Biosynthesis of the Antitumour Catharanthus Alkaloids. Conversion of Anhydrovinblastine into Vinblastine

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Summary Administered [21′-^{3}H]anhydrovinblastine (**8**) is incorporated into vinblastine (**1**) by cell-free preparations of *Catharanthus roseus*.

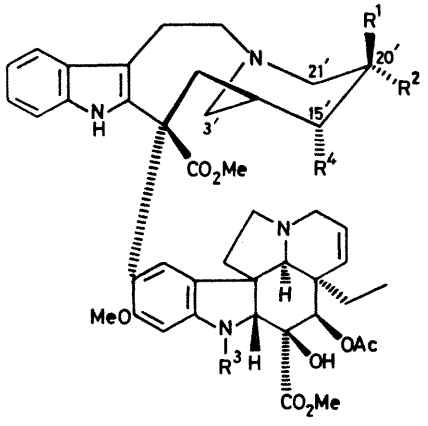
THE biosynthetic origins of the *Catharanthus sp.* antitumour alkaloid dimers, exemplified by vinblastine (VLB, **1**), vincristine (VCR, **2**), leurosidine (**3**), and leurosine (**4**), have been the subject of numerous studies over the past decade.[1] While the incorporations of the monomeric indole alkaloids vindoline (**6**) and catharanthine (**7a**) into the *Aspidosperma* and *cleavamine* segments of the dimers, respectively, are typically low (< 0·1%),[1,2] we have recently shown that these compounds are efficiently incorporated into anhydrovinblastine (anhydro-VLB, **8**), a compound not previously recognized as a natural product, by differentiated shoots of *C. roseus*.[3] While the formation of biologically significant quantities of (**8**) suggests that it is a likely intermediate in the biosynthesis of (**1**), administration of labelled (**8**) to *C. roseus* shoots did not result in any measurable incorporation into (**1**) or (**3**). We here describe the conversion of (**8**) into (**1**) by cell-free extracts of *C. roseus* plants (Table).

TABLE. Incorporation of [21′-^{3}H]anhydrovinblastine (anhydro-VLB, **8**) into vinblastine (VLB, **1**) by cell-free extracts[a] of *C. roseus*

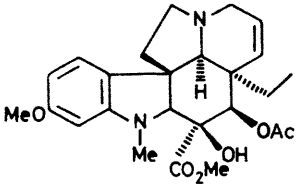
Expt.	Time/h	[21′-^{3}H]anhydro-VLB fed (d.p.m.)[c]	[21′-^{3}H]anhydro-VLB recovered (d.p.m.)[d]	Leurosine (**4**) isolated (d.p.m.)[d]	VLB isolated (d.p.m.) [% incorp.[e]][d]
1	3·0	$6{\cdot}80 \times 10^7$	$7{\cdot}37 \times 10^3$	$3{\cdot}25 \times 10^3$	$9{\cdot}63 \times 10^5$ [1·42]
2	3·0	$3{\cdot}27 \times 10^7$	$3{\cdot}10 \times 10^6$	$4{\cdot}67 \times 10^5$	$6{\cdot}07 \times 10^5$ [1·87]
3	3·5	$4{\cdot}07 \times 10^7$	—	—	$5{\cdot}24 \times 10^5$ [1·29]
4[b]	3·0	$2{\cdot}21 \times 10^7$	$3{\cdot}22 \times 10^6$	$2{\cdot}70 \times 10^5$	$6{\cdot}91 \times 10^4$ [0·31]

[a] Cell-free extracts were prepared as described (A. I. Scott and S.-L. Lee, *J. Amer. Chem. Soc.*, 1975, **97**, 6906). Incubations were carried out with 62 μM [21′-^{3}H]anhydro-VLB in enzyme preparations containing 1·2—1·5 mg of protein ml^{-1}. In expt. 3 leaves were ground with alumina under liquid N_2 prior to Polyclar treatment. [b] Extract boiled for 2 min prior to feeding. [c] Specific activity 15·88 mCi mmol^{-1}. [d] At the end of each expt. VLB-sulphate was added, the extract adjusted to pH 11 (NH_4OH) and extracted ($CHCl_3$); the compounds (**1**), (**4**), and (**8**) were separated by t.l.c. (silica gel), (**8**) was diluted with unlabelled material and crystallized to constant specific activity, (**4**) and (**1**) were further purified by h.p.l.c., and (**1**) was crystallized to constant specific activity as its 0·5 MeOH·0·5 Et_2O solvate (m.p. 205—211 °C). [e] No adjustment made for recovered precursor.

