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BIOTRANSFORMATION OF LIGNANS: A SPECIFIC MICROBIAL OXIDATION OF (+)-EUDESMIN AND (+)-MAGNOLIN BY *ASPERGILLUS NIGER*

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Key Word Index—*Aspergillus niger*; microbes; lignan; (+)-eudesmin; (+)-magnolin; (+)-yangabin; (+)-de-4′-*O*-methyleudesmin; (+)-pinoresinol; (+)-5′-hydroxypinoresinol; (+)-de-*O*-methylmagnolin; (+)-de-4′-*O*-methyl-5′-hydroxymagnolin.

Abstract—Biotransformation of the lignans, (+)-eudesmin, (+)-magnolin and (+)-yangabin, by *Aspergillus niger* has been investigated. (+)-Eudesmin was metabolized and transformed to (+)-de-4′-*O*-methyleudesmin and (+)-pinoresinol. Additionally, (+)-pinoresinol was examined and oxidized to (+)-5′-hydroxypinoresinol. (+)-Magnolin was transformed to (+)-de-*O*-methylmagnolin and (+)-de-4′-*O*-methyl-5′-hydroxymagnolin. In these metabolic processes, other products were not generated, although (+)-yangabin and (+)-de-4′-*O*-methyl-5′-hydroxymagnolin were hardly metabolized by this fungus. This suggested that the veratryl and guaiacyl groups of these lignans were possibly metabolized preferentially, with oxidation proceeding predominantly through de-*O*-methylation at the *p*-position of veratryl groups. By contrast, 3,4,5-trimethoxyphenyl and 4,5-dihydroxy-3-methoxyphenyl groups of this type of lignan were stable and not attacked by *A. niger*. The structures of metabolic products were determined by spectroscopic methods as well as by comparison of spectral data with those of known related compounds.

INTRODUCTION

Lignans are widely distributed in terrestrial plants and exhibit a variety of biological activities, e.g. antitumour, antimitotic and antiviral [1]. Neutral lignans, (+)-eudesmin (**1**), (+)-magnolin (**2**) and (+)-yangabin (**3**), also possess biological activities [2, 3]. In the course of our studies to generate bioactive compounds from natural products by biotransformation, we reported biotransformation of (+)-magnolin (**2**) and (+)-yangabin (**3**) by rats [4]. Consequently, we established that de-*O*-methylation of the veratryl and 3,4,5-trimethoxyphenyl groups of both (+)-magnolin (**2**) and (+)-yangabin (**3**) proceeded specifically at the *p*-position, to yield their mono-de-*O*-methyl derivatives.

Microbial transformation possesses the advantage of proceeding under mild conditions and with high regio- and enantioselectivity. The degradation of lignans by fungi has been investigated by wood researchers. Degradation of pinoresinol by intact cells of *Fusarium* spp. was studied and it was revealed that pinoresinol was degraded through α-carbonyl compounds [5]. The degradation of syringaresinol by *Fusarium solani* M-13-1 also proceeded via α-carbonyl compounds [6] and *Phanerochate chrysosporium* converted syringaresinol to its oxidized derivatives [7]. These degradations were catalysed by fungal phenol oxidizing enzymes and this was confirmed by chemical investigation of the degradation products of the fungi [6, 7]. Recently, conversion of deoxypodophyllotoxin by microbes was studied and established that a hydroxyl group was specifically added to the benzyl carbon by microbes [8]. Up to now, however, there is no report that the non-phenolic lignans, (+)-eudesmin (**1**), (+)-magnolin (**2**) and (+)-yangabin (**3**) can be metabolized by microbes. This paper deals with the microbial oxidation of (+)-eudesmin (**1**) to (+)-de-4′-*O*-methyleudesmin (**4**) and (+)-pinoresinol (**5**), (+)-pinoresinol (**5**) to (+)-de-5′-hydroxypinoresinol (**6**), and (+)-magnolin (**3**) to (+)-de-4′-*O*-methylmagnolin (**7**) and (+)-de-4′-*O*-methyl-5′-hydroxymagnolin (**8**) by *Aspergillus niger*.

RESULTS AND DISCUSSION

The transformation of (+)-eudesmin (**1**) by *A. niger* was examined first. (+)-Eudesmin (**1**) was metabolized and two metabolic products (**4** and **5**) generated by static cultivation. The time-courses of each metabolic product were observed by TLC and quantitatively calculated by TLC/FID (flame ionization detector) analysis (Fig. 1). After incubation of **1** (R_f 0.62) for 24 hr, it was metabolized and the amount decreased gradually. In proportion to this, **4** (R_f 0.44) was produced and accumulated for 18 hr, and then slowly disappeared. Compound **5** (R_f 0.26) was then produced gradually and increased in proportion to the decline in **4**. The time-courses of **1** and

