Biosynthesis of the Antitumour Catharanthus Alkaloids: The Fate of the 21′α-Hydrogen of Anhydrovinblastine

Robert L. Baxter,* Mashooda Hasan, Neil E. Mackenzie, and A. Ian Scott*
Department of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ

[21′α-^{3}H,*methyl*-^{14}C]Anhydrovinblastine is incorporated into vinblastine by cell-free preparations of *Catharanthus roseus* without loss of ^{3}H.

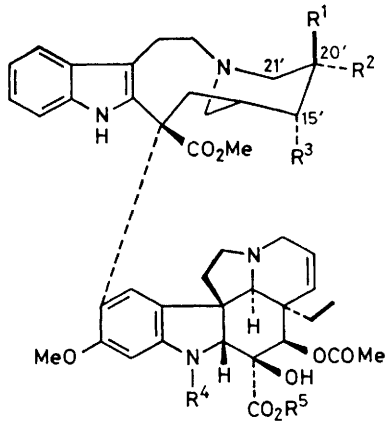
The chemotherapeutic importance of the bis-indole dimeric alkaloids, vinblastine (**1**) and vincristine (**2**), from *C. roseus* has given impetus to both synthetic[1,2] and biosynthetic[3–8] studies of these and the related alkaloids, leurosidine (**3**) and leurosine (**4**). Incorporation experiments with whole plants[3,4] and cell-free systems[6b] have established the role of vindoline (**8**) and catharanthine (**10**) as precursors of the *Aspidosperma* and *Iboga* segments of the dimers, respectively. Anhydrovinblastine (**5**), radiolabelled in either, but not both, of the *Aspidosperma* or *Iboga* derived segments, has been shown to be incorporated into (**1**) by cell-free preparations.[5,6a] In addition, singly-labelled 20′-deoxyleurosidine (**6**) has been incorporated into (**1**) by intact *C. roseus* plants.[7]

While these results suggest a biosynthetic route (**8**) + (**10**) → (**5**) → (**6**) → (**1**) [path (a) in Scheme 1] similar to that previously proposed by Potier[2] on the basis of synthetic analogy,

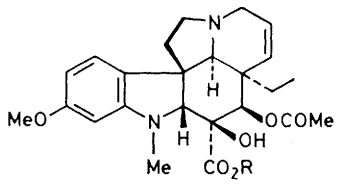
Table 1. Incorporation of anhydrovinblastine (**5**) into vinblastine (**1**) by cell-free extracts[a] of *C. roseus*.

Expt	Precursor	Anhydrovinblastine fed (d.p.m.)		$^3H/^{14}C$ ratio	(**1**) isolated (d.p.m.)[e]		$^3H/^{14}C$ ratio	% incorp.[f]
		^3H	^{14}C		^3H	^{14}C		
1	[21′α-^{3}H,*methyl*-^{14}C](**5**)[c]	5.03×10^6	7.37×10^5	6.83 ± 0.05	8.59×10^4	1.23×10^4	6.98 ± 0.24	1.67
2	[21′α-^{3}H,*methyl*-^{14}C](**5**)[c]	4.70×10^6	5.70×10^5	8.25 ± 0.05	3.69×10^4	4.56×10^3	8.10 ± 0.30	0.8
3[b]	[21′α-^{3}H][d](**5**)	8.24×10^7	—	—	1.32×10^4	—	—	0.02

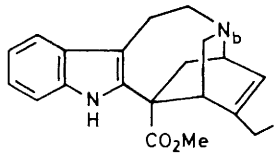
[a] Cell-free extracts were prepared as described (A. I. Scott and S-L. Lee, *J. Am. Chem. Soc.*, 1975, **97**, 6906). Approximately 10 g fresh leaves/10 ml of 0.05 M tris-maleate buffer (pH 7.0) were used in expts 1 and 3. The concentration of plant material was halved in expt 2. [b] Extract boiled for 5 min prior to feeding. [c] Specific activities 4.01 mCi mmol^{-1} for ^{3}H; 0.22 mCi mmol^{-1} for ^{14}C. [d] Specific activity 38.25 mCi mmol^{-1}. [e] At the end of each expt vinblastine sulphate (8—10 mg) was added, the extract was adjusted to pH 11 (NH_4OH) and extracted (CH_2Cl_2); (**5**) and (**1**) were separated by t.l.c., (**1**) was further purified by h.p.l.c. and crystallised to constant activity as its 0.5 MeOH : 0.5 Et_2O solvate. [f] No adjustment was made for recovered precursor.



