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DIHYDROPYRIDINES IN BIOSYNTHESIS AND SYNTHESIS*.

James P. Kutney,

Department of Chemistry, University of British Columbia,

2075 Wesbrook Place, Vancouver, B.C.

Canada, V6T 1W5

A summary of experiments relating to the role of dihydropyridines as possible intermediates in the biosynthesis of indole alkaloids and performed in the author's laboratory is presented. In relation to these interests a series of investigations concerning the stabilization of dihydropyridine systems by means of chromium carbonyl complex formation is described. Some examples of the utilization of such intermediates in synthetic areas are also presented.

* This article is dedicated to Professor R.B. Woodward on the occasion of his sixtieth birthday.

It is well established that certain types of dihydropyridines play a very important role in biological processes. For example 1,4-dihydropyridine derivatives are important intermediates in biological oxidation-reduction reactions involving the NAD-NADH system. The nicotinamides which possess the electron-withdrawing and stabilizing carboxamide group are relatively stable systems and their chemistry as well as that of other dihydropyridines which contain such electron-withdrawing substituents is well documented in the literature[1]. On the other hand the biological role (if any) of less stable dihydropyridines which lack these substituents is not well understood and the chemistry of such systems is similarly limited. Clearly the greater experimental difficulties associated with the preparation and manipulation of such intermediates are at least in part a reason for this lack of knowledge. This article summarizes some of our investigations which relate to the possible implications of these types of unstable dihydropyridines as intermediates in certain areas of biosynthesis and their utilization in synthesis.

Our own interests in indole alkaloid biosynthesis, as summarized in two recent reviews[2], relate to the later stages of the various biosynthetic pathways put forth for the different alkaloid families. It is at these stages that certain dihydropyridine derivatives have been involved as possible biosynthetic intermediates. Experiments designed to provide some information in this area were initiated in our laboratory some years ago but in view of the recent reviews[2] the present discussion will be brief and is intended to provide only an introduction and rationale for the investigations presently underway.

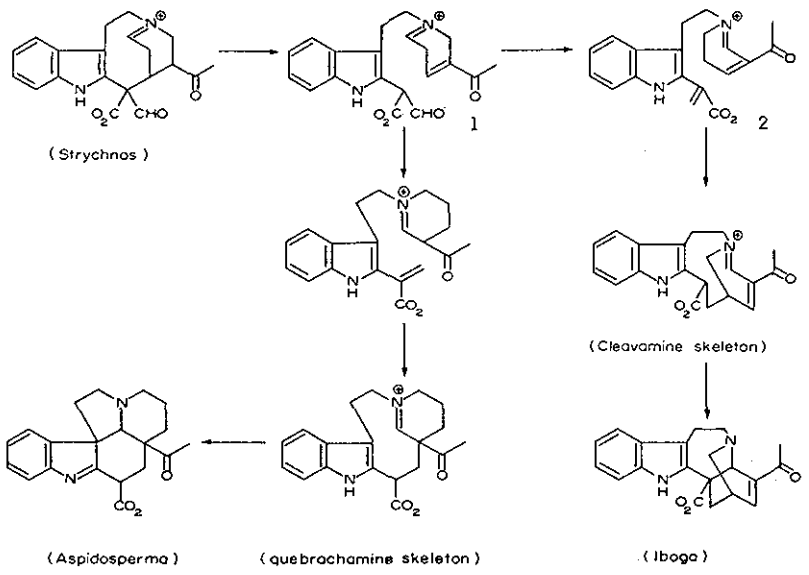


Figure 1. Wenkert's postulates as they relate to later stages of Aspidosperma and Iboga alkaloid biosynthesis.

In the early 1960's various postulates were advanced for the biosynthesis of the indole alkaloids. The proposal which is most pertinent to the present discussion was put forward by Wenkert[3] and is summarized in Figure 1. It is seen that in this postulate a Strychnos system is envisaged to undergo a retro-Michael reaction to provide the dihydropyridinium intermediates 1 and 2 which, in turn, can be elaborated to the Aspidosperma and Iboga systems. Some experimental support for the possible intermediacy of such dihydropyridines came forth from Scott's experiments with germinated Catharanthus roseus seedlings[4,5] and our investigations with C. roseus plants[6]. Scott established the sequential formation of alkaloidal types according to the scheme, Strychnos → Aspidosperma → Iboga, while both of our groups were able to demonstrate the

incorporation of tabersonine (an Aspidosperma alkaloid) to catharanthine, a well-known member of the Iboga family. In order to explain these and other published experiments, a rationale, as shown in Figure 2, was put forth by our respective groups. The dotted lines illustrate proposed conversions still lacking experimental verification.

