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

## Kenneth L. Stuart,

Department of Chemistry, University of the West Indies, Kingston 7, Jamaica.

and

James P. Kutney, Toshio Honda and Brian R. Worth,

2075 Wesbrook Place, Vancouver, B.C.

Canada, V6T 1W5

Radiolabelling experiments have demonstrated the incorporation by <u>Catharanthus roseus</u> 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 <u>C</u>. <u>roseus</u><sup>1</sup>. Furthermore, similar preparations were shown to mimic the action of horseradish peroxidase in catalysing the conversion of 3',4'-dehydrovinblastine (I) to leurosine (II)<sup>2</sup>. 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-<sup>3</sup>H]3',4'-dehydrovinblastine was incubated at ambient temperature in solutions of the cell free extracts at pH 6.3<sup>1</sup>. 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  $[Ar-^{3}H]3',4'$ -dehydro-vinblastine. 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<sup>3</sup>.

The very low values for vincristine are worthy of comment. The low incorporations could be <u>rationalised</u> 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 <u>C</u>. <u>roseus</u> 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<sup>4</sup>.

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.

Expt.	Substrate	Alkaloid	Time (h.)	Specific Incorporations % <sup>g</sup>			
1	Ic	IV	3	< 0.0047 <sup>f</sup>			
2	ľc	IV	50	< 0.03 <sup>h</sup>			
3	ıc	III	3	1.84			
4	Ic	II	2	8.15			
5	ľ	V	3	15.15			
6	$II^d$	v	3	12.08			
7 <sup>e</sup>	ιıd	v	3	1.17			
a) b)	At ambient temperature; Oxidants (e.g. $H_2O_2$ ) and antioxidants (e.g. $\beta$ -mercaptoethanol)						
c)	$1.40 \times 10^{11} \text{ dpm/m mole;}$						
d)	$1.15 \times 10^9 \text{ dpm/m mole;}$						
e)	Blank reaction, i.e. identical to (b) but without enzymes;						
f)	Effectively zero;						
	Factors accounting for the diversity of possible biochemical and						
g)	Factors accounts	ing for the di	versity of pos	ssible biochemical and			
g)	dilution changes	ng for the di s have <u>not</u> bee	en used to adj	ssible biochemical and ust these values <sup>7</sup> ;			

	TAI	<u>BLE I</u>		
Incubations <sup>a</sup>	With	Cell	Free	Extracts <sup>b</sup> .

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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.

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