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BIOSYNTHETIC INCORPORATION OF L-LEUCINE INTO THE INDOLE ALKALOIDS. THE EFFECT OF PUROMYCIN.

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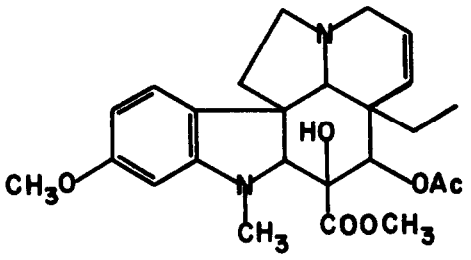
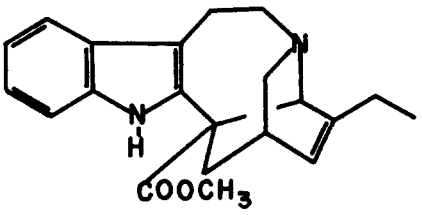
Although the history of the results of attempts to incorporate acetate into the indole alkaloids has been conflicting and confused, it has become clear that acetate can be incorporated, but in low yield and with randomization of the ^{14}C label (1). This phenomenon, concerning the very early stages of the biosynthetic pathway, is in direct contrast with the results on that major portion of the pathway from mevalonic acid on to the final alkaloids, which as a result of spectacular and rapid progress by several groups (2), have clearly delineated the route with ambiguities only of fine detail.

In an effort to shed more light on this intriguing puzzle, we have considered the possibility that, in this particular biosynthetic route, there is a precursor of mevalonate other than acetate, the obvious alternative candidate being leucine, whose metabolic breakdown in some systems has been shown to lead to mevalonic acid (3).

Accordingly, we have investigated whether or not leucine can act as a precursor of the major indole alkaloids, vindoline and catharanthine, in Vinca Rosea plants, and, if so, what are the optimum conditions for its incorporation.



Vindoline



Catharanthine

Our first experiments concerned the determination of the best method of feeding leucine, and the hydroponic method of placing freshly cut shoots of the plant in an aqueous solution of the radioactive precursor emerged as superior to various other methods, the only drawback being that ajmalicine, the major alkaloid having the Corynanthe skeleton (as opposed to the Iboga skeleton of Catharanthine and the Aspidosperma skeleton of Vindoline) could not be investigated, since it is primarily present in the roots of the plant. The results obtained using the hydroponic method as a function of the age of the plant and the time between feeding and isolation are summarized in the Table.

From these results a number of points are clear. Firstly, there is no doubt that leucine is incorporated into both Catharanthine and Vindoline, although the incorporation is low and, interestingly, rather close to the values obtained for the incorporation of acetate (1). Secondly, the age of the plant does not appear to greatly affect the level of incorporation, and thirdly, in common with experience of administration of other precursors, a longer feeding time, up to 2 weeks, seems desirable. We considered that one possible cause of the low incorporation might be the predominant use of leucine in protein synthesis and, in an effort to prevent this, the effect of the protein biosynthesis inhibitor puromycin (4) was investigated. As shown in the Table (entries 7, 8), administration of puromycin together with leucine did cause a measurable, although not dramatic, increase in incorporation; however, when puromycin was fed 24 hours prior to the feeding of leucine, no transfer of radioactivity from leucine to the alkaloids could be detected.

TABLE

Incorporation[a] of uniformly labelled-C^{14}-L-leucine into Vinca Rosea.

Plant age	Feeding time	% incorporation into	
		Vindoline	Catharanthine
6 months	2 days	.002	.002
6 months	7 days	.007	.003
10 months	7 days	.003	.002
10 months	2 weeks	.006	.003
3 years	7 days	.003	.002
3 years	2 weeks	.005	.004
10 months	2 weeks[b]	.008	.004
10 months	2 weeks[c]	.000	.000

[a] Hydroponic method of feeding.

[b] 1.5 mg puromycin fed simultaneously with leucine.

[c] 1.5 mg puromycin fed 24 hours prior to leucine feeding.

Although these results appear to indicate that leucine may be acting as a precursor of the monoterpene portion of the indole alkaloids, their full significance can be evaluated only when two questions are answered: these are (a) whether leucine is being incorporated into the tryptophan portion or into the monoterpene portion of the alkaloids and (b) whether incorporation is specific. Although the known metabolism of leucine (3) and biosynthesis of tryptophan (5) render the possibility of incorporation into the tryptophan portion exceedingly remote, definite answers to both these questions can only be obtained by specific degradation of the radioactive alkaloids. In view of the paucity of published data on the degradation of these alkaloids, it is probable that considerable time will elapse before these results are complete.

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