Biological Stereoselective Reduction of 4-Propylcyclohexanone and 4-Isopropylcyclohexanone by Anthracnose Fungi

Shigeaki OKAMURA, Mitsuo MIYAZAWA, Masashi YAMAGUCHI and Hiromu KAMEOKA

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University (3-4-1, Kowakae, Higashiosaka-shi, Osaka 577-8502)

Abstract: The biotransformation of 4-propylcyclohexanone and 4-isopropylcyclohexanone was examined using ten different anthracnose fungi as biocatalysts. 4-Propylcyclohexanone and 4-isopropylcyclohexanone were reduced to the corresponding cis- and trans-alcohols, respectively, and transformed mainly to trans-4-alcohols by all ten fungi enumerated as follows; Colletotrichum lagenarium, C. dematium MAFF410046, C. trifolii MAFF305389, C. fragariae, C. atramentarium MAFF712102, C. lindemuthianum (C-1), C. lindemuthianum (C-3), C. lindemuthianum (C-13), C. graminicola MAFF305460 and Glomerella cingulata. In particular, the ratio of cis- and trans-alcohol products was shown to be 1:13 (4-propylcyclohexanol) and 1:11 (4-isopropylcyclohexanol) with high stereoselectivity by C. lagenarium after a 7-day incubation period.

Key words: biotransformation, 4-propylcyclohexanone, 4-isopropylcyclohexanone, biological stereoselective reduction, anthracnose fungi

1 Introduction

We have investigated the microbial transformations of terpenoids and relative cyclic compounds using a plant pathogenic fungus. A previous study described the biotransformation of the cyclic ketones 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, 4-tert-butylcyclohexanone, 4-tert-pentylcyclohexanone, 2,6- and 3,5-dimethylcyclohexanone by anthracnose fungi. These compounds were reduced the corresponding cis- and trans-alcohols. With regard to enzymic reduction of 4-propyl- and 4-isopropylcyclohexanone by horse liver alcohol dehydrogenase has been reported. For example, the 4-isopropylcyclohexanone was reduced to mainly trans-4-isopropylcyclohexanol. However, there are no report of microbial reduction of 4-propylcyclohexanone and 4-isopropylcyclohexanone. In the present paper, we report the biological stereoselective reaction of 4-propylcyclohexanone and 4-isopropylcyclohexanone by ten kinds of anthracnose fungi.

2 Materials and Methods

2.1 Microorganisms

Glomerella cingulata, Colletotrichum fragariae, C. lindemuthianum (C-1), C. lindemuthianum (C-3), C. lindemuthianum (C-13) and C. lagenarium used in this study were obtained from Gifu University, where it was prepared by Prof. Dr M. Hyakumachi. C. atramentarium MAFF712102, C. trifolii MAFF305389, C. dematium MAFF410046 and C. graminicola MAFF305460 were purchased from the Genetic Resources Center National Institute of Agrobiological Resources Ministry of Agriculture, Forestry and Fisheries (MAFF), Japan.

2.2 Culture Conditions

Spores of microorganisms which had been preserved at low temperature (10°C) were inoculated into sterilized culture media: 1.5% (w/v) sucrose, 1.5% (w/v) glucose, 0.5% (w/v) polypeptone, 0.1% (w/v) K₂HPO₄, 0.05% (w/v) KCl, 0.05% (w/v) MgSO₄·7H₂O, 0.001% (w/v) FeSO₄·7H₂O and distilled water, pH 7.2 in an
Erlenmeyer flask which was autoclaved at 121°C for 15 min and shaken at 27°C for 3 d. The mycelia were transplanted in a 1 L stirred fermenter containing 800 ml of a medium. Cultivation was carried out at 27°C with stirring for 3 d with aeration. After the growth of fungi the oil substrate (400 mg) was added directly into the medium and cultivated for 7 d.

