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Studies in biotechnology. Important routes to the synthesis and biosynthesis of biologically active natural products

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Abstract - Studies involving plant cell cultures of Catharanthus roseus for the synthesis and biosynthesis of the clinical anti-cancer drugs vinblastine and vincristine are presented. Experiments utilizing these cell cultures for biosynthetic studies in both whole cells and enzyme systems obtained from such cultures are detailed. Evidence presented reveals important information about the later stages of the biosynthetic pathways leading to these alkaloids. These data, when combined with chemical studies, afford a highly efficient and commercially important method for the synthesis of these clinical drugs.

INTRODUCTION

Plant cell cultures represent an excellent medium for studies relating to the biosynthesis of complex natural products. Control of growth parameters during propagation of such cultures affords a reproducible medium for the production of such secondary metabolites.

With a stable cell line on hand, studies involving different ages of cultures, varying enzyme levels within the cells, etc. can indeed refine the necessary parameters for the optimum production of a target compound. In particular, the isolation of relevant enzymes from the cells and subsequent enzyme immobilization on solid supports, can afford a particularly powerful method for detailed studies involving the synthesis and biosynthesis

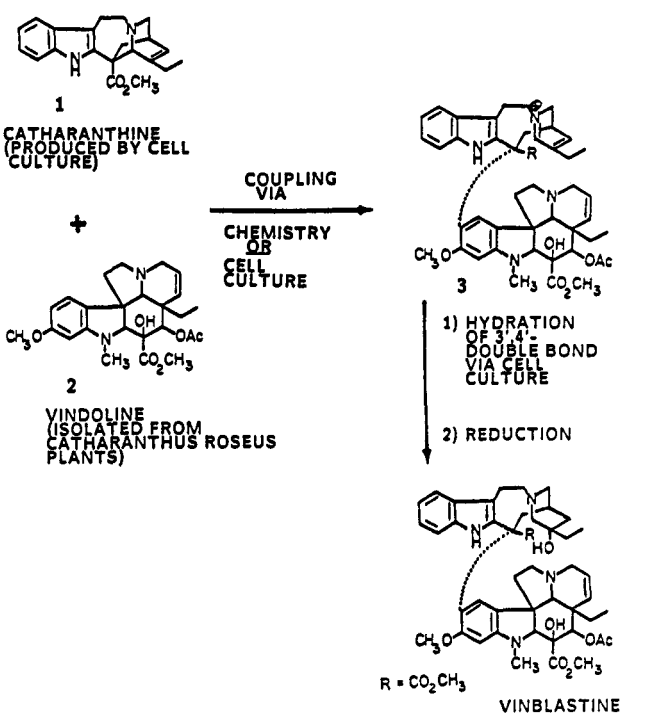


Fig. I. Biotechnological approach to production of vinblastine-vincristine.

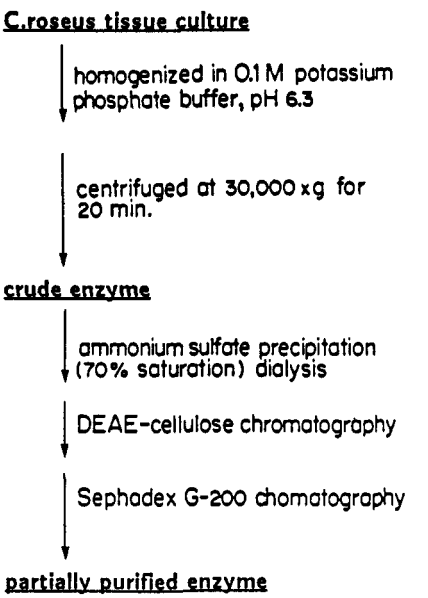


Fig. 2 Purification procedure.

of complex systems. A particularly effective approach involves a "team" effort in which "biological" and "chemical" information is coupled to achieve these objectives. Our earlier studies are summarized in a recent review article (ref. 1) while some of the most recent results are presented in ref. 2-4. The present discussion will summarize some of these latter studies and focus on the most recent and, as yet, unpublished results.

BIOTECHNOLOGICAL APPROACH TO PRODUCTION OF VINBLASTINE-VINCRISTINE

Figure 1 summarizes the approach which has been utilized in our program. Well developed stable cell lines (see ref. 1) have been employed to study the production of catharanthine (1) and subsequently for the enzymatic coupling of 1 and vindoline (2) to the highly unstable dihydropyridinium intermediate (3). The latter, available from a carefully controlled laboratory synthesis, is shown to be the first formed biointermediate in the enzyme-catalyzed coupling of 1 and 2. Enzyme isolation and purification (Fig. 2) affords enzyme mixtures which, after immobilization on solid supports (ref. 3), can achieve coupling of 1 and 2 to 3 in 90% yield.

