INDOLE ALKALOID BIOSYNTHESIS

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Abstract — The early and late stages of indole alkaloid biosynthesis in Catharanthus roseus were studied by cell-free enzymes isolated from the plant and its tissue culture. The intermediacy of strictosidine (isovincoside), geissoschizine and other compounds in the formation of ajmalicine is discussed. Vinblastine, the antineoplastic dimeric alkaloid, was synthesized enzymically from a semi-synthetic precursor, anhydrovinblastine.

Although the ultimate goal of biosynthetic studies is the isolation and purification of the enzymes responsible for each of the steps leading to the final product, the development of enzymological studies in plants has been much slower than had been anticipated.[1] In this paper we discuss how, in the field of indole alkaloid research, a cell-free system of Catharanthus roseus[2] provided the first entrée into the enzymology of the formation of these biologically important compounds. We shall see that although many steps remain to be defined, the prognosis for the study of the biosynthesis of dimeric alkaloids is now good.

One of the major obstacles in cell-free studies of indole alkaloid biosynthesis to be overcome was the analytical problem of identifying target molecules from an array of more than 80 alkaloids in the mature plant. The second difficulty lay in the rapid inactivation of the biosynthetic enzymes by the phenolic compounds when the cells are ruptured. The tissue culture of C. roseus produces approximately ten alkaloids, mainly of the Corynanthé type, greatly simplifying the analytical problem. At the same time, a low abundance of phenolic compounds contributes to a higher retention of enzyme activity. We believe that these advantageous factors contributed to the disclosure of a

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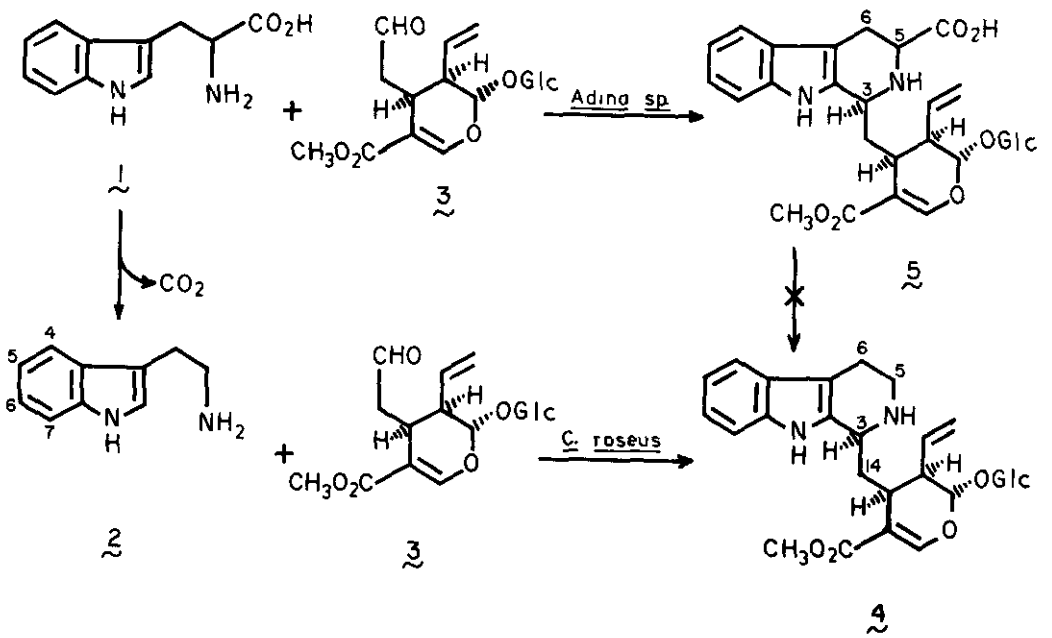
method for the cell-free study in C. roseus in 1975,[2] which not only led to substantial understanding of the early stages of indole alkaloid biosynthesis, but as promising cell lines were selected, provided a burgeoning source of the "later" alkaloids, e.g., catharanthine.[3]

Tryptamine and Secologanin as Precursors

The immediate precursors to the Catharanthus alkaloids are tryptamine (2) and secologanin (3). In whole plant feedings, the fact that tryptamine (2) was less effective than tryptophan as a precursor to the alkaloids[4] and the isolation of 5α-carboxystrictosidine (5)[5] in Adina cordifolia might have led to a premature conclusion that the glycoalkaloid (4) is a decarboxylation product of (5). However, when [^{14}C]-tryptophan and [^{14}C]-tryptamine were incubated with the cell-free preparation, the amount of alkaloids radio-labeled from tryptamine was at least five times higher than for tryptophan, showing that (2) is a more efficient precursor. We suggest that nonspecific and/or specific tryptophan transport and low permeability of tryptamine across the cell membrane are likely reasons for the low incorporation of tryptamine in vivo.



Scheme 1

Incorporation of [2-^{14}C]-tryptamine and [O-C$^{3}H_3$]-secologanin into ajmalicine (6) was 20 to 40 times more efficient with a cell-free enzyme preparation from the callus culture (18%, 28%, respectively) than from the seedlings or mature plants (0.5%, 0.2%, respectively). Inclusion of NADPH was found to promote alkaloid formation in the crude enzyme system and the absolute requirement for this reduced pyridine nucleotide coenzyme was confirmed subsequently.[6]

Secologanin, the iridoid glucose precursor, is derived from mevalonic acid through the well-defined terpenoid pathway. The biosynthesis of this compound has been studied in some detail[7] and will not be discussed here.

Strictosidine (isovincoside)

When tryptamine was condensed non-enzymically with secologanin at pH 4.5, the Pictet-Spengler products were a diastereomer (60%) first called "vincoside", and a minor diastereomer (40%), "isovincoside". Vincoside was found to be incorporated into ajmalicine (6a), serpentine (7), vindoline (8), catharanthine (9) and perivine (10).[8]

However, a later revision[9,10] of the C-3 stereochemistry of vincoside to [4; 3β-H] called for an epimerization mechanism[11] at C-3 to be consistent with the stereochemistry of all derived 3α indole alkaloids.