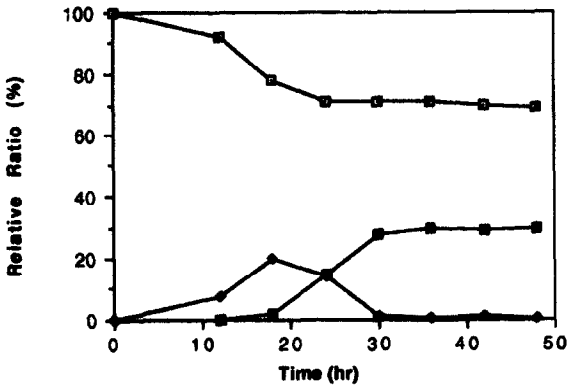


Fig. 1. Time-course of (+)-eudesmin (1) metabolism and (+)-de-4′-*O*-methyleudesmin (4) and (+)-pinoresinol (5) formation after incubation with *Aspergillus niger*. □ = (+)-eudesmin (1); ◆ = (+)-de-4′-*O*-methyleudesmin (4); ■ = (+)-pinoresinol (5).

its metabolic products (4 and 5) indicated that 4 was a first metabolic product of 1; compound 5 was subsequently transformed from 4.

In order to isolate metabolic products, cultivation media were combined after 24 hr incubation and extracted with ether. The ether extract was fractionated into neutral and phenolic portions by treatment with 5% NaOH solution in the usual way. The phenolic portion was then chromatographed on silica gel repeatedly and metabolic products (4 and 5) were isolated. The neutral portion was investigated by TLC, but no metabolic products of 1 could be detected.

Compound 4 had a molecular formula $C_{21}H_{24}O_6$ as determined by a high resolution mass spectrum and NMR data. The IR spectrum contained a hydroxyl band 3418 cm^{-1}, and the specific rotation showed 4 had the (+)-form. The ^1H and ^{13}C NMR spectra contained signals for a complete 2,6-diaryl-3,7-dioxabicyclo[3,3,0]octane skeleton, a veratryl group, and a guaiacyl group. These spectral data allowed us to identify 4 as (+)-de-4′-*O*-methyleudesmin.

The major metabolic product 5 had a molecular formula $C_{20}H_{22}O_6$, which was 14 mu less than 4. Its IR spectrum contained a wide hydroxyl band at 3410 cm^{-1} and the specific rotation showed the (+)-form. The ^1H NMR spectrum revealed the existence of two completely overlapped phenolic hydroxyl protons at δ 5.59, two methoxyl groups, and a same basal skeleton as those of 1 and 4. The ^{13}C NMR spectral data contained signals for two guaiacyl groups and completely overlapped symmetrical aliphatic carbons. The NMR data corresponded with those of (+)-pinoresinol [9].

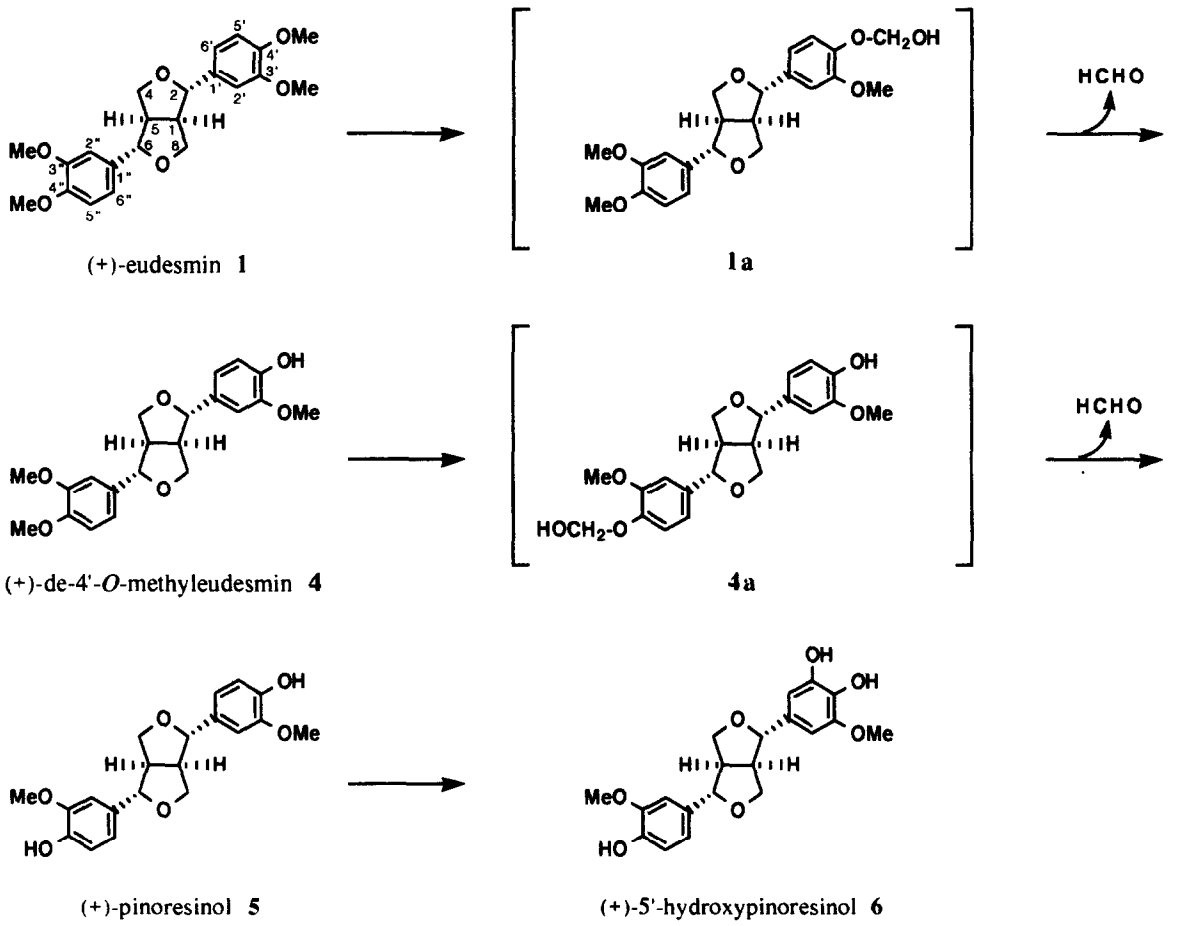
Consideration of the time-courses of substrate 1 and its metabolic products (4 and 5) along with their structures, allowed us to conclude the transformation sequence of 1 by *Aspergillus niger* was as shown in Scheme 1. As a first step, oxidation of (+)-eudesmin (1) occurred preferentially at the *p*-position, produced presumably via intermediate 1a. Intermediate 1a was then immediately de-*O*-methylated to afford (+)-de-4′-*O*-methyleudesmin

