The Rearrangement Process in Indole Alkaloid Biosynthesis

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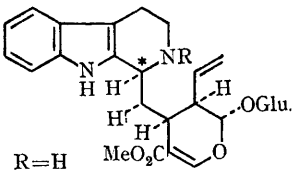
IT was recently proved[1] that when doubly-labelled vincoside (I) in admixture with isovincoside (V) is fed to *Vinca rosea* plants, intact incorporation occurs into representatives of all three major classes of indole alkaloids, *viz.*, *Corynanthe*, *Aspidosperma*, and *Iboga* families. Fractionation of the glycosides from *Vinca rosea* has now yielded *N*-acetylvincoside (II), (19 mg. from 1·5 kg. fresh plant material) in addition to crystalline samples or derivatives of the four other glucosides identified earlier;[1,2] (II) was identified by m.p. and full spectroscopic comparison with partially synthetic material.[1]

Separation of the [O-*methyl*-^{3}H]-labelled glucosides[1] (I) and (V) by partition chromatography allowed feeding experiments with the pure isomers to be carried out on *Vinca rosea* shoots; vincoside (I) was found to be the specific precursor of alkaloids in this plant, giving incorporations as follows: serpentine (VII) 3·9%, ajmalicine (VI) 0·51%, vindoline (VIII) 0·78%, catharanthine (IX) 0·89%, and perivine (X) 0·05%. Incorporations from isovincoside (V) ranged from negligible to zero [0·002% into serpentine (VII)].

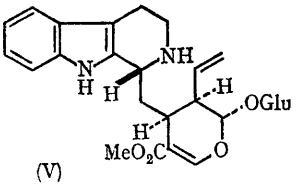
The biological conversion of vincoside (I) into the *Aspidosperma* and *Iboga* systems [*e.g.* (VIII) and (IX)] involves rearrangement of the original *Corynanthe*-type skeleton [*cf.* non-tryptamine units of (I) and (VI)]. Current thinking[3,4] about the mechanism of these processes focusses attention on (*a*) the role of the side-chain unsaturation in (I) and (*b*) the oxidation level at the starred carbon of (I) which corresponds to C-5 of loganin (XII).

Experimental work on (*a*) involved reduction of [O-*methyl*-^{3}H]vincoside[1] to [O-*methyl*-^{3}H]dihydrovincoside (III), characterised as its penta-acetate, $[\alpha]_D$ − 134°, and its *N*-acetyl derivative, m.p. 197—198°, $[\alpha]_D$ − 216° (MeOH). When (III) was fed to *Vinca rosea* shoots, no significant incorporation (< 0·001%) occurred into any member of the same set of alkaloids examined in the foregoing parallel experiment with vincoside (I). It is thus established that side-chain unsaturation is essential for the further biosynthetic steps. Stemmadenine (XI) or some very close relative is known[3] to lie after vincoside on the pathway to vindoline (VIII) and catharanthine (IX). The work with dihydrovincoside is in keeping with the view that isomerisation of the double-bond from the ethylidene to the 20,21-position of skeleton (XI) occurs during its biological conversion into the rearranged skeletons (VIII) and (IX).

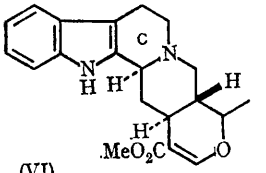
The study of (*b*) required the preparation of [5-^{3}H]loganin (XII) in the following way. Borotritiide reduction of the tetra-*O*-acetate (XIV) of dehydrologanin[5] afforded [5-^{3}H]-5-epiloganin tetra-acetate[6] (XV). The corresponding methanesulphonyl derivative (XVI) reacted with tetraethylammonium acetate to generate [5-^{3}H]loganin penta-acetate (XIII) from which [5-^{3}H]loganin was obtained by mild hydrolysis followed by re-esterification of the carboxy-function; this



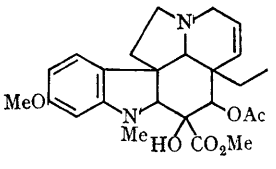
(I) R=H
(II) R=Ac
(III) R=H, vinyl reduced
(IV) R=Ac vinyl reduced



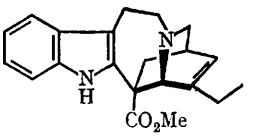
(V)



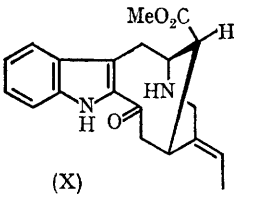
(VI)
(VII) Ring c aromatised



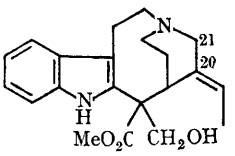
(VIII)



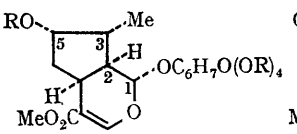
(IX)



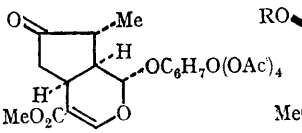
(X)



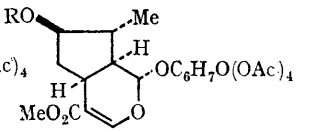
(XI)



(XII) R=H
(XIII) R=Ac



(XIV)



(XV) R=H
(XVI) R=$MeSO_2$

product was mixed in known proportion with [O-*methyl*-^{3}H]loganin.[2] Administration of the doubly-labelled loganin (80% of total activity at C-5) to *Vinca rosea* shoots gave the following incorporations and % of total activity at the skeletal ^{3}H-label: serpentine (VII) 2·0, 0%, ajmalicine (VI) 0·38, 77%, vindoline (VIII) 0·78, 79%, and catharanthine (IX) 1·5, 82%. Apart from the expected elimination in the case of serpentine (VII), there is clearly no loss of ^{3}H from the carbon corresponding to C-5 of loganin (XII) throughout the biosynthetic steps leading to all three families of indole alkaloids.

The foregoing results and those reported earlier[3,4] impose strict requirements on the nature and mechanism of the biosynthetic stages beyond vincoside (I); the sequences previously outlined[3,4] still meet these requirements and further tightening of the experimental test is in hand.

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[1] A. R. Battersby, A. R. Burnett, and P. G. Parsons, *Chem. Comm.*, 1968, 1282; see also A. R. Battersby, *Pure Appl. Chem.*, 1967, **14**, 117.
[2] A. R. Battersby, R. T. Brown, R. S. Kapil, J. A. Martin, and A. O. Plunkett, *Chem. Comm.*, 1966, 890; see G. N. Smith, *Chem. Comm.*, 1968, 912 concerning strictosidine.
[3] A. A. Qureshi and A. I. Scott, *Chem. Comm.*, 1968, 945, 947, 948.
[4] A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. G. Payne, D. Arigoni, and P. Loew, *Chem. Comm.*, 1968, 951; A. R. Battersby, *Chimia (Switz.)*, 1968, **22**, 313.
[5] K. Sheth, E. Ramstad, and J. Wolinsky, *Tetrahedron Letters*, 1961, 394.
[6] See also S. Brechbuhler-Bader, C. J. Coscia, P. Loew, Ch. von Szczepanski, and D. Arigoni, *Chem. Comm.*, 1968, 136; H. Inouye, T. Yoshida and S. Tobita, *Tetrahedron Letters*, 1968, 2945.