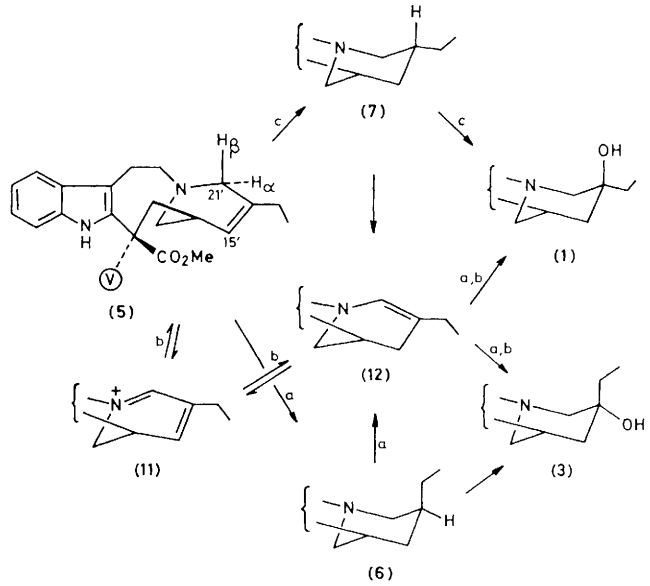
(**1**) R^1 = OH, R^2 = Et, R^3 = H, R^4 = R^5 = Me
(**2**) R^1 = OH, R^2 = Et, R^3 = H, R^4 = CHO, R^5 = Me
(**3**) R^1 = Et, R^2 = OH, R^3 = H, R^4 = R^5 = Me
(**4**) R^1 = Et, R^2, R^3 = –O–, R^4 = R^5 = Me
(**5**) R^1 = Et, $\Delta^{15'(20')}$, R^4 = R^5 = Me
(**6**) R^1 = Et, R^2 = H, R^3 = H, R^4 = R^5 = Me
(**7**) R^1 = H, R^2 = Et, R^3 = H, R^4 = R^5 = Me



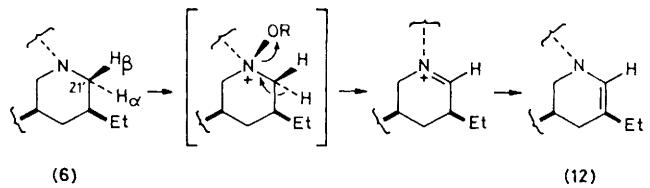
(**8**) R = Me
(**9**) R = H



(**10**)
(**10a**) N_b-oxide of (**10**)



Scheme 1



Scheme 2

Guéritte[7] has argued that they are equally compatible with the existence of a biogenetic grid in which the conjugated immonium salt (**11**), derived from a Polonovski type reaction of (**8**) and the *N*-oxide (**10a**) is in equilibrium with the 1,2-reduction product anhydrovinblastine (**5**) and the 1,4-reduction product (**12**) [path (b)]. Hydration of the enamine (**12**) could then give rise to (**1**) and (**3**). A feature common to both of the suggested pathways from (**5**) to (**1**) is the loss of one of the 21′-hydrogens of (**5**) by a process which could be mediated by the biological equivalent of Polonovski elimination of the corresponding *N*-oxide, a possibility advanced earlier.[2] If this were the case then loss of the 21′α-H should be expected, as only this hydrogen can adopt an antiperiplanar orientation relative to the oxygen of (**6**) [or (**5**)] *N*-oxide (Scheme 2). To test the possible intervention of an *N*-oxide (albeit indirectly) it was required to prepare anhydrovinblastine stereospecifically labelled with ^{3}H in the 21′α-position.

Inspection of models of the coupling reaction product (**11**)[1] indicated that the steric congestion of the β-face of the dihydropyridinium ring might force hydride reduction to occur predominantly from the less hindered α-face. Gratifyingly, treatment of (**11**) with NaB[^{2}H]$_4$ in methanol afforded monodeuterio-(**5**), the ^{1}H n.m.r. spectrum (360 MHz) of which showed absence of the 21′α-H doublet at δ 3.52 and the collapse of the 21′β-H doublet at δ 3.27 (*J* 16 Hz) to a singlet. [21′α-^{3}H]Anhydrovinblastine (**5**) was prepared in a similar manner using NaB[^{3}H]$_4$.[5]

Administration of [21′α-^{3}H, *methyl*-^{14}C](**5**) (R^5 = $^{14}CH_3$),† to cell-free extracts of mature *C. roseus* leaves followed by

† [*methyl*-^{14}C](**5**) (R^5 = $^{14}CH_3$) was prepared by treatment of vindolic acid (**9**) with [^{14}C]diazomethane, coupling[1] of the resultant [*methyl*-^{14}C](**8**) (R = $^{14}CH_3$) with (**10a**) and reduction of the product with $NaBH_4$.

isolation, afforded (**1**) with no significant change in $^{3}H/^{14}C$ ratio (expts 1 and 2, Table 1), showing that the 21′α-^{3}H was retained in the transformation.

These results provide the first unambiguous demonstration of intact incorporation of anhydrovinblastine into (**1**). Retention of the tritium at 21′α indicates that the transformation (**6**) → (**12**) or (**5**) → (**11**) by *trans*-elimination involving the corresponding *N*-oxide (Scheme 2) are unlikely steps in the pathway.‡ While this does not preclude the possibility that *cis*-elimination might be involved by a different mechanism of dehydrogenation, we suggest that the sequences (**5**) → (**6**) → (**12**) → (**1**) [path (a)] and (**5**) → (**11**) → (**12**) → (**1**) [path (b)] mediated by the *N*-oxide route shown in Scheme 2 do not appear likely as the major pathways from (**5**) to (**1**) *in vivo*.

A number of possible pathways compatible with the above results still remain: (a) direct hydration of the $\Delta^{15'(20')}$ double bond of (**5**), (b) reduction of (**5**) to 20′-deoxyvinblastine (**7**) and hydroxylation with *retention* of configuration [path (c) in Scheme 1], and (c) reduction to 20′-deoxyleurosidine (**6**) followed by hydroxylation with *inversion* of configuration.[9]

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‡ Complete loss of ^{3}H might be expected for such a mechanism only if loss of the 21′α-H were not a rate determining step. For the antithetical case an abnormal ^{3}H isotope effect of $>25:1$ would be required to invalidate this result (ref. 10).

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