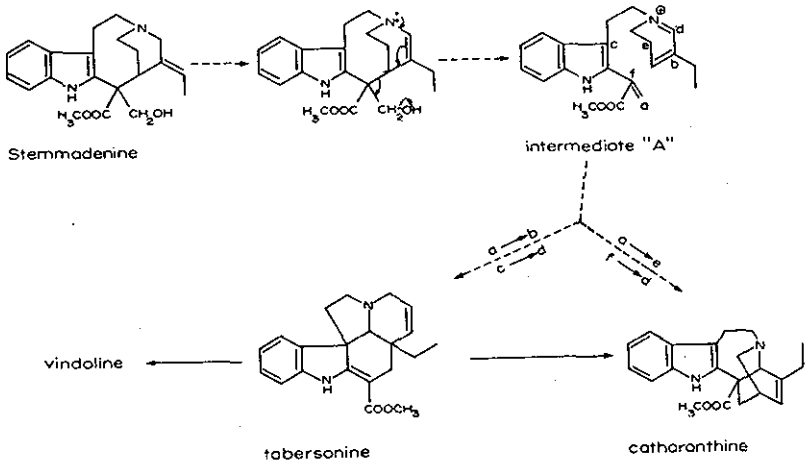


Figure 2. A possible rationale for the implication of intermediate "A" in the biosynthesis of Aspidosperma and Iboga alkaloids.

Clearly intermediate "A" in Figure 2 represents a dihydropyridinium system closely related to the intermediates proposed by Wenkert and shown in Figure 1. It was now appropriate to consider experiments which would provide some information about the role of such dihydropyridines in the later stages of indole alkaloid biosynthesis.

The expected instability of these types of dihydropyridines led us to consider a synthetic program which would allow the preparation of the appropriate tetrahydropyridines. It was felt that the latter compounds

could be more easily utilized in the various biosynthetic experiments and that hopefully in vivo oxidation to the dihydropyridines could be performed by the plant enzymes. After much frustration and difficulty, a successful sequence was developed and is summarized in Figure 3.

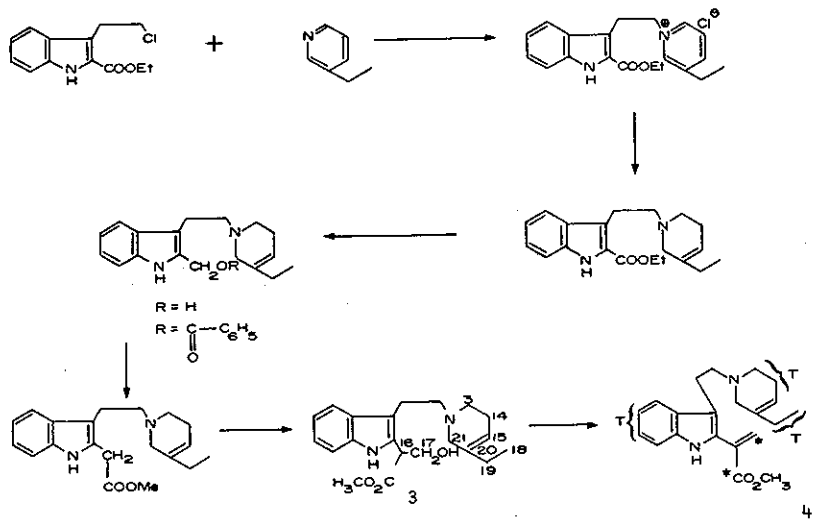
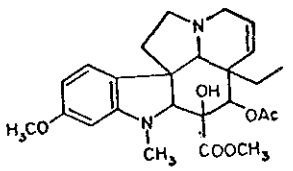


Figure 3. Synthesis of 16,17-dihydrosecodin-17-ol (3) and secodine (4).

At the time that the synthetic program was initiated the presence of such systems in natural sources was unknown but fortunately the Manchester group[7] were able to demonstrate the occurrence of such alkaloids in plants. These workers proposed the name secodine to the tetrahydropyridine derivative 4 and provided results which were of great value in evaluating the chemistry of 4 and related synthetic derivatives in the sequence shown. Our synthetic efforts provided secodine with tritium and/or carbon-14 labels at various sites as shown in 4 thereby allowing the opportunity for the biosynthetic evaluation of this compound.



