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BIOTRANSFORMATION OF THE SESQUITERPENOID (+)-γ-GURJUNENE USING A PLANT PATHOGENIC FUNGUS, *GLOMERELLA CINGULATA*, AS A BIOCATALYST

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Key Word Index—*Glomerella cingulata*; plant pathogenic fungus; biotransformation; biocatalyst; sesquiterpene hydrocarbon; (+)-γ-gurjunene; (1*S*,4*S*,7*R*,10*R*)-5-guaien-11,13-diol; (1*S*,4*S*,7*R*,10*S*)-5-guaien-10,11,13-triol.

Abstract—The biotransformation of a sesquitepenod which possesses a guaiane skeleton, (+)-γ-gurjunene has been investigated using the plant pathogenic fungus, *Glomerella cingulata* as a biocatalyst. (+)-γ-Gurjunene was oxidized at the double bond of the isopropenyl group to (1*S*,4*S*,7*R*,10*R*)-5-guaien-11,13-diol and in addition oxidized at the C–10 position to (1*S*,4*S*,7*R*,10*S*)-5-guaien-10,11,13-triol. The structures of the metabolic products have been elucidated on the basis of their spectral data.

INTRODUCTION

We have investigated the biotransformation of terpenoids using a plant pathogenic fungus, *Glomerella cingulata* as a biocatalyst. In our previous papers, some cyclic sesquiterpenoids {(−)-globulol [1], (−)-α-bisabolol [2], (+)-cedrol [3], (+)-aromadendrene [4], (−)-alloaromadendrene [4], β-selinene [5]} were transformed to novel terpenes via stereoselective oxidation by *G. cingulata*. In a previous report [5], we described the oxidation of β-selinene (sesquiterpene hydrocarbon) at the double bond and the hydroxylation at one of the methylenes on the eudesmane skeleton ring to form the triol [5]. We were interested in the stereospecific oxidation of cyclic sesquiterpene hydrocarbons. In particular we were interested in the oxidation of the isopropenyl group of the bicyclic sesquiterpenoids by *G. cingulata*. (+)-γ-Gurjunene (**1**) is a sesquiterpene hydrocarbon with a guaiane skeleton. There was a report for the isolation of **1** from the gurjun balsams of several species of *Dipterocarpus* [6]. Oxidation of **1** by reaction with *m*-chloroperbenzoic acid was also reported [7]. Until now there have been no reports of the biotransformation of **1**. Therefore, as part of our programme, we investigated the biotransformation of **1** by *G. cingulata*. This report describes the biotransformation of **1** into (1*S*,4*S*,7*R*,10*R*)-5-guaien-11,13-diol and (1*S*,4*S*,7*R*,10*S*)-5-guaien-10,11,13-triol by *G. cingulata*.

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RESULTS AND DISCUSSION

To investigate the time-course of the biotransformation of **1** by *G. cingulata*, a small amount of **1** was incubated with *G. cingulata* for ten days. Two main products (**2** and **3**) and many minor products were detected by TLC and GC analysis. These products were not detected by TLC or GC analysis of the culture of *G. cingulata* to which substrate was not fed, and in which the mixture of **1** and culture medium were stirred for eight days. From the above result, it was demonstrated that *G. cingulata* transformed **1** into **2**, **3** and many minor products. The time-course of the concentration change of **1**–**3** was monitored by TLC and quantitatively measured by a GC method (Figure 1). After ten days, *ca* 90% of the starting substrate **1** was transformed. The metabolites **2** and **3** accounted for *ca* 10% and *ca* 55%, respectively, of recovered materials after ten days. In order to isolate these metabolites, a large scale incubation of using *G. cingulata* was carried out for ten days. After the biotransformation, the culture was extracted as described in the Experimental, and metabolites **2** and **3** were isolated from the extract. The structures of **2** and **3** were determined by spectral data.

