nm and an increasing absorption of base line of the UV spectrum as shown in Fig. 2.

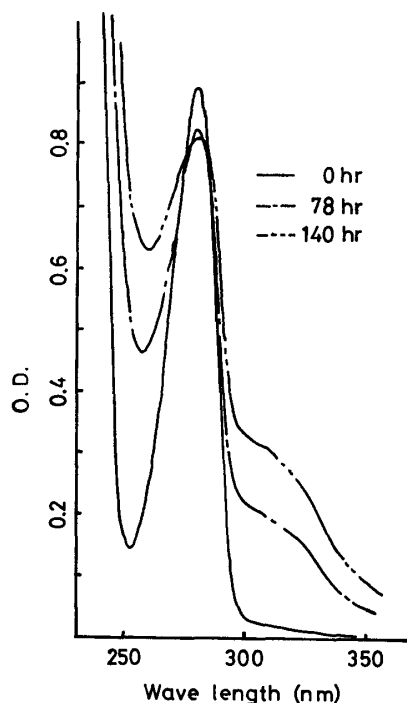


Fig. 2 Changes in UV absorption of 1,2-diguaiacylpropane-1,3-diol during shake culture of *Fusarium Solani*-M-13-1.

From the culture filtrate after 144 hr of incubation a small amount (ca. 1 mg) of 2-guaiacylpropane-1,3-diol (2) was isolated and identified as follows.

Methylene chloride extract and ethyl acetate extract were combined and submitted to silica gel TLC (solvent, 8% MeOH/methylene chloride, ethyl acetate/*n*-hexane, 1:1, 4 times). The product separated was acetylated with acetic anhydride and pyridine at room temperature, and the acetylated product was submitted to silica gel TLC (solvent, ethyl acetate/*n*-hexane, 1:3, twice, 1% MeOH/methylene chloride). The purified acetate was identified by MS spectrometry as shown in Fig. 3. MS m/z : 324 (M^+), 282, 264, 223, 222 (base ion), 209, 180, 167, 151, 150, 149, 147, 135, 131, 124, 119, 107, 103, 91, 43. Relative abundance of the respective ion peaks were identical with those of authentic compound.

3.1 Degradation of 2-guaiacylpropane-1,3-diol (2)

2-Guaiacylpropane-1,3-diol as a degradation intermediate was incubated with

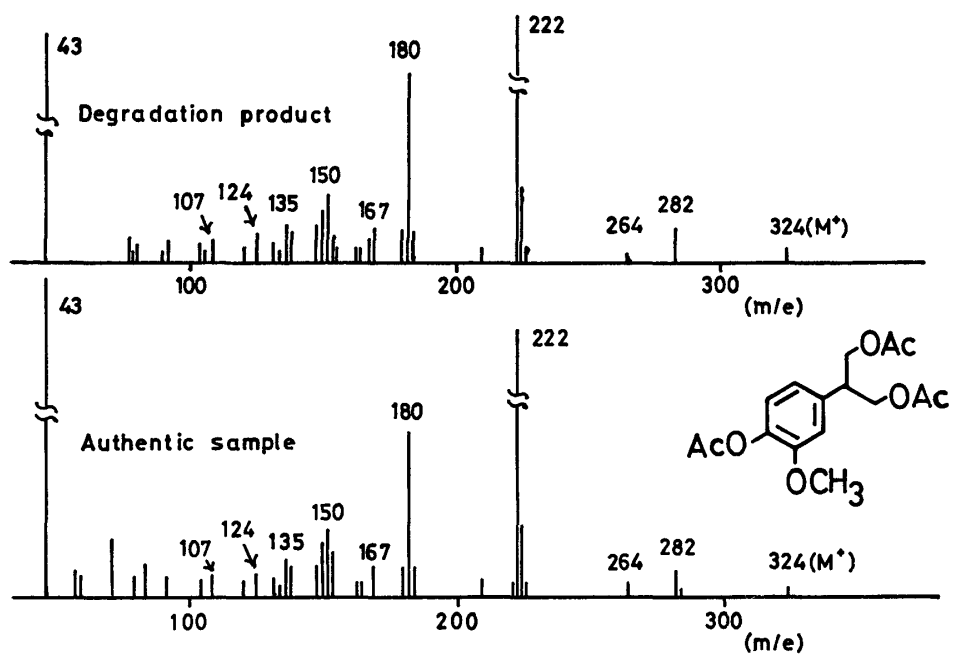


Fig. 3 Mass spectrum of 2-guaiacylpropane-1,3-diol isolated as a degradation product of 1,2-diguaiacylpropane-1,3-diol.

