Concerning the Terpenoid Origin of Indole Alkaloids: Biosynthetic Mapping by Direct Mass Spectrometry

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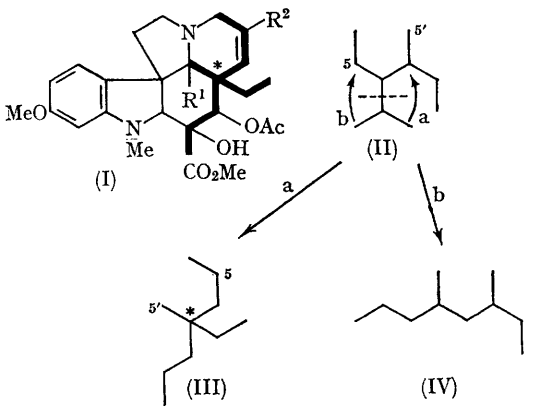
WE have previously shown [1,2] that the Aspidosperma type alkaloid, vindoline (I; $R^1 = R^2 = H$), is of mevalonoid origin, a finding which was independently confirmed in two other laboratories.[3,4] Several theories[2,5] have been proposed to rationalise the intimate details of rearrangement of a "C_{10}" precursor pattern (II) into the *Aspidosperma* [(III) and thickened lines in (I)] and *Iboga* (IV)

skeletons. Recent work with ^{14}C-labelled mevalonates[4] supports the earlier theories[5] summarised in (II) → (III) + (IV).

We have now confirmed certain of these radiochemical experiments by administration of 5-[$^2H_2$]-mevalonic acid lactone (V)[6] to *Vinca rosea* plants, followed by direct mass-spectrometric analysis of the resultant purified deuterovindoline



(I; $R^1 = R^2 = D$). The enrichment data (Table, column 2) obtained by comparison of the spectrum with that of vindoline obtained under identical operating conditions show that both incorporated deuterium atoms are found in the fragments ***P*** (parent molecular ion), (a), (d), and (e).[7] (See Fig. 1). The average enrichment of 1·6% of the (m/e + 2) peaks of these species corresponds to an incorporation of 0·2% of deuteromevalonate (V) into vindoline, whilst the absence of any enrichment in fragments (b) and (c) (the tryptophan-derived segment of vindoline) reveals that, given incorporation of the order of 0·2% and higher, the mass-spectrometric method provides an immediate and reasonably accurate analysis† without recourse

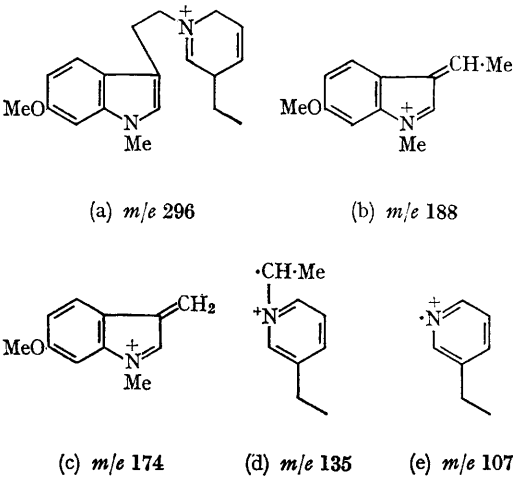


FIGURE 1

Principal fragments of vindoline[7]

to degradative chemistry. The above results are compatible with mevalonoid dimerisation to the C_{10} level (*e.g.*, 1-[$^2H_2$]-5[$^2H_2$]-geraniol; VI) followed by cyclisation to a cyclopentanoid[5] system (as IX), cleavage to (5,5'-[$^2H_4$]-II), rearrangement to the deuterated vindoline skeleton (5,5'-[$^2H_4$]-III) and thence to (I; $R^1 = R^2 = D$). An alternative theory[2] would require enrichment of (m/e + 3) peaks in the fragments *P*, (a), (d), (e) which is not observed.

