THE ROLE OF ISOVINCOSIDE (STRICTOSIDINE) IN THE BIOSYNTHESIS OF THE INDOLE ALKALOIDS

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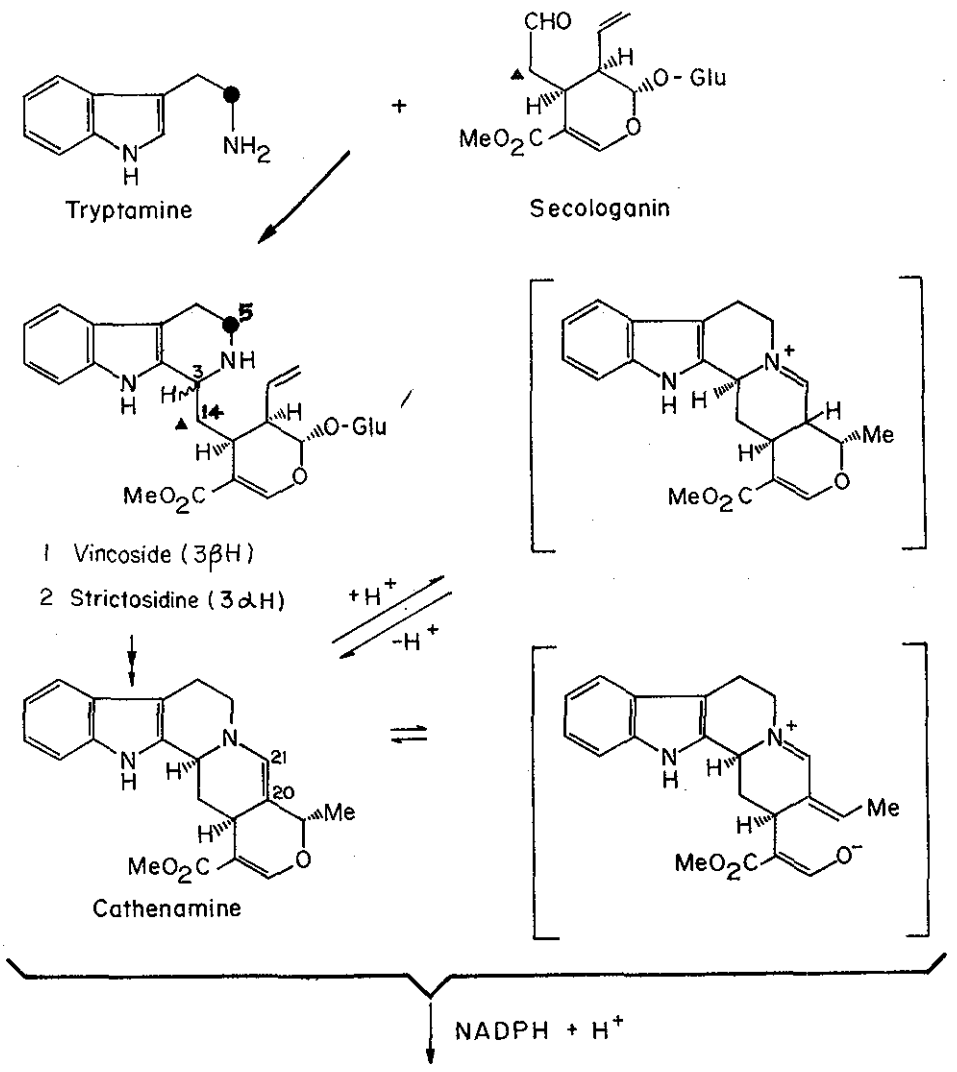
For the last decade it has been assumed[1] that the obligatory precursor for the indole alkaloids of monoterpene derivation is vincoside, the 3β(R) epimer (1) which during conversion to members of the Corynanthé, Aspidosperma and Iboga series in whole plant feeding experiments suffers inversion of the 3β stereochemistry with retention of hydrogen.[2] The situation had been rendered more complex by the revision of the original stereochemistry from 3α(S)(2) to that of the 3β(R) diastereomer (1).[3,4,5] Meanwhile the absolute stereochemistry (1) of vincoside was confirmed by X-ray diffraction.[6] Since no bioconversion of the 3α-isomer (2) to the more complex alkaloids could be observed[1] in differentiated Catharanthus roseus plants, the assumption was made that vincoside represents the pivotal intermediate for the three major classes of indole alkaloids in Nature.

