STUDIES ON INDOLE ALKALOID BIOSYNTHESIS IX[1].
THE LATER STAGES OF IBOGA ALKALOID BIOSYNTHESIS.

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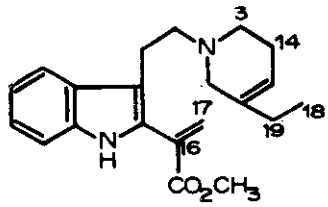
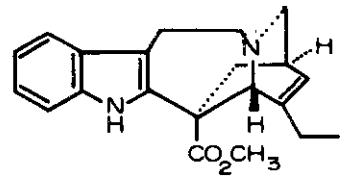
Biosynthetic experiments with Vinca rosea L. plants employing radioactively labelled forms of secodine (I) reveal that this substance is incorporated intact during the biosynthesis of catharanthine (II). These results provide some information about the later stages of biosynthesis of this alkaloid.

A considerable body of data is now available on the earlier stages of Iboga alkaloid biosynthesis but experimental results on many aspects of the later stages of the pathway are still lacking[2-4]. It is generally accepted that the structural template for the bio-intermediate which is important in these late phases is of the secodine type (I) and that a higher oxidized form of secodine, namely dehydrosecodine (I, 3,14-double bond) is presently the best representative of this class. Evaluation of the possible role of these systems is complicated by the high instability associated with them, secodine being stable for only a few hours, and dehydrosecodine, as yet an unknown compound, is expected to be highly unstable. Previous investigations in our laboratory[1,3-5] have provided some results on the role of secodine in the Aspidosperma and

Hunteria families and we now wish to report the first series of investigations which reveal the role of this intermediate in the Iboga alkaloids.



I



II

In order to maintain consistency with previous studies[1,3,4], Vinca rosea L. was the plant employed and catharanthine (II), one of the major alkaloids, was isolated as the representative of the Iboga series. Unfortunately, apart from the instability of the secodine system, the present studies were further complicated by the frustratingly low incorporations into the catharanthine molecule. In spite of numerous attempts to overcome this problem we have been unable to demonstrate the level of incorporation shown in vindoline, the Aspidosperma alkaloid previously studied in this same plant system.[1,6]

To determine optimum conditions for the incorporation studies, a series of experiments were performed with [2-^{14}C]-tryptophan as the precursor and time periods varying from 1-20 days. The percentage of incorporation into catharanthine was shown to vary from 0.08-1.0%, the latter value being obtained at the longer time periods. On this basis, radioactively labelled forms of secodine (I) available from the synthetic sequence already presented[3], were administered over a period of 20 days, via the cotton wick technique to plants varying in age from 1-2 years. The secodine molecule was incorporated in the form of its acetate salt and the isolated alkaloid carefully purified to constant activity. In all instances the individual experiments were repeated with different plants and different secodine preparations so as to eliminate any doubt about the validity of the obtained data. The results of the various

experiments are presented in Table 1.

Table 1. Results of Incorporation of Secodine into Catharanthine.[a]

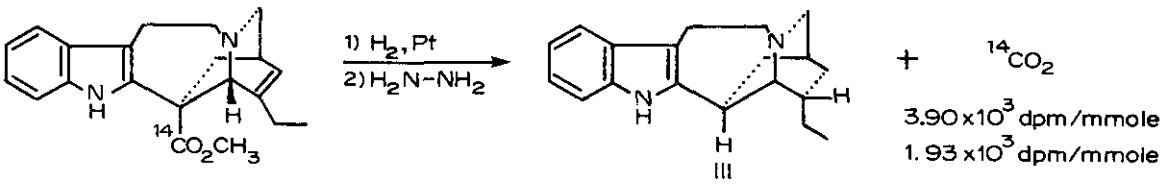
Expt.	Compound Fed	% Incorporation	Ratio of Activity Fed ($^3H/^{14}C$)	Ratio of Activity Isolated ($^3H/^{14}C$)
1.	[ar-^{3}H]-secodine (I)	0.005		
2.	[ar^3-H]-secodine	0.002		
3.	[^{14}COOCH$_3$]-secodine	0.006		
4.	[3,14,15,21-^{3}H,^{14}COOCH$_3$]-secodine	0.003 (^{14}C) 0.001 (^{3}H)	5.02	1.93
5.	[ar^{3}H,^{14}COOCH$_3$]-secodine	0.0008 (^{14}C) 0.0007 (^{3}H)	3.93	3.39
6.	[ar^{3}H,^{14}COOCH$_3$]-secodine	0.001 (^{14}C) 0.0009 (^{3}H)	3.96	3.53
7.	[19-^{3}H,^{14}COOCH$_3$]-secodine	0.001 (^{14}C) 0.001 (^{3}H)	1.91	1.95
8.	[19-^{3}H,^{14}COOCH$_3$]-secodine	0.0006 (^{14}C) 0.0005 (^{3}H)	5.55	4.58

[a] See Table 2 for other experimental details.

As Table 1 reveals, the secodine molecule is incorporated into the alkaloid system and when this data is coupled with the degradation studies indicated it is clear that the incorporation is rather specific. The degradation of catharanthine via dihydrocatharanthine and epiibogamine (III), from several different experiments, are presented for purposes of comparison. The radioactive carbon dioxide was isolated as the hyamine salt.

Table 2. Specific Activities Associated with the Experiments in Table 1.