Table 1. ^1H NMR spectral data for lignans (1–3) and metabolic products (4–8) (δ, TMS, in $CDCl_3$ at 500 MHz)

H	1 (270 MHz)	2 (270 MHz)	3	4	5	6	7	8
1	3.12 *m*	3.11 *m*	3.10 *m*	3.11 *m*	3.10 *m*	3.08 *m*	3.11 *m*	3.08 *m*
2	4.76 *d* (4)	4.77 *d* (5)	4.75 *d* (4)	4.74 *d* (4.5)	4.74 *d* (4.5)	4.71 *d* (4)[a]	4.75 *d* (3.5)	4.73 *d* (4)[b]
4_{ax}	3.90 *dd* (4, 9)	3.92 *dd* (4, 9)	3.94 *dd* (4, 9)	3.89 *dd* (4, 9)	3.88 *dd* (4, 9)	3.87 *dd* (4, 9)	3.91 *dd* (4, 9)	3.91 *dd* (4, 9)
4_{eq}	4.26 *dd* (7, 9)	4.28 *dd* (7, 9)	4.31 *dd* (7, 9)	4.26 *dd* (7, 9)	4.25 *dd* (7, 9)	4.23 *dd* (7, 9)	4.30 *dd* (7, 9)	4.26 *dd* (7, 9)
5	3.12 *m*	3.11 *m*	3.10 *m*	3.11 *m*	3.10 *m*	3.08 *m*	3.10 *m*	3.08 *m*
6	4.76 *d* (4)	4.75 *d* (5)	4.75 *d* (4)	4.76 *d* (4.5)	4.74 *d* (4.5)	4.72 *d* (4)[a]	4.75 *d* (3.5)	4.71 *d* (4)[b]
8_{ax}	3.90 *dd* (4, 9)	3.92 *dd* (4, 9)	3.94 *dd* (4, 9)	3.89 *dd* (4, 9)	3.88 *dd* (4, 9)	3.87 *dd* (4, 9)	3.91 *dd* (4, 9)	3.90 *dd* (4, 9)
8_{eq}	4.26 *dd* (7, 9)	4.29 *dd* (7, 9)	4.31 *dd* (7, 9)	4.26 *dd* (7, 9)	4.25 *dd* (7, 9)	4.26 *dd* (7, 9)	4.28 *dd* (7, 9)	4.29 *dd* (4, 9)
OH	—	—	—	5.63 *s*	5.59 *s*	5.30 *s*	5.80 *s*	5.50 *s*
					5.59 *s*	5.34 *s*		5.57 *s*
						5.58 *s*		
2′	6.91 *d* (2)	6.91 *d* (2)	6.57 *d* (2)	6.90 *d* (2)	6.90 *d* (2)	6.51 *d* (1.5)	6.90 *d* (2)	6.51 *d* (2)
5′	6.84 *d* (8)	6.84 *d* (8)	—	6.89 *d* (8)	6.89 *d* (8)	—	6.89 *d* (8)	—
6′	6.89 *dd* (2, 8)	6.89 *dd* (2, 8)	6.57 *s*	6.82 *dd* (2, 8)	6.82 *dd* (2, 8)	6.57 *d* (1.5)	6.82 *dd* (2, 8)	6.56 *d* (2)
2″	6.91 *d* (2)	6.58 *s*	6.57 *s*	6.91 *d* (2)	6.90 *d* (2)	6.89 *d* (2)	6.58 *s*	6.57 *s*
5″	6.84 *d* (8)	—	—	6.84 *d* (8)	6.89 *d* (8)	6.89 *d* (8)	—	—
6″	6.89 *dd* (2, 8)	6.58 *s*	6.57 *s*	6.88 *dd* (2, 8)	6.82 *dd* (2, 8)	6.82 *dd* (2, 8)	6.58 *s*	6.57 *s*
OMe								
3′	3.88 *s*	3.88 *s*	3.88 *s*	3.90 *s*	3.91 *s*	3.89 *s*	3.89 *s*	3.88 *s*
4′	3.91 *s*	3.91 *s*	3.84 *s*	—	—	—	—	—
5′	—	—						
3″	3.88 *s*	3.88 *s*	3.88 *s*	3.87 *s*	3.91 *s*	3.91 *s*	3.88 *s*	3.87 *s*
4″	3.91 *s*	3.84 *s*	3.84 *s*	3.90 *s*	—	—	3.84 *s*	3.84 *s*
5″	—	3.88 *s*	3.88 *s*	—	—	—	3.88 *s*	3.87 *s*

