BIOORGANIC CHEMISTRY 1, 157–173 (1971)

Mechanisms of Indole Alkaloid Biosynthesis. Recognition of Intermediacy and Sequence by Short-Term Incubation[1]

A. IAN SCOTT,[2] P. B. REICHARDT, M. B. SLAYTOR,[3] AND J. G. SWEENY

Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut 06520

Received March 26, 1971

Autoradiography and radiochemical analysis of two-dimensional chromatograms of the alkaloids of *Vinca rosea* seedlings have been used to determine the activity of various postulated intermediates after administration of 2-^{14}C-DL-tryptophan. The technique holds promise as a method for (a) distinction between "dynamic" and "static" metabolites, (b) determination of the sequence of occurrence of alkaloids from 5 min to 8 days, (c) the discovery of hitherto unknown intermediates, and (d) the attainment of specific incorporations of up to 30%.

INTRODUCTION

The state of the art of higher plant biosynthesis can fairly be said to be entering a new phase of development. Progressing from speculations throughout the first half of the century, pioneered in large measure by Sir Robert Robinson (*1*), the advent of tracer methods and the establishment of criteria for biointermediacy during the last two decades have led to an understanding of many of the pathways by which complex natural products are formed in whole plant systems. However, it is clear, especially in the alkaloid series, that the demonstration of specific incorporation without randomization of label together with the proof for the presence of a postulated intermediate are frequently difficult to obtain with plant material (*2*). In particular, it is felt that low specific incorporations (0.001–0.01%), although satisfying acceptable conventions of constant radioactivity and in many (but not all) cases supported by degradative and/or multiple labeling data, may not distinguish between obligatory biointermediacy and nonspecific biotransformation. Problems such as nonpermeability of complex intermediates and large, variable pool sizes in mature biological material are often more serious in plant systems than in mammalian and fungal metabolism where so much is known of controlled culture conditions.

Thus, the third phase of the study of plant biosynthesis is now under way and methods are being sought for the elucidation of pathways at the cell-free level. Mention may be made of recent terpenoid studies (*3*), a study of *N*- and *O*-methylation (*4*), and earlier experiments with $^{14}CO_2$ (*5*). However, by comparison with our knowledge of the path of carbon in photosynthesis (*6*), the subject of short-term analysis of the biosynthesis of alkaloids and other complex plant products is virtually unexplored.

Thus, it became of interest to devise methods for distinguishing between the static and dynamic constituents of a plant such as *Vinca rosea*, which at maturity contains as

[1] Supported by Grant CA 11095 from the National Institutes of Health. One of us (J.G.S.) thanks N.I.H. for a postdoctoral fellowship (1969–1971).

[2] To whom correspondence should be addressed.

[3] Present address: Department of Biochemistry, The University, Sydney, Australia.

many as 100 alkaloids yet whose seeds are virtually devoid of alkaloidal material (*7*). Some progress in obtaining a cell-free system using acetone powders from homogenates of 8–12-day-old seedlings of *V. rosea* has been made, but the activity of this preparation for alkaloid synthesis is still very low and so far the only reproducible partial reaction has been the decarboxylation of tryptophan to tryptamine (*8*). During the course of these studies however, it was found that specific incorporations of 2-^{14}C-DL-tryptophan into the alkaloids of young seedlings was extremely high (ca. 30%). It was, therefore, decided to develop the technique of short-term incubation of tryptophan with young seedlings (9–17 days) grown from a mixed strain of *V. rosea* at 92°F using full illumination (see Experimental section). Promising intermediates can, in principle, be detected by autoradiography of chromatograms corresponding to frequent sampling of the alkaloidal content. Provided that small pool sizes are present during experiments with "early" biological material and that a linear uptake of tryptophan is in operation during the times of assay, we could anticipate that pivotal intermediates should at appropriate points in the sequence contain a relatively large proportion of the total radioactivity which would then decay as the counts are transferred to the next intermediate with a similar "radio-profile." At the same time the less dynamic constituents of *V. rosea* should be gradually gaining radioactivity as they are (irreversibly) laid down in labeled form.

The technique has the additional advantage that rapid evaluation on a qualitative basis for several related species can be made. Quantitative experiments require development of two-dimensional tlc systems, removal and collection of the alkaloids, and recrystallization (after dilution with authentic material) to constant radioactivity. For the isolation and identification of unknown metabolites with "dynamic" radio-profiles, it will probably be necessary to carry out large-scale cell-free incubations to accumulate sufficient material for structural determination. Refeeding experiments can be used to decide whether the unknown occupies a crucial position and in fact justifies further analysis.

Although our experiments in this field are still incomplete, it has become evident that biointermediates can in fact be distinguished from other less dynamic constituents and further that a 100-fold increase over previous specific incorporations can be demonstrated. In spite of the crude nature of the kinetic data which emerge from our experiments, we shall see that a number of conclusions regarding the early stages of alkaloid synthesis in *V. rosea* can be made.

Uptake of 2-^{14}C-DL-Tryptophan

The incorporation of DL-tryptophan by the seedlings is shown in Fig. 1 where 0.06 μmole was fed to four seedlings for each indicated time interval. It can be seen that the maximum uptake (90%) is complete within 2–3 days. Reference to Fig. 2 reveals that quite large amounts (5 mg/g) can be absorbed in a 10-hr experiment.

Incorporation of tryptophan into the alkaloids takes place in two distinct phases. For the first 2 hr of incubation it is linear (Figs. 3 and 4) but after this interval a rapid increase in the rate of incorporation is observed. Between 12 and 48 hr a maximum of 3% is reached and this level is maintained during the full time of the experiment (up to 8 days). It is concluded from these results that there is present no large pool of tryptophan and also that, since after 8 days vindoline (**18**) (a major alkaloid of *V. rosea*) contains only 7% of all the activity, 8 days is insufficient time for the distribution of radioactivity to approximate to the "normal" percentage of alkaloid distribution in the seedlings. Shoots of mature *V. rosea*, which have been hitherto used for most of the

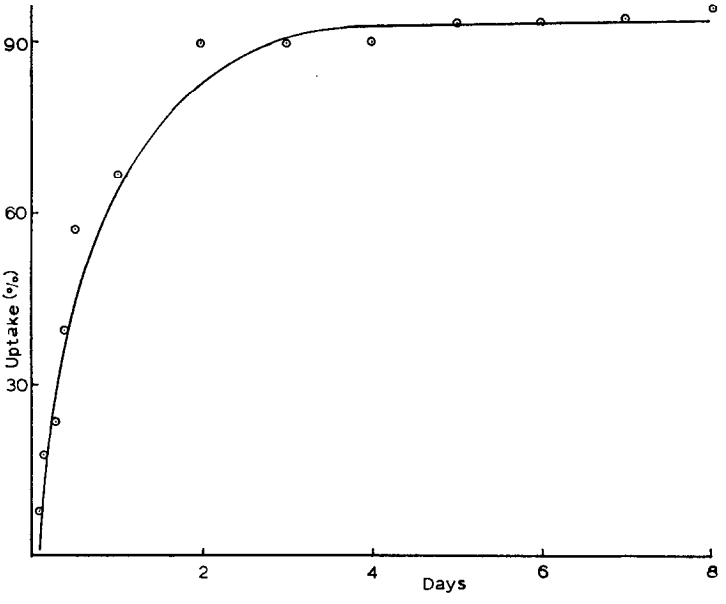


FIG. 1. Uptake of ^{14}C-DL-tryptophan by *V. rosea* seedlings.

