INTERMEDIACY OF 3',4'-DEHYDROVINBLASTINE IN THE BIOSYNTHESIS OF VINBLASTINE-TYPE ALKALOIDS.

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Cell free extracts from Catharanthus roseus plants have been used to demonstrate the formation of 3',4'-dehydrovinblastine and leurosine from radiolabelled catharanthine and vindoline. 3',4'-Dehydrovinblastine has been established as a precursor to the vinblastine group of alkaloids and a biosynthetic pathway is proposed.

Investigations of the biosynthetic pathway(s) to vinblastine (I) and related compounds have provided a source of both curiosity and understanding of the behaviour and mode of formation of these complex molecules. Even in recent work, much of the biosynthetic investigation of alkaloid biogenesis has been plagued by the inconclusive nature of very low incorporations of radiolabelled precursors. The use of plant derived tissue cultures enabled Zenk and Stöckigt[1-5] to demonstrate alkaloid interrelationships as high as 71% while, earlier, Scott and *co-workers*[6-8] achieved successful results with cell free extracts from calluses and young plants. Recent efforts in these laboratories, using cell free extracts from mature *Catharanthus roseus* plants, have enabled similar, efficient, transformations[9-11]. Although the exact meaning of precursor incorporation must always be evaluated with caution, the present *in vitro* work provides good insight into the probable, gross biosynthetic derivation of the vinblastine-type alkaloids.

A recent report[12] described the use of apical cuttings of *C. roseus* to give an absolute incorporation of catharanthine (II) and vindoline (III) into vinblastine (I) to the extent of 0.006%. In view of these and similar low incorporations with intact plants[13,14], our approach has been to test the validity of various segments of a proposed biosynthetic pathway to I (Figures 1 and 2). Exploitation of cell free extracts from *C. roseus* plants demonstrated significant incorporation of tryptamine into vindoline (III) (1.36%)[9], and of a proposed, later stage intermediate (IV) into the natural products leurosine (V) (8.22%), catharine (VI) (15.15%) and vinblastine (I) (1.84%)[10,11].

Figure 1.

