Further Evidence Concerning the Biosynthesis of Indole Alkaloids and Quinine

By A. R. BATTERSBY, R. T. BROWN, R. S. KAPIL, J. A. KNIGHT, J. A. MARTIN, and A. O. PLUNKETT

(*Robert Robinson Laboratories, University of Liverpool*)

EARLIER work[1–3] established that the C_{9-10} unit of the indole alkaloids [thickened bonds in (I—IV)] is of mevalonoid origin. Degradation of the labelled catharanthine (I) and dehydroaspidospermidine gave results[3] in agreement with head-to-tail combination of the two C_5 units and the logical deduction that geraniol is a precursor of the indole alkaloids was proved to be correct for the *Iboga*,[4] *Corynanthe*[4,5] and *Aspidosperma*[4–6] groups of bases. Still further confirmation has recently been added.[7] The labelling patterns were consistent with the annexed scheme[8] in which structures (XI), (XII), and (XIII) represent the C_{10} units of the three alkaloidal families without indication of oxidation level. Further progress requires (a) examination of sufficient positions in the residues (XI), (XII), and (XIII) to establish that the illustrated rearrangements, and only those, occur, and (b) evidence for the intermediacy of cyclopentane monoterpenes. In this and the following Communication we outline experiments bearing on both these points.

Sodium [2-^{14}C]-, [3-^{14}C]-, [4-^{14}C]-, and [5-^{14}C] mevalonates were administered in separate experiments to *Vinca rosea* plants. Degradation of the isolated alkaloids then gave the results collected in the Table. The methods were those used earlier,[3,4] together with cleavage[9] of serpentine (IV) to harman (VI) which was methylated and reduced to *N*(b)-methyltetrahydroharman. Kuhn–Roth degradation then gave carbons 3 and 14 of the alkaloid as acetic acid suitable for Schmidt degradation.

When the tabulated results[10] are combined with our earlier findings,[3,4] they provide almost a complete pattern of labelling for the unit (XI) which is in quantitative accord with the annexed scheme. Further, a bond is proved to be generated between the C-4 carbons of the two mevalonate residues in agreement with cyclopentane ring closure (see X). Finally, the patterns derived so far for the *Iboga* and *Aspidosperma* types (XII) and (XIII) support their formation at some stage from the unit (XI) in the ways illustrated.

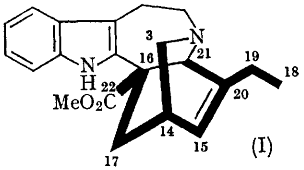
Though specific incorporation of activity from [2-^{14}C]geraniol into ajmalicine (V) had been established,[4] the exact location of the label remained to be determined. The isolation of perivine (III; *Corynanthe* unit XI) from this feeding experiment (0·04% incorp.) allowed the definitive experiment. Hydrogenation of perivine followed by Kuhn–Roth oxidation gave propionic acid (93% of total activity) and radio-inactive acetic acid; essentially all the labelling is thus at C-20 in keeping with the scheme.

The degree of steric specificity at the acyclic monoterpene stage of the biosynthetic process was studied by preparing [1-^{3}H]geraniol and [1-^{3}H]-nerol; g.l.c. showed the former to contain less than 15% of the latter and the latter to contain less than

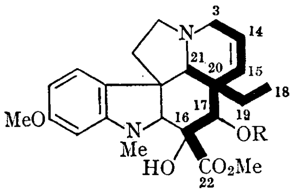
TABLE. *Degradation of labelled alkaloids*

Position of ^{14}C-label in mevalonate	Alkaloid	Position of carbon(s) isolated*	% of total activity Theory	% of total activity Found
2	Serpentine (IV)	3	50	43
2	Ajmalicine (V)	16—17	25	22
3	Catharanthine (I)	18—19	50	44
3	Vindoline (II; R = Ac)	19	50	45
4	Serpentine (IV)	15	50	45
4	Perivine (III)	20	50	44
4	Catharanthine (I)	20	50	48
4	Vindoline (II; R = Ac)	20	50	45
5	Serpentine (IV)	14	50	43

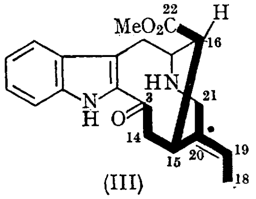
* The numbering scheme is that of J. LeMen and W. I. Taylor, *Experientia*, 1965, **21**, 508.



