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ENANTIOSELECTIVITY IN THE HYDROLYSIS OF BICYCLIC MONOTERPENE ACETATES WITH THE CULTURED CELLS OF *NICOTIANA TABACUM*

TAKAYUKI SUGA*, TOSHIFUMI HIRATA and SHUNSUKE IZUMI

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730, Japan

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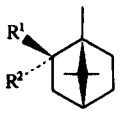
Key Word Index—*Nicotiana tabacum*; Solanaceae; tissue culture; biotransformation; hydrolysis; enantioselectivity; bicyclic monoterpene acetates.

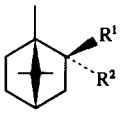
Abstract—The enantioselectivity in the hydrolysis of bornyl acetate, isobornyl acetate and isopinocampheyl acetate with the cultured cells of *Nicotiana tabacum* was investigated. The cultured cells were found to have the ability to hydrolyse enantioselectively the acetates, of which the configuration at the carbon atom bearing the acetoxyl group is *R*.

INTRODUCTION

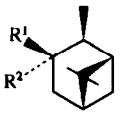
The cultured cells of *Nicotiana tabacum* have the ability to hydrolyse the acetoxyl group of linaloyl acetate [1] and the hydroxyimino group of carvoxime [2]. It was observed that the acetoxyl group of (+)- and (−)-α-terpinyl acetates tends to experience enantioselective hydrolysis in the cultured cells [3]. We have now investigated the enantioselectivity in the hydrolysis of the acetates having a rigid skeleton using a suspension of the cultured cells of *N. tabacum*. The acetates used as a substrate were (1*R*,2*S*,4*R*)-(+)- and (1*S*,2*R*,4*S*)-(−)-2-acetoxy-1,7,7-trimethylbicyclo[2.2.1]heptanes [trivial names: (+)- and (−)-bornyl acetates, **1a** and **1b**], (1*S*,2*S*,4*S*)-(−)- and (1*R*,2*R*,4*R*)-(+)-2-acetoxy-1,7,7-trimethylbicyclo-[2.2.1]heptanes [(−)- and (+)-isobornyl acetates, **2a** and **2b**], and (1*S*,2*S*,3*S*,5*R*)-(+)- and (1*R*,2*R*,3*R*,5*S*)-(−)-3-acetoxy-2,6,6-trimethylbicyclo [3.1.1] heptanes [(+)- and (−)-isopinocampheyl acetates, **3a** and **3b**].



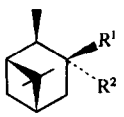
1a R^1 = H, R^2 = OAc
2a R^1 = OAc, R^2 = H
4a R^1 = H, R^2 = OH
5a R^1 = OH, R^2 = H
7a R^1, R^2 = O



1b R^1 = H, R^2 = OAc
2b R^1 = OAc, R^2 = H
4b R^1 = H, R^2 = OH
5b R^1 = OH, R^2 = H
7b R^1, R^2 = O



3a R^1 = H, R^2 = OAc
6a R^1 = H, R^2 = OH



3b R^1 = H, R^2 = OAc
6b R^1 = H, R^2 = OH

RESULTS AND DISCUSSION

The acetates were incubated with a suspension of the cultured cells of *N. tabacum* in a similar manner as described in refs [1, 4]. Figure 1 shows the results of the time-course experiments in the biotransformation of bornyl acetates (**1a** and **1b**), isobornyl acetates (**2a** and **2b**) and isopinocampheyl acetates (**3a** and **3c**). These acetates were hydrolysed to their corresponding alcohols by the cultured cells, but the extent of hydrolysis was quite different between their enantiomers. The hydrolysis of (1*S*,2*R*,4*S*)-(−)-bornyl acetate (**1b**) predominated over that of (1*R*,2*S*,4*R*)-(+)-enantiomer (**1a**), as given in Fig. 1, A and B. Similarly, the hydrolysis of (1*R*,2*R*,4*R*)-(−)-isobornyl acetate (**2a**) was more predominant rather than that of its enantiomer (**2b**) (C and D of Fig. 1). In the case of isopinocampheyl acetate, (1*R*,2*R*,3*R*,5*S*)-(−)-enantiomer (**3b**) was hydrolysed in preference to its enantiomer (**3a**) (E and F of Fig. 1).

In the biotransformation of (+)-bornyl acetate (**1a**) and (−)-isobornyl acetate (**2b**) (A and C of Fig. 1), the yield of the respective hydrolysed products (**4a** and **5a**) decreased gradually as an increase in the yield of camphor (**7a**) with the lapse of the incubation time. This indicates the formation of **7a** is due to the successive oxidation of **4a** and **5a** with the cultured cells. No formation of camphor was found in the biotransformation of (−)-bornyl acetate (**1b**) and (+)-isobornyl acetate (**2a**). Such a difference in the formation of camphor might be due to the enantioselectivity in the oxidation of the respective hydro-

*To whom correspondence should be addressed.

