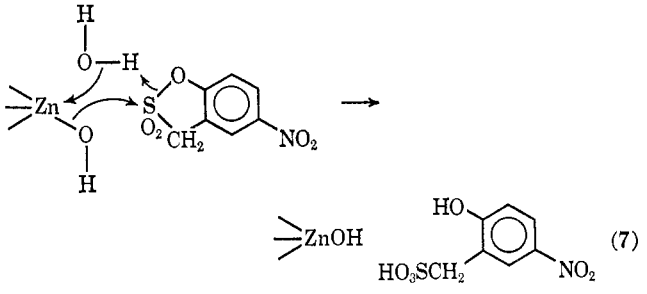
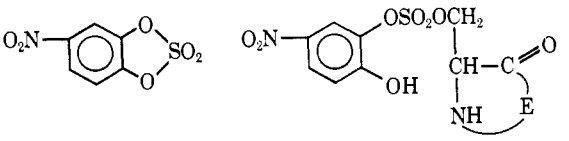
dration of CO_2,[34a] hydration of carbonyl compounds,[34b] and hydrolysis of nitrophenyl esters of carboxylic acids.[34c] The carbonic anhydrase catalyzed hydrolysis of **12** is subject to sulfonamide inhibition, as are the other reactions just mentioned. On the basis of our observations taken in conjunction with those of other investigators we have proposed the cyclic mechanism of eq 7 in which a zinc-bound hydroxide ion is the active catalytic species.[35]



(7)

The enzymatic reactions of other cyclic sulfate and sulfonate esters are also being studied. For instance, the cyclic sulfate **23** rapidly sulfonates the active site of α-chymotrypsin to produce **24**. Unlike the sulfonyl-enzyme **18**, however, the rate of decomposition of **24** is negligible over a wide pH range.[36]



23 **24**

Many additional aspects of the enzymatic hydrolyses of the reactive five-membered cyclic sulfates and sulfonates are being explored,[37] but to maintain the moderate length of this Account further discussion of our findings must be deferred. At this stage of our work, however, it certainly appears that these compounds and related cyclic esters will have wide applicability to the investigation of enzymatic reaction mechanisms.

The research reported in this review was supported in part by Agricultural Research Service, U. S. Department of Agriculture, Grant No. 12-14-100-9145(71), administered by Northern Utilization Research and Development Division, Peoria, Ill., and by grants from the National Institute of General Medical Sciences and the National Science Foundation. The author wishes to express his appreciation to the students and postdoctoral fellows whose work is summarized in this review.

(34) (a) J. C. Kernohan, *Biochim. Biophys. Acta*, **81**, 346 (1964); (b) Y. Pocker and J. E. Meany, *Biochemistry*, **4**, 2535 (1965); (c) Y. Pocker and J. T. Stone, *ibid.*, **6**, 668 (1967).

(35) We favor the cyclic reaction pathway of eq 7 because it avoids the difficulty of postulating a net proton transfer from the enzyme to the solvent, a reaction which may be too slow to be consistent with the high catalytic efficiency of carbonic anhydrase. Although a step equivalent to that shown in eq 7 can be written for the carbonic anhydrase catalyzed attack of water on carbon dioxide, we do not mean to imply that additional steps might not be needed to account for the overall enzyme catalyzed equilibration of carbon dioxide and bicarbonate in solution.

(36) The reasons for this difference in the behavior of **18** and **24** are under examination.

(37) The use of the sultone **12** as an active-site titrant has been described: J. H. Heidema and E. T. Kaiser, *Chem. Commun.*, 300 (1968); F. J. Kézdy and E. T. Kaiser, "Principles of Active Site Titration of Proteolytic Enzymes," a review which will appear in a forthcoming volume of *Methods Enzymol.*

Biosynthesis of the Indole Alkaloids

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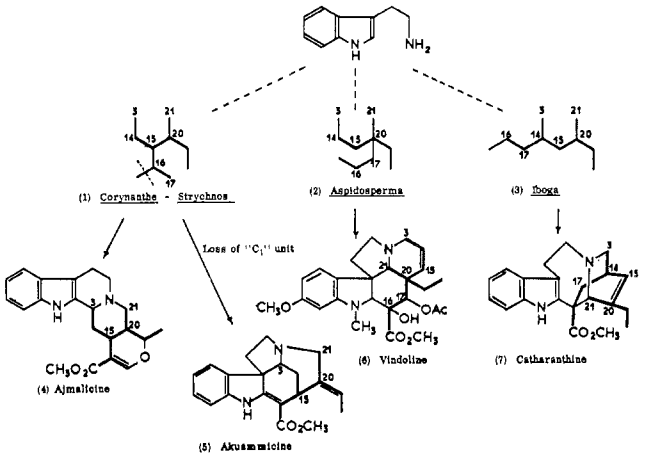
In the annals of biogenetic theory perhaps no single class of natural product has enjoyed more ingenious speculation from the organic chemist than the family of indole alkaloids, which are formally derived from the combination of tryptamine and an ubiquitous "C_9–C_{10}" unit. Not only the biochemical origin of the latter species but its appearance in the well-known *Corynanthe–Strychnos* pattern (**1**) have provoked stimulating comment ever since Barger[1] drew attention to a possible biogenesis of yohimbine in 1934.

