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Hydroxylation of benzylic and allylic sites by plant cultured suspension cells

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Abstract—A novel transformation of α-substituted benzylcyanides by hydroxylation of benzyl sites using cultured cells of cotton, *Gossypium hirsutum*, producing alkano- or benzophenones via presumably α-cyanohydrin is demonstrated. Also regioselective hydroxylation of 4*S*-(−)-perillyl alcohol and 3*S*-(−)-citronellol by cultured suspension cells of *Catharanthus roseus* is reported.

Many investigators have studied biotransformation of organic compounds by plant cultured suspension cells, reporting selective and/or specific conversion ability including enantioselective oxidation, stereoselective reduction, regioselective hydroxylation, enantioselective glycosylation, esterification, methylation, and isomerization.[1,2] In practice, the ability of plant cultured cells to transform foreign substrates into potentially useful substances is of great interest, as products may be formed which are difficult to prepare by synthetic chemical methods. Hitherto we have studied the hydroxylation ability for foreign substrates such as testosterone,[3] carvone,[4] geraniol,[4] and piperitone[5] by plant cultured suspension cells. Our interest is now focused on hydroxylation of α-positions of nitriles due to the high synthetic utility of α-cyanohydrins,[6] and at allylic position of 4*S*-(−)-perillyl alcohol and 3*S*-(−)-citronellol. We now report a novel transformation of α-substituted benzylcyanides by hydroxylation of benzyl sites by the plant cultured suspension cells of cotton, *Gossypium hirsutum*, into alkano- or benzophenones via presumably an α-cyanohydrin intermediate and regioselective hydroxylation of allylic positions by cultured suspension cells of periwinkle, *Catharanthus roseus*.

The cotton and periwinkle cultured suspension cells used were prepared as described in our previous papers and feeding and incubation experiments were carried out in a manner similar to that reported in Refs. 3–5. Just prior to use for this work, a part of the callus tissues (fresh weight 50 g) was transplanted to freshly prepared Murashige and Skoog's medium (100 mL in a 300 mL conical flask, pH 5.8) containing 2 ppm of 2,4-dichlorophenoxyacetic acid and 3% sucrose and grown with continuous shaking for 1 week at 25°C in the light (2000 lux). The substrate (20 mg/flask) was added to the suspension cultures and the cultures were incubated at 25°C for 3–5 days on a rotary shaker (120 rpm) in the light.

The incubation mixture was filtered and the culture medium was extracted with EtOAc. Then, transformation products were isolated by preparative TLC and were identified using ^{1}H and ^{13}C NMR, GC/MS, and IR. We began with the hydroxylation of 1-(4-methoxybenzo)cyclobutenecarbonitrile (**1**) with cultured suspension cells of cotton, which produced the benzoketone **2** in 23.1% yield instead of the predicted cyanohydrin **3** of **1**. Interestingly, ketone **2** was known as a key intermediate in the synthesis of 14α-hydroxyestrone.[7] In order to obtain **2** via **3**, so far, attempts to hydroxylyze **1**

Table 1. Biotransformation of benzylcyanides

Substrate	Product	Yield[a] (%)
1	**2**	23.1
4	**7**	37.5
5	**8**	9.1
6	**9**	58.8

[a] Weight (%) of product relative to the substrate administered.

Keywords: regioselective hydroxylation; α-cyanohydrin; (−)-perillyl alcohol; cotton; *Catharanthus roseus*; biocatalyst.
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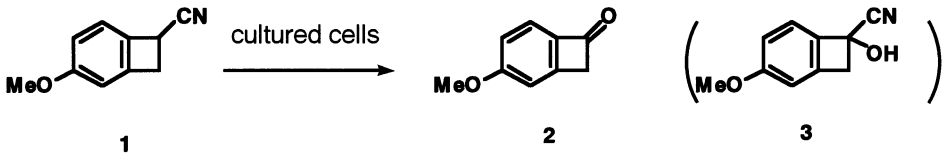
using chemical methods remain unsuccessful. Accordingly, the synthesis of **2** required a four-step sequence from **1** via another route.[8]