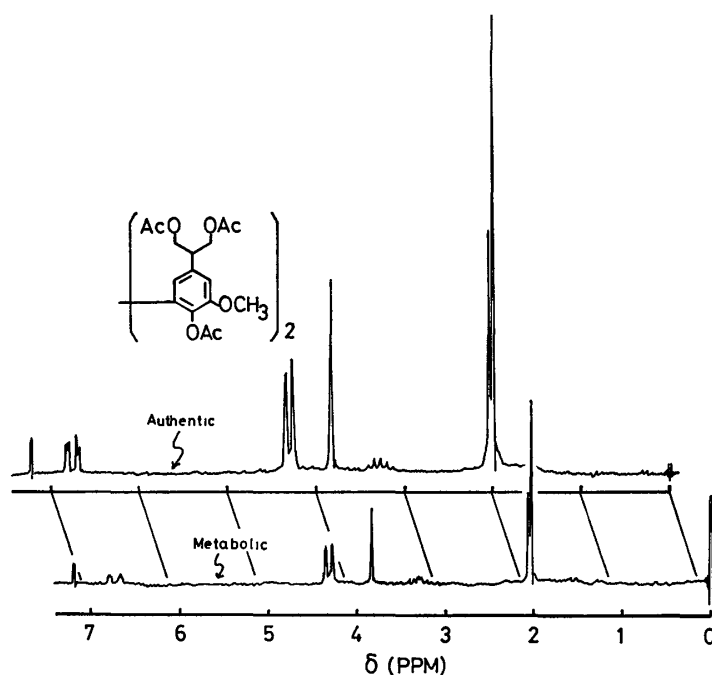


Fig. 4 NMR spectrum of 2,2'-dihydroxy-3,3'-dimethoxy-5,5'-di-(1,3-dihydroxy-2-propyl)-biphenyl as a degradation product of 1,2-diguaiacylpropane-1,3-diol.

the *Fusarium* and the degradation process was monitored by measurement of UV spectra of the culture filtrates during shake-culture.

From the culture filtrate after 84 hr of incubation, about 2 mg of 5-5' conjugate of the substrate (3) was isolated by HPLC (column, Micro bondapack C-18, MeOH/H₂O, 1:9, 3 ml/min.). The compound isolated was acetylated with acetic anhydride and pyridine at room temperature, and purified by silica gel TLC (solvent, methylene chloride). Fig. 4 shows the NMR spectra of isolated compound and authentic sample. The peak of phenolic acetate shifted to the higher magnetic field and aromatic protons showed a meta-coupling, which indicated that 2-guaiacylpropane-1,3-diol was connected by 5-5' linkage. MS spectrum of the compound showed a peak at 604 ($M^+ - 42$) and relative abundance of respective peaks were the same as those of authentic sample. MS m/z : 604 ($M^+ - 42$), 562, 544, 502, 460, 442, 400, 382, 369, 349, 340, 339, 327, 322, 309, 307, 295, 283, 279, 277, 267, 265, 263, 43 (base ion).

3.2 Degradation of 1,2-disyringylpropane-1,3-diol (1')

UV spectra of the culture filtrates with 1' at various incubation times are shown in Fig. 5. The methylene chloride extract of the culture filtrates after 60, 69, 81 and 102 hr of incubation were combined and submitted to silica gel TLC (solvent, 5% MeOH in methylene chloride). The following four fractions were separated. F-1, R_f 1.0–0.29, 19.5 mg; F-2, R_f 0.29–0.19, 2.4 mg; F-3, R_f 0.19–0.12, 6.5 mg; F-4, R_f 0.12–0.0, 1.0 mg.

Fraction-1 was purified by silica gel TLC (solvent, ethyl acetate/*n*-hexane, 1:1) and 2,6-dimethoxy-*p*-benzoquinone (4) was identified by MS spectrometry in com-

