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BIOTRANSFORMATION OF (+)-CEDROL BY PLANT PATHOGENIC FUNGUS, *GLOMERELLA CINGULATA*

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Key Word Index—*Glomerella cingulata*; plant pathogenic fungus; biotransformation; microbial transformation; (+)-cedrol; (+)-3α-hydroxycedrol; (+)-3β-hydroxycedrol; (+)-12-hydroxycedrol; (−)-8-cedren-3α-ol.

Abstract—Microbial transformation of (+)-cedrol has been investigated by using plant pathogenic fungus, *Glomerella cingulata*. (+)-Cedrol was hydroxylated at the C-3 position, and transformed mainly to 3α-hydroxycedrol and with a smaller amount of 3β-hydroxycedrol. 3α-Hydroxycedrol was further transformed to 8-cedren-3α-ol by dehydration at the C-8 position. In addition, *G. cingulata* produced a small amount of 12-hydroxycedrol from (+)-cedrol with stereoselective hydroxylation of the methyl group at the C-12 position. The structures of the metabolic products were determined by their spectroscopic data.

INTRODUCTION

As part of our continuing programme to investigate the biotransformation of terpenoids by plant pathogenic fungus, we have examined the conversion of the terpenoids 1,8-cineole [1], (−)-α-bisabolol [2, 3], (+)-cedrol (**1**) [4], (−)-globulol [5], (+)-ledol [5] and (−)-nopol [6] using *Glomerella cingulata*. The biotransformation of **1** by *G. cingulata* into 12-hydroxycedrol (**3**) was reported previously [4].

The biotransformation of **1** by *Aspergillus niger* [7] and *Beauveria sulfurescens* [8] resulted in hydroxylation at the C-3 position to give 3α-hydroxycedrol (**2**). *Rhizopus stolonifer*, *Streptmyces bikiniensis*, *Verticillium tenerum*, *Streptoverticillium reticuli* and *Corynespora cassiicola* hydroxylated **1** at the C-2, C-3, C-4, C-9, C-10 and C-12 positions [9]. *Cephalosporium aphidicola* mainly hydroxylated at the C-3 or C-12 positions to give either 3β-hydroxycedrol (**4**) or 12-hydroxycedrol (**3**); **4** was further transformed to 8-cedren-3β-ol by dehydration at the C-8 position [10]. On the other hand, the biotransformation of **1** by rabbit gave 8-cedren-3β-ol, 8-cedren-3α-ol (**5**) and 3-hydroxycedrol [11], and the biotransformation of **1** by dog gave **2**, **3**, 4β-hydroxycedrol and 4β,8β-dihydroxy-15-cedranoic acid [12].

However, there is no detailed report on the bioconversion of **1** using *Glomerella* sp. Accordingly, as described in this report, the biotransformation of **1** by *G. cingulata* was investigated. In a 14-day incubation of **1** with *G. cingulata*, four major metabolites, including **3** [4], were detected together with three other metabolites, **2**, **4** and **5**. This is the first report of **5** in the microbial transformation of **1**.

RESULTS AND DISCUSSION

For time-course experiments, a small amount of (+)-cedrol (**1**) was incubated with *Glomerella cingulata* for 14 days. Four major metabolites (**2**–**5**) were detected by TLC and GC analysis. Compounds **2**–**5** were not detected on TLC and GC analysis of the culture of *G. cingulata* to which no substrate was fed, and they were absent after a mixture of **1** and the medium were stirred for 14 days. Thus it is concluded that *G. cingulata* transformed **1** to **2**–**5**. Figure 1 shows the time course for appearance of the metabolites; about 65% of **1** was metabolized after 14 days. The major metabolite **2** was about 30% after eight days, and then decreased after 10 days. Compounds **3** and **4** were about 10–12% at eight days while compound **5** appeared at six days and was about 10% at 14 days.

In order to isolate these metabolites a large-scale incubation of **1** with *G. cingulata* was carried out and the culture was extracted as described in the Experimental. Metabolites **2**–**5** were isolated from the CH_2Cl_2 extract and their structures determined by spectral data. Metabolites **2**–**4** were identified to be 3α-hydroxycedrol (**2**), 12-hydroxycedrol (**3**) and 3β-hydroxycedrol (**4**) by spectroscopic data.