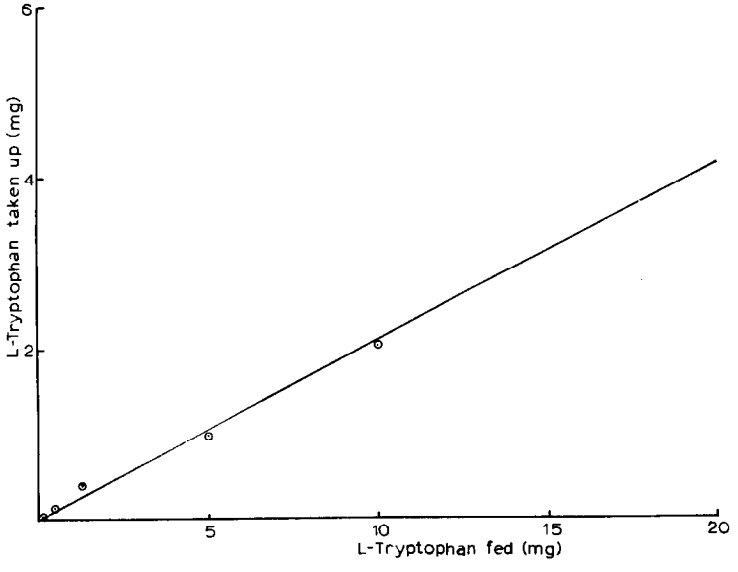


FIG. 2. Uptake of L-tryptophan by 1 g of seedlings.

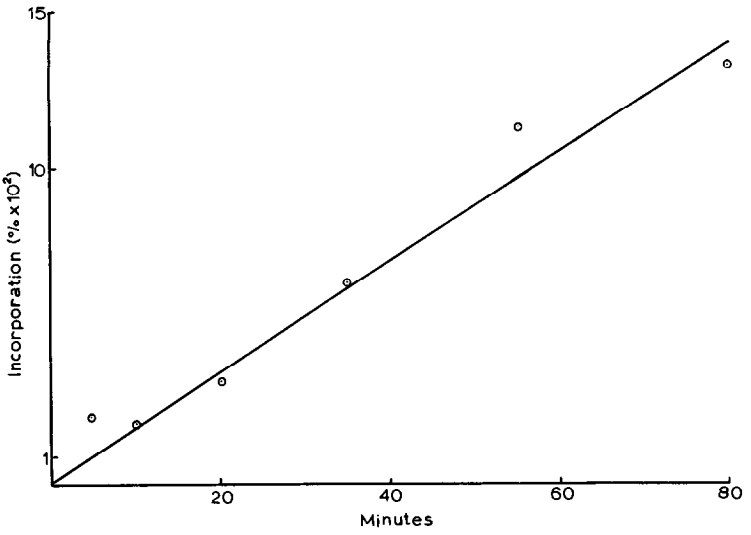


FIG. 3. Incorporation (%) of ^{14}C-DL-tryptophan into alkaloids in *V. rosea* seedlings during 80 min.

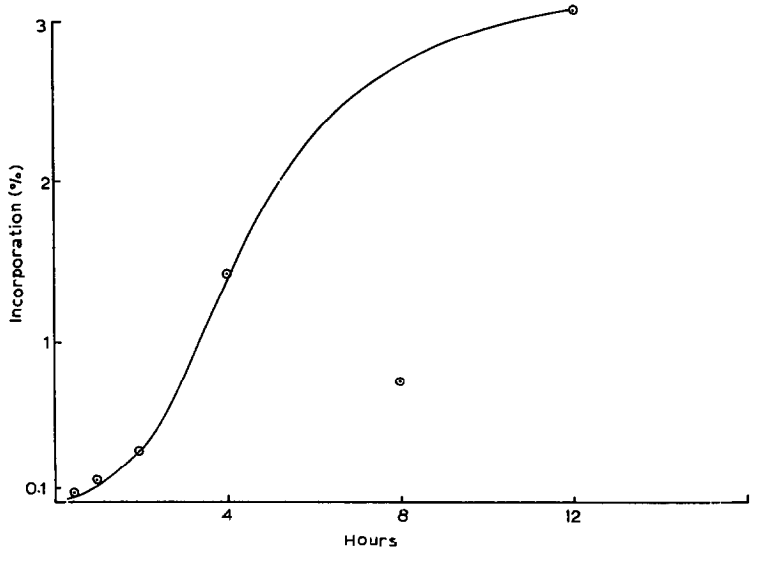


FIG. 4. Incorporation (%) of ^{14}C-DL-tryptophan into alkaloids in *V. rosea* seedlings during 12 hr.

published feeding data, incorporate tryptophan but at a reduced initial rate, and the final (constant) percentage is about 1.0 (Fig. 5). The latter observation is of particular interest for comparative studies with diversely aged biological material.

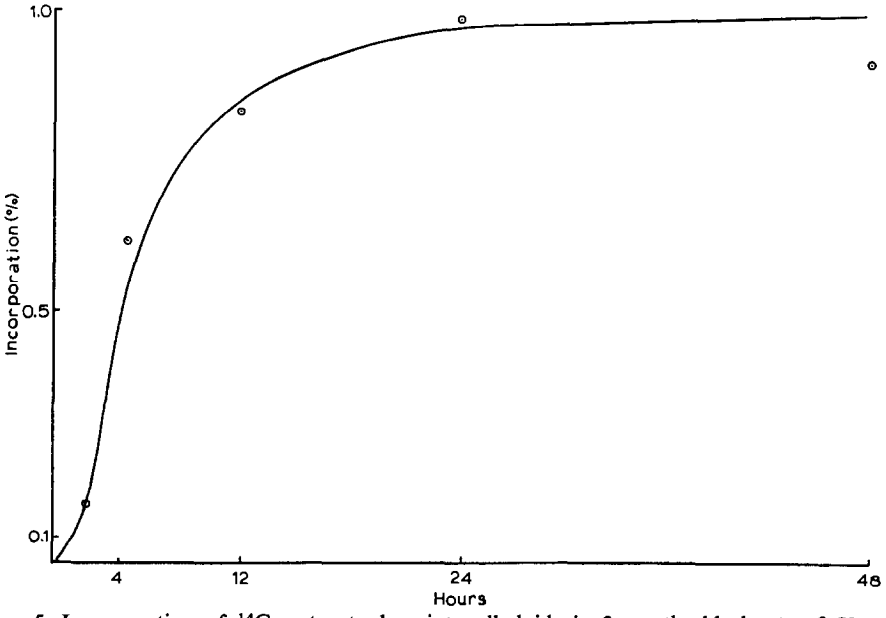


FIG. 5. Incorporation of ^{14}C-DL-tryptophan into alkaloids in 3-month-old shoots of *V. rosea* during 48 hr.

