Note

The Conversion of Deoxypodophyllotoxin to Epipodophyllotoxin in Resting Cell System of *Penicillium* F-0543[†]

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Podophyllotoxin-related compounds, naturally occurring lignan lactone, showing specifically inhibit mitosis^{1~6)} and have significant antitumor activities.^{7~11)} Among them, etoposide (VP-16-213), a podophyllotoxin glycoside derivative, is used in the treatment of lung cancer, *etc.*^{12~19)} because of its selective toxicity. Therefore, it is worthwhile to obtain epipodophyllotoxin (EPT) which is the basic skeleton in etoposide in good yield. We have microbially transformed deoxypodophyllotoxin (DPT), a major constituent (2.4%) of the seeds of *Hernandia ovigera* L. to EPT, and found that *Penicillium* F-0543 (unidentified) converted DPT to EPT in 100% yield.^{20,21)} These microbial transformation may be useful for the mass-production of etoposide. Furthermore, our attempt has been focused on a simple conversion of DPT to EPT. Immobilized mycelium is very effective for obtaining the object in an enzyme reaction in good yield. Thus,

as a preliminary step to immobilize the mycelium, the conversion of DPT to EPT was examined in a resting cell system of *Penicillium* F-0543. As a result, non-proliferating mycelium of *Penicillium* F-0543 was shown to convert DPT to EPT in 100% yield (Fig. 1).

This fungus was grown in modified *Corticium* medium²²⁾ (1 l in a 5 l flask) at 28 °C in a reciprocal shaker (120 rpm, amplitude, 7 cm). After 2 days of incubation, the mycelium was harvested by centrifugation at 3000 rpm for five minutes and washed several times with phosphate buffer (m/5 NaH₂PO₄-Na₂HPO₄, pH 7.0). Five ml of mycelium suspension in phosphate buffer (m/5 NaH₂-Na₂HPO₄, pH 7.0) was placed in two hundred and twelve test tubes (18 mm × 20 cm). DPT (1 mg, 0.25 mmol) in ethanol (0.1 ml) was added to these test tubes and shaking was continued for 7 days. The filtrate of the mycelium suspension was then extracted with ethyl acetate (100 ml × 3), and the extract was evaporated to dryness under reduced pressure. The residue (210 mg) was chromatographed on a silica gel (20 g) column, developed with hexane-ethyl acetate (1:1). The homogenous fractions on thin layer chromatogram were combined and recrystallized from H₂O-EtOH to give the product (1) as colorless needles, mp 160~162 °C (170 mg), $[\alpha]_D^{25}$ -60.0° (*c*=0.5, CHCl₃). The *R_f*-value on thin layer chromatogram, optical rotation, ¹H-NMR, IR and MS spectra of an authentic sample [reference value: $[\alpha]_D^{25}$ -69.0° (*c*=0.5, CHCl₃)] of EPT.²³⁾ Therefore, the product (1) was identified as EPT.

Next, the mycelium (wet, 40 g) obtained by the filtration of the mycelium suspension was extracted with methanol (100 ml × 3) and the extract was evaporated to dryness under reduced pressure. The residue (288 mg) was extracted with hexane-ethyl acetate (1:1) to yield EPT (60 mg). The sum of EPT indicate that this conversion in resting cell system proceeded in 100% yield.

From these results, the conversion of DPT to EPT was also found to be achieved by a resting cell system of *Penicillium* F-0543. This conversion in a resting cell system is noteworthy from the following four points; 1) the

Fig. 1. Conversion of Deoxypodophyllotoxin to Epipodophyllotoxin in Resting Cell System of *Penicillium* F-0543.

[†] Microbial Transformation of Antitumor Substance, Deoxypodophyllotoxin. Part III.

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conversion of lignan lactone with severe cytotoxicity such as DPT is reported for the first time in this paper, 2) this enzyme reaction has not been inhibited by DPT having strong toxicity, 3) it seems to lead to the immobilization of the mycelium and 4) we are hopeful it can be a method for the mass-production of etoposide.

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