Hydroxylation of Two Saturated Acyclic Monoterpenoids, Tetrahydrogeraniol and Tetrahydrolavandulol, by the Plant Pathogenic Fungus *Glomerella cingulata*

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Received June 5, 1996®

The microbial transformations by *Glomerella cingulata* of two saturated acyclic monoterpenoids, tetrahydrogeraniol (**1**) and tetrahydrolavandulol (**3**), were investigated. Both compounds were hydroxylated regioselectively at the isopropyl group. Tetrahydrogeraniol was transformed to hydroxycitronellol (**2**), while tetrahydrolavandulol was transformed to 5-hydroxytetrahydrolavandulol (**4**). This is the first report describing the microbial transformation of compounds **1** and **3**.

Saturated terpenoids have few activated carbon atoms. Therefore, if such a compound were to be regioselectively and stereoselectively oxidized by microbial transformation, this would represent an interesting general method in synthetic organic chemistry. Although there are many reports on the microbial transformation of unsaturated acyclic terpenoids, no such procedures have appeared for saturated acyclic terpenoids.

We have previously reported the biotransformation of the unsaturated acyclic terpenoids, (±)-*cis*-nerolidol,[1] nerylacetone,[1] (±)-*trans*-nerolidol,[2] geranylacetone,[2] (*E*,*E*)-farnesol,[3] and (±)-citronellol[4] using the plant pathogenic fungus *Glomerella cingulata* as a biocatalyst. These acyclic terpenoids were mainly oxidized by *G. cingulata* regioselectively at the double bond distant from the hydroxyl or carbonyl group (the remote double bond). To further study the metabolism of acyclic terpenoids by *G. cingulata* and specifically to investigate the influence of the presence of the remote double bond on the microbial transformation of acyclic terpenoids by *G. cingulata*, we studied the microbial transformation of two saturated acyclic terpenoids: tetrahydrogeraniol (**1**) and tetrahydrolavandulol (**3**) (Scheme 1). Compound **1** is used in the fragrance industry[5] and as an intermediate in the synthesis of α-tocopherol.[6] Compound **3** has been reported as an intermediate in the synthesis of tetradesoxybacterioruberin.[7]

To investigate the capacity of *G. cingulata* to metabolize **1**, a time−course experiment was carried out as described in the Experimental Section. One major metabolite (**2**) and some minor metabolites were detected by TLC and GC analysis. The time−course of relative abundance of metabolites was qualitatively observed by TLC and quantitatively measured by GC (Figure 1). The starting substrate **1** was mainly transformed to **2** (70%), and >95% of **1** was consumed after 5 days.

To isolate metabolite **2**, a large-scale incubation of **1** using *G. cingulata* was carried out for 5 days. After the biotransformation, the culture was extracted, and metabolite **2** was isolated from the CH_2Cl_2 extract using chromatography as described in the Experimental Section. The structure of **2** was identified to be hydroxycitronellol (3,7-dimethyl-1,7-octanediol) on the basis of its spectral data, especially by comparison of its ^{13}C-NMR chemical shifts with literature values.[8]

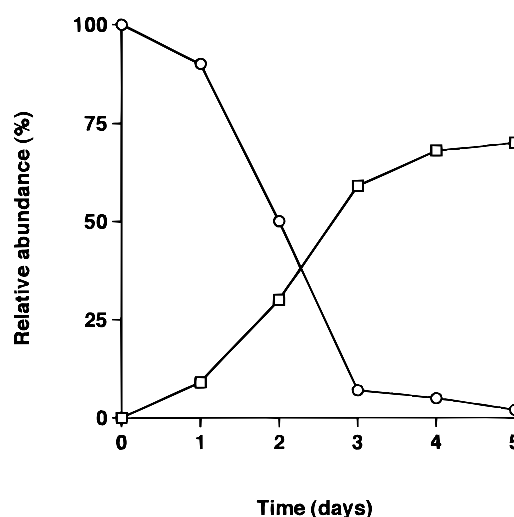
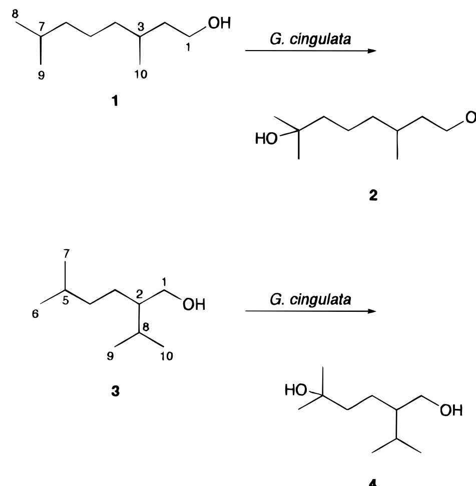


