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THE BIOTRANSFORMATION OF CARVOXIME AND DIHYDROCARVOXIME WITH CELL SUSPENSION CULTURES OF *NICOTIANA TABACUM*

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tissue culture; biotransformation; hydrolysis; carvoxime, dihydrocarvoxime.

Abstract—In newly initiated cell suspension cultures of *Nicotiana tabacum*, (4*R*)-(−)- and (4*S*)-(+)-carvoximes and (1*S*,4*R*)-(+)-dihydrocarvoxime were hydrolysed to the corresponding ketones and then the resultant ketones were reduced to the corresponding alcohols.

INTRODUCTION

In our investigations concerning the dependence of the biotransformation patterns of foreign substrates on their structures [1–5], we have shown that cell suspension cultures of *Nicotiana tabacum* L. bring about the stereospecific reduction of the C=C double bond adjacent to the carbonyl group of carvone [2], whereas they bring about the regio- and stereo-selective hydroxylation of the isolated C=C double bond in the case of terpineols [3–5]. We have now examined the biotransformation of (4*R*)-(−)- and (4*S*)-(+)-carvoximes (**1a** and **1b**) and (1*S*,4*R*)-(+)-dihydrocarvoxime (**2**) in cultured cells of *N. tabacum* to elucidate the metabolic behaviour of the cells toward a molecule having the C–C double bond adjacent to a hydroxyimino group.

RESULTS AND DISCUSSION

On incubation with a suspension of tobacco cells in a similar manner to that described in ref. [2], (4*R*)-(−)- and (4*S*)-(+)-carvoximes (**1a** and **1b**) were transformed to carvone (**3**), dihydrocarvone (**4**), isodihydrocarvone (**5**), neodihydrocarveol (**6**), dihydrocarveol (**7**) and neoisodihydrocarveol (**8**)* (Table 1). However, the total yield of products was quite low and a large amount of the unchanged substrate was recovered. In spite of careful analyses of the reaction mixture by a combination of TLC and GLC, not even a trace of dihydrocarvoxime (**2**) was found. The biotransformation patterns of **1a** and **1b** were similar to those of (4*R*)-(−)- and (4*S*)-(+)-carvones (**3a** and **3b**) [2], respectively. The time-courses in the biotransformation of **1a** and **1b** with the suspension cells were followed and are shown in Fig. 1. The results suggest that product **3** is the precursor of products **4**–**8** [2].

(1*S*,4*R*)-(+)-Dihydrocarvoxime (**2**) was transformed to **4**–**7** (Table 1). This pattern was also similar to that seen in the biotransformation of dihydrocarvone (**4**) [2]. It was found that suspension cells of *N. tabacum* have the ability to hydrolyse oximes to ketones and then to reduce the ketones to the corresponding alcohols. No reduction of the C=C double bond adjacent to the hydroxyimino group of carvoxime occurred, although reduction of the C=C double bond adjacent to the carbonyl group was observed in the biotransformation of carvone [2] and pulegone†. The conjugation of a carbonyl group with the C=C double bond might be essential for reduction of the C=C double bond with suspension cells of *N. tabacum*. Thus, it was confirmed that the transformation patterns of foreign substrates in the cultured cells are determined by the functional group of the foreign substrate and the structural features around this functional group.

EXPERIMENTAL

TLC: silica gel (0.5 mm) developed with (i) EtOAc–hexane (3:7), (ii) MeOH–hexane (1:9) and (iii) C_6H_6–Me_2CO (7:3); GLC: FID, glass column (3 mm × 2 m) packed with 15% DEGS, 15% PEG and 2% OV-17 on Chromosorb W (AW-DMCS; 80–100 mesh) at 110°, 130° and 80–200° (3°/min), respectively.

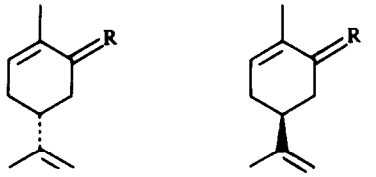
Substrates. (4*R*)-(−)-Carvoxime (**1a**), (4*S*)-(+)-carvoxime (**1b**) and (1*S*,4*R*)-(+)-dihydrocarvoxime (**2**) were prepared from (−)-carvone ($[\alpha]_D^{25}$ −60.1° (neat), d_4^{25} 0.9611), (+)-carvone ($[\alpha]_D^{25}$ +59.0° (neat), d_4^{25} 0.9592) and (+)-dihydrocarvone ($[\alpha]_D^{25}$ +13.0° (EtOH; *c* 0.6)), respectively, with $NH_2OH \cdot HCl$ and

Table 1. Biotransformation of carvoximes (**1a** and **1b**) and dihydrocarvoxime (**2**) by suspension cells of *N. tabacum*

Substrate	Yield of products (% wt of substrate)					
	3	**4**	**5**	**6**	**7**	**8**
1a	0.49	0.23	<0.01	4.9	0.59	
1b	1.6	<0.01	0 11	<0.01	0.19	0 33
2		0.26	<0.01	4.5	1 3	

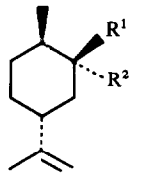
*The formulae depicted represent only one enantiomer.

