Strictosidine (Isovincoside): the Key Intermediate in the Biosynthesis of Monoterpenoid Indole Alkaloids

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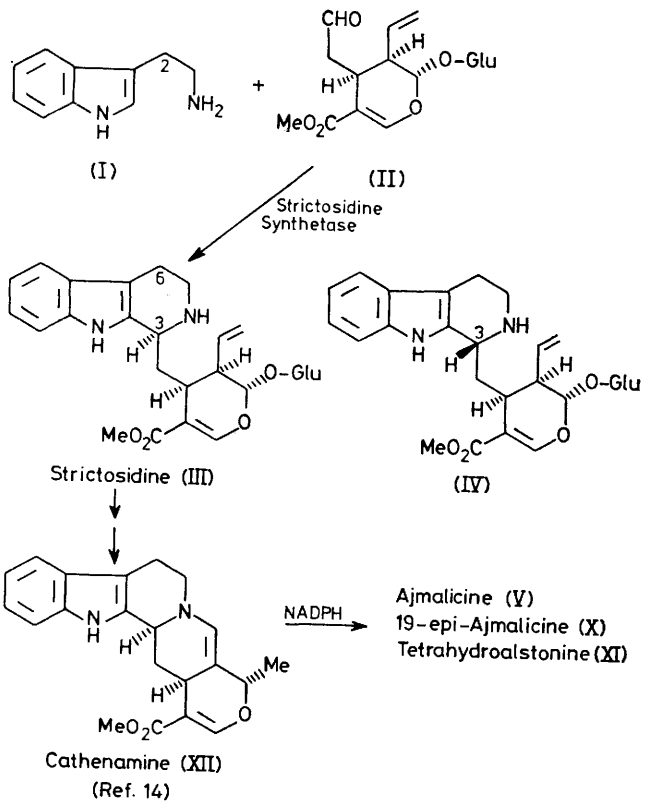
Summary In contrast to previous assumptions the precursor of monoterpenoid *Catharanthus* alkaloids, which arises from the enzymatic condensation of tryptamine (I) and secologanin (II), is 3α(*S*)-strictosidine (isovincoside) (III) and not its 3β(*R*)-epimer vincoside (IV).

FROM biosynthetic studies on indole alkaloids it has been proposed that the exclusive precursor for *Aspidosperma*, *Iboga*, and even for *Corynanthe*-type alkaloids with 3α(*S*) stereochemistry, is the 3β(*R*) epimer[1,2] (IV) and that the subsequent inversion at C-3 occurs with retention of hydrogen.[3] Compound (IV) was originally assumed to have 3α(*S*) stereochemistry[2] but subsequently this was revised to 3β(*R*)[4–6] while its epimer (III) was shown to possess the 3α(*S*) configuration.[4] The revision of the absolute stereochemistry of (IV) to 3β(*R*) was affirmed subsequently by *X*-ray diffraction.[7] The precursor role of (IV) was deduced from feeding experiments,[2] which demonstrated the exclusive incorporation of labelled (IV) into the three classes of monoterpenoid alkaloids in differentiated *Catharanthus roseus* (syn. *Vinca rosea*) plants, while the labelled epimer (III) was not converted under these conditions. The correct stereochemical relationship of the precursor to the products has, however, been a matter of much controversy and confusion.[8] Recently, using a cell-free enzyme system[9] from *C. roseus* cell suspension cultures[10] and (I) and (II) as substrates, we were able to isolate the transient intermediate tetrahydro-β-carboline alkaloidal glucoside and identified it, as (III) with the correct 3α(*S*) configuration[11] required for the biosynthesis of *Corynanthe*-type alkaloids.

An enzyme preparation[9] from *C. roseus* cells was incubated with [2-^{14}C]-(I) and (II) at pH 6·5 in the presence of δ-D-gluconolactone, a glucosidase inhibitor, which blocks[12] the cell-free synthesis of ajmalicine (V) and its isomers and causes the accumulation of the intermediate nitrogenous glucoside.[11] Under these conditions, 40% of the label accumulated as the β-carboline. The incubation mixture was added to a chemically synthesized[2] mixture of (III) and (IV) (390 mg), solvent evaporated off and the residue was acetylated; pure penta-acetates of (IV) (232 μmol; 8 d.p.m. μmol^{-1}) and (III) (172 μmol; 2·57 × 10^3 d.p.m. μmol^{-1}) were isolated. Hydrogenation (Pd–C) of the penta-acetate of (III) (162 μmol) yielded the penta-acetate (VI) which was isolated as needles (m.p. 117—120 °C, lit. 120—121 °C,[2] 74 μmol; 2·34 × 10^3 d.p.m. μmol^{-1}, *m/e* 742 *M*$^+$). The acetate (VI) (32 μmol) was deacetylated (NaOMe) and (VII) (18 μmol; 2·35 × 10^3 d.p.m. μmol)$^{-1}$ was isolated. This dilution analysis shows clearly that (IV), previously assumed to be the intermediate *in vivo*, has no label present, while the 3α(*S*) epimer (III) contained exclusively the label from the β-carboline metabolite.