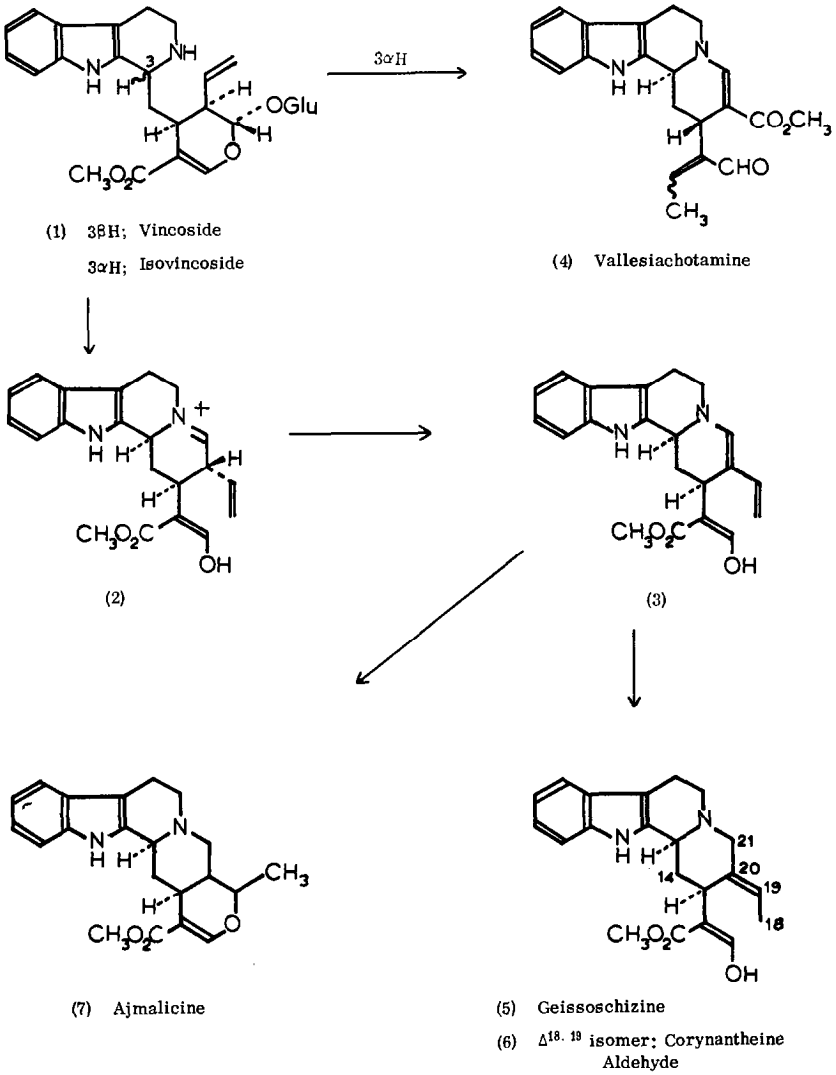
From Vincoside to the Corynanthé Alkaloids

In spite of considerable progress in the determination of the sequence of events in *V. rosea* whereby tryptophan and secologanin are condensed and transformed to both simple and complex members of the three major families of alkaloid (*7*), autoradiograms of two-dimensional tlc systems disclose (Fig. 6) that the earliest alkaloid into which tryptophan is incorporated is (as yet) an unknown base. Thus, at our first sampling time (5 min) this metabolite contains 35% of the total counts of the alkaloidal fraction. Within 1 hr the unidentified compound contains virtually no radioactivity. These results are consistent with the view that the plot is characteristic of an active intermediate with a constant pool size corresponding to 35% of the radioactivity at 5 min at which time a linear incorporation into the alkaloids is being maintained (Figs. 3 and 4). Comparison of the data of Fig. 6 with the profile for geissoschizine (**5**) shows (Fig. 7) that the unknown lies between vincoside (**1**) (not extracted from aqueous solution) and geissoschizine (**5**). The nearest (but nonidentical) alkaloid (R_f in two solvent systems) is corynantheine aldehyde (**6**).

A second possibility that the new intermediate might be vallesiachotamine (*9*) (**4**) could also be ruled out by tlc comparison with an authentic sample. Since it had been shown (*10*) that isovincoside (strictosidine) is transformed to vallesiachotamine during the normal work-up procedure, it seemed possible that the appearance and disappearance of isovincoside (**1**) from the system could be monitored in this way. In fact, the recent revision (*11*) of stereochemistry at C_3 in (**1**) for that epimer which is incorporated into the main classes of alkaloids appears to be in accord with our observation, although the time scale may have to be shortened still further (1–250 sec) before such species as vallesiachotamine can be eliminated completely from consideration.

Another attractive candidate for the unidentified precorynanthe alkaloid is the unknown diene, $\Delta^{20:21}$ corynantheine aldehyde (**3**). The structure of this dieneamine represents a link between the highly reactive aglucone of vincoside, the corresponding

Vincoside and Corynanthe Alkaloids



(1) 3βH; Vincoside
3αH; Isovincoside

(4) Vallesiachotamine

(2)

(3)

(7) Ajmalicine

(5) Geissoschizine

(6) $\Delta^{18,19}$ isomer; Corynantheine Aldehyde

immonium species (**2**) and (by enamine–imine tautomerism) the dehydro version (**5**; $\Delta^{N:21}$) of geissoschizine. This would imply an indirect connection between the corynantheine ($\Delta^{18,19}$) and geissoschizine ($\Delta^{19,20}$) series and is in accord with the similar but nonidentical tlc behavior of the new intermediate and that of corynantheine aldehyde (**6**). So far, only microgram quantities of this alkaloid are available and further isolation experiments are in hand.

Turning now to Figs. 7 and 8, it can be seen that within the limits of the technique the next detectable intermediate is geissoschizine (**5**) which bears a profile expected from previous feeding experiments (*7*), viz., a rapid rise to ca. 4% of total radioactivity in the alkaloids over the first 60–90 min followed by a gradual decline over the 8-day experiment (Fig. 7). This curve is to be contrasted with the behavior of ajmalicine (**7**)

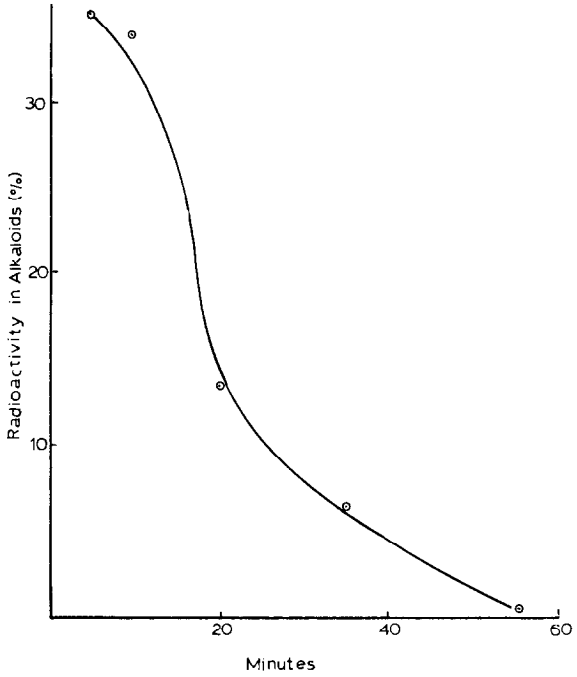


FIG. 6. Radioactivity of unknown alkaloid ($t = 5$–60 min) of *V. rosea* after administration of ^{4}C-DL-tryptophan to seedlings.

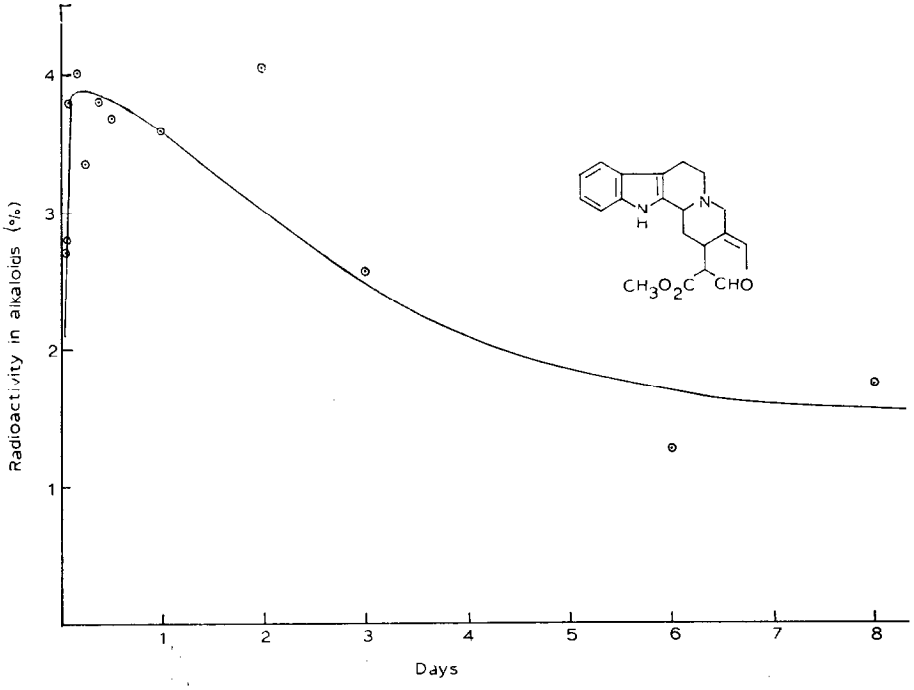


FIG. 7. Radioactivity of geissoschizine during 8 days after administration of ^{14}C-DL-tryptophan.

