

## ENANTIOSELECTIVITY IN THE REDUCTION OF $\alpha$ -DICARVELONE BY CELL SUSPENSION CULTURES OF *NICOTIANA TABACUM*

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**Key Word Index**—*Nicotiana tabacum*; Solanaceae; tissue culture; biotransformation; reduction; enantioselectivity; dicarvelones.

**Abstract**—The biotransformation of the enantiomeric pairs of  $\alpha$ -dicarvelone by cell suspension cultures of *Nicotiana tabacum* was investigated. It was found that the cultured cells transform only (1R,1'R,4R,4'R,6R,6'R)-(+)- $\alpha$ -dicarvelone to the corresponding alcohol.

### INTRODUCTION

The ability of callus tissues to convert regio- and stereoselectively foreign substrates is of considerable interest [1-3]. Recent studies on the biotransformation of foreign substrates by cultured cells of *Nicotiana tabacum* showed that they have the ability to hydrolyse enantioselectively the acetoxy group of bornyl acetate [4] and oxidize enantioselectively the hydroxyl group of bicyclic monoterpene alcohols [5-8]. We have now investigated enantioselectivity in the reduction of the carbonyl group of dicarvelones with a suspension of the cultured cells of *N. tabacum*. (1R,1'R,4R,4'R,6R,6'R)-(+)- and (1S,1'S,4S,4'S,6S,6'S)-(-)- $\alpha$ -Dicarvelones (**1a** and **1b**) were used as substrates.

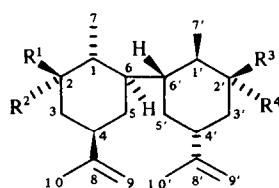
### RESULTS AND DISCUSSION

Tobacco callus tissues were derived from the stem of *Nicotiana tabacum* 'Bright Yellow'. The feeding experiment and the work-up were carried out as described in our previous paper [2].

(1R,1'R,4R,4'R,6R,6'R)-(+)- $\alpha$ -Dicarvelone (**1a**) was converted to (1R,1'R,2R,2'R,4R,4'R,6R,6'R)-(+)-6,6'-*p*-menth-8-en-2-ol (**2a**) in a 53.8% yield whereas the conversion of the corresponding enantiomer, (1S,1'S,4S,4'S,6S,6'S)-(-)- $\alpha$ -dicarvelone (**1b**), to (1S,1'S,2S,2'S,4S,4'S,6S,6'S)-(-)-6,6'-*p*-menth-8-en-2-ol (**2b**) did not occur. In spite of careful and repeated TLC and GC/MS analyses, no alcohols (**3a** and **4a**) were found in the reaction mixture obtained, and no products other than these transformation products were found even by careful and repeated TLC and GLC analyses. This indicates that the reduction of the carbonyl group of  $\alpha$ -dicarvelones with cell suspension cultures of *N. tabacum* is enantioselective. The formation of **2a** in the biotransformation of **1a** shows that (+)- $\alpha$ -dicarvelone (**1a**) is stereoselectively reduced to the compound with the chirality of *R* at C-2 and *R* at C-2'. Reduction to the carbonyl group takes place stereospecifically from the *si*-face at C-2 and the *si*-face at C-2'.

### EXPERIMENTAL

$^1\text{H}$  and  $^{13}\text{C}$  NMR were obtained at 60 and 22.6 MHz, respectively, with TMS as int. std. EI and HR-MS were measured at 70 eV. Merck silica gel (230-400 mesh) was used for flash chro-

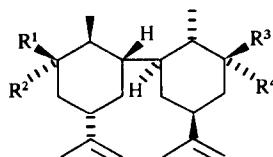


**1a**  $\text{R}^1, \text{R}^2 = \text{O}$ ,  $\text{R}^3, \text{R}^4 = \text{O}$

**2a**  $\text{R}^1 = \text{OH}$ ,  $\text{R}^2 = \text{H}$ ,  
 $\text{R}^3 = \text{H}$ ,  $\text{R}^4 = \text{OH}$

**3a**  $\text{R}^1$  or  $\text{R}^2 = \text{OH}$ ,  
 $\text{R}^3, \text{R}^4 = \text{O}$

**4a**  $\text{R}^1, \text{R}^2 = \text{O}$   
 $\text{R}^3$  or  $\text{R}^4 = \text{OH}$



**1b**  $\text{R}^1, \text{R}^2 = \text{O}$ ,  $\text{R}^3, \text{R}^4 = \text{O}$

**2b**  $\text{R}^1 = \text{H}$ ,  $\text{R}^2 = \text{OH}$ ,  
 $\text{R}^3 = \text{OH}$ ,  $\text{R}^4 = \text{H}$

**3b**  $\text{R}^1$  or  $\text{R}^2 = \text{OH}$ ,  
 $\text{R}^3, \text{R}^4 = \text{O}$

**4b**  $\text{R}^1, \text{R}^2 = \text{O}$   
 $\text{R}^3$  or  $\text{R}^4 = \text{OH}$

Table 1.  $^{13}\text{C}$  NMR spectral data of compounds, **1a** and **2a** (22.6 Hz,  $\text{CDCl}_3$ , TMS int std)

