Phytochemistry, 1971, Vol. 10, pp. 2661 to 2669. Pergamon Press. Printed in England.

BIOSYNTHESIS OF QUINAZOLINE ALKALOIDS OF *PEGANUM HARMALA*

D. R. LILJEGREN

Department of Agricultural Biochemistry, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, South Australia

(*Received* 25 *September* 1970)

Abstract—Evidence has been found supporting the proposal that synthesis of vasicine in *Peganum harmala* occurs from anthranilic acid and a compound closely related to ornithine. Specificity of incorporation of varying degree was observed after feeding glutamic acid-5-^{14}C, proline-5-^{14}C, ornithine-2-^{14}C and -5-^{14}C, and putrescine-1,4-^{14}C. The approximately equal distribution of label between C-1 and C-10 of vasicine showed a symmetrical intermediate to be involved in the biosynthetic pathway. Catabolism of tryptophan to anthranilic acid was demonstrated by the specific incorporation of tryptophan [benzene ring-^{14}C(U)] into vasicine. Experiments designed to examine interconversions between the quinazoline alkaloids were unsuccessful due to the rapid synthesis and degradation of these compounds within the plant.

INTRODUCTION

SEVERAL theories have been proposed to account for the biosynthesis of the pyrroloquinazoline alkaloids such as vasicine (I), vasicinone (II) and desoxyvasicinone (III) found in *Peganum harmala*. The pathway shown in Figure 1 was considered most likely, whereby anthranilic acid condensed with proline or a related metabolite to give the alkaloid skeleton.[1,2] This proposal was supported by the involvement of ornithine in the biosynthesis of nicotine, hyoscyamine, and other compounds having monocyclic or fused pyrrolidine ring systems.[3] Chemical evidence for the pathway was supplied when *o*-aminobenzaldehyde was shown to condense with γ-aminobutyraldehyde[4] and α-keto-δ-aminovaleric acid[5] to yield the pyrroloquinazoline system. A similar condensation using α-hydroxy-γ-aminobutyraldehyde led ultimately to vasicine.[6]

A part of this pathway has been proven correct by the specific incorporation of ^{14}C-labelled anthranilic acid into vasicine in *P. harmala*[7] and *Adhatoda vasica*.[8] Experiments using ^{15}N-labelled precursors have shown that anthranilic acid also supplies N-9 of the alkaloid molecule.[9] Whether the anthranilic acid used *in vivo* is derived from shikimic acid or tryptophan has not been definitely established, though it has been shown that when tryptophan [benzene ring-^{14}C(U)] is fed to *P. harmala*, radioactive quinazoline alkaloids can be isolated.[10]

[1] R. ROBINSON, *The Structural Relations of Natural Products*, Clarendon Press, Oxford (1955).
[2] E. LEETE, in *Biogenesis of Natural Compounds* (edited by P. BERNFELD), 2nd edn., Pergamon Press, Oxford (1967).
[3] I. D. SPENSER, *Comprehensive Biochemistry* (edited by M. FLORKIN and E. H. STOTZ), Vol. 20, Elsevier, Amsterdam (1968).
[4] C. SCHÖPF and F. OECHLER, *Ann. Chem.* **523**, 1 (1936).
[5] L. MACHOLAN, *Collection Czech. Chem. Commun.* **24**, 550 (1959).
[6] N. J. LEONARD and M. J. MARTELL, *Tetrahedron Letters* 44 (1960).
[7] D. GROGER and K. MOTHES, *Arch. Pharm.* **293**, 1049 (1960).
[8] D. GROGER, S. JOHNE and K. MOTHES, *Experientia* **21**, 13 (1965).
[9] S. JOHNE, D. GROGER and G. RICHTER, *Arch. Pharm.* **301**, 721 (1968).
[10] D. R. LILJEGREN, *Phytochem.* **7**, 1299 (1968).

