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BIOSYNTHESIS OF THE DIMERIC INDOLE ALKALOIDS. II. CATHARANTHINE AS A PRECURSOR OF VINCALEUKOBLASTINE.

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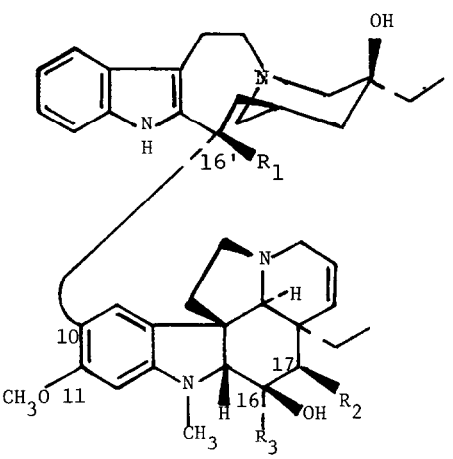
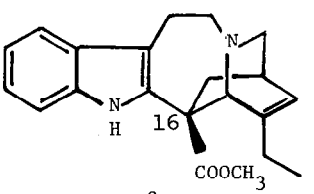
While the biosynthesis of the monomeric alkaloids of Catharanthus roseus G. Don (Apocynaceae) is quite well understood[1,2], that of the dimeric alkaloids such as vincaleukoblastine (VLB), 1, is less precisely known. A plausible biogenetic path is one involving coupling of catharanthine, 2, and vindoline, 3, two major alkaloids occurring in C. roseus. The incorporation of loganin, 4, in vivo, into 1, 2, and 3[3], and the successful synthetic coupling of catharanthine N_b-oxide, 5, or its derivatives with vindoline to give anhydrovinblastine[4,5], supports such an idea. However, extensive feeding experiments with intact C. roseus plants by the research group of A. I. Scott[2,6] and by us, gave either low or no incorporations of labelled 2 or 3 into VLB. Based on a previous observation that the catabolic turnover of catharanthine and vindoline is faster in apical cuttings than in intact plants[7], it was thought that apical cuttings of 3-4 month old plants might provide a more suitable system for studying the biosynthesis of VLB. That such may be the case is evident from the incorporations we have observed, as described below.



	R_1	R_2	R_3
VLB, 1	$COOCH_3$	$OCOCH_3$	$COOCH_3$
6	$COOCH_3$	OH	$COOCH_3$
7	H	OH	$CONHNH_2$
8	CH_2OH	OH	CH_2OH



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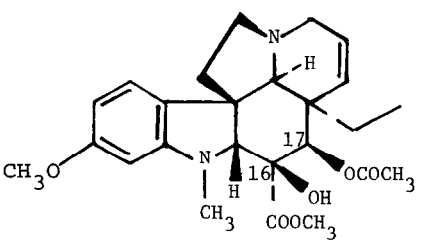

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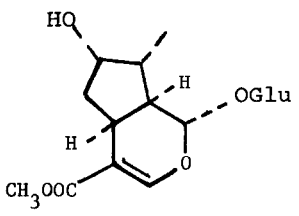

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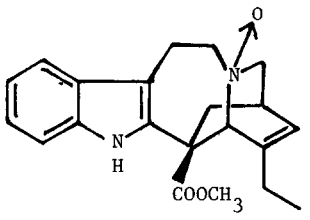

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The radioactive precursors [^{3}H-CO_2CH_3]-2 and [^{14}C-O$\underline{C}$OCH_3]-3 were synthesized using established methods[6,8], and were fed as an admixture for experiments A, B, and C (Table I). Experiment A was terminated after 3 days because a commercial Hoagland nutrient caused severe tissue necrosis at the recommended concentration. Experiments B (3 days), C and D (7 days each), were maintained on distilled water. In all four experiments, 1 was isolated after dilution with radioinactive VLB (5-6 mg), while 2 and 3 were isolated by established procedures[9]. Radioactive VLB was obtained from experiments A, C, and D after recrystallization to constant specific radioactivity; the ^{3}H/^{14}C ratios of the VLB isolated from experiments A and C were quite similar, 0.43:1 and 0.36:1, respectively.

Table I. Incorporation of 2 and 3 into VLB in C. roseus apical cuttings.

Expt	Alkaloid fed		Isolated VLB		Absolute Incorporation (%)[e]	
	2[a]	3[b]	^{3}H	^{14}C	2	3
A	7.16×10^9 (2.13×10^8)[c]	1.65×10^{10} (3.55×10^8)[d]	1.89×10^{6a} (1.40×10^4)[c]	4.45×10^{6b} (3.29×10^4)[d]	6.6×10^{-3}	9.3×10^{-3}
B	7.16×10^9 (3.51×10^7)[c]	1.85×10^{10} (6.67×10^7)[d]	ca. zero		ca. zero	
C	7.16×10^9 (1.60×10^8)[c]	1.85×10^{10} (3.04×10^8)[d]	1.39×10^{6a} (1.02×10^4)[c]	3.87×10^{6b} (2.86×10^4)[d]	6.3×10^{-3}	9.3×10^{-3}
D	7.16×10^9		8.14×10^{5a} (1.68×10^4)[c]		9.3×10^{-3}	

(a) dpm ^{3}H/mmole. (b) dpm ^{14}C/mmole. (c) dpm ^{3}H. (d) dpm ^{14}C. (e) dpm ^{3}H or ^{14}C of isolated VLB/dpm ^{3}H or ^{14}C precursor fed x 100.