When the problem was reinvestigated in a cell-free system,[2] independent work in Texas and Wisconsin[12] and at Bochum[13] reached the same conclusion in 1977 that strictosidine (isovincoside) (4), the 3α-H alkaloid,[14] is the key intermediate leading to all the Catharanthus alkaloids bearing a 3α-H as well as other known indole alkaloids bearing 3β-H.[15]

[5-^{14}C,14-^{3}H]-Strictosidine (isovincoside) incubated with the callus enzymes yielded ajmalicine (4.9%), 19-epiajmalicine (1.3%) and tetrahydroalstonine (1.2%) whilst under the same conditions doubly labeled vincoside did not label any of these alkaloids.[12] Incorporation of strictosidine into ajmalicine (6a) (0.149%), catharanthine (9) (0.247%) and vindoline (8) (0.486%) by in vivo feeding confirmed the intermediacy of strictosidine. The results of this experiment are shown in Table 1. (This Table amends and supplements the data published in a preliminary report[12] on this topic.) The Bochum group found that [6-^{14}C] and/or [O-methyl-^{3}H]-strictosidine fed[13] to C. roseus plants labeled ajmalicine (6a) (1.53% - 5.20%), serpentine (7) (0.51% - 1.4%), vindoline (8) (1.31% - 1.90%) and catharanthine (9) (2.08% - 4.51%). Incubation of [2-^{14}C]-tryptamine and secologanin at pH 6.5 in the presence of α-D-gluconolactone which stops alkaloid synthesis at the glycoalkaloid stage yielded[13] only strictosidine. All cell-free systems from Amsonia tabernaemontana, Rhazia

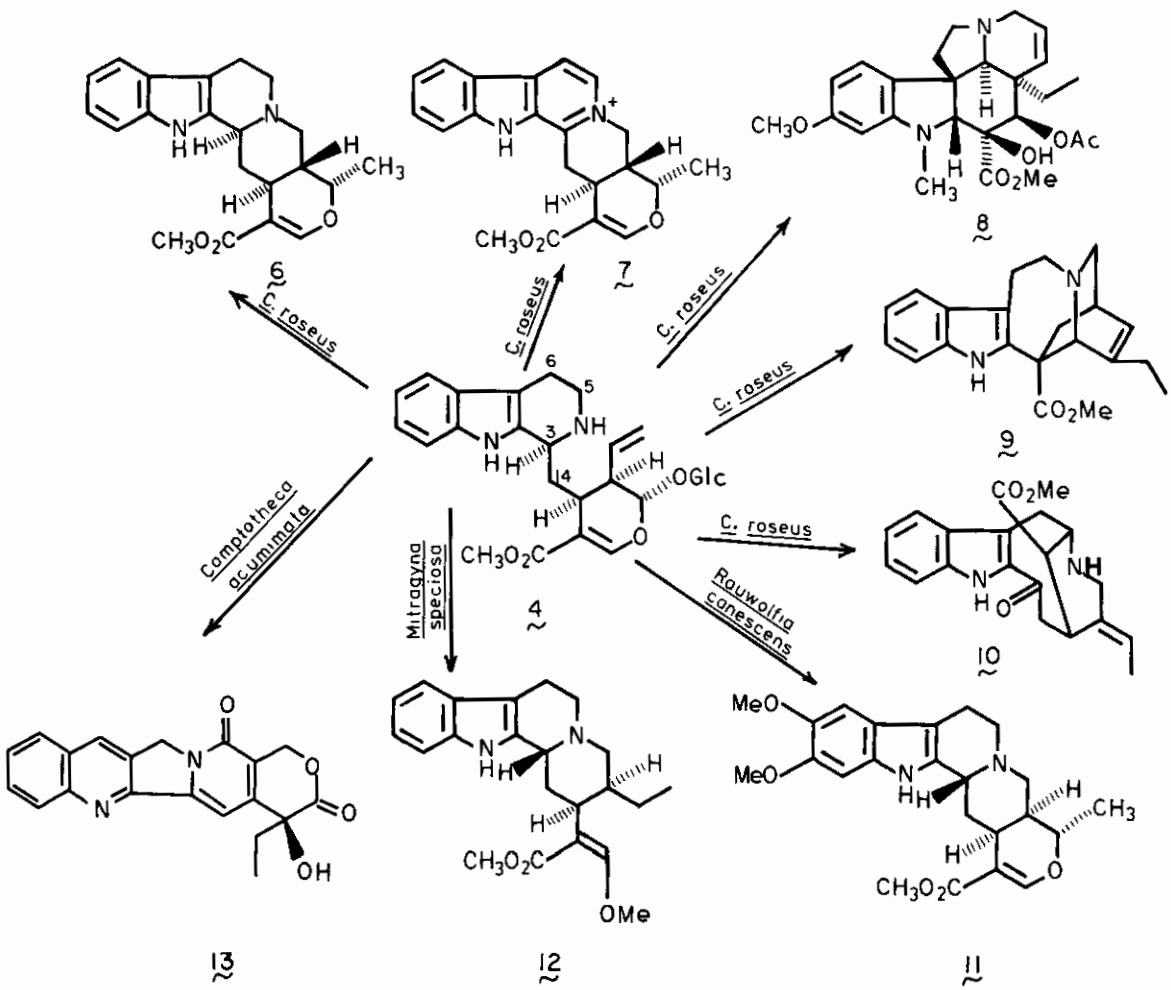
Table 1. Incorporation of Strictosidine[a] into Catharanthus Alkaloids

products	specific radioact ^{3}H	specific radioact ^{14}C	$^{3}H/^{14}C$ ratio[b]	incorpn[c] (%)
Ajmalicine	2.95×10^{4} dpm	2.58×10^{3} dpm	11.4	0.099
Akuammicine	2.62×10^{5} dpm	2.43×10^{4} dpm	10.8	0.93
Catharanthine	1.35×10^{4} dpm	2.45×10^{3} dpm	5.5	0.094
Vindoline	7.42×10^{4} dpm	1.28×10^{4} dpm	5.8	0.49

[a] Radioactivity of [5-^{14}C,14-^{3}H]-strictosidine (isovincoside) is 2.61×10^{6} dpm for ^{14}C and 3.00×10^{7} dpm for ^{3}H (T/C = 11.5), fed for 24 h.

[b] Standard deviation is ca. ± 0.5.

[c] Incorporation was calculated on the basis of ^{14}C.



Scheme 2

orientalis, Rhazia stricta and Vinca minor utilize strictosidine (but not vincoside) as a precursor of the indole alkaloids. 3β-H alkaloids from Rauwolfia canescens (e.g., Reserpiline 11) and Mitragyna speciosa (e.g., speciociliatine 12),[15] and the rearranged indole alkaloid, camptothecin 13,[16] in Camptotheca amninata have been found to be derived from the same intermediate, strictosidine.