Compound **5** had a molecular formula $C_{15}H_{24}O$ based on its EI-mass spectrum ($[M]^+$ at m/z 220). Its ^1H and ^{13}C NMR signals indicated the presence of a second-

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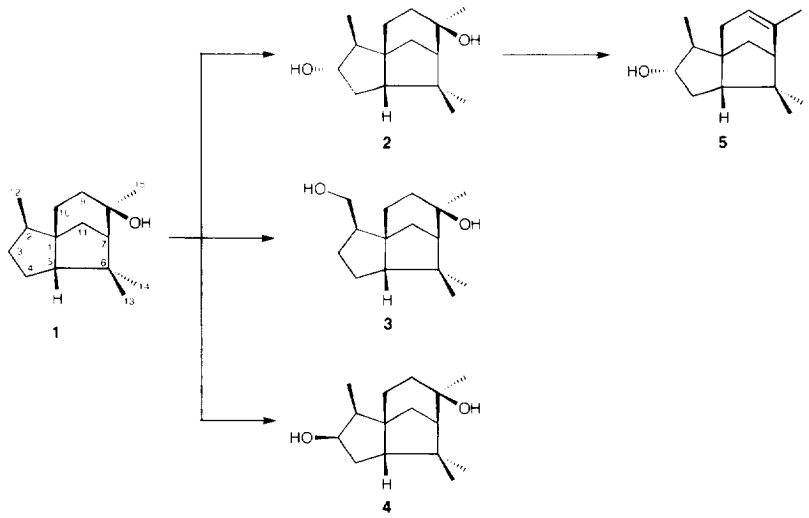

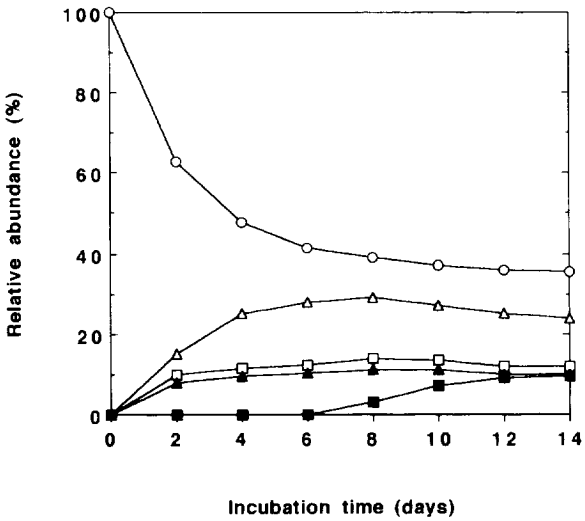

Fig. 1. Time-course in the bitransformation of **1** by *Glomerella cingulata*. ○ = (+)-cedrol (**1**); △ = (+)-3α-hydroxycedrol (**2**); □ = (+)-12-hydroxycedrol (**3**); ▲ = (+)-3α-hydroxycedrol (**4**); ■ = (−)-8-cedren-3α-ol (**5**).

ary hydroxyl group (δ_H 3.70; δ_C 81.3) and a trisubstituted double bond [(δ_H 5.18; δ_C 118.4 (CH) and 140.4 (C)] bearing a methyl group (δ_H 1.67; δ_C 24.7). Comparison of the ^{13}C NMR spectral data of **5** and 8-cedrene [13] suggested that the double bond was in the C-8 position and comparison of the ^{13}C NMR spectral data of **5** and **2** suggested that the hydroxyl group was in the 3-α position of **5**. Thus, the structure of **5** was elucidated to be 8-cedren-3α-ol. This compound was obtained previously by biotransformation of **1** in a rabbit [11], but the detailed ^{1}H and ^{13}C NMR data were not described. The ^{1}H NMR data of **5** are now assigned as described in the Experimental and the ^{13}C NMR data are presented in Table 1.