Figure 1. Time−course of the biotransformation of **1** by *Glomerella cingulata*: (○) tetrahydrogeraniol (**1**); (□) hydroxycitronellol (**2**).

Scheme 1



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® Abstract published in *Advance ACS Abstracts,* February 1, 1997.

S0163-3864(96)00502-2 CCC: $14.00 © 1997 American Chemical Society and American Society of Pharmacognosy

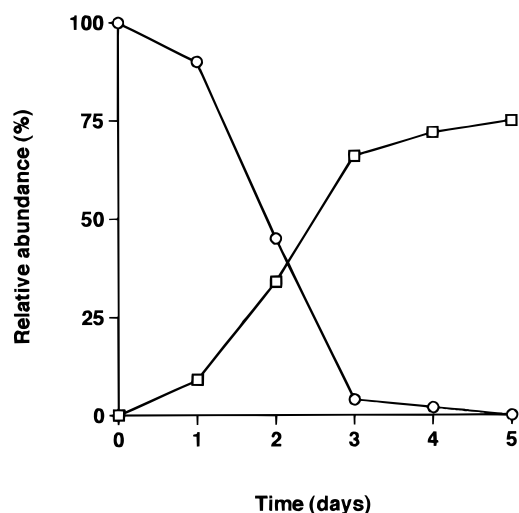

Figure 2. Time–course of the biotransformation of **3** by *Glomerella cingulata*: (○) tetrahydrolavandulol (**3**); (□) 5-hydroxytetrahydrolavandulol (**4**).

Compound **2** is used as a cosmetic and perfume ingredient[9] and has been obtained by chemical reaction (hydration of citronellol)[10,11] and by biotransformation (from citronellal using *Pseudomonas*;[12] from 3,7-dimethyl-7-hydroxy-1-octanal and 3,7-dimethyl-2-octene-1,7-diol using a cell suspension culture of *Catharanthus roseus*;[13] and from citronellol using *Botrytis cinerea*[14]). However, there has been no report of the preparation of **2** by biotransformation or chemical reaction of **1**. The present method is an easy way to produce this commercially available compound because **2** is obtained as a major product under mild conditions.

To determine the time–course of the microbial transformation of tetrahydrolavandulol (**3**) by *G. cingulata*, a small amount of **3** was incubated with this organism for 5 days. One major metabolite (**4**) and some minor metabolites were detected by TLC and GC analysis. As in the case of **1**, the time–course of the relative abundance of the metabolites was qualitatively observed by TLC and quantitatively measured by GC (Figure 2). The starting substrate **3** was mainly transformed to **4** (75% of all metabolites), and **3** was completely consumed after 5 days.