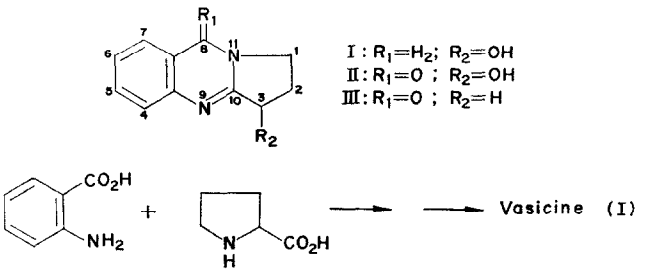


FIG. 1. POSSIBLE BIOSYNTHETIC ROUTE TO VASICINE.

Administration of compounds related to ornithine has thus far led to inconclusive results. Activity from putrescine-1,4-^{14}C and ornithine-2-^{14}C and -5-^{14}C is incorporated into vasicine by *P. harmala*.[10] Similarly putrescine-1,4-^{14}C, proline-U-^{14}C and γ-hydroxyglutamic acid-α-^{14}C gave labelled vasicine in *A. vasica*,[11] but degradation showed this incorporation to be non-specific.[12,13] Randomly-labelled alkaloids were also isolated when ornithine-2-^{14}C and 4-hydroxyproline-2-^{14}C were used with the same system.[12]

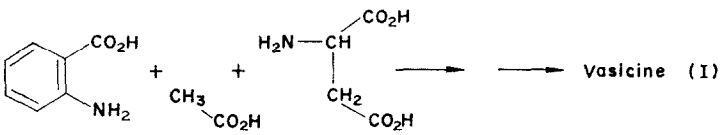


FIG. 2. POSSIBLE PRECURSORS OF VASICINE.

The failure of these experiments to show specific incorporation of the ornithine group of compounds led to the proposal that aspartic acid and a 2-carbon fragment might be used for the 'non anthranilic acid' portion of vasicine (Fig. 2).[12] This was supported experimentally[9,12] with *A. vasica* by the incorporation of aspartic acid-3-^{14}C, malic acid-3-^{14}C and succinic acid-2,3-^{14}C, with localization of the label at C-1 and C-2 of vasicine. Some specificity was also shown in the incorporation of acetate into C-3 and C-10.

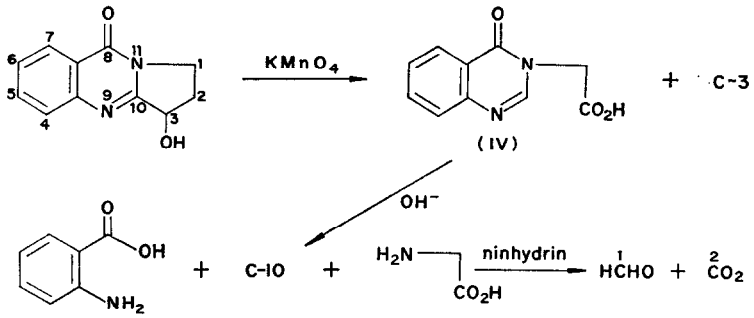
This paper reports the results of feeding a number of precursors related to ornithine to *P. harmala*. The stability and interconversions of the alkaloids are also described.

RESULTS

The rates of incorporation of radioactivity into the quinazoline alkaloids following administration of the possible precursors ornithine-2-^{14}C, ornithine-5-^{14}C, putrescine-1,4-^{14}C and tryptophan [benzene ring-^{14}C(U)] have been reported.[10] Results of similar experiments with glutamic acid-5-^{14}C and proline-5-^{14}C are shown in Table 1.

