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BIOTRANSFORMATION OF A SYNTHETIC COMPOUND, 1,5-DIPHENYLSULPHINYL-3-METHYL-3-NITROPENTANE, BY CELL SUSPENSIONS OF *CATHARANTHUS ROSEUS*

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Key Word Index—*Catharanthus roseus*; Apocynaceae; cell suspension cultures; biotransformation; oxidation; sulphoxide.

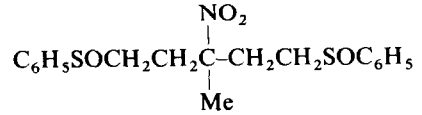
Abstract—Biotransformation of a synthetic substrate, 1,5-diphenylsulphinyl-3-methyl-3-nitropentane, by cell suspension cultures of *Catharanthus roseus* was investigated. It was found that this substrate was (i) incorporated into cells and (ii) converted within three days into a new product, 1-phenylsulphonyl-5-phenylsulphinyl-3-methyl-3-nitropentane through a regioselective oxidizing process not previously described. This original organic substrate used for bioconversion is entirely synthetic and the functions (nitro, sulphoxide) are rarely involved in biotransformation studies. Such a biotechnological process could be of great interest for the production of new chemical compounds.

INTRODUCTION

The goal of producing useful products by employing cultured plant cells to effect specific biotransformations represents a potentially important application of plant cell biotechnology. Since the reviews by Furuya in 1978 [1] and Reinhard and Alfermann in 1980 [2], numerous biotransformations by various plant cell cultures have been described: hydroxylation [3–5], oxidation [3, 6, 7], reduction [8, 9], acetylation [10], deacetylation [3], methylation [11], *O*-glucosylation [3, 11–14], deglucosylation [3], *C*-glucosylation [11], transglucosylation [15, 16], glucuronylation [11]. Substrates added to cell suspension cultures were often natural compounds, and usually common to the plant species chosen for the experiments. More rarely, synthetic compounds were added to cell suspension cultures [17–20]. This last biotechnological process could be a new original approach for producing chemical compounds by hemisynthesis.

As part of the study [21] of the scope of biotechnological process [22, 23] and oxidation [24] using *Catharanthus roseus* cell cultures, it seems of interest to investigate biotransformation by cell plants, of 1,5-diphenylsulphinyl-3-methyl-3-nitropentane (**1**) [25] which is an entirely synthetic product possessing aliphatic nitro group and is known to be devoid of toxicity to animal cells. Even though nitro groups are present in plants, phenylsulphoxide is quite unknown in nature but very useful in organic synthesis.

The present paper reports the first results of specific and regioselective oxidation of **1**, by cell suspension cultures of *Catharanthus roseus* G. Don (strain C20).



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RESULTS AND DISCUSSION

In order to investigate the toxicity of **1** towards cell suspension cultures of *Catharanthus roseus* G. Don, cells were inoculated in the liquid medium containing various concentrations: 0, 30, 50, 100, 200, 500 and 1000 mg/l. of **1**. After incubation for seven days (Fig. 1), the negative effect of **1** on the fresh weight of the cells was evident when more than 50 mg/l had been added to culture medium. This result was confirmed by microscopic examination of cells: by comparison with suspension cultures without **1**, the dead cell ratio did not increase, except for cultures containing more than 50 mg/l of **1**. So, in further experiments, we added 30 mg/l (79 mM) of **1** into cell suspension cultures of *Catharanthus roseus*. Morever, addition of DMSO to the cell cultures did not influence cell growth.

In an attempt to study the aptitude of the cells to metabolize **1**, several experiments were simultaneously carried out: cell suspension cultures of *Catharanthus roseus* with **1** dissolved in DMSO, cell suspension cultures of *Catharanthus roseus* without **1** (control culture), liquid medium with **1** dissolved in DMSO, but without cells, cell suspension cultures of *Catharanthus roseus* with

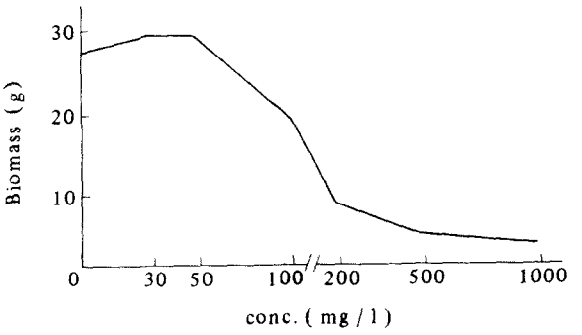


Fig. 1. Toxicity of 1,5-diphenylsulphinyl-3-methyl-3-nitropentane (1) towards *Catharanthus roseus* cell suspensions (strain C20) after seven days.

DMSO. After an incubation time of seven days, both in darkness and in the light, cells and liquid medium were analysed separately.

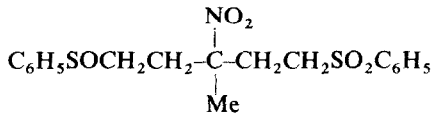
Product **1** remained unchanged after seven days of incubation, when it was added to culture medium without cells. However, **1** was partially metabolized when it was added to cell suspension cultures; three spots were detected in TLC: **1** (R_f=0.20), **2** (R_f=0.42) and **3** (R_f=0.61) both from cells and culture medium, indicating that **1** has been absorbed by the cells and converted into products **2** and **3**. No difference could be noted between experiments performed in darkness and in the light.

The time-course study of the biotransformation of **1** showed that the product **2** was detected from the first day of the experiment, both from the cells and the culture medium, its yield increasing up to day three and remaining unchanged until day seven. Throughout the study, the amount of **2** was higher in the medium than in the cells. Product **3** formed more slowly and was clearly detected only after day six, principally in the medium.