Coupling constants in Hz.

[a–b] Values are interchangeable within each column.



Scheme 1. A specific oxidation process to (+)-de-4'-*O*-methyleudesmin (**4**) and (+)-pinoresinol (**5**) from (+)-eudesmin (**1**), and to (+)-5'-hydroxypinoresinol (**6**) from (+)-pinoresinol (**5**) by *Aspergillus niger*.

(**4**) as a secondary reaction. Subsequently, transformation of **4** to (+)-pinoresinol (**5**) occurred, affording **5** via **4a** in the same step as **1** to **4**. These results indicated that the veratryl group of (+)-eudesmin (**1**) could be oxidized predominantly at the *p*-position by *A. niger*.

For further investigation of metabolism of (+)-eudesmin (**1**) by this fungus, (+)-pinoresinol (**5**) was incubated with *A. niger*. Compound **5** was incubated under the same conditions as those used for the transformation of **1**. After 24 hr incubation, the combined media were extracted with ether as before. The ether extract was chromatographed on silica gel repeatedly, and unchanged **5** and compound **6** (R_f 0.06) were isolated.

Metabolic product **6** had one oxygen mass larger than **5**, with a molecular formula $C_{20}H_{22}O_7$, established by HR mass spectrometry and NMR data. The specific rotation showed **6** was the (+)-form. The mass spectrum showed an ion at *m/z* 374 $[M]^+$, two ions at *m/z* 151 $[ArCO]^+$ and 137 $[ArCH_2]^+$ assignable to a guaiacyl group ion (Ar=4-hydroxy-3-methoxyphenyl) and two other ions at *m/z* 167 and 153. The ^1H NMR spectral data coincided with those of **5** except for the disappearance of one aromatic proton and the existence of three hydroxyl protons. Three aromatic protons at δ 6.82 (*dd*, $J=2$, 8 Hz), 6.89 (*d*, $J=8$ Hz) and 6.89 (*d*, $J=2$ Hz), were assignable to those of a guaiacyl group. The remaining two aromatic protons at δ 6.52 and 6.57 had a coupling constant 1.5 Hz and two hydroxyl groups had to be linked to the aryl group, suggesting that **6** possessed a 4,5-dihydroxy-3-methoxyphenyl group. Therefore, the specific ions at *m/z* 167 $[ArCO]^+$ and 153 $[ArCH_2]^+$, where Ar=4,5-dihydroxy-3-methoxyphenyl, in the mass spectrum could be assigned, respectively. These spectral data supported **6** as (+)-5'-hydroxypinoresinol.

These results suggested that incorporation of an oxygen to the 5'-position of **1** and **4** was not intermediated by *A. niger*. However, when both *p*-positions of the aryl groups were hydroxylated as in (+)-pinoresinol (**5**) an oxygen was incorporated at the 5'-position. Therefore, it is clearly supported that the oxidation process of **1** to **4**, **5** and **6** by this fungus was completely regularized.