Samples of vasicine from each experiment were degraded as shown in Scheme 1 to give the distribution of activity among the skeletal atoms. Vasicine isolated after feeding tryptophan [benzene ring-^{14}C(U)] had 87 per cent of the original activity localized in the anthranilic acid derived from the aromatic portion of the alkaloid molecule. The remaining 13% was randomly scattered (C-3, 5·9%; C-1 + C-2, 2·2%; C-10, 5·3%). This specific incorporation

[11] D. GROGER, S. JOHNE and K. MOTHES, *Abhandl. Deut. Akad. Wiss. Berlin Kl. Chem. Geol. Biol.* **581** (1966).
[12] S. JOHNE and D. GROGER, *Phytochem.* 7, 429 (1968).
[13] S. JOHNE and D. GROGER, *Z. Pflanzenphysiol.* **61**, 353 (1969).



SCHEME 1. DEGRADATION OF VASICINE.

TABLE 1. SPECIFIC ACTIVITIES OF ALKALOIDS ISOLATED FROM *Peganum harmala* FOLLOWING ADMINSTRATION OF LABELLED PRECURSORS

Precursor*	Specific activity (dis/min/mM × 10^{-5}; % incorporation in brackets) Vasicine†	Vasicinone	Desoxy-vasicinone
Glutamic acid-5-^{14}C	12·5 (0·66)	2·13 (0·02)	15·9 (0·47)
Proline-5-^{14}C	8·63 (0·49)	1·24 (0·01)	14·2 (0·56)

* 100 μCi of each.

† Specific activities of vasicine samples measured after conversion to vasicinone.

Values refer to alkaloids from stems and leaves. Duration of both experiments was 5 days. % incorporation = 100 × total dis/min isolated/total dis/min fed.

suggests the existence in *P. harmala* of a catabolic pathway from tryptophan to anthranilic acid, which is used, at least in part, for the synthesis of the quinazoline alkaloids. The rate of incorporation (0·071%)[10] is however much lower than that of ornithine (0·39% and 1·6%)[10] putrescine (1·2%),[10] glutamic acid and proline (Table 1), indicating that the majority of the anthranilic acid used in alkaloid synthesis is obtained via the shikimic acid pathway.

The distribution of activity in the vasicine samples from 5 other feeding experiments is shown in Table 2. Following the feeding of ornithine-2-^{14}C, 84 per cent of the activity in vasicine was found to be localized at C-1 and C-10, and was equally divided between these 2 atoms. The remaining 16 per cent was randomly scattered throughout the molecule. The result from the experiment using ornithine-5-^{14}C as precursor is similar, with some 65 per cent of the original activity being equally divided between C-1 and C-10. Again, administration of the symmetrical putrescine-1,4-^{14}C gave a localization of 69 per cent of the activity at C-1 and C-10, but in this case the latter atom was more heavily labelled. It is significant that in these three cases very little radioactivity was recovered as anthranilic acid, the original aromatic portion of vasicine.

The labelling pattern became more random when glutamic acid-5-^{14}C and proline-5-^{14}C were used as precursors. Some 23 per cent of the activity was recovered as anthranilic acid although in both cases C-1 and C-10 remained the most heavily labelled individual atoms.

Experiments were also designed to examine the possible interconversions of the three quinazoline alkaloids within the plant. Vasicine, vasicinone and desoxyvasicinone were

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN VASICINE AFTER PRECURSOR FEEDING

Precursor	Specific activity (dis/min/mM × 10^{-5}) Vasicinone	4-Quinazolone-3-acetic acid	C-3 (difference)	Anthranilic acid	Glycine C-1 + C-2
Ornithine-2-^{14}C	12·1 (100%)	11·4 (94·1)	(5·9)	0·698 (5·8)	5·63 (46·7)
Ornithine-5-^{14}C	0·657 (100%)	0·501 (76·2)	(23·8)	0·050 (7·6)	0·225 (34·2)
Putrescine-1,4-^{14}C	1·4 (100%)	1·08 (76·6)	(23·4)	0·071 (5·0)	0·426 (30·4)
Glutamic acid-5-^{14}C	2·39 (100%)	2·04 (85·5)	(14·5)	0·596 (23·4)	0·947 (39·7)
Proline-5-^{14}C	0·885 (100%)	0·719 (81·3)	(18·7)	0·208 (23·5)	0·317 (35·8)