C	<b>1a</b>	<b>2a</b>
1,1'	45.1 (d)	41.2 (d)
2,2'	211.2 (s)	76.1 (d)
3,3'	46.5 (t)	41.8 (t)
4,4'	46.9 (d)	42.9 (d)
5,5'	29.7 (t)	30.0 (t)
6,6'	44.2 (d)	40.8 (d)
7,7'	11.0 (q)	11.2 (q)
8,8'	147.2 (s)	147.3 (s)
9,9'	110.2 (t)	109.7 (t)
10,10'	20.4 (q)	20.9 (q)

matography [9]. Analytical and prep. TLC were carried out on Merck 60 GF<sub>254</sub> silica-gel plates with 0.25 and 0.75 mm layers of adsorbent, respectively.

*Substrates.* (+)- $\alpha$ -Dicarvelone (**1a**) { $\nu_{\text{max}}^{\text{CCl}_4}$  nm ( $\epsilon$ ): 292 (88.3), mp 148–149°,  $[\alpha]_D^{25} + 74.9^\circ$  ( $\text{CHCl}_3$ ;  $c$  1.0), lit. [10] mp 148–149°,  $[\alpha]_D^{20} - 77^\circ$  ( $\text{CHCl}_3$ ;  $c$  1.42)} and (−)- $\alpha$ -dicarvelone (**1b**) { $[\alpha]_D^{25} - 74.8^\circ$  ( $\text{CHCl}_3$ ;  $c$  1.0)} were prepared from (−)- and (+)-carvones, respectively, according to the method given in ref. [10]. All substrates used were >99.5% pure by GC.

*Feeding of substrates to tobacco suspension cells.* Callus tissues [1] of *N. tabacum* were transplanted to freshly prep'd Murashige and Skoog's medium [11] (100 ml/300 ml conical flask) containing 2 ppm of 2,4-D and 3% sucrose, and then grown with continuous shaking for 3–4 weeks at 25° in the dark. After this time substrate (10 mg) was added to the flask containing the suspension cells (*ca* 60–80 g fr wt/flask) and the cultures incubated at 25° for a further 10 days on a rotary shaker (70 rpm) in the dark.

*Isolation and identification of products.* The cultured mixt was worked-up in a similar manner to that described in refs [1, 2]. The product was extd with  $\text{Et}_2\text{O}$  and identified by comparison of

its TLC and GC/MS with an authentic sample. The physical constants and spectral data are shown below.

(1R,1'R,2R,2'R,4R,4'R,6R,6'R)-(+)-6,6'-*p*-menth-8-en-2-ol (**2a**).  $[\alpha]_D^{25} + 17.4^\circ$  ( $\text{CHCl}_3$ ;  $c$  1.20); IR  $\nu_{\text{max}}^{\text{liquid}}$   $\text{cm}^{-1}$ : 3200 (OH) and 1640 (C=C);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  1.08 (6H, *brs*, 1,1'-Me), 1.77 (6H, *s*, 8,8'-Me), 3.20 (2H, *br*,  $>\text{CH}-\text{OH} \times 2$ ), 4.73 (4H, *brs*,  $>\text{C}=\text{CH}_2 \times 2$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ): see Table 1; EIMS  $m/z$  (rel. int.): 306 (6,  $[\text{M}]^+$ ) 288 (55,  $[\text{M}-\text{H}_2\text{O}]^+$ ), 270 (10,  $[\text{M}-\text{H}_2\text{O}-\text{H}_2\text{O}]^+$ ), 255 (8), 135 (100), 107 (40), 93 (45); HRMS  $m/z$  (rel. int.): 306.2542 (15,  $[\text{M}]^+$ ),  $\text{C}_{20}\text{H}_{34}\text{O}_2$  requires  $m/z$  306.2526, 288.2446 (18,  $[\text{M}-\text{H}_2\text{O}]^+$ ),  $\text{C}_{20}\text{H}_{32}\text{O}$  requires  $m/z$  288.2438, 270.2357 (3,  $[\text{M}-\text{H}_2\text{O}-\text{H}_2\text{O}]^+$ ),  $\text{C}_{20}\text{H}_{30}$  requires  $m/z$  270.2348.

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