Recent structural studies have increased the number of these alkaloids to more than 800, and two further main groups (with many subdivisions) can be discerned in which the C_9–C_{10} unit conforms to the *Aspidosperma* (**2**) and *Iboga* (**3**) skeletons.[2] Typical examples of these categories are ajmalicine (*Corynanthe*) (**4**), akuammicine (*Strychnos*) (**5**), vindoline (*Aspidosperma*) (**6**), and catharanthine (*Iboga*) (**7**), all of which occur (together with at least 70 other indole alkaloids) in the tropical periwinkle, *Vinca rosea*.[2] It is perhaps surprising that such prolonged biogenetic stimulation had, until 5 years ago, provided the experimental facts that although tryptophan served as a specific precursor for the appropriate (tryptamine) segment of the alka-

(1) G. Barger, IXth Congress Internacional de Quimica Pura y Applica, Madrid, Conferencias de Introduccion, 1934, p 177.

(2) (a) M. Hesse, "Indolalkaloide in Tabellen," Springer, Berlin, Vol. I, 1964, Vol. II, 1968; (b) *Alkaloids*, **11**, 1 (1968).



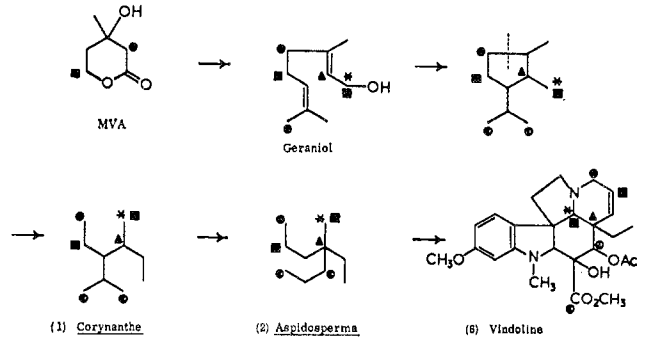
loids of several higher plants,[3] the biochemical building blocks for the "C_9–C_{10}" unit remained obscure and not one of the several theories tested had received support or seemed likely to survive.

Origin of the C_9–C_{10} Unit

The ready availability of *V. rosea*, its robust hydroponic behavior, and the diversity of its alkaloidal constituents[4] gave us the opportunity to reexamine experimentally the proposal which Thomas had discussed with us in 1961 concerning the mevalonoid origin of the indole alkaloids. Thus it could be shown that (*RS*)-mevalonate-2-^{14}C[5,6] and (5*RS*)-mevalonate-*d*[6] were specifically incorporated into the C_{10} section of vindoline (**6**) with equipartition of the terminal ^{14}C label, as indicated in Scheme I,[7] after hydroponic administration to *V. rosea* shoots.[6] The next logical precursor, geraniol, was then administered with both 2-^{14}C and 1-*d* labels,[6c,d] and biological conversion without randomization of either label into vindoline could be demonstrated as summarized in Scheme I. These results were obtained and independently confirmed[8,9]

Scheme I

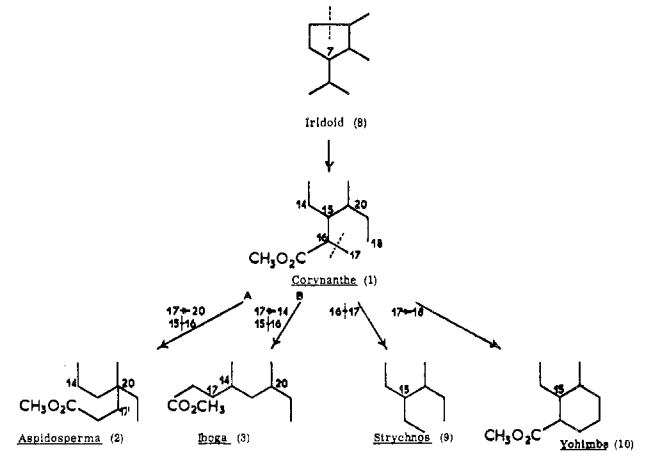
Incorporation of (*RS*)-Mevalonate and Geraniol into the *Aspidosperma* Alkaloid Vindoline[a]



[a] ●, MVA-2-^{14}C; ■, MVA-5-*d*; *, geraniol-1-*d*; ▲, geraniol-2-^{14}C; ◐ indicates one-half of the original ^{14}C label at this position.

Scheme II

Thomas–Wenkert Hypothesis



at a time when popular enthusiasm for the monoterpene hypothesis of Thomas[10] and Wenkert[11] seemed to have languished.[12]

In the Thomas–Wenkert hypothesis (Scheme II) the *Corynanthe* skeleton (**1**) is derived formally by cleavage of the generalized iridoid pattern (**8**). Loss of one carbon (indicated by the broken line in **1**) rationalizes the *Strychnos* "C_9" unit (**9**) as found in akuammicine (**5**). The *Corynanthe* skeleton is schematically related to the *Aspidosperma* (**2**) and the *Iboga* (**3**) series by cleavage of the C_{15}–C_{16} bond[13] in **1** and formation of C_{17}–C_{20} (path A) or C_{17}–C_{14} (path B). The *Yohimbe* class (**10**) is also reached from **1**, this time by ring closure *via* C_{17}–C_{18} bond formation.

A most ingenious idea was adduced by Wenkert[11] in order to rationalize type A and B transformations at the full alkaloidal level, *i.e.*, after the combination of

(3) (a) Tryptamine has recently been shown (ref 3b-e) to be specifically incorporated into several alkaloids of *V. rosea* with considerable variation in efficiency, suggesting that decarboxylation may be delayed in some cases. (b) E. Leete, *Accounts Chem. Res.*, **2**, 59 (1969); (c) A. I. Scott and P. C. Cherry, unpublished observations; (d) I. D. Spenser, *Compr. Biochem.*, **20**, 231 (1968); (e) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc.*, 1187 (1969); P. Loew and D. Arigoni, *Chem. Commun.*, 137 (1968), and references cited in these papers.