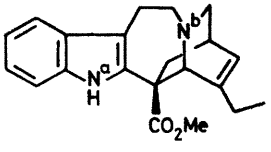
Labelled anhydro-VLB was prepared by the modified Polonovski reaction of (**6**) and catharanthine *N*-oxide (**7b**),[4] the intermediate imine being reduced with NaB^3H_4 in methanol affording [21′-^{3}H]-(**8**) which was purified by successive t.l.c. and crystallization to constant specific activity (15·88 mCi mmol^{-1}). The location of the introduced ^{3}H at C-21′ was supported by spectroscopic examination of ^{2}H labelled (**8**) prepared in a similar manner



(1) R^1=OH; R^2=Et; R^3=Me; R^4=H
(2) R^1=OH; R^2=Et; R^3=CHO; R^4=H
(3) R^1=Et; R^2=OH; R^3=Me; R^4=H
(4) R^1=Et; R^2R^4=—O—; R^3=Me
(5) R^1=H; R^2=Et; R^3=Me; R^4=H



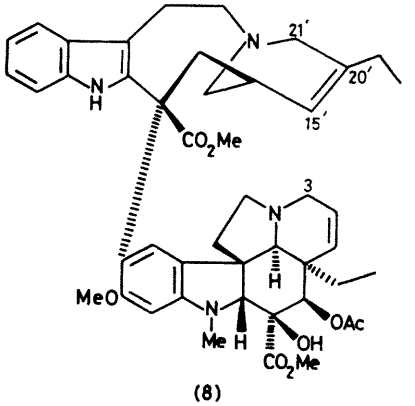
(6)



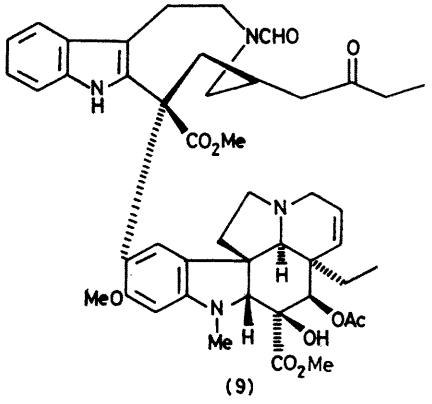
(7a) Catharanthine
(7b) N^b-oxide of (7a)

using NaB^2H_4. [^{1}H n.m.r. spectroscopy showed diminution of the complex multiplet centred at δ 3·10 (C-21′ and C-3 methylenes), the ^{2}H n.m.r. spectrum showed a broad peak at δ 3·5, and in the ^{13}C n.m.r. spectrum[5] the signal at δ 51·10 p.p.m. (C-21′) was considerably reduced in intensity relative to the spectrum of the undeuteriated (**8**) (other components of the expected triplet were obscured by adjacent resonances)].

Administration of [21′-^{3}H]-(**8**) to cell-free extracts of *C. roseus* afforded VLB (**1**) with radiochemical incorporations of up to 1·87% (Table). Evidence that no randomisation of the ^{3}H label from the C-21′ position had occurred was obtained by $KMnO_4$ oxidation of (**1**), isolated from



(8)



(9)

feeding experiments, to the lactam [3′-oxo-(**1**)] and catharinine (**9**).[6,7] While the former showed no significant loss of radioactivity, (**9**) retained only 34% of the ^{3}H present in (**1**).

The observation of a lower but significant incorporation of (**8**) into (**1**) in a boiled enzyme control (expt. 4, Table) might suggest that some non-enzymic conversion of (**8**) into (**1**) occurs. However, as blank incubations over several days at pH 2—6 afforded no evidence of VLB formation, this result may reflect a high degree of thermal stability of the enzyme system involved. In contrast, the incorporations of (**8**) into leurosine (**4**) must be interpreted

with caution in view of the ready oxidation of (**8**) to (**4**) *in vitro*.[8]

The question remains as to whether the transformation of (**8**) into (**1**) involves direct enzymatic hydration or reduction to 20′*R* deoxyvinblastine (**5**) followed by hydroxylation. The nature of this process, which could also involve stereospecific tritium loss at C-21′, is under investigation with appropriately labelled substrates. In view of the clinical value of compounds (**1**) and (**3**) and their low yields from natural sources, coupled with the relative availability of the anhydro dimer (**8**), the conversion of (**8**) into (**1**) in significant yield by cell-free preparations may prove of great importance. We conclude that the previous failure[3] to incorporate (**8**) in whole plants reflects both the known[4] instability of (**8**) and the non-permeability of the complete plant system.

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