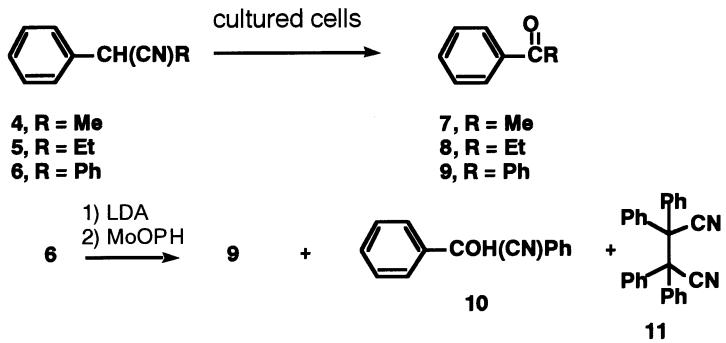


1 **2** **3**

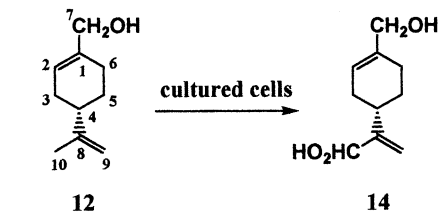
Therefore, the above procedure resulted in a new one-step synthesis of **2**. In a similar manner, the α-alkyl substituted benzylcyanides **4** and **5** were converted into the corresponding ketones **7** and **8**, respectively (Table 1). As shown in Table 1, the yield of **8** was very low in the case of the biotransformation of **5** and substrate **5** was recovered unchanged from the medium. Although it was known that the hydroxylation of diphenylacetonitrile **6** with molybdenum peroxide MoO_2-Py-HMPA (MoOPH) using lithium diisopropylamide as a base provided a mixture of a cyanohydrin **10**, benzophenone (**9**), and tetraphenylsuccinonitrile (**11**),[9] treatment of **6** with cotton cultured suspension cells interestingly gave only benzophenone (**9**) (Table 1).

In the case of the biotransformation of **13** after 24 h incubation **13** was converted to 8- and 10-hydroxy citronellol (**15** and **16**) in 13.0 and 50.0% yield, respectively. These results indicate that the cultured suspension cells of *C. roseus* regioselectively hydroxylate the allylic position of 4*S*-(−)-perillyl alcohol and 3*S*-(−)-citronellol in good yields.

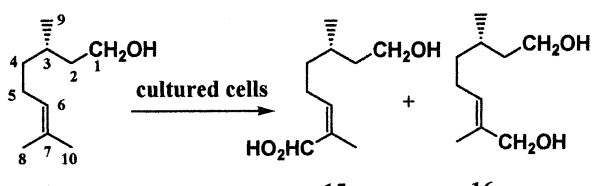
In summary, it was found that the cultured suspension cells of cotton selectively converted benzylnitriles to the corresponding ketones in moderate yields. This procedure is a simple operation and is environmentally friendly. Also, the cultured suspension cells of *C. roseus* have the ability of regioselective hydroxylation. Furthermore, substrate specificity and generality of this reaction are in progress and we are now using plant cultured suspension cells in organic synthesis as biocatalysts.



We also studied the biotransformation of 4*S*-(−)-perillyl alcohol (**12**) and 3*S*-(−)-citronellol (**13**) by cultured suspension cells of *C. roseus* and the results are shown in Table 2. After 48 h incubation **12** was converted to 10-hydroxy perillyl alcohol (**14**) in 72.0% yield.



12 **14**



13 **15** **16**

Table 2. Biotransformation of monoterpens

Substrate	Product	Yield[a] (%)
12	**14**	72.0
13	**15**	13.0
	16	50.0

[a] Weight (%) of product relative to the substrate administered.

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