Biosynthesis of Antitumour Alkaloids from *Catharanthus roseus*. Conversion of 20′-Deoxyleurosidine into Vinblastine

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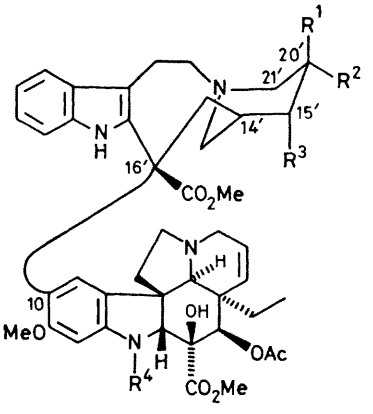
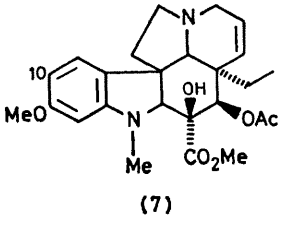
Summary Administered [*aromatic*-^{3}H]20′-deoxyleurosidine and [*acetyl*-^{14}C]20′-deoxyleurosidine are incoporated into vinblastine in *Catharanthus roseus*.

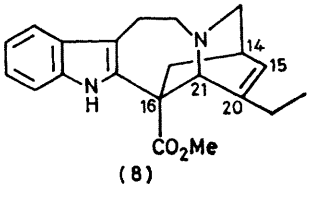
THE discovery in our laboratory of a biomimetic method[1] for the partial synthesis of the antitumour bis-indole alkaloids, related to the vinblastine group, has induced much chemical study resulting in the synthesis of vinblastine (**1**), vincristine (**2**), leurosidine (**3**), and leurosine (**4**).[2] The biosynthetic origin of these antitumour alkaloids has also been investigated in experiments either involving direct incorporation in the whole plant[3,4] or using cell-free preparations.[5,6] From these experiments it is now well established that vindoline (**7**) and catharanthine (**8**) are precursors of $\Delta^{15'(20')}$-20′-deoxyvinblastine [anhydrovinblastine (**5**)].[4,7] In spite of the incorporation of $\Delta^{15'(20')}$-20′-deoxyvinblastine (**5**) into vinblastine (**1**) in cell free extracts,[5,6] however, some experiments had still to be done to investigate the last stages of the biosynthesis of these alkaloids.



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



(**7**)



(**8**)

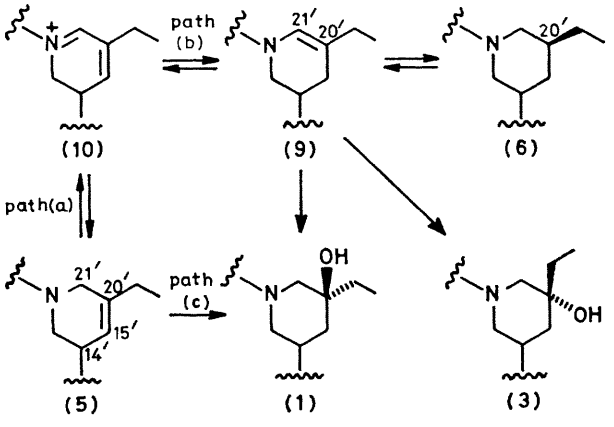
The *in vitro* partial synthesis of vinblastine (**1**) and leurosidine (**3**) *via* $\Delta^{20'}$ 20′-deoxyvinblastine (**9**)[2] led us to propose a biogenetic scheme for the formation of these dimeric alkaloids. The conjugated immonium salt (**10**) can be considered as a pivotal intermediate leading either to $\Delta^{15'(20')}$-20′-deoxyvinblastine (**5**) by 1,2-reduction [path (a), Scheme] or to vinblastine (**1**), leurosidine (**3**), and 20′-deoxyleurosidine (**6**) by 1,4-reduction (and subsequent 1,2-reduction for the latter) [path (b)]. Another possibility proposed by Scott[5] and Stuart[6] involves a direct enzymatic hydration of $\Delta^{15'(20')}$-20′-deoxyvinblastine (**5**) [path (c)].

We report here that 20′-deoxyleurosidine (**6**) can also act as a precursor of vinblastine. Thus, the feeding of [*aromatic*-^{3}H]20′-deoxyleurosidine† and [*acetyl*-^{14}C]20′-deoxyleurosidine‡ to 5—7 month-old *Catharanthus roseus* plants for seven days was followed by the extraction, purification, dilution, and crystallization of the vinblastine fraction to constant activity. [*aromatic*-^{3}H]Vinblastine and [*acetyl*-^{14}C]vinblastine were isolated with significant radiochemical incorporations (Table). On the other hand, an *in vitro* blank experiment afforded no evidence for vinblastine formation from [*acetyl*-^{14}C]20′-deoxyleurosidine.

TABLE. Incorporation of (**6**) into (**1**).

	(**6**) d.p.m.	(**6**) dpm mmol^{-1}	(**1**) isolated/ % incorp.[c]
[*aromatic*-^{3}H] (**6**)[a]	$3{\cdot}9 \times 10^8$	$9{\cdot}2 \times 10^{10}$	0·6
[*acetyl*-^{14}C] (**6**)[a]	$1{\cdot}89 \times 10^7$	$3{\cdot}9 \times 10^9$	0·32
[*acetyl*-^{14}C] (**6**)[a]	$1{\cdot}41 \times 10^7$	$2{\cdot}14 \times 10^9$	0·42
[*acetyl*-^{14}C] (**6**)[b]	$4{\cdot}5 \times 10^5$	$2{\cdot}14 \times 10^9$	0·01

[a] Incorporated in the plant as a water solution of (**6**) hydrochloride. Feeding time 7 days. [b] Blank experiment. [c] The tlc purification (SiO_2: MERCK) (eluent: AcOEt–MeOH: 90/10; C_6H_6–AcOEt–MeOH: 60/20/20; Ether–AcOEt–MeOH: 80/10/15) was monitored by autoradiography.



(**10**) (**9**) (**6**) (**5**) (**1**) (**3**)

SCHEME

These results suggest that at least two paths are available in the biogenesis of vinblastine (**1**), the first one using $\Delta^{15'(20')}$-20′-deoxyvinblastine (**5**) as precursor[5,6] and the

† This compound was prepared by direct tritiation of 20′-deoxyleurosidine in the presence of $CF_3CO_2[^3H]$.
‡ This compound was prepared by saponification (MeONa) and re-acetylation $(H_3[^{14}C]O)_2O$–pyridine of 20′-deoxyleurosidine.

second using 20′-deoxyleurosidine (**6**). The question remains whether or not these two paths are independent.

If the unstable $\Delta^{20'}$ 20′-deoxyvinblastine (**9**) is an intermediate in the transformation of 20′-deoxyleurosidine (**6**) into vinblastine (**1**), the immonium salt (**10**) could well be formed from the tetrahydropyridine derivative (**9**) by oxidation or disproportionation (Scheme). If this is the case, the two routes leading to vinblastine (**1**) should be interconnected. Further experiments are necessary to ascertain if this is so. However, this experiment concerning the biosynthesis of vinblastine (**1**), carried out for the first time in the whole plant, answers the question[8] as to whether or not alkaloid (**1**) is an artefact.

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