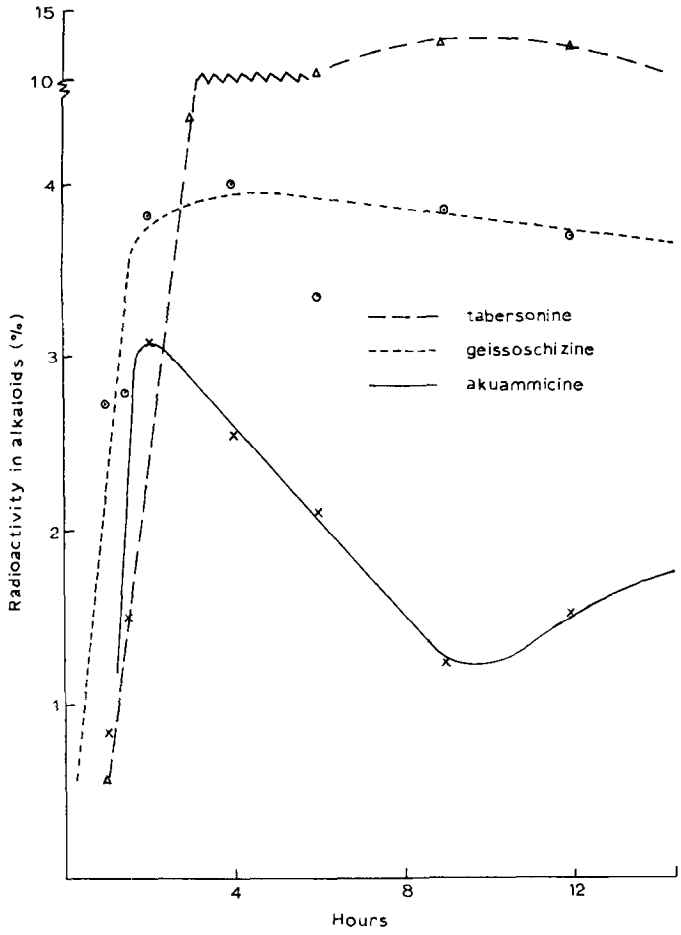


FIG. 8. Relative radioactivities in geissoschizine (--⊙--), akuammicine (—×—), and tabersonine (—▲—) over 12 hr.

(Fig. 9), which, although isomeric with geissoschizine, no longer exhibits the required dynamic structure for further transformation, a fact reflected in its rather slow climb and flat profile over the 8-day incubation.

The Corynanthé-Strychnos Relationship

Previous work in these laboratories demonstrated that geissoschizine (*7*) serves as a good precursor both for akuammicine (**10**) (*Strychnos*) and coronaridine (**17**) (*Iboga*). When the profile of the akuammicine was examined (Figs. 8 and 10) it at first seemed surprising that a definite maximum (3%) occurs after about 2 hr. Yet thereafter the amount of radioactivity in both akuammicine and its phenolic derivative vinervine (**11**) remains virtually unchanged from 9 hr to 8 days. The latter part of the akuammicine profile suggests that both (**10**) and (**11**) are shunt products formed from a rapidly metabolizing intermediate at a constant rate during the early part of the biosynthesis. The rapidly changing segment of the curve (0–9 hr) then may well represent the flow of radioactivity through the labile intermediate preakuammicine (*7*) (**8**). It is known

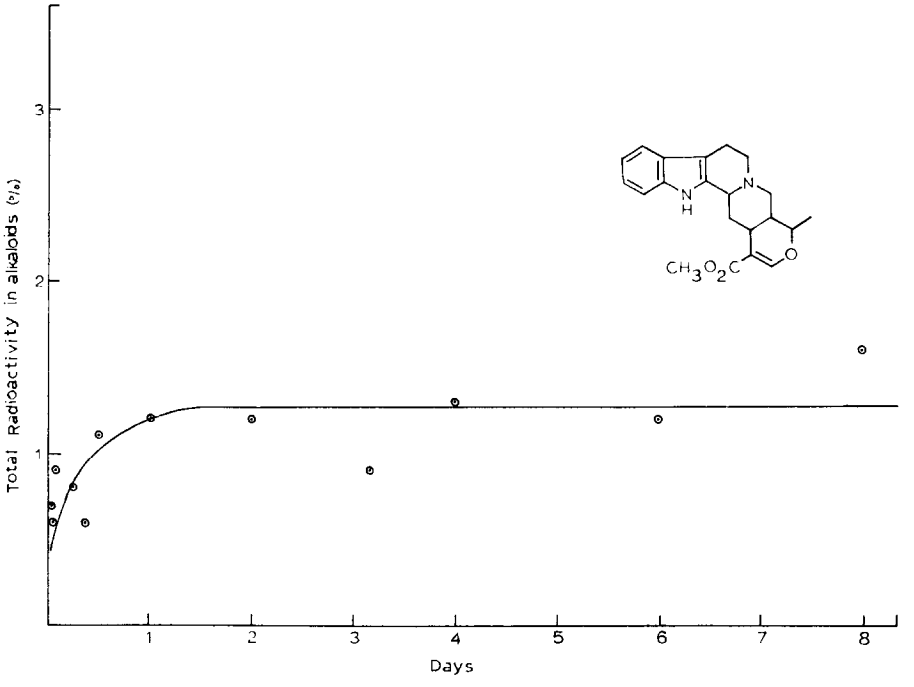


FIG. 9. Radioactivity in ajmalicine after administration of ^{14}C-DL-tryptophan during 8 days.

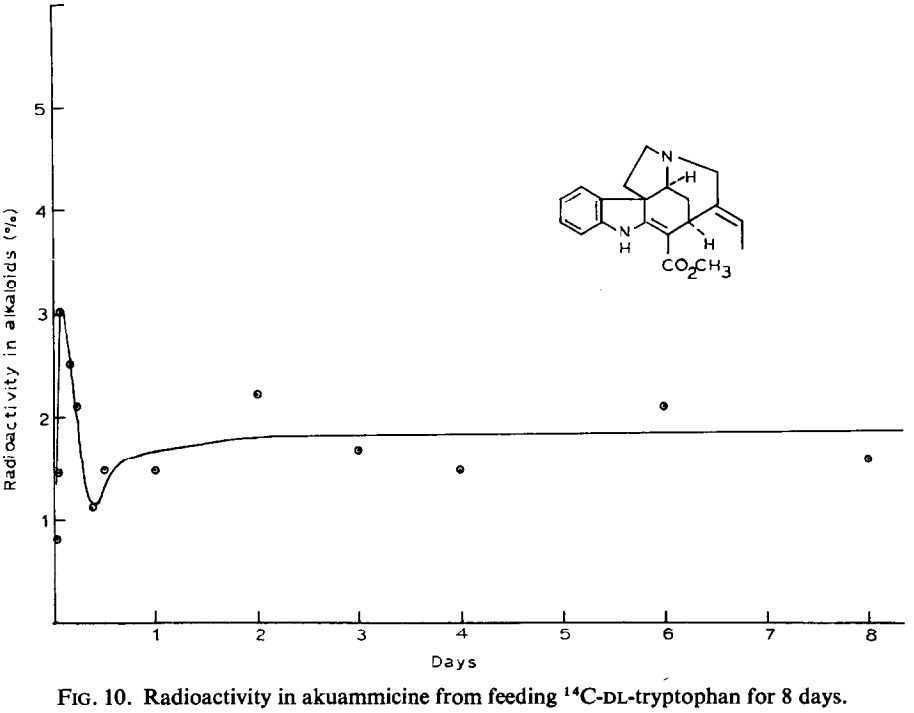


FIG. 10. Radioactivity in akuammicine from feeding ^{14}C-DL-tryptophan for 8 days.

from the chemistry of this compound that, under the normal conditions of work-up, almost quantitative conversion to akuammicine occurs. Thus, the first 9 hr of the experiment may constitute an assay for preakuammicine which is analyzed as the nor-derivative, akuammicine. The latter, of course, does not serve as a mainstream intermediate but is presumably the precursor of vinervine (**11**).

