$Ru(NH_3)_5NO^{3+}$ during the initial phase of the reaction. If HNO_2 and $Ru(NH_3)_6^{2+}$ form the same intermediate as $Ru(NH_3)_6^{3+}$ and NO, we must conclude that the usual fate of the intermediate is decomposition to $Ru(NH_3)_6^{3+}$ and NO, rather than formation of $Ru(NH_3)_5NO^{3+}$.

The ion $Ru(NH_3)_5NO^{3+}$ is also formed from $Ru(NH_3)_5OH_2^{3+}$ with NO. The reaction is of a rapidity comparable to that of $Ru(NH_3)_5Br^{2+}$ or $Ru(NH_3)_6^{3+}$ as the starting materials. The rate of formation of $Os(NH_3)_5NO^{3+}$ (the NO stretching band of $[Os(NH_3)_5NO](ClO_4)_3$ in KBr is split: weak at 1895; strong, sharp at 1875 cm^{-1}) from $Os(NH_3)_5OH_2^{3+}$ and NO seems to be even somewhat greater.

When $Fe(CN)_6^{3-}$ in excess acid is treated with NO, the yellow solution slowly begins to turn brown. At the end of 24 hr, the solution is red-brown. Upon addition of S^{2-} ion, the solution immediately turns violet. This result is consistent with that of Schwarzkopf,[8] who claimed that he obtained nitroprusside on bubbling ferri- or ferrocyanide solutions with nitric oxide.

Further work is in progress on these and related reactions of ruthenium and osmium.

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(8) P. Schwarzkopf, *Abhandl. Deut. Naturw., Med. Ver. Böhmen*, **3**, 1 (1911); *Chem. Abst.*, **8**, 1106 (1914).

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Studies on Indole Alkaloid Biosynthesis. II[1]

Sir:

In a previous communication[1] we reported some of our results relating to the later stages of indole alkaloid biosynthesis. In particular it was suggested that the transannular cyclization of nine-membered ring intermediates as had been previously postulated[2] was probably not a biosynthetically significant reaction in the Aspidosperma and Iboga series. This communication describes further results which strongly support such a suggestion, at least in the Aspidosperma alkaloids, and which in addition yield novel information about the later steps in the biosynthesis of these alkaloids.

In the hope of obtaining more distinctly positive results to those reported previously[1] we have studied a completely different approach to this problem. It is clear that the transannular cyclization process as illustrated in the conversion of the alkaloid vincadine (I) to vincadifformine (II), a reaction easily accomplished in the laboratory,[3] is only one of a number of alternative pathways in the plant elaboration of Aspidosperma alkaloids. An equally attractive and plausible scheme could invoke the reverse process, namely the ring opening of the pentacyclic system to yield the nine-membered ring alkaloids (*i.e.*, II → I).[4] This latter process would imply that Aspidosperma alkaloids of type II are

I, R = H
III, R = CH_3

II, R = H
IV, R = CH_3
VII, R = H; 6,7-double bond

biosynthetic precursors of type I. In order to obtain information on the relationship, if any, between these alternatives we have initiated some studies in *Vinca minor* L., a plant which possesses a wonderful array of Aspidosperma alkaloids.[5]

A detailed investigation involving the incorporation of DL-tryptophan-3-^{14}C into *V. minor* L. over different time intervals was undertaken, and some of the results are summarized in Table I. The method involved

Table I. Results of Incorporation of DL-Tryptophan-3^{14}C into *Vinca minor* L. at Various Time Intervals

Time	Total % incorporation: Vincadine (I) + vincaminoreine (III) (*A*)	Vincadifformine (II) + minovine (IV) (*B*)	*B*/*A*
4 hr	0.003	0.057	19
1 day	0.015	0.24	16
2 days	0.010	0.21	21
4 days	0.010	0.22	22
7 days	0.009	0.13	14
14 days	0.003	0.06	20

