4605) and by the college's Fund for the Coordination of the Sciences.

(27) Alfred P. Sloan Research fellow, 1964–1968.

Frank B. Mallory[27]
Department of Chemistry, Bryn Mawr College
Bryn Mawr, Pennsylvania 19010

Robert L. Conner, Josephine R. Landrey
Department of Biology, Bryn Mawr College
Bryn Mawr, Pennsylvania 19010

J. M. Zander, J. B. Greig, E. Caspi
The Worcester Foundation for Experimental Biology
Shrewsbury, Massachusetts 01545

Received March 25, 1968

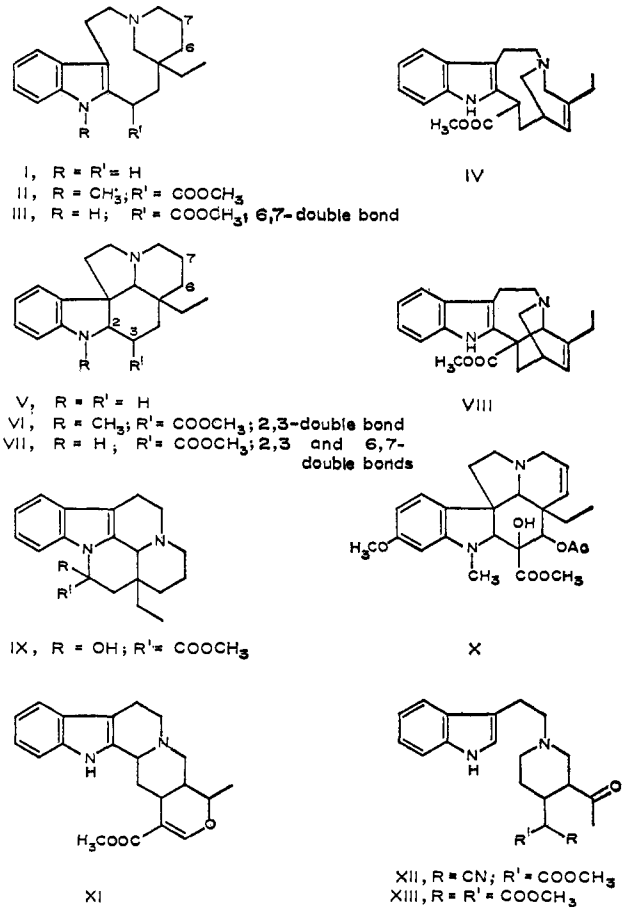
Studies on Indole Alkaloid Biosynthesis

Sir:

In recent years the biosynthesis of indole alkaloids has stimulated considerable interest in various laboratories.[1] Almost without exception these investigations have concentrated on the nature of the "non-tryptophan" unit necessary in the biosynthesis, and numerous elegant experiments are now in hand which establish the monoterpene loganin as playing an important role in this regard.[2–5] Our own interests in this area have been concerned with the later stages of the biosynthetic pathway, *i.e.*, the steps involved *after* the tryptophan–C_{10} "complex" has been formed. Such questions as (a) the structure of this "complex(es)" and (b) the pathways which it follows to elaborate the various families in the indole and dihydroindole series were of prime consideration. This communication presents some of our results in this area.

Of the various postulates which were available, the one proposed by Wenkert[6] was of particular interest in our initial considerations, since it relates directly to our synthetic work in this area. The transannular cyclization reaction developed in our laboratories provides a general entry into Aspidosperma, Iboga, and Vinca alkaloids.[7–10] The fundamental similarity of this latter process to the later steps in Wenkert's postulates provides the stimulus for its evaluation as a biosynthetically significant reaction.

For this purpose, the appropriate nine-membered ring intermediates represented by quebrachamine (I), vincadine (I, R = H; R′ = $COOCH_3$), and vincaminoreine (II) were evaluated as possible precursors of the Aspidosperma and Vinca alkaloids, while the corresponding carbomethoxycleavamine derivative (IV) was studied for its possible role in the biosynthesis of the Iboga family. Numerous experiments were conducted



in *Vinca rosea* Linn and *Vinca minor* Linn plants, and a brief resumé of the results is presented in Tables I and II.