From the above results, the biotransformation of **1** by the plant pathogenic fungus *G. cingulata* proceeds with stereoselective hydroxylation at the C-3 and C-12 positions to yield **2**–**4**, then **2** is further transformed to 8-cedren-3α-ol (**5**) by dehydration at C-8. However, 8-cedren-3β-ol (epimer of **5**) was not detected in this conversion and thus **4** (epimer of **2**) was not further transformed by *G. cingulata*. The difference in the dehydration at C-8 of 3-hydroxy-cedrol (**2** and **4**) is due to the configuration of the C-3 position. The microbial dehydration by *G. cingulata* at C-8 of 3-hydroxycedrol proceeded only with the 3α-form (**2**). The conversion pattern of **1** by *G. cingulata* (hydroxylation at C-3 and C-12 of **1**) is similar to that of other microorganisms [7–10]. However, there is no previous report on the microbial transformation of **1** to produce **5**. *G. cingulata* is the first example of the production of **5** by microbial transformation.

EXPERIMENTAL

Pre-culture of Glomerella cingulata. Spores of *G. cingulata* which had been preserved at low temp. were inoculated into sterilized culture medium (1.5% saccharose, 1.5% glucose, 0.5% polypeptone, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.1% K_2HPO_4, 0.001% $FeSO_4 \cdot 7H_2O$ and distilled water, pH 7.2) in a shaking flask at 27° for 3 days.

Time-course experiment. Pre-cultured *G. cingulata* were transferred into a 100-ml Erlenmeyer flask containing 50 ml of medium and stirred for 3 days. After the growth of *G. cingulata*, **1** (50 mg) was added to the medium and the culture continued for 14 more days. Every other day 5 ml of the culture medium was removed, acidified to pH 2 with 1M HCl, satd with NaCl and then extracted with Et_2O. The extract was analysed by GC (OV-1 column, 0.25 mm × 25 m, 140 to 240°, 2° min^{-1}) and TLC. The ratios between the substrate (**1**) and the

Table 1. ^{13}C NMR spectral data of compounds **1**–**5** (125.65 MHz, $CDCl_3$, residual $CDCl_3$ used as int. ref. δ77.00), chemical shifts in ppm multiplicites were determined by the DEPT pulse sequence

C	1	2	3	4	5
1	54.1 (C)	50.7 (C)	53.3 (C)	52.6 (C)	50.0 (C)
2	41.1 (CH)	50.1 (CH)	50.4 (CH)	45.9 (CH)	50.8 (CH)
3	37.0 (CH_2)	81.3 (CH)	32.1 (CH_2)	79.8 (CH)	81.3 (CH)
4	25.3 (CH_2)	32.1 (CH_2)	25.8 (CH_2)	32.7 (CH_2)	35.2 (CH_2)
5	56.5 (CH)	52.6 (CH)	57.4 (CH)	54.2 (CH)	56.3 (CH)
6	43.4 (C)	42.5 (C)	43.0 (C)	42.8 (C)	47.1 (C)
7	61.0 (CH)	60.9 (CH)	61.2 (CH)	61.4 (CH)	55.2 (CH)
8	75.3 (C)	74.8 (C)	74.9 (C)	74.9 (C)	140.4 (C)
9	35.3 (CH_2)	35.2 (CH_2)	34.9 (CH_2)	34.9 (CH_2)	118.4 (CH)
10	31.6 (CH_2)	34.2 (CH_2)	30.9 (CH_2)	33.8 (CH_2)	39.3 (CH_2)
11	42.0 (CH_2)	43.0 (CH_2)	42.6 (CH_2)	42.3 (CH_2)	41.3 (CH_2)
12	15.6 (Me)	12.3 (Me)	63.7 (CH_2)	9.6 (Me)	12.7 (Me)
13	27.6 (Me)	27.3 (Me)	27.5 (Me)	27.4 (Me)	27.1 (Me)
14	28.9 (Me)	29.5 (Me)	29.0 (Me)	29.0 (Me)	26.5 (Me)
15	30.2 (Me)	30.1 (Me)	30.2 (Me)	30.1 (Me)	24.7 (Me)

metabolic products were determined on the basis of the peak areas of GC (Fig. 1).

Biotransformation of (+)-cedrol (**1**) *for 14 days.* Precultured *G. cingulata* were transferred into a 3 l stirred fermentor containing 2 l medium. Cultivation was carried out at 27° with stirring for 3 days under aeration. After the growth of *G. cingulata*, **1** (2.50 g) was added into the medium and the culture continued for 14 more days.

