Biosynthesis of Indole Alkaloids: Sequential Precursor Formation and Biological Conversion in *Vinca rosea*

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RECENT experiments[1] have defined the monoterpenoid glucoside loganin as an important intermediate in the biosynthesis of the three main groups of indole alkaloid in *Vinca rosea*. A major problem inherent in the remaining unknown steps between loganin and the many alkaloids of *V. rosea*.[2] concerns the timing and mechanism of the transformations whereby the cleaved[3] loganin molecule [as (I)] is sequestered by tryptophan not only to form *Corynanthe* (II—V) and *Strychnos* alkaloids but rearranged to the *Aspidosperma* (VII, IX) and *Iboga* (VIII) templates. We have studied the technique of short-term (1—300 hr.) germination of *V. rosea* seeds on a scale sufficient to allow isolation and identification of possible alkaloidal intermediates between loganin and the major alkaloids of the mature plant such as catharanthine (VIII), vindoline (IX), and ajmalicine (X).

Batches of sterilised *V. rosea* seed were moistened with distilled water and germinated in artificial light at 32°. Preparative t.l.c. of the extracts of aliquots of the seedlings revealed that onset of the formation of recognisable alkaloids could be detected after about 26 hours and that the subsequent appearance and disappearance of alkaloids followed conveniently and reproducibly (Table 1). In each case the identity of the alkaloid was confirmed by mass spectrometry and by direct comparison with an authentic sample. Since stemmadenine (V) was presented in insufficient amounts for complete spectroscopic characterisation its presence was confirmed by the radiochemical dilution method after administration of (±)-[3-^{14}C]tryptophan (50 hr. incubation), addition of authentic (V) and crystallisation to constant radioactivity (0·8% specific incorporation). Significantly, with the exception of ajmalicine, none of the first six alkaloids observed at 0—72 hr. has previously been described as a constituent of mature *V. rosea*[2] and the sequence of their occurrence strongly suggests, but does not prove, the order *Corynanthe* → *Aspidosperma* → *Iboga*. In particular the structure of stemmadenine (V), which appears as the principal alkaloid at 50 hr. between corynanthine (II) and tabersonine (VII), contains an important feature of the rearrangement involving formation of a bond between C* (V) and the α-indolic position while still retaining the unrearranged *Corynanthe* skeleton. Satisfactory mechanisms *via* the labile intermediate (VI) are suggested by *in vitro* experiments[4] leading from (V) to the *Aspidosperma* alkaloid tabersonine (VII) and to catharanthine (VIII) (*Iboga* series). If the germination is allowed to proceed for 240 hr.,

TABLE 1

Sequence of alkaloid formation in V. rosea

Time (hr.)	Alkaloid detected and isolated	Type
0	None	—
28—32	(II)[a], (III), (IV)	Corynanthe
45	(X)	Corynanthe
50	(V)[b]	Corynanthe
72	(VII)[c]	Aspidosperma
168	(VIII)[d], (IX)[d]	Iboga, Aspidosperma

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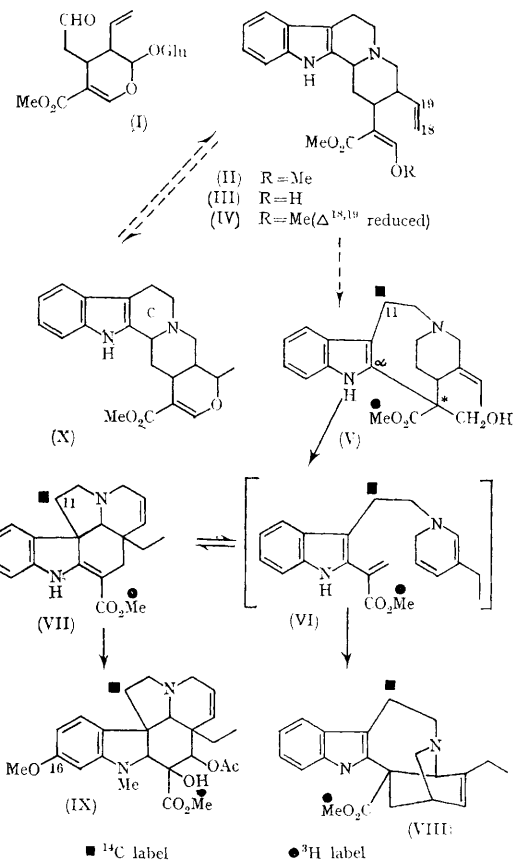
TABLE 2

Precursor	Label	Specific activity (mc/mmole) (^{3}H)	^{3}H/^{14}C %	Alkaloid isolated	Specific incorporation %	^{3}H/^{14}C %
Stemmadenine (V)	*O*-methyl-^{3}H	2·95	—	(VII)	0·27	—
				(VIII)	0·56	—
				(IX)	1·76	—
	O-methyl-^{3}H; 11-^{14}C	4·6	92·8/7·2	(VII)	0·10	92·3/7·7
				(VIII)	0·30	91·8/8·2
				(IX)	0·95	91·9/8·1
Tabersonine (VII)	*O*-methyl-^{3}H	0·52	—	(VIII)	0·80[a]	—
				(IX)	4·80[a]	—
	O-methyl-^{3}H; 11-^{14}C	1·32	95·8/4·2	(VIII)	0·14[b]	95·6/4·4
				(IX)	1·10[b]	96·0/4·0
Catharanthine (VIII)	*O*-methyl-^{3}H	1·50	—	(VII)	<0·001	—