(4) We are grateful to Dr. C. T. Beer, Cancer Research Institute, University of British Columbia, Vancouver, Canada, for drawing our attention to these attributes of periwinkle and for providing us with our first cutting in Oct 1964.

(5) A. I. Scott, Karl Folkers Lecture Series, University of Wisconsin, Dec 1964.

(6) T. Money, I. G. Wright, F. McCapra, and A. I. Scott, *Proc. Nat. Acad. Sci. U.S.*, **53**, 901 (1965); (b) F. McCapra, T. Money, A. I. Scott, and I. G. Wright, *Chem. Commun.*, 537 (1965); (c) E. S. Hall, F. McCapra, T. Money, K. Fukumoto, J. R. Hanson, B. S. Mootoo, G. T. Phillips, and A. I. Scott, *ibid.*, 348 (1966); (d) T. Money, I. G. Wright, F. McCapra, E. S. Hall, and A. I. Scott, *J. Amer. Chem. Soc.*, **90**, 4144 (1968).

(7) This randomization is now known to be a common phenomenon. For recent examples see H. Schmid, *Chimia*, **22**, 312 (1968); M. Biollaz and D. Arigoni, *Chem. Commun.*, 633 (1969).

(8) H. Goeggel and D. Arigoni, *ibid.*, 538 (1965); A. R. Battersby, R. T. Brown, R. S. Kapil, A. O. Plunkett, and J. B. Taylor, *ibid.*, 46 (1966).

(9) For reviews see (a) A. R. Battersby, *Pure Appl. Chem.*, **14**, 117 (1967); (b) ref 3b; (c) ref 3d.

(10) R. Thomas, *Tetrahedron Lett.*, 544 (1961).

(11) E. Wenkert, *J. Amer. Chem. Soc.*, **84**, 98 (1962); E. Wenkert and B. Wickberg, *ibid.*, **87**, 1580 (1965).

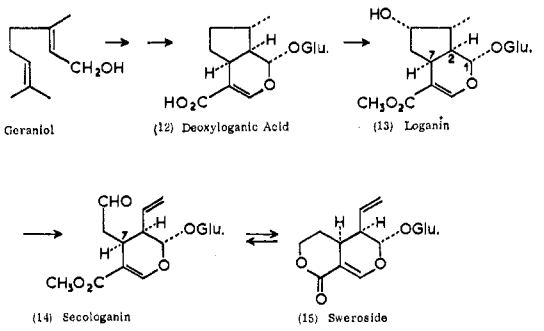
(12) For interesting accounts of this era see, *inter alia*, E. Leete in "Biogenesis of Natural Compounds," P. Bernfeld, Ed., Pergamon, Oxford, Chapter 17: 1st ed, 1963; 2nd ed, 1967; A. R. Battersby, ref 9a, and Summer School in Biogenesis (Donegani Lectures), Milan (1962); R. Robinson, *Pure Appl. Chem.*, **6**, 601 (1963).

(13) The biogenetic numbering system followed is that of W. I. Taylor and J. Le Men, *Experientia*, **21**, 508 (1965).

tryptamine with the monoterpene unit (as **1**), and later modifications of the theory included the prediction that the *Corynanthe* alkaloids would act as progenitors of the more complex systems. The rearrangements suggested for paths A and B[11] required the presence of the 1,5-dicarbonyl function in order to operate the reverse Michael reaction implicit in the cleavage of C_{15}–C_{16}. As discussed later, an alternative theory for these changes obviates the requirement of a carbonyl function at C_{19}. With the knowledge that the indole alkaloids were in fact rather elaborate monoterpenoids, we were intrigued by the second major problem posed by the structures before us: how are the rearrangements summarized in A and B (Scheme II) carried out in nature, and where would we begin to test the virtual myriad of possible substrates designed to undergo the A and B transformations? We decided that in spite of many problems associated with the controlled growth and analysis of *V. rosea* from seed, the insight gained from a knowledge of the sequence of alkaloid production in a biological system which eventually yields all three types of structure (and yet whose seeds contain virtually no alkaloid) would be of the greatest value in suggesting the dynamics of the biosynthetic mechanism. While the necessary techniques of germination and analysis of *V. rosea* were being developed during 1966–1967 our colleagues in Liverpool and Zurich were rapidly establishing the structural and stereochemical details of the monoterpene–iridoid sequence in mature *Vinca* and other species. Thus loganin (**13**) and secologanin (**14**)[14] were found to be the real biointermediates corresponding to **8** and **1**, respectively, in the Thomas–Wenkert hypothesis (Scheme II).

A most gratifying outcome of these elegant labeling experiments was the finding that the stereochemical integrities of C_7 in loganin and its seco derivative **14** are maintained at C_{15} in the *Corynanthe* alkaloids and, as indicated in our earlier experiment with geraniol-1-*d*, C_1 of loganin is carried through to the alkaloids at the aldehyde level, *i.e.*, the proton marked with an asterisk in geraniol in Scheme I survives all subsequent rearrangements. The latter requirement assumes prime importance in formulating and testing the mechanisms developed below. The summary of the geraniol → secologanin pathway[14] is shown in Scheme III and includes the recently described iridoid **12**[15] as well as the "secolactone" sweroside (**15**) which has been biologically converted[16] to the three main classes of indole alkaloid. In an experiment which simulates the first biological encounter of tryptamine (≡tryptophan) with the C_{10} unit, Battersby[17] was able to carry out the laboratory condensation of secologanin (**14**) and tryptamine to form vincoside (**16**). The latter was found to serve as a precursor for the three classes

Scheme III



Geraniol (12) Deoxyloganic Acid (13) Loganin (14) Secologanin (15) Sweroside

of alkaloid. We shall see presently how our work with the "early" form of *V. rosea* converges with the iridoid–vincoside experiments in a most gratifying way.