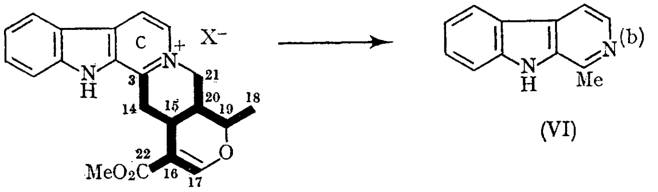
(I)



(II)



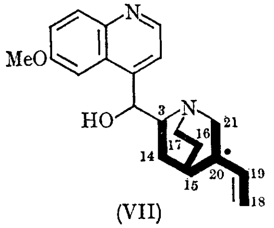
(III)



(IV)

(V) Ring c reduced

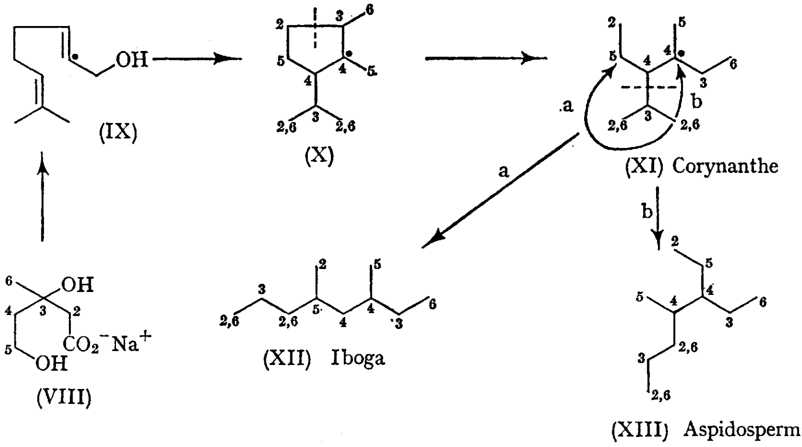
(VI)



(VII)

5% of the former. These were fed separately as emulsions in aqueous Tween to *Vinca rosea* plants to give the following incorporations showing the two isomers to be about equally effective; the geraniol result is reported first in each case, catharanthine (I) 0·25%, 0·21%, serpentine (IV) 0·14%, 0·11%, and ajmalicine (V) 0·05%, 0·03%.

Quinine (VII) and its relatives have long been considered to be derived from indolic precursors[11] and the specific incorporation of tryptophan into quinine has been established.[12] On the basis of current knowledge in the indole series, [2-^{14}C]-geraniol should be incorporated into quinine to label C-20. When this precursor was fed to *Cinchona ledgeriana* plants, radioactive quinine was obtained (0·001% incorp.). Degradation of the derived dihydroquinine by the methods used in the indole series gave propionic acid (98% of total



(IX)

(X)

(XI) Corynanthe

(VIII)

(XII) Iboga

(XIII) Aspidosperma

activity) and radio-inactive acetic acid. This establishes specific labelling at C-20 and quinine is shown to be a monoterpene derivative.

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[1] T. Money, I. G. Wright, F. McCapra, and A. I. Scott, *Chem. Comm.*, 1965, 537.

[2] H. Goeggel and D. Arigoni, *Chem. Comm.*, 1965, 538.

[3] A. R. Battersby, R. Binks, W. Lawrie, G. V. Parry, and B. R. Webster, *J. Chem. Soc.*, 1965, 7459; A. R. Battersby, R. T. Brown, R. S. Kapil, A. O. Plunkett, and J. B. Taylor, *Chem. Comm.*, 1966, 46.

[4] A. R. Battersby, R. T. Brown, J. A. Knight, J. A. Martin, and A. O. Plunkett, *Chem. Comm.*, 1966, 346.

[5] P. Loew, H. Goeggel, and D. Arigoni, *Chem. Comm.*, 1966, 347.

[6] E. S. Hall, F. McCapra, T. Money, K. Fukumoto, J. R. Hanson, B. S. Mootoo, G. T. Phillips, and A. I. Scott, *Chem. Comm.*, 1966, 348.

[7] E. Leete and S. Ueda, *Tetrahedron Letters*, 1966, 4915.

[8] R. Thomas, *Tetrahedron Letters*, 1961, 544; E. Wenkert, *J. Amer. Chem. Soc.*, 1962, **84**, 98.

[9] E. Leete, *Tetrahedron*, 1961, **14**, 35.

[10] Outlined in part at the IUPAC 4th International Symposium on The Chemistry of Natural Products, Stockholm, June, 1966.

[11] R. Goutarel, M.-M. Janot, V. Prelog, and W. I. Taylor, *Helv. Chim. Acta.*, 1950, **33**, 150.

[12] N. Kowanko and E. Leete, *J. Amer. Chem. Soc.*, 1962, **84**, 4919.