BIOMIMETIC CHEMICAL TRANSFORMATION OF 3',4'-ANHYDROVINBLASTINE TO VINBLASTINE AND RELATED BISINDOLE ALKALOIDS.

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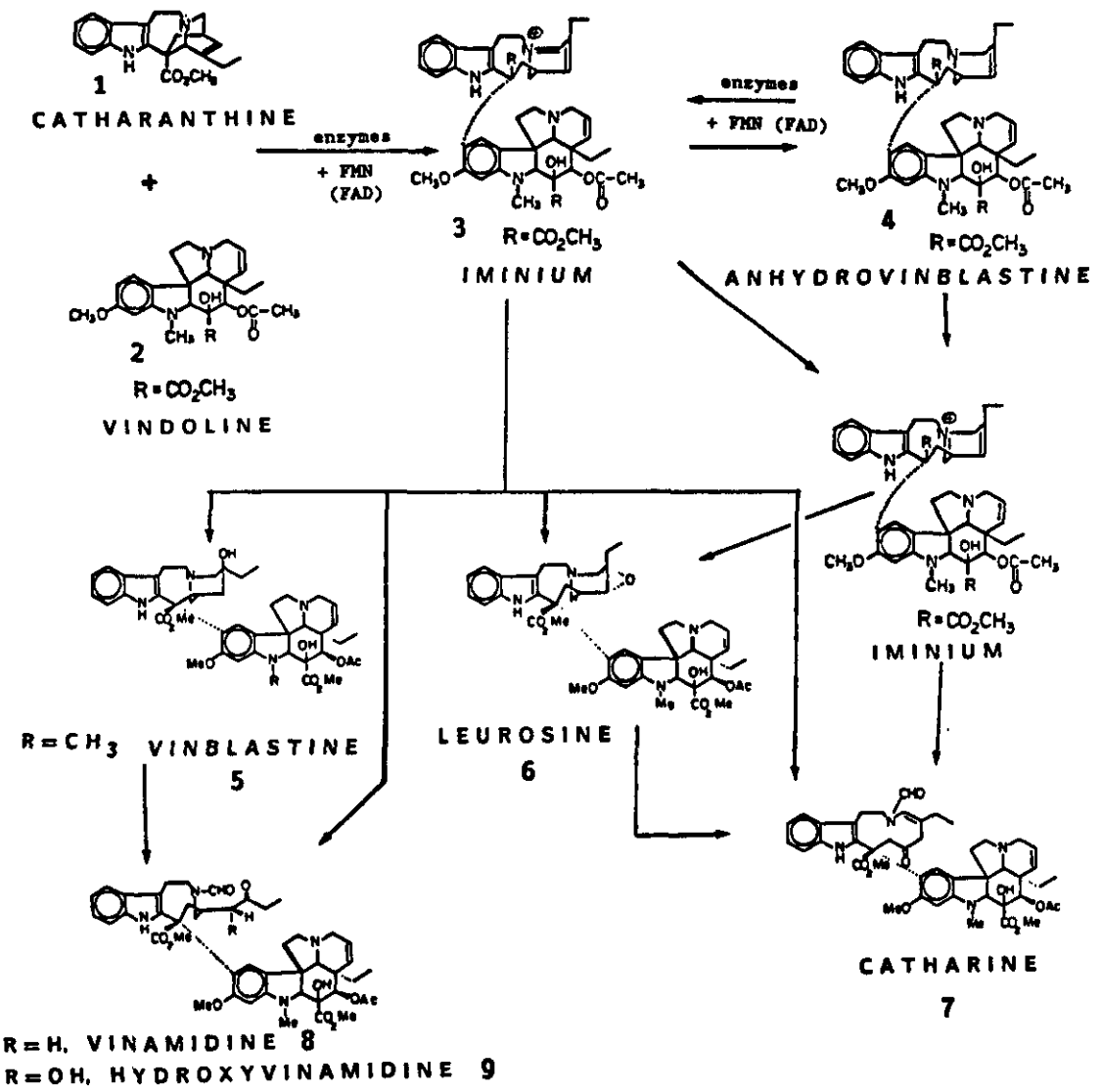
Abstract:- Employing flavine co-enzyme mediated photooxidation and reduced nicotinamide-adenine dinucleotide (β-NADH) as reactant, 3',4'-anhydrovinblastine (4) is transformed into vinblastine (5), 4'-deoxy-leurosidine (14), vinamidine (8) and its reduction product (16). In this biomimetic transformation of 4 to the end products, unstable intermediates are recognized and their structures established by appropriate deuterium labelled experiments. The results obtained provide important information on the nature of biointermediates involved in the later stages of the biosynthetic pathway of vinblastine and related bisindole alkaloids. Contrary to earlier speculations proposed from various laboratories, 4 is not a biosynthetic precursor for the above-mentioned alkaloids. Its formation is the result of regiospecific 1,2-reduction of the true biosynthetic precursor, the highly unstable dihydropyridinium intermediate 3. The latter, after enzymatically controlled conversion to 10, 11 and 12, then affords routes to the alkaloids 5, 8 and 14.