The crucial enzyme, strictosidine synthetase, which is presumably present in all plants containing indole alkaloids, has been purified more than 700 times to homogeneity by gel electrophoresis and isoelectric focusing. It has a molecular weight of 38,000 and an isoelectric point at 4.6. Michaelis constants for tryptamine and secologanin are 0.8 mM and 0.5 mM, respectively.[17a]

Hydrolysis of Strictosidine

In an attempt[17b] to purify the "ajmalicine synthetase" system, four β-glucosidases (more correctly, glycosidases owing to their nonspecificity) were found in the seedling and the mature plant, and two in the callus system, as separated by gel filtration (Table 2). Two of the glycosidases

Table 2. Glycosidases from Seedlings and Plants of C. roseus

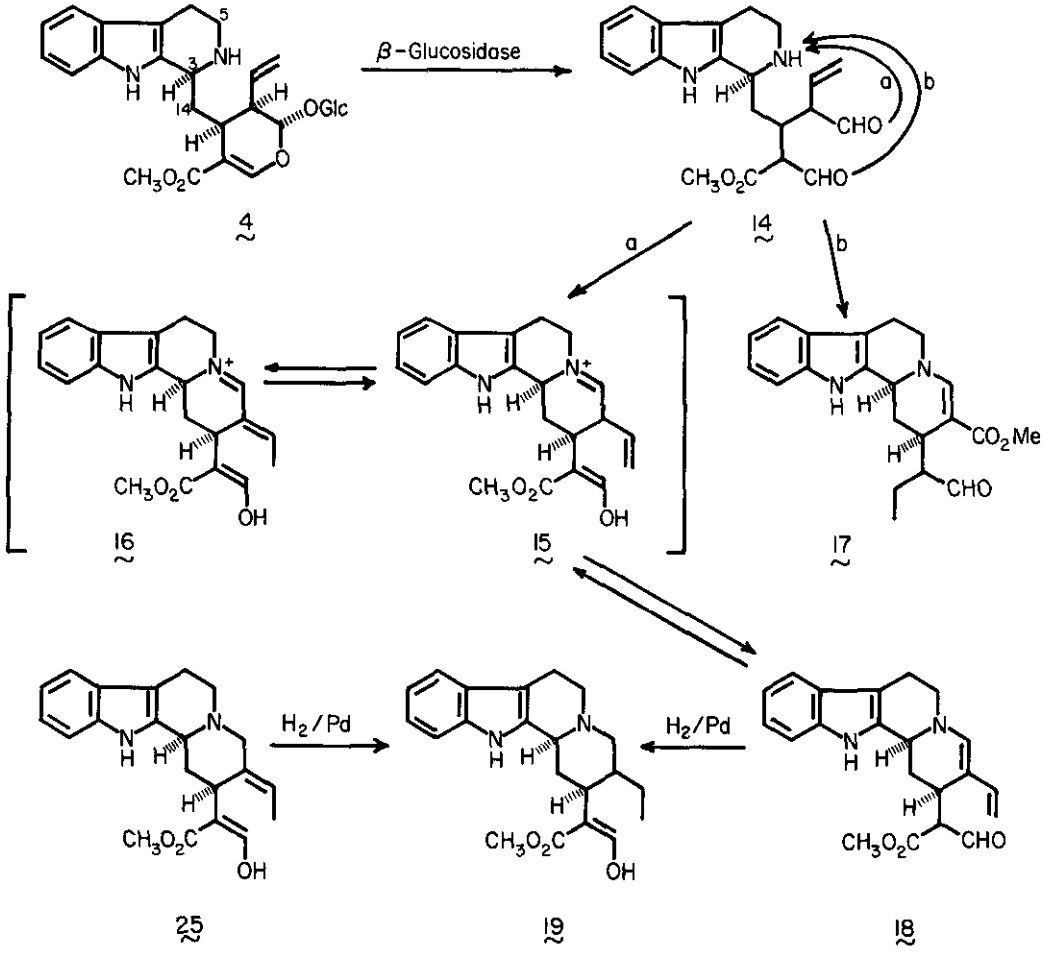
isozyme	mol. wt.	Km value for pNP-β-Glc*	pNP-α-Glc*	pNP-β-Gal*	pNP-β-Fuc*
A	182,000	2.17 mM	6.25 mM	0.125 mM	1.33 mM
B	120,000	0.71 mM	0.95 mM	0.46 mM	2.63 mM
C	55,000	0.51 mM	0.98 mM	0.63 mM	9.10 mM
D	8,000	1.72 mM	12.5 mM	2.17 mM	22.2 mM

*pNP-β-Glc = p-nitrophenyl-β-D-glucoside; pNP-α-Glc = p-nitrophenyl-α-D-glucoside; pNP-β-Gal = p-nitrophenyl-E-galactoside; pNP-β-Fuc = p-nitrophenyl-β-D-fucoside.

(B and C) from the plant were activated slightly by the addition of tryptamine when p-nitrophenyl-glycosides were used as substrates, while the other two (A and D) were not. Present in both the plant and the callus system was a glycosidase of M.W. ~55,000 daltons, which coincides with the alkaloid biosynthetic activity. The significant loss of activity for alkaloid biosynthesis after gel filtration and the later purification of strictosidine synthetase suggest that the "alkaloid synthetase" system is neither a multienzyme complex nor a polyfunctional polypeptide. Rather, the activity obtained on gel filtration was that of a reconstituted heterogeneous mixture of enzymes of similar molecular weights. The proximity of molecular weights of strictosidine synthetase (38,000

daltons) and the glycosidase (55,000 daltons) agrees with this argument. Judging from its activity toward p-nitrophenyl derivatives of β-D-glucose, α-glucose, β-galactose and β-fucose, it is unlikely that this glycosidase is specific towards strictosidine. Further purification and tests for specificity are in progress.