[a] 300 hr. incubation [b] 168 hr. incubation

analysis of the derived mixture of alkaloids reveals a closer resemblance to the content of the 3-month old plant.[2]

Radiochemical evidence in support of this sequence was obtained by administration of alkaloids labelled as shown in Table 2 to germinating seeds, followed by isolation of the appropriate compounds at the times indicated in Table 1. [*O-methyl*-^{3}H]-labels were introduced by treatment of the corresponding acid with diazomethane/tritiated water and 11-[^{14}C] by isolation after feeding (±)-[3-^{14}C]tryptophan to seedlings. Each alkaloid derived from [*O-methyl*-^{3}H]-labelled precursor was hydrolysed to the corresponding inactive carboxylic acid,† showing that all of the radioactivity was retained specifically in the *O*-methyl function. In the case of doubly labelled precursors, the maintenance of the ^{3}H/^{14}C ratio in each case provides further compelling evidence for incorporation without randomisation, or transfer of intact methoxy-groups. Thus the operation of the sequence stemmadenine (V) → tabersonine (VII) → catharanthine (VIII) is clearly demonstrated. Furthermore the irreversible nature of the change from *Aspidosperma* to *Iboga* is indicated by the lack of incorporation of [*O-methyl*-^{3}H]catharanthine into tabersonine. The relatively high specific incorporation (4·8%) of tabersonine into vindoline (IX) after 300 hr., reveals that introduction of the 16-methoxy-, N(α)-methyl, and acetoxy-groups into vindoline occurs at a late stage in the biosynthesis. As a consequence of the above results stemmadenine (V) and tabersonine (VII) can be regarded as probable intermediates in the biosynthesis of *Aspidosperma* and *Iboga* alkaloids in many other species, and the mechanisms



suggested by these and the accompanying *in vitro* experiments[4] used as a working hypothesis[5] for the

† Details of these degradative experiments will be published in the full paper.

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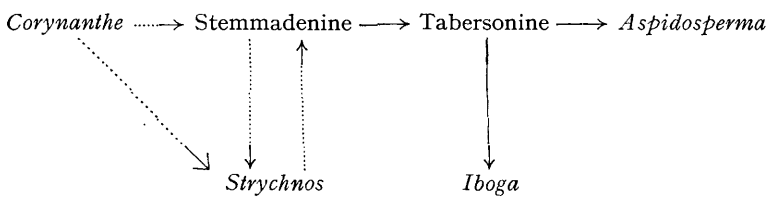

prediction of the biosynthetic interrelationships of all indole alkaloids. Thus, the overall sequential pattern for the *Vinca* alkaloids summarised in the Scheme and extended to include possible connections (– – – →) with the *Strychnos* series can now be tested as a general pathway.‡

Further comment must await appropriate experiments with multiply labelled (III) and (X) but assuming no randomisation of label, a considerable drop in specific incorporation between seedling and plant is evident. These results with mature plants are in close agreement with those

TABLE 3

Specific incorporations of Corynanthe *precursors in* V. rosea

	Incorporation (%)	
Precursor	Seedlings	Plant
(III)-[*O-methyl*-^{2}H]	Corynantheine (13) Catharanthine (0·3) Vindoline (0·1)	Vindoline (0·003)
(X)-[*ring*-C-^{3}H]	Catharanthine (0·3) Vindoline (0·6)	Vindoline (0·004)

Preliminary feeding experiments (with Drs. C. R. Bennett and G. T. Phillips) using [*O-methyl*-^{3}H]corynantheine aldehyde (III) (0·1 mc) and [*ring*-C-^{3}H]ajmalicine (X) (0·1 mc) gave the incorporations shown in Table 3.

of Battersby, Arigoni, and their colleagues (accompanying Communication).

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‡ *Added in proof:* These suggestions together with the incorporation data summarised herein were first adumbrated at the Natural Products Symposium Jamaica, January 1968. More recently Professor J. P. Kutney has confirmed the incorporation of labelled tabersonine into catharanthine and vindoline in *V. rosea* plants. We thank Professor Kutnes for informing us of his results prior to publication (J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson, and D. C. Wigfield, *J. Amer. Chem. Soc.*, 1968, in the press).

[1] A. R. Battersby, R. S. Kapil, J. A. Martin, and Mrs. Lucy Mo, *Chem. Comm.*, 1968, 133; P. Loew and D. Arigoni, *ibid.*, p. 137.

[2] Some 65 alkaloids have been identified in extracts of mature *V. rosea* and *Catharanthus*, see *e.g.* N. R. Farnsworth, R. N. Blomster, D. Damratoski, W. A. Meer, and L. V. Cammarato, *Lloydia*, 1964, **27**, 202.

[3] Review: A. R. Battersby, *Pure Appl. Chem.*, 1967, **14**, 117.

[4] A. A. Qureshi and A. I. Scott, accompanying Communication.

[5] The intermediates and mechanisms derived from these experiments represent an important modification (ref. 4) of Wenkert's original theory (E. Wenkert, *J. Amer. Chem. Soc.*, 1962, **84**, 98; E. Wenkert and B. Wickberg, *ibid.*, 1965, **87**, 1580) particularly with regard to sequence, oxidation level and mechanism, without however detracting from the essential correctness of his views on the interrelationships of the main classes of indole alkaloid.