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THE STEREOSPECIFIC HYDROXYLATION OF ENDOCYCLIC ETHYLENIC LINKAGE IN THE BIOTRANSFORMATION OF α-TERPINYL ACETATE WITH CULTURED SUSPENSION CELLS OF NICOTIANA TABACUM

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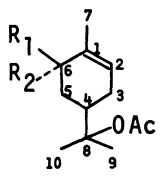
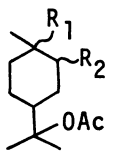
The biotransformation of (±)-8-acetoxy-p-menth-1-ene (α-terpinyl acetate) with the cultured cells of Nicotiana tabacum was found to result in the predominant formation of 8-acetoxy-c-4-p-menthane-r-1,t-2-diol. This experimental result indicates that the hydroxylation of the endocyclic ethylenic linkage with the cultured suspension cells is stereospecific.

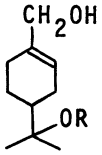
Recently, increasing interest has been focused on the biochemical capability of plant cells to metabolize foreign substrates and/or convert the substrates into highly valued substances.[1] In such a status, we have recently found that the suspension cells of Nicotiana tabacum have the ability not only to reduce stereospecifically the carbon-carbon double bond adjacent to the carbonyl group as well as the carbonyl group of carvone,[2] but also to hydroxylate the allylic positions of the carbon-carbon double bond of linalool[3,4] and terpineols[5] as well as the carbon-carbon double bond of β-terpinyl acetate.[5] However, the



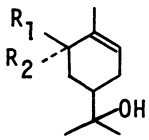
1: $R_1=R_2=H$
4: $R_1=H$, $R_2=OH$
5: $R_1=OH$, $R_2=H$
11: $R_1,R_2=O$



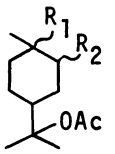
2: R_1=—OH, R_2=···OH
3: R_1=···OH, R_2=—OH



6: R=Ac
10: R=H



7: $R_1=R_2=H$
8: $R_1=H$, $R_2=OH$
9: $R_1=OH$, $R_2=H$



12: $R_1=R_2$=···OH
13: $R_1=R_2$=—OH

stereochemistry of the hydroxylation of carbon-carbon double bond could not be elucidated, because of the free rotation of the newly formed hydroxymethyl group. We now have investigated the stereoselectivity in the hydroxylation of the endocyclic ethylenic linkage of (±)-8-acetoxy-p-menth-1-ene (α-terpinyl acetate) (1)[6] with the cultured suspension cells of Nicotiana tabacum, and here wish to communicate a new finding that the tobacco suspension cells have the ability to hydroxylate stereospecifically the endocyclic ethylenic linkage.

The suspension cells of Nicotiana tabacum subcultured for about 8 years were used for this work, as in our previous work.[4,5] The feeding experiment and working-up were carried out in the same manner as described in our previous paper.[4] On the basis of a combination of TLC, GLC, and GC-MS analyses of a transformation product obtained from the incubation mixture, the product was found to be composed of ten components. Then, the time-course of the biotransformation was followed, and its result is shown in Fig. 1. Product 2 became a major component after incubation for 5 days. After incubation for 9 days, the yield of 2 was about 20 times that of 3 (0.5%), reaching to 10.3%(wt) for the α-terpinyl