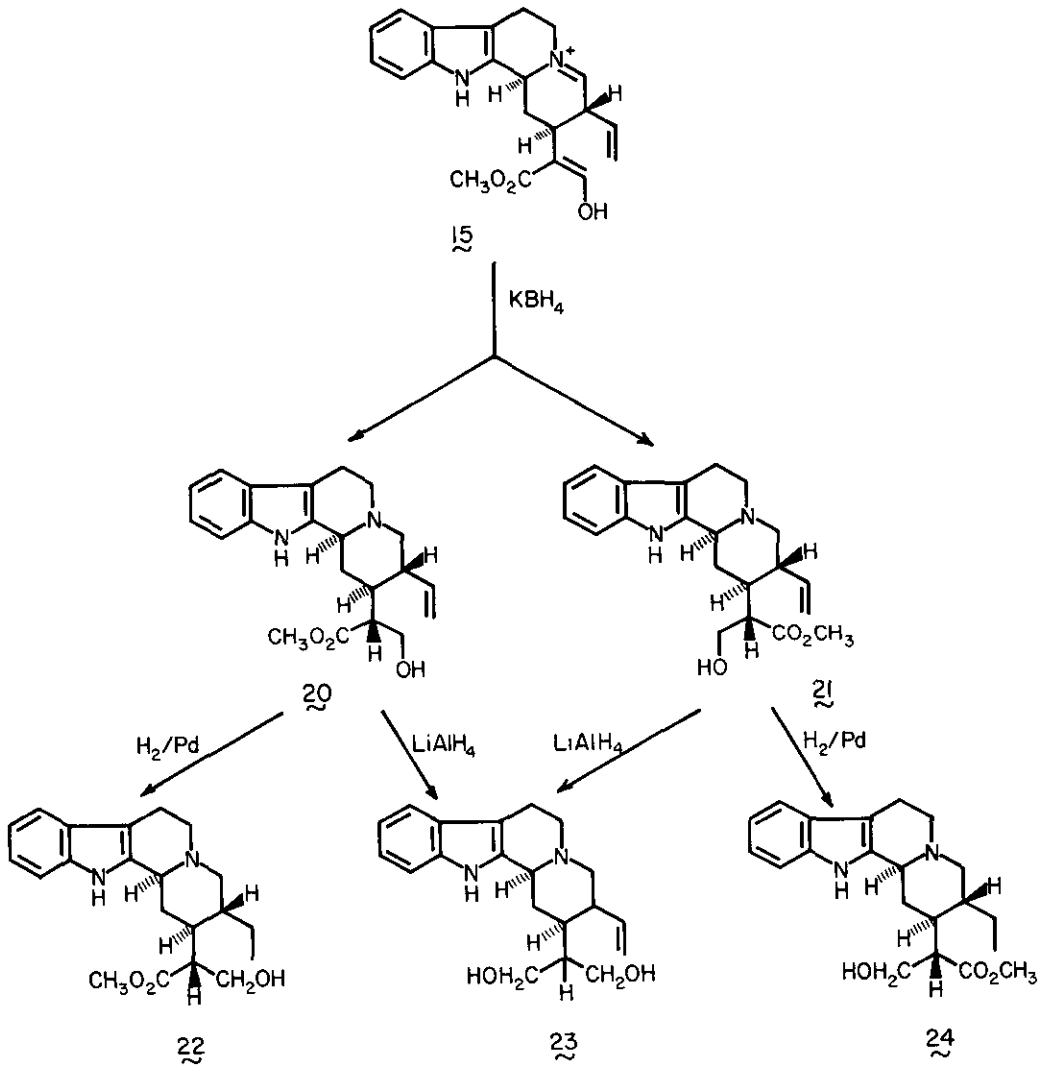
Hydrolysis of strictosidine exposes two aldehyde functions (Scheme 3). In considering plausible



Scheme 3

mechanisms connecting the latter species with the observed products, condensation via pathway a yields an immonium species 15 (presumably in equilibrium with 16). Pathway b gives rise to vallesiachotamine (17) which was found to be a major product on hydrolysis of strictosidine by almond β-glucosidase. The immonium species 15 has not yet been isolated, but an unstable isomerization product dieneamine (18) could be isolated in 2-3% yield by hydrolysis of isovincoside using almond β-glucosidase.[18] Its structure was assigned by reduction (catalytic hydrogenation) to the

tetrahydro compound 19 identical with the reduction product of geissoschizine 25. Further evidence was obtained[19] from incubation of tryptamine and secologanin with the gel-filtered enzyme from C. roseus. In addition to vallesiachotamine (17), a compound was isolated with the following mass spectrometric fragmentation: M^+ 350, 322 (M^+ - CO), 281 (M^+ - COOMe), 247 and 221. The non-polar nature of this compound (R_f = 0.75 Silicagel G/tlc/$CHCl_3$/MeOH, 9:1) suggests the dieneamine (18) rather than the immonium structure (15) for this species. It is of interest to note the recent isolation of 16[19c] from natural sources.



15 20 21 22 23 24

Scheme 4

(After Stöckigt et al 1978)

Stöckigt et al.[20] subsequently identified the immonium compound 15 (which again may be isomerized to 18) by reducing it to the isomeric substances 20 and 21, which in turn were further reduced by catalytic hydrogenation and $LiAlH_4$ to 22, 23 and 24 (Scheme 4).

It is particularly gratifying to record that in spite of some differences (both kinetic and equilibrium) occasioned by the techniques used by the main laboratories engaged in this work, the principal steps of the enzyme-catalyzed reactions now being uncovered are in excellent accord with some of the speculations in this field of a decade ago.[21b]

