Our first experiments with 2-year old plants involved the evaluation of tryptophan, a progenitor of many indole alkaloids, and the establishment of satisfactory feeding methods. Using tryptophan labeled with tritium in the indole nucleus, significant incorporation into apparicine, but not into uleine, was observed by feeding either the whole plant (by the cotton wick method) or just the roots (severed from the plant) (by the hydroponic method). Both feeding methods gave similar values of incorporation, and the results are summarized in Table I.

Table I. Results of Incorporation of [*ar*-^{3}H]DL-Tryptophan into *Aspidosperma pyricollum* Using Different Feeding Methods

Expt	Feeding method	Activity of tryptophan fed, dpm	Activity of alkaloids, dpm	% incorporation
1	Wick into stem	1.25×10^8	Apparicine, 1.3×10^4	0.010
			Uleine, <120	<0.0001
2	Roots, hydroponic	1.28×10^8	Apparicine, 2.3×10^4	0.018
			Uleine, <120	<0.0001

These figures suggested that (1) apparicine and uleine are not interrelated *via* a common precursor such as **5**, and (2) tryptophan could act as a precursor for apparicine. Both results are at variance with the above-mentioned postulate.[4]

Since, on the basis of the structural relationship between tryptophan and apparicine, it seemed likely that at least one of the two side-chain carbon atoms of tryptophan should be lost during biosynthesis, it became of great importance to determine whether either of these atoms was incorporated into apparicine. For this purpose, double-label feeding experiments were performed utilizing mixtures of tryptophan tritiated in the aromatic portion and tryptophan labeled with carbon-14 either at C-2 or at C-3. The tritium label provided an internal check that biosynthesis of the alkaloid from tryptophan had occurred during each experiment, while the ratio of activity between the two nuclei provided evidence as to the retention or loss of each of the two labeled carbon atoms. The results, summarized in Table II, clearly indicate that C-3 of

Table II. Results of Incorporation of Double-Labeled DL-Tryptophan into *Aspidosperma pyricollum*

Expt	Label distribution	Ratio of activity (^{14}C/^{3}H) Tryptophan fed	Apparicine isolated	Uleine isolated
3	*ar*-^{3}H,^{14}C$_3$	1.1	1.5	Inactive
4	*ar*-^{3}H,^{14}C$_2$	1.0	<0.03	Inactive

tryptophan is *incorporated* into apparicine, presumably at C-6, while over 97% of the activity of C-2 is removed, showing *loss* of this carbon atom during biosynthesis. The relatively constant ratio of ^{14}C to ^{3}H activity in expt 3 also demonstrates that feeding experiments with materials labeled with tritium in the indole ring are valid in the sense that significant exchange or loss of tritium does not occur during biosynthesis. Furthermore, it provides evidence that appreciable degradation of tryptophan does not occur during its incorporation into apparicine.

With respect to uleine, the above experiments served to confirm the initial results, that tryptophan is not incorporated into this alkaloid. Although negative results must be viewed with some caution, it seems likely that a progenitor of this amino acid as suggested by Wenkert[7] is involved. Experiments in this direction are now in progress.

Further experiments designed to determine at what stage in the biosynthesis of apparicine the tryptamine unit cleaves to lose C-2 are reported in the accompanying communication.

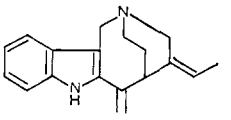
Acknowledgment. Financial aid from the National Research Council of Canada is gratefully acknowledged. We are very grateful to Dr. C. Djerassi, Stanford University, Dr. J. Joule, Manchester University, Dr. J. Schmutz, Research Institute, A. Wander, S.A., Bern, and Dr. B. Gilbert, Laboratorio de Quimica Organica, Faculdade Nacional de Farmacia, Rio de Janeiro, for samples of uleine and apparicine.