Results of our recent investigations of the biosynthetic pathways to vinblastine (5) and the related bisindole alkaloids have established the pivotal role of the dihydropyridinium intermediate 3 (Scheme 1) linking the two monomeric units, catharanthine (1) and vindoline (2) to the various bisindole alkaloids[1,2]. The same dihydropyridinium system (3) was also found to be the initial product in the biotransformation of 3',4'-anhydrovinblastine (4)[3]. However, under the conditions employed, the apparently more stable and predominating oxidase and/or peroxidase type enzyme systems obtained from the C. roseus cell cultures, resulted in further

oxidation of 3 and/or subsequent biointermediates with predominant formation of the higher oxidation state metabolites, leurosine (6), catharine (7), vinamidine (8), and hydroxy-vinamidine (9) (Scheme 1). The yield of vinblastine (5) obtained in these studies was very low

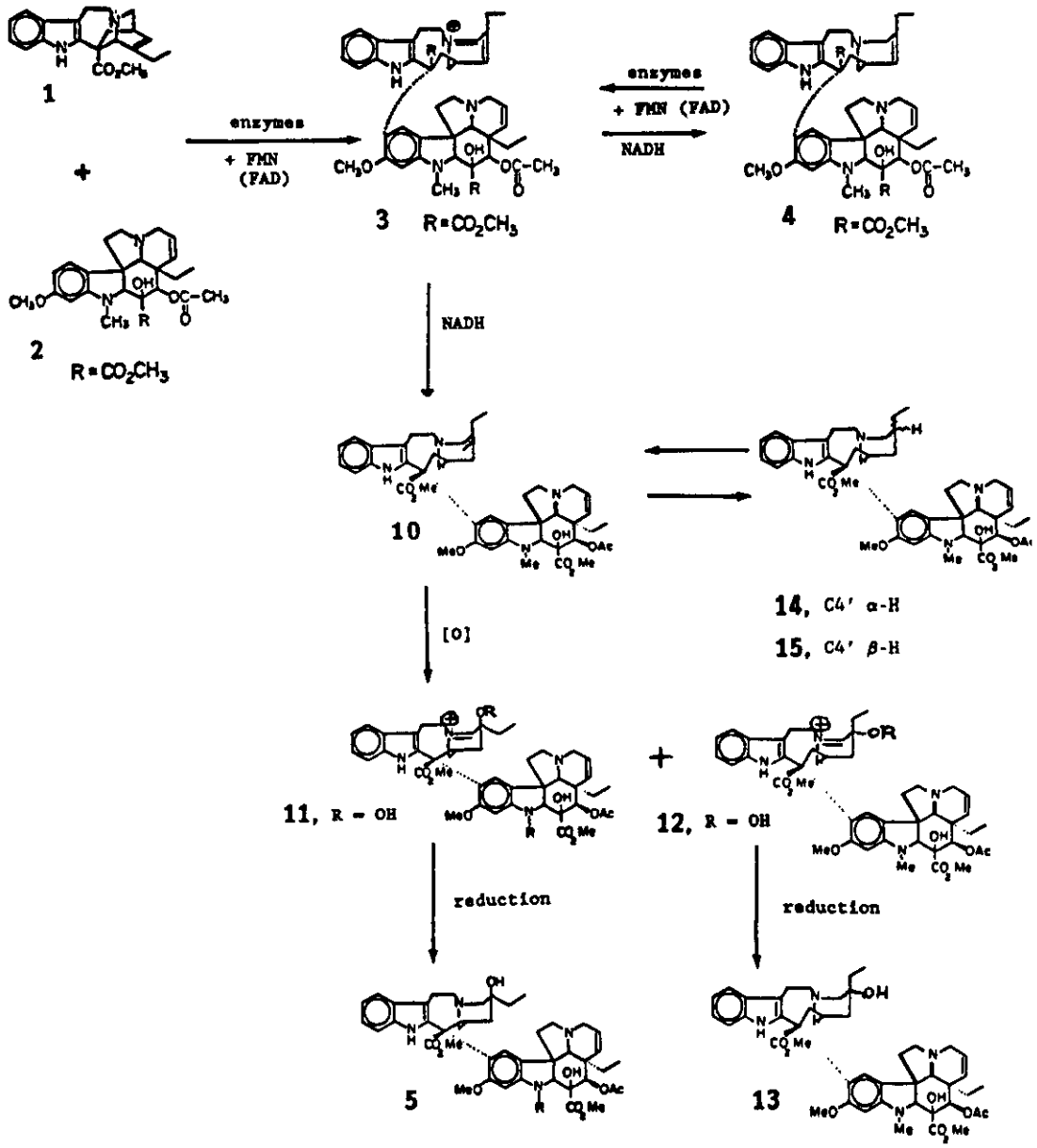


Scheme 1 Overall Summary of Enzymatic Coupling of Catharanthine (1) and Vindoline (2) and Biotransformation of 3',4'-Anhydrovinblastine (4) to Various Bisindole Alkaloids.

(generally 1-3%). It was clear that if higher yields of 5 were to be realized, serious consideration must be given toward the understanding of the various biointermediates which must be involved in the enzymatic bioconversion of 3 to vinblastine. The studies presented here address this aspect of the biosynthetic pathway.

In considering the bioconversion of 3 to 5, we envisaged that the former biointermediate once formed must be preferentially reduced in an initial regiospecific manner (1,4-reduction), to an enamine intermediate (10) (Scheme 2). The latter upon selective oxidation (hydroxylation) at C4' could afford the iminium species 11 and/or 12 which then in a 1,2-reduction process would provide, respectively, the alkaloids vinblastine (5) and leurosidine (13). Evaluation of this postulate could proceed via the FMN-mediated photooxidation of 3',4'-anhydrovinblastine (4), as described in the accompanying