to the DCC–H_2SO_4 mixture within 30 sec after the DCC and H_2SO_4 were combined, under the "dilute" conditions. The DCC and H_2SO_4 react in the DMF to form dicyclohexylurea. With the disappearance of DCC, the reaction mixture can no longer sulfate a nucleophile. Therefore, DCC, H_2SO_4, and DMF do not react to form a stable sulfating agent under the "dilute" conditions, such as DMF–SO_3,[21] which would not lose its sulfating ability in 30 sec. Thus the nucleophile appears to be reacting with a DCC–sulfuric acid complex, rather than with a product produced by the dehydration of H_2SO_4, such as sulfur trioxide. Since solvation is one of the most important factors controlling nucleophilicity,[22] DMF may play an active role in the mechanism of the reaction under the "dilute" conditions, when nucleophilic selectivity is found. A reasonable mechanism would involve the formation of a solvolyzed protonated DCC–H_2SO_4 intermediate, followed by a hydroxyl nucleophilic attack to produce a monosulfate ester and dicyclohexylurea.

(21) M. L. Wolfrom and T. M. Shen Han, *J. Am. Chem. Soc.*, **81**, 1764 (1959).
(22) R. F. Hudson, *Chimia*, **16**, 173 (1962).

Analogous intermediates have been postulated[10] for reactions of phosphoric acids and of sulfonic acids (RSO_3H) with DCC under similar reaction conditions. It is reasoned that a protonated adduct enhances the subsequent nucleophilic attack, for it weakens the high π-electron density of the oxygens surrounding the sulfur atom. This hydroxyl attack is further promoted by a slight positive charge residing on the sulfur atom owing to the sulfur–oxygen semipolar bonds of the adduct.

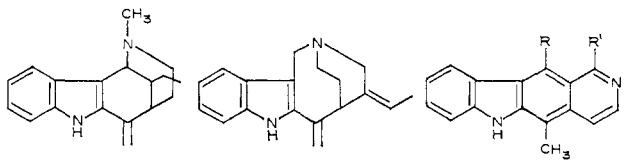
The results of these experiments raise a number of questions which are currently under investigation in our laboratory. The ratio of the reactants used was chosen for comparison purposes; therefore, additional studies are necessary to determine the ratio of reactants for optimal yields of monosulfates. DCC was chosen because of its common use and its availability; however, other carbodiimides might give better yields and perhaps a different selectivity. The techniques of the isolation of gram quantities of the ^{35}S-labeled products are being developed. Additional studies are necessary to determine what role DMF may play in the mechanism and to determine whether solvents other than DMF will also show a selectivity in sulfation.

Communications to the Editor

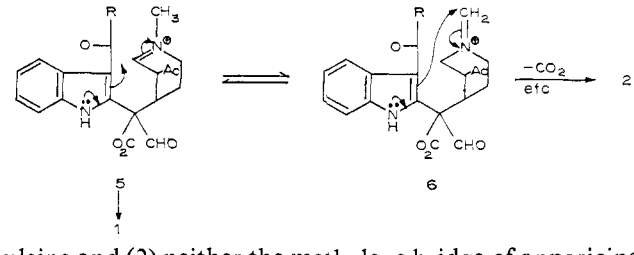
Studies on Indole Alkaloid Biosynthesis. III[1]

Sir:

In previous communications[1,2] we have reported results relating to the later stages of the biosynthesis of Aspidosperma and Iboga alkaloids. Another highly interesting family of indole alkaloids is that which, in common with other Aspidosperma, Iboga, and Corynanthe alkaloids, possesses the ubiquitous C_{10} "non-tryptophan" unit, yet does *not* possess an obvious tryptophan portion. Alkaloids in this family include uleine (**1**)[3] and apparicine (**2**),[4] in which only one carbon atom separates the indole nucleus and the basic nitrogen atom, as opposed to the normal two-carbon bridge of tryptamine and most indole alkaloids. Olivacine (**3**)[5] and ellipticine (**4**),[6] on the other hand, have three carbon atoms between the indole and the nitrogen functions.



No data are presently available on the biosynthesis of these interesting alkaloids. Wenkert[7] has proposed that a progenitor of tryptophan, rather than tryptophan itself, condenses with the C_{10} unit to give **5**, from which uleine, olivacine, and ellipticine could be derived. Djerassi and coworkers,[4] in reporting the structure of apparicine, postulated that a prototropic rearrangement, followed by appropriate cyclization, etc. (**5** → **6** → **2**), would yield the apparicine system. These postulates imply (1) the same precursor for both apparicine and uleine and (2) neither the methylene bridge of apparicine nor the N-methyl of uleine is derived from the tryptophan side chain.



In the hope of obtaining some biosynthetic data relating to these interesting aspects, we have initiated some studies on *Aspidosperma pyricollum*, a plant which has been reported to contain both alkaloids.[8,9]

(1) Part II: J. P. Kutney, C. Ehret, V. R. Nelson, and D. C. Wigfield, *J. Am. Chem. Soc.*, **90**, 5929 (1968).
(2) J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson, and D. C. Wigfield, *ibid.*, **90**, 3566 (1968).
(3) G. Buchi and E. W. Warnhoff, *ibid.*, **81**, 4433 (1959).
(4) J. A. Joule, H. Monteiro, L. J. Durham, B. Gilbert, and C. Djerassi, *J. Chem. Soc.*, 4773 (1965).
(5) (a) G. B. Marini-Bettolo and J. Schmutz, *Helv. Chim. Acta*, **42**, 2146 (1959); (b) J. Schmutz and H. Wittmer, *ibid.*, **43**, 793 (1960); (c) E. Wenkert and K. G. Dave, *J. Am. Chem. Soc.*, **84**, 94 (1962).
(6) R. B. Woodward, G. A. Iacobucci, and F. A. Hochstein, *ibid.*, **81**, 4434 (1959).
(7) E. Wenkert, *ibid.*, **84**, 98 (1962).
(8) B. Gilbert, L. D. Antonaccio, A. A. P. G. Archer, and C. Djerassi, *Experientia*, **16**, 61 (1960).
(9) R. R. Arndt and C. Djerassi, *ibid.*, **21**, 566 (1965).

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.

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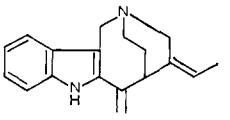
Chemistry Department, University of British Columbia
Vancouver 8, Canada
Received May 12, 1969

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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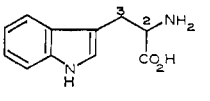
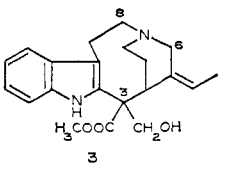


2

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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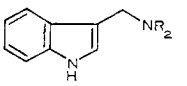

4, R = H
5, R = CH_3

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).