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INDOLE ALKALOID BIOSYNTHESIS IN CATHARANTHUS ROSEUS — INVOLVEMENT OF GEISSOSCHIZINE AND 19-EPIAJMALICINE

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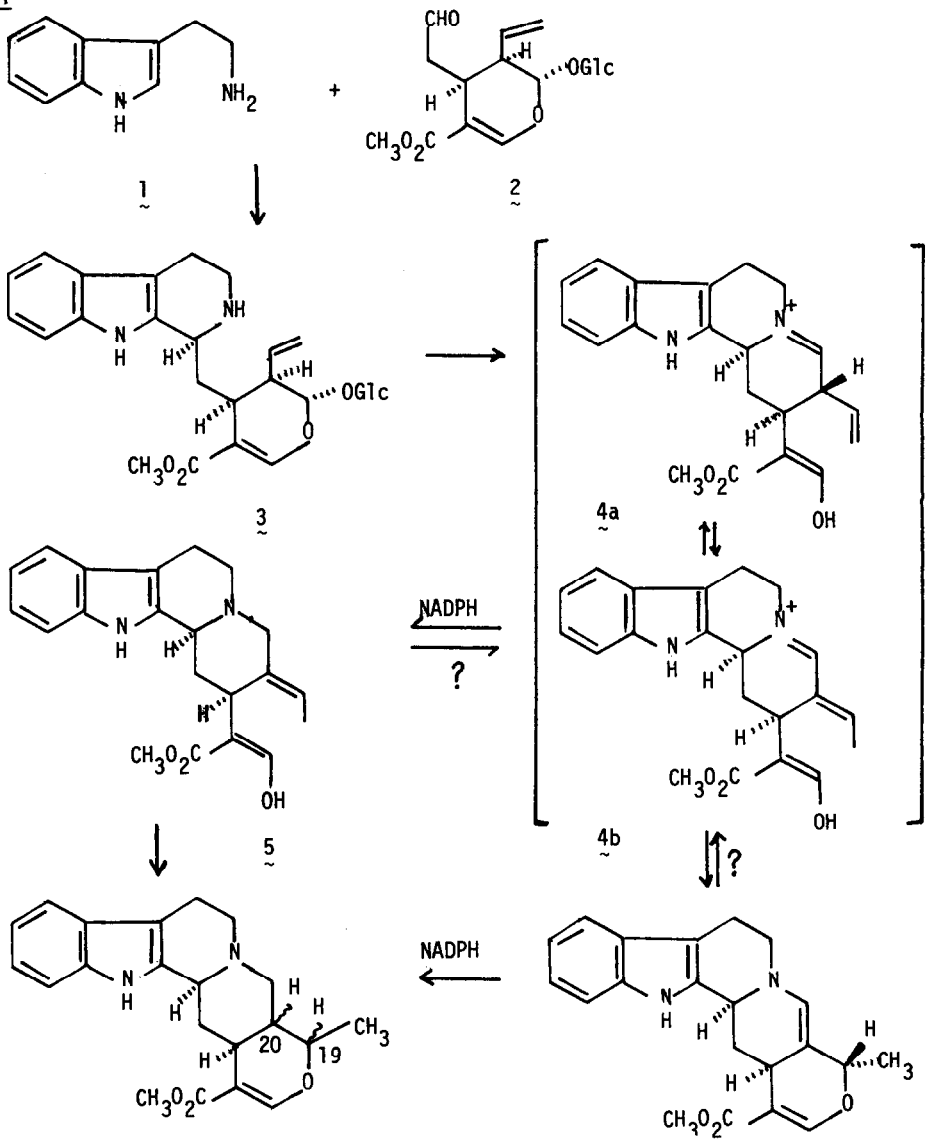
The isolation[1] of a cell-free system from Catharanthus roseus has opened up the study of indole alkaloid biosynthesis in vitro. This cell-free system converts tryptamine (1) and secologanin (2), as well as geissoschizine (5), into ajmalicine (7a). Stöckigt and Zenk[2] later confirmed the conversion of (1) and (2) in to (7a) plus two diastereomeric alkaloids 19-epiajmalicine (7b) and tetrahydroalstonine (7c). They also isolated[3], in an NADPH - deprived enzyme incubation, 20, 21-dehydroajmalicine (cathenamine) (6) and proposed that this compound is a precursor of ajmalicine (7a). Quantitative reduction[3] of cathenamine (6) to tetrahydroalstonine (7c) by $NaBH_4$ requires a stereochemistry of 19β-H as in ajmalicine (7a) and tetrahydroalstonine (7c). In contrast the predominant product obtained[2] in an enzymic incubation of tryptamine and secologanin is 19-epiajmalicine (7b), a 19α-H alkaloid never before reported in C. roseus. To clarify the intermediacy of geissoschizine (5), which has been already supported by in vivo[4] and in vitro[1] experiments, we have carried out a time-dependent study of the incorporation of a mixture of [2-^{14}C] tryptamine and [aryl-^{3}H]-geissoschizine (5) into ajmalicine (7a) in the cell-free system[1] (Scheme 1). The results are shown in Figure 1.

The data clearly demonstrate the intermediacy of geissoschizine (5) in the biosynthesis of ajmalicine (7a) and tetrahydroalstonine (7c). No significant amount of 19-epiajmalicine (7b) was formed in these incubations. Cyclization of the E ring thus appears to be carried out by proton attack at C-20 on the si face (→ ajmalicine) or at a much reduced rate at the re face (→ tetrahydroalstonine) generating a new carbonium ion at C-19 which in turn is quenched by the enolate from the si face. Geissoschizine is not converted to ajmalicine in the absence of enzyme(s) (Scheme 2).

