Pergamon

Tetrahedron Letters 40 (1999) 6595–6598

TETRAHEDRON LETTERS

Synthesis of styrenes through the decarboxylation of *trans*-cinnamic acids by plant cell cultures

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Received 21 May 1999; revised 21 June 1999; accepted 25 June 1999

Abstract

A new method has been developed for the synthesis of styrenes through the decarboxylation of *trans*-cinnamic acids by plant cell cultures at room temperature. 4-Hydroxy-3-methoxystyrene (**2a**), 3-nitrostyrene (**2d**) and furan (**2e**) were synthesized quantitatively.

Keywords: decarboxylation; enzymes and enzyme reactions; plants; carboxylic acids and derivatives.

Decarboxylation of *trans*-cinnamic acids is the most widely used chemical method for preparing styrenes or stilbenes. A typical decarboxylation is carried out by heating under reflux at 200–300°C for 4–5 h in quinoline in the presence of a Cu powder (Y>50%). Quinoline is useful as a solvent for the decarboxylation of unsaturated acids because it is basic enough to form the required carboxylate anion and also because it boils at a temperature favorable for decarboxylation. This method, however, needs a high temperature.

On the other hand, the known decarboxylative enzymes are mainly as follows: (i) pyruvate decarboxylase;[1] (ii) oxalate decarboxylase;[2] (iii) glutamate decarboxylase;[3] (iv) benzoylformate decarboxylase;[4] (v) aconitate decarboxylase;[5] and (vi) aspartate 4-decarboxylase.[6]

In the case of *trans*-cinnamic acids, β-phenylacrylic acid was decarboxylated by *Aspergillus niger* to give styrene.[7] *Aerobacter* has been found to decarboxylate *trans*-4-hydroxycinnamic acid to the corresponding 4-hydroxystyrene.[8] However, only a few attempts for the decarboxylation of other *trans*-cinnamic acids by a decarboxylase have been reported.

Here we report a novel method for decarboxylation of *trans*-cinnamic acids (**1a–g**) by plant cell cultures to the corresponding styrenes or furan (**2a–g**). This decarboxylation takes advantage of the mild reaction conditions for preparing styrenes.

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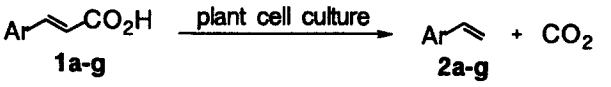
0040-4039/99/$ - see front matter
PII: S0040-4039(99)01281-2

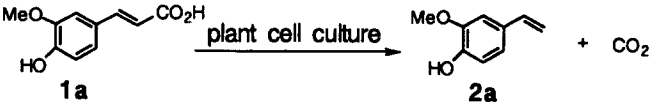


1a-g **2a-g**

a; Ar = 4-hydroxy-3-methoxyphenyl b; Ar = phenyl
c; Ar = 4-hydroxyphenyl d; Ar = 3-nitrophenyl
e; Ar = 2-furyl f; Ar = 4-methoxyphenyl
g; Ar = 4-chlorophenyl

When *trans*-ferulic acid (**1a**) was subjected to plant cell culture in a medium, 4-hydroxy-3-methoxystyrene (**2a**)[9] was given quantitatively as shown in Table 1. The decarboxylative reaction was performed by two methods, that is: (A) with freely suspended plant cells in the stationary phase after 10 days of incubation (10 g of cells in 20 ml of a medium); and (B) with homogenized plant cell culture in 0.1 M phosphate buffer solution (pH 6.4). In the case of *Catharanthus roseus*, **1a** was quantitatively decarboxylated to **2a** not only with method (A), but also with (B) (entries 1, 2). But in the cases of *Nicotiana tabacum* and *Daucus carota*, the decarboxylation proceeded with only method (B) (entries 4, 6).

