THE BIOSYNTHESIS OF VINDOLINE USING CELL FREE EXTRACTS FROM MATURE CATHARANTHUS ROSEUS PLANTS.

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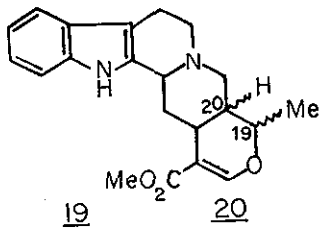
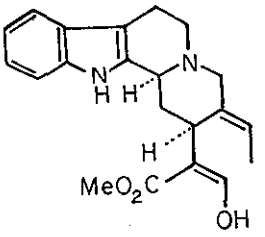
Cell free extracts from mature Catharanthus roseus plants can biosynthesise vindoline (7, R = Ac) utilising ^{14}C-tryptamine, secologanin (6) and S-adenosyl-methionine (methyl-^{14}C).

Recent investigations using cell free enzyme mixtures from Catharanthus roseus (Vinca rosea) seedlings, 3-month old plants, calluses, and cell suspension cultures have demonstrated some of the stages relating to the biosynthesis of the Corynanthe-type indole alkaloids ajmalicine (1), 19-epiajmalicine (2), tetrahydroalstonine (3) and geissoschizine (4)[1-8]. These compounds were formed by the initial condensation of tryptamine (5) with secologanin (6) in the presence of NADPH as a co-factor.



	19	20
1	β	β
2	α	β
3	β	α



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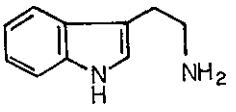

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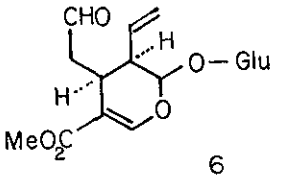

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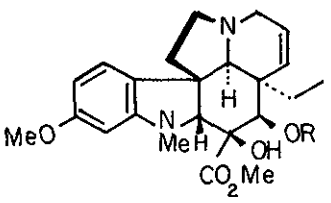
In the past, acetone powders from mature C. roseus plants have been unable to catalyse alkaloid formation[9], but in the light of recent findings, one of us (KLS) initiated a reinvestigation of this problem and we now report that we have utilized cell free extracts from mature plants to demonstrate certain aspects of the biosynthesis of the complex

alkaloid vindoline (7, R = Ac). These crude preparations therefore contained the different enzymes which catalyse the various steps necessary for the formation of this and possible related alkaloids[10].



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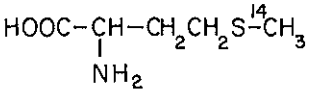

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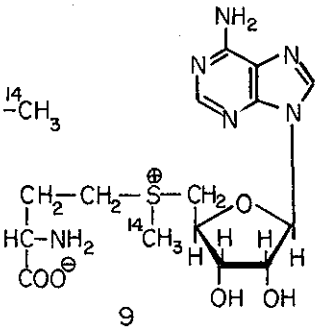

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Leaves from mature, and in some cases flowering plants were homogenised in Tris-maleate buffer (0.05M, pH 7, and containing β-mercaptoethanol) in the presence of an equal weight of Polyclar AT and a supernatant produced by centrifuging the extract at 30,000 g. This cell free extract was incubated with 2-^{14}C-tryptamine bisuccinate and secologanin for 2 hr at 34°C in the presence of NADPH and FAD. The alkaloids were extracted with chloroform after adjusting the solution to pH 9. Autoradiography of the chromatograms of the extract showed the presence of several radioactive compounds. This experiment was repeated on two other occasions using leaves from different plants. Vindoline was isolated by tlc, diluted with inactive compound and further purified by tlc in a different solvent system. Vindoline hydrochloride was then formed and recrystallised to constant activity. Conversion to desacetylvindoline (7, R = H) followed by tlc purification and in one case conversion to desacetyl-

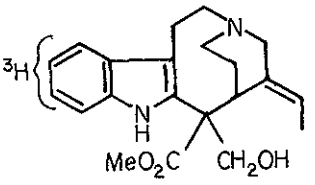
vindoline hydrochloride showed that tryptamine was incorporated into vindoline to the extent of 1.10% and 1.36%. Parallel experiments with boiled enzymes gave unlabelled vindoline. Similar experiments were carried out with ^{14}C-methionine (8) and S-adenosyl-methionine (methyl-^{14}C) (9). Whereas S-adenosyl-methionine (methyl-^{14}C) was incorporated (0.04%; 0.04%), ^{14}C-methionine was not utilised. The fairly low incorporation of the ^{14}C-methyl group of S-adenosyl-methionine is worthy of comment in view of the observation that vindoline itself was shown to exert significant inhibition of a 18-fold purified loganic acid methyl transferase[11]. It would be of interest to see if an enzyme preparation freed of all the pre-formed vindoline would more efficiently use this precursor.

^{3}H-Stemmadenine (10) (labelled in the aromatic ring) did not show significant incorporation into vindoline when compared to a boiled enzyme experiment. The above results are summarised in Table I.

TABLE I

Incorporations into vindoline (7, R = Ac) with cell free extracts.

Substrates	% incorporation
^{14}C-tryptamine	1.10
"	1.36
" (boiled enzyme)	0.00
^{14}C-methionine	0.00
S-adenosyl-methionine (methyl-^{14}C)	0.04
" "	0.04
^{3}H-stemmadenine	0.006
" (boiled enzyme)	0.007



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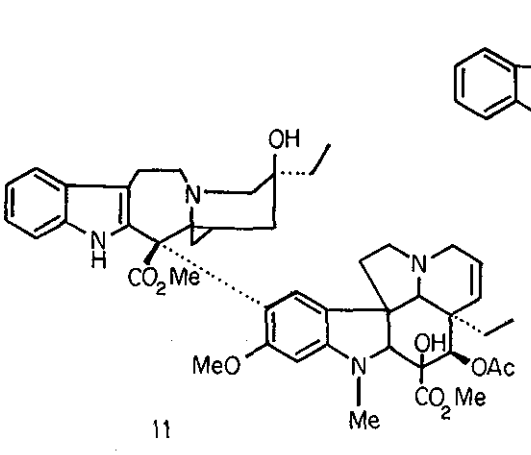

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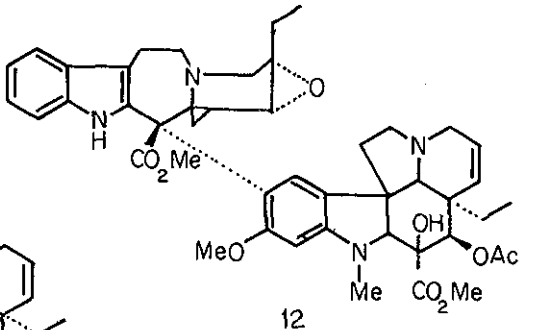

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Studies are now underway to see if these crude enzyme preparations can be used to demonstrate the formation of the clinically important indole alkaloid VLB (11) although in one experiment to date leurosine (12) did not show any incorporation of ^{14}C-tryptamine. The use of these preparations as synthetic tools is now also being investigated.

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