Isolation of metabolites. After the the fermentation, culture medium and mycelia were sepd by filtration. The medium was acidified to pH 2 with 1 M HCl, satd with NaCl and extracted with CH_2Cl_2. The mycelia were also extracted with CH_2Cl_2. Both CH_2Cl_2 extracts were combined, the solvent was evapd and the crude extract (2.83 g) was obtained. The extract was sepd into the neutral part (2.57 g) and the acidic part (0.17 g) in the usual manner. The neutral part was chromatographed on silica-60 columns with hexane–EtOAc; the substrate and four metabolites were isolated and purified (**1**: 483 mg, **2**: 392 mg, **3**: 135 mg, **4**: 120 mg and **5**: 115 mg).

Compound **2**. Crystals; mp 159.5–162.7°; $[\alpha]_D^{20}$ + 12.87° ($CHCl_3$; $c = 1.0$); EIMS *m/z*: 238 ($[M]^+$ $C_{15}H_{26}O_2$); IR ν_{max} cm^{-1}: 3258 (OH); ^1H NMR (500.00 MHz, in $CDCl_3$, TMS as int. standard): δ0.96 (3H, *d*, $J = 7.0$ Hz, H-12), 1.01 (3H, *s*, H-13), 1.26 (3H, *s*, H-14), 1.34 (3H, *s*, H-15), 3.61 (1H, *dt*, $J = 10.0$, 5.5 Hz, H-3); ^{13}C NMR: see Table 1.

Compound **3**. Crystals; mp 124.9–127.2°; $[\alpha]_D^{20}$ + 9.78° ($CHCl_3$; $c = 1.0$); EIMS *m/z*: 238 ($[M]^+$ $C_{15}H_{26}O_2$); IR ν_{max} cm^{-1}: 3327 (OH); ^1H NMR (500.00 MHz, in $CDCl_3$, TMS as int. standard): δ1.02 (3H, *s*, H-13), 1.27 (3H, *s*, H-14), 1.33 (3H, *s*, H-15), 3.49 (1H, *dd*, $J = 7.5$, 10.5 Hz, H-12), 3.68 (1H, *dd*, $J = 7.0$, 10.5 Hz, H-12'); ^{13}C NMR see Table 1.

Compound **4**. Crystals; mp 149.5–152.4°; $[\alpha]_D^{20}$ + 3.21° ($CHCl_3$; $c = 1.0$); EIMS *m/z*: 238 ($[M]^+$ $C_{15}H_{26}O_2$); IR ν_{max} cm^{-1}: 3247 (OH); ^1H NMR (500.00 MHz, in $CDCl_3$, TMS as int. standard): δ0.91 (3H, *d*, $J = 7.0$ Hz, H-12), 1.00 (3H, *s*, H-13), 1.26 (3H, *s*, H-14), 1.34 (3H, *s*, H-15), 2.16 (1H, *t*, $J = 9.0$ Hz, H-5), 4.26 (1H, *dt*, $J = 5.0$, 4.0 Hz, H-3); ^{13}C NMR: see Table 1.

Compound **5**. Crystals; mp 101.5–104.0°; − 48.13° ($CHCl_3$; $c = 0.65$); EIMS *m/z*: 220 ($[M]^+$ $C_{15}H_{24}O$); IR ν_{max} cm^{-1}: 3215 (OH); ^1H NMR (500.00 MHz, in $CDCl_3$, TMS as int. standard): δ0.96 (3H, *d*, $J = 7.0$ Hz, H-12), 0.99 (3H, *s*, H-13), 1.05 (3H, *s*, H-14), 1.67 (3H, *m*, H-15), 1.36 (1H, *ddd*, $J = 10.0$, 11.5, 11.5 Hz, H-4), 1.52 (1H, *dq*, $J = 10.0$, 7.0 Hz, H-2), 1.56 (1H, *d*, $J = 10.5$ Hz, H-11), 1.59–1.65 (3H, H-5, 10, OH), 1.70 (1H, *dd*, $J = 3.8$, 10.5 Hz, H-11), 1.78 (1H, *d*, $J = 3.8$ Hz, H-7), 1.88 (1H, *ddd*, $J = 5.5$, 7.5, 11.5 Hz, H-4), 2.20 (1H, *m*, H-10), 3.70 (1H, *ddd*, $J = 5.5$, 10.0, 10.0 Hz, H-3), 5.18 (1H, *br s*, H-9); ^{13}C NMR: see Table 1.

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