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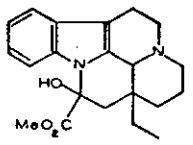

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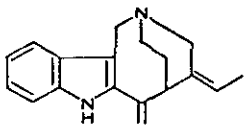

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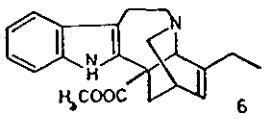

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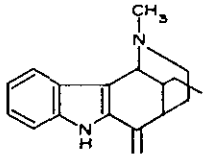

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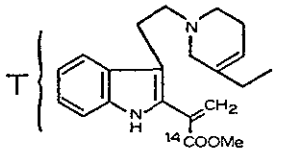
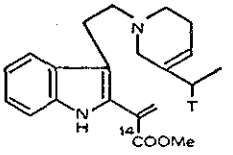
A large number of experiments were performed in different plant systems containing various representatives of different indole alkaloid families. All of these are detailed elsewhere[2] so only a very brief resumé will be provided here. In these studies an attempt was made to incorporate secodine into a variety of alkaloid systems in order to provide some information about the general utilization of this type of intermediate in various biosynthetic routes. Thus vindoline (5, Aspidosperma) and catharanthine (6, Iboga), were studied in Catharanthus roseus (Vinca rosea L.), vincamine (7, Hunteria) in Vinca minor, apparicine (8) and uleine (9) in Aspidosperma pyricollum. Negative incorporations were observed in the case of uleine while low, but definite incorporations were seen in the other alkaloids. Tables 1 and 2 summarize the results of several of the important experiments with doubly labelled secodine in the above mentioned plants. From these investigations it is noted that the entire secodine molecule is incorporated intact and insignificant losses of tritium occur when the label is placed in the aromatic portion or the ethyl side chain. Appropriate degradations of the isolated alkaloids

TABLE 1

Incorporation Studies with 

Expt	Plant fed	Ratio of activity fed ($^3H/^{14}C$)	Ratio of activity isolated ($^3H/^{14}C$)	
1	V. rosea	8.8	Vindoline 8.3	
2	V. minor	8.4	Vincamine 8.6	Minovine 10.1
3	A. pyricollum	8.7	Apparicine 8.4	

TABLE 2

Incorporation Studies with. 

Expt	Plant fed	Ratio of activity fed ($^3H/^{14}C$)	Ratio of activity isolated ($^3H/^{14}C$)	
1	V. rosea	1.54	Vindoline 1.35	
2	V. minor	1.84, 1.94	Vincamine 1.88, 2.02	Minovine
3	A. pyricollum	1.91	Apparicine 2.60	Uleine

(Figures 4 - 7) were performed to establish that the ester group of secodine is retained in the various alkaloids. On the other hand when the tritium label is located in the piperideine unit of the secodine molecule (see Figure 4, for example) significant losses of tritium are observed: 60% in the study with vindoline, 61.5% with catharanthine and 48% in the case of apparicine. Thus a higher oxidized form of secodine,

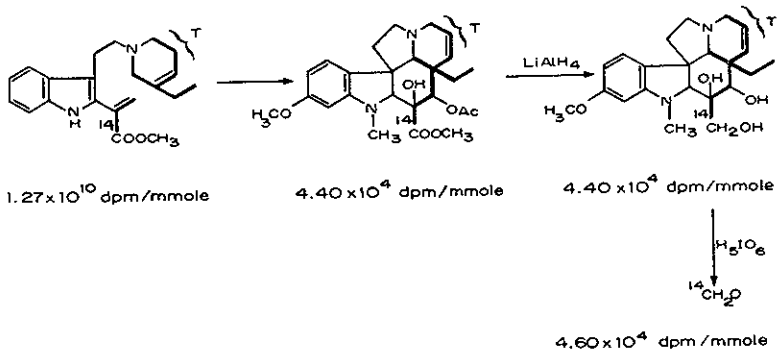


Figure 4. Degradation of vindoline from [$^{14}COOCH_3$,3,14,15,21-^3H]-secodine.

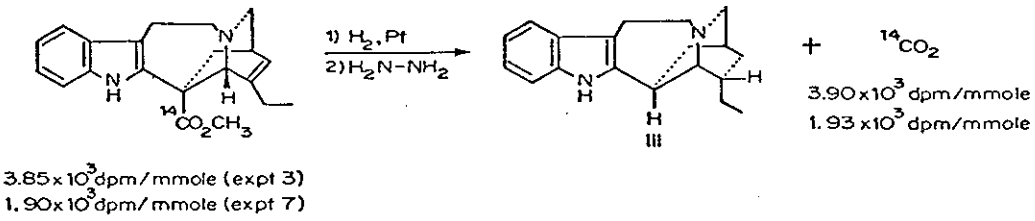


Figure 5. Degradation of catharanthine isolated from the secodine incorporation experiments.