Expt.	Activity Fed		Specific Activity Fed		Specific Activity Isolated	
	^{3}H	^{14}C	$^{3}H\left(\frac{dpm}{mM}\right)$	$^{14}C\left(\frac{dpm}{mM}\right)$	$^{3}H\left(\frac{dpm}{mM}\right)$	$^{14}C\left(\frac{dpm}{mM}\right)$
1.	1.05×10^{8}		1.62×10^{10}		7.78×10^{4}	
2.	1.05×10^{8}		1.62×10^{10}		2.83×10^{4}	
3.		4.56×10^{7}		4.37×10^{9}		3.85×10^{3}
4.	8.82×10^{7}	1.75×10^{7}	1.98×10^{10}	4.94×10^{9}	2.90×10^{4}	1.50×10^{4}
5.	8.03×10^{7}	2.02×10^{7}	1.62×10^{10}	4.37×10^{9}	6.18×10^{3}	1.82×10^{3}
6.	7.92×10^{7}	$2\ 00 \times 10^{7}$	1.62×10^{10}	4.37×10^{9}	9.26×10^{3}	2.64×10^{3}
7.	3.69×10^{7}	1.95×10^{7}	1.22×10^{10}	4.94×10^{9}	3.70×10^{3}	1.90×10^{3}
8.	1.92×10^{8}	3.44×10^{7}	2.34×10^{10}	4.37×10^{9}	1.82×10^{4}	3.98×10^{3}



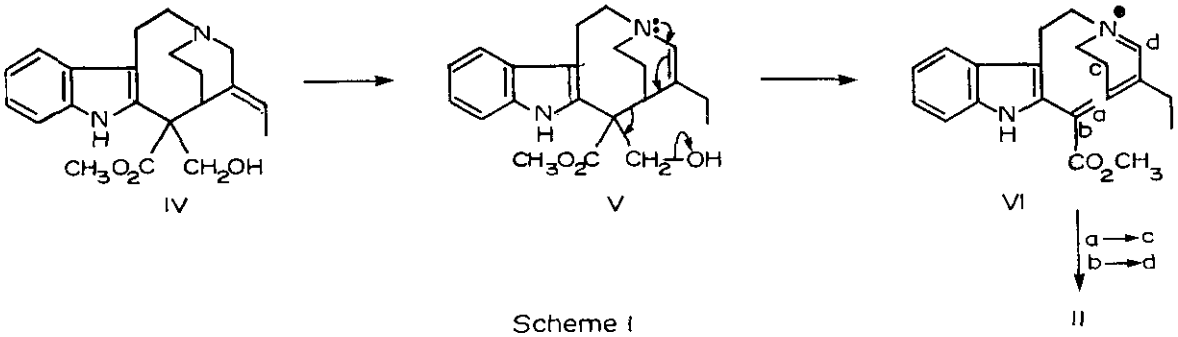
III

3.85×10^{3} dpm/mmole (expt 3)
1.90×10^{3} dpm/mmole (expt 7)

First of all it is clear that the carbomethoxy group of the secodine molecule becomes the ester function in the alkaloid. Secondly the various experiments with doubly labelled secodine provide some information in terms of the biosynthetic conversion of secodine to catharanthine. Thus the results of experiments 5 and 6 when coupled with the degradation data, show that little

or no alteration of the indole portion of the secodine system occurs during its conversion to the alkaloid. A small loss of tritium (10-14%) from the aromatic ring of I appears but this is presumably due to simple exchange with the medium. In the case of experiments 7 and 8, it is clear that the entire secodine molecule becomes incorporated into the alkaloid system since again only a small loss of label from the side chain is observed. On the other hand, the ratio of activity isolated in the alkaloid from experiment 4 reveals that 61.5% of the tritium is removed from the piperideine unit of the secodine molecule. This is the expected result if, as already mentioned above, an oxidation of I to a dehydrosecodine or a similar bio-intermediate is to prevail before it can be utilized by the plant. It is interesting to note that a similar result had been obtained in a previous series of investigations involving the Aspidosperma alkaloid, vindoline[1]. In the latter instance, a 60% loss of tritium was observed. These results tend to support the previous postulates that there appears to be an intermediate, possessing the structural features of the secodine system, which is common to both the Iboga and Aspidosperma families. In summary, the above results are entirely consistent with the pathway outlined in Scheme 1 in which the Strychnos series, here exemplified by stemmadenine (IV) and isostemmadenine (V), undergoes bond fission to a dehydrosecodine derivative (VI) which is merely a double bond isomer of the 3,14-dehydro analog of I. This type of system by a series of bond-forming processes, then elaborates to the Iboga alkaloids[2-4]. Obviously a great deal of additional data must be acquired before further details of these later phases can be ascertained. Undoubtedly, some of these anticipated studies must entail the evaluation of a highly unstable dehydrosecodine derivative with regard to its role as a true bio-intermediate. Methods designed specifically for the preparation and isolation of such systems must first be developed and we hope to present one

possible solution to this problem in the not too distant future.



Scheme I

ACKNOWLEDGEMENT Financial aid from the National Research Council of Canada is gratefully acknowledged.

REFERENCES

1 Part VIII, J. P. Kutney, J. F. Beck and G. B. Fuller, Heterocycles, 1973, 1, accompanying communication.

2 A. I. Scott, Acc. Chem. Res., 1970, 3, 151, and references cited therein.

3 J. P. Kutney, J. F. Beck, C. Ehret, G. A. Poulton, R. S. Sood and N. D. Westcott, Bioorg. Chem., 1971, 1, 194, and references cited therein.

4 J. P. Kutney, J. Het. Chem., 1972, 9, Suppl. Issue, S-1 and references cited therein.

5 J. P. Kutney, J. F. Beck, V. R. Nelson and R. S. Sood, J. Amer. Chem. Soc., 1971, 93, 255.

6 In private discussions with Professor A. I. Scott, Yale University, similar difficulties have been encountered in their studies. We are grateful to Professor Scott for informing us of his results.

Received, 14th May, 1973