In order to determine the structure of **2**, 192 mg of **1** were added to culture medium of the suspension cultures of *Catharanthus roseus* (density of cells and concentration of **1** being similar to those used in previous experiments). The culture was stopped after three days. Preparative TLC of a purified methylene chloride extract from suspensions cultures afforded 75 mg of starting product **1** and 20 mg of compound **2** (conversion =21%).

EIMS spectra of **2** showed a peak at m/z=396 (MH^+) and gave characteristic fragments of functional phenylsulphonyl group at m/z=143 ($C_6H_5SO_2H_2^+$, 100%), 142 ($C_6H_5SO_2H^+$) and 141 ($C_6H_5SO_2^+$), of the nitro group at 349 [M−46] and of phenylsulphinyl group at 126 ($C_6H_5SOH^+$), 125 ($C_6H_5SO^+$) and 109 ($C_6H_5S^+$). ^{1}H NMR of compound **2** indicated the presence of five aromatic protons very differentiated at δ7.83 (AB, J=7, 2H *ortho*), δ7.63 (*t*, 1H *para*) and 7.55 (*t*, 2H *meta*).

On the basis of these chemical (C, H, N analysis) and spectroscopic data, conversion product **2** was concluded to be 1-phenylsulphonyl-5-phenylsulphinyl-3-methyl-3-nitropentane.



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In conclusion, a regioselective enzymatic oxidizing activity has been detected in *Catharanthus roseus* (strain C20) cell suspensions. If oxidation reactions had been previously shown in *Catharanthus roseus* (L.) G. Don [24] no sulphur containing metabolites had been detected until now in the plant, making the regioselective bioconversion observed in the related cell suspension cultures somewhat unexpected. So it can be anticipated that other plant cell cultures (other strains of *Catharanthus roseus* or other plant species) will lead to different new bioconversions useful in producing chemical compounds by hemisynthesis.

EXPERIMENTAL

Culture methods. The cell suspension cultures of *Catharanthus roseus* G. Don (strain C20) were cultivated in Gamborg's B5 medium [26] with 2,4 D (10^{-4} M), kinetin (10^{-4} M) and sucrose (20 g/l). They were subcultured every 7 days into 250 ml conical flasks (5 ml of cell suspension into 45 ml of fresh medium) on a giratory shaker (100 rpm) at 25° under 16 hr photoperiod (2500 erg/cm^2/sec). Cell fr. wt was determinated by weighing the cells separated from culture medium by filtration through nylon cloth (diameter 50 μm). Dead cell ratio was determined by erythrosine coloration.

The standard protocol for the bioconversion experiments was as follows: 1.5 mg of **1** dissolved in 50 ml of DMSO were added into 50 ml of cell suspension culture (i.e. 30 mg/l) at the time of subculture and incubated for 7 days. In order to isolate conversion product **2** for identification, 192 mg of **1** were added to 6.4 l of culture medium distributed in six 400 ml and four 1 l conical flasks, the density of cells and the concentration of **1** being similar to those used in previous experiments.

Extraction and purification procedure. After filtration of cell suspension cultures, cells were crushed with fine sand in a mortar and extracted twice with CH_2Cl_2 for 30 min at ambient temp. Also, the culture medium was extracted ×3 by agitation with CH_2Cl_2 vol. for vol. at ambient temp. After concn of the combined organic layers the residue was analysed by silica gel TLC (solvent, EtOAc); spots (**1** R_f=0.20, **2** R_f=0.42 and **3** R_f=0.61) were detected by UV light (254 nm). In order to isolate conversion product **2**, the CH_2Cl_2 residues from 6.4 l of cell suspension cultures were taken up in 100 ml of CH_2Cl_2 and filtered through celite. The filtrate was dried ($MgSO_4$) and freed from solvent under red. pres. Prep. TLC (silica gel 60F-254, Merck Art. 5717, eluent Et_2O) of the residue (120 mg) afforded 75 mg of **1** and 20 mg of **2** (conversion: 21%).

General. NMR spectra were determined at 400 MHz with TMS as int. standard. EIMS were taken with a Kratos MS50 (70 eV, 8 kV). All analyses (C, H and N) were within ±0.3% of the calcd. values.

1,5-(Diphenylsulphinyl)-3-methyl-3-nitropentane (**1**). Prepared according to ref. [25]: $C_{18}H_{21}NO_4S_2$=379.48, analysis C, H, N. mp: 110–113°; ^{1}H NMR ($CDCl_3$, δ): 1.45 (*s*, 3H, Me), 2.30 (*m*, 4H, $2CH_2$–CH_2–C); 2.75 (*m*, 4H, $2CH_2SO$), 7.50 (*s*, 10H, aro.); MS *m/z*: 380 (MH^+), 333, 254 (100%), 223, 218, 207, 126, 125, 107, 81, 78, 77.

1-Phenylsulphonyl-5-phenylsulphinyl-3-methyl-3-nitropentane (**2**). $C_{18}H_{21}NO_5S_2$=395.35, analysis C, H, N. Amorphous solid; ^{1}H NMR ($CDCl_3$, δ): 1.46 (*s*, 3H, Me), 2.26 (*m*, 4H, $2CH_2$–CH_2–C), 2.75 (*m*, 2H, CH_2SO), 2.98 (*m*, CH_2SO_2), 7.50 (*s*, 5H, aro.), 7.52 (*t*, 2H, aro.), 7.63 (*t*, 1H, aro.), 7.80 (*d*, 2H, aro.); MS *m/z*: 396 (MH^+), 349, 270, 240, 239, 223, 218, 172, 143 (100%), 142, 141, 126, 125, 109, 99, 97, 81, 78, 77.

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