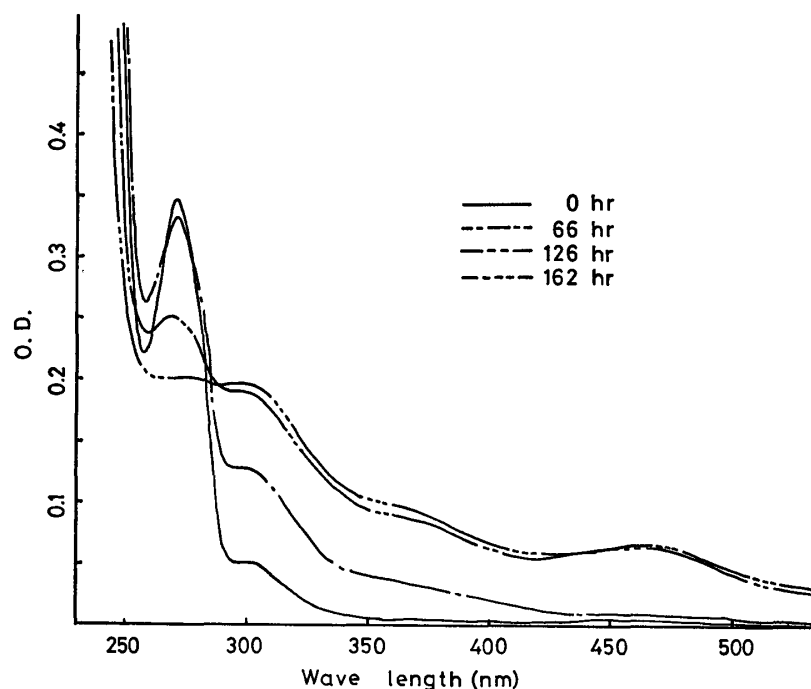


Fig. 5 Changes in UV absorption of 1,2-disyringylpropane-1,3-diol during shake culture of *Fusarium solani* M-13-1.

parison with authentic compound. MS m/z : 168 (M^+), 153, 140, 138, 125, 112, 97, 80, 69 (base ion), 59, 57, 55, 53. Relative abundance of the respective ion peaks of the isolated compound were identical with those of authentic compound.

Fraction-2 was separated and purified by silica gel TLC (solvent, 4% MeOH in methylene chloride). About 1 mg syringaldehyde (7) and 2-syringylpropane-1,3-diol (2') were identified by MS spectrometry. Relative abundance of the ion peaks of both compounds are identical with those of authentic compounds, respectively. Syringaldehyde, MS m/z : 182 (M^+ base ion), 181, 167, 153, 139, 135, 125, 123, 121, 111, 96, 93, 81, 79, 67, 65. Orange color with 2,4-dinitrophenylhydrazine, and blue color with $FeCl_3$ -potassium ferricyanide. 2-Syringylpropane-1,3-diol, MS m/z : 228 (M^+), 197, 181, 169, 154, 149, 138, 125, 123, 113, 97, 95, 85, 83, 81, 73, 71, 69, 57, 55, 43 (base ion). Blue color with $FeCl_3$ -potassium ferricyanide.

Fraction-3 was separated into five fractions (F-3-1 to F-3-5) by silica gel TLC (solvent, ethyl acetate/*n*-hexane, 1:3, three times). Fraction-3-2 was separated by silica gel TLC (solvent, 3% MeOH in methylene chloride, three times) to 1-syringylethane-1,2-diol (6) and an epoxide (5), respectively. 1-Syringylethane-1,2-diol, MS m/z : 214 (M^+), 196, 183, 168, 167 (base ion), 155, 140, 123, 95, 77, 65. Blue color: $FeCl_3$ -potassium ferricyanide, and quinonemonochlorimide/1N NaOH. Relative abundance of the ion peaks of the isolated compound were identical with those of authentic compound. Epoxide, MS m/z : 378 (M^+), 360, 348, 329, 317, 315, 303, 182, 181, 180, 167, 153, 138, 123, 110, 108, 95, 93, 82, 78, 77, 67, 65. Three compounds in Fig. 6 were supposed to have the molecular ion peak at m/z 378. The isolated compound was found to be easily hydrolyzed to a glycerol derivative with 1N HCl in acetone at room temperature, and could not be visualized under a long wave UV ($\lambda_{max}=365$), which indicated that the compound is not a stilbene (5') but an epoxide. Compound (5'') with epoxide structure between C_α and C_β would be more unstable than (5), because both C_α and C_β are in the benzylic positions. Based on these results the compound was tentatively identified as epoxide (5).

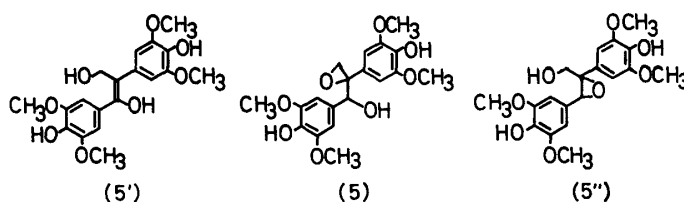


Fig. 6 Possible structures for the $M^+=378$ compounds detected by Mass spectra.