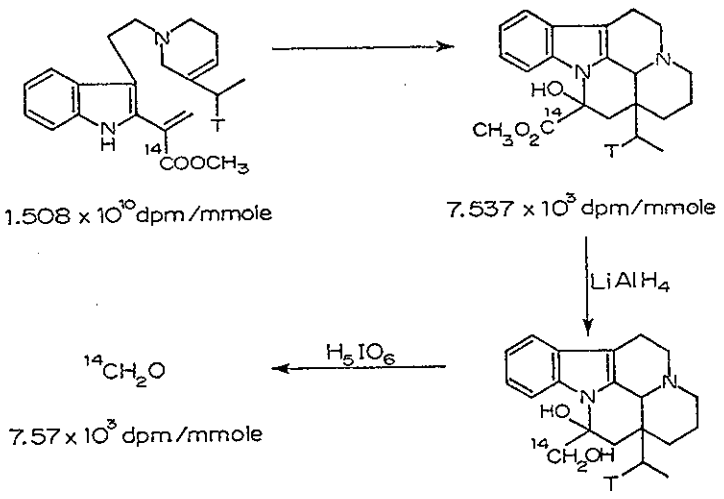


Figure 6. Degradation of vincamine from incorporation of [$^{14}COOCH_3$,19^3H]-secodine.

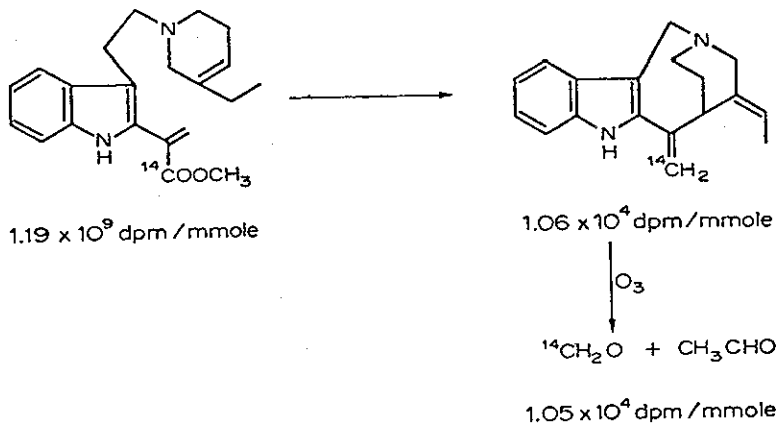


Figure 7. Degradation of apparicine from incorporation of [$^{14}COOCH_3$]-secodine.

perhaps a dehydrosecodine is involved in the later stages and would account for the observed tritium losses. Figure 2 illustrates the possible role of such an intermediate (intermediate "A") for the Aspidosperma and Iboga bases while Figure 8 summarizes a similar role in the apparicine series.

Apart from the low levels of incorporation obtained with secodine, experimental difficulties associated with the instability of this molecule continued to provide frustrations with investigations in this area. It could be demonstrated, for example, that secodine undergoes dimerization to an extent of about 40% in the time period (2-4 hours) required for this precursor to be absorbed into the plants. Thus the levels of incorporation observed with secodine (usually about 0.01%) are <u>not</u> a true indication of the relative efficiency of its utilization by the plant enzymes. Since the above results provide a suggestion that dehydrosecodine may be a more appropriate representation of the late stage bio-intermediate, its evaluation in this regard becomes particularly interesting. The high reactivity and instability expected for the dehydrosecodine system poses an even more demanding experimental difficulty in the types of investigation discussed above and therefore the development of techniques for stabilizing such systems became a prime consideration in our laboratory.

As mentioned earlier, certain areas of dihydropyridine chemistry have been extensively investigated but unfortunately little was known about the specific types of systems which were required for the present purpose. Clearly the dehydrosecodine system does not possess any stabilizing electron-withdrawing substituents but rather electron-donating groups.