The experimental method associated with the incorporation of large molecular weight compounds in terms of permeability, etc., was appreciated, and the initial experiments dealt with an evaluation of various techniques for the incorporation of such compounds. Table I illustrates the results from the various methods of feeding. It soon became apparent that no particular technique showed any obvious advantage over the others. The most frustrating aspect of these results was our inability to delineate what might be construed as a "positive" demonstration of the transannular cyclization process from the rather trivial oxygen-catalyzed conversion of the intermediates to the alkaloids during the period of incorporation.[11]

In an attempt to obtain internally consistent data which may shed more light on the cyclization reaction, we then turned our attention to a series of experiments in which identical conditions were maintained throughout the entire series. For this purpose, 6-month-old *V. rosea* L. plants were selected, and the incorporation of the appropriate precursor was administered by the cotton wick technique into the stems of the plant. In each instance, the number of plants fed was sufficient to provide the direct isolation of the alkaloids which, without any further dilution with "cold" material, could be crystallized to constant activity. Conversion of each of these into the corresponding hydrochloride

(1) For a recent survey, see A. R. Battersby, *Pure Appl. Chem.*, **14**, 117 (1967).
(2 A. R. Battersby, R. S. Kapil, and R. Southgate, *Chem. Commun.*, 131 (1968).
(3) A. R. Battersby, R. S. Kapil, J. A. Martin, and L. Mo, *ibid.*, 133 (1968), and references cited therein.
(4) S. Brechbühler-Bader, C. J. Coscia, P. Loew, Ch. von Szczepanski, and D. Arigoni, *ibid.*, 136 (1968).
(5) P. Loew and D. Arigoni, *ibid.*, 137 (1968), and references cited therein.
(6) E. Wenkert, *J. Am. Chem. Soc.*, **84**, 98 (1962).
(7) J. P. Kutney and E. Piers, *ibid.*, **86**, 953 (1964).
(8) J. P. Kutney, R. T. Brown, and E. Piers, *ibid.*, **86**, 2286, 2287 (1964).
(9) J. P. Kutney, N. Abdhurahman, P. Le Quesne, E. Piers, and I. Vlattas, *ibid.*, **88**, 3656 (1966).
(10) J. P. Kutney, W. J. Cretney, P. Le Quesne, B. McKague, and E. Piers, *ibid.*, **88**, 4756 (1966).

(11) The conversion of these compounds to the appropriate alkaloids by oxygen in the presence of a metal catalyst was already demonstrated in our laboratory. It was hoped that a much higher level of incorporation in the plants relative to the blank experiment could be obtained.

Table I. Results of Incorporation of Nine-Membered Ring Intermediates into *V. rosea* L. and *V. minor* L. by Various Techniques

Expt	Compd[a] fed	Plant	Feeding method	Alkaloid isolated	% incorporation—Plant	% incorporation—Blank[b]
1	[*ar*-^{3}H]Carbomethoxycleavamine (IV, HCl salt)	*V. rosea* L.	Cotton wick into stems, 8 days	Catharanthine (VIII)	<0.015	0.04
2	[*ar*-^{3}H]Carbomethoxycleavamine (IV, HCl salt)	*V. rosea* L.	Vacuum infiltration of leaf discs, 42 hr	Catharanthine	<0.008	
3	[*ar*-^{3}H]Carbomethoxycleavamine (IV, acetate salt)	*V. rosea* L.	Leaf vein injection,[c] 6 days	Catharanthine	<0.05	
4	[*ar*-^{3}H]Carbomethoxycleavamine (IV, HCl salt)	*V. rosea* L.	Hydroponic, cut stems, 46 hr	Catharanthine	<0.011	
5	[*ar*-^{3}H]Quebrachamine (I)	*V. minor* L.	Hydroponic, cut stems, Tween 20 emulsion, 4 days	Aspidospermidine (V)	0.48	3.4
6	[*ar*-^{3}H]Vincaminoreine (II, acetate salt)	*V. minor* L.	Absorption through leaf sections, 4 days[d]	Vincamine (IX) Aspidospermidine Minovine (VI)	<0.001 <0.008 0.7	 0.3

[a] The preparation of the various precursors employed in these studies will be discussed in our detailed paper. [b] In all instances, blank experiments were run under similar conditions to those involved in the plant feedings. [c] In these experiments, incorporation of precursors was performed by capillary injection into leaves of growing plants. [d] In these experiments, fine incisions were made on the leaves of the growing plant and the precursor was absorbed from plastic bags carefully fitted over these sections.