To isolate metabolite **4**, a large-scale incubation of **3** using *G. cingulata* was carried out for 5 days. After the biotransformation, the culture was extracted as described in the Experimental Section and the metabolite **4** was isolated from the CH_2Cl_2 extract using chromatographic procedures. The structure of **4** was determined by its spectral data. This compound had the molecular formula $C_{10}H_{22}O_2$ based on its HRFABMS. Other spectral data indicated that compound **4** had a tertiary hydroxyl group [δ_C 71.2 (C)] as well as a primary hydroxyl group [δ_H 3.57, 3.66; δ_C 63.6 (CH_2)]. Two of the four methyl signals in the ^{1}H-NMR spectrum were shifted to low magnetic field and appeared as a singlet (δ_H 1.23). The ^{13}C-NMR chemical shifts of C-1 (δ 63.6), C-9 (δ 19.6), and C-10 (δ 19.8) were not shifted in comparison with those of **3** [C-1 (δ 63.8), C-9 (δ 19.3), and C-10 (δ 19.7)]. From an analysis of its spectral data, the structure of **4** was therefore determined to be the novel compound, 5-hydroxytetrahydrolavandulol.

On the basis of the above results, compounds **1** and **3** were hydroxylated regioselectively by *G. cingulata* at the methine (C-7 of **1** and C-5 of **3**) of the isopropyl moiety that was located at the more remote position from the hydroxyl group. This result and those from our previous reports[1–4] indicate that *G. cingulata* is able to oxidize the isopropyl or isopropylidene moiety distant from the hydroxyl or carbonyl group of acyclic terpenoids.

Experimental Section

General Experimental Procedures. TLC was performed on precoated plates (Si gel 60 F_{254}, 0.25 mm, Merck). The mobile phase was hexane–EtOAc (1:1). Compounds were visualized by spraying plates with 1% vanillin in 96% H_2SO_4 followed by brief heating. GC was performed on a HP 5890 Series ll Plus gas chromatograph equipped with a flame-ionization detector (FID). The column was a fused silica capillary column [DB-5 (5% diphenyl and 95% dimethylpolysiloxane), 30 m × 0.25 mm i.d. (J&W Scientific, Folsom, CA)]. Chromatographic conditions were as follows: column temperature, 80–260 °C at 4 °C min^{-1}; injector temperature, 270 °C; detector temperature, 280 °C; carrier gas, He at 40 cm s^{-1}. Yields of individual constituents were determined by peak areas as measured by a HP 3396 integrator. EIMS measurements were obtained using gas chromatography–mass spectrometry (GC–MS). GC–MS was performed on a HP 5972A mass selective detector interfaced with a HP 5890 Series ll Plus gas chromatograph fitted with a column [HP-5MS (5% diphenyl and 95% dimethylpolysiloxane), 30 m × 0.25 mm i.d. (Hewlett-Packard)]. Chromatographic conditions were the same as described above. FABMS was obtained on a JEOL JMS-HX 100 mass spectrometer, and the matrix was glycerin. IR spectra were determined with a Perkin-Elmer 1760-x IR Fourier-transform spectrometer. NMR spectra were recorded on a JEOL GSX 270 NMR spectrometer (^{1}H NMR, 270.05 MHz; ^{13}C NMR, 67.80 MHz). Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^{1}H-NMR spectra measured in $CDCl_3$. Residual $CHCl_3$ was used as internal reference (δ 77.00) for ^{13}C-NMR spectra measured in $CDCl_3$. Multiplicities were determined by the DEPT pulse sequence.

Preculture of *G. cingulata*. Spores of *G. cingulata* (the strain isolated from diseased grape was a gift from Dr. M. Hyakumachi, Gifu University, Gifu, Japan), which had been preserved on potato dextrose agar (PDA) at 4 °C, were inoculated into 200 mL of sterilized culture medium (1.5% saccharose, 1.5% glucose, 0.5% polypeptone, 0.05% $MgSO_4·7H_2O$, 0.05% KCl, 0.1% K_2HPO_4, and 0.001% $FeSO_4·7H_2O$ in distilled H_2O) in a 500-mL shaking flask, and the flask was shaken (reciprocating shaker, 100 rpm) at 27 °C for 3 days.