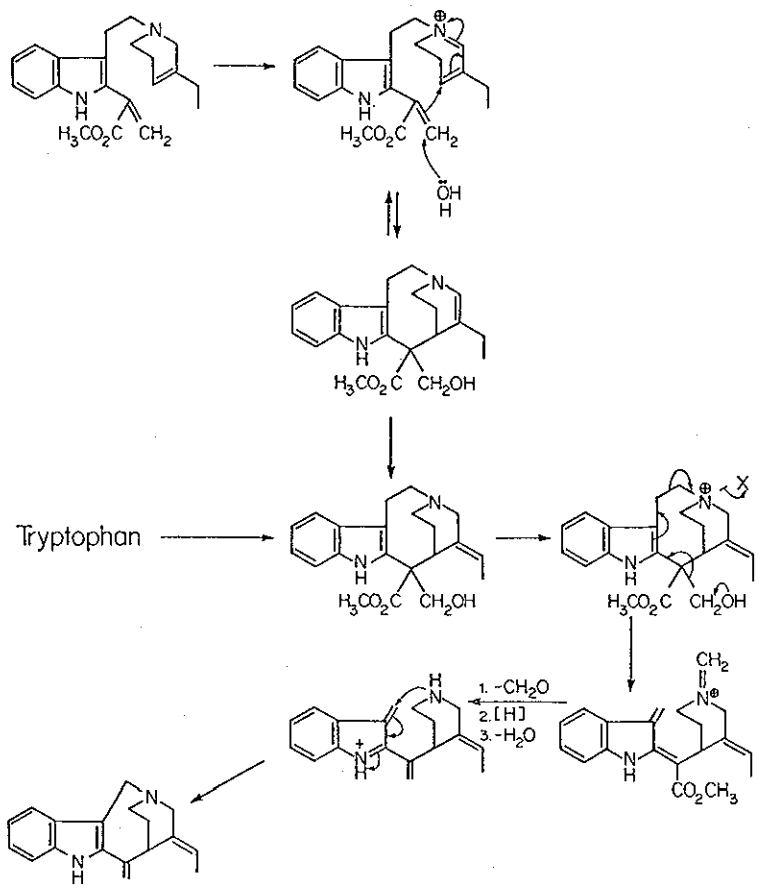


Figure 8. A postulate which is consistent with our experiments involving incorporation of tryptophan, stemmadenine and secodine into apparicine in A. pyricollum.

A more fundamental study of such unknown dihydropyridines was therefore warranted and a simpler but yet appropriate model system was selected.

The reduction of pyridinium salts to tetrahydropyridines, as shown in Figure 9 (10 → 11), is a well known process[1]. Mechanistic speculation concerning this conversion suggests that under appropriate conditions the intermediate dihydropyridines should be isolable. After considerable effort, success in this direction was achieved and the conversion, 10 → 12, could be accomplished in a two-phase system in high overall yield (about 85%)[8]. Initial efforts to stabilize this novel dihydropyridine system were directed toward the preparation of complexes involving various metal carbonyls. Indeed crystalline complexes could be obtained when 12 was reacted with the reagent $(CH_3CN)_3Cr(CO)_3$, *available from* $Cr(CO)_6$ *and* acetonitrile[9-11] (Figure 10). Although it was originally thought[8] that the borohydride reduction of 10 provides a mixture of the expected 1,2- and 1,6-dihydropyridines, it has been established in our more recent detailed studies that N-methyl-3-ethyl-1,2-dihydropyridine (12) is essentially the exclusive product. The latter compound, provides the 1,2- and 1,6-dihydropyridine complexes (13 and 14) during reaction with the complexing reagent as shown in Figure 10. X-ray analyses on these complexes[8] provided the necessary structural data and left no doubt about their isomeric nature.

The availability of stable, crystalline complexes would provide the opportunity for development of the chemistry of these novel systems if successful removal of the organic ligand could be achieved. After much experimentation, it was found that reaction of the complexes with pyridine at room temperature afforded the pure 1,2- and 1,6-dihydropyridines (Figure 11).

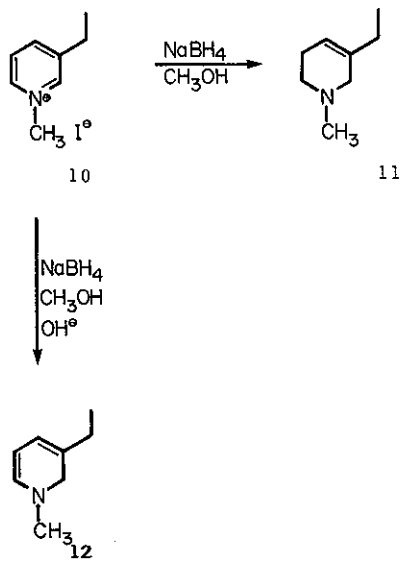


Figure 9. Sodium borohydride reduction of N-methyl-3-ethylpyridinium iodide (10).