Incubation of (+)-magnolin (**2**) with precultivated mycelia of *A. niger* was first examined. The decline of (+)-magnolin (**2**, R_f 0.58) and the accumulation of two metabolic products **7** (R_f 0.40) and **8** (R_f 0.22) were

Table 2. ^{13}C NMR spectral data for compounds **1**–**5**, **7** and **8** (δ, TMS, in $CDCl_3$ at 500 MHz)

C	1 (270 MHz)	2	3	4	5	7	8
1	54.0	54.1	54.4	54.1	54.2	54.0	54.1
2	85.6	85.7	86.0	85.9	85.9	85.7	86.0[d]
4	71.6	71.7	72.0	71.7	71.7	71.6	71.7
5	54.0	54.4	54.4	54.1	54.2	54.3	54.3
6	85.6	86.0	86.0	85.8	85.9	86.0	85.7[d]
8	71.6	71.9	72.0	71.7	71.7	71.8	72.1
1′	133.4	133.5	136.7	132.9	132.9	132.7	132.9
2′	109.1	109.2	102.9	108.6	108.6	108.6	100.9
3′	148.5[a]	148.7[b]	153.5	146.7	146.7	146.7	147.1
4′	149.1[a]	149.0[b]	137.6	145.2	145.2	145.2	131.9
5′	110.9	111.1	153.5	114.3	114.3	114.3	144.0
6′	118.1	118.2	102.9	118.9	119.0	118.8	106.4
1″	133.4	136.8	136.7	133.5	132.9	136.7	136.8
2″	109.1	102.8	102.9	109.2	108.6	102.8	102.9
3″	148.5[a]	153.4	153.5	148.6[c]	146.7	153.3	153.4
4″	149.1[a]	137.5	137.6	149.2[c]	145.2	137.4	137.5
5″	110.9	153.4	153.5	109.3	114.3	153.3	153.4
6″	118.1	102.8	102.9	118.2	119.0	102.8	102.9
OMe	55.8	55.9	56.2	55.9	56.0	55.9	56.2
	55.8	55.9	56.2	55.9	56.0	56.1	56.2
	55.8	56.2	56.2	55.9	—	56.1	56.2
	55.8	56.2	56.2	—	—	60.7	60.8
	—	60.9	60.9	—	—	—	—
	—	—	60.9	—	—	—	—

[a–d]Values are interchangeable within each column.

observed by TLC and quantified by TLC/FID analysis (Fig. 2). In order to isolate metabolic products, 1.2 g of (+)-magnolin (**2**) was incubated with mycelia for 24 hr. The cultivation media were collected and extracted with ether. The ether extract was subjected to silica gel column chromatography several times, and **7** (140 mg) and **8** (201 mg) were isolated.

Metabolic product **7** had a molecular formula $C_{22}H_{26}O_7$, determined by HR-mass spectrometry and NMR data. The specific rotation showed that **7** had the (+)-form. The NMR spectral data were consistent with those of (+)-de-*O*-methylmagnolin [4, 9], as were the mass and IR spectra.

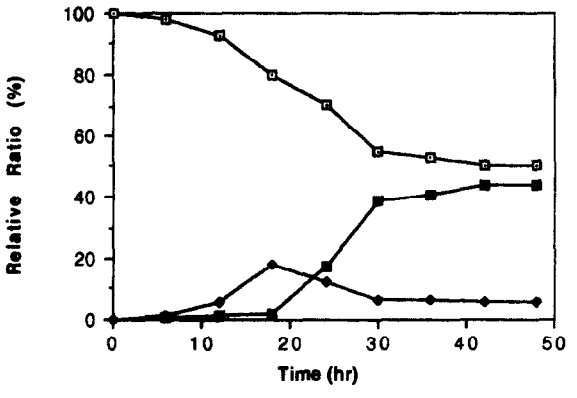
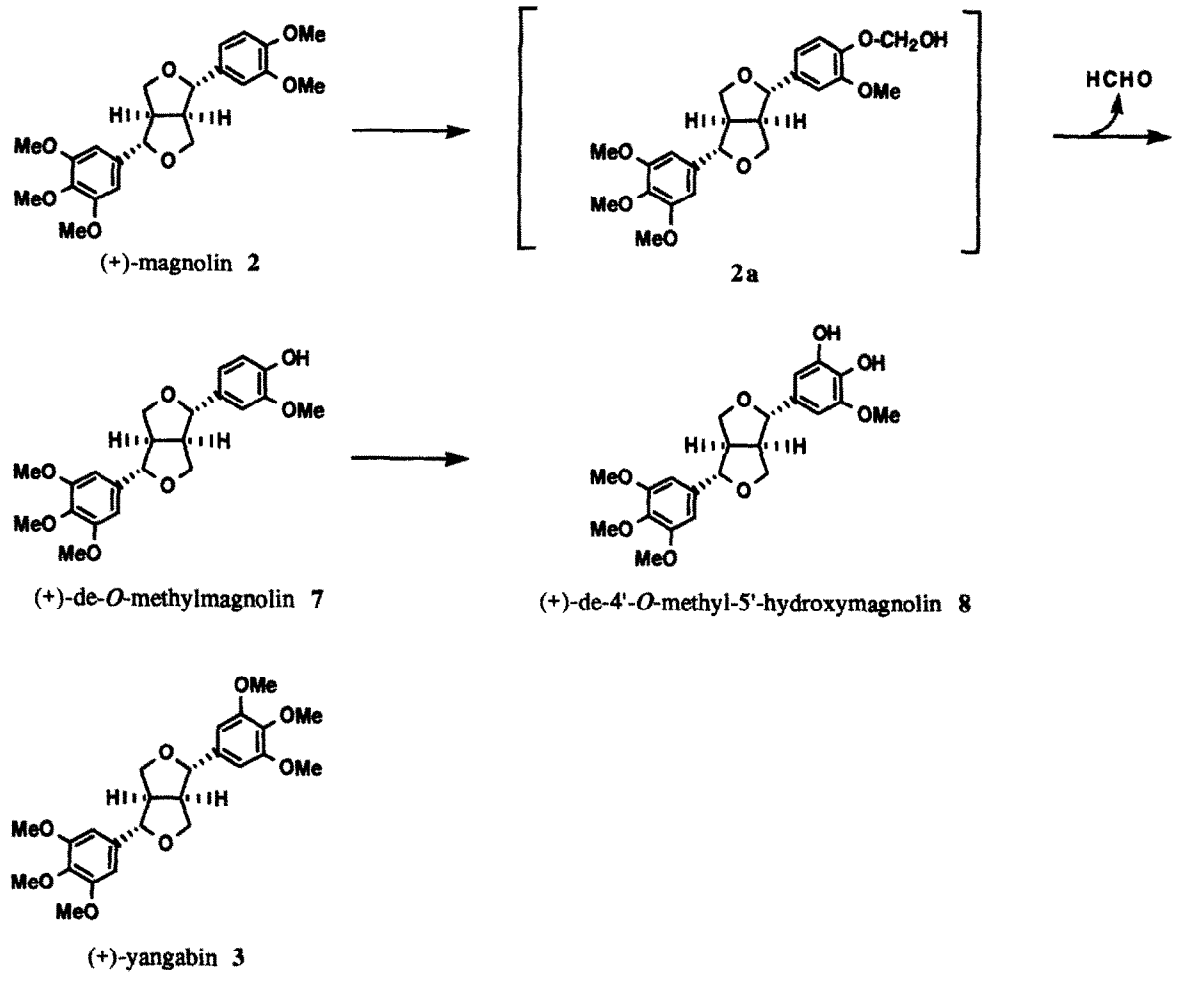


