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THE ENANTIOSELECTIVE BIOTRANSFORMATION OF α-TERPINEOL AND ITS ACETATE WITH THE CULTURED CELLS OF NICOTIANA TABACUM

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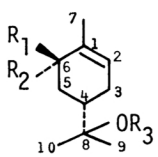
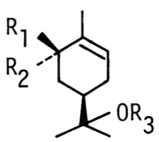
In the biotransformation of the enantiomers of p-menth-1-en-8-ol (α-terpineol) and 8-acetoxy-p-menth-1-ene (α-terpinyl acetate) with the cultured suspension cells of Nicotiana tabacum, it was clarified that the cultured cells effected the hydroxylation at the 6-position of (4R)-(+)-enantiomer in preference to the (4S)-(-)-enantiomer, whereas the cells did the hydrolysis of the acetoxyl group and the hydroxylation of the ethylenic linkage of (4S)-(-)-α-terpinyl acetate in preference to the (4R)-(+)-enantiomer.

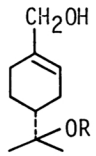
Our recent studies on the biotransformation of foreign substrates with the cultured cells of Nicotiana tabacum have shown that the cells effect not only the regio- and stereoselective hydroxylations at the allylic positions of the carbon-carbon doule bond of (-)-linalool[1,2] and terpineols,[3] but also the stereospecific hydroxylation of the endocyclic ethylenic linkage of (±)-α-terpinyl acetate.[4] The cells also effected the hydrolysis of (-)-linalyl



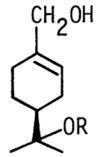
1a: $R_1=R_2=R_3=H$
2a: $R_1=R_2=H$, $R_3=Ac$
3a: $R_1=OH$, $R_2=R_3=H$
4a: $R_1=R_3=H$, $R_2=OH$
6a: $R_1=OH$, $R_2=H$, $R_3=Ac$
7a: $R_1=H$, $R_2=OH$, $R_3=Ac$



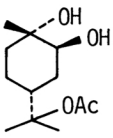
1b: $R_1=R_2=R_3=H$
2b: $R_1=R_2=H$, $R_3=Ac$
3b: $R_1=R_3=H$, $R_2=OH$
4b: $R_1=OH$, $R_2=R_3=H$
6b: $R_1=H$, $R_2=OH$, $R_3=Ac$
7b: $R_1=OH$, $R_2=H$, $R_3=Ac$



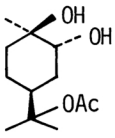
5a: R=H
8a: R=Ac



5b: R=H
8b: R=Ac



9a



9b

acetate[1,2] and (±)-α-terpinyl acetate[4] in preference to β-terpinyl acetate[3] and γ-terpinyl acetate.[5] We interested in exploring whether the cultured suspension cells of Nicotiana tabacum effect the enantioselective transformation or not, and now have tested the biotransformations of such enantiomeric pairs as (4R)-(+)- and (4S)-(-)-p-menth-1-en-8-ols (α-terpineols) (**1a** and **1b**) and (4R)-(+)- and (4S)-(-)-8-acetoxy-p-menth-1-enes (α-terpinyl acetates) (**2a** and **2b**). We here wish to communicate a new finding that the cultured suspension cells effect the enantioselective transformation of these foreign substrates.

Callus tissues induced from the stem of Nicotiana tabacum "Bright Yellow" were used in this investigation. The feeding experiment and working-up were carried out in the same manner as described in our previous paper.[2,6] The biotransformation of the substrates, **1a** ($[\alpha]_D^{25}$ +100.3° (neat)), **1b** ($[\alpha]_D^{25}$ -100.8° (neat)), **2a** ($[\alpha]_D^{25}$ +74.9° (neat)), and **2b** ($[\alpha]_D^{25}$ -76.2° (neat)), with the suspension cells resulted in the formation of the products qualitatively similar to those in the previously described biotransformation of racemic α-terpineol and its acetate.[3,4] The products were identified by direct comparisons of their TLC, GLC, and GC-MS with those of the products obtained in the cases of the racemic substrates.[3,4] The configurations of the products shown in **3**—**9** were assigned by relating them to the configuration at C-4 of the substrates.

The time-course studies on the biotransformation of the enantiomers were then carried out as follows. Each sample of the enantiomers was administered to seven flasks (10 mg per one flask containing 100 ml of the medium). The cultured mixtures were worked up at a regular time interval. The experiments for the enantiomeric pair were simultaneously carried out under the same conditions as possible. The yields of the products were determined on the basis of the peak area on the GLC and are expressed as relative percentage to the whole reaction mixture obtained. Figure 1 (A)—(D) show the result.

As is shown in Fig. 1 (A) and (B), the hydroxylation at the 7-position took place for both the enantiomers of α-terpineol to a comparative extent, but the hydroxylation at the 6-position predominates for the (4R)-(+)-enantiomer (**1a**). The hydroxylation at the 6-position predominates for the (4R)-(+)-α-terpinyl acetate (**2a**), compared with the (4S)-(-)-enantiomer (**2b**), though this