G. cingulata oxidized **1** at the double bond at the

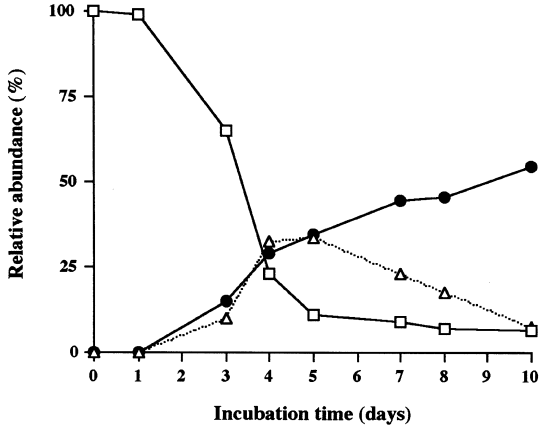


Fig. 1. Times course for the biotransformation of **1** by *G. cingulata*: □, (+)-*γ-gurjunene* (**1**); △. 5-guaien-11,13-diol (**2**); ●, 5-guaien-10,11,13-triol (**3**).

isopropenyl group to form a diol **2** and in addition hydroxylated at the methyne proton to form a triol **3**. The ^{1}H NMR spectrum of **2** displayed no signals for the olefinic protons of an isopropenyl group, but there was the appearance of two signals at δ3.54 (1H, *d*, $J=10.5$ Hz) and δ3.77 (1H, *d*, $J=10.5$ Hz) due to a primary alcohol. From the ^{13}C NMR spectral data of **1** and **2** (Table 1), hydroxyl groups were revealed at C–11 and C–13 as tertiary and primary, respectively. The metabolite **2** was identified as 5-guaien-11,13-diol.

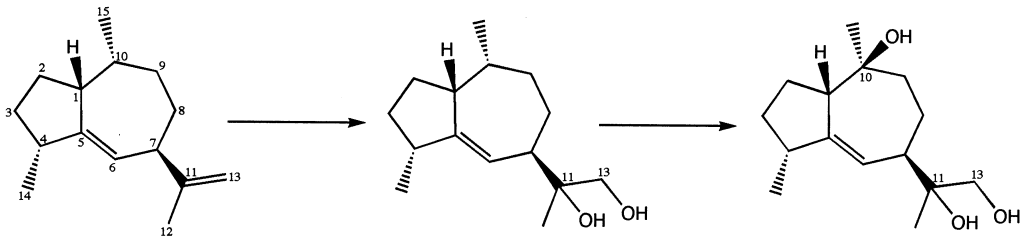
The ^{1}H NMR spectrum of **3** contained no signals for the olefinic protons of an isopropenyl group. There were, however, the two new signals at δ3.44 (1H, *d*, $J=10.5$ Hz) and 3.58 (1H, *d*, $J=10.5$ Hz) of the methylene proton due to a primary alcohol. One of two methyl groups which appeared as a doublet in the starting material changed to singlet and moved downfield from δ0.84 to δ1.11. To elucidate the position of the hydroxyl group which was introduced by fermentation, the metabolite **3** was acetylated to yield monoacetate **4** (pyridine-acetic anhydride on room temp.). The ^{1}H NMR spectrum of **4** displayed one signal of a methyl proton at δ2.11 due to the acetyl group and a methyl signal at C–12 was moved downfield from δ1.16 to δ1.21. In the ^{13}C NMR spectral data for **1**, **3** and **4** Table 1, hydroxyl groups were revealed at C–10, C–11 and C–13 as tertiary, tertiary and primary alcohols, respectively. The data from the HRFAB mass spectrum showed the composition of **3** was $C_{15}H_{26}O_3$. Thus, the metabolite **3** was determined as 5-guaien-10,11,13-triol. As a result of comparison with the ^{1}H NMR and ^{13}C NMR data of (−)-globulol and (+)-ledol [1], the shifts of H–15, C–10 and C–15 of **3** were nearly identical with those (−)-globulol. Because the *trans*-orientation of the hydrogen at C–1 to the methyl group at C–10 was suggested, compound **8** was the (1*S*,4*S*,7*R*,10*S*)-form.