Fig. 2. Time-course of (+)-magnolin (**2**) metabolism and (+)-de-*O*-methylmagnolin (**7**) and (+)-de-4′-*O*-methyl-5′-hydroxymagnolin (**8**) formation after incubation with *Aspergillus niger*; □ = (+)-magnolin (**2**); ◆ = (+)-de-*O*-methylmagnolin (**7**); ■ = (+)-de-4′-*O*-methyl-5′-hydroxymagnolin (**8**).

The product **8** was one oxygen mass larger than **7**, with a molecular formula $C_{22}H_{26}O_8$, indicated by HR mass and NMR spectral data. The mass spectrum showed principal ions at *m/z* 418 $[M]^+$, 195, 181, 167 and 153. The ions *m/z* 195 $[ArCO]^+$ and 181 $[ArCH_2]^+$ (Ar = 3,4,5-trimethoxyphenyl) were assignable. The remaining signals *m/z* 167 and 153 were coincident with those of (+)-5′-hydroxypinoresinol (**6**), indicating that **8** also had a 4,5-dihydroxy-3-methoxyphenyl group. The ^1H NMR spectral data contained signals for a 2,6-diaryl-3,7-dioxabicyclo[3,3,0]octane skeleton, a 3,4,5-trimethoxyphenyl group, two aromatic protons and two phenolic hydroxyl protons. The signals for the two aromatic protons at δ 6.51 and 6.56 and the two hydroxyl protons were assignable to those of the 4,5-dihydroxy-3-methoxyphenyl group. The ^{13}C NMR spectral data supported **8** having the same basic skeleton as **2** and **7**, with a 3,4,5-trimethoxyphenyl group and a 4,5-dihydroxy-3-methoxyphenyl group. Interpretation of the COSY spectrum established that **8** is de-4′-*O*-methyl-5′-hydroxymagnolin. The specific rotation showed **8** has the (+)-form.

For further investigation, (+)-de-4′-*O*-methyl-5′-hydroxymagnolin (**8**) was incubated with *A. niger* for 36 hr; no metabolic products were obtained. Consequently, the veratryl and guaiacyl groups of **2** and **7** could be



Scheme 2. A specific oxidation process to (+)-de-*O*-methylmagnolin (**7**) and (+)-de-4'-*O*-methyl-5'-hydroxymagnolin (**8**) from (+)-magnolin (**2**), by *Aspergillus niger*, and non-oxidized lignans, (+)-yangabin (**3**) and **8**.

oxidized by *A. niger* as well as those of **1**, **4** and **5**. Therefore, the metabolic process of (+)-magnolin (**2**) is that proposed in Scheme 2. Incubation of (+)-yangabin with *A. niger* was also examined, but again no metabolic products were detected by TLC and TLC/FID analysis. These results indicated that the trisubstituted phenyl groups, 3,4,5-trimethoxyphenyl and 4,5-dihydroxy-3-methoxyphenyl, were stable and not attacked by this fungi.

