# STUDIES ON INDOLE ALKALOID BIOSYNTHESIS VIII[1].

## THE LATER STAGES OF THE BIOSYNTHESIS OF APPARICINE.

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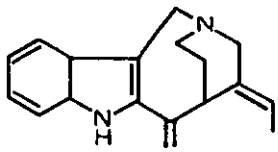
Biosynthetic experiments with Aspidosperma pyricollum plants employing radioactively labelled forms of 16,17-dihydrosecodin-17-ol (2) and secodine (3) reveal that the latter is incorporated intact during the biosynthesis of apparicine (1). These results provide important information about the later stages of biosyntheses of this alkaloid.

The alkaloid apparicine (1) possesses an interesting skeleton which portrays the isoprenoid $C_{10}$ unit commonly found in the Strychnos family but which lacks the ethanamine side chain of the indole unit normally associated with tryptophan derivation. Consequently investigations directed at evaluating the biosynthetic pathway of the indolic portion of this alkaloid are of considerable interest. In previous communications[2] we provided evidence which established that in contrast to published speculation[3] the one carbon bridge which separates the indole nucleus and the basic nitrogen atom is derived from $C_3$ of the tryptophan side chain and that extrusion of the other carbon atom ($C_2$) occurs at a late stage in the biosynthetic pathway. We now wish to report our most recent experiments which provide further information about the biosynthesis of this alkaloid.

Various radioactively labelled forms of 16,17-dihydrosecodin-17-ol (2) and secodine (3) available from synthetic investigations in our laboratory[4] were

administered via a hydroponic feeding technique to root cuttings of Aspidosperma pyricollum.



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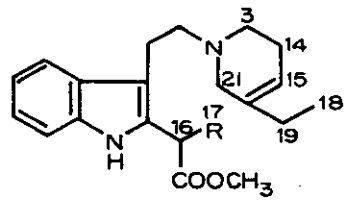
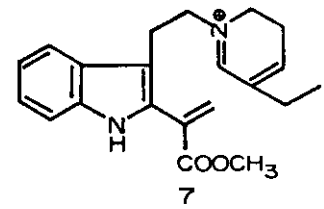


2, R = $CH_2OH$

3, R = = $CH_2$



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This method was adopted in view of the presence of apparicine (I) in the roots and because of the slow growth rate of this tropical plant species making the feeding of an entire plant or the aerial portions thereof costly in terms of plants as well as the time required to grow suitable specimens. The experiments were conducted over a 5 day period and the acetate salts of the labelled compounds were employed. The results of the various experiments are presented in Tables I and II.

Table I. Results of Incorporation of 16,17-Dihydrosecodin-17-ol and Secodine into Apparicine[a].

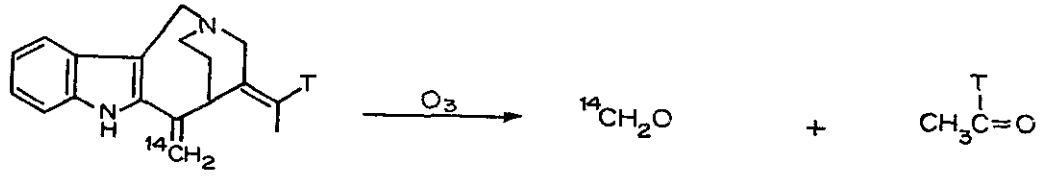
| Experiment Number | Compound Fed | % Incorporation | Ratio of Activity Fed $^3H/^{14}C$ | Ratio of Activity Isolated $^3H/^{14}C$ |
|---|---|---|---|---|
| 1 | [ar-$^3$H]-16,17-dihydro-secodin-17-ol (2) | <0.001 | | |
| 2 | [ar-$^3$H] secodine (3) | 0.01 | | |
| 3 | [$^{14}COOCH_3$] secodine | 0.01 | | |
| 4 | [ar-$^3$H,$^{14}COOCH_3$] secodine | 0.015 | 8.7 | 8.4 |
| 5 | [3,14,15,21-$^3$H,$^{14}COOCH_3$]-secodine | 0.009 | 4.2 | 2.2 |
| 6 | [19-$^3$H,$^{14}COOCH_3$]-secodine | 0.024 | 3.98 | 2.05 |

[a] See Table II for other experimental details.

Table II. Specific Activities Associated with the Experiments in Table I.

| Expt. No. | Activity Fed | | Specific Activity Fed | | Specific Activity Isolated | |
|---|---|---|---|---|---|---|
| | $^{3}H$ | $^{14}C$ | $^{3}H$ dpm/mmol | $^{14}C$ dpm/mmol | $^{3}H$ dpm/mmol | $^{14}C$ dpm/mmol |
| 1 | $9.20 \times 10^{7}$ | | $1.82 \times 10^{10}$ | | $1.43 \times 10^{4}$ | |
| 2 | $2.57 \times 10^{8}$ | | $1.82 \times 10^{10}$ | | $7.68 \times 10^{5}$ | |
| 3 | | $7.06 \times 10^{6}$ | | $1.19 \times 10^{9}$ | | $1.06 \times 10^{4}$ |
| 4 | $1.11 \times 10^{8}$ | $1.28 \times 10^{7}$ | $1.28 \times 10^{9}$ | | $1.6 \times 10^{5}$ | $1.94 \times 10^{4}$ |
| 5 | $4.42 \times 10^{7}$ | $1.07 \times 10^{7}$ | $1.27 \times 10^{9}$ | | $3.03 \times 10^{4}$ | $1.35 \times 10^{4}$ |
| 6 | $6.2 \times 10^{6}$ | $1.56 \times 10^{6}$ | $1.18 \times 10^{10}$ | $1.98 \times 10^{9}$ | $2.13 \times 10^{3}$ | $1.04 \times 10^{3}$ |