The next isolated alkaloid of *V. rosea* which (like geissoschizine) has been demonstrated to give rise to both the *Aspidosperma* and *Iboga* alkaloids is stemmadenine (**9**). Stemmadenine and preakuammicine are related by oxidation of the former at position

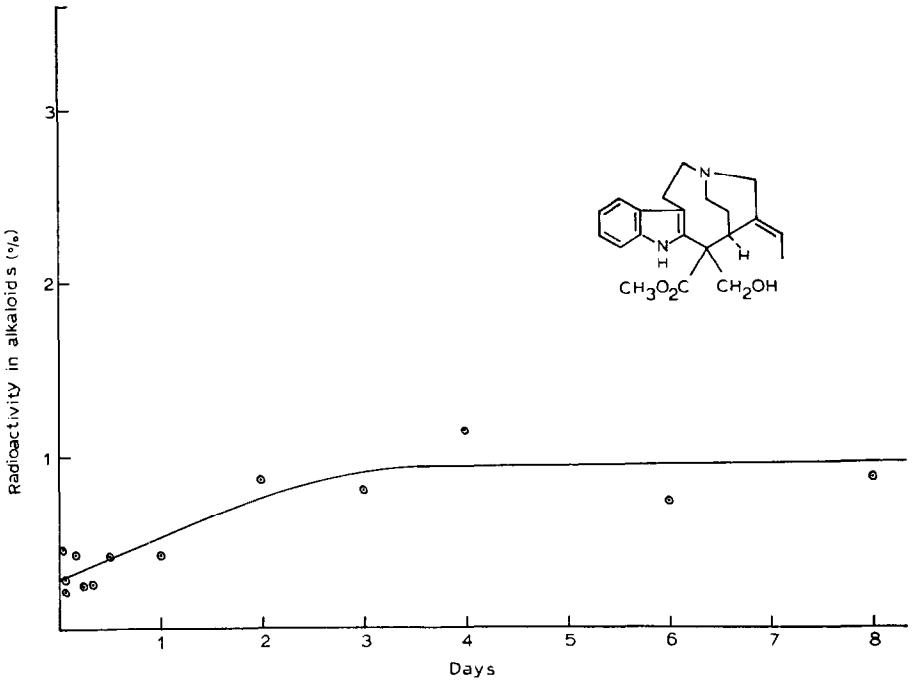


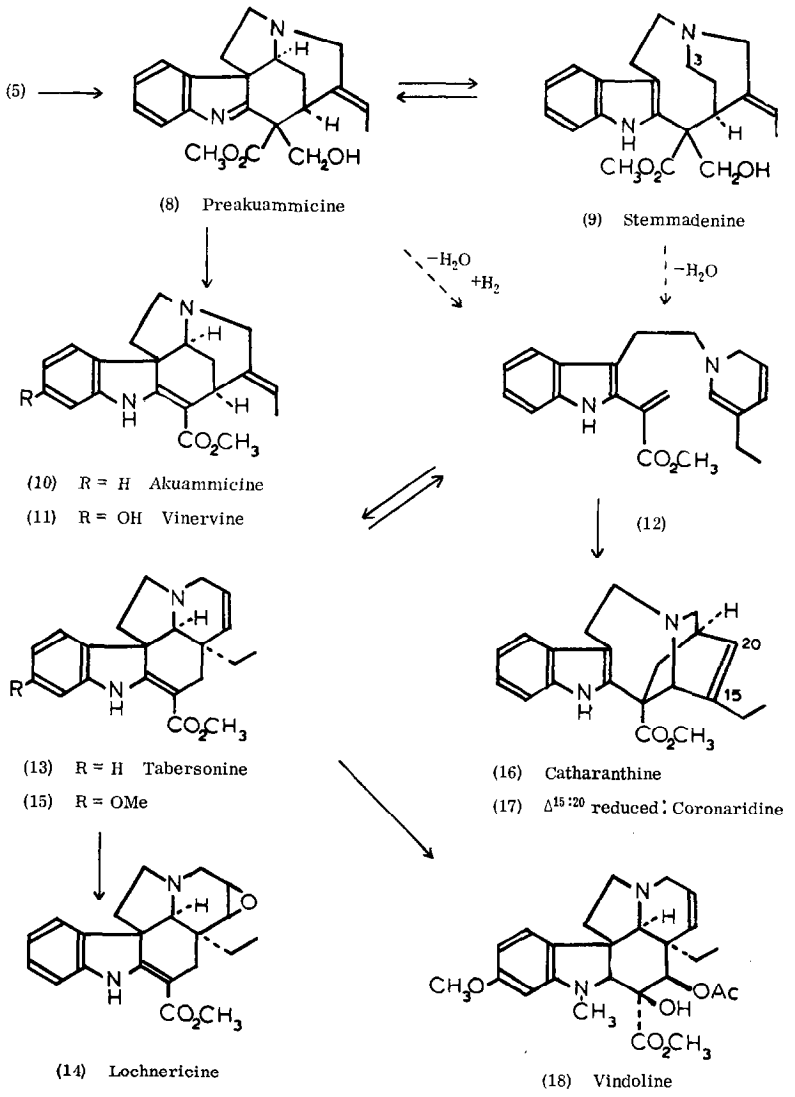
FIG. 11. Radioactivity in stemmadenine during 8 days.

3 while reduction of preakuammicine has been used to illustrate the reversal of this process in the laboratory. In spite of the successful, nonrandomized bioconversion of stemmadenine to both vindoline (**18**) and catharanthine (**16**) in *V. rosea* (*7*) and to related alkaloids (*12*) in *V. minor*, the radio-profile for stemmadenine (Fig. 11) does not reveal any dramatic rise in radioactivity at the expected time interval, i.e., between (pre)akuammicine (2 hr) and tabersonine (9 hr). The possibility of course exists that the preakuammicine–stemmadenine equilibrium is an enzyme-bound process which precludes analysis of a dynamic intermediate such as stemmadenine.[4] In the same category may be included the next plausible intermediate, viz., the labile acrylic ester

[4] An alternative interpretation of the stemmadenine profile implicates this intriguing alkaloid as a reduced, stabilized version of preakuammicine (**8**) which (especially in young seedlings (*7*)) can be brought into the biogenetic scheme by oxidative cyclization (**9** → **8**). Bioconversion and isolation studies (*7*) leave no doubt that the "normal" criteria of intermediacy are satisfied. However, the radio-profile (together with the extremely low [0.001 %] incorporation of ^{14}C-tryptophan into stemmadenine) serves as a third criterion which is not met in the dynamic sense by (**9**) under our experimental conditions. The answer to this problem must await development of the cell-free technique with *V. rosea*.