The time-course of the change in pH value of the medium and the weight of mycelia are shown in Fig. 3. In these experiments, time course of pH values of the medium with each substrate and those of the medium without substrate were unchanged. The incremental increase of weight of mycelia was also the same in these experiments.

So far, there are no reports of the oxidation of this type of neutral lignans by microbes. But we found that (+)-cudesmin (**1**) and (+)-magnolin (**2**) could be oxidized, whereas (+)-yangabin (**3**) was stable to *A. niger*. The oxidation of **1** and **2** occurred on their veratryl groups with de-*O*-methylation of the *p*-methoxyl group preceeding. The metabolic product, (+)-pinoresinol (**5**) was further oxidized and one oxygen was incorporated at the 5-position of the guaiacyl group. In this metabolic process, incorporation of oxygen at the 5-position did not

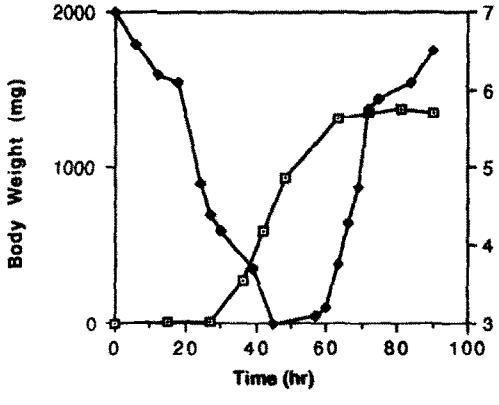


Fig. 3. Time-course of pH change of cultivation medium and increase in weight of mycelia; ◆ = pH; □ = weight of mycelia.

occur before de-*O*-methylation of the *p*-position, indicating that the oxidation order was completely regularized. By contrast, the 3,4,5-trimethoxyphenyl group of **2** was not oxidized and the 4,5-dihydroxy-3-methoxyphenyl group of **8** also was not attacked by this fungus, suggesting that the free 5-position of the aryl group is reserved for oxidation. This suggestion was supported by the stability of (+)-yangabin (**3**) to *A. niger*. Comparison of the metabolism of **2** and **3** by *A. niger* with those of the rat [4] revealed that de-*O*-methylation at the *p*-methoxyl group is a common initial reaction; incorporation of one oxygen at the 5′-position is a reaction specific to *A. niger*. Additionally, the transformation process of the phenolic lignan (+)-pinoresinol (**5**) to (+)-5′-hydroxypinoresinol (**6**) by *A. niger* is clearly different from that of other microbes [5].

EXPERIMENTAL

Preparation of lignans. (+)-Eudesmin (**1**), (+)-magnolin (**2**) and (+)-yangabin (**3**) were isolated from flower buds of *Magnolia fargesii* by previously reported methods [4, 9].

Microorganism and culture conditions. Spores of *A. niger* IFO 4414 (purchased from Institute of Fermentation of Osaka) maintained on nutrient agar slants at 10° were inoculated into the autoclaved culture medium (50 ml in a 200 ml conical flask): sucrose 15 g, glucose 15 g, polypeptone 5 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, K_2HPO_4 1 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, H_2O 1 l. The culture was maintained for 2 days in the incubator (28° under static conditions). Mycelia were then transplanted to the culture medium (15 ml in a 50 ml Petri dish) and incubated for 36–42 hr (until mycelia occupied 60–80% of the surface area of a culture medium) under the same conditions. (+)-Eudesmin (**1**) (1.2 g) was dissolved in 7.5 ml of DMSO and added to the culture medium (corresponding to 35–40 mg of substrate per Petri dish). Petri dishes were incubated at 28° under static situation, together with two controls which contained either mycelia with medium or substrate dissolved in DMSO with medium. (+)-Magnolin (**2**) (1.2 g) and (+)-yangabin (**3**) (1.1 g) were also dissolved in DMSO and added to the culture medium in the same way.

Time-course of substrates and metabolic products. Cultivation media from Petri dishes were acidified to pH 2 with 1 M HCl and extracted Et_2O several times at various intervals. Metabolic products of each substrate were confirmed by preliminary examination. Et_2O extracts (2 mg) were dissolved in 300 μl of CH_2Cl_2 and 2 μl of each soln applied to silica gel-coated rods. The rods were dried and then developed in $CHCl_3$–Me_2CO (9:1) to a distance of 10 cm from the point of application. The rods were dried again and subjected to TLC/FID. The conditions for analyses were as follows: H_2 flow rate 160 ml min^{-1}, air flow rate 2 l min^{-1} and scan speed 200 mm min^{-1}. The ratio between the substrate and metabolic products was quantified and is shown in Figs 1 and 2.