Table 1 portrays that **16,17-dihydrosecodin-17-ol** (2) is not incorporated into appariciné to any significant extent while secodine is utilized effectively. Experiments 4-6 provide results which pertain to the manner in which secodine is being involved in its biosynthetic elaboration to the alkaloid. First of all these experiments reveal incorporation of carbon-14 from the appropriate carbomethoxy labelled form of secodine. In addition the employment of a doubly labelled precursor (experiment 4) in which its tritium/carbon-14 ratio is retained in the isolated alkaloid implied that the ester function of secodine became the exocyclic methylene moiety of apparicine. Degradation of this alkaloid from several different experiments established this prediction and provided conclusive results for the specific and intact incorporation of secodine. Thus the formaldehyde obtained from the radioactive alkaloid isolated in experiment 3 revealed that the vinyl methylene carbon atom contained all of the carbon-14 activity. This result when coupled with the essentially constant $^{3}H/^{14}C$ ratio shown in experiment 4 established that the indole portion of secodine was being utilized with little or no alteration during the biosynthetic conversion.

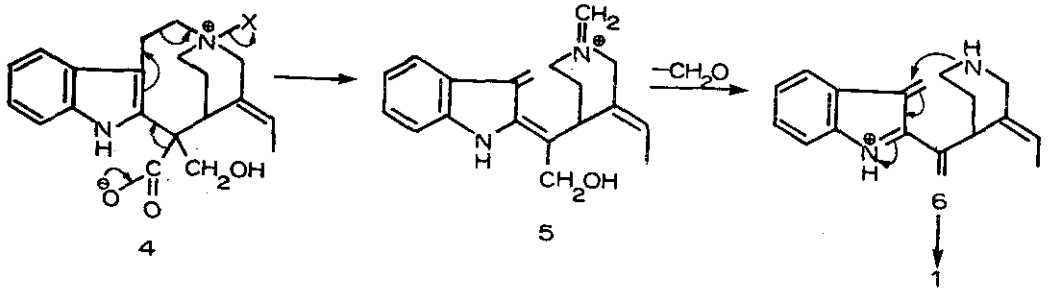


1.06x10$^4$dpm/mmol (expt 3)
1.04x10$^3$dpm/mmol ($^{14}$C, expt 6)
2.13x10$^3$dpm/mmol ($^3$H, expt 6)

1.05x10$^4$dpm/mmol (expt 3)
1.04x10$^3$dpm/mmol (expt 6)

2.13x10$^3$dpm/mmol (expt 6)

It is also pertinent to note that these results demand alteration in another recently proposed postulate by Potier and coworkers[5]. In this proposal it is suggested that an activated form of stemmadenine (4) undergoes fragmentation to 5 which then converts to apparicine in the manner indicated (5 → 6 → 1). The necessary loss of the ester group implied in this suggestion is not compatible with the results as obtained above.



Additional information regarding the utilization of secodine came forth from results in experiment 6. Isolation of both formaldehyde and acetaldehyde (as dimedone derivatives) from the degradation established firstly the specific incorporation of the entire secodine molecule into the alkaloid. Secondly the observed reduction in the $^3H/^{14}C$ ratio in the isolated alkaloid (3.98 vs 2.05) revealed that approximately 50% of the tritium label in the side chain of the incorporated secodine had been lost in the formation of the ethylidene side chain of apparicine. This result is in accord with expectation since the method of synthesis (to be discussed elsewhere) employed for the introduction

of the side chain tritium in the secodine molecule would be expected to place the tritium atoms in both R and S configurations. Since enzymic removal of $^{3}H$ at $C_{19}$ of secodine might be stereospecific a partial loss of this isotope is therefore expected.

Finally experiment 5 provides preliminary information about the fate of the piperideine unit in the conversion of secodine to apparicine. The loss of tritium in this portion of the secodine molecule (reduction in ratio from 4.2 to 2.2) is clearly associated with the bond forming processes which must prevail during the biosynthetic conversion to 1. These results suggest that a secodine derivative having a higher state of oxidation, as for example 7, is probably a better representative for the biointermediate. However, until isolation techniques for such highly unstable intermediates can be devised their role in the biosynthetic pathway must remain an open question.

In conclusion it is of interest to note that the above results portray a similar pattern in the utilization of secodine in the biosynthesis of apparicine to that already observed in two completely different indole families, exemplified by vindoline in Vinca rosea[1,4] and vincamine in Vinca minor[6]. These investigations provide a strong suggestion that there probably exists a rather common pathway in the early stages of the biosynthesis of many indole alkaloids. Enzymatic control at approximately the secodine level would then provide the necessary divergence in the pathways of the different alkaloids in the various plant species. We hope to present more definite information in this direction in future communications.

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