The low absolute incorporation of 2 and 3 into 1 probably reflects the possibility that only some fraction of the fed precursors contributes to VLB biosynthesis, while the rest may undergo some other biochemical changes. For example, from the data in Table II for experiment C, the expected dilution of 2 on the basis of amounts fed vs. isolated is 3.5-fold, while the observed dilution is 31.6-fold, 9-fold greater. This factor raises the absolute incorporation for 2 into 1 to 0.056%. Similarly the absolute incorporation of 3 into 1 is raised to 0.049%.

To show that the labelling in 1 was regiospecific, the isolated VLB was deacetylated via the Zemplen reaction (0.011 mmoles VLB, 1.13 mmoles $NaOCH_3$)[4]. Under these conditions the sole product is desacetyl VLB, 6 [^{1}H NMR ($CDCl_3$), δ, 2.75 (N-Me); 3.60 (C-16' COOMe); 3.78 (C-16

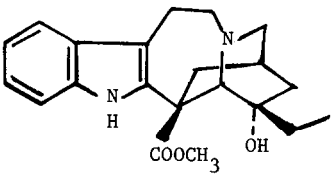
Table II. Data relating to the in vivo fate of precursors 2 and 3.

Expt	Amount (mg) of alkaloid fed [isolated] 2	3	Radioactivity of isolated alkaloid 2[a]	3[b]	Radioactivity recovered (%) 2[e]	3[f]	Dilution in vivo 2[g]	3[h]
A	9.54 [9.89]	9.77 [50.70]	5.11×10^7 (1.48×10^6)[c]	1.75×10^8 (1.95×10^7)[d]	0.69	5.5	147	94.3
B	1.57 [2.05]	1.64 [11.50]	1.38×10^8 (8.41×10^5)[c]	3.99×10^8 (1.00×10^7)[d]	2.39	14.9	51.9	46.3
C	7.17 [25.14]	7.49 [97.18]	2.26×10^8 (1.69×10^7)[c]	2.69×10^8 (5.73×10^7)[d]	10.5	18.8	31.6	68.8
D	8.49 [22.95]		4.68×10^8 (3.32×10^7)[c]		18.4		14.7	

(a) dpm ^{3}H/mmole. (b) dpm ^{14}C/mmole. (c) dpm ^{3}H. (d) dpm ^{14}C. (e) dpm 2 isolated/dpm 2 fed x 100. (f) dpm 3 isolated/dpm 3 fed x 100. (g) sp. act. 2 fed/sp. act. 2 isolated. (h) sp. act. 3 fed/sp. act. 3 isolated.

COOMe); 3.84 (C-11 OMe)]. VLB from experiments A and C was combined, radioinactive VLB added to give 0.011 mmoles VLB, 8.70×10^5 dpm ^{3}H/mmole, 2.20×10^6 dpm ^{14}C/mmole, ^{3}H/^{14}C ratio = 1:2.53. On deacetylation the product (6) had ^{3}H/^{14}C ratio of 1:0.110 (3390 dpm ^{3}H, 374 dpm ^{14}C), indicating a loss of 96% of the ^{14}C radioactivity. Thus at least 96% of the ^{14}C radioactivity was present in the C-17 acetate of VLB, confirming that [^{14}C-O$\underline{C}$OCH$_3$]-vindoline was incorporated into VLB intact. To remove the ^{3}H label that was expected to be in the C-16' carbomethoxy group of VLB, the radioactive desacetyl VLB was refluxed in N_2H_4/CH_3OH[10]. The reaction however failed to give the usually observed product, desacetyl VLB hydrazide, 7. The major product isolated was radioinactive (2 cpm ^{3}H/mg, 4 cpm ^{14}C/mg) and low resolution mass spectrometry indicated the compound had the basic skeleton of VLB-type dimers[4,5,10] [m/e 764, 736, 710, 695, 650, 593, 509, 294, 239, 225, 221, 197, 188, 154, 144, 135, 122, 107). Comparison with the mass spectrum of 7 showed a great deal of structural similarity [m/e 710 (M^+), 695, 593, 509, 295, 221, 197, 188, 154, 135, 122, 107] although its exact structure could not be defined. Consequently, proof that the ^{3}H label was at the C-16' carbomethoxy group of VLB was shown by $LiAlH_4$ reduction of diluted VLB from experiment D (0.009 mmoles VLB, 4.26×10^5 dpm ^{3}H/mmole)[11]. The resulting pentahydroxy derivative, 8, was virtually radioinactive as was its diacetate (10 cpm/mg). The identity of 8 was confirmed by ^{1}H NMR [($CDCl_3$), δ, 3.81 (C-11 OMe), 3.05 (N-Me)][11] and low resolution mass spectrometry (m/e 712 (M^+), 694, 682, 593, 513, 295, 212, 194, 188, 154, 144, 135, 122, 107].

While the above data support the role of catharanthine as a precursor to the velbanamine moiety of VLB, they do not preclude the involvement of monomeric alkaloids such as 5, 9, or 10, which might also serve as precursors of VLB via a metabolic grid of several competing biosynthetic pathways. It is of interest to note that 5 and 10 were not incorporated into VLB in intact plants in feeding experiments done by A. I. Scott's group[6], although the low incorporations of radioactivity into VLB from any monomeric precursor tested to date by either research group make secure biosynthetic deductions difficult. Future work will thus be directed at the feeding of such precursors to apical cuttings, in the hope of further clarifying the biosynthesis of VLB.[12]



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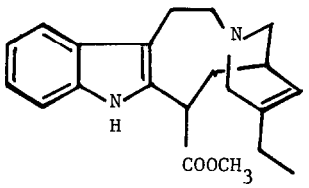

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12. For example, it is possible that the in vivo coupling of catharanthine with vindoline gives initially anhydrovinblastine,[4,5] whose presence in C. roseus should be ascertained.