Table II. Results of Incorporation of Various Intermediates into *V. rosea* L. under Identical Conditions

Expt	Compd[a,b] fed	% incorporation—Catharanthine (VIII)	% incorporation—Vindoline (X)	% incorporation—Ajmalicine (XI)
7	[3-^{14}C]DL-Tryptophan	0.05	0.15	0.8
8	[*ar*-^{3}H]Tryptamine	0.01	0.003	0.4
9	[*ar*-^{3}H]III	<0.001	<0.001	<0.001
10	[*ar*-^{3}H]Tabersonine (VII)	0.05	0.03	<0.001
11	[*ar*-^{3}H]Carbomethoxycleavamine (IV)	0.03	<0.001	Inactive
12	[*ar*-^{3}H]Carbomethoxycleavamine (IV) blank experiment	0.04	Inactive	Inactive
13	[*ar*-^{3}H]-XII	<0.001	<0.001	0.004
14	[*ar*-^{3}H]-XIII	<0.001	<0.001	<0.001

[a] The preparation of the various precursors employed in these studies will be discussed in our detailed paper. [b] In all instances, the acetate salts were utilized.

salts confirmed the level of radioactivity. By this sequence even very low levels of incorporation could be easily detected. The pertinent results for catharanthine (VIII), vindoline (X), and ajmalicine (XI) are summarized in Table II, and a brief analysis of these is appropriate.

Experiments 7 and 8 illustrate that (a) the age of the plants selected for this study was suitable for biosynthesis and (b) the experimental method chosen at least provides positive incorporation of established precursors. Experiments 9 and 10 provide an important comparison between two closely related compounds in their role as potential precursors in the biosynthetic pathway. While the alkaloid tabersonine (VII) is converted into catharanthine and vindoline, the 6,7-dehydrovincadine derivative (III) is not incorporated. The latter compound is the immediate precursor of this alkaloid (VII) in the laboratory conversion which utilizes the transannular cyclization reaction.[12] The incorporation of tabersonine into these alkaloids furthermore establishes that the experimental method employed allows the incorporation of higher molecular weight "precursors" into the plant system. The level of incorporation of the cleavamine derivative IV into the Iboga system is also negligible, as shown in expt 11 and 12. All of these experiments strongly suggest that the transannular cyclization reaction is probably not significant in either Aspidosperma or Iboga biosynthesis, although it is clear that negative results must be interpreted with caution.

One of the most interesting results obtained in these investigations concerns the conversion of tabersonine to catharanthine in the plant.[13] In order to accommodate this transformation, it is necessary to rearrange the carbon skeleton of the tabersonine molecule. The manner in which this latter process occurs is of distinct relevance, and experiments in this direction are now in progress.

Finally, expt 13 and 14 reveal briefly the results of some of our initial investigations which utilize totally synthetic substances as potential precursors. It is noted that the cyanoacetic ester analog XII is incorporated at a low level into ajmalicine, while the malonic ester intermediate XIII is not utilized. Clearly the former substance cannot be seriously considered as a precursor, although the nitrile function is probably converted into an aldehyde or similar grouping in the living plant. Further experiments in this area are now being conducted.

Acknowledgment. Financial aid from the National Cancer Institute of Canada, National Research Council of Canada, and Medical Research Council of Canada is gratefully acknowledged.

(12) The facile cyclization of vincadine and its derivatives to the pentacyclic vincadifformine family is now well documented in our laboratory.

(13) At a recent Natural Products Symposium at the University of West Indies, Professor A. I. Scott, Sussex University, reported the conversion of tabersonine to vindoline and catharanthine in germinated seeds of *V. rosea* L.

James P. Kutney, Walter J. Cretney, John R. Hadfield
Ernest S. Hall, Vern R. Nelson, Donald C. Wigfield
Chemistry Department, University of British Columbia
Vancouver 8, British Columbia, Canada

Received April 12, 1968

On the Structure of the Liquid Crystalline State of Cholesterol Derivatives

Sir:

It is well established that liquid crystals in the cholesteric phase have a helical structure.[1] The long axes

(1) G. W. Gray, "Molecular Structure and The Properties of Liquid Crystals," Academic Press Inc., New York, N. Y., 1962.