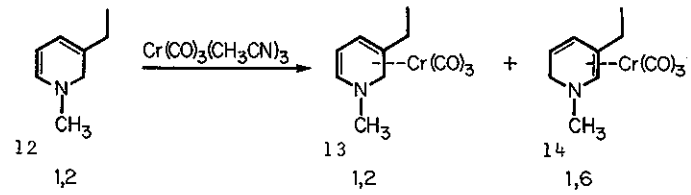


Figure 10. Preparation of stable chromium carbonyl complexes in the dihydropyridine series.

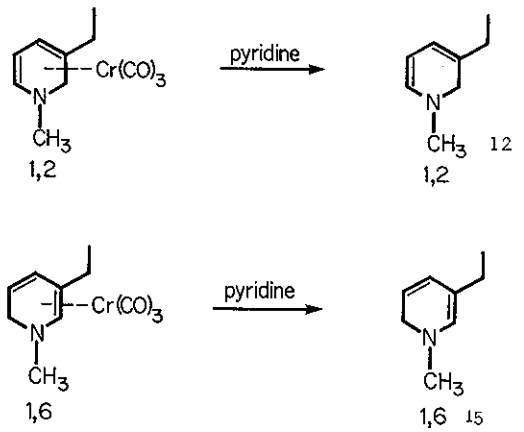


Figure 11. The synthesis of N-methyl-3-ethyl-1,2-dihydropyridine (12) and N-methyl-3-ethyl-1,6-dihydropyridine (15) from the chromium complexes.

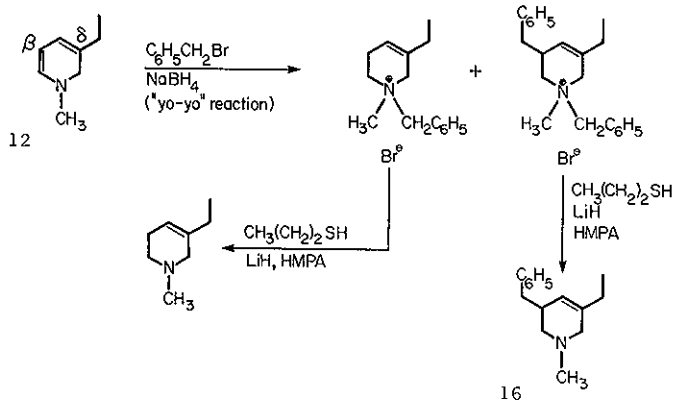


Figure 12. Alkylation studies with N-methyl-3-ethyl-1,2-dihydropyridine (12).

It was now of interest to establish the site of substitution (alkylation or acylation) with these novel dihydropyridine systems since such information would be of importance in subsequent studies. The 1,2-dihydropyridine derivative 12 was selected for this purpose and the alkylation studies were performed with benzyl bromide as the reagent (Figure 12)[12]. Considerable difficulties were encountered in the initial experiments since only tarry reaction product mixtures were obtained. However refinement of reaction conditions employing the principle of transferring reagents between the layers in a two-phase system ("yo-yo" reaction) did allow isolation of alkylation products, for example 16, in an overall yield of 48%. Alkylation occurs exclusively at the β-position (see 12) and 3,5-disubstituted tetrahydropyridines are obtained. This process provides an attractive synthesis of 3,5-disubstituted pyridines from readily available 3-substituted pyridines (Figure 13). The intermediate 17 obtained from β-alkylation is exposed to an oxidation (silver nitrate for example) and the resulting pyridinium salt is dealkylated with triphenylphosphine to provide the final product. In this study both alkyl and acyl halides can be employed.

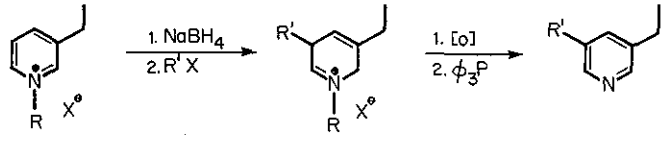


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Figure 13. The synthesis of 3,5-disubstituted pyridines via alkylation or acylation of dihydropyridines.

The alkylation of 3,5-lutidine[12] via the dihydropyridine derivative 18 (Figure 14) illustrates that introduction of an alkyl group can be achieved at a carbon atom already bearing a substituent.