If the normal biological conversion of mevalonate[8] is followed, the logical precursor of the C_{10} unit would be geraniol. This has now been verified by two independent methods. Thus, when 1-[$^2H_2$]-geraniol (VII) was fed to *V. rosea* the derived vindoline (0·8% incorporation of deuterogeraniol) showed (Table, column 3) an average enrichment of the (m/e + 1) peaks of the ions corresponding to *P*-, (a), (d), and (e) of 1·6%. The (m/e + 2) peaks and fragments (b) and (c) were virtually devoid of enrichment. On the basis

TABLE*. *Enrichment data for deuterovindolines*

Fragmentation (see Fig. 1)	% Enrichment (I; $R^1=R^2=D$)	% Enrichment (I; $R^1=D; R^2=H$)
P + 1	0·00	1·50
P + 2	1·55	0·00
(a) + 1	0·30	1·90
(a) + 2	1·55	0·00
(b) + 1	0·00	0·00
(b) + 2	0·00	0·00
(c) + 1	0·00	0·00
(c) + 2	0·00	0·00
(d) + 1	0·40	1·20
(d) + 2	1·50	0·00
(e) + 1	0·30	1·90
(e) + 2	1·90	0·00

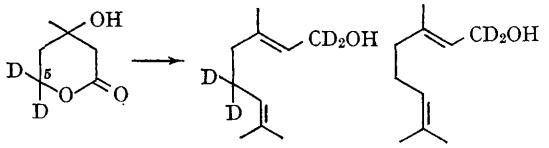
* Spectra measured on an AEI-MS9 spectrometer. Percentage enrichment is measured from the appropriate fragment peak heights in "cold" vindoline run under identical conditions.

† With the important *proviso* that the fragmentation processes under study do not involve extensive hydrogen transfers and that a number of runs are made with "hot" and "cold" samples under identical conditions. The accuracy of the present study was determined by at least 10 runs of each sample to be within 0·3% and suggests an enrichment of at least 1% as the most convenient level for direct-measurement studies using deuterated precursors.

of the previous ^{14}C results with mevalonate, the deuterium atom [which must be attached to one of seven carbons of the fragment (e)] corresponds to R^1.

(VII → 5′-[$^2H_2$]-II → 5′-[$^2H_2$]-III →
I; R^1 = D; R^2 = H)

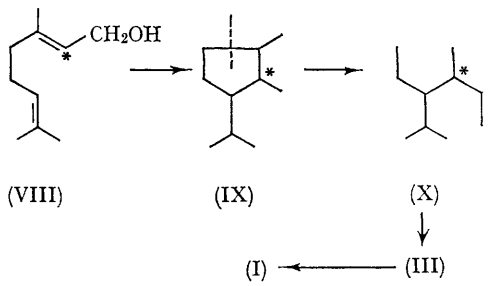
At the same time 2-^{14}C-geraniol (VIII) (0·28 mc) was fed to *V. rosea*. The radioactive vindoline was



(V) (VI) (VII)

degraded by Kuhn–Roth oxidation to a separable mixture of acetic and propionic acids. The entire radioactivity (99·3%) was located in the propionic acid and none in the acetic acid showing that C-2 of the geraniol becomes the starred atom (C-5) in (I) without measurable spread of label. This may be rationalised in terms of current theory[1–4,6] as (VIII) → (IX) → (X) → (III) → (I).

The above results indicate that experiments (now in progress) with various labelled deuterogeraniols should, in principle, lead to complete and rapid evaluation by direct mass spectrometry* of the biosynthesis of indole alkaloids and other terpenoids. Recent, independent investigations by Battersby and by Arigoni (see accompanying Communications) show that both [2-^{14}C]geranyl pyrophosphate and [2-^{14}C]geraniol are specifically



incorporated into several classes of indole alkaloid in accord with the mevalonoid theory.

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