(12), which has served as a satisfactory mechanistic concept to connect the preakuammicine series with *both Aspidosperma* and *Iboga* alkaloids, and for which a considerable body of circumstantial evidence now exists in terms of its generation both *in vivo* (*7*) and *in vitro* (*13*).

Strychnos, Aspidosperma. and Iboga Alkaloids



Aspidosperma and Iboga Alkaloids

One of the earliest isolation experiments in which new metabolites of immature *V. rosea* were identified concerned the characterization of (–) tabersonine (**13**) as an abundant constituent of 3-day-old seedlings, an observation which contrasts with the virtual absence of tabersonine in the mature plant. The possibility was tested that

tabersonine (**13**) (the simplest of the *Aspidosperma* alkaloids) could serve not only as a precursor for the major *V. rosea* constituent, vindoline (**18**), and related highly oxygenated *Aspidosperma* alkaloids, but also as a precursor of the isomeric *Iboga* alkaloid catharanthine (**16**) once again via the *chano*-intermediate (**12**). Since tabersonine and catharanthine are formed at different rates (they are isolated at 72 and 120 hr, respectively) from geissoschizine, this implied a special role for tabersonine in *V. rosea* and hence in many other species as a prototype of *Aspidosperma*, *Iboga*, and *Hunteria* bases. The profile of tabersonine reveals that (in accord with these

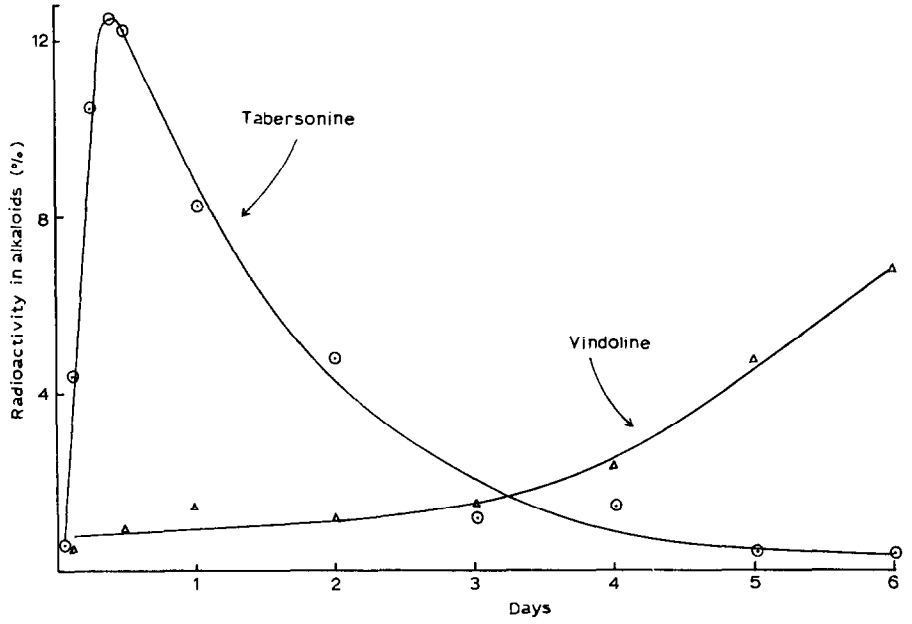


FIG. 12. Relative radioactivities in tabersonine (—⊙—) and vindoline (—▲—) during 6 days.

expectations) a remarkably dynamic role must indeed be ascribed to the alkaloid. Thus, after 9 hr the tabersonine pool has attained maximum radioactivity (Fig. 8). At this time 2-^{14}C-tryptophan of specific activity 52 mCi/mmole gave rise to tabersonine of specific activity 15.6 mCi/mmole (30% specific incorporation), indicating the extremely small pool sizes of the tryptophan available for alkaloid synthesis and of tabersonine. Autoradiograms clearly showed that tabersonine was metabolized almost as rapidly as it was formed, and in fact its activity falls (Figs. 12 and 13) from 12% to 2% in 3 days. This behavior is similar to that of the unknown alkaloid (Fig. 6), but is complicated by the falling rate of incorporation of tryptophan into the alkaloids after 1 day. Refeeding ^{14}C-labeled tabersonine isolated after feeding tryptophan for 9 hr gives a number of labeled metabolites. After 1 day, when there is still a large amount of unchanged tabersonine (**13**), it is found that epoxytabersonine (**14**), methoxytabersonine (**15**), and coronaridine (**17**) are all labeled. After 6 days there is almost no tabersonine remaining while vindoline (**18**) has gained activity (Fig. 12).

The role of epoxytabersonine [(–)lochnericine] (**14**) in the overall biosynthetic map is at present obscure. It is a minor constituent of the normal alkaloid pattern of *V. rosea*, but after 9 hr it has 4.3% of all the activity (Fig. 13), a specific activity of 10 mCi/mmole

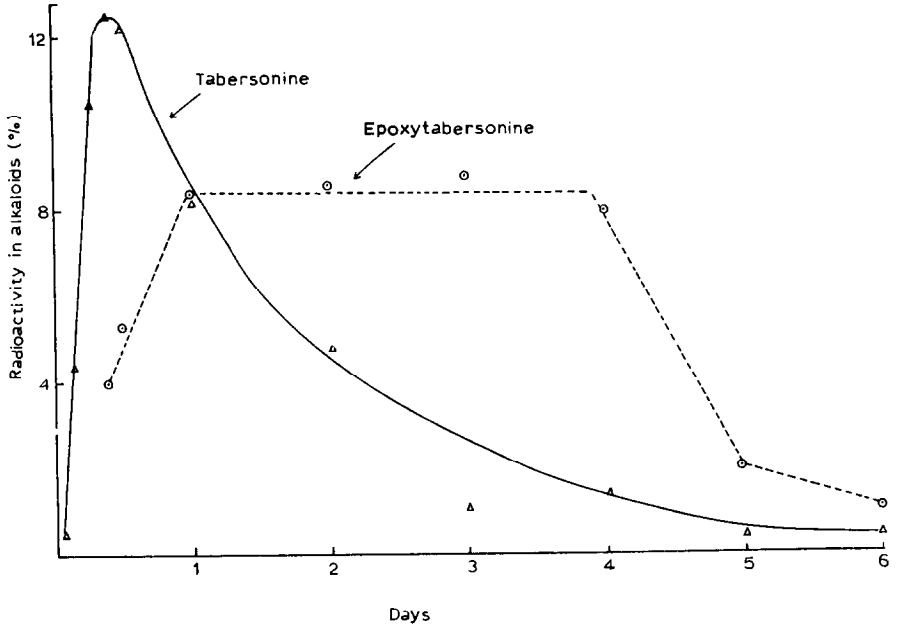


FIG. 13. Radioactivities in tabersonine (—▲—) and its epoxide lochnericine (--⊙--) over 6 days.

