STUDIES ON THE BIOSYNTHESIS OF BISINDOLE ALKALOIDS. THE FINAL STAGES IN BIOSYNTHESIS OF VINBLASTINE, LEUROSINE AND CATHARINE.

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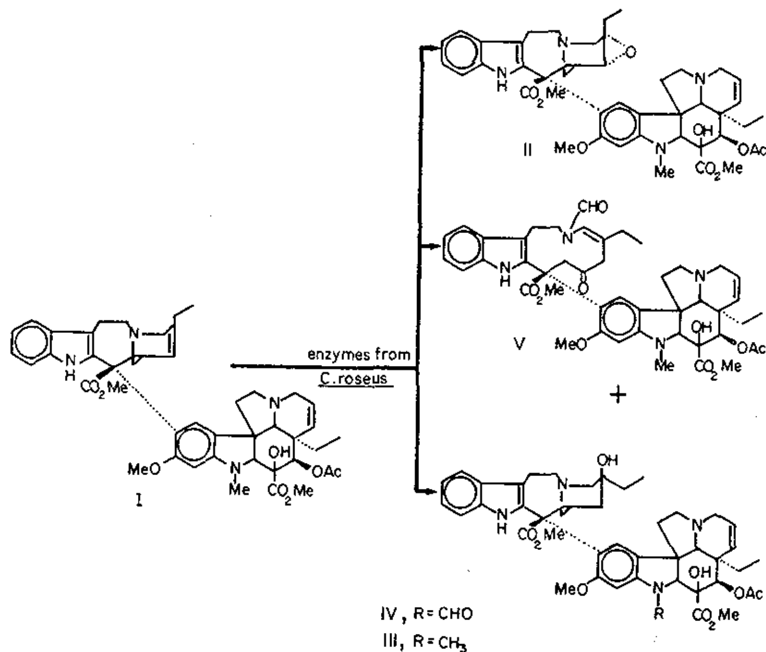
Radiolabelling experiments have demonstrated the incorporation by *Catharanthus roseus* cell free extracts of 3',4'-dehydrovinblastine into vinblastine, leurosine and catharine. The enzyme catalysed formation of catharine from leurosine indicated that the former is not an artefact.

Recently we reported the incorporation of tryptamine and secologanin into vindoline using cell free extracts from *C. roseus*[1]. Furthermore, similar preparations were shown to mimic the action of horseradish peroxidase in catalysing the conversion of 3',4'-dehydro-vinblastine (I) to leurosine (II)[2]. The success of this latter investigation immediately prompted a consideration of the possible role of I as the pivotal intermediate in the biosynthesis of "bisindole" alkaloids such as vinblastine (III) and vincristine (IV). To this end [Ar-^{3}H]3',4'-dehydrovinblastine was incubated at ambient temperature in solutions of the cell free extracts at pH 6.3[1]. Dilution with cold alkaloid and rigorous purification to constant specific activity gave the results listed in Table I.



Direct incorporation of I into leurosine and catharine (V) was high, and that into vinblastine still very gratifying. After a 50 hour incubation, negligible activity was transformed into vincristine. Combining the figures from entries 3, 4 and 5 accounts for the metabolic fate of 25% of the [Ar-^{3}H]3',4'-dehydrovinblastine. Incubation of labelled leurosine afforded a 12% incorporation into catharine (V) while in a blank experiment, only 1% conversion was noted. This strongly suggests that V is indeed a "natural product" and not an artefact[3].

The very low values for vincristine are worthy of comment. The low incorporations could be rationalised in several ways, but clearly a more extensive stepwise approach to this problem will be necessary.

Although the conversion of I to II has been shown possible with both cell free extracts from *C. roseus* and with horseradish peroxidase, there is no proof at this stage that the mechanism of action is the same or even similar. The extracts used here have however been shown to exhibit peroxidase type activity and natural formation of some epoxides has been shown to occur by haloperoxidase action and by way of halohydrin intermediates[4].

Earlier biosynthetic studies evaluating the incorporation of loganin, vindoline and catharanthine into vinblastine (III) have been plagued by low specific incorporation values, such as those experienced here with vincristine (IV)[5-7]. Here the value of IV (entry 2 in table) approaches the limit of experimental error and "incorporations" in this region must be considered in this light.

TABLE I

Incubations[a] With Cell Free Extracts[b].

Expt.	Substrate	Alkaloid	Time (h.)	Specific Incorporations %[g]
1	I[c]	IV	3	< 0.0047[f]
2	I[c]	IV	50	< 0.03[h]
3	I[c]	III	3	1.84
4	I[c]	II	2	8.15
5	I[c]	V	3	15.15
6	II[d]	V	3	12.08
7[e]	II[d]	V	3	1.17

a) At ambient temperature;

b) Oxidants (e.g. H_2O_2) and antioxidants (e.g. β-mercaptoethanol) were not added to these preparations;

c) 1.40×10^{11} dpm/m mole;

d) 1.15×10^{9} dpm/m mole;

e) Blank reaction, i.e. identical to (b) but without enzymes;

f) Effectively zero;

g) Factors accounting for the diversity of possible biochemical and dilution changes have not been used to adjust these values[7];

h) Insufficient material prevented purification to constant activity.

Further investigations to determine the formation of the proposed intermediate I from catharanthine and vindoline using cell free extracts are underway in these laboratories.

In summary, significant incorporations of I into vinblastine, leurosine and catharine strongly support the theory that I is in fact the key biointermediate of the vinblastine-type dimeric alkaloids.

Acknowledgements:

Financial aid from the National Research Council of Canada is gratefully acknowledged. One of us (KLS) wishes to express his sincere thanks to the International Development Research Centre for a Research Associateship. The authors also wish to thank the Lilly Research Laboratories, Indianapolis for alkaloid samples and Professor Neil Towers, Botany Department, UBC for use of research facilities.

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Received, 3rd July, 1978