The Mechanism of Indole Alkaloid Biosynthesis

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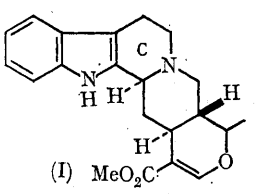
THE non-tryptamine units of the three main types of indole alkaloid, exemplified by (I), (II), and (III), are all derived from geraniol[1] (IV) *via* the key intermediate loganin[2] (V). In a plausible scheme, the putative seco-loganin[3] (VI) is considered to condense with tryptamine, initially[4] to form (VIII) from which the *Corynanthe* and *Strychnos* types [as in (IX and (X)] can be derived by reasonable steps. Suggestions have been made[5,6] for further elaboration to generate the *Aspidosperma* and *Iboga* types. Earlier work proved that rearrangement of the C_{10}-units must occur by an intramolecular mechanism,[3] and quite recently the conversions stemmadenine (XI) → tabersonine (XII) → vindoline (II) and catharanthine (III)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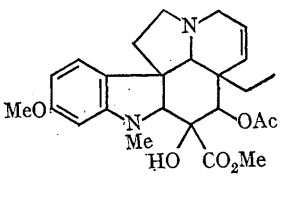
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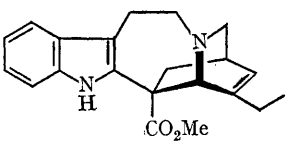
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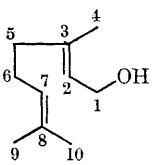
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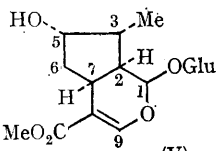
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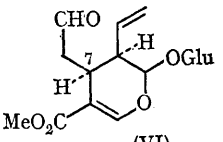
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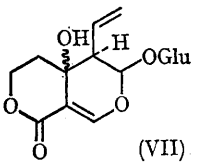
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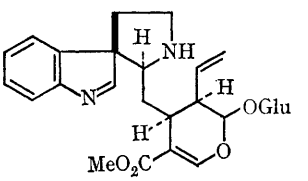

(VIII)

have been demonstrated in *Vinca rosea*,[6] implying a probable role for the hypothetical acrylic ester (XIII). Stemmadenine (XI) and its close relatives could be derived by fragmentation of the indolenine (X; see arrows). We now report evidence from experiments *in vivo* which impose strict requirements on the mechanism of the formation of loganin and its conversion into the three classes of indole alkaloids. The results obtained from the incorporation of various doubly labelled specimens of geraniol and of other substrates into young plants of *V. rosea* are summarised in the Table.

[1-^{3}H]Geraniol [*cf.* IV] was prepared by reduction of geranial with sodium borotritiide; [2-^{3}H]geraniol and [2-^{3}H]nerol were obtained from methyl [2-^{3}H]geranate and methyl [2-^{3}H]nerate derived in turn from methylheptenone and [2-^{3}H]bromoacetic acid. [2-^{14}C]Geraniol and [2-^{14}C]nerol were used in admixture with the ^{3}H-labelled materials as internal standards for the feeding experiments. Expt. 1 (Table) establishes that close to 50% of the ^{3}H activity of [1-^{3}H]geraniol is retained during its conversion into loganin (V) in agreement with stereospecific oxidation of C-1 to the aldehydic state. Further, the ^{3}H label at C-1 of loganin is then retained throughout all subsequent steps leading to the three classes of alkaloid. If stemmadenine (XI) is an obligatory intermediate on the way to (II) and (III),[6] it follows that the required removal of a proton from the starred carbon must occur in a completely stereospecific manner.

No significant loss of ^{3}H occurs from C-2 of geraniol in the course of loganin biosynthesis (Expt. 2) but almost complete loss is observed during the subsequent steps of alkaloid formation for all three types. Similar results were obtained for the alkaloids derived from [2-^{3}H,2-^{14}C]nerol (Expt. 3).

We next established in a feeding experiment with

TABLE

Tracer experiments on Vinca rosea

Expt. No.	Precursor	% Retention ^3H with respect to ^{14}C (or incorpn.) Loganin (V)	Ajmalicine (I)	Serpentine (I, ring c aromatised)	Perivine (XIV)	Catharanthine (III)	Vindoline (II)
1[a]	[1-$^3H_2$,2-^{14}C]Geraniol (IV)	45	44	43	49	48	47
2[a]	[2-^{3}H,2-^{14}C]Geraniol (IV)	95	<5	<5	<5	<5	<5
3[a]	[2-^{3}H,2-^{14}C]Nerol	101	<5	<5	<5	<5	<5
4[b]	Sodium (±)-[4-^{3}H,2-^{14}C]-(3*R*;4*R*)-mevalonate	109	46	—	—	56	57
5[a]	Sodium (±)-[4-^{3}H,2-^{14}C]-(3*R*;4*R*)-mevalonate	98	47	49	50	42	47
6[a]	Sodium (±)-[4-^{3}H,2-^{14}C]-(4*S*)-mevalonate	10 ± 5[d]	<5	<5	<5	<5	<5
7[a]	[*ring*-c-^{3}H]Corynantheine aldehyde (IX; R = H)		<0·001 incorpn.			<0·001 incorpn.	
8[a]	[*O-methyl*-^{3}H]Corynantheine aldehyde (IX; R = H)		<0·001 incorpn.			<0·001 incorpn.	
9[a]	[*ring*-c-^{3}H]Ajmalicine (I)			>1·8[c] incorpn.		0·007 incorpn.	
10[a]	[*O-methyl*-^{3}H]Ajmalicine (I)						0·001 incorpn.