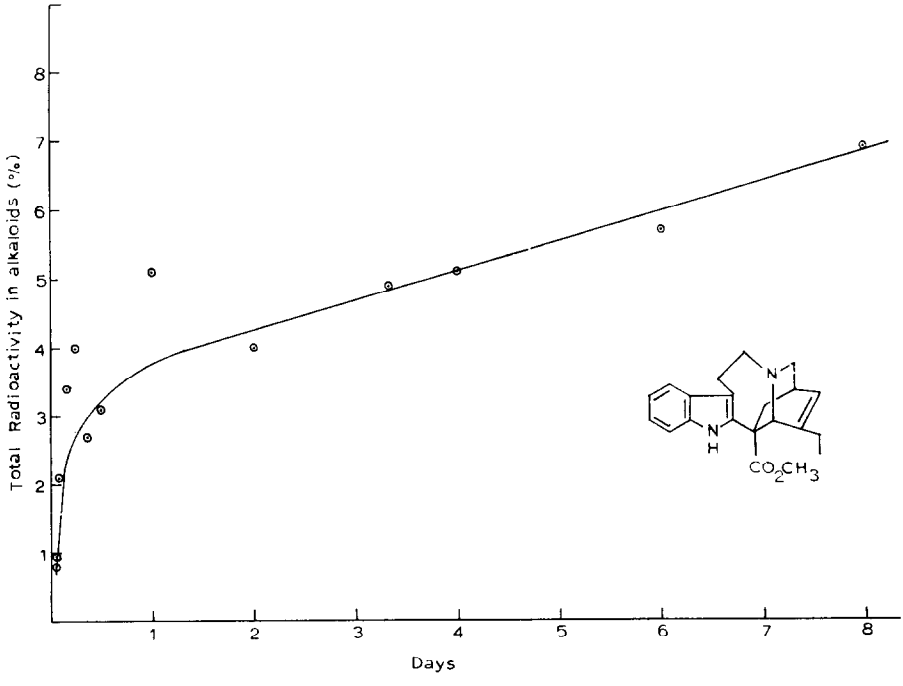


FIG. 14. Radioactivity in catharanthine (8 days).

(i.e., 20% specific incorporation) and is formed from tabersonine as shown from refeeding the latter labeled with ^{14}C from ^{14}C-tryptophan. Refeeding (**14**) gave rise to several unidentified alkaloids.

Although vindoline can be detected on autoradiograms after 1 day, its activity does not begin to increase rapidly until after 3 days (Fig. 12). At this stage the amount of radioactivity in the alkaloids has reached a maximum and the activity in vindoline will presumably increase rapidly to a maximum value which will not alter unless vindoline is broken down.

Soon after the onset of the decline in radioactivity in tabersonine, the principal *Iboga* alkaloid, catharanthine (**16**), is labeled, and a linear increase is observed from the first to eighth day to reach a value of 6% of the radioactivity in the alkaloids at the

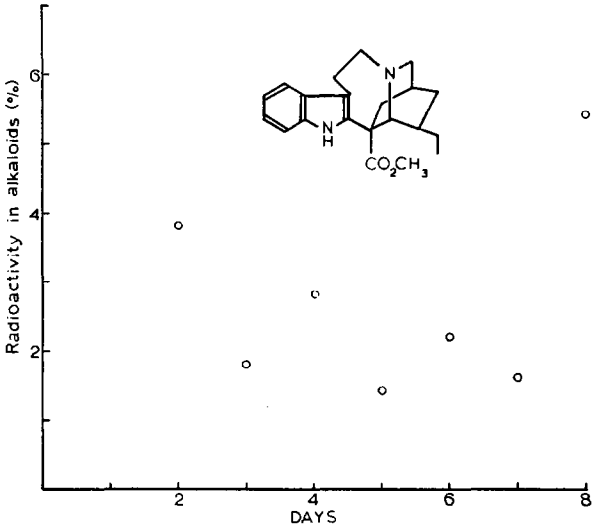


FIG. 15. Radioactivity in coronaridine (8 days).

end of the experiment (Fig. 14). The dihydro form of catharanthine, coronaridine (**17**) exhibits a fluctuating profile (Fig. 15), which may reflect a control by its (presumed) precursor, catharanthine. Complementary experiments with a tritiated version of coronaridine indicate that this precursor relationship is valid, for there is no evidence for the dehydrogenative return of coronaridine to catharanthine.

In summary, a set of dynamic precursors has been detected by short-term incubation. Several, but not all, of these correspond to previously discovered intermediates. In addition to the unknown alkaloid which is maximally labeled after 5 min, the appearance of the "radio-profiles" of geissoschizine (**5**) (1.5–2 hr), (pre)akuammicine (**8**) (2–2.5 hr), and tabersonine (**13**) (9 hr), distinguish these alkaloids from ajmalicine (**7**), catharanthine (**16**), coronaridine (**17**), lochnericine (**14**), and vindoline (**18**). Confirmation of the intermediacy of the former set has been secured for (**5**) and (**13**) in separate feeding experiments (*7*). The specific incorporations of tryptophan into the alkaloids reaches a most satisfactory level in these experiments, e.g., tabersonine (30%) and its epoxide (20%). Again the precursor role of tabersonine not only for the *Aspidosperma* but also for the *Iboga* series is confirmed. However, in view of the large differences (1-2 orders of magnitude) observed between the specific incorporations of tabersonine into vindoline and the *Iboga* alkaloids, the mechanism of the latter process may in fact involve the generation of both (+) and (−) forms of tabersonine, one of which

[the (–) form] leads to vindoline, the other, hitherto unknown (+) form undergoing transformation to catharanthine and coronaridine (*14*). The details of this mechanism and the stemmadenine–preakuammicine–acrylic ester relationship will be discussed elsewhere (*15*).

Application of the technique to several other putative precursors and the development of rapid sampling methods to evaluate a shorter time scale [0–300 sec] with substrates of very high specific activity are in progress.

EXPERIMENTAL

Plant Material

Seedlings of *V. rosea* (Burpee mixed strain) were grown in sand at 92°F in an environmental chamber (Biotronette Mark III; Lab-Line Instruments Inc.) in full light for 9–17 days. The rate of growth was very dependent on temperature and if temperature fell to 86°, the seedlings took longer (~3 weeks) to reach a suitable size. Seedlings were carefully removed intact, excess sand removed by gently dipping them into water, and then placed in groups of four in $\frac{1}{4}$-dram vials (9 × 20 mm) (Kimble Glass Company). Average weight of seedlings was 16 mg. The vials were placed on Scotch tape (preferably double surfaced), on 20 cm × 20 cm glass plates and allowed to grow at 92° in an environmental chamber for as long as required. When necessary the vials were topped up with tap water, requiring about 2 ml/day. Under these conditions the seedlings were healthy for about 7 days when the leaves began yellowing. By 10 days the seedlings looked distinctly yellow and wilted.

Alkaloid Extraction

The seedlings (sand free) were homogenized in a Ten Broeck glass homogenizer with methanol, allowed to stand overnight at room temperature, and the filtrate was evaporated to dryness. The residue was partitioned between ethyl acetate (discarded) and two portions of 1 *N* HCl. The acid solution was adjusted to pH 7.5–8.0 with ammonia (conveniently estimated by a pigment changing to yellow in the aqueous fraction) and extracted twice with ethyl acetate. The ethyl acetate extracts were washed once with water and dried with anhydrous $MgSO_4$.

The alkaloids were analyzed by chromatography using Merck precoated tlc plates (Silica Gel F254; 0.25-mm thickness). The main solvent systems used were ether:pet. ether, 1:9, developed five times for resolution of the tabersonine alkaloids and coronaridine; and for two-dimensional separations ether (1st direction) and either chloroform:methanol (95:5) or chloroform:methanol (9:1) (2nd direction). Chloroform:methanol (9:1) rather than 95:5 was better for resolving akuammicine and vinervine from the most polar alkaloids.

Alkaloids were located by spraying with ceric ammonium sulfate(1.5% in phosphoric acid) after adding standards where necessary. The radioactive areas indicated on the autoradiograms were scraped off and counted in toluene scintillant.

Autoradiograms

These were made by placing X-ray film (Kodak Royal Blue: folder wrapped; 8 × 20 in.) against a tlc plate using another plate to hold the film tightly in contact. The plates and film were made light-proof by folding twice in aluminum foil and kept in the darkroom for 4 days (for 10,000 dpm), or proportionately longer or shorter according to the amount of radioactivity on the plate. The X-ray film was developed and fixed using regular Kodak X-ray developer and fixer.