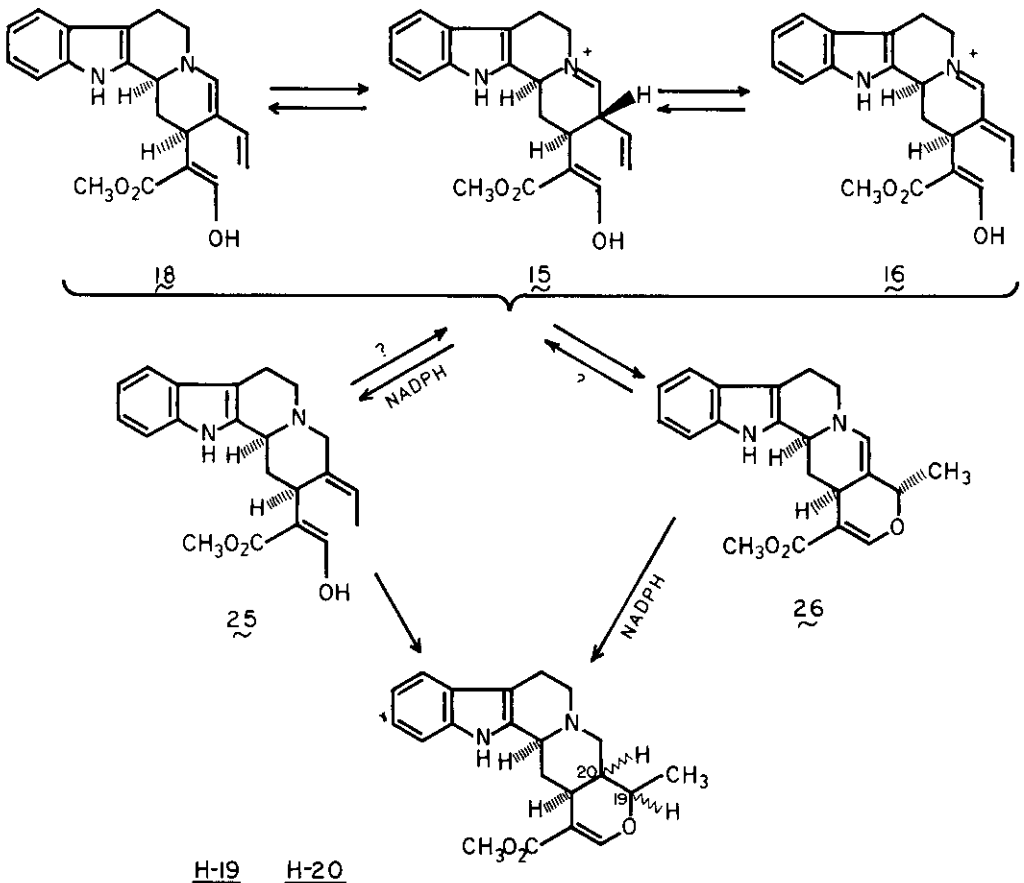
Geissoschizine and Cathenamine

Geissoschizine (25) was first observed to be incorporated into ajmalicine (6a) by *in vivo* feedings[21a-c] and later confirmed by a cell-free study.[2] Geissoschizine was formed from [2-^{14}C]-tryptamine (1%) and [OC$^3H_3$]-secologanin (0.21%) enzymically, and [aryl-^{3}H]-geissoschizine was incorporated into ajmalicine appreciably (7.7%).

Stöckigt et al.[22] isolated, in an NADPH-deprived enzyme incubation, cathenamine (26) (20,21-dehydroajmalicine), identical with material from *Guettarda eximia* (Rubiaceae).[23] This compound on reincubation with the *C. roseus* enzyme and NADPH gave an isomeric mixture of 6a, 6b and 6c in a total yield of 68%. The stereochemistry at C-19 of cathenamine was assigned as S(α-methyl) based on its quantitative reduction to tetrahydroalstonine (6c) by $NaBH_4$. Its formation from 15 has also been considered.[22] However, the formation of 19-epiajmalicine 6b from cathenamine 26 cannot be explained without a reversion to the immonium intermediates, since there is no isomerization observed between the three heteroyohimbine isomers 6a, 6b and 6c. Evidence for the indirectness of cathenamine intermediacy is provided by the following experiments.

To reinvestigate the question of geissoschizine (25) as an intermediate, a time-dependent study was carried out by incubating a mixture of [aryl-^{3}H]-geissoschizine, [2-^{14}C]-tryptamine and secologanin. Ajmalicine and geissoschizine were isolated at two hourly intervals, purified and recrystallized. The results shown in Figure 1 clearly demonstrate an increasing incorporation of tryptamine into geissoschizine and the latter into ajmalacine. Tetrahydroalstonine (6c) was also found to be doubly labeled, but 19-epiajmalicine (6b) was only marginally detectable.[19b]

In a second experiment[19b] [^{14}C]-tryptamine was incorporated into ajmalicine, 19-epiajmalicine and tetrahydroalstonine to the extent of 11.9%, 0.35% and 4.5%, respectively (ratio 1:0.03:0.38), whilst [^{3}H,^{14}C]-cathenamine gave 3.8%, 7.1% and 1.3% (ratio 1:1.9:0.35), similar to that obtained by Stöckigt et al.[6] Thus tryptamine and geissoschizine form ajmalicine and its isomers in a



	H-19	H-20
6a	β	β
6b	α	β
6c	β	α

Scheme 5

"natural" ratio while cathenamine behaves "abnormally" in forming 19-epiajmalicine which has never been reported as a natural alkaloid from C. roseus. The isolation of this compound from Corynanthé mayumbensis[24] nevertheless demonstrates its presence in Nature. The situation is further complicated by a recent publication describing the cell-free conversion of geissoschizine to 19-epiajmalicine.[22b]

The incorporation of geissoschizine into ajmalicine was found to fluctuate over a wide range between 5-10%, and occasionally (for unknown reasons) up to 70%.[19a] The low incorporations of tryptamine into geissoschizine (compared with ajmalicine) and of geissoschizine into ajmalicine (compared with tryptamine as substrate) are presumably a result of enzyme-bound geissoschizine being rapidly turned over to ajmalicine.