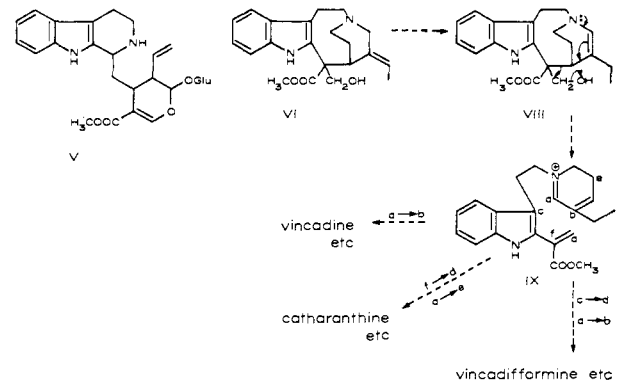
incorporation of a solution of the amino acid in 0.1 *N* acetic acid containing a few drops of methanol, and after the appropriate time the isolation of the alkaloids was carried out by chromatographic techniques. In each time interval reported the experiment was repeated at least twice. There was surprisingly good agreement between the results obtained in the individual experiments, and Table I gives the average values obtained in these studies. For the purposes of this discussion, the *total* per cent incorporation into the nine-membered ring alkaloids, vincadine (I) and vincaminoreine (III), and their respective cyclic relatives, vincadifformine (II) and minovine (IV), is presented. The fourth column in Table I shows the relative ratio of activities between these two groups.

A critical analysis of these results reveals several interesting features. These are: (1) activity in the alkaloids is noted even after a short exposure of 4 hr, (2) the activity in the pentacyclic alkaloids (II and IV) is consistently higher than in the nine-membered ring system, and (3) the relative ratio of activities (*B*/*A*) is *remarkably similar* over the time interval, 4 hr–2 weeks. This latter finding is certainly the most important in terms of providing information about the later stages of Aspidosperma alkaloid biosynthesis. The lack of any tendency for the ratio *B*/*A* to progressively increase or decrease with time speaks strongly against any direct biosynthetic relationship between the two groups of alkaloids. In other words, the previous suggestion[1]

(1) Part I: J. P. Kutney, W. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson, and D. C. Wigfield, *J. Am. Chem. Soc.*, **90**, 3566 (1968).
(2) E. Wenkert, *ibid.*, **84**, 98 (1962).
(3) J. P. Kutney, K. K. Chan, A. Failli, J. M. Fromson, C. Gletsos, and V. R. Nelson, *ibid.*, **90**, 3891 (1968).
(4) We have recently shown that such a ring-opening process can readily occur in the laboratory.
(5) J. Mokry and I. Kompis, *Lloydia*, **27**, 428 (1964).

that the transannular cyclization process is not biosynthetically important is now strongly supported by the above results. Furthermore, the ring-opening process (II → I) as mentioned above is similarly unimportant in providing a pathway to the nine-membered ring alkaloids found in *V. minor* L. It is clear that such an interpretation of our results is valid only if we are able to exclude any equilibration in the plant between the two alkaloid groups. The rather constant *B*/*A* could be obtained if an equilibrium mixture were enriched with respect to the pentacyclic alkaloids vincadifformine (II) and minovine (IV) to the extent of approximately 20:1. An evaluation of this process was thereby in order. The incorporation of various nine-membered ring alkaloids into *V. minor* L. was studied, and some experiments in this direction have been already discussed.[1] An approach from the opposite direction was also studied more recently when the radioactive alkaloid minovine (IV) was incorporated into this plant. In a typical experiment, during which radioactive IV was administered over a 1-week period, the subsequent investigation of the isolated plant alkaloids showed essentially *no* activity in the nine-membered ring compounds (*B*/*A* > 2500). On this basis we must propose that the above equilibration process is not significant in *V. minor* L. In turn we must conclude that the above results strongly support the situation that the genesis of the quebrachamine–vincadine family is *independent* of the pathway leading to the rigid pentacyclic Aspidosperma series (II, IV, etc.).