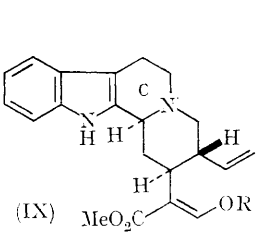
[a] Carried out in Liverpool; [b] Carried out in Zürich; [c] Not corrected for loss of ^3H in biological conversion; [d] Low incorpn. resulting in reduced accuracy.

optically pure sodium [5-^{14}C]-(3*R*)-mevalonate,† that this isomer is a specific precursor of the alkaloids in *V. rosea*. This result allowed sodium [4-^{3}H; 2-^{14}C]-(3*R*:4*R*)-mevalonate to be used safely in admixture with its antipode. It is known that the 3*R*-form of this compound is converted biologically into all-*trans* polyprenols having unchanged $^3H/^{14}C$ ratios.[7] Expts. 4 and 5 show complete retention of ^3H activity through to loganin and then close to 50% retention over the subsequent steps to all the alkaloid types. Since ^3H at C-2 of loganin is consistently lost in alkaloid formation, it follows that ^3H at C-7 of loganin is retained as the alkaloids are formed. When sodium (±)-[4-^{3}H; 2-^{14}C]-(4*S*)-mevalonate was fed, almost all the ^3H was lost (Expt. 6).

From the results of Expts. 2—6 we conclude that: (*a*) The stereospecificity established for the formation of the two geraniol double-bonds in other biological systems[7] also holds good in *V. rosea* (Expts. 4 and 5). (*b*) If saturation of the 2,3-double bond of geraniol is a prerequisite for the formation of loganin, then both the reduction process and the subsequent removal of the proton from C-2 must occur in a stereospecific fashion (Expts. 2 and 3). (*c*) In accord with a previous suggestion,[5] the configuration of loganin (V) at C-7 is determining for the stereochemistry of the corresponding centre in ajmalicine (I), and by extension for all other *Corynanthe* and *Strychnos* compounds (Expts. 2, 3, 4, and 5). (*d*) The recen suggestion[8] that swertiamarin (VII) is an intert mediate for the indole alkaloids is not in accord with Expts. 4 and 5. (*e*) The stereochemical correlation of C-2 of loganin (V) with the corresponding centre of ajmalicine (I) is fortuitous; the observed loss of a proton from this position supports the idea of an enamine intermediate.

We have also tested whether corynantheine aldehyde (IX; R = H) and ajmalicine (I) can undergo the skeletal change required for conversion into the *Aspidosperma* and *Iboga* types. The former precursor was prepared by reduction of dehydrocorynantheine[9] (IX; R = Me, ring c aromatised) with sodium borotritiide followed by hydrolysis[10] of the enol ether group. A specimen of [*O-methyl*-^{3}H]-corynantheine was made by treatment of corynantheic acid[11] with tritiated diazomethane and the corresponding [*O-methyl*-^{3}H] corynantheine aldehyde (IX; R = H) was again derived by hydrolysis. No significant incorporation occurred into the *Iboga*-type alkaloid catharanthine (III) from either labelled form of this aldehyde (Expts. 7 and 8). Further, ring-c labelled ajmalicine (I) and [*O-methyl*-^{3}H]ajmalicine were prepared in ways similar to those used in the corynantheine series. These materials gave only very low incorporations of activity into

† The preparation of this compound as carried out by F. Von der Mühll and U. Trepp will be reported elsewhere.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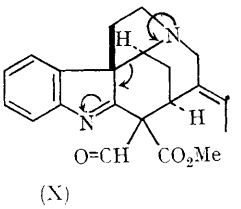
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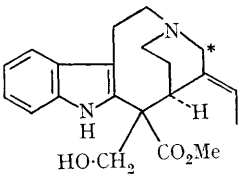
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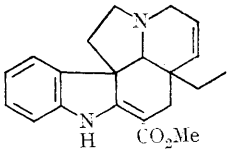
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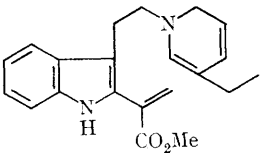
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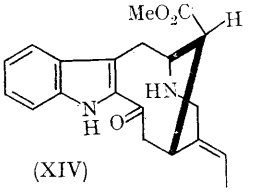
(XIV)

catharanthine (III) and vindoline (II), though satisfactory conversion into serpentine (I; ring c aromatised) was observed (Expts. 9 and 10). Two interpretations of these results are possible: (i) that corynantheine aldehyde (IX; R = H) is a precursor of the *Iboga* and *Aspidosperma* types but is not penetrating to the site of further biosynthesis or (ii) that (IX; R = H) lies beyond the branching point leading to the rearranged systems. These two possibilities also hold for the work with ajmalicine (I). At present (ii) seems the more probable and we are exploring the possibility that the pentacyclic *Strychnos* system is the parent from which the rearranged types are formed.

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