The Intermediacy of Geissoschizine in Indole Alkaloid Biosynthesis: Rearrangement to the *Strychnos* Skeleton

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Summary Geissoschizine (VI) is proved to undergo rearrangement in *Vinca rosea* plants to form akuammicine (XIII) together with members of the other three major classes of indole alkaloids.

REPRESENTATIVES from the three major classes of indole alkaloids, *viz.* the *Corynanthe* (*e.g.* VIII), *Aspidosperma* (*e.g.* X) and *Iboga* (*e.g.* XI) families, have been shown[1] to be biosynthesised in *Vinca rosea* plants from vincoside having the β-carboline skeleton (I). This biological conversion could reasonably involve cleavage of glucose to form vincoside aglucone (II) which should be in equilibrium with, or convertible into, the aldehydes (III). Ring-closure to N(b), see (IV), and reduction could then lead to corynantheine aldehyde (V) and/or geissoschizine[2] (VI). Current thinking[3–5] on the subsequent stages of the pathway implicates the *Strychnos* skeleton (*e.g.* XII) as an important intermediate, yet no biosynthetic studies had been carried out on the *Strychnos* system using precursors beyond geraniol. Akuammicine (XIII) was therefore selected for our present work to represent the *Strychnos* group; it is present in *Vinca rosea*.[6]

Geissospermine[2] (VII) was cleaved to yield geissoschizine (VI) which was converted into [*Ar*-^{3}H]geissoschizine by exchange with [^{3}H]-trifluoroacetic acid. The rigorously purified product was fed to *V. rosea* shoots and led to the incorporations reported in the Table (Expt. 1). A further portion of the labelled geissoschizine was mixed with ajmalicine (VIII), catharanthine (XI), and akuammicine (XIII). Re-isolation of these alkaloids and purification gave the blank "incorporations" reported as Expt. 2. A fresh sample of geissoschizine was labelled as before and the product was purified by a different sequence. This was administered in buffer to *V. rosea* shoots and the incorporations are collected as Expt. 3. A second blank run was carried out which checked the entire experiment including the period in buffer (Expt. 4).

Treatment of geissospermine (VII) with [^{3}H]methoxide in [*methyl*-^{3}H]methanol yielded [O-*methyl*-^{3}H]geissospermine which was cleaved. The [O-*methyl*]geissoschizine formed was mixed with [*Ar*-^{3}H]-labelled material to give [O-*methyl*-^{3}H; *Ar*-^{3}H]geissoschizine having the ratio O-*methyl* label : total [^{3}H]-label of 1 : 2·25. Again, good incorporations were observed into all four alkaloidal types (Expt. 5)

Incorporation into alkaloids of Vinca rosea

Expt.	Precursor (or blank experiment)	Ajmalicine (VIII)	Serpentine (IX)	Vindoline (X)	Catharanthine (XI)	Akuammicine (XIII)
1	[*Ar*-^{3}H]Geissoschizine (VI)	0·12	0·58	0·13	0·21	0·63
2	Blank for Expt. 1	Inactive			Inactive	<0·04
3	[*Ar*-^{3}H]Geissoschizine (VI)	0·11	0·65	0·14	0·31	0·84
4	Blank for Expt. 2	Inactive			Inactive	<0·05
5	[O-*methyl*-^{3}H; *Ar*-^{3}H]Geissoschizine (VI); ratio of labels 1 : 2·25[a]	0·12	0·82	0·41	0·47	2·0
6	Ratio found in alkaloids from Expt. 5	1 : 2·1	1 : 2·1	1 : 1·7	1 : 2·0	1 : 2·25
7	[*Ar*-^{3}H]Vincoside (I)	0·95	1·6	0·11	0·35	0·76