2-3 Time-course Experiment
Sample (10 ml) of the culture medium were removed daily and saturated with sodium chloride extracted with dichloromethane (3 ml) and the extract analysed by GC. The substrate to metabolite product ratios were determined from the areas of the peaks separated by GC and are shown in Figs. 1-4, respectively.

2-4 Isolation of Metabolites
After incubation time of 7 d, culture medium and mycelia were separated by filtration, and the filtrate (730 ml) was saturated with sodium chloride and then extracted with dichloromethane (200 ml). The extracts were evaporated and analyzed by GC and GC-MS. The products were identified by comparison of retention times (4-propylcyclohexanol; cis-alcohol; 16.8 min, trans-alcohol; 17.4 min, 4-isopropylcyclohexanol; cis-alcohol; 16.5 min, trans-alcohol; 17.4 min) and mass spectral data; cis-4-propylcyclohexanol: EIMS m/z 124(M+)(0.5), 109(13), 82(28), 81(100), 55(30), 43(40), 41(27).

The extracts were subjected to chromatography on Si-60 columns with a hexane/diethyl ether gradient (from 99:1 to 8:2) to give the products: C. atramentarium MAFF712102 (2; 118 mg, 3; 189 mg, 5; 136 mg, 6; 150 mg), C. dematium MAFF410046 (2; 115 mg, 3; 196 mg, 5; 74 mg, 6; 222 mg), C. fragariae (2; 62 mg, 3; 249 mg, 5; 92 mg, 6; 185 mg), C. graminicola MAFF305460 (2; 119 mg, 3; 191 mg, 5; 80 mg, 6; 202 mg), C. lagenarium (2; 22 mg, 3; 297 mg, 5; 24 mg, 6; 269 mg), C. lindemuthianum (C-1) (2; 93 mg, 3; 186 mg, 5; 87 mg, 6; 175 mg), C. lindemuthianum (C-3) (2; 124 mg, 3; 187 mg, 5; 82 mg, 6; 164 mg), C. lindemuthianum (C-13) (2; 144 mg, 3; 159 mg, 5; 79 mg, 6; 158 mg), C. trifolii MAFF305389 (2; 120 mg, 3; 156 mg, 5; 87 mg, 6; 174 mg), G. cingulata (2; 111 mg, 3; 144 mg, 5; 92 mg, 6; 147 mg), respectively.

2-5 GC and GC-MS Conditions
GC and GC-MS were carried out on previously reported conditions².
2-6 Chemicals

4-Propylcyclohexanone (1) and 4-isopropylcyclohexanone (4) was purchased from Lancaster Synthesis Ltd.

2-7 NaBH₄ Reduction of Ketone

A solution of 1 and 2 (1.3 mmol) in methanol (2 ml) were treated in portions at 25°C with NaBH₄ (95 mg, 2.5 mmol) respectively. After stirring for 60 min the mixture were diluted with water (5 ml) and extracted three times with diethyl ether (10 ml). The combined organic phases were washed with water (5 ml), dried (Na₂SO₄), and the solvent was then removed at reduced pressure.

The analysis of alcohol was carried out GC-MS. 4-Propylcyclohexanol and 4-isopropylcyclohexanol were obtained by NaBH₄ reduction of 4-propylcyclohexanone and 4-isopropylcyclohexanone with a 1 : 3.4 ratio of the cis/trans-isomers respectively.