Precursor	Specific activity (dis/min/mM × 10^{-5}) C-10 (difference)	Formaldehyde C-1	C-2 (difference)
Ornithine-2-^{14}C	(41·6)	5·15 (42·7)	(4·0)
Ornithine-5-^{14}C	(34·4)	0·199 (30·4)	(3·8)
Putrescine-1,4-^{14}C	(41·2)	0·389 (27·7)	(2·7)
Glutamic acid-5-^{14}C	(22·3)	0·901 (37·7)	(2·0)
Proline-5-^{14}C	(22·0)	0·282 (31·8)	(4·0)

tritiated and administered to *P. harmala* plants. The specific activities of the isolated alkaloids in each case are shown in Table 3. On feeding vasicine-T, the majority of the activity was recovered unchanged after a 7-day period although some conversion to vasicinone had occurred. In contrast, however, very little of the original activity was recovered in the form of alkaloids following the feeding of vasicinone-T and desoxyvasicinone-T. In both cases vasicinone was the most active of the alkaloids labelled.

TABLE 3. SPECIFIC ACTIVITIES OF ALKALOIDS ISOLATED AFTER FEEDING TRITIATED ALKALOIDS

Alkaloid fed	Specific activity (dis/min/mM × 10^{-5}; % incorporation in brackets) Vasicine*	Vasicinone	Desoxyvasicinone
Vasicine-T (103 μCi)	1800 (57·3)	943 (7·7)	2·69 (0·05)
Vasicinone-T (100 μCi)	1·07 (0·04)	123 (1·61)	0·655 (0·03)
Desoxyvasicinone-T (100 μCi)	1·84 (0·06)	64·0 (0·45)	3·62 (0·12)

% incorporation = 100 × total dis/min isolated/total dis/min fed. Duration of experiments was 7 days.

* Specific activities of vasicine samples measured after conversion to vasicinone.

Information on the specificity of the interconversions observed in these experiments was gained by degrading the alkaloids to anthranilic acid. The tritiated precursors fed to the plants were randomly labelled, but a constant ratio of anthranilic acid activity–alkaloid activity should be observed if there is a simple conversion of one alkaloid to another. From the results in Table 4 the only apparent simple conversion is from vasicine to vasicinone. The constant activity ratio observed may be fortuitous, resulting from similar non-specific metabolism of the two alkaloids. In view of the high recovery of activity in the form of vasicine (Table 3) this is considered unlikely.

TABLE 4. DISTRIBUTION OF ACTIVITY IN ALKALOIDS ISOLATED AFTER FEEDING TRITIATED ALKALOIDS

Alkaloid fed	Specific activities (dis/min/mM × 10^{-5})			Ratio $\frac{\text{acid}}{\text{alkaloid}}$
	Alkaloid isolated*		Anthranilic acid	
Vasicine-T	Vasicine	1800	1550	0·863
	Vasicinone	943	837	0·888
Vasicinone-T	Vasicine	1·07	0·356	0·332
	Vasicinone	123	54·4	0·442
Desoxyvasicinone-T	Vasicine	1·84	1·27	0·688
	Vasicinone	64·0	31·2	0·488

* Specific activities of vasicine samples measured after conversion to vasicinone.

Ornithine-5-^{14}C was administered to excised stems of *P. harmala* in an attempt to discover which of the three quinazoline alkaloids was formed first from a common precursor. The specific activities of alkaloids isolated from individual stems harvested over a 23-hr period are illustrated in Fig. 3. Synthesis of all three bases is rapid, but evidence of one acting as a precursor for another is not apparent.