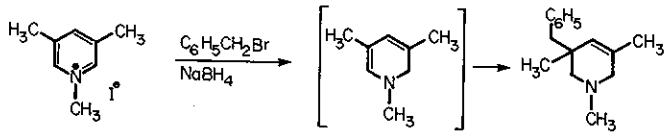

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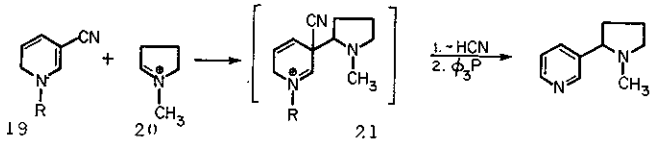
Figure 14. The alkylation of 3,5-lutidine via N-methyl-3,5-dimethyl-1,2-dihydropyridine (18) as intermediate.

The above alkylation studies reveal various synthetic possibilities within the alkaloid area. For example, the nicotine series is generally considered to arise biosynthetically from nicotinic acid and an ornithine-derived pyrrolinium salt[13]. The above-mentioned studies provide a close laboratory analogy since the important coupling reaction between the pyridine and pyrrolidine units required for the generation of the tobacco alkaloids could well involve such an alkylation process. Indeed a preliminary study in our laboratory, as summarized in Figure 15, indicates that such a route can provide an attractive synthesis of these alkaloid systems.



19 20 21

Figure 15. A biogenetic type synthesis of the nicotine series.

The known N-benzyl (or N-methyl)-3-cyano-1,6-dihydropyridine (19)[14,15] was coupled with N-methylpyrrolinium chloride (20)[16]. The expected intermediate 21 is not isolated but converted to the pyridinium salt which upon reaction with triphenylphosphine affords the nicotine series. Refinement of reaction conditions is essential but there appears little doubt that this sequence could provide an interesting synthetic entry into this family of compounds.

The above investigations in the dihydropyridine series also provided a basis for experiments directed toward the synthesis of dehydrosecodine, the compound suggested earlier as a late stage intermediate in various biosynthetic pathways in the indole alkaloids. It was now appropriate to initiate studies in this area. Figure 16 summarizes a successful route to the chromium carbonyl complex 24. The purpose of this investigation was to determine whether the chromium complex could be formed with the dihydropyridine unit when the electron rich indole ring was present and whether the indole chromophore forms a complex as well.

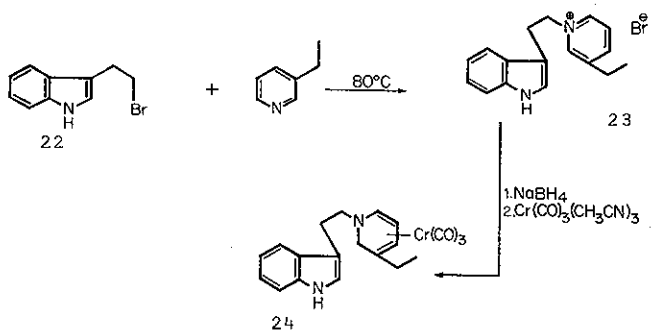


Figure 16. The synthesis of the chromium carbonyl complex 24.

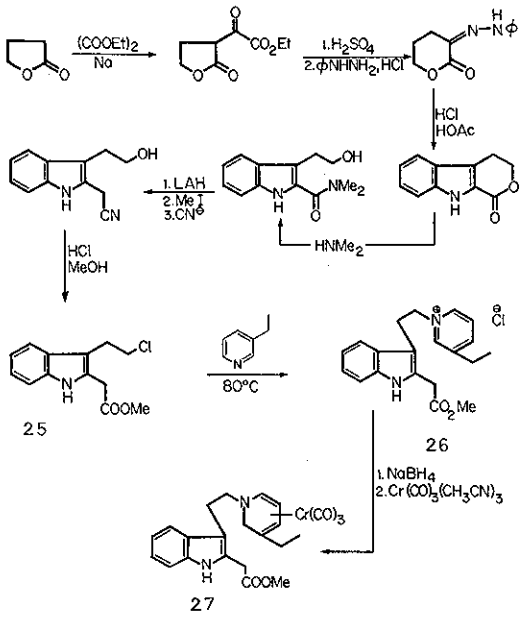


Figure 17. The synthesis of the chromium carbonyl complex 27.