Radioactive Counting

Radioactive counting was measured in a Packard Tri-Carb liquid scintillation counter (Model 3320) using either toluene scintillant (15 ml) or dioxan scintillant (15 ml) for aliquots from the feed water. Toluene scintillant: PPQ 16 g; BBOT, 0.4 g; toluene to make 4 liters. Dioxan scintillant: naphthalene, 60.0 g; PPO, 4.0 g; BBOT, 0.2 g; absolute methanol, 100 ml; ethylene glycol, 20 ml; dioxan to 1 liter.

Radioactive Feeding

All the DL-^{14}C tryptophan used (purchased from the Radiochemical Centre, Amersham) was of the same specific activity, 52 mCi/mmole. It was dissolved in water and aliquots (33 μCi each) added to 14 groups of 4 seedlings in glass vials (total 462 μCi). The total amount of radioactivity administered was largely determined by the length of the feeding experiment once it was established that maximum conversion was approximately 3%.

Identification of Alkaloids

This was established by coincidence of radioactivity and ceric ammonium sulfate (CAS) positive areas on autoradiogram and tlc plate. There are only five alkaloids present in sufficient concentrations in four seedlings to give a positive CAS color. These are vindoline, coronaridine, akuammicine, vinervine, and an unidentified alkaloid more polar ($CHCl_3$:MeOH, 9:1) than vinervine with a tabersonine-like CAS color sequence. Coronaridine, with its vague blue-gray rapidly fading color could not always be positively identified.

With the alkaloids shown in Table 1 the radioactive areas were scraped off and the alkaloids eluted and rechromatographed with standards; identification was established by coincidence of radioactivity and CAS-positive areas on autoradiogram and tlc plate.

TABLE 1

Alkaloid	Solvent system	
	First	Second
Akuammicine	Benzene–methanol (9:1)	$CHCl_3$:methanol (9:1)
Ajmalicine	Ether	$CHCl_3$:methanol (9:1)
Catharanthine	Ether	$CHCl_3$:methanol (9:1)
Geissoschizine	Ether	$CHCl_3$:methanol (9:1)
Coronaridine	Ether–pet. ether (1:9) (5 times)	Ethyl acetate:hexane (1:9)
Epoxytabersonine	Ether–pet. ether (1:9) (5 times)	Ethyl acetate:hexane (1:9)
Methoxy tabersonine	Ether–pet. ether (1:9) (5 times)	Ethyl acetate:hexane (1:9)
Vindoline	Ether	$CHCl_3$:methanol (95:5)
Vinervine	$CHCl_3$:methanol (9:1)	$CHCl_3$:methanol (4:1)
Stemmadenine	$CHCl_3$:methanol (9:1)	$CHCl_3$:methanol (85:15)

Refeeding Experiments

Thirty-two vials of 4 seedlings (10 day) were each (vial) fed 33 μCi of ^{14}C-DL-tryptophan for 9 hr. The feed water was reused for a further 18 vials for 9 hr and the feed water from these 18 vials was used for another 10 vials for 9 hr feeding. Initially 1 mCi was used and the total amount of tryptophan taken up by all the three groups was 85%. The alkaloids were extracted as described previously [total activity 2.7×10^7 dpm (1.5%)] and chromatographed on a single 20 cm $\times$ 20 cm tlc plate developed five

times with ether: pet. ether, 1:9. The radioactive bands were located by overnight autoradiogram, and then scraped off and eluted with ether.

Tabersonine: uv spectrum (ether) λ_{max} 225, 297, 325 nm. Total radioactivity 2.87×10^6 dpm, 14.3% of total activity. Specific activity 15.6 mCi/mmole.

Coronaridine: radioactivity 1.4×10^6 dpm (5.1%). This was rechromatographed on the same solvent system. uv spectrum (ether) 227, 285 nm. Recovered radioactivity 1.02×10^6 dpm: sp act 0.49 mCi/mmole.

Epoxytabersonine: radioactivity 1.3×10^6 dpm (4.3%). This was rechromatographed on the same solvent system. uv spectrum (ether) 225, 297, 325 nm; recovered radioactivity 6.4×10^5 dpm: sp act 10 mCi/mmole.

For the refeeding experiments, the alkaloid was dissolved in a drop of ethanol, diluted with water, and added to groups of four seedlings (six groups with tabersonine, two each for coronaridine and epoxytabersonine). The seedlings were then allowed to grow for 1, 3, and 6 days (tabersonine), 6 days (coronaridine), and 5 days (epoxytabersonine). The alkaloids were then extracted and analyzed as described previously.

REFERENCES

1. R. ROBINSON, *J. Chem. Soc.* **111**, 876 (1917); "The Structural Relations of Natural Products," Oxford Univ. Press, Oxford, 1955.
2. I. D. SPENSER, "Comprehensive Biochemistry" (M. Florkin and E. H. Stotz, Eds.), Vol. 20, Chap. 6. Elsevier, New York, 1968.
3. D. V. BANTHORPE AND A. WRIZ-JUSTICE, *J. Chem. Soc.* (C), 541 (1969); J. BATTAIL, A. J. BURBOTT, AND W. D. LOOMIS, *Phytochemistry*, 1159 (1968); I. SCHECHTER AND C. A. WEST, *J. Biol. Chem.* **244**, 3200 (1969).
4. S. H. MUDD, *Biochim. Biophys. Acta* **38**, 354 (1960); H. M. FALES, J. MANN, AND S. H. MUDD, *J. Amer. Chem. Soc.* **85**, 3820 (1963).
5. H. RAPOPORT, F. R. STERMITZ, AND D. R. BAKER, *J. Amer. Chem. Soc.* **82**, 2765 (1960).
6. For a recent survey see D. A. WALKER AND A. R. CROFTS, *Annu. Rev. Biochem.* **39**, 389 (1970).
7. A. I. SCOTT, *Acc. Chem. Res.* **3**, 151 (1970), and References cited therein.
8. M. B. SLAYTOR, unpublished work.
9. C. DJERASSI, H. J. MONTEIRO, A. WALSER, AND L. J. DURHAM, *J. Amer. Chem. Soc.* **88**, 1792 (1966).
10. G. N. SMITH, *Chem. Commun.*, 912 (1968).
11. M. PINAR, M. HANAOKA, M. HESSE, AND H. SCHMID, *Helv. Chim. Acta* **54**, 15 (1971), and footnote 13.
12. J. P. KUTNEY, V. R. NELSON, AND D. C. WIGFIELD, *J. Amer. Chem. Soc.* **92**, 4278 (1969); J. P. KUTNEY, J. F. BECK, V. R. NELSON, AND R. S. SOOD, *J. Amer. Chem. Soc.* **93**, 255 (1971).
13. A. I. SCOTT AND P. C. CHERRY, *J. Amer. Chem. Soc.* **91**, 5872 (1969).
14. This is only one of several possible mechanisms to be considered for the conversion of the *Corynanthe* to both the *Aspidosperma* and *Iboga* series. In view of recent experiments in these laboratories (*15*), at Zurich and Cambridge (Professors D. Arigoni and A. R. Battersby, private communications), a separate fate must be ascribed to each of the prochiral protons at C_{14} in geissoschizine during biotransformation to vindoline (**18**) and catharanthine (**16**), respectively. In other words, as adumbrated by one of us (*16*) in more general terms, the *Aspidosperma* and *Iboga* alkaloids in *V. rosea* are derived by an enantiotopic process at some stage in the biosynthesis. Detailed proposals will be made elsewhere (*15*).
15. A. I. SCOTT, P. B. REICHARDT, AND J. G. SWEENY, in preparation.
16. A. I. SCOTT, 21st National Organic Symposium, Amer. Chem. Soc., 1969, Abstr., p. 50; *Chem. Commun.*, 948 (1968).