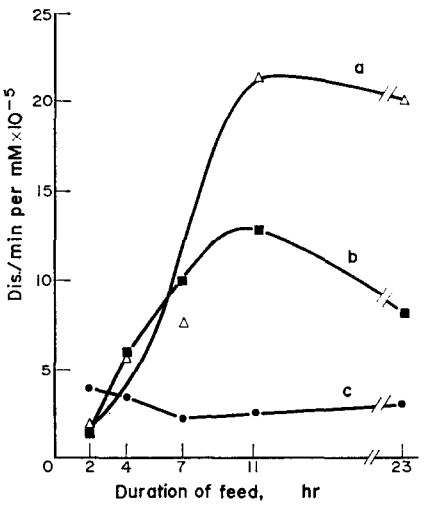


FIG. 3. SPECIFIC ACTIVITIES OF ALKALOIDS ISOLATED AFTER FEEDING ORNITHINE-5-^{14}C TO EXCISED STEMS OF *P. harmala*.

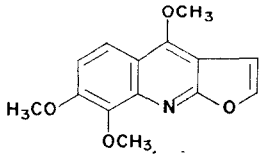
A: Vasicine; B: Desoxyvasicinone; C: Vasicinone.

DISCUSSION

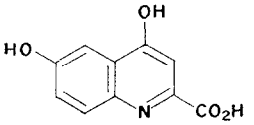
Incorporation of label from the benzene ring of tryptophan into vasicine demonstrates that a catabolic pathway to anthranilic acid does operate in *Peganum harmala*. Whether this is the same as the well known 'kynurenine pathway' found in mammals and micro-organisms is not known; e.g. tryptophan pyrrolase has not been isolated from a plant system.[14] There are, however, two other examples of tryptophan degradation and further

[14] P. K. MACNICOL, *Biochem. J.* **107**, 473 (1968).

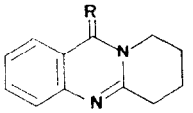
metabolism in plants. Matsuo and Kasida[15] report a specific incorporation of tryptophan-3-^{14}C into the alkaloid skimmianine (V) and tryptophan-1-^{14}C leads to 6-hydroxykynurenic acid (VI) in *Nicotiana tabacum*.[16] It would be of interest to determine whether tryptophan acts as a precursor for the pyridoquinazoline alkaloids VII and VIII,[17] as for the pyrroloquinazoline vasicine.



(V)



(VI)



(VII) R = O
(VIII) R = H_2

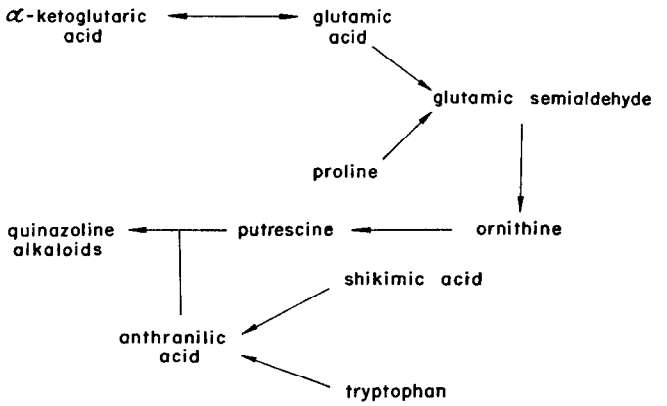
Results from feeding experiments using ornithine and related compounds (Table 2) support the theory that it is a member of this group that supplies carbon atoms 1, 2, 3 and 10 and N-11 of vasicine. In each case greater than 75 per cent of the vasicine activity is distributed among these atoms, with the majority located at C-1 and C-10. Ornithine and putrescine are incorporated more specifically than proline or glutamic acid, which suggests that they are closer to the immediate alkaloid precursor.