The question as to how the present results fit into the biosynthetic pattern which is rapidly evolving from the combined data of other investigations is worthy of comment. Wenkert[6] suggested that "all indole alkaloids of the tryptamine + C_{10} structure type may be derived from corynantheinoid or closely related progenitors." Based on elegant experiments in his and other laboratories, Battersby[7] was able to postulate the possible role of structures such as V in the biosynthesis of corynantheinoid as well as Aspidosperma and Iboga bases. Strong support for this latter postulate is available from very recent reports.[8] The possible intermediacy of units similar in structure to the alkaloid stemmadenine (VI) was invoked in the sequence between the corynantheinoid and Aspidosperma bases,[2,6] and again recent work[9] on germinated *Vinca rosea* L. seeds supports its role in this regard. The conversion of the alkaloid tabersonine (VII) to vindoline and most interestingly to the Iboga alkaloid catharanthine has been demonstrated in *V. rosea* L. plants in our laboratories[1] and independently by Scott in germinated seeds.[9] This latter result suggests a possible relationship between the Aspidosperma and Iboga alkaloids. In summation, all of these results strongly suggest *but do not prove* that the sequential formation of the indole alkaloids may follow the order: Corynanthe → Aspidosperma → Iboga. The results of the present investigation relate to the possible structural units which bear on the Corynanthe → Aspidosperma pathway. An attractive sequence which is in accord with present findings may be postulated (VI → IX → vincadine, vincadifformine, etc.). The rearrangement of stemmadenine (VI) to an isomer (VIII) provides an



attractive mechanistic rationale for bond fission to the intermediate IX.[10] This latter intermediate may then elaborate to the Aspidosperma and Iboga bases by the formal bond formations as indicated. The relative order of the latter processes relates directly to the results presented in this and the previous communication.[1] From the tryptophan incorporations discussed above, it is attractive to postulate that the independent biosynthetic pathways which lead to the vincadine and vincadifformine groups may initiate from a common intermediate such as IX. The eventual elaboration of the alkaloid systems depends merely on the relative order in which the bonds are formed. Thus the process a → b converts IX to vincadine, etc. The relative insignificance of the transannular cyclization process now suggests that the process c → d occurs *prior* to or *simultaneously* with a → b in the elaboration of IX to vincadifformine. In similar fashion, the conversion of unit IX to the alkaloid catharanthine is unlikely to proceed initially *via* the process a → e since this would lead to a carbomethoxycleavamine system. Previous results[1] have shown that we were unable to demonstrate the conversion of the latter to this Iboga alkaloid.

The relative importance of intermediates such as IX in the biosynthesis of indole alkaloids must await further investigations with appropriately designed experiments which will serve to test this postulate.

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(6) E. Wenkert and B. Wickberg, *J. Am. Chem. Soc.*, **87**, 1580 (1965).
(7) A. R. Battersby, *Pure Appl. Chem.*, **14**, 117 (1967).
(8) At the IUPAC Fifth International Symposium on the Chemistry of Natural Products, London, England, July 1968, two groups of workers (G. N. Smith and R. T. Brown, Manchester University, and A. R. Battersby and coworkers, University of Liverpool) reported the isolation of the alkaloidal glycoside V and mentioned its biosynthetic conversion to these various alkaloid families.
(9) A. A. Qureshi and A. I. Scott, *Chem. Commun.*, in press. We are very grateful to Professor A. I. Scott for providing us with results prior to publication.
(10) A similar postulate for the formation of IX has been advanced by A. I. Scott and coworkers.

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Tri-μ-hydrido- and Tri-μ-alkoxy-hexacarbonyldirhenate(I)

Sir:

The known carbonyl hydrides of rhenium are formed by reducing dirhenium decacarbonyl with sodium amal-