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STUDIES ON THE SYNTHESIS OF BISINDOLE ALKALOIDS. XIV[1]
ENZYME CATALYSED FORMATION OF LEUROSINE.

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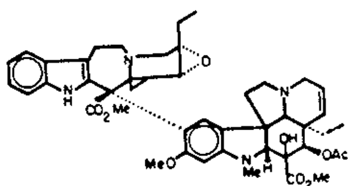
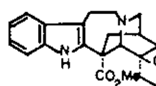
Enzyme catalysed oxidation of 3',4'-dehydrovinblastine using either horseradish peroxidase or cell free extracts from *Catharanthus roseus* plants provided leurosine, suggesting that the latter is in fact a natural product.

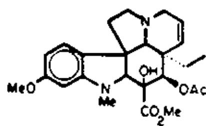
The alkaloid leurosine (I)[2] is probably the most widespread of the vinblastine-type, "bisindole" natural products. In the last few years, work from several laboratories has resulted in numerous syntheses of (I). Coupling of the epoxide (II) with vindoline (III) provided leurosine[3-5] and although this route was inefficient, it did provide an unambiguous stereochemical assignment[3] for the oxiran function in (I). Alternatively, and certainly more efficiently, the anhydro-derivative (IV) of vinblastine, directly available by modified Polonovski coupling between catharanthine and vindoline[6,7], was converted to (I) under a variety of oxidative conditions, including; THF/TFA/O_2[8,9], t-BuOOH[8,9], $Hg(OAc)_2$[9], peracids[5], OsO_4[9], $Pb(OAc)_4$[10], *and aerial oxidation*[10].



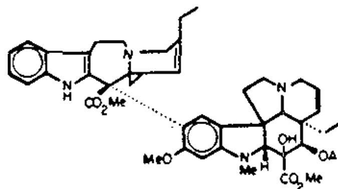
I.



II.



III.



IV.

If one assumes that (IV) may well be the biological precursor of many (if not all) of these "bisindole" alkaloids, then consideration of the above facts leads logically to the suggestion that leurosine may be an artefact as previously mentioned[10]. Similar comments regarding catharine, a further oxidation product, have also been made[1].

Cell free extracts from C. roseus were used in these[11] laboratories to demonstrate the biosynthesis of vindoline (III) from recognised precursors[12]. Simultaneously, an investigation into the utility of enzyme systems for the conversion of (IV) to leurosine (I), and perhaps other alkaloids, was initiated. The results of this latter study are presented here.

It was found initially that the sulphate salt of (IV) remained stable in aqueous buffer, in the absence of added oxidant, for prolonged periods (see table). Since conversion of (IV) to many of the natural products under consideration (vinblastine, leurosidine, leurosine, vincristine, vincadioline etc.) would probably necessitate an oxidation process, the commercially available enzyme, horseradish peroxidase (ICN, Lot No. 7738, RZ = 3.2) was chosen for the preliminary experiments. This choice was also influenced by the fact that peroxidase-type activity was detected in cell free extracts of C. roseus mature leaves, using two independent assay methods[13,14].

Table 1. Enzyme Catalysed Conversions of 3',4'-Dehydrovinblastine Sulphate to (I)[a].

	Enzyme[b]	H_2O_2 (mol. equiv.)[c]	Time (h)	Yield[d]	
				% IV	% I
1	None	0	100	> 98	–
2	A	0	100	> 98	–
3	None	1.3	1.5	87	5
4	A	1.3	1.5	28	65
5	None	0	3	> 98	–
6	B	0	3	46	22
7	None	1.3	3	49	26
8	B	1.3	3	5	25
9	B	1.3	1.5	5	27

a At 23°C, pH 6.3, *ca* 5 x 10^{-4} M.

b A: horseradish peroxidase 17% by weight with respect to substrate.
B: cell free extracts from *C. roseus*.

c With respect to the substrate.

d As measured by HPLC on the isolated product.

As shown by entry 2 in the table, addition of HRP, without activation, did not catalyse the formation of (I). However, on addition of a small excess of hydrogen peroxide, leurosine was formed at a rate twelve times that observed for the blank experiment (entries 3 and 4). Assuming a molecular weight of 4×10^4 for HRP[15] and a maximum of two oxidising sites[16], this conversion was possible with a substrate : catalyst ratio of ca 100.

The reactions with the enzymes from C. roseus plants were more complicated, not surprisingly in view of the number of enzymes probably present in the cell free extract. In fact after a 3 hour incubation and without added H_2O_2, only 46% of the substrate remained and a 22% conversion to leurosine was observed (entry 6). When H_2O_2 was added (entry 8), almost complete depletion of IV was observed after 3 hours, with a 25% yield of leurosine noted. However, when compared to the corresponding blank experiment (entry 7) this conversion to leurosine is meaningless.

More definitive results were obtained with a 1½ hour incubation (entry 9) after which a 27% yield of leurosine was obtained, compared with 5% for the blank experiment (entry 3). Here also a 95% depletion of the substrate IV was observed. Thus within the conditions of the experiment (entry 9), a rate of formation of leurosine approximately 5 fold that of the corresponding blank, was observed.

The measured yields of leurosine seem affected by at least two factors. Firstly, the rapid consumption of IV possibly by the peroxidase-type enzyme(s) as well as by other enzymes in the mixture and secondly, the

probable further conversion of leurosine[11] may explain these observations.

These results strongly suggest that leurosine (I) is a natural product, possibly formed from 3',4'-dehydrovinblastine (IV) by a peroxidase-type enzyme. Clearly utilisation of (IV) by the enzyme mixture involves its conversion to products other than leurosine, which were in fact detectable by t.l.c. and h.p.l.c. Investigations towards their separation and characterisation are continuing.

These enzyme studies indicate the strong probability that 3',4'-dehydrovinblastine (IV) is a biosynthetic precursor of the vinblastine-type alkaloids. Details of the work presented here, together with further, related investigations, will be reported at a later date.

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