Previous work[10] has shown that a similar amount of ^{15}N was incorporated into the quinazoline alkaloids when either ornithine-α-^{15}N or ornithine-δ-^{15}N was supplied to *P. harmala* plants. This suggested that a symmetrical intermediate such as putrescine was involved in the pathway from ornithine to vasicine. The approximately equal degree of labelling at C-1 and C-10 after feeding ornithine-2-^{14}C and -5-^{14}C supports this suggestion. Theoretically each of these atoms should show 50 per cent of the incorporated activity, and the feeding of putrescine-1,4-^{14}C should give a similar result. The fact that atoms other than C-1 and C-10 become labelled in these experiments can be explained by the rapid degradation and metabolism of the alkaloids once formed in the plant (see Table 3). In this situation short feeding times should result in a more specific incorporation than that shown in Table 2, and conversely a long feeding time should give a more random labelling pattern. Experiments to test this theory are in progress.

The results obtained support Scheme 2 as representing part of the pathway leading to quinazoline alkaloids in *P. harmala*. However, Groger *et al.* have been unable to find evidence for its operation in *Adhatoda vasica*; random incorporation resulted when γ-hydroxyglutamic acid-α-^{14}C, ornithine-2-^{14}C, 4-hydroxyproline-2-^{14}C and putrescine-1,4-^{14}C were fed to the plants.[12,13] The difference between the two systems can be seen in Table 5, which illustrates the variation in the specificity of incorporation of common precursors. This, together with evidence that aspartic acid and acetate act as vasicine precursors in *A. vasica* leads to the conclusion that two different pathways exist for the synthesis of the quinazoline alkaloids.

The pathway shown in Scheme 2 does not indicate which of the alkaloids is formed first as a result of the condensation yielding the pyrroloquinazoline system. Nor is anything known of any interconversions that occur between the alkaloids of similar structure. The

[15] M. MATSUO and Y. KASIDA, *Chem. Pharm. Bull.* **14**, 1108 (1966).
[16] M. SLAYTOR, L. COPELAND and P. K. MACNICOL, *Phytochem.* **7**, 1779 (1968).
[17] J. S. FITZGERALD, S. R. JOHNS, J. A. LAMBERTON and A. H. REDCLIFFE, *Austral. J. Chem.* **17**, 151 (1966).



SCHEME 2. SECTION OF THE PATHWAY LEADING TO QUINAZOLINE ALKALOIDS IN *Peganum harmala*.

TABLE 5. LABELLING PATTERNS OF VASICINE AFTER PRECURSOR FEEDING TO *Peganum harmala* AND *Adhatoda vasica**

Precursor	Plant	Percentage vasicine activity			
		Anthranilic acid	Glycine	C-1	C-3 + C-10
Ornithine-2-^{14}C	*P. harmala*	5·8	46·7	42·7	47·5
Ornithine-2-^{14}C	*A. vasica*	40	33	19	—
Putrescine-1,4-^{14}C	*P. harmala*	5	30·4	27·7	64·6
Putrescine-1,4-^{14}C	*A. vasica*	62·5	9·9	—	27·6

* Values for *A. vasica* from Johne and Gröger.[12]

two approaches selected to examine these questions—the separate feeding of tritiated alkaloids and an examination of individual alkaloid synthesis with time—gave inconclusive results. This was due to the rapid synthesis and metabolism of the alkaloids within the plant. Table 3 shows that vasicinone and desoxyvasicinone in particular are degraded and the fragments used for the synthesis of non-alkaloidal compounds. There is evidence for similar metabolism of other classes of alkaloids and secondary plant products. Fairbairn *et al.* have demonstrated rapid alkaloid turnover in *Papaver somniferum*[18] and *Conium maculatum*.[19] Ricinine is extensively degraded in *Ricinus communis*,[20] and alkaloid concentration varies over both the short and the long term in several *Solanum* species.[21]

Evidence of specific interconversions between alkaloids in *P. harmala* was masked by their rapid metabolism. In only one case—after feeding vasicine-T—was a similar distribution of activity found in the vasicine and vasicinone isolated (Table 4). From this experiment it is possible to say that *in vivo* oxidation of vasicine does occur; evidence of other alkaloid interconversions will only be gained from feeding experiments over shorter periods.