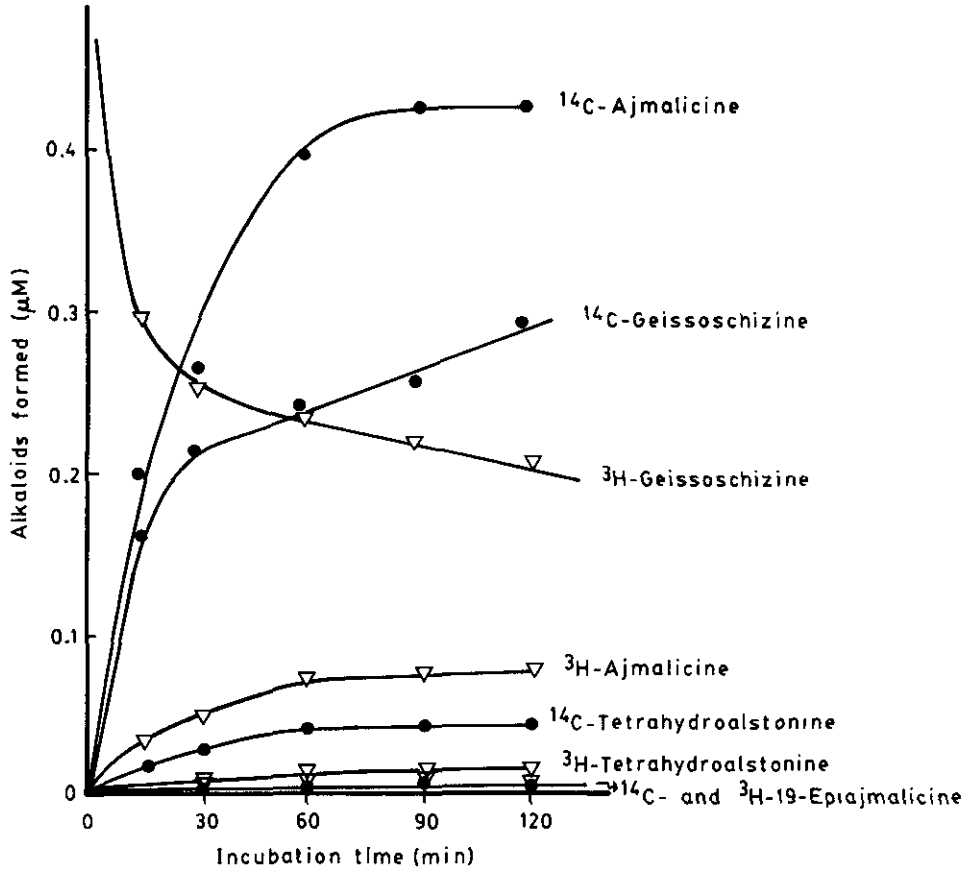
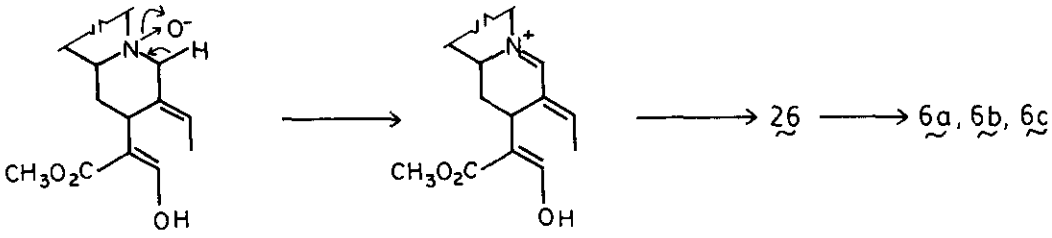


FIGURE 1. Incorporation of geissoschizine and tryptamine into ajmalicine and isomers. Incubations were carried out with a mixture of 2 μCi [2-^{14}C]-tryptamine (4.0 μM) and 15 μCi [aryl-^{3}H]-geissoschizine (2.0 μM) at 37°C in 0.05 M citrate buffered solution (pH 7.2) containing 10 mM mercaptoethanol, 20 mM 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.

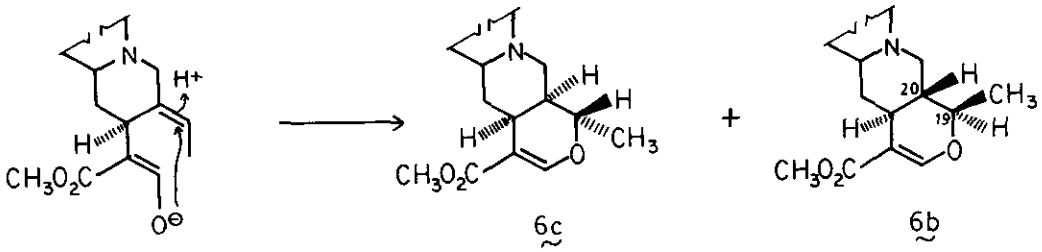
The interconversion of cathenamine and geissoschizine has been examined by incubating labeled geissoschizine with the C. roseus enzymes and trapping the cathenamine formed as its 21-cyano-adduct,[25] or by isolation of geissoschizine with cathenamine as substrate. Neither of these trapping experiments gave completely conclusive results.

Several mechanisms for the conversion of geissoschizine to ajmalicine have been considered (Scheme 6).

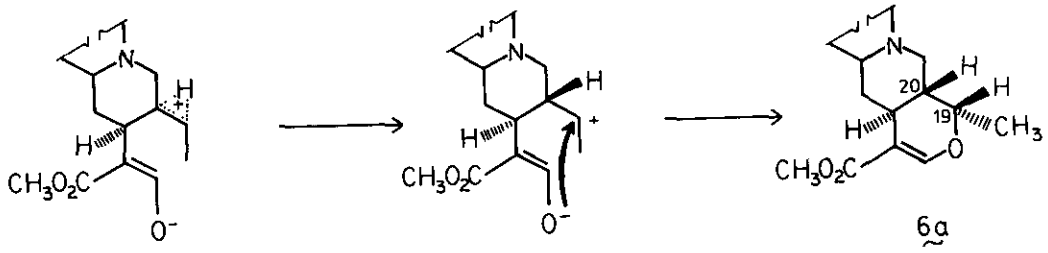
A. N-oxide mediation



B. Concerted mechanism



C. Stepwise mechanism



Scheme 6

The N-oxide mechanism (A) was first postulated to link geissoschizine with cathenamine. The concerted mechanism (B), should give rise to tetrahydroalstonine and 19-epiajmalicine. Mechanism C involves proton attack at C-20 on the si face (→ ajmalicine) or at the re face (→ tetrahydroalstonine). Recent work with ^{2}H labeled geissoschizine[22b] appears to support mechanism A, but in our hands the main product of the reaction is ajmalicine, not the 19-epimer.

The roles of geissoschizine and cathenamine as true intermediates in the natural synthesis of the major alkaloids are still somewhat obscured by the differing results in vivo and in vitro and, as in other pathways, these differences could be explained by involving a metabolic grid or could be due to variations in the cell lines of C. roseus. Further work is obviously needed in this difficult area.