James P. Kutney, Vern R. Nelson, Donald C. Wigfield
Chemistry Department, University of British Columbia
Vancouver 8, Canada
Received May 12, 1969

Studies on Indole Alkaloid Biosynthesis. IV[1]

Sir:

In the accompanying communication[1] we have unequivocally demonstrated that in *Aspidosperma pyricollum* biosynthesis of apparicine (**1**) from tryptophan (**2**) occurs by loss of C-2 and retention of C-3. We now



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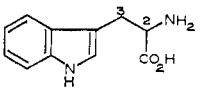

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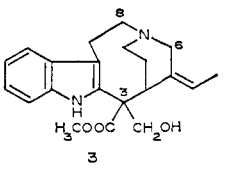
report results of experiments relating to the biosynthetic pathway involved and possible mechanisms by which the carbon atom may be lost.

Of the wide variety of compounds that could be tested as biosynthetic precursors of apparicine (**1**), two which were felt to be the greatest potential sources of information were stemmadenine (**3**) and 3-aminomethylindole (**4**).



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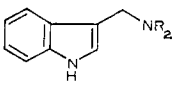


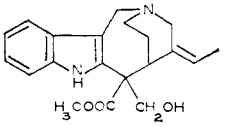
4, R = H
5, R = CH_3

Stemmadenine (**3**) was selected owing to its close structural relationship to apparicine (**1**) and also because of the crucial biosynthetic position this alkaloid has recently been shown to occupy in the rearrangement of the Corynanthe skeleton to the Aspidosperma and

(1) Part III: J. P. Kutney, V. R. Nelson, and D. C. Wigfield, *J. Am. Chem. Soc.*, **91**, 4278 (1969).

Iboga alkaloids.[2] Furthermore, it was encouraging that stemmadenine (**3**) has been shown to coexist with apparicine (**1**), being present in the fruits of *Aspidosperma pyricollum*.[3] If the biosynthesis of apparicine (**1**) were to proceed through the intermediacy of stemmadenine (**3**), then clearly the degradation of the two-carbon bridge to the one-carbon unit present in this alkaloid, with loss of the original C-2 of tryptophan, much occur as one of the very *last* steps in the biosynthetic pathway. In direct contrast to this, it was possible that this carbon atom was lost as one of the *first* transformations from tryptophan. Indeed, our results showing loss of C-2 and retention of C-3 are highly reminiscent of the results of the elegant experiments of Leete and Marion[4,5] regarding the biosynthesis of the alkaloid gramine (**5**), in which compound **4** is presumably the penultimate precursor. Regarding **4** as "nortryptamine," it was attractive to envisage an alternative biosynthetic route to the apparicine family of alkaloids as paralleling the biosynthesis of other indole alkaloids except that they originated from this "nortryptamine" compound instead of from tryptamine (or tryptophan) itself.

In order to test these hypotheses, stemmadenine (**3**), 3-aminomethylindole (**4**) (prepared as reported previously by the reduction of indole-3-carboxaldehyde oxime),[6] and vallesamine (**6**),[7] an alkaloid with structural featues intermediate between stemmadenine (**3**) and apparicine (**1**), were labeled with tritium in the aromatic portion of the molecule and fed to the roots of



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Aspidosperma pyricollum. A summary of the results of the various experiments is presented in Table I.

Table I. Results of Incorporation into Apparicine and Uleine of Various Intermediates under Identical Conditions in *Aspidosperma pyricollum* Roots

Expt	Compound fed	% incorporation Apparicine	Uleine
1	[*ar*-^{3}H]DL-Tryptophan (**2**)	0.02	<0.0001
2	[*ar*-^{3}H]Stemmadenine (**3**)	0.55	<0.0007
3	[*ar*-^{3}H]Vallesamine (**6**)	0.01	<0.003
4	[*ar*-^{3}H]3-Aminomethylindole (**4**)	<0.001	<0.0001