SCHEME 1



1 + 2 → 3 → 4a ⇄ 4b ⇄ 5 ⇄ 6 → 7a, 7b, 7c

	H-19	H-20
7a	β	β
7b	α	β
7c	β	α

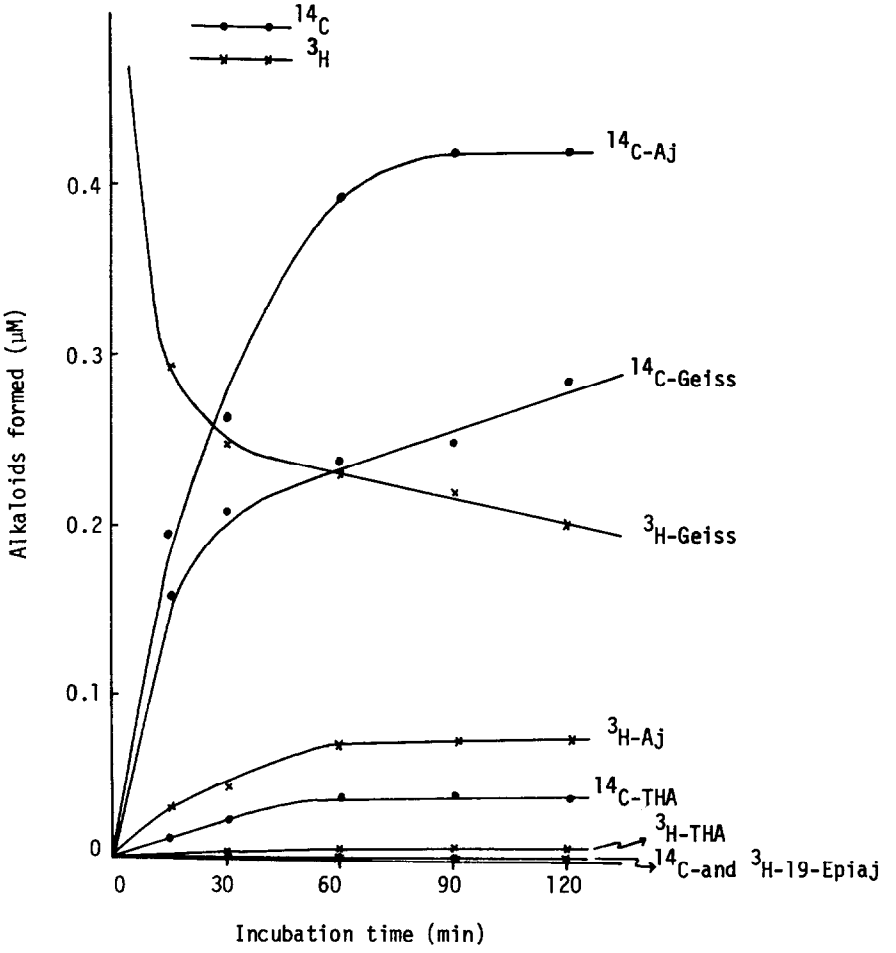
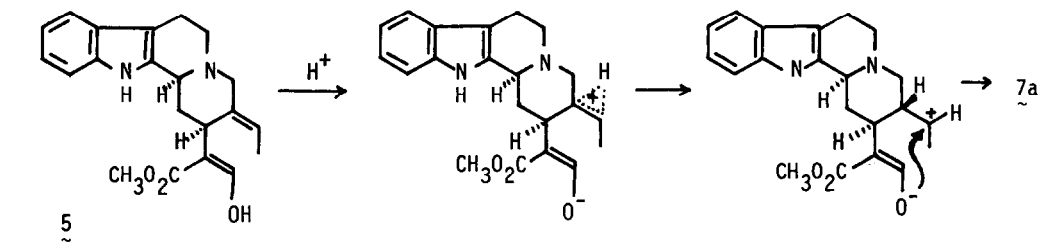


Fig. 1 Incorporation of Geissoschizine and Tryptamine into Ajmalicine and Isomers.

Incubations were carried out with a mixture of 2 μCi [2-^{14}C] tryptamine (4.0 μM) and 15 μCi [aryl-^{3}H] geissoschizine (2.0 μM) at 37°C in 0.05 M citrate buffered solution (pH 7.2) containing 10 mM mercaptoethanol, 20 mM NADPH, 20 mM NADP, and 4.0 μM secologanin. Alkaloids were isolated at intervals and purified by tlc systems ($CHCl_3$/MeOH, 85:15; Hexane/acetone/diethylamine, 7:2:1; $CHCl_3$/acetone/hexane, 5:4:8). Ajmalicine and geissoschizine in each case were recrystallized repeatedly (minimum 4 times) to constant specific activity with the authentic compounds. Abbreviations: Aj = Ajmalicine; Geiss = Geissoschizine; THA = Tetrahydroalstonine; 19-Epiaj = 19-Epiajmalicine.

SCHEME 2



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The lower incorporation of [^{3}H]-geissoschizine into ajmalicine and its low abundance in the plant might reflect its role as an enzyme-bound intermediate, rapidly converted to other alkaloids.

This experiment does not disprove the intermediacy of cathenamine which, as an equilibrium product of the immonium species 4b, could be converted to ajmalicine and tetrahydroalstonine. However we cannot account for the formation of 19-epiajmalicine which Zenk[2] and we also observed, since no isomerization of ajmalicine to the other two isomers occurs and oxidation of 5 would be expected to lead via 4b and 6 to a detectable amount of 7b in our incubations.

In summary we firmly believe that geissoschizine plays a role in the intermediary metabolism leading to ajmalicine.

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