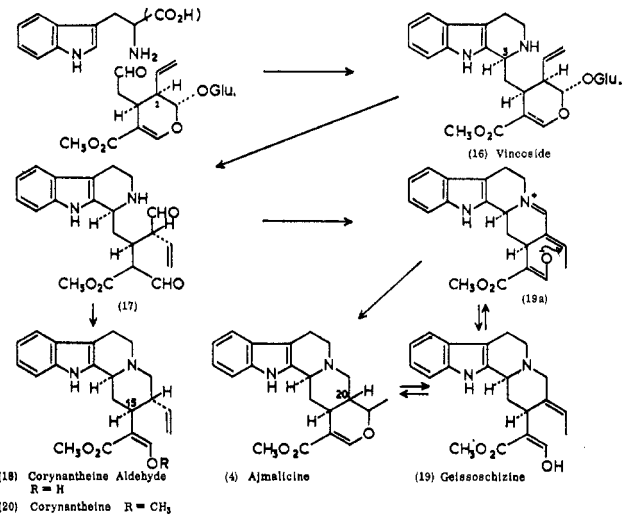
Sequential Studies in Germinating Seedlings

After many frustrations it was found that germination of *V. rosea* seeds in sterile media at 32° afforded reproducible variations of alkaloidal content with time. It could also be shown that, while the dry seeds were initially devoid of alkaloids, after about 10–12 days the mixture of indole alkaloids formed by the rapidly growing seedlings closely resembled that isolated from the mature plant.[18] The onset of alkaloid production could not be detected before the 24–26-hr aliquots, but when preparative thin-layer chromatography and spectroscopic techniques were applied to these fractions a small quantity (11 mg from 500 g of seeds) of a glucoside, $C_{27}H_{34}O_9N_2$, was isolated (as the pentaacetate). The structure of the glucoside (**16**) forges the link between tryptamine and the secoiridoids (*e.g.*, **14**). The "primordial" alkaloid, vincoside, had just become available as a partially synthetic biointermediate for the indole alkaloids proper, as mentioned above, and comparison of the synthetic and natural materials confirmed their identity. Although C_3 of vincoside is easily epimerized, it could be shown[17] that one isomer, almost certainly **16**, serves as a good precursor of the three main classes of alkaloid in mature *V. rosea*. The isolation of "strictosidine" (with the same gross structure) from *Rhazya stricta*[19] and of vincoside from mature *Vinca*[17] were also reported at this time.

The bioconversion of vincoside to corynantheine aldehyde (**18**) or geissoschizine (**19**) would entail unexceptional enzymic hydrolysis of the glucosidic residue followed by reductive condensation of the nascent aldehyde as shown in Scheme IV (**17** → **18**), while ajmalicine (**4**), an abundant *Corynanthe* representative of *V. rosea*, could be reached by cyclization of **19** or the species **19a** since the C_{20} proton of **4** is not labeled by loganin-2-*t*.[20] In support of this premise, examination of the 35-hr seedling fraction afforded a separable mixture of the aldehydes **18** and **19**, the methyl ether

(14) See ref 3e.
(15) H. Inouye, S. Ueda, Y. Aoki, and Y. Takeda, *Tetrahedron Lett.*, 2351 (1969).
(16) H. Inouye, S. Ueda, and Y. Takeda, *ibid.*, 3453 (1968).
(17) A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc.*, 1193 (1969).
(18) A. I. Scott, 2nd Natural Product Symposium, Jamaica, Jan 1968; A. A. Qureshi and A. I. Scott, *Chem. Commun.*, 948 (1968).
(19) G. N. Smith, *ibid.*, 912 (1968).
(20) A. R. Battersby, J. C. Byrne, R. S. Kapil, J. A. Martin, T. Payne, D. Arigoni, and P. Loew, *ibid.*, 951 (1968).

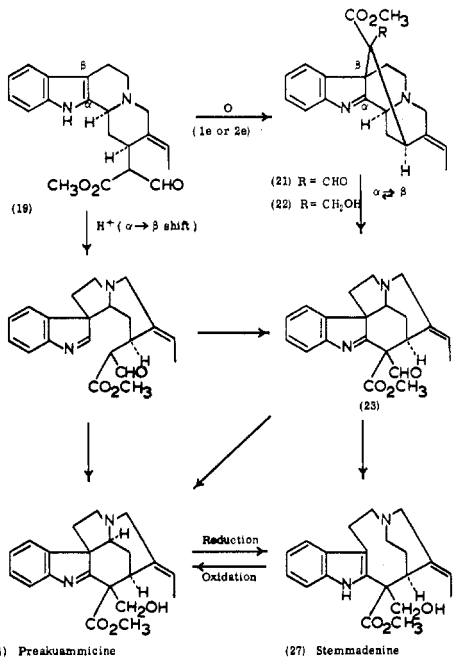
Scheme IV



corynantheine (**20**), and ajmalicine (**4**). Of these compounds only **4** was a previously authenticated *V. rosea* constituent, and while the reactivity of corynantheine (**20**) as a precursor for the more complex alkaloids seemed limited, the β-aldehydo ester function in both geissoschizine (**19**) and the aldehyde **18** is endowed with a most favorable reactivity whence the subsequent skeletal rearrangements might evolve.