The most striking feature of the results was the astonishingly high incorporation of stemmadenine (**3**), strongly indicating that this alkaloid, which plays such an important role in the biosynthesis of other indole alkaloids, is also of crucial importance in the biosynthesis of apparicine (**1**). This result, together with the failure of **4** to be incorporated, demonstrates that fragmentation of the tryptophan moiety and loss of C-2 occur as *one of the final steps* in the biosynthesis. Since initial evidence bearing on the biosynthesis of stemmadenine (**3**) is already available,[2] interest in the present instances is immediately directed toward the way in which stemmadenine (**3**) is converted to apparicine (**1**). In this conversion, essentially two distinct changes are required—loss of C-8 of stemmadenine (**3**) and decarboxylation of the ester function at C-3 to the exocyclic methylene group. Clearly these two changes can be considered *a priori* to occur in one of three ways: (1) loss of C-8 preceding decarboxylation, (2) decarboxylation preceding loss of C-8, and (3) both changes occurring simultaneously. In this regard, the very low incorporation of vallesamine (**6**) (with respect to that of stemmadenine (**3**)) would tend to rule out consideration 1, since vallesamine (**6**) can be regarded as that structure in which loss of C-8 of stemmadenine (**3**) has occurred without decarboxylation. That some incorporation (0.01%) is observed is perhaps not very surprising in view of the very close structural relationship between vallesamine (**6**) and apparicine (**1**), but if vallesamine (**6**) were on the true biosynthetic pathway, an incorporation comparable to, or even higher than, that observed for stemmadenine would have been expected. We wish to emphasize that these experiments were conducted on roots of the same plant so as to completely minimize any differences between them.

The present data do not allow distinction between choices 2 and 3, and one can envisage mechanisms by which either choice could be involved. Experiments designed to distinguish between these possibilities are now in progress.

As expected, none of the above compounds was incorporated into uleine. These results are consistent with the negative incorporation of tryptophan as noted in the accompanying communication.

Acknowledgment. Financial aid from the National Research Council of Canada is gratefully acknowledged. We are very grateful to Dr. C. Djerassi, Stanford University, and Dr. A. Hofmann and Dr. D. Stauffacher, Sandoz, Ltd., Basle, for samples of stemmadenine and vallesamine.

James P. Kutney, Vern R. Nelson, Donald C. Wigfield
Chemistry Department, University of British Columbia
Vancouver 8, Canada
Received May 12, 1969

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(3) R. R. Arndt, S. H. Brown, N. C. Ling, P. Roller, C. Djerassi, J. M. Ferreira, B. Gilbert, E. C. Miranda, S. E. Flores, A. P. Duarte, and E. P. Carrazzoni, *Phytochemistry*, **6**, 1653 (1967).
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The Acetolysis of the 7-Chloro-2-tosyloxynorbornanes

Sir:

The anomalous results obtained in our laboratory in relation to the acetolysis of 7-oxygenated 2-norbornyl tosylates have demonstrated the complexity of utilizing electron-withdrawing functions containing oxygen to destabilize the norbornyl cation.[1] In order to test whether a nonoxygenated electron-withdrawing function could be used to determine the effect of an electron deficiency on the developing 2-norbornyl cation, we have investigated the solvolysis of the four epimeric 7-chloro-2-tosyloxynorbornanes.[2] We wish to report at

(1) P. G. Gassman and J. L. Marshall, *J. Am. Chem. Soc.*, **88**, 2822 (1966); P. G. Gassman and J. L. Marshall, *Tetrahedron Letters*, 2429, 2433 (1968); P. G. Gassman and J. G. Macmillan, *J. Am. Chem. Soc.*, in press.
(2) *syn*-7-Chloro-*exo*-2-tosyloxynorbornane (**1**) and *anti*-7-chloro-*exo*-2-tosyloxynorbornane (**3**) have been studied previously.[3] However, these studies only provided rate data on the *exo*-tosylates and product studies were based on infrared comparisons of reaction mixtures.