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# A NOVEL ENZYMATIC DEHYDROGENATION OF PODOPHYLLOTOXIN CONGENERS BY YEAST CELLS

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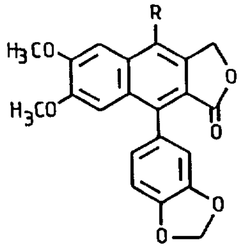
**Abstract:** The biotransformation of aryltetralin lignans to arylnaphthalene lignans in presence of yeast is described. Podophyllotoxone, an oxidation product of podophyllotoxin on incubation with yeast from different sources produced dehydropodophyllotoxin, an important representative of arylnaphthalene lignans. © 1997 Elsevier Science Ltd. All rights reserved.

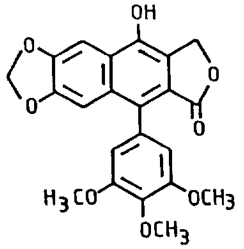
The aryltetralin lignan lactones isolated from various species of *Podophyllum* (May Apple, American Mandrake) have been the subject of much chemical and biochemical study[1-3] due to their widespread occurance in nature and broad range of biological activities.[4,5] The majority of natural arylnaphthalenes are lactones which represent a significant subclass of lignans.[6] 1-Arylnaphthalene lignan lactones, for example, diphyllin(**1**), justicidinB(**2**) and dehydropodophyllotoxin(**3**) have attracted considerable interest with the discovery of their intriguing biological activities such as antitumour and antihyperlipidimic activities.[7] Deoxypodophyllotoxin(**4**) and podophyllotoxin(**5**) are two well known naturally occuring aryltetralin lignans. Both compounds are cytotoxic,[8] and their derivatives have potential clinical use as antitumour agents. Etoposide (**6**)[9,10] and teniposide(**7**)[10b,10d] are derivatives of podophyllotoxin which are currently being used for treating variety of tumours including small cell lung carcinoma, testicular cancer, and malignant lymphoma. One of the proposed mechanism of action of these drugs is the inhibition of catalytic activity of DNA topoisomerase II and concurrent enzyme-mediated production of lethal DNA strand breaks leading to DNA damage and cytotoxicity.[11] In contrast, podophyllotoxin itself and some of its congeners act by the mechanism of inhibition of microtuble polymerization.[9]

In the light of our recent exploratory investigations of biocatalytic reactions,[12] we became interested in biotransformations that would produce new lignan derivatives. Consequently, we probed the biotransformations of podophyllotoxone(**8a**) and its stereoisomer, picropodophyllone (**8c**). In this paper, we report a novel enzymatic dehydrogenation of aryltetralin lactone lignans by yeast cells.