publication[4], since this method affords an excellent procedure to obtain the highly unstable 3 under enzyme-free conditions, and subsequent studies of 1,2- versus 1,4-reduction of 3 under enzyme-like conditions, for example, with reduced nicotinamide-adenine dinucleotide (β-NADH). We had already shown in our studies involving enzymes isolated from C. roseus cell cultures[1,2], that β-NADH and NADPH can play important roles as cofactors in the enzyme-catalyzed synthesis of various bisindole alkaloids. Indeed, as will be shown below, this approach has been highly informative in evaluating the biotransformation of 3 to vinblastine (5) and leurosidine (13). The information thus obtained, casts important light on the later stages of the biosynthetic pathway and, in parallel with chemical studies to be described in the accompanying publication[5], has led to the development of a highly efficient chemical synthesis of the clinical drug vinblastine (5) from catharanthine (1) and vindoline (2).

A Tris-HCl buffer solution of reduced nicotinamide-adenine dinucleotide (β-NADH, 8 equiv) was added to a methanol-Tris-HCl buffer solution of FMN-generated 3[4] and the reduction (4.5 h) afforded two major products (85% yield). Direct reverse-phase (Waters C-18 and CN columns, methanol:water, 27:23) HPLC monitoring revealed a 4:1 ratio of products. The minor component was identified as 4, regenerated by 1,2-reduction of 3. The major product identified as the enamine (10), (Scheme 2), was found to be unstable under the conditions for actual isolation and was characterized by further reduction (direct addition of $NaBH_4$ to reaction mixture, 0° C) to the known compound, 4'-deoxyleurosidine (14). The structural assignment of the enamine was further confirmed by a deuterium incorporation experiment ($NaBD_4$ added directly to reaction mixture, 0° C), which afforded 14 containing one deuterium atom at C5' (mass spectrometric analysis, HRMS: m/z 795.4275, M^+, calcd. for $C_{46}H_{57}N_4O_8D$ = 795.4317; 139.1348, calcd. for $C_9H_{15}ND$ = 139.1345).