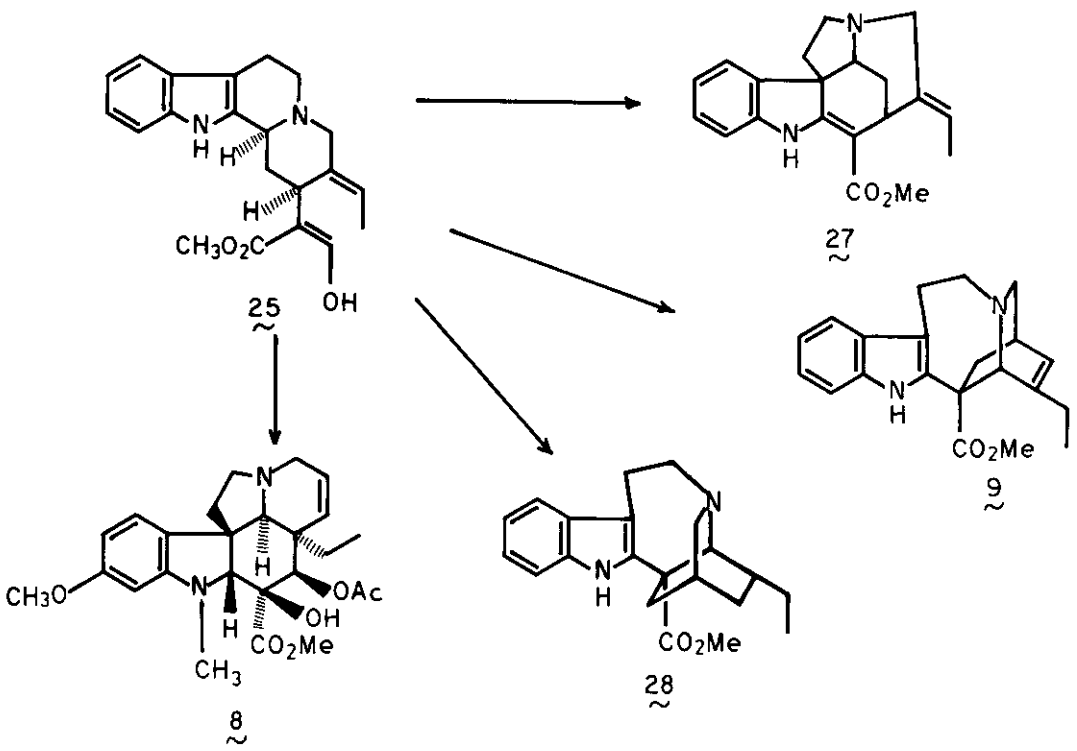
Nonspecificity of the Alkaloid Synthetase System

In spite of the specificity of tryptamine compared with tryptophan, the enzyme system can tolerate substitutions on the indole ring. Thus tryptamine analogs with 4-, 5-, 6-, 7-methyl- and 5-fluoro-substitution were converted to, presumably, ajmalicine analogs which gave red and purple colors (instead of yellow as for the natural Corynanthé alkaloids) with ceric ammonium sulfate spray.[19a] Immuno-positive substances were also detected[26] when 5-fluoro, 6-fluoro-, 5-hydroxy-, 5-methoxy- and 7-methyl-tryptamine were used as substrate. This nonspecificity can be exploited to synthesize alkaloid analogs which might have different pharmacological value.

The Missing Link between Corynanthé and Other Alkaloids

In vivo feeding experiments[21c] have shown that [O-methyl-^{3}H,aryl-^{3}H]-geissoschizine (25) is converted to akuammicine 27 (Strychnos), vindoline 8 (Aspidosperma), and catharanthine 9 (Iboga). [Aryl-$^{2}H_4$]-geissoschizine was also incorporated into akuammicine 27 and coronaridine 28 as shown by mass spectrometric analysis.[27]

Unfortunately, C. roseus tissue culture (until recently) has not produced substantial amounts of the alkaloids beyond the Corynanthé type. Attempts to demonstrate the cell-free conversion of geissoschizine to later alkaloids have been so far unsuccessful with whole plants, thus leaving various hypothetical pathways[21b] untested. This situation has recently been changed by the discovery of Iboga alkaloids in a cell line of C. roseus.[3] Further developments can now be anticipated.



Scheme 7

Vinblastine

A major component of the effort spent on indole alkaloid research has been aimed at an improved yield of vinblastine (VLB; 29) and vincristine (VLC; 30), the two most effective antineoplastic plant products, which have natural abundance at parts per million.

Although vindoline 8 and catharanthine 9 had long been assumed to be the precursors for VLB, *in vivo* feeding results were only marginally positive (0.005%).[28,29] However, the discovery of an unstable dimeric alkaloid, anhydrovinblastine (31), in these feedings suggested the latter as an intermediate. The incorporation of [acetyl-^{14}C]-vindoline into AVLB was increased to 2.63% when a supplement of catharanthine (9) was added to the feeding solution, implying that an enriched catharanthine pool is essential for the isolation of this compound (Table 3). Similar incorporations of vindoline and catharanthine into 31 have been reported by Stuart et al.[38]

Table 3. Incorporation of Vindoline 8 and Catharanthine 9 into Dimeric Alkaloids in C. roseus Plants

expt.	precursor	feeding time, days	alkaloid isolated (% incorpn) anhydro-VLB (31)	VLB (28)	leurosine (32)
1	[aryl-^{3}H]-9[a]	7	0.037	0.005	0.072
2	[-$CO_2C^3H_3$]-9[b]	6	0.031	0.001	0.055
3	[-$CO_2C^3H_3$]-9[c]	9	0.47		
4	[acetyl-^{14}C]-8[d]	7	0.04	0.005	0.13
5	[acetyl-^{14}C]-8 + 9[e]	6[f]	2.63	~0.005	0.50

[a]Prepared by treatment of catharanthine hydrochloride (30 mg) in $^{3}H_2O$ (0.1 mL, 100 mCi)/H_2O (0.09 mL) with $(CF_3CO)_2O$ (0.93 mL) (5°C, 5 h); 14 mg (6.6×10^7 dpm/mg) were fed to 4 shoots of C. roseus followed by isolation and dilution with 32, 29 and 31 and crystallization to constant activity.

[b]Prepared by hydrolysis of catharanthine (KOH-EtOH, reflux, 8 h) and re-esterification (DMF/T_2O/CH_2N_2/Et_2O); 13.6 mg (6.99×10^7 dpm/mg) were administered as described in footnote a.

[c]As in footnote b but longer feeding time.

