ALKALOID PRODUCTION IN CATHARANTHUS ROSEUS CELL CULTURES. IX.[1] BIOTRANSFORMATION STUDIES WITH 3',4'-DEHYDROVINBLASTINE.

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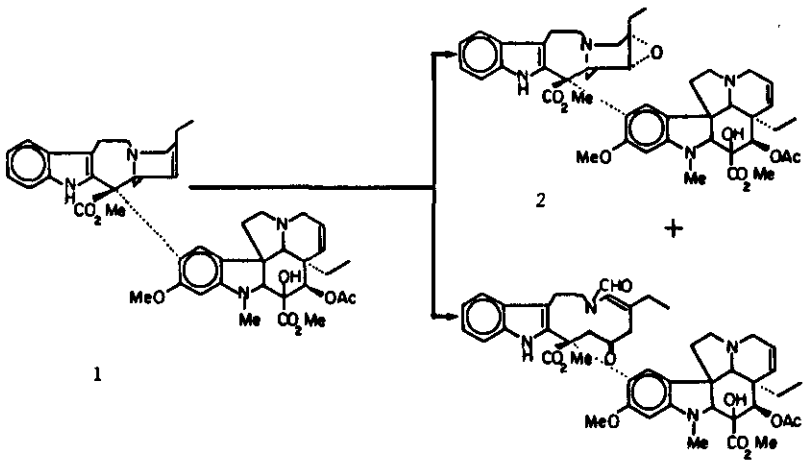
Abstract - The biotransformation of 3',4'-dehydrovinblastine by suspension cultures of the "916" cell line from Catharanthus roseus is reported. It is shown that leurosine, catharine and several other bisindole alkaloids are the products formed.

In previous publications[1-8] from our laboratories involving studies with Catharanthus roseus cell cultures we have described the production of various types of alkaloids within the Corynanthé, Strychnos, Aspidosperma and Iboga families. It was shown that serially cultured callus and cell suspension cultures derived from highly uniform explants (anthers of buds identical in developmental stage) can produce a different spectrum of alkaloids and with optimization of growth parameters etc these lines could be utilized for the production of such alkaloids. Another avenue of potential interest with such culture lines involves biotransformation of appropriate substrates introduced into the culture medium at different stages of culture growth etc. Studies involving the transformation of various functional groups of organic compounds by plant cell cultures have been reported[9]. We would now like to present the first observations with a C. roseus cell line and the synthetic bisindole intermediate, 3',4'-dehydrovinblastine (1). 3',4'-Dehydrovinblastine (1), available from the coupling of vindoline and catharanthine[10] was selected as the first substrate in our biotransformation studies since its chemistry, in view of our previous investigations, was well understood. Of the various cell lines of C. roseus which could have been selected from our program we chose a line coded as "916". This cell line was somewhat unique in exhibiting satisfactory growth characteristics etc but did not produce any of

the alkaloids normally found in the other lines within our program.

After several preliminary experiments to determine the conditions of the experiment (amount of substrate, time period etc) 3',4'-dehydrovinblastine sulfate (500 mg) was incubated with the 916 line of C. roseus cell suspension cultures (5.5 liters) in a Microferm bioreactor for a period of 24 hours. The freeze-dried sample (152 g) was then extracted with methanol and partitioned between neutral, acidic and basic fractions. The basic alkaloid fraction (396 mg) was subjected to semi-preparative high pressure liquid chromatography separation (Waters Associates HPLC system employing silica gel in stainless steel 1" columns and Waters radial compression module) to provide two major and two minor components. Spectral and chromatographic comparisons of the major components with authentic samples of leurosine (2) and catharine (3) established their identity. The amounts of 2 and 3 obtained in this experiment were 106 and 30 mg respectively while unreacted 1 recovered was 164 mg. Based on recovered 1 the yields of 2 and 3 in this biotransformation study are 31% and 9% respectively. Several other minor bisindole products as yet uncharacterized have been isolated. It should be noted that the conditions employed have not been optimized so that these yields may vary with subsequent refinements in experimental conditions. Appropriate blank experiments employing 1 in the growth medium but in the absence of cells provided only a very low yield of leurosine (4%) and traces of catharine (<1%) thereby confirming that these compounds are true biotransformation products and not artefacts formed in the medium.



1 2 + 3

It is of interest to note that the 916 line, although presently lacking the ability to produce alkaloids characteristic in C. roseus plants, does contain enzyme systems capable of biotransforming 1 into several typical bisindole alkaloids. Also it appears that there may be a close parallel between the enzymic systems present in 916 and enzymes present in cell free extracts from C. roseus plants obtained earlier in our laboratory[11-14]. Further studies are underway.

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