Since it is necessary to oxidize the *Corynanthe* series in order to reach the *Strychnos* level it was suggested[11] that this process applied to geissoschizine (**19**) involves one-electron oxidative coupling to give strictamine (**21**; R = CHO). Precedent for the rearrangement of compounds such as **22** to the *Strychnos* representative, akuammicine (**5**), is available,[21] so

Scheme V

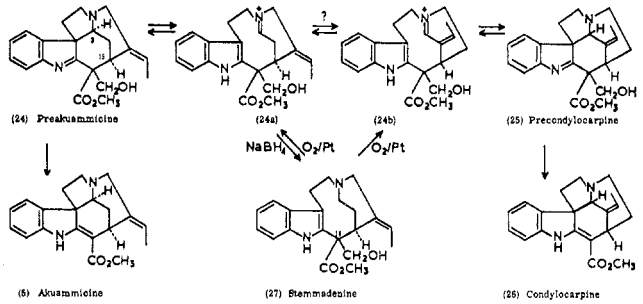


that the indolenine (**23**) or its reduced form (**24**) (Scheme V) could be reached by such a mechanism.

(21) J. E. Saxton, *Alkaloids*, **10**, 501 (1968).

An alternative to this process also with *in vitro* analogy[22] is α protonation of the indole nucleus followed by the α → β rearrangement summarized in Scheme V.

Our search in the 40–50-hr fractions was ultimately justified when 1 kg of 45-hr-old seedlings afforded a homogeneous if amorphous fraction (8 mg), whose spectroscopy and chemistry revealed that the long-sought "C_{10}-strychnos" alkaloid, preakuammicine (**24**), was an isolable, though labile, species.[23] Storage of its solution at room temperature led to decomposition to (−)-akuammicine (**5**), an alkaloid which, not surprisingly, could also be separated from the 45-hr fraction.



The same transformation was quantitatively effected in methanolic sodium methoxide, thus demonstrating the stereochemistry of **24** at C_3 and C_{15}. The complete carbon skeleton and the stereochemistry of the remaining chiral center were defined by sodium borohydride reduction of **24** *via* **24a** to the dihydro compound which was identical with yet another rare alkaloid, stemmadenine[24] (**27**). The latter alkaloid could be catalytically oxidized[23b] (Pt–O_2) back to a separable mixture of preakuammicine (**24**) and precondylocarpine (**25**),[25] *via* **24a,b** respectively, a process which also resulted in the formation of the "C_9" compounds akuammicine (**5**) and condylocarpine (**26**).

Returning to the chronological isolation studies which are fully revealed in Table I we see that stemmadenine[24] ($C_{20}H_{24}O_3N_2$) is a predictable but welcome arrival at 50 hr and furthermore that formal dehydration links this *Corynanthe–Strychnos* hybrid to the next hitherto undiscovered *V. rosea* metabolite, the *Aspidosperma* alkaloid (−)-tabersonine ($C_{20}H_{22}O_2N_2$) (**28**).

The isolation and identification of substantial quantities (20 mg/100 g of seeds) of (−)-tabersonine (**28**) in the 72-hr fraction were to constitute a vital piece of evidence in extending our ideas to embrace the *Iboga* alkaloids. Thus catharanthine (**7**), the principal *Iboga* alkaloid of *Vinca*, although isomeric with tabersonine, does not appear to be formed until germination has proceeded for 100 hr (Table I). A most attractive mechanism linking stemmadenine (**27**), tabersonine (**28**), and catharanthine (**7**) involves the common

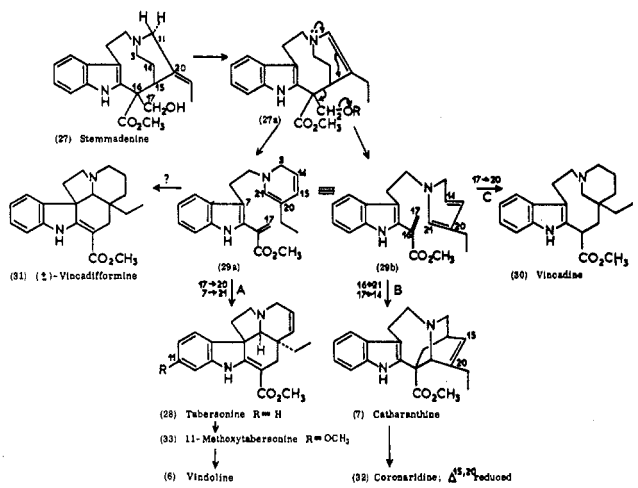
(22) J. Harley-Mason and W. Waterfield, *Tetrahedron*, **19**, 65 (1963); A. J. Gaskelle and J. A. Joule, *ibid.*, **23**, 4053 (1967).

(23) (a) A. I. Scott and A. A. Qureshi, *J. Amer. Chem. Soc.*, **91**, 5874 (1969); (b) A. A. Qureshi, unpublished.

(24) The stereochemistry of stemmadenine and therefore of **24** at C_{15} remains to be defined: D. Stauffacher, *Helv. Chim. Acta*, **44**, 2006 (1961); A. Sandoval, F. Walls, J. N. Shoolery, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *Tetrahedron Lett.*, 409 (1962).

(25) A. Walser and C. Djerassi, *Helv. Chim. Acta*, **48** 391 (1965).