Table 1
Decarboxylation of *trans*-ferulic acid (**1a**) with plant cell culture



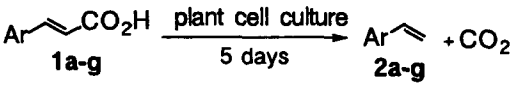
1a **2a**

Entry	Plant cell culture	Method	Time (day)	Product **2a** C.Y. (%)	Recovery **1a** C.Y. (%)
1	*C. roseus*	A	5	quant.	0
2	*C. roseus*	B	3	quant.	0
3	*N. tabacum*	A	5	trace	84
4	*N. tabacum*	B	5	quant.	0
5	*D. carota*	A	5	0	100
6	*D. carota*	B	5	30	65
7	*C. sinensis*	A	5	0	100
8	*C. sinensis*	B	5	0	100

Next we tried the decarboxylation of other *trans*-cinnamic acids (**1b–g**) using method (B) as shown in Table 2. The decarboxylation of **1d** and **1e** with *Camellia sinensis* gave 3-nitrostyrene **2d** and furan **2e** quantitatively (entries 11, 12). In the case of **1c**, **2c** was given in 30–32% yield by *C. roseus* or *D. carota* (entries 7, 9). In the case of **1b**, **1f** and **1g**, the corresponding products **2b**, **2f** and **2g** were given in low chemical yields (entries 4, 13, 16). These styrenes (**2b**,[10] **2d**,[11] **2f**[11] and **2g**[11]) and furan **2e**[12] were chemically synthesized by the decarboxylation of *trans*-cinnamic acids (**2b**, **2d–g**) in the presence of a copper powder in quinoline at 185–195°C for 2–4 h (Y>50%). A major advantage of our method is that the decarboxylation with plant cell culture proceeds mildly at room temperature. Studies are now in progress to shorten the reaction time.

For a typical experiment, we used suspension-cultured cells which had originally been isolated from *C. roseus*, *N. tabacum* 'Bright Yellow-2', *D. carota*, and *C. sinensis* as described in our previous papers.[13–15]

Table 2
Decarboxylation of cinnamic acids (**1a–g**) with plant cell cultures



Entry	Substrate	Plant cell culture	Product **2a-g** C.Y. (%)	Recovery **2a-g** C.Y. (%)
1	**1a**	*C. roseus*	quant.	0
2		*N. tabacum*	quant.	0
3		*D. carota*	30	65
4	**1b**	*C. roseus*	10	80
5		*N. tabacum*	trace	86
6		*D. carota*	trace	85
7	**1c**	*C. roseus*	30	63
8		*N. tabacum*	5	84
9		*D. carota* (root [a])	32	55
10		*C. sinensis*	trace	83
11	**1d**	*C. sinensis*	quant.	0
12	**1e**	*C. sinensis*	quant.	0
13	**1f**	*C. sinensis*	10	76
14		*D. carota* (seed [b])	trace	85
15		*D. carota* (root)	trace	87
16	**1g**	*D. carota* (seed)	10	78
17		*C. sinensis*	trace	82

a) *D. carota* cell line derived from root.
b) *D. carota* cells derived from seedling.

In the case of method (A), a substrate (50 mg) was added to the freely suspended *C. roseus* (B-5 medium pH 5.5), *N. tabacum* 'Bright Yellow-2' (MS medium, pH 5.8), *D. carota* (MS medium, pH 5.8), and *C. sinensis* (B-5 medium, pH 5.8). The mixture was shaken at 25°C on a rotary shaker (110 rpm) in the dark. At the conclusion of the reaction, the incubation mixture was filtered, the filtered cells were washed with CH_2Cl_2, and the filtrates were combined. The combined mixture was extracted with CH_2Cl_2. The organic layer was dried over anhydrous $MgSO_4$ and subjected to column chromatography. In the case of method (B), 10 g of plant cells were homogenized in 10 ml 0.1 M phosphate buffer (pH 6.4). A substrate (50 mg) was added to the homogenate. The subsequent procedure was the same as for method (A).

Acknowledgements

This work was supported in part by the Research Foundation for Pharmaceutical Sciences.

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