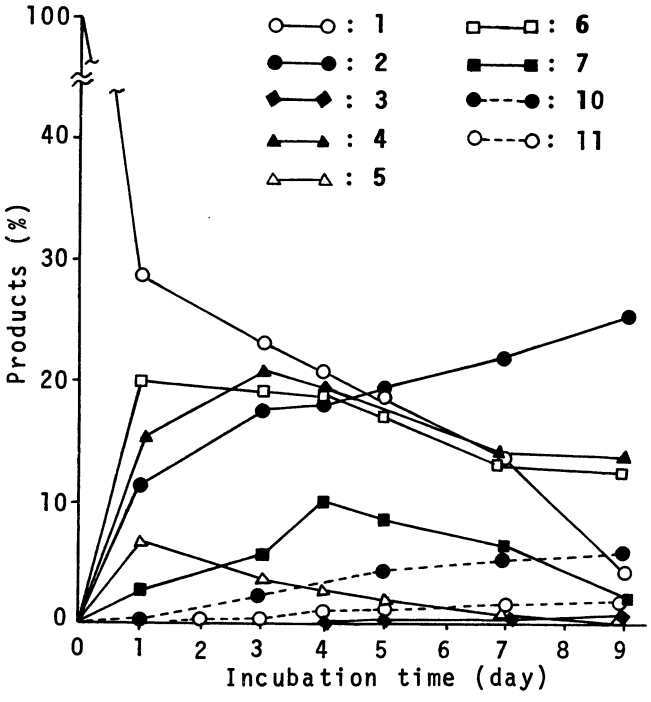


Fig. 1. The time-course in the biotransformation of α-terpinyl acetate (1) by the cultured suspension cells of Nicotiana tabacum.

acetate administered.

The CI-MS of the product **2** (n_D^{25} 1.4723) showed an ion peak at m/z 231 assigned to the $[M+H]^+$ ion. The ^1H NMR of **2** exhibited the signal at δ 3.63 due to >CH-OH instead of the signal at δ 5.32 due to >C=CH- of **1**, while the other signals at δ 1.96 and 1.43 were similar to the signals due to 1-acetoxy-1-methylethyl group of **1**. The IR spectrum of **2** in 0.002M CCl_4 solution exhibited two free-hydroxyl bands at 3635 and 3621 cm^{-1} assigned to hydroxyl groups attached to a secondary carbon atom and a tertiary one, respectively.[7] These observations indicated that **2** may be a glycol resulted from the hydroxylation of the double bond of **1**. The EI-MS fragmentation patterns of both the products **2** and **3** were similar to those of cis-glycols (**12** and **13**), which were prepared by oxidizing α-terpinyl acetate (**1**) with OsO_4. However, neither **2** nor **3** was consistent with the cis-glycols on the basis of TLC and GLC analyses. These observations indicate that the products **2** and **3** may be trans-glycols.[8] In the IR spectrum of **2** in the above-described CCl_4 solution, no band being assignable to the intramolecularly hydrogen bonded hydroxyl group was observed. This shows that the orientation of the two hydroxyl groups newly introduced into the endocyclic ethylenic linkage is diaxial, and hence the product **2** should be 8-acetoxy-c-4-p-menthane-r-1,t-2-diol, and inductively the product **3** may be 8-acetoxy-t-4-p-menthane-r-1,t-2-diol.

The other minor products were identified as 8-acetoxy-t-4-p-menth-1-en-r-6-ol (**4**) (5.7% yield after 9-days incubation), 8-acetoxy-c-4-p-menth-1-en-r-6-ol (**5**) (0.2%), 8-acetoxy-p-menth-1-en-7-ol (**6**) (4.8%), 8-hydroxy-p-menth-1-ene (**7**) (0.4%), t-4-p-menth-1-ene-r-6,8-diol (**8**) (0.2%), c-4-p-menth-1-ene-r-6,8-diol (**9**) (0.1%), p-menth-1-ene-7,8-diol (**10**) (2.6%), and 8-acetoxy-p-menth-1-en-6-one (**11**) (0.8%), on the basis of interpretation of their spectral data and/or direct comparison of their TLC, GLC, and spectral data with those of authentic samples.[5,9,10] The amounts of **8**—**11** increase as the decrease in those of **4**—**7** with the lapse of the incubation time, as shown in Fig. 1. This fact shows that the products **8**—**11** may be formed by further transformation of **4**—**7**; the dihydroxy compounds **8**—**10** may be derived from **4**—**6** by the hydrolysis of their acetoxyl groups and/or from **7** by the hydroxylation of its C-6 or C-7 position, and the ketone **11** was probably formed by the oxidation

of the hydroxyl group of **4** and **5**.

Thus, it was clarified that the hydroxylation of the endocyclic ethylenic linkage of α-terpinyl acetate (**1**) with the cultured suspension cells of Nicotiana tabacum takes place stereospecifically, resulting in the predominant formation of its trans-diaxial-diol (**2**).

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