Reaction of tryptophyl bromide (22) with 3-ethylpyridine in a manner similar to that employed in the secodine synthesis (Figure 3), afforded the pyridinium salt 23. The latter intermediate is readily converted to the chromium carbonyl complex 24 utilizing the procedures developed earlier in the dihydropyridine series.

Extensions of our studies to intermediates more directly on the dehydrosecodine pathway are summarized in Figures 17 and 18. Figure 17 portrays a successful sequence from butyrolactone to the chloroester 25 and, in turn, the pyridinium salt 26. As before, elaboration of 26 to the chromium carbonyl complex 27 followed the established route although refinement of reaction conditions is essential in this latter conversion since the yield is low.

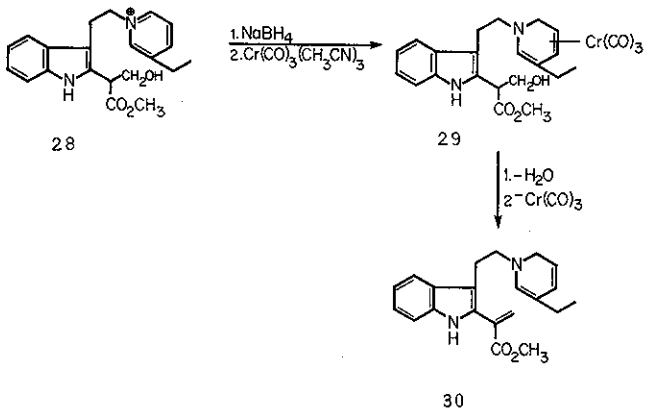


Figure 18. A possible synthesis of dehydrosecodine (30) from the pyridinium salt 28.

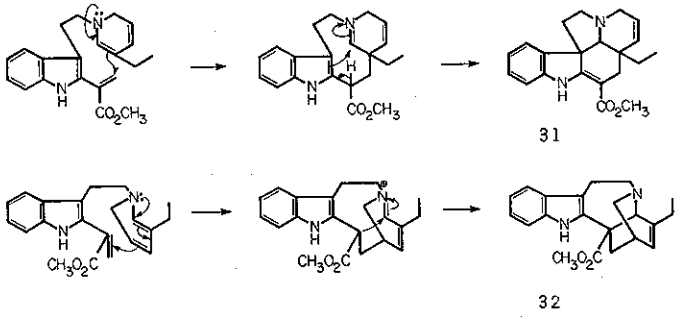


Figure 19. Possible cyclizations of dehydrosecodine to the Aspidosperma and Iboga alkaloid systems.

The final steps in the synthesis of dehydrosecodine involve the introduction of the methylene group in the ester side chain, a reaction sequence which has been successfully accomplished in a closely related indole system in the secodine synthesis (Figure 3).

An alternative sequence is outlined in Figure 18 where the pyridinium salt 28 is converted to the chromium carbonyl complex 29 and the latter is elaborated to dehydrosecodine (30) by means of dehydration and final removal of chromium. This sequence is presently under investigation in our laboratories.

Apart from the biosynthetic interests noted above for the dehydrosecodine series, it would be of considerable interest to evaluate the chemistry of this system. Figure 19 illustrates the possible cyclizations of dehydrosecodine to the Aspidosperma (31) and Iboga (32) alkaloid families.

It is hoped that this article will serve to illustrate that the chemistry of certain dihydropyridines is still in the infancy stage and that interesting synthetic opportunities are still available with such intermediates. It is felt that their role in synthetic and biosynthetic areas can only be properly evaluated when such systems can be prepared and isolated in some stable form. Hopefully the area of metal carbonyl complexes will present one possible avenue to achieve these various objectives.

Acknowledgement

The experiments presented above have been performed by a group of hard working and enthusiastic colleagues and it is certainly a pleasure for the author to acknowledge their efforts. They are: J. Beck, W. Cretney, N. Eggers, C. Ehret, G. Fuller, R. Greenhouse, D. Grierson, J. Hadfield, E.S. Hall, H. Hanssen, G. Krasny, V. Nelson, G. Poulton, V. Ridaura, P. Salisbury, P. Singh, R. Sood, N. Westcott, D. Wigfield and A. Zanarotti.

I would also like to express our sincere thanks to Dr. B. Gilbert and his colleagues in Rio de Janeiro, Brazil, for their assistance in making collections of seeds and acquiring numerous plant extracts from which the required alkaloids could be isolated. Professor Carl Djerassi, Stanford University, kindly provided samples of vallesamine and apparicine.

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