With the advent of a cell-free system from C. roseus callus[7] the problem could be reinvestigated in vitro. We have found that incubation, with the previously described system, of synthetic samples of [5-^{14}C, 14-^{3}H]-vincoside (1) and isovincoside (2) under identical conditions (See Table 1) reveals that the Corynanthé alkaloids ajmalicine (3), 19-epi-ajmalicine (4) and tetrahydroalstonine (5) are formed exclusively from isovincoside (=Strictosidine[4] 2) and



		19-H	20-H
Ajmalicine	3	β	β
19-epi-Ajmalicine	4	α	β
Tetrahydroalstonine	5	β	α

that no radioactivity can be detected when vincoside (1)is used as a potential precursor.

In order to compare these results with previous experiments in whole plants, the experiments were repeated with [5-^{14}C, 14-^{3}H], (1) & (2) in aqueous solution feedings to 18 day old C. roseus shoots. The results of these experiments (Table 2) leave no doubt that isovincoside (2) is indeed the precursor of the

TABLE 1

Cell-free Conversion of Isovincoside/Vincoside
C. roseus Alkaloids

	Ajmalicine	19-epiajmalicine	tetrahydroalstonine
Isovincoside* (T/C = 13.3)	T/C = 12.8 % incorp. = 4.9	T/C = 13.3 % incorp. = 1.3	T/C = 13.2 % incorp. = 1.2
Vincoside** (T/C = 11.06)	---***	---	---

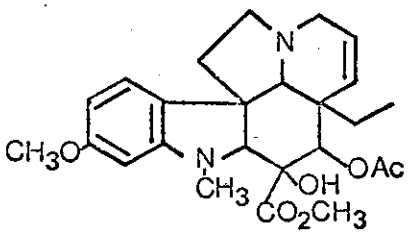
* Sp. act. of [5-^{14}C, 14-^{3}H]isovincoside = 1.330mCi of ^{3}H/0.10 mCi of ^{14}C/mmole,

** Sp. act. of [5-^{14}C, 14-^{3}H]vincoside = 2.72 mCi of H/0.246 mCi of ^{14}C/mmole,

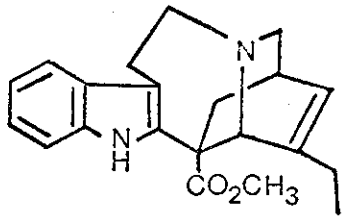
*** Background counts only.

Incubation contained 1.75 mg protein (from callus 11 days after transfer), 1 mg of doubly labeled isovincoside or vincoside, 3 mg NADPH in a total volume of 7 ml of 0.1 M sodium phosphate buffer at pH 7.0 containing 10 mM 2-mercaptoethanol. Incubations were carried out at 34°C for 2 hrs.

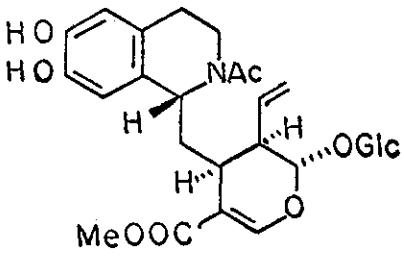
major alkaloids ajmalicine (3), vindoline (6) and catharanthine (7), representing the Corynanthé, Aspidosperma, and Iboga families respectively, and that with both cell-free and whole plant systems vincoside (1) is not metabolized to the natural alkaloids of C. roseus. While this work was in progress similar results