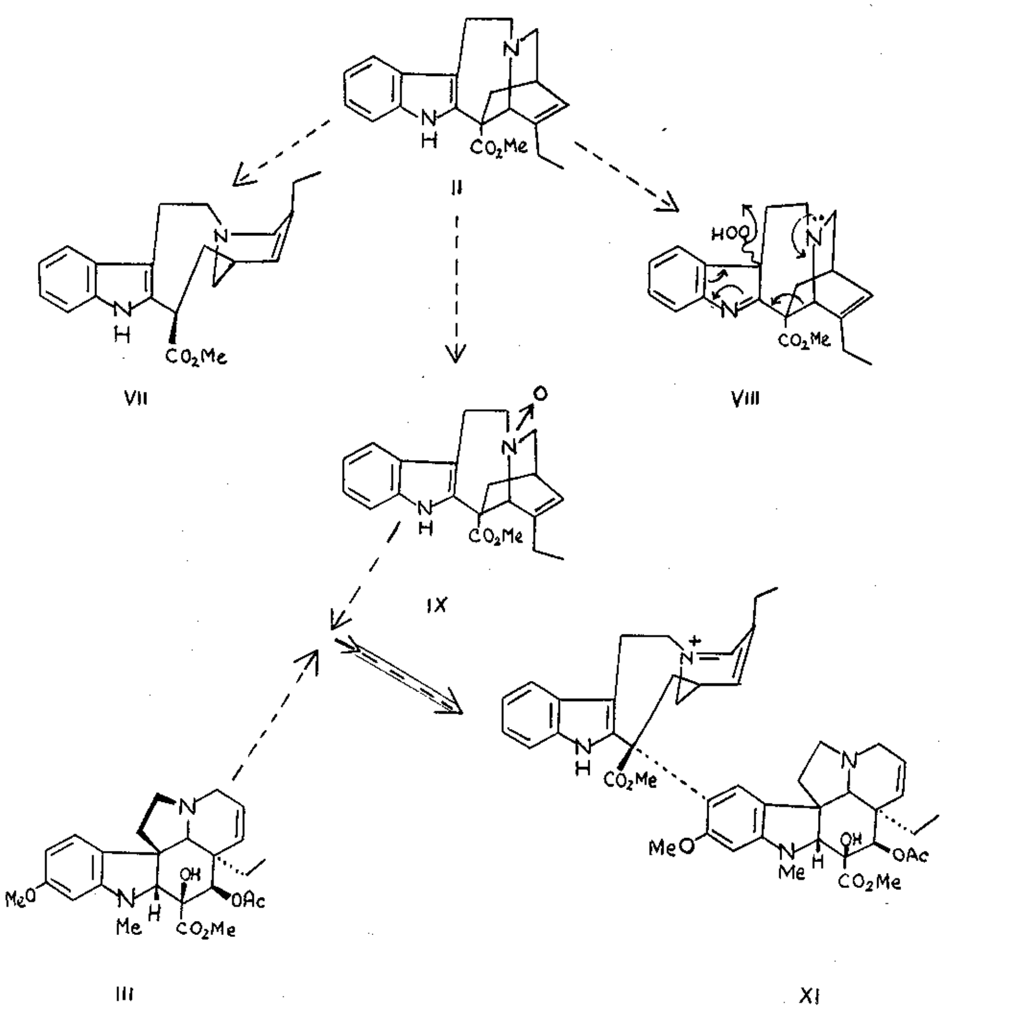
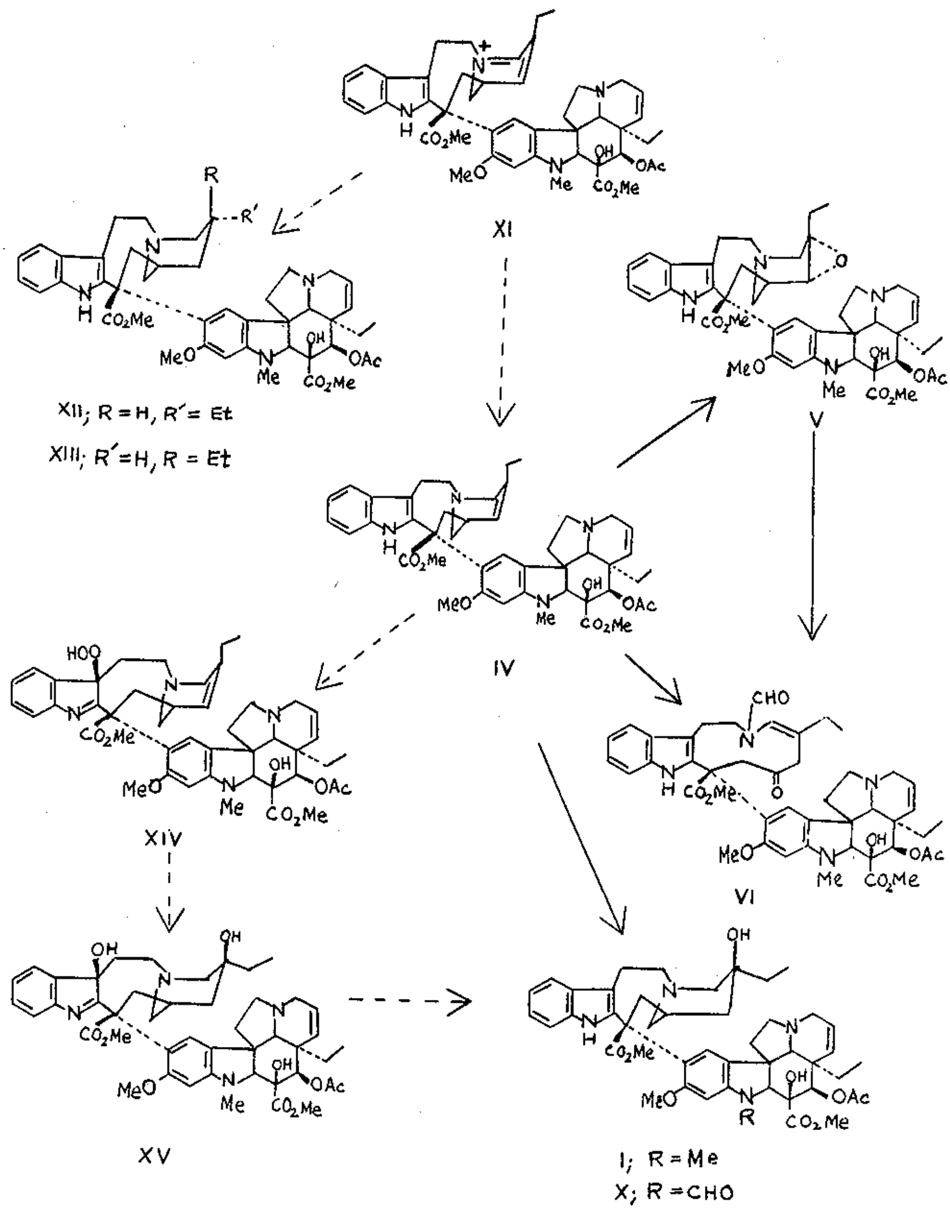
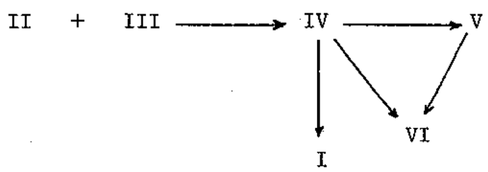


Figure 2.