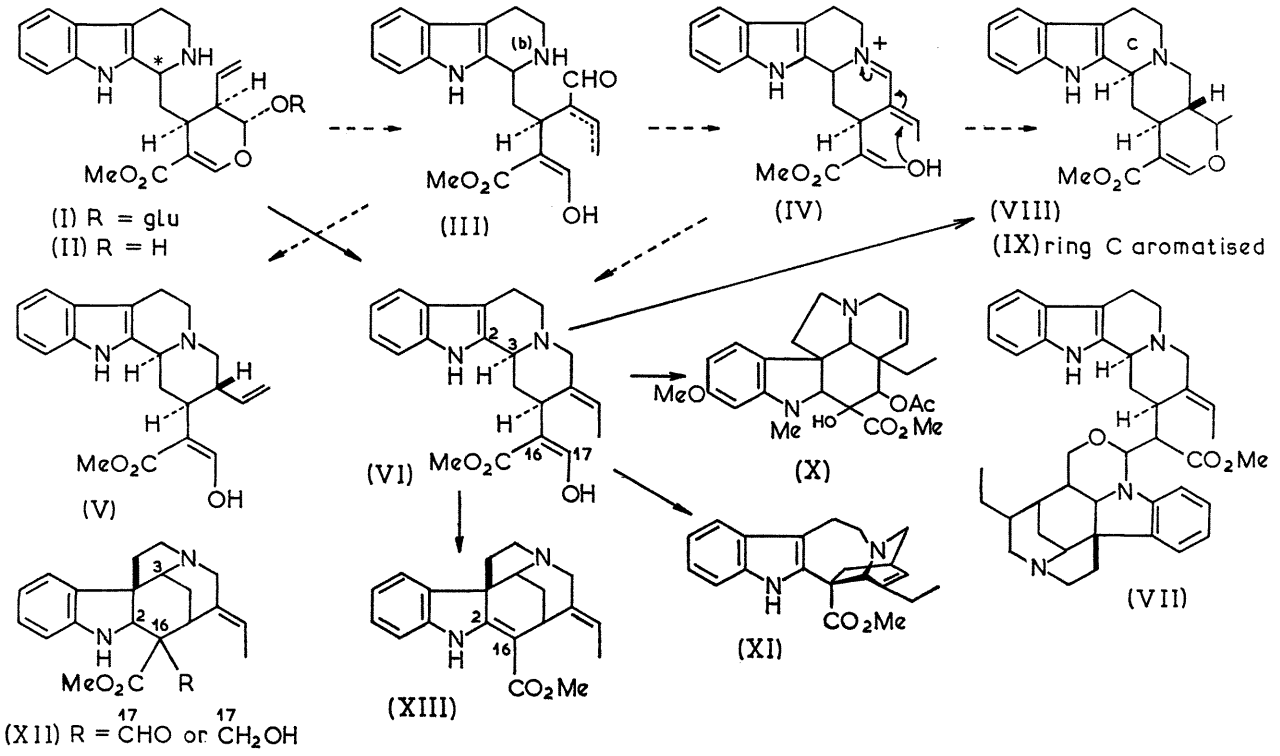
[a] Ratio reported is *O*-methyl label : total ^{3}H label.

Earlier experiments[4,5] established that corynantheine aldehyde (V) does *not* act as precursor of (VIII), (XI), and (X) in mature *V. rosea* plants. We now outline studies with geissoschizine (VI) and the β-carboline system (I) in relation to all four alkaloidal types (VIII), (X), (XI), and (XIII).

and the ratio of labels in the isolated alkaloids was determined as earlier by degradation[1] (Expt. 6). The significant loss of tritium when geissoschizine is converted into vindoline (X) is of interest in relation to the "NIH shift"[7] and is being further investigated.

The foregoing results prove: (a) the intact incorporation

of geissoschizine (VI) into the *Corynanthe*, *Aspidosperma*, and *Iboga* families (b) the rearrangement[8] ($\alpha \rightarrow \beta$) of geissoschizine to form the *Strychnos* skeleton of akuammicine (XIII). Particular importance is attached to (b) since this rearrangement generates the bond between C-2 and C-16 (see VI and XII) which is present in the rearranged *Aspidosperma* and *Iboga* skeletons.



Interlocking evidence on the $\alpha \rightarrow \beta$ rearrangement came from feeding the plants with a mixture of [*Ar*-^{3}H]vincoside and [*Ar*-^{3}H]isovincoside[1,9] (I, epimers at starred centre). Good incorporations were observed as earlier[1,9] (Expt. 7) into (VIII), (IX), (X), and (XI). Further, the β-carboline system (I) is shown now to act as precursor of akuammicine (XIII). The higher incorporations of (I) relative to those from (VI) into (VIII) and (IX) are understandable in that (IV) is expected as an intermediate between (I) and ajmalicine (VIII) (see arrows on IV). Geissoschizine (VI) can enter this path only by reversal of the reductive step which is necessary to form it from (IV) or its equivalent.

The incorporation of corynantheine aldehyde (V) into (X) and (XI) has been reported in *V. rosea* seedlings,[4] in sharp contrast to the insignificant incorporations observed with this substance in mature plants.[4,5] Degradations and/or multiple labelling experiments will be necessary to decide whether the incorporation in seedlings is specific. However, it is possible that seedlings are able to convert (V) into (VI).

Geissoschizine has not been isolated from *V. rosea* and it was therefore sought by dilution analysis in plants which had taken up [5-^{3}H]loganin.[9,10] The radioactive geissoschizine isolated (1·3% incorp.) was shown to be radiochemically pure by conversion into two derivatives having the same constant molar activity. A large scale extraction of *V. rosea* plants yielded crystalline geissoschizine identified by direct comparison. Both requirements for a true precursor are thus met and geissoschizine (VI) stands as a key *Corynanthe* alkaloid beyond vincoside on the biosynthetic pathway.

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