Scheme VI



achiral intermediate **29a** ≡ **29b**[26a,c] which can, in principle, be generated (see **27a**) by dehydration of stemmadenine (Scheme VI). In order to explain the observed sequence, it is suggested that enzymatic folding of **29** in mode A would give tabersonine (**28**), and later, at another enzyme site, cyclization in mode B forms catharanthine (**7**). Yet a third possibility (C) explains the genesis of the vincadine set[26b] (**29** → **30**). This theory places stemmadenine in a key position between *Strychnos* and the other families not only in *V. rosea* but predictably in all species, and furthermore rationalizes the formation of racemic *Aspidosperma* alkaloids such as (±)-vincadifformine (**31**) mediated by the achiral ester **29**. The absolute minimum of functionality has been used for all of these postulated interconversions, and we suggest that the proposed biogenesis is common to all species. Thus the galaxy of complex, oxygenated, fragmented, and rearranged structures which constitute the complete series of indole alkaloids in fact stem from these few fundamental alkaloids. To illustrate further, coronaridine (**32**), another new isolate (100 hr), is probably formed from **7**, while 11-methoxytabersonine (72 hr; **33**) and vindoline (200 hr; **6**) represent further oxygenative transformation products of tabersonine.

Thus far we have not commented on the detection of metabolites which might reflect the detailed mechanistic connections of the *Corynanthe* (35 hr) and *Strychnos* (40 hr) set in accord with Scheme VI. Although alkaloids such as strictamine (**21**) have been isolated from *Rhazya stricta*, no such alkaloids predicted on the basis of Scheme V have yet been discovered in the 35–45-hr range, nor would we expect to find intermediates other than geissoschizine or the indolenine (**23**) in support of the acid-catalyzed rearrangement (Scheme V). More recently, however, we suggested[28,27] a third mechanism (Scheme VII) which imputes an intermediary role to an unknown alkaloid, geissoschizine oxindole (**34**). The formation of such an oxindole from geissoschizine (**19**) has ample *in vitro* precedent and might take place *in vivo* by the steps indicated in Scheme VII where the β-hydroxindolenine (**35**) is rearranged directly or *via* the dihydroxyindoline ("diol" **36**) to **34**. Oxindoles as such have never received serious consideration as intermediates, but conversion to the imino ether **34a** (R = alkyl or enzyme-bound functionality) would endow **34** with the reactivity required[28] to form preakuammicine (**24**), as shown. Accordingly the oxindole (**34**) with the requisite stereochemistry was prepared by partial synthesis from geissoschizine (**19**). Although the details of this synthesis and of the establishment of the absolute configuration **34** for the oxindole[29] cannot properly be discussed in this Account, suffice it to say that from the most recently examined 45-hr fractions a few milligrams of geissoschizine oxindole identical with synthetic material was isolated.

Table I

Isolation of Alkaloids from *V. rosea* Seedlings[8c,18,29,30]

Germination time, hr	Alkaloid isolated	Type
0	None	
26	Vincoside (**16**)	*"Corynanthe"*
	Ajmalicine	
	Corynantheine (**20**)	
28–40	Corynantheine aldehyde (**18**)	*Corynanthe*
	Geissoschizine (**19**)	
	β-Hydroxyindolenine (**35**)	
	"Diol" (**36**)	
	Geissoschizine oxindole (**34**)	*Corynanthe*
40–50	Preakuammicine (**24**)	*"Corynanthe–Strychnos"*
	Akuammicine (**5**)	*Strychnos*
	Stemmadenine (**27**)	*"Corynanthe–Strychnos"*
	Tabersonine (**28**)	
72	11-Methoxytabersonine (**33**)	*Aspidosperma*
100–160	Catharanthine (**7**)	
	Coronaridine (**32**)	*Iboga*
200	Vindoline (**6**)	*Aspidosperma*

At this juncture the various predictions made as to the exact nature of the intermediates of indole alkaloid biosynthesis may be compared with the full sequential analysis (Table I).[18,30] As these isolation experiments were maturing we had also begun the task of establishing criteria for true intermediacy, namely the incorporation of those labeled isolates which bore the requisite reactivity in terms of the schemes outlined above.

Results of Incorporation Experiments

The most recent incorporation data[18,29] using *V. rosea* seedlings are summarized in Table II together with some comparative data using mature plants.

(26) (a) See A. A. Qureshi and A. I. Scott, *Chem. Commun.*, 945, 947 (1968); (b) J. P. Kutney, C. Ehret, V. R. Nelson, and D. C. Wigfield, *J. Amer. Chem. Soc.*, **90**, 5930 (1968); (c) an additional pathway open to biochemical test involves the collapse of stemmadenine to the acrylic ester (**29**) by way of the *chano*-iminium species (**24a**). Appropriate labeling experiments are in hand.