Direct evidence as to the chemical nature of the intermediate came from a large scale isolation of the intermediate as the lactam. The incubation[11] (37 ml) produced 19·9 μmol of the glucoalkaloid; Na_2CO_3 was added (1·4 M) and the mixture was heated (75 °C, 120 min). The lactam was extracted into ethyl acetate and purified by chromatography [6 mg; R_f identical to authentic strictosamide (VIII)]. It was then acetylated and yielded the tetra-acetate (4·2 mg), *m/e* (80 eV) 666 (M^+), which was unambiguously identified by n.m.r. spectroscopy to be strictosamide tetra-acetate (IX); δ ($CDCl_3$, Me_4Si, 90 MHz) 2·06, 1·98, and 1·87, (9H, s, OCOMe) and the characteristic anomalous acetate signal at 1·23.[13] The n.m.r. spectrum is in every respect identical to that of an authentic sample.[13] Further c.d. spectroscopic evidence also showed the intermediate to be (III); from a large scale enzyme incubation experiment, 7 mg of the β-carboline metabolite were isolated as the penta-acetate. Prior to this it was conclusively shown that no inversion at C-3 occurs during isolation or acetylation, even in the presence of protein, which supports previous findings.[2] C.d. spectra of authentic samples of the penta-aetate of (III) showed λ_{max} 216·6 nm ($\Delta\epsilon$ − 9·8), the penta-acetate of (IV) λ_{max} 217·4 nm ($\Delta\epsilon$ +10·1), and the penta-acetate of the enzymic product λ_{max} 216·8 nm ($\Delta\epsilon$ −9·5). The c.d. curves obtained from the penta-acetates of (III) and that of the metabolite were superimposable. These data, taken together, furnish definite proof for the β-carboline formed in cell-free extracts of *C. roseus* having structure (III).

Cell-free preparations[11] of cell-suspension cultures of other monoterpenoid alkaloids containing *Apocynaceae* species also catalysed the formation of (III), rather than of (IV), from [2-^{14}C]-(I) and (II). The following conversions of (I) into (III) were obtained: *Amsonia tabernaemontianum* 8%; *C. roseus* 71%; *Rhazia orientalis* 14%; *Rhazia stricta* 64%; and *Vinca minor* 17%. In each case the radioactivity was exclusively localized in (III), and the formation of (IV) was never observed. We propose the name strictosidine synthetase for this widely distributed enzyme which catalyses the condensation of (I) and (II) to yield (III).

To prove that (III), which accumulates in cell-free preparations under our experimental conditions, is indeed the natural precursor for monoterpenoid alkaloids its metabolism was studied in *C. roseus in vitro*. Enzymatically formed [6-^{14}C]-(III) (160 pmol) was incubated with a cell-free extract and NADPH,[9] and the formation of ajmalicine (V) (16 pmol), 19-epi-ajmalicine (X) (26 pmol), and tetrahydroalstonine (XI) (6 pmol) was observed. In the absence of NADPH, cathenamine[14] (XII) accumulated (37%). A similar experiment conducted in the presence of NADPH with synthetic unlabelled (III) (81 nmol) as substrate and estimation of the amounts of (V), (X), and (XI) formed using a very sensitive radioimmunoassay technique[15] (lower detection limit 0·1 ng/0·1 ml sample) and antibodies directed against (V), showed that a total of 27 nmol of alkaloids had been formed.

In vivo feeding experiments provided the final confirmation. Enzymatically formed [6-^{14}C]-(III) and chemically[2] prepared [*O*-methyl-^{3}H]-(III) and [*O*-methyl-^{3}H]-(IV) were fed in 1·75 mM aqueous solutions to 30 day old *C. roseus* shoots (with similar results the compounds were fed *via* the root system). The plants used were selected[10] and bred for a high content of *Corynanthe*-type alkaloids. These were identified and analysed by co-chromatography, radioscanning, and by dilution with unlabelled material and crystallisation to constant specific activity. Satisfactory incorporation into representatives of all three families of indole alkaloids was obtained exclusively from the 3α(*S*)-epimer (III) (expt. 1, Table). As evident from the double label experiment (expt. 4), the three types of alkaloids had been biosynthesized without appreciable change in the ratio of labels, which were localized at well separated positions in (III).

It should be noted that if labelled (III) is fed in concentrations of *ca.* 0·1 mM no significant incorporation into alkaloids is observed. Obviously, in low concentration the compound is degraded by unspecific glucosidases, and thus (III) is not available for alkaloid synthesis. This might explain why incorporation of (III) was not previously observed when fed to *C. roseus*.[16] Subjected to high concentrations of (III), the degrading capacity of the tissue is presumably saturated and some quantity of (III) escapes unspecific transformation and is incorporated into the alkaloids, while the epimer (IV), even under these conditions, is not a precursor.

These results prove that the 3α(*S*)-epimer (III) is the necessary intermediate in the biosynthesis of these alkaloids in *C. roseus*. The 3β(*R*)-epimer (IV) is biologically inert in this plant as well as in the related *Apocynaceae*, at least as far as the biosynthetic pathway under examination is concerned. Therefore strictosidine[17] (III) rather than vincoside (IV) occupies the crucial position in the biosynthesis of the monoterpenoid alkaloids.

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TABLE

Incorporations into alkaloids of differentiated *Catharanthus roseus* plants (%)

Experiment	Precursor	Ajmalicine	Serpentine	Vindoline	Catharanthine
(1)	[6-^{14}C]Strictosidine	5·20	0·70	1·80	4·51
(2)	[*O*-methyl-^{3}H]Strictosidine	2·85	0·51	1·90	2·08
(3)	[*O*-methyl-^{3}H]Vincoside	<0·001	<0·001	<0·001	<0·001
(4)	Doubly labelled [^{3}H : ^{14}C] strictosidine; ratio of label 7·4 : 1	1·53	1·40	1·31	—
	Ratio found in alkaloids from expt. 4	7·0 : 1	6·9 : 1	7·3 : 1	—

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