The possible metabolic pathway for modification of **1** is shown in Scheme 1. From the result of the time-course experiment Fig. 1, **1** was transformed into **2** in which the isopropenyl group was oxidized. Furthermore, the metabolite **2** was hydroxylated to yield metabolite **3**. The oxidation by *G. cingulata* of the double bond at the isopropenyl group gave two metabolites of *β*-selinene which were diastereomers [4]. The biotransformation of **1** by *G. cingulata* with oxidation of the 11(13)-double bond did not proceed stereoselectively and diastereomers of metabolites were not separated. This distinction originates from a difference in their skeleta.

Table 1. ^{13}C NMR spectral data for compounds **1**–**4** (recorded at 67.80 MHz, residual $CHCl_3$ used as int. reference, $\delta=77.00$)

C	1	2	3	4
1	45.75 (CH)	46.00 (CH)	49.26 (CH)	49.49 (CH)
2	29.95 (CH_2)[a]	29.70 (CH_2)	26.15 (CH_2)	26.15 (CH_2)
3	33.78 (CH_2)	33.51 (CH_2)	33.53 (CH_2)	33.48 (CH_2)
4	40.99 (CH)	38.13 (CH)	40.44 (CH)	40.51 (CH)
5	150.54 (C)	134.10 (C)	150.88 (C)	151.52 (C)
6	122.20 (CH)	109.69 (CH)	119.45 (CH)	119.01 (CH)
7	47.44 (CH)	48.57 (CH)	47.75 (CH)	47.85 (CH)
8	25.66 (CH_2)	25.30 (CH_2)	21.07 (CH_2)	21.01 (CH_2)
9	33.21 (CH_2)[a]	32.93 (CH_2)	41.41 (CH_2)	41.45 (CH_2)
10	33.75 (CH)	33.76 (CH)	77.25 (C)	76.87 (C)
11	147.01 (C)	80.50 (C)	76.08 (C)	75.83 (C)
12	21.32 (CH_3)	21.15 (CH_3)	20.65 (CH_3)	20.91 (CH_3)
13	110.55 (CH_2)	78.80 (CH_2)	68.54 (CH_2)	70.07 (CH_2)
14	19.65 (CH_3)	20.27 (CH_3)	19.79 (CH_3)	19.80 (CH_3)
15	15.50 (CH_3)	14.57 (CH_3)	25.58 (CH_3)	25.55 (CH_3)
$COCH_3$				171.14 (C)
$COCH_3$				21.38 (CH_3)

Chemical shifts in ppm; multiplicities were determined by the DEPT pulse sequence.
[a] The assignments for these signals within the same column may be interchanged.



Scheme 1. The possible metabolic pathway of **1** by *G. cingulata*.

EXPERIMENTAL

Compound **1** was purchased from Fluka Chem.

Preculture 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 and 0.001% $FeSO_4 \cdot 7H_2O$ in distilled water) in a flask which was shaken at 27° for 3 days.

Time-course experiment. Precultured *G. cingulata* was transferred into a 200 ml Erlenmeyer flask containing 100 ml of medium, and stirred for 3 days. After the growth of *G. cingulata*, compound **1** (50 mg) was added to the medium and the organism cultivated for 10 more days. Every day, 5 ml of the culture medium was removed, satd with NaCl, extracted with EtOAc and the solvent then evapd. The crude extract was analyzed by TLC, GC and GC-MS. The relative concns of substrate **1** and metabolites were determined on the basis of GC peak area Fig. 1.

Large-scale biotransformation of **1**. Precultured *G. cingulata* was transferred into a stirred fermentor containing 3 l. of medium. Cultivation was carried out at 27° with stirring for 5 days and under aeration. After the growth of *G. cingulata*, compound **1** (1.2 g) was added to the medium and then cultivation was continued for 8 days.