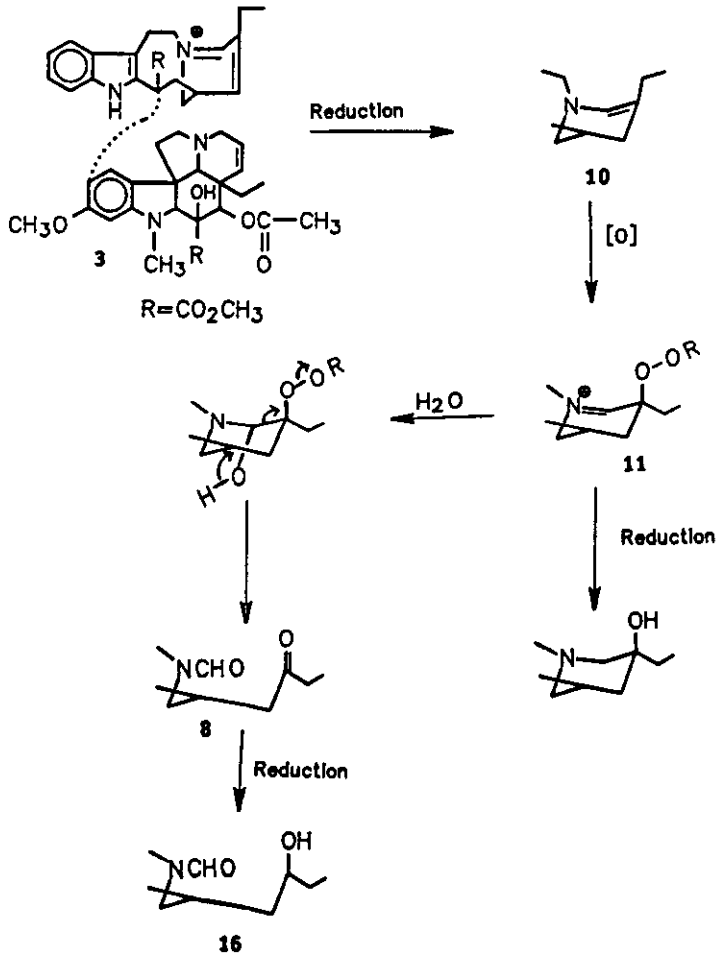


Scheme 2

The enamine (10) obtained in the above reduction and without isolation, was subjected to various oxidation conditions (air, oxygen, H_2O_2, horseradish peroxidase/air, horseradish peroxidase/H_2O_2). In all instances, the reaction was performed in the dark. The most convenient and best conversion, in terms of overall yield to vinblastine (5), was direct aeration although the rate of oxidation of 10 was higher with horseradish peroxidase/air or

horseradish peroxidase/H_2O_2 (3 times faster). The resultant unstable intermediate, assigned structure 11 (Scheme 2), was directly reduced ($NaBH_4$ directly added to reaction mixture, room temperature) and afforded vinblastine (5). Formation of the iminium intermediate 11 was further supported by deuterium incorporation ($NaBD_4$) to afford deuterium labelled vinblastine (mass spectrometric determination, HRMS: m/z 811.4260, M^+, calcd. for $C_{46}H_{57}N_4O_9D$ - 811.4266). Furthermore, when the borohydride reduction step was omitted, no vinblastine (5) was obtained, instead the major product isolated was identified as vinamidine (8) (52%).

Formation of 8 can be rationalized by hydrolysis of iminium 11 and subsequent ring cleavage (Scheme 3). Confirmatory evidence to support the hydrolytic pathway shown in Scheme 3 was obtained when the resultant mixture containing the intermediates 11/12 was treated with $NaBH_4$. Under these conditions, a significant amount of a reduction product of vinamidine (see 16 in Scheme 3) (20%) was obtained in addition to vinblastine (5) (23%).



Scheme 3

Although leurosidine was not detected in the final product mixture, formation of its precursor (12) cannot be excluded entirely. Hydrolysis of 12, followed by ring opening similar to that shown in Scheme 3 for the vinblastine precursor (11), will also lead to 8. The rate of this process for 12 may be much faster than for 11, under the reaction conditions employed.

In conclusion, the above results provide considerable support for the overall biogenetic scheme for the later stages of the biosynthetic pathway for vinblastine (5) and the other bisindole alkaloids as outlined in Scheme 1. Thus, 3',4'-anhydrovinblastine (4) is formed by 1,2-reduction of the initially formed dihydropyridinium intermediate 3 while the route to vinblastine (5) involves sequential 1,4-reduction of 3, followed by oxidation (hydroxylation) to 11 and finally reduction to (5). The isomeric alkaloid leurosidine (12) is similarly formed from 12. If reduction of the enamine 10 occurs prior to oxidation, the known compounds 4'-deoxyvinblastine (15) and 4'-deoxyleurosidine (14) are the products formed. It is of interest to note that 4 and 15 have been isolated from C. roseus plants[6,7] and 4 and 15 were implicated previously as biosynthetic precursors to vinblastine[8-10]. The present data reveal that these alkaloids are formed from the true biosynthetic intermediates 3, 10 and 11/12 via stepwise and enzyme controlled reductions and oxidations. Consideration of all of these factors has, in a simultaneous study in our laboratory and reported in the accompanying publication[5], led to the development of a highly efficient synthesis of vinblastine.

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