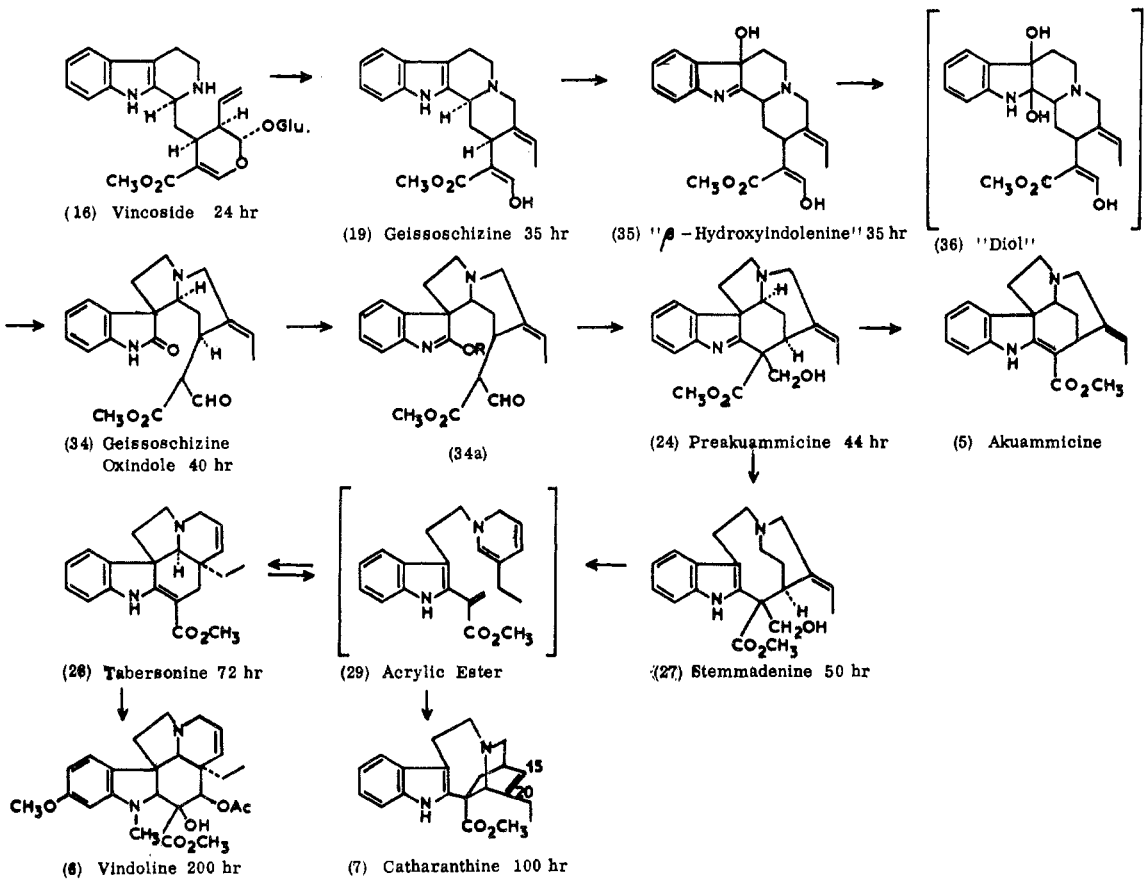
(27) A. I. Scott, 21st National Organic Symposium, Salt Lake City, Utah, June 1969, Abstracts, p 50. These ideas were based on model experiments carried out by Dr. C. R. Bennett.

(28) T. Oishi, M. Nagai, and Y. Ban, *Tetrahedron Lett.*, 491 (1968).

(29) A. I. Scott and C. R. Bennett, manuscript in preparation. Dr. Bennett has very recently obtained evidence for the presence of both the indolenine **35** and the diol **36** in *V. rosea* seedlings (see Table II).

(30) A. I. Scott, P. C. Cherry, and A. A. Qureshi, *J. Amer. Chem. Soc.*, **91**, 4932 (1969).

Scheme VII



In general the incorporations in the seedlings follow the same trend as in the plant, but with frequently greater efficiency of substrate incorporation. The results confirm the postulated sequence outlined in Scheme VII. Thus in the seedlings all three *Corynanthe* alkaloids **4**, **18**, and **19** are biologically converted to the *Aspidosperma* and *Iboga* alkaloids. The essentially qualitative nature of comparative specific incorporation data is quite well recognized, but the experiments with geissoschizine-*ar*-*d* (Table II) are illustrative of the dangers involved. Feeding 10 mg of geissoschizine (containing the following percentages of deuterated species: d_1, 10; d_2, 31; d_3, 34; d_4, 20%) to 100 g of seedlings gave a 1.53% incorporation into akuammicine (**5**) with retention of the same deuterium ratio, thus providing the first evidence[30] for the suspected *Corynanthe–Strychnos* relationship. However, when 40 mg of the same species (**19**) was incubated with only 20 g of seeds the mass spectrum of the resultant akuammicine was greatly enriched (5.2% specific incorporation),[3c] with preservation of the deuterium ratios. Similarly, it was found that the relationship between the quantity of tryptophan-*ar*-*d* fed and its specific incorporation into akuammicine[3c] (which can be "raised" to 2.7%) leaves little doubt that the size of the pool of endogenous alkaloid at any one time in mature plant and seedling experiments is critical in determining the percentage incorporation. We feel that unless this pool size is taken into account comparative incorporation data must be treated with reserve. Once again the need for cell-free systems of known stoichiometry is clearly evident.

Turning again to the relationships within the *Corynanthe* series (Table II; Scheme V) it appears that although corynantheine (**20**) is formed from aldehyde **18** with high (13%) incorporation, a low conversion (0.2%) of geissoschizine (**19**) to ajmalicine (**4**) is observed. This may reflect a minor interconnecting pathway for the *Corynanthe* set (see Scheme IV), a manifestation of the "pool size" effect or the fact that each alkaloid may be formed separately by obvious although unproven changes at the vincoside aglycone level (**17**).[20] Further work is clearly required to clarify this situation.

