

Enantioselective Introduction of Oxygen Functions into Alkenes —Oxidation of Limonene by the Cultured Suspension Cells of *Nicotiana tabacum*

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Chiral introduction of oxygenated functional group into alkenes is one of the important reactions for asymmetrization of alkenes in chemical syntheses. However, there are only a few reports of chiral introduction of the oxygenated functional group into unfunctionalized alkenes, which do not bear a specific functional group at the position adjacent to the C-C double bond, by use of chemical reagent¹⁻⁴). In connection with the development of a suitable methodology for the introduction of the oxygenated functional groups into alkenes by use of plant cultured cells as a biocatalyst⁵), we have investigated the biotransformation of terpene hydrocarbons such as limonene by the cultured suspension cells of *Nicotiana tabacum*.

The suspension cells of *N. tabacum* were prepared as described in ref. 6. The cultured cells were added to a 300-ml conical flask containing 100 ml of Murashige and Skoog's medium⁷). To the flask containing the suspension cells, the substrates (10 mg), such as (4*S*)-(–)-limonene (**Fig. 1-1a**) ($[\alpha]_D^{25} - 93.5^\circ$ (*c* 5.08, EtOH) (lit.⁸). $[\alpha]_D^{25} - 94^\circ$); >99.5% pure on GC) and (4*R*)-(+)-limonene (**Fig. 1-1b**) ($[\alpha]_D^{25} + 88.7^\circ$ (*c* 4.96, EtOH); >99.5% pure on GC), were administered; the total amount of

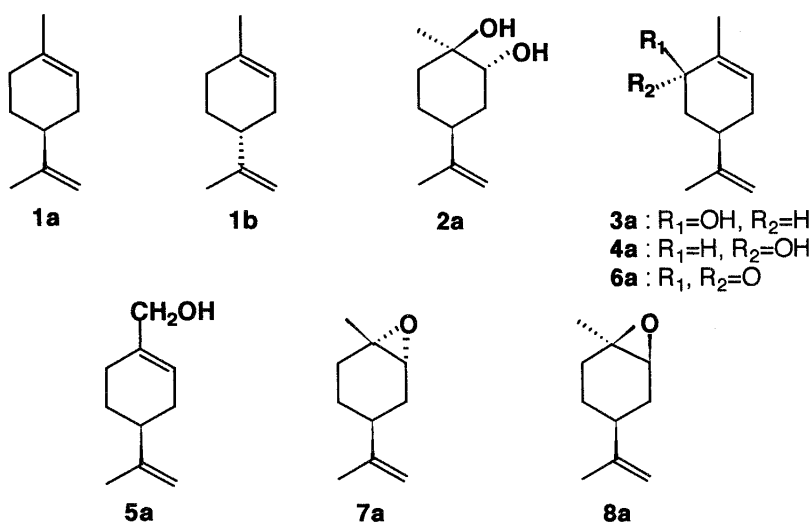


Fig. 1 Structures 1a-8a.

Table 1. Biotransformation of (4*S*)- and (4*R*)-limonenes (**Fig. 1-1a** and **1b**) with the suspension cells of *N. tabacum*.

Products* ¹	Yield* ² /%	
	1a	1b
Limonene-1, 2-diol (2a)	7.4	tr.* ³
<i>cis</i> -Carveol (3a)	10.6	2.6
<i>trans</i> -Carveol (4a)	9.4	2.7
Perillyl alcohol (5a)	5.7	0.6
Carvone (6a)	9.5	1.2
Limonene- <i>trans</i> -epoxide (7a)	0.9	tr.
Limonene- <i>cis</i> -epoxide (8a)	1.8	tr.

*¹Products derived from **Fig. 1-1b** are the corresponding enantiomers of the products, **Fig. 1-2a-8a**, from **Fig. 1-1a**.

*²Weight (%) of products relative to the substrate administered.

*³tr. denotes trace amount (<0.1 %).

the substrate used was 100 mg in each run. The transformation of the substrate was brought about by incubating the cultured mixture at 25°C for 16 hr on a rotary shaker (70 r.p.m.) in the dark. The cultured mixture was worked up in a manner similar to that described in ref. 6. The products were identified by comparison of their TLC, GC, and MS with those of authentic samples^{9,10}. Yields of the products were determined on the basis of the GC analyses.

(4*S*)-Limonene (**Fig. 1-1a**) was converted into limonene-1, 2-diol (**Fig. 1-2a**), *cis*-carveol (**Fig. 1-3a**), *trans*-carveol (**Fig. 1-4a**), perillyl alcohol (**Fig. 1-5a**), carvone (**Fig. 1-6a**), limonene-*trans*-epoxide (**Fig. 1-7a**), and limonene-*cis*-epoxide (**Fig. 1-8a**), as shown in **Table 1**. However, transformation of (4*R*)-limonene (**Fig. 1-1b**) into the corresponding products, **2b-8b**, hardly occurred. Specific transformation of (4*S*)-limonene (**Fig. 1-1a**) indicates that the cells discriminate the enantiomer of limonene and introduce the oxygen to the enantiomer selectively. In view of our previous experiment and based on literature data^{11,12}, the diol **Fig. 1-2a** may be produced by hydrolysis of the resultant epoxides, **Fig. 1-7a** and **8a**. On the other hand, carvone (**Fig. 1-6a**) may be formed by further oxidation of the resultant carveols (**Fig. 1-3a** and **4a**)¹⁰. These observations indicate that limonene was first oxidized at the C-C double bond and its allylic position.

Thus, it was established that the cultured suspension cells of *N. tabacum* have the ability to introduce enantioselectively the oxygenated functional groups, such as hydroxyl and epoxy groups, into alkene; the hydroxylation occurs regioselectively at the allylic position of the endocyclic C-C double bond of limonene and the formation of diol occurs at the endocyclic C-C double bond via the corresponding epoxides. The mechanism of the oxidation with the cells and the enzymes participating in the oxidation is under further investigation.

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《和文要約》

アルケン類への鏡像体選択的な酸素官能基導入 ——タバコ培養細胞によるリモネンの酸化

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アルケン類へのキラルな酸素官能基導入法の開発に関連して、タバコ培養細胞を生体触媒とする (4*S*)-および (4*R*)-リモネンの変換を調べた。タバコ培養細胞は鏡像体選択的に (4*S*)-リモネンの環内二重結合をエポキシドを経てジオールに変換する機能と、二重結合のアリル位を位置選択的に水酸化する機能を有することがわかった。