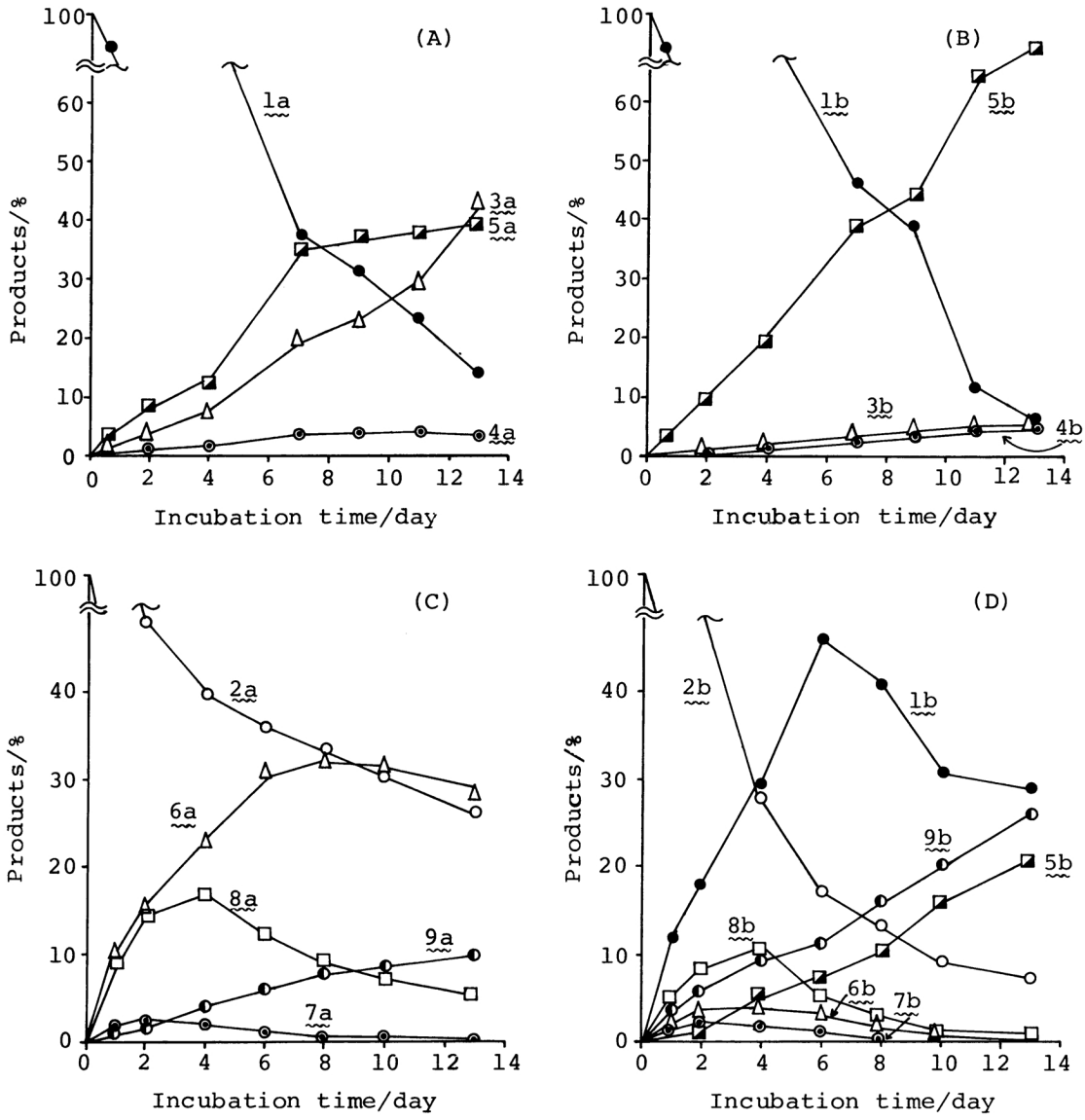


Fig. 1. The time-courses in the biotransformation of the following substrates with Nicotiana tabacum suspension cells: (A) (4R)-(+)-α-terpineol (**1a**), (B) (4S)-(-)-α-terpineol (**1b**), (C) (4R)-(+)-α-terpinyl acetate (**2a**), and (D) (4S)-(-)-α-terpinyl acetate (**2b**).

enantiomer (**2b**) suffers the hydrolysis of the acetoxyl group followed by the transformation of the hydrolyzed product, as is shown in Fig. 1 (C) and (D). The hydrolysis was preferential for **2b**. In contrast with the biotransformation of α-terpineols (**1a** and **1b**), the hydroxylation of the endocyclic ethylenic linkage was noted for both the acetates **2a** and **2b**,

resulting in the formation of (1S,2S,4R)-8-acetoxy-p-menthane-1,2-diol (**9a**) and (1R,2R,4S)-8-acetoxy-p-menthane-1,2-diol (**9b**), but this hydroxylation was preferential for the (4S)-(-)-enantiomer (**2b**) to yield **9b** predominantly.

Thus, it was clarified that the cultured suspension cells of Nicotiana tabacum have the ability to transform enantioselectively α-terpineol and its acetate; that is, (i) the cultured cells prefer (4R)-(+)-enantiomers (**1a** and **2a**) to (4S)-isomers (**1b** and **2b**) for the hydroxylation at their 6-position, whereas (ii) the cells do (4S)-(-)-enantiomer (**2b**) to (4R)-isomer (**2a**) for the hydrolysis of its acetoxyl group and also the hydroxylation of its endocyclic ethylenic linkage. However, the cells were not enantioselective for the hydroxylation at the 7-position of the substrates.

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References

1) T. Suga, T. Hirata, Y. Hirano, and T, Ito, Chem. Lett., 1976, 1245.
2) T. Hirata, T. Aoki, T. Ito, and T. Suga, Bull. Chem. Soc. Jpn., 54, 3527 (1981).
3) T. Suga, T. Aoki, T. Hirata, Y. S. Lee, O. Nishimura, and M. Utsumi, Chem. Lett., 1980, 229.
4) T. Hirata, Y. S. Lee, and T. Suga, Chem. Lett., 1982, 671.
5) Y. S. Lee, T. Hirata, and T. Suga, Presented at the 43rd Annual Meeting of the Chemical Society of Japan, Tokyo, Japan, April 1981, The Abstract (II), p. 689.
6) T. Hirata, H. Hamada, T. Aoki, and T. Suga, Phytochemistry, 21 (1982), in press.

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