The present work describes the enzyme catalysed formation of 3',4'-dehydrovinblastine (IV) from naturally occurring alkaloids catharanthine (II) and vindoline (III), together with the formation of leurosine (V). Here the use of cell free extracts has simplified the methodology and, as quoted in Table 1, necessitated only short reaction times to give satisfying results. In fact, [Ar-^{3}H]-catharanthine was transformed to labelled (IV) to the extent of *ca* 0.54%, and to the alkaloid leurosine (V) in *ca* 0.36% yield. Furthermore, simultaneous incubation of [Ar-^{3}H]-II and [Ac-^{14}C] vindoline (III) afforded doubly labelled IV and V. The ^{3}H/^{14}C ratio of an equimolar ratio of the substrates was 23.6 while that of the products was 20.8 for IV and 21.1 for V; in good agreement with the obvious stoichiometry of the reaction. These results, together with the transformations of IV to the alkaloids I, V and VI, strongly support the gross biogenesis of "bisindole" alkaloids as:



Although no conclusive, direct evidence is available for the exact route of each transformation, plausible suggestions for various intermediates are given in Figures 1 and 2. It is generally accepted that a precursor with the cleavamine skeleton, such as VII, is not involved in the biosynthesis since related monomeric compounds are not found in the plant. Moreover, initial cleavage of an indole-3-substituted catharanthine, such as VIII, would also be expected to

TABLE 1

Exp	Substrate		Product		% Incorporation	
	catharanthine	vindoline	anhydro-vinblastine	leurosine	anhydro-vinblastine	leurosine
A	1.667×10^{10} [a] (1.791×10^{8}) [b]		8.563×10^{5} [b]	1.857×10^{5} [b]	0.48 [e]	0.10 [e]
B	1.667×10^{10} [a] (1.671×10^{8}) [b]		8.955×10^{5} [b]	5.925×10^{5} [b]	0.54 [e]	0.36 [e]
C	1.667×10^{10} [a] (1.748×10^{8}) [b]	7.077×10^{8} [c] (9.451×10^{6}) [d]	6.201×10^{5} [b] 2.979×10^{4} [d]	5.936×10^{5} [b] 2.812×10^{4} [d]	0.36 [e] 0.32 [f]	0.34 [e] 0.30 [f]

Exp. A = 3 hrs; Exp. B = 8 hrs; Exp. C = 6 hrs.

a = dpm ^{3}H/mmole; b = dpm ^{3}H; c = dpm ^{14}C/mmole; d = dpm ^{14}C;

e = % incorporation for ^{3}H; f = % incorporation for ^{14}C

yield monomeric alkaloids, e.g. VII. Intermediacy of the N-oxide IX in a biological equivalent of the modified Polonovski reaction (used for the synthesis of IV) seems highly probable. Feeding of labelled IX to C. roseus plants has been carried out in these laboratories[14], but the specific incorporation of only 0.008% into vincristine (X) was considered insignificant. However, the actual use of an intermediate such as IX on coupling with III could lead to the iminium species XI which on enzymic reduction, using NADPH as co-factor, would give IV. Similar, conjugate reduction could account for the alkaloids 4'-deoxy-vinblastine (XII) and 4'-deoxyleurosidine (XIII).

The formation of I, V and VI from IV has already been demonstrated, but significant incorporation of IV into vincristine (X) was not shown[11]. Several indirect routes to X can be proposed and one of these, involving initial N-demethylation and subsequent formylation gains some credence from the reported cleavage/oxidation of tertiary aromatic amines by peroxidase enzymes[15]. An interesting proposal for the elaboration of vinblastine (I) is shown in Figure 2. Molecular models show that the hydroperoxyindolenine (XIV) is ideally oriented for conversion to XV which would represent an immediate precursor to vinblastine. A discussion of the synthetic application of this route will be reported elsewhere.

Thus the use of cell free extracts of C. roseus plants has enabled a demonstration of the probable biogenesis of vinblastine-type alkaloids from catharanthine and vindoline, via 3',4'-dehydrovinblastine (or the corresponding iminium species XI) a late stage, pivotal intermediate. The synthetic utility of enzyme mixtures, such as those used here, on solid support is currently under investigation (KLS).

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