3 Results and Discussion

For time-course experiments, analytical amount of 4-propylcyclohexanone (1) was incubated with ten kinds of anthracnose fungi for 7 d respectively. 4-Propylcyclohexanone (1) was used as starting material, and the medium was analyzed at one day intervals for 7 d. Two metabolites (2 and 3) were detected by TLC and GC analysis. Compound (2 and 3) were not detected on TLC and GC analysis of the culture of anthracnose fungi to which no substrate was fed, nor were they produced in a mixture of 1 and the medium which were stirred for 7 d. After one day of cultivating the biological reduction was detectable. The mass and GC data of the products were the same as those of synthetic compounds by NaBH₄ reduction 4-propylcyclohexanols, the predominant isomer being trans-4-propylcyclohexanol (3). Compound 1 was reduced the corresponding cis- and trans-alcohol (Scheme 1). As shown in Fig. 1, the time course for appearance of the metabolites; 99.6% of 1 was metabolized after 7 d by Colletotrichum lindenmuthianum (C-13). The metabolite 3 was about 52.2% at the end of 7 d. Compound 2 was about 47.4% at 7 d. The time course of 1 by C. lagenarium was shown Fig. 2. Compound 1 had completely disappeared after 7 d incubation period and the corresponding cis-alcohol (7.1%) and trans-alcohol (92.9%) were generated. The ratio of cis- and trans-alcohol was 1:13. Compound 1 was

![Scheme 1 The Reduction of 4-Propylcyclohexanone (1).](image)
transformed to \textit{trans}-4-propylcyclohexanol as the major product at the end of 7 d. In the case of \textit{C. lagenarium}, \textit{trans}-alcohol was formed with stereoselectivity.

The behavior of the fungi toward 4-isopropylcyclohexanone (4) was also studied. The mass and GC data of the products from 4 were compared with those of synthetic compounds by NaBH\textsubscript{4} reduction. Compound 4 was reduced to the corresponding \textit{cis}- and \textit{trans}-alcohol (Scheme 2). As shown in Fig. 3, during 7 d incubation with \textit{C. dematiium} MAFF 410046, a major portion (92.5\%) of compound 4 was metabolized to yield the corresponding two metabolites, \textit{cis}-alcohol (5; 23.1\%) and \textit{trans}-alcohol (6; 69.4\%). The ratio of \textit{cis}- and \textit{trans}-alcohol was 1 : 3. As shown in Fig. 4, during 7 d incubation with \textit{C. lagenarium}, the 92.0\% of compound 4 was metabolized to give the corresponding two metabolites, 5 and 6. The main product (6) was generated to give a yield of 84.3\% (\textit{trans}-alcohol). The ratio of \textit{cis-} and \textit{trans}-alcohol was 1 : 11. In the case of other fungi, the reduction of 4-isopropylcyclohexanone was low stereoselectivity. For example, in the case of \textit{C. atramentarium} and \textit{G. cingulata}, the main metabolic product was obtained to \textit{trans}-alcohol, the ratio of \textit{cis-} and \textit{trans}-alcohol was 1 : 1.1 and 1 : 1.6 at the end of 7 d, respectively.

\begin{center}
\textbf{Scheme 2} The Reduction of 4-Isopropylcyclohexanone (4).
\end{center}

The ketone : alcohol ratio after 7 d microbiological reduction of 4-propylcyclohexanone also the \textit{cis} : \textit{trans} ratios of the 4-alkylcyclohexanols with 10 fungi are shown in Table 1. The results in Table 1 show that the major metabolized compound was the \textit{trans}-isomer of 4-propylcyclohexanol (3) and 4-isopropylcyclohexanol (6). Both of cyclic ketones were reduced by all of fungi, the ratio of \textit{cis} : \textit{trans}-alcohol was quite different by each fungus. Particularly, compared with the other fungi, \textit{C. lagenarium} showed high stereoselectivity to produce \textit{trans}-4-forms (3 and 6).

Thus, the main metabolite of the biotransformation of 4-propylcyclohexanone (1) and 4-isopropylcyclohexanone was obtained \textit{trans}-4-propylcyclohexanol (3) and \textit{trans}-4-isopropylcyclohexanol (6) in all of fungi respectively. A noteworthy feature of stereoselective reductions were the produce of \textit{trans}-alcohol (3 and 6) as main product by \textit{C. atramentarium}.

\begin{center}
\textbf{Table 1} The Ratio of \textit{cis}/\textit{trans}-cyclohexanols after 7 Days Cultivation of Cyclohexanols with 10 Fungi.
\end{center}

\begin{table}[h]
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\begin{tabular}{lccccc}
\hline
\textbf{microorganisms} & \textbf{Alcohol Yield(\%)} & \textbf{Alcohol Yield(\%)} \\