Time–Course Experiment. Precultured *G. cingulata* (1 mL) was transferred into two 200-mL Erlenmeyer flasks containing 100 mL of medium and was stirred (ca. 100 rpm) for 3 days. After the growth of *G. cingulata*, **1** (50 mg) or **3** (50 mg) was added into the medium, respectively, and cultivated for 5 more days. Each day, 5 mL of the culture medium were removed. The culture medium was extracted with EtOAc. This extract was analyzed by GC and TLC. The ratios between the substrate and metabolic products were determined by GC and as shown in Figures 1 and 2.

Biotransformation of Tetrahydrogeraniol (1) or Tetrahydrolavandulol (3) for 5 Days. Precultured

G. cingulata (5 mL) was transferred into a 3-L stirred fermenter containing 2 L of medium. Cultivation was carried out at 27 °C with stirring (ca. 100 rpm) for 3 days under aeration (200 mL min^{-1}). After the growth of *G. cingulata*, **1** (1.02 g) or **2** (1.01g) was added into the medium and cultivated for an additional 5 days.

Isolation and Identification of the Metabolites 2 and 4. After the fermentation, the culture medium and mycelia were separated by filtration. The medium was saturated 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 evaporated, and a crude extract (880 mg) was obtained. The extract was chromatographed on a Si-60 column with a hexane−EtOAc gradient (19:1 to 1:1) to yield metabolite **2** (260 mg). The crude extract (920 mg) obtained from the biotransformation of **3** was chromatographed to yield metabolite **4** (290 mg).

Compound 2: colorless oil; $[\alpha]^{20}_{D}$ +0.38° (*c* 1.0, $CHCl_3$), [lit.[13] 3*R*-form $[\alpha]_D$ +2.07° (*c* 6, $CHCl_3$), 3*S*-form $[\alpha]_D$ −2.00° (*c* 6, $CHCl_3$)]; IR ν_{max} 3344, 2942, 1656, 1460, 1377, 1217, 1159, 1053, 1015, 938, 911 cm^{-1}; ^1H NMR δ 0.90 (3H, d, J = 7 Hz, H_3-10), 1.21 (6H, s, H_3-8, H_3-9), 3.67 (2H, m, H_2-1); ^{13}C-NMR data identical to those recorded in the literature[9] [literature ^{13}C-NMR assignments at δ 44.1 (C-2) and 39.7 (C-6) should be revised to δ 39.7 (C-2) and 44.1 (C-6), respectively]; EIMS *m/z* 159 ($M^+ - CH_3$) (2), 141 ($M^+ - H_2O - CH_3$) (1), 123 (11), 98 (7), 83 (10), 70 (14), 59 (100), 55 (23), 43 (37), 31 (21).

Compound 4: colorless oil; $[\alpha]^{20}_{D}$ −1.23° (*c* 0.35, $CHCl_3$); IR ν_{max} 3342, 2960, 1656, 1467, 1381, 1367, 1216, 1158, 1036, 909 cm^{-1}; ^1H NMR δ 0.91 (6H, d, J = 7 Hz, H_3-9, H_3-10), 1.23 (6H, s, H_3-6, H_3-7), 3.57 (1H, dd, J = 6,11 Hz, H-1), 3.66 (1H, dd, J = 5,11 Hz, H-1′); ^{13}C NMR δ 19.6 (CH_3, C-9), 19.8 (CH_3, C-10), 22.2 (CH_2, C-3), 28.3 (CH, C-8), 29.26 (CH_3, C-6), 29.31 (CH_3, C-7), 41.3 (CH_2, C-4), 46.9 (CH, C-2), 63.6 (CH_2, C-1), 71.2 (C, C-5); EIMS *m/z* 159 ($M^+ - CH_3$) (2), 156 ($M^+ - H_2O$) (0.1), 141 ($M^+ - H_2O - CH_3$) (2), 123 (12), 98 (4), 89 (14), 83 (15), 69 (27), 59 (100), 56 (27), 43 (50), 31 (17); HRFABMS (pos.) *m/z* 175.1691 [MH^+], calcd for $C_{10}H_{23}O_2$, 175.1698.

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NP960502J