The subsequent enzymatic conversions of 3 to the various bisindole alkaloids (4-8) are summarized in Figure 3.

Finally, and in conjunction with the above studies, a highly efficient "one-pot" process for the synthesis of these clinical drugs has been developed (Fig. 4). The high overall yield of vinblastine from the starting alkaloids (5 step overall process) requires that each reaction proceeds in yields in excess of 80%.

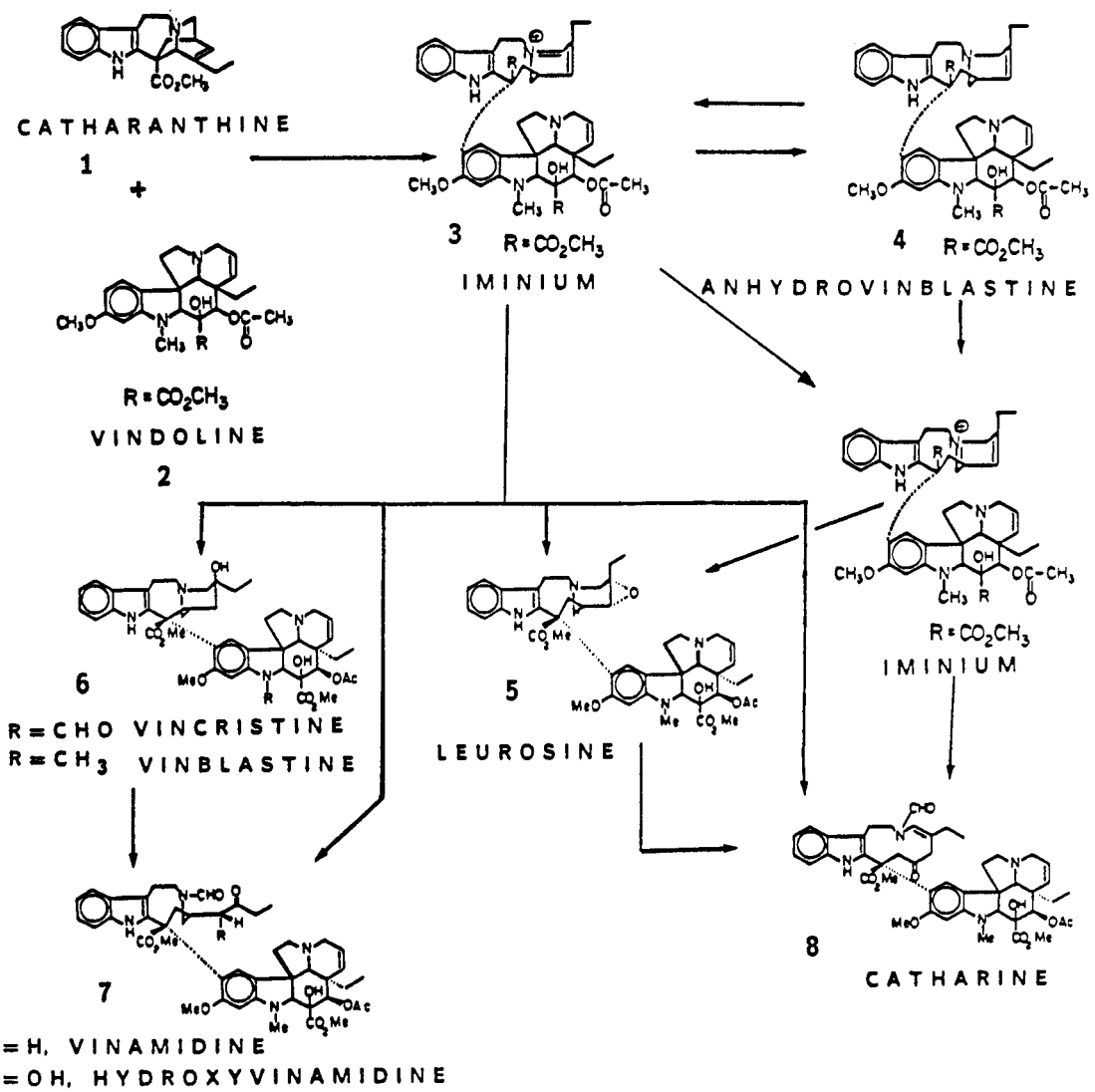
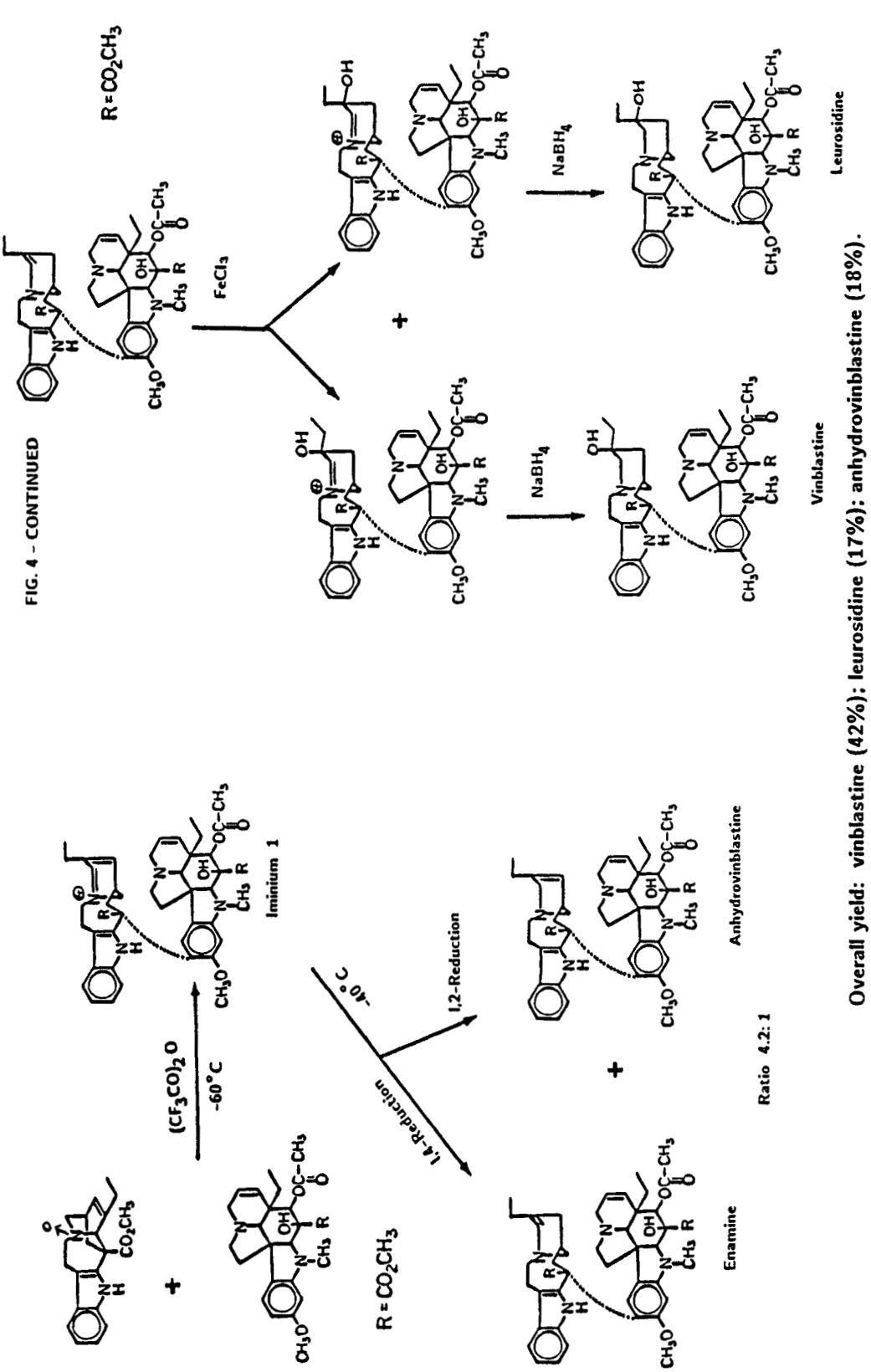


Fig. 3. Overall summary - growing cells and cell free extracts.



Overall yield: vinblastine (42%); leurosidine (17%); anhydrovinblastine (18%).

Fig. 4. Optimum procedure for one-pot process – vinblastine from catharanthine and vindoline.

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