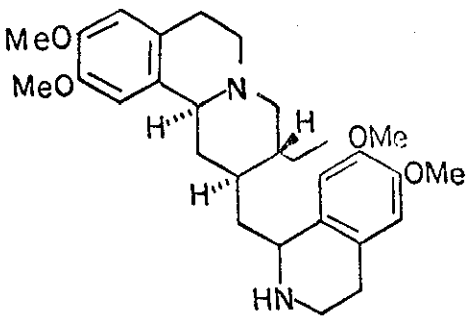
6 VINDOLINE



7 CATHARANTHINE



8 Ipecoside



9 Emetine

TABLE 2

Feeding of Isovincoside/Vincoside to
3-Week Old C. roseus Seedlings

Isovincoside*			
(T/C = 13.3)	T/C = 13.3	T/C = 14.1	T/C = 14.1
	% incorp. = 0.149	% incorp. = 0.247	% incorp. = 0.486
Vincoside**			
(T/C = 11.06)	---***	---	---

* Sp. act. of [5-^{14}C, 14-^{3}H] isovincoside = 1.330mCi of ^{3}H/0.10mCi of ^{14}C/mmole,

** Sp. act. of [5-^{14}C, 14-^{3}H] vincoside = 2.72 mCi of ^{3}H/0.246 mCi of ^{14}C/mmole,

*** Background counts only. $^{+}T/C = {}^{3}H/{}^{14}C$

Three-week-old seedlings were fed with either vincoside (T/C = 1.358 x 10^{7}dpm/1.425 x 10^{6}dpm) or isovincoside (T/C = 4.096 x 10^{6}dpm/8.81 x 10^{4}dpm) for 36 hrs. Uptake of radioactivity was 35% in both cases.

using single labeled vincoside and doubly labeled isovincoside were obtained by Stöckigt and Zenk[8] who have also shown that isovincoside (2) accumulates in a cell-free system when alkaloid synthesis is inhibited by δ-gluconolactone. The revision of the stereochemistry of the central intermediate of indole alkaloid synthesis in Apocyanaceae spp., confirmed independently and simultaneously by differently labeled substrates[8], brings into line the bio-intermediacy of (2) in the formation of camptothecin[9] and also suggests evaluation of the role of ipecoside (8) in the biosynthesis of the Ipecac alkaloids[10] such as emetine (9) at the cell free level, in order to avoid incorporation problems associated with whole plant feeding experiments.

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REFERENCES

1. A. R. Battersby, A. R. Burnett, and P. G. Parsons, J. Chem. Soc. (C) 1969, 1193.

2. A. R. Battersby and K. H. Gibson, J.C.S. Chem. Comm. 1971, 902.

3. A. R. Battersby, A. R. Burnett, E. S. Hall and P. G. Parsons, J.C.S. Chem. Comm. 1968, 1582.

4. K. T. Desilva, G. N. Smith, and K. E. H. Wassen, J.C.S. Chem. Comm. 1971, 905.

5. O. Kennard, P. J. Roberts, N. W. Isaacs, F. H. Allen, W. D. S. Motherwell, K. H. Gibson, A. R. Battersby, J.C.S. Chem. Comm. 1971, 899.

6. K. C. Mattes, C. R. Hutchinson, J. P. Springer and J. Clardy, J. Amer. Chem. Soc. 1975, 97, 6270.

7. A. I. Scott, and S. L. Lee, J. Amer. Chem. Soc. 1975, 97, 6906.

8. J. Stockigt and M. H. Zenk, FEBS Lett, 1977, 79, 233; idem J.C.S.Chem.Comm. 1977,646.

9. C. R. Hutchinson, A. H. Heckendorf. P.E. Doddona, E. Hagaman, and E. Wenkert, J. Amer. Chem. Soc. 1974, 96, 5609.

10. A. R. Battersby and R. J. Parry, J.C.S. Chem. Comm., 1971, 901.

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