4. Discussion

Fig. 7 shows the proposed degradation pathway for the 1,2-diguaiacylpropane-

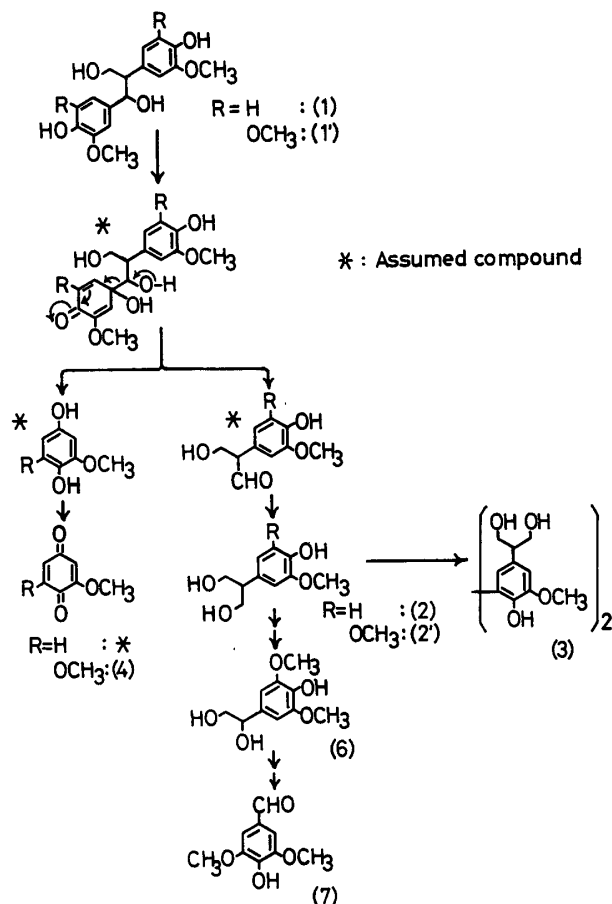


Fig. 7 Proposed degradation pathway for 1,2-diguaiacylpropane-1,3-diol by *Fusarium solani* M-13-1.

1,3-diol (1) by *Fusarium solani* M-13-1 based on the identification of the degradation products. From the culture filtrate of (1), 2-guaiacylpropane-1,3-diol (2) and, 2,2'-dihydroxy-3,3'-dimethoxy-5,5'-di-(1,3-dihydroxy-2-propyl)-biphenyl (3) were identified. The results indicated that compound (1) was cleaved at alkyl-aryl C-C bond. The alkyl-aryl cleavage could be catalyzed by phenol oxidizing enzymes. 2-Methoxy-*p*-benzoquinone could not be identified. It seems that the quinone is oxidatively condensed or metabolized further.

On the other hand, from the culture filtrate of 1,2-disyringylpropane-1,3-diol (1'), 2,6-dimethoxy-*p*-benzoquinone (4), syringaldehyde (7), 2-syringylpropane-1,3-diol (2'), 1-syringylethane-1,2-diol (6), and an epoxide (5) were identified.

It has been found that the non-phenolic substrates, 1,2-diarylpropane-1,3-diols are cleaved to C6-C1 aldehyde and phenylglycol by *Phanerochaete chrysosporium*^{4,5}. The formation of syringaldehyde, 1-syringylethane-1,2-diol and epoxide as degradation product of 1,2-disyringylpropane-1,3-diol seems to indicate that a similar but not the same degradation pathway shown in Fig. 8 is operative in both fungi.

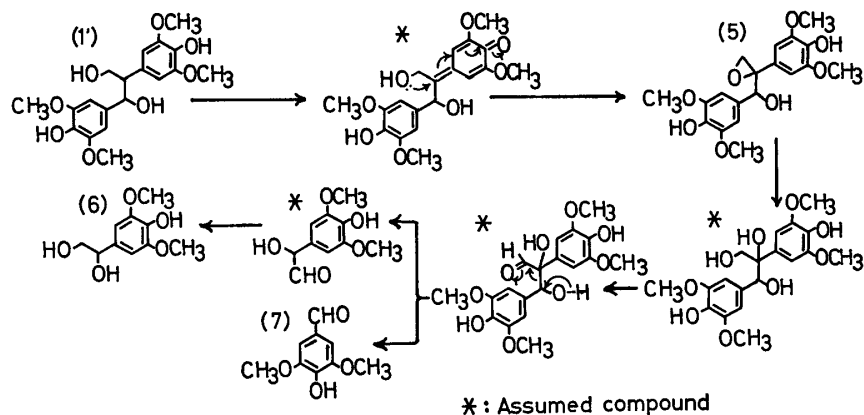


Fig. 8 Alternative degradation pathway for 1,2-disyringylpropane-1,3-diol by *Fusarium solani* M-13-1.

References

- 1) T. HIGUCHI: *Experientia*, **38**, 159 (1982).
- 2) S. IWAHARA, M. KUWAHARA and T. HIGUCHI: *Hakko Kogakushi*, **55**, 325 (1977).
- 3) F. NAKATSUBO and T. HIGUCHI: *Holzforsch.*, **29**, 193 (1975).
- 4) F. NAKATSUBO, I. D. REID and T. K. KIRK: *Biochem. Biophys. Res. Comm.*, **102**, 484 (1981).
- 5) A. ENOKI and M. H. GOLD: *Arch. Microbiol.*, **132**, 123 (1982).