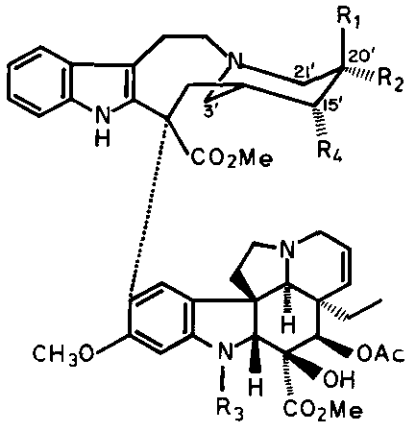
[d]Vindoline (100 mg) hydrolyzed with concentrated HCl (3 mL) (5 min, reflux) and the purified deacetyl compound (21 mg) reacetylated with [1-^{14}C]-Ac_2O (250 μCi) in dry pyridine; 6.5 mg (2.13×10^7 dpm/mg) were administered as in footnote a.

[e]As in footnote d using 11.8 mg (5.71×10^7 dpm/mg) with the addition of catharanthine (11 mg).

[f]Total workup time, 3 h.

Owing to the facile oxidation of anhydrovinblastine to leurosine (32) in air,[30] feeding it to the intact plant proved unfruitful. However, feeding of [21'-^{3}H]-anhydrovinblastine to the cell-free system gave consistent incorporations of between 1.3 and 1.9% into vinblastine.[31] A similar result has also been reported by Kutney et al.,[32] who have developed a cell free extract capable of forming vindoline from tryptamine[32,39].

The transformation of 31 to vinblastine can be regarded as arising by a) direct hydration of the 15',20' double bond, b) oxidation to 32 followed by reductive opening of the 15',20' epoxide, c) reduction to deoxyvinblastine (33) followed by hydroxylation or d) reduction to deoxyleurosidine (34) followed by Polonovski type elimination of the corresponding N-oxide affording the $\Delta^{14',15'}$ enamine which is then oxidized. While sequence d has been carried out chemically,[33] efforts to effect hydration of the 15',20' double bond under a variety of conditions have been unsuccessful.



29	VLB	R_1 = OH, R_2 = Et, R_3 = CH_3, R_4 = H
30	VLC	R_1 = OH; R_2 = Et, R_3 = CHO, R_4 = H
31	AnhydroVLB	R_1 = Et; R_3 = CH_3; R_4 = H; $\Delta^{15',20'}$
32	Leurosine	R_1 = Et; R_2 - R_4 =  , R_3 = CH_3
33	DeoxyVLB	R_1 = H, R_2 = Et; R_3 = CH_3, R_4 = H
34	Deoxyleurosidine	R_1 = Et; R_2 = H; R_3 = CH_3; R_4 = H
35		R_1 = Et, R_3 = CH_3; R_4 = H, $\Delta^{20',21'}$

Scheme 8

Whereas [21'-^{3}H]-leurosine is not incorporated into vinblastine,[34] [aryl-^{3}H]-deoxyleurosidine is *incorporated to the extent of 0.6% by intact plants.*[35] *While the latter result is indicative of* sequence d *in vivo*, it should be noted that [acetyl-$CH_3{}^{14}CO$]-deoxyleurosidine showed approximately half the above incorporation in this system. When [21'-^{3}H,acetyl-$CH_3{}^{14}CO$]-anhydrovinblastine was fed to cell-free preparations a drop in $^{14}C/^3H$ ratio from 1/10 (for anhydrovinblastine) to 1/22 (for vinblastine) was observed.[34] Although the observed lability of the acetyl group of 31 and 34 *in vivo may merely reflect the presence of a non-specific acylase, the possibility of desacetyl* precursors being involved in a biosynthetic grid cannot be ignored. Obviously, further multiple labeled experiments are required to settle this point.

Since vindoline and catharanthine, which are readily available as the major alkaloids of C. roseus, can be chemically coupled by the modified Polonovski reaction and reduced to anhydrovinblastine in good yield,[36,37] the enzymatic hydration of this compound may prove important as a final step in the future production of the dimeric antitumor alkaloids.

EXPERIMENTAL

Preparation of crude enzyme system was carried out as previously indicated.[2] The plant material was homogenized (Omnimixer) with either 0.05 M tris or phosphate buffer at pH 7.0 in the proportion of 1:3 (w/v) and in the presence of 10 mM 3-mercaptoethanol and Polyclar AT (50% by weight of plant). This can be omitted when callus or suspension is used. After centrifuging at 37,000 g for 20 min, the supernatant was used for most of the incubations. When necessary, treatment with active charcoal (5 mg/100 ml supernatant) or gel filtration through Sephadex G25 was used to eliminate alkaloids and cofactors. Fractionation of the glycosidases was carried out by gel filtration through Ultrogel AcA 34.

[Aryl-^{3}H]-geissoschizine was prepared by catalytic isotopic exchange of aromatic hydrogen atoms in tritiated water with trifluoroacetic acid (48 h at 0°C). The tritiated product was purified by preparative tlc. [5-^{14}C,14-^{3}H]-Strictosidine and [5-^{14}C,14-^{3}H]-vincoside were gifts of Dr. C. R. Hutchinson. [2-^{14}C]-Tryptamine was purchased from New England Nuclear. [O-C^{3}H$_3$]-Secologanin was prepared by $C^{3}H_2N_2$ treatment of secologanic acid obtained by careful hydrolysis of secologanin. [^{3}H]-Anhydrovinblastine was synthesized by Polonovski coupling of catharanthine-N_b-oxide with vindoline in the presence of trifluoroacetic anhydride. The product was reduced by $NaBT_4$.[32]

Identifications of alkaloids were carried out by at least two or more of the following chromatographic systems. (1) TLC: (a) $CHCl_3$-MeOH (9:1), (b) ether, (c) acetone-pet. ether-diethylamine (2:7:1), (d) acetone-MeOH-diethylamine (7:2:1), (e) $CHCl_3$-acetone-n-hexane (10:8:1.5), (f) 7% MeOH in $CHCl_3$. Systems (a), (b) and (c) were for general alkaloid separation, (d) for strictosidine and vincoside, (e) for ajmalicine and isomers, (f) for dimeric alkaloids. (2) HPLC: (g) μC_{18}, eluted with MeOH-H_2O (55:45) with 0.14 M $(NH_4)_2CO_3$, for Corynanthé alkaloids, (h) μ-alkylphenyl, MeOH-H_2O (4:1) with 0.14 M $(NH_4)_2CO_3$, for dimeric alkaloids. Except for 19-epiajmalicine and tetrahydroalstonine, which were not available in sufficient quantities, identity of all other product alkaloids was confirmed by repeated co-crystallization with authentic samples to constant specific activity.

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