†We have recently established that the C=C double bond adjacent to a carbonyl group in the s-*cis*-α,β-unsaturated carbonyl group of pulegone is stereoselectively reduced with suspension cells of *N. tabacum*, in the same manner as the s-*trans*-α,β-unsaturated carbonyl group of carvone [2].

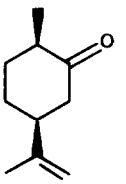


1a R = NOH
3a R = O

1b R = NOH
3b R = O



2 R^1, R^2 = NOH
4 R^1, R^2 = O
6 R^1 = OH, R^2 = H
7 R^1 = H, R^2 = OH



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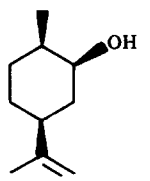

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KOAc. The physical properties of these oximes are as follows: **1a**, mp 72–73°, $[\alpha]_D^{25}$ −39.6° (EtOH; *c* 2.0) (lit. [6] −39.3°); **1b**, mp 72–73°, $[\alpha]_D^{25}$ +39.5° (EtOH; *c* 1.2) (lit. [6] +39.6°); **2**, mp 89–90°, $[\alpha]_D^{25}$ +3.5° (EtOH, *c* 0.5) (lit. [7] +2.9°).

Incubation of the oximes, **1a**, **1b** *and* **2**, *with tobacco suspension cells.* The callus tissues [1] of *N. tabacum* were transplanted to freshly prepared Murashige and Skoog's medium [8] (100 ml/300 ml conical flask) containing 2 ppm of 2,4-dichlorophenoxyacetic acid and 3% sucrose and then grown with continuous shaking for 3–4 weeks at 25° in the dark. After this time the substrate (5 mg) was added to the flask containing the suspension cells (about 50–70 g fr. wt/flask) and the cultures incubated at 25° for a further 10 days on a rotary shaker (70 rpm) in the dark.

To determine the time-courses of the biotransformations of **1a** and **1b** one flask was taken for analysis at each of the time points shown in Fig. 1.

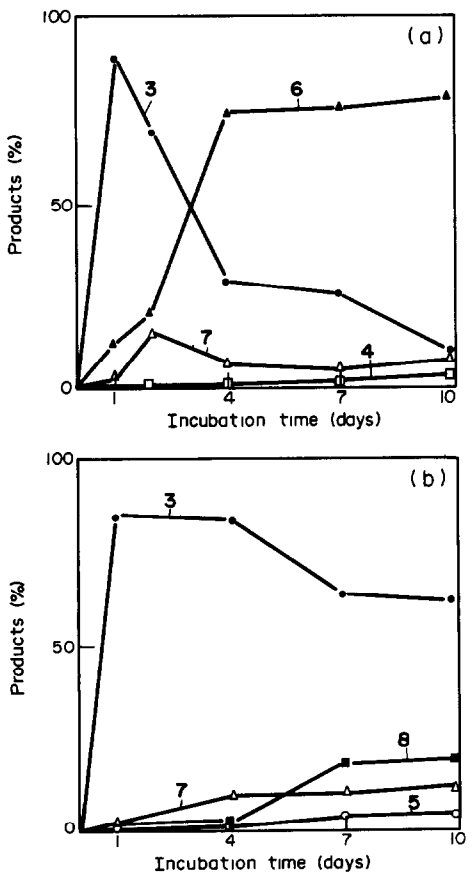

Fig. 1 The time-courses of the biotransformations of (a) (4*R*)-(−)-carvoxime (**1a**) and (b) (4*S*)-(+)-carvoxime (**1b**).

Isolation and identification of the products. The cultured mixture was worked up in a similar manner to that described in refs. [1, 2]. The products were extracted with Et_2O and identified by comparison of their TLC and GLC with those of authentic samples [2]. The amounts of the products (Table 1) were determined from the GLC trace by use of a standard curve prepared with carvone

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