\textit{Colletotruchum atramentarium} MAFF712102 & 96.1 & 1 & 1.6 & 89.8 & 1 & 1.1 \\
\textit{Colletotruchum dematiium} MAFF410046 & 97.6 & 1 & 1.7 & 92.5 & 1 & 3 \\
\textit{Colletotruchum fragariae} & 97.6 & 1 & 4 & 86.9 & 1 & 2 \\
\textit{Colletotruchum graminicola} MAFF305460 & 97.1 & 1 & 1.6 & 88.5 & 1 & 2.5 \\
\textit{Colletotruchum lagenarium} & 100 & 1 & 13 & 92.0 & 1 & 11 \\
\textit{Colletotruchum lindemithianum} (C-1) & 87.2 & 1 & 2 & 82.2 & 1 & 2 \\
\textit{Colletotruchum lindemithianum} (C-3) & 97.6 & 1 & 1.5 & 77.2 & 1 & 2 \\
\textit{Colletotruchum lindemithianum} (C-13) & 99.6 & 1 & 1.1 & 74.2 & 1 & 2 \\
\textit{Colletotruchum trifolii} MAFF305389 & 86.3 & 1 & 1.3 & 81.6 & 1 & 2 \\
\textit{Glomerella cingulata} & 80.1 & 1 & 1.3 & 75.1 & 1 & 1.6 \\
\hline
\end{tabular}
\end{table}

\begin{center}
a)The relative intensities (\%) were calculated on the basis of peak areas in GC
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lagenarium at the end of 7 d.

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[報文] 塩化ヘキサデシルおよび塩化ドデシルピリジニウム混合ミセルのグリコールオリゴマー水溶液中での挙動

Mandeep Singh BAKSHI
Department of Chemistry, Guru Nanak Dev University (Amritsar–143005, (Punjab) INDIA.)

塩化ヘキサデシルおよび塩化ドデシルピリジニウム（HPyCl）と塩化ドデシルピリジニウム（DDPCI）の混合比を変えた混合ミセルの電気伝導度を測定した。ミセル濃度と電気伝導度の相関はいずれの混合比でも一致的であった。その伝導率曲線の挙動から、臨界ミセル濃度、ミセル誘電率等を算出した。HPyCl、DDPCIモル分率を変えた時のミセル形成の結果からその時のグリコール添加効果を見た。DDPCIの混合比が大きい時がHPyClの混合比が小さい時に比べ、構造変化への影響が顕著であることがわかった。

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[報文] 炭疽病菌による4-propylcyclohexanoneおよび4-isopropylcyclohexanoneの立体選択的還元

岡村 茂昭・宮澤 三雄・山口 眞司・亀岡 弘
近畿大学理工学部応用化学科
（〒577–8502 大阪府東大阪市小若江3–4–1）

テルペノイドおよびそれらの関連化合物の微生物変換に関する研究の一環として、10種の炭疽病菌、Colletotrichum atramentarium MAFF712102、C. dematium MAFF410046、C. fragariae、C. graminicola MAFF305460、C. lagenarium、C. lindemuthianum (C-1)、C. lindemuthianum (C-3)、C. lindemuthianum (C-13)、C. trifolii MAFF305389およびGlomerella cingulata を生体触媒として用い、4-propylcyclohexanoneおよび4-isopropylcyclohexanoneの変換を行った。その結果、ケトン基の還元反応が進行し、それぞれ対応するcisおよびtrans-alcoholを生成した。全菌種において主要変換物はtrans-alcoholであり、特にC. lagenariumを用いた場合、立体選択性が高い。4-propylcyclohexanoneの変換においては、cis体およびtrans体の生成比は1:13(変換7日目)、4-isopropylcyclohexanoneの変換ではcis体およびtrans体の生成比は1:11(変換7日目)であった。

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