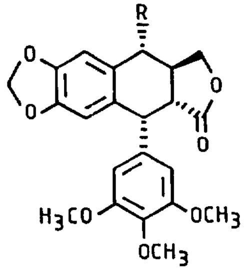


**1** R = OH , Diphyllin

**2** R = H , Justicidin B

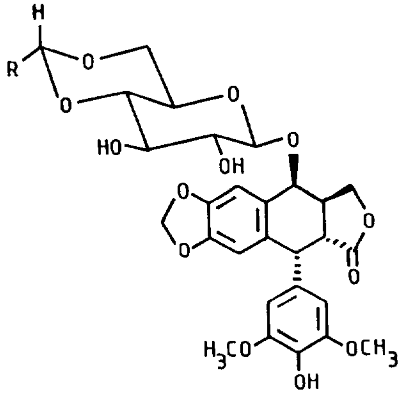


**3** Dehydropodophyllotoxin

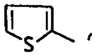


**4** R = H , Deoxypodophyllotoxin

**5** R = OH , Podophyllotoxin

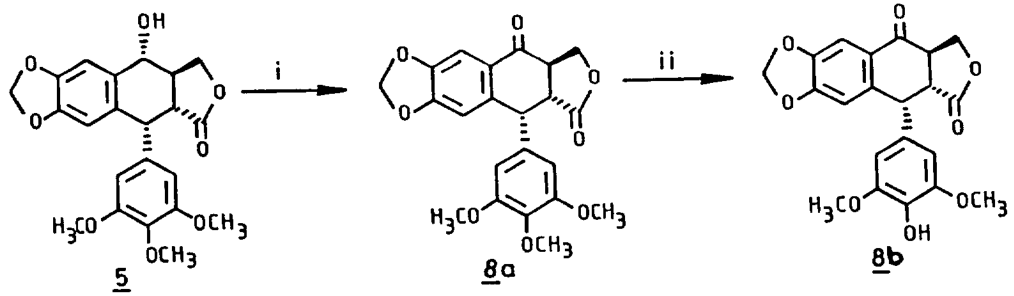


**6** R = $CH_3$ , Etoposide

**7** R =  , Teniposide

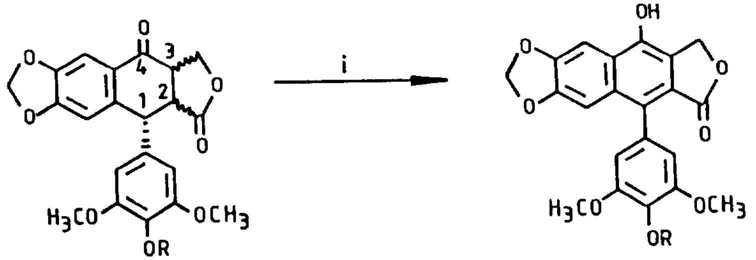
Podophyllotoxone(**8a**), an oxidation product of podophyllotoxin, is a naturally occuring compound of *podophyllum*[13] species. It can also be synthesized by the oxidation of podophyllotoxin (**5**) with freshly prepared pyridinium chlorochromate (PCC) or manganese dioxide[14] in 68% yield (Scheme 1). The chemical reduction of **8a** with sodium borohydride ($NaBH_4$) in methanol gave picropodophyllotoxin instead of the regeneration of podophyllotoxin. It is reported in the literature that $NaBH_4$ when used in excess often causes the opening of the lactone ring thus resulting in a mixture of compounds wherein picropodophyllin is formed as one of the minor products[15,16]. However, in our study, the equimolar addition of $NaBH_4$ at 0°C exclusively produced picropodophyllin from **8a** in about 98% yield. This prompted us to explore the microbial reduction of **8a** with a view to produce podophyllotoxin selectively. Whereas the incubation of **8a** in the presence of baker's yeast gave a C-ring aromatized product, dehydropodophyllotoxin(**3**), an important arylnaphthalene lignan. This was possibly because of the enzymatic dehydrogenation at the 1,2-position of the C-ring which further enolized spontaneously to produce a complete

## Scheme 1



i) PCC or $MnO_2$/DCM, RT, 6hrs ; ii) HBr / 0°C, 15 min.

## Scheme 2



8a-c

a, R = $CH_3$(2α,3β)

b, R = H (2α,3β)

c, R = $CH_3$(2β,3β)

3a,b

a, R = $CH_3$

b, R = H

i) Yeast / EtOH/$H_2O$

aromatic system (Scheme 2). Attempts were carried out to dehydrogenate **5** by yeast. However, no biotransformation was observed in this case as dehydrogenation alone cannot produce aromatization of C-ring. Therefore, the driving force of this yeast-mediated dehydrogenation may be due to stabilization of the entire ring system by the aromatization of the C-ring.

In the beginning, the yeast-mediated biotransformations were carried out by using dextrose (1gm/1gm of yeast) as an energy source for the yeast and found that the product was dehydropodophyllotoxin instead of the expected podophyllotoxin. The reaction time was almost 3 weeks. Once it was realized that the product was not a simple carbonyl reduced compound, these transformations were performed in the absence of sugar and obtained the same product. Infact, under these conditions the dehyrdogenation process was complete in less than half the time as compared to the time required in the presence of sugar. Initially, this unusual biocatalytic dehydrogenation was observed by baker's yeast (*Saccharomyces cerevisiae*) which prompted us to examine the yeast from other sources as well such as *Candida pseudotropicalis* and *Candida* *utilis*. In case of *C. pseudotropicalis* and *S. cerevisiae*, this bioconversion was complete in one and two weeks, and the yields were 85 and 75% respectively. In case of *C. utilis* the conversion was negligible. In addition, it was observed that this bioconversion was facilitated by the addition of $NADP^+$, however, there was no transformation with $NADP^+$ alone.