Isolation of metabolic products of (+)-eudesmin. After 24 hr cultivation, the culture medium was collected and acidified to pH 2 with 1 M HCl and satd with NaCl. The culture was then extracted with Et_2O several times, the Et_2O extract washed with a satd NaCl soln, dried (Na_2SO_4) and evapd under red. pres. The extract was dissolved in Et_2O and shaken with 5% aq. NaOH soln three times. After removal of the neutral part, the aq. part was acidified with 1 M HCl and extracted with Et_2O several times. Removal of Et_2O under red. pres. afforded a neutral part (721 mg) and a phenolic part (310 mg). The phenolic part was chromatographed on silica gel repeatedly and **4** (119 mg) and **5** (127 mg) were isolated.

Isolation of metabolic product of (+)-pinoresinol. (+)-Pinoresinol (127 mg) was incubated with *A. niger* under the conditions described above. After 24 hr incubation, the cultivation media were combined and acidified to pH 2 with 1 M HCl and satd with NaCl. The culture was then extracted with Et_2O several times, the Et_2O extract washed with satd NaCl soln, dried (Na_2SO_4) and evapd under red. pres. The Et_2O extract (135 mg) was chromatographed on silica gel repeatedly. Compound **6** (4 mg) was isolated as the metabolic product of **5**.

Isolation of metabolic products of (+)-magnolin. After 42 hr cultivation, the culture medium was collected. It was treated in the same way as that from (+)-eudesmin. The Et_2O extract (1.179 g) was chromatographed on silica gel repeatedly and **7** (140 mg) and **8** (201 mg) were isolated.

Incubation of (+)-de-4′-O-methoxy-5′-hydroxymagnolin. Isolated and purified (+)-de-4′-*O*-methyl-5′-hydroxymagnolin (**8**) (60 mg) was incubated with *A. niger* as described above. After 36 hr cultivation, the medium was collected and extracted with Et_2O several times; no metabolic products were found by TLC.

Incubation of (+)-yangabin. (+)-Yangabin (**3**) (1.1 g) was incubated with *A. niger* as described above. Four days of incubation afforded no detectable metabolite.

(+)-De-4′-O-methyleudesmin (**4**). Oil. HRMS *m/z*: 372.1602 ($[M]^+$, calcd for $C_{21}H_{24}O_6$: 372.1573). MS *m/z* (rel. int.): 372 ($[M]^+$, 100), 341 (15), 294 (50), 205 (19), 177 (42), 165 (50), 151 (68), 137 (28). $[\alpha]_D$ +40.66° ($CHCl_3$; *c* 0.75). IR ν_{max} cm^{-1}: 3418, 1594, 1516, 1464, 1267, 1237, 1160, 1142, 1030. NMR see Tables 1 and 2.

(+)-Pinoresinol (**5**). Oil. HRMS *m/z*: 358.1411 ($[M]^+$, calcd for $C_{20}H_{22}O_6$: 358.1417). MS *m/z* (rel. int.): 358 ($[M]^+$, 100), 327 (11), 205 (27), 163 (40), 151 (100), 137 (55). $[\alpha]_D$ +33.95° ($CHCl_3$; *c* 0.25). IR ν_{max} cm^{-1}: 3410, 1604, 1517, 1465, 1434, 1367, 1160. NMR see Tables 1 and 2.

(+)-5′-Hydroxypinoresinol (**6**). Oil. HRMS *m/z*: 374.1366 ($[M]^+$, calcd for $C_{20}H_{22}O_7$: 374.1366). MS *m/z* (rel. int.): 374 ($[M]^+$, 100), 167 (41), 153 (35), 151 (69), 137 (58). $[\alpha]_D$ +39.6° ($CHCl_3$; *c* 0.05). IR ν_{max} cm^{-1}: 3500–3100, 1606, 1518, 1464, 1274, 1212, 1159, 1034. NMR see Tables 1 and 2.

(+)-De-O-methylmagnolin (**7**). Oil. HRMS *m/z*: 402.1696 ($[M]^+$, calcd for $C_{22}H_{26}O_7$: 402.1679). MS *m/z* (rel. int.): 402 ($[M]^+$, 100), 207 (20), 195 (22), 181 (25), 151 (28), 137 (20). $[\alpha]_D$ +51.86° ($CHCl_3$; *c* 0.45). IR ν_{max} cm^{-1}: 3419, 1593, 1515, 1464, 1272, 1216. NMR see Table 1.

(+)-*De*-4'-O-*methyl*-5'-*hydroxymagnolin* (**8**). Oil. HRMS *m/z*: 418.1606 ($[M]^+$, calcd for $C_{22}H_{26}O_8$: 418.1628). MS *m/z* (rel. int.): 418 ($[M]^+$, 100), 387 (4), 207 (20), 195 (16), 181 (26), 167 (20), 153 (17). $[\alpha]_D$ +20.59° ($CHCl_3$; *c* 0.9). IR ν_{max} cm^{-1}: 3500–3100, 1594, 1509, 1464, 1329, 1235, 1129, 1097. NMR see Tables 1 and 2.

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