The rapid nature of the alkaloid synthesis is illustrated in Fig. 3. There is no indication of one compound being formed first in any sequential pathway, and such a result makes it likely that any interconversions that do occur take place rapidly.

[18] J. W. FAIRBAIRN and S. EL-MASRY, *Phytochem.* **6**, 499 (1967).
[19] J. W. FAIRBAIRN and P. N. SUWAL, *Phytochem.* **1**, 38 (1961).
[20] G. R. WALLER, M. S. TANG, M. R. SCOTT, F. J. GOLDBERG, J. S. MAYES and H. AUDA, *Plant Physiol.* **40**, 803 (1965).
[21] D. C. LEWIS and D. R. LILJEGREN, *Phytochem.* **9**, 2193 (1970).

EXPERIMENTAL

Source of materials. Tritiated water, DL-tryptophan [benzene ring-^{14}C(U)] (19·8 mc/mM) and putrescine-1,4-^{14}C (23·7 mc/mM) were obtained from the Radiochemical Centre, Amersham, England; DL-ornithine-5-^{14}C (11 mc/mM), DL-proline-5-^{14}C (5 mc/mM) and DL-glutamic acid-5-^{14}C (8·6 mc/mM) from Commissariat à l'Energie Atomique, France, and DL-ornithine-2-^{14}C (3·3 mc/mM) from Baird Atomic Inc., U.S.A.

Established *Peganum harmala* plants were collected in the field, planted in large pots and grown in the open. Labelled compounds were administered to the plants by the wick-feeding method.[10]

Synthesis of tritiated alkaloids. Preparation of tritiated vascine has been reported previously.[10] Samples of tritiated vasicinone ($1{\cdot}11 \times 10^{10}$ dis/min/mM) and desoxyvasicinone ($5{\cdot}74 \times 10^{9}$ dis/min/mM) were similarly prepared using a reduced platinum catalyst and tritiated water (0·2 c/ml) and were purified by chromatography and sublimation.

Isolation of alkaloids. A number of general methods have been described.[10] For separation of the alkaloids, the crude mixture was dissolved in water-saturated $CHCl_3$ and applied to the top of column of Supercell containing 50% by weight of 10% phosphoric acid. Elution with water-saturated $CHCl_3$ yielded vasicinone and desoxyvasicinone in one fraction (A) and application of $CHCl_3$ saturated with 10% NH_4OH gave vasicine (fraction B).

TABLE 6. ALKALOIDS ISOLATED FROM PRECURSOR FEEDING EXPERIMENTS

Precursor	Alkaloids isolated (mg) Vasicine	Vasicinone	Desoxyvasicinone
Glutamic acid-5-^{14}C	222	36	117
Proline-5-^{14}C	238	52	162
Vasicinone-T	182	58	178
Desoxyvasicinone-T	141	31	134

Fraction A was reduced to dryness, dissolved in 1:1 benzene–$CHCl_3$ and added to the top of an alumina column (Woelm neutral, activity 1) packed in the same solvent mixture. Continued elution with this system gave deoxyvasicinone. Vasicinone was eluted when $CHCl_3$ containing 10% MeOH was passed through the column.

Fraction B, containing vasicine, was allowed to stand in sunlight in a container open to the air for a period of approximately 1 week. Additional $CHCl_3$ was added to compensate for evaporation and the progress of the auto-oxidation followed by chromatography (Whatman No. 1 chromatography paper; solvent system *n*-BuOH saturated with 5% HOAc). The resulting vasicinone was purified by chromatography on alumina as described above. The yields of alkaloids from precursor feeding experiments not previously described are shown in Table 6.