In a typical procedure, to a preincubated suspension of yeast (2 g) in tap water (20 ml) was added the podophyllotoxone (50mg, 0.125mmol) dissolved in 50% aqueous ethanol (3 ml). The overall ethanol concentration should not be more than 10%. The reaction mixture was incubated at 37°C in an orbital shaker and the conversion was monitored by TLC. Upon completion of the reaction, it was extracted with ethylacetate. The combined organic layer was concentrated to dryness and the residue was purified on a silica column using $CHCl_3$:MeOH (9:1) as eluent to afford the pure product in quantitative yields. The product was identified as dehydropodophyllotoxin by spectral analysis[17] and by comparision to authentic sample.

The scope of this biotransformation was investigated using substrates like 4'-demethylpodophyllotoxone[18](**8b**) and picropodophyllone[19](**8c**). These also underwent yeast-mediated dehydrogenation and no carbonyl reduced products were obtained. In these substrates also, *C. pseudotropicalis* gave the best results among the three species of yeast examined.

This investigation also gives some insight into the biosynthesis of such lignans. There has been continued pursuit of biosynthetic studies for these biologically important podophyllotoxin family of natural products.[20] A wide range of aryltetralin lactone lignans like podophyllotoxin, 4'-demethylpodophyllotoxin, 4'-demethyldesoxypodophyllotoxin, podophyllotoxone and also arylnaphthalene lignans like dehydropodophyllotoxin[21] have been isolated from the same podophyllin resin of *Podophyllum* species. A recent study has shown that dibenzylbutyrolactone lignan, matairesinol as precursor of aryltetralin lactone lignans.[22] Arylnaphthalene lignans like dehydropodophyllotoxin and diphyllin have been chemically synthesized by oxidation of aryltetralin lignans and it appears that a similar sequence occurs in nature.[23] Our present finding about the biooxidative transformation of podophyllotoxone by yeast closely resembles this hypothesis.

In conclusion, we have demonstrated a new and potential application of yeast in the dehydrogenation of *Podophyllum* lignans. This biotransformation by yeast could be a viable methodology for the preparation of different arylnaphthalene lactone lignans in large quantities. Further, this could be a facile and concise route for the synthesis of these lignans starting from naturally occuring aryltetralin lignans.

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17. **Dehydropodophyllotoxin(4a):** m.p.: 265-268°C. $^1$HNMR ($CDCl_3$ + DMSO - $d_6$) (200MHz):$\delta$3.8(s,6H,3&5$OC\underline{H}_3$),3.9(s,3H,4'-$OC\underline{H}_3$),5.35(s,2H,Ar-$C\underline{H}_2$), 6.09 (s,2H, -$OC\underline{H}_2O$-),6.5(s,2H, Ar-$\underline{H}$),6.95(s,1H, Ar-$\underline{H}$),7.65(s,1H, Ar-$\underline{H}$),9.9(bs,1H, Ar-$O\underline{H}$). Mass m/e: 410 ($M^+$ 100%).

18. Preparation of **4'-demethyl podophyllotoxone(8b):** HBr gas was passed through a solution of podophyllotoxone (100 mg, 0.25 mmol) in dry DCM (20ml) for 10 minutes at 0°C. Then the reaction mixture was allowed to stir at 0°C for 30 minutes and the solvent was evaporated under reduced pressure at room temperature. The residue was taken in ethylacetate and washed with sodiumthiosulphate solution. The organic layer was dried ($MgSO_4$) and concentrated to dryness to afford dark coloured viscous material which upon purification on a silica column (EtOAc : hexane 2:1) resulted a pale yellow coloured crystals. Yield : 65%. $^1$HNMR($CDCl_3$): $\delta$3.5-3.6(m,2H),3.75(s,6H),3.85(s,1H),4.4-4.6(m,2H),5.4-5.6(bs,1H),6.05(d,2H,$J_{H\text{-}H}$=2Hz),6.25(s,2H),6.65(s,1H),7.4(s,1H). Mass m/e 398($M^+$).

19. Picropodophyllone is prepared from picropodophyllin by the oxidation with freshly prepared PCC.[14]

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