Of various stereoisomers of geissoschizine oxindole tested only that with the configuration shown (**34**) was converted to both the *Strychnos* (0.55%) and the *Aspidosperma* (0.05%) series. Thus the oxindole hypothesis is shown to represent a viable pathway in *V. rosea*.[29] All of this interlocking evidence so far is also in accord with the elegant experiments[14,20] with stereospecifically labeled monoterpene precursors in that the protons C_1 and C_7 in loganin (**13**) and secologanin (Scheme III) must survive multistage transformation into all three classes of alkaloid (Schemes IV–VII). However, in interpreting the next result, *viz.*, the positive incorporation of doubly labeled stemmadenine (**27**) into tabersonine (**28**), vindoline (**6**), and catharanthine (**7**) it must be assumed that stereospecific proton loss at C_{21} implicit in the formation of

Table II

Specific Incorporation (%) of Precursors in *V. rosea* Seedlings

Precursor	Alkaloids isolated: *Corynanthe* Ajmalicine (4)	*Strychnos* Akuammicine (5)	*Aspidosperma* Vindoline (6)	*Iboga* Catharanthine (7)	Ref
Vincoside-*ar-t*[a] (16)	0.95	0.76	0.11	0.35	17, *b*
Geissoschizine-OC^{3}H$_3$,*ar-t*[a] (19)	0.12	2.0	0.47	0.47	*b*
^{3}H ratio (1:2.25)	(1:2.1)	(1:2.25)	(1:1.7)	(1:2.0)	
Geissoschizine-*ar-d* (19)					
expt 1[c]		1.53		0.35[e]	30
expt 2[d]		5.0			3c
Geissoschizine-OC^{3}H$_3$ (19)	0.22		0.35	0.41	23b
Corynantheine-OC^{3}H$_3$ aldehyde (18)					
expt 1			0.1	0.3	18
expt 2[a]			0.003		18, 20
Ajmalicine-*ring C-t* (4)					
expt 1			0.6	0.3	18
expt 2[a]			0.004		18, 20
Geissoschizine oxindole-*ar-t* (34)		0.55	0.05		29
Stemmadenine-OC^{3}H$_3$,6-^{14}C (27)			0.95	0.30	18
(Ratio 92.8:7.2)			(91.9:8.1)	(91.8:8.2)	
Tabersonine-OC^{3}H$_3$,6-^{14}C (28)			4.80	0.80	18
(Ratio 95.8:4.2)			(96:4)	(95.6:4.4)	
Catharanthine-OC^{3}H$_3$ (7)			0.001		18

[a] Mature plant. [b] A. R. Battersby and E. S. Hall, *Chem. Commun.*, 793 (1969). [c] 10 mg of precursor/100 g of seeds. [d] 200 mg of precursor/100 g of seeds. [e] As coronaridine (**32**).

27a (Scheme VI) takes place, in conformity with the retention of the C_1 proton of loganin in the *Aspidosperma* alkaloids. This evidence is also in harmony with the intervention of the acrylic ester **29**, although it can be seen that while tabersonine (**28**) serves[31,32] as a good precursor (0.35%) for catharanthine (**7**) the reverse reaction is not observed (Table II; Scheme VII). It can also be inferred that tabersonine is the prototype of the *Aspidosperma* class since almost all of the other members, *e.g.*, vindoline (**6**), may be reached from it, the incorporation of **28** into **6** being excellent (*ca.* 5%). The biochemical pathway from tabersonine to the *Iboga* series is intriguing since the genesis of ester **29** must be invoked at different control points in the early stages of *Vinca* germination.[32,33]

The above results indicate that the steps from vincoside to stemmadenine will be quite general, that most species will utilize at least the first part of this pathway, and that stemmadenine will fulfill a pivotal role in the genesis of the various families.

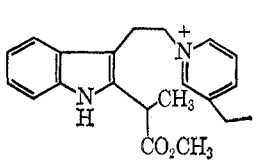
Summary

The sequential isolation and feeding experiments suggest that the biosynthesis of the indole alkaloid family proceeds in the order shown in Scheme VII. Laboratory analogies for almost all of the suggested processes are now available, and with the establishment of the *Corynanthe–Strychnos–Aspidosperma–Iboga* relationship the various further subclasses should fall into place. Thus preakuammicine (**24**) and precondylocarpine (**25**) could equilibrate *via* **23a** and **23b**, thus connecting the *Akuammicine* and *Condylocarpine* series. Cyclization of type 1 alkaloids formally generates the *Yohimbé* family (**10**; Scheme II), indicating another area for experiment, while further adjustments of *Aspidosperma* alkaloids lead to the *Hunteria*, *Kopsia*, and related families.[2,11] As mentioned above, one-electron oxidation might generate the *Pleiocarpa–Mavacurine* (**22**) and/or *Akuamma* (**21**) series,[11] but again the ubiquitous loss of one of the carbons at C_{16} in the *Pleiocarpa* alkaloids (**22**) could mark the site of C_{16} oxygenation by processes other than radical coupling. All of these and many other possibilities can now be tested with whole plants and seedlings, but surely the most important, and perhaps difficult, phase of the investigation—the cell-free biosynthesis of the alkaloids—must now begin. Only then may a distinction be made between obligatory precursor activity and the successful bioconversion of "close relatives."

(31) This result has been independently confirmed in mature *V. rosea:* J. P. Kutney, N. J. Cretney, J. R. Hadfield, E. S. Hall, V. R. Nelson, and D. C. Wigfield, *J. Amer. Chem. Soc.*, **90**, 5566 (1968).

(32) The possibility of a connection between the dihydro series, *i.e.*, vincadifformine (**31**) and coronaridine (**32**), also must be explored. This would require **28** → **31** → **32** → **7**.

(33) *In vitro* evidence for the formation of the acrylic ester **29** and its isomeric *salt* (i) has recently been secured by Dr. Peter Cherry: A. I. Scott and P. C. Cherry, *J. Amer. Chem. Soc.*, **91**, 5672 (1969).



i

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