All alkaloid samples were then sublimed and/or recrystallized to constant specific radioactivity. Concentrations of solutions used in these determinations were measured by UV absorption. The physical constants of the alkaloids have been reported previously.[10]

Degradation of vasicinone. In a typical experiment $KMnO_4$ (130 mg) was added to a mixture of vasicinone (65 mg) dissolved in acetone (50 ml). The mixture was stirred at room temp. for 4 hr and then the solvent removed under reduced pressure. The solid residue was suspended in H_2O and the solution clarified by the addition of a small amount of a saturated solution of $Na_2S_2O_3$. After adjusting the pH to 2 with HCl, 4-quinazolone-3-acetic acid was extracted with Et_2O over a period of 2–3 days. Purification by preparative scale paper chromatography (Whatman No. 3 mm; solvent system 95% EtOH–0·88 NH_4OH, 99:1) yielded the acid as small colourless needles, m.p. 239–240°, λ_{max} 267, 275 (sh), 303, 315 nm, log ϵ 3·88, 3·83, 3·55, 3·47 λ_{min} 283, 310 nm, log ϵ 3·42, 3·41.

4-Quinazolone-3-acetic acid was hydrolysed by heating with 20% KOH (100°, 30 min) under an atmosphere of N_2. The cooled solution was adjusted to pH 3 and extracted with Et_2O. Evaporation of the Et_2O yielded anthranilic acid which was purified by sublimation. The aqueous phase was added to a column of Amberlite CG-120 (H^+ form), and after desalting by elution with H_2O, glycine was recovered by the addition of 1 N NH_4OH. Samples were stored over P_2O_5 and purified by preparative scale paper chromatography (Whatman 3 mm; *n*-BuOH–AcOH–H_2O, 50:12:50). For the determination of specific activities, glycine samples were dissolved in H_2O and the concentrations measured using ninhydrin.

Glycine was decomposed by heating with ninhydrin at pH 5·5. A stream of N_2 was passed through the boiling mixture, and the liberated HCHO and CO_2 were collected as the dimedone derivative and Ba salt, respectively.

Feeding of ornithine-5-^{14}C to excised stems of Peganum harmala. Stems at an approximately equal stage of development were cut from one plant and the ends dipped into a vial containing ornithine-5-^{14}C (50 μc; 11 mc/mM) in water (*ca.* 2 ml). H_2O was added to the vial at times to maintain the volume. Samples were removed after 2, 4, 7, 11 and 23 hr, immediately ground with MeOH and extracted successively with 3 × boiling MeOH. The solution was evaporated under reduced pressure and the individual alkaloids isolated as described above. Yields are shown in Table 7. All final alkaloid concentrations were determined by UV absorption, aliquots of these solutions being used for determination of radioactivity.

TABLE 7. ALKALOIDS ISOLATED FROM ORNITHINE-5-^{14}C FEEDING TO EXCISED STEMS OF *P. harmala*

Time of sampling (hr)	Stem wet wt. (g)	Crude alkaloids (mg)	Vasicine (mg)	Vasicinone (mg)	Desoxy-vasicinone (mg)
2	1·6	17·7	9·4	0·5	2·6
4	1·6	15·8	8·9	0·8	3·1
7	1·8	18·6	9·9	0·8	4·1
11	1·9	19·6	12·5	0·6	5·0
23	2·1	22·2	9·5	0·6	4·9

Determination of radioactivity. Specific activities of alkaloids were measured using a Beckman Lowbeta II gas flow counter (background 2 counts/min) or a Packard Tricarb liquid scintillation spectrometer model 3375. Aliquots of MeOH or 95% EtOH solutions were dried on 2 in. stainless-steel planchets or added to toluene scintillation fluid containing 2,5-diphenyloxazole (PPO, 7 g) and 1,4-bis-2(5-phenyloxazolyl)benzene (POPOP, 0·3 g) per 1. Glycine samples in H_2O were added to toluene scintillant containing 50% by volume of 0·6 N NCS solubilizer (Amersham/Searle Corporation). All determinations were carried out to at least a 5% confidence level.

Acknowledgement—I am grateful to Mr. P. Walsh for skilled technical assistance.