Isolation of metabolites **2** *and* **3**. After the fermentation the culture medium and mycelium were sepd by filtration. The medium was satd with NaCl and extracted continuously with EtOAc. The mycelium was also extracted with EtOAc. The EtOAc extracts were mixed and dried over Na_2SO_4 and the solvent was evapd to yield a crude extract (1.52 g). The extract was subjected to CC on silica gel with a $CHCl_3$-MeOH gradient. The pure metabolites **2** (202 mg) and **3** (68 mg) were isolated. No metabolite in the acidic fr. was identified by GC-MS analysis.

Compound **2**. Oil. $[\alpha]_D^{24} + 30.1°$ ($CHCl_3$; *c* 0.25). EIMS *m/z* (rel.int.): $[M]^+$ 238(1), $[M - H_2O]^+$ 220(17), $[M - CH_3OH]^+$ 206(8), 184(3), 162(17), 159(20), 147(24), 105(45), 91(46), 79(35), 55(34), 43(100). IR ν_{max} cm^{-1}: 3501, 2930, 2899, 1210, 1068. ^{1}H NMR (500.00 MHz, $CDCl_3$, TMS as int. standard): δ0.85 (3H, *s*, H–15), 1.01 (3H, *d*, *J* = 6.5 Hz, H–14), 1.16 (3H, *s*, H–12), 3.44 (1H, *d*, *J* = 10.5 Hz, H–13), 3.58 (1H, *d*, *J* = 10.5 Hz, H–13). ^{13}C NMR data: Table 1.

Compound **3**. Oil. $[\alpha]_D^{24} + 25.2°$ ($CHCl_3$; *c* 0.5). EIMS *m/z* (rel.int.): $[M-H_2O]^+$ 236(1), $[M-H_2O-CH_2OH]^+$ 205(9), $[M-2H_2O-CH_2OH]^+$ 187(5), 162(66), 147(55), 120(26), 105(46), 91(43), 79(41), 57(31), 43(100); HRFABMS(neg.) *m/z* 253.1785 $[MH^+-H]^-$, calcd for $C_{15}H_{25}O_3$, 253.1804. IR ν_{max} cm^{-1}: 3285, 2940, 2899, 1475, 1034. ^{1}H NMR (500.00 MHz, $CDCl_3$, TMS as int. standard): δ1.06 (3H, *d*, *J* = 6.5 Hz, H–14), 1.11 (3H, *s*, H–15), 1.16 (3H, *s*, H–12), 3.09 (1H, *m*, H–7), 3.44 (1H, *d*, *J* = 10.5 Hz, H–13), 3.58 (1H, *d*, *J* = 10.5 Hz, H–13), 5.44 (1H, *ddd*, *J* = 1.0, 2.5, 2.5 Hz, H–6). ^{13}C-NMR data: Table 1.

Acetylation of **2**. Pyridine (ml) was added to a soln of **3** (5 mg) in Ac_2O (ml), and the soln was stirred for 7 hr at room temp (25°). The products were isolated in the usual manner and sepd by silica gel CC with a hexane-EtOAc gradient. The monoacetate **4** (4 mg) was obtained.

Compound **4**. Oil. EIMS *m/z* (rel.int.): $[M-H_2O]^+$ 278(1), $[M-2H_2O]^+$ 260(1), 218(1), 205(4), 185(2), 162(38), 147(22), 133(8), 117(46), 105(24), 91(17), 79(15), 75(17), 55(9), 43(100). ^{1}H NMR (500.00 MHz, $CDCl_3$, TMS as int. standard): δ1.06 (3H, *d*, *J* = 6.5 Hz, H–14), 1.11 (3H, *s*, H–15), 1.21 (3H, *s*, H–12), 2.11 (3H, *s*, $COCH_3$) 3.09 (1H, *m*, H–7) 4.01 (1H, *d*, *J* = 11.0 Hz, H–13), 4.07 (1H, *d*, *J* = 11.0 Hz, H–13), 5.44 (1H, *ddd*, *J* = 1.0, 2.5, 2.5 Hz, H–6). ^{13}C NMR data: Table 1.

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