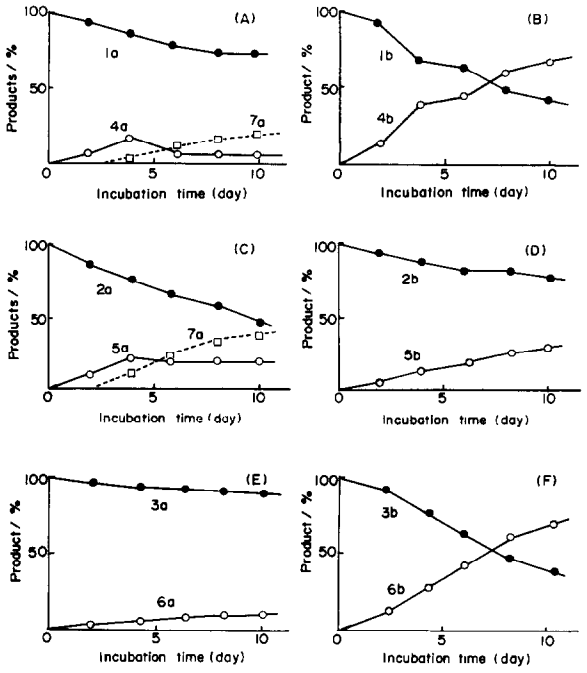


Fig. 1. The time-courses in the biotransformation of (A) (1*R*,2*S*,4*R*)-(+)-bornyl acetate (**1a**), (B) (1*S*,2*R*,4*S*)-(−)-bornyl acetate (**1b**), (C) (1*R*,2*R*,4*R*)-(−)-isobornyl acetate (**2a**), (D) (1*S*,2*S*,4*S*)-(+)-isobornyl acetate (**2b**), (E) (1*S*,2*S*,3*S*,5*R*)-(+)-isopinocampheyl acetate (**3a**) and (F) (1*R*,2*R*,3*R*,5*S*)-(−)-isopinocampheyl acetate (**3b**).

lysed products, because the enantioselectivity in the oxidations of borneol and isoborneol with the cultured cells of *N. tabacum* is in preference to (1*R*,2*S*,4*R*)- and (1*R*,2*R*,4*R*)-enantiomers (**4a** and **5a**), respectively, as described in our previous paper [5].

Thus, it was established that a suspension of the cultured cells of *N. tabacum* have the ability to hydrolyse enantioselectively the acetoxyl groups of bornyl acetate, isobornyl acetate and isopinocampheyl acetate; the cultured cells prefer the acetates with the *R*-configuration at the carbon atom bearing an acetoxyl group.

EXPERIMENTAL

Preparative TLC: silica gel (0.5 mm) developed with EtOAc–hexane (3:7). GLC: FID, glass column (3 mm × 2 m) packed with 15% DEGS or 2% OV-17 on Chromosorb W (AW-DMCS; 80–100 mesh) at 120° or 80–150° (3°/min), respectively.

Substrates. (+)-Bornyl acetate (**1a**) {$[\alpha]_D^{25}$ +40.0° (*c* 1.00; EtOH)} (lit. [6] +44.1°). (−)-Bornyl acetate (**1b**) {$[\alpha]_D^{25}$ −39.0° (*c* 1.00; EtOH)} (lit. [7] −44.5°). (+)-Isobornyl acetate (**2b**) {$[\alpha]_D^{25}$ +1.5° (*c* 1.00; EtOH)} (lit. [8] +1.7°) and (−)-isobornyl acetate (**2a**) {$[\alpha]_D^{25}$ −2.0° (*c* 1.00; EtOH)} (lit. [9] −1.8°) were prepared from the corresponding alcohols by acetylation with Ac_2O in pyridine, followed by purification by preparative TLC. The alcohols, such as (+)- and (−)-borneols (**4a** and **4b**) and (−)- and (+)-isoborneols (**5a** and **5b**), were prepared by reduction of (+)- and (−)-camphors (**7a** and **7b**) (Tokyo Kasei Kogyo Co. Ltd.) with $LiAlH_4$.

(+)-Isopinocampheyl acetate (**3a**) {$[\alpha]_D^{25}$ +35.0° (*c* 1.00; EtOH)} (lit. [10] +37.5°) and (−)-isopinocampheyl acetate (**3b**) {$[\alpha]_D^{25}$ −35.0° (*c* 1.00; EtOH)} were synthesized by the acetylation of the corresponding alcohols, which had been previously prepared in our laboratory [11] by the hydroboration–oxidation [12] of (−)- and (+)-α-pinene, respectively. All of the substrates used were > 99.7% pure on GLC.

Time-courses in the biotransformation of the acetates. The callus tissues [1] of *N. tabacum* was transplanted to freshly prepared Murashige and Skoog's medium [13] (100 ml per a 300-ml conical flask) containing 2 ppm of 2,4-dichlorophenoxyacetic acid and 3% sucrose and then grown for 3–4 weeks at 25° on a rotary shaker (70 rpm) in the dark. To the flask containing a suspension of the cells (about 50–70 g fr. wt/flask), the substrate (10 mg) was administered, and cultures were incubated under continuous shaking at 25° for 10 days in the dark. At a regular time interval, a part (10 ml) of the incubated mixture was pipetted out under sterile conditions and extracted with Et_2O. Each Et_2O extract was subjected to GLC. The products were identified by comparison (TLC, GLC and co-GLC) with authentic samples. The yields of the products were determined on the basis of the peak area on GLC and are expressed as a relative percentage to the total amount of the whole reaction mixture extracted.

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