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THE BIOSYNTHESIS OF QUINAZOLINE ALKALOIDS OF *PEGANUM HARMALA* L.

D. R. LILJEGREN

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

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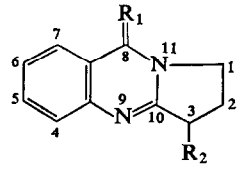
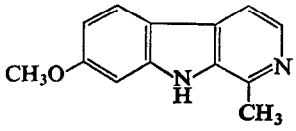
Abstract—Controlled isolation and purification methods have shown vasicinone and desoxyvasicinone to be natural products. Administration of precursors *in vivo* has led to the incorporation of ornithine, putrescine and to a lesser degree tryptophan into the alkaloid skeleton. The significance of these findings is discussed.

INTRODUCTION

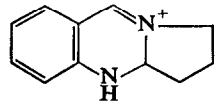
ALKALOIDS based on the quinazoline ring system have been isolated from members of six plant families.[1] In particular pyrroloquinazolines are present in *Adhatoda vasica* Nees, *Peganum harmala* L., several *Linaria* species[2] and in *Daemonorops draco*. The main alkaloid is vasicine (I), which in *P. harmala* is accompanied by vasicinone (II), desoxyvasicinone (IV) and harmine (V) based on the indole ring system.[3]



I: $R_1=H_2$; $R_2=OH$
II: $R_1=O$; $R_2=OH$
III: $R_1=H_2$; $R_2=H$
IV: $R_1=O$; $R_2=H$



(V)



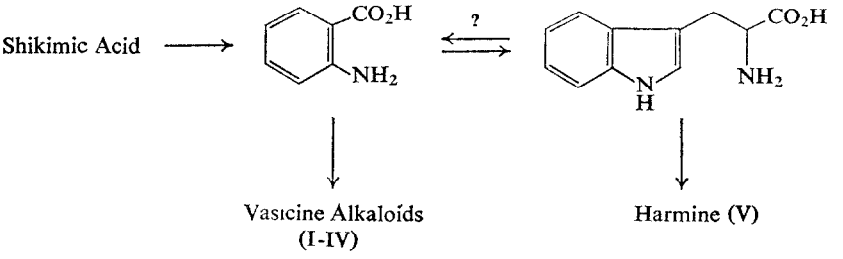
(VI)

Speculation on the biosynthesis of this group of compounds began when Robinson suggested[4] that vasicine may be derived from anthranilic acid and proline, or closely related metabolites. This possibility was strengthened when Schopf and Oechler[5] synthesized desoxyvasicine under "physiological conditions". The condensation of *o*-aminobenzaldehyde with γ-aminobutyraldehyde (Δ^1-pyrroline) at pH 5 yielded a quaternary salt (VI) that underwent double-bond migration under reducing conditions to give desoxyvasicine (III) and 9,10-dihydrodesoxyvasicine.

[1] H. G. BOIT, *Ergbnisse der Alkaloid Chemie*. Akademie Verlag, Berlin (1961).
[2] D. GROGER and S. JOHNE, *Planta Med.* **13**, 182 (1965).
[3] N. I. KORETSKAYA, *Zh. Obshch. Khim.* **27**, 3361 (1957), *Chem. Abs.* **52**, 9163 (1958).
[4] R. ROBINSON, *The Structural Relations of Natural Products*. Clarendon Press, Oxford (1955).
[5] C. SCHOPF and F. OECHLER, *Ann. Chem.* **523**, 1 (1936).

More recently tracer studies with *in vivo* systems have been reported. When anthranilic acid carboxyl-^{14}C was fed to *P. harmala* labelled vasicine was isolated.[6] A similar experiment with *A. vasica* also yielded labelled vasicine, and chemical degradation showed a specific incorporation since all the activity was localized at C-8 of the alkaloid.[7] In addition the administration of ^{14}C-labelled proline, putrescine and γ-hydroxyglutamic acid to *A. vasica* has led to the isolation of radioactive vasicine in each case.[8]

The coexistence in *P. harmala* of alkaloids derived from anthranilic acid (vasicine) and tryptophan (harmine) offers an opportunity to study the interrelationship that may exist between these compounds. There is no evidence for the catabolism of tryptophan to nicotinic acid in higher plants,[9] but degradation to anthranilic acid is a possibility, as shown in Scheme 1.



SCHEME 1. POSSIBLE INTERRELATIONSHIP OF VASICINE AND HARMINE ALKALOIDS.

This paper describes the results of an investigation on the biosynthesis of the vasicine group of alkaloids from tryptophan and ornithine, which are used in the plant to form the alkaloid skeleton.

RESULTS AND DISCUSSION

Isolation of Alkaloids

We have confirmed a previous finding[10] that vasicine in dilute benzene or chloroform solution is auto-oxidized to vasicinone. It was important therefore to establish that desoxyvasicinone and vasicinone were not merely degradation products resulting from the original isolation procedure.[3]

Skursky has observed[11] that desoxyvasicine is not oxidized if kept in acid medium. Further, it is shown in this paper that vasicine is stable in aqueous solution up to pH 8·3. Extraction of the alkaloids from *Peganum harmala* and chromatography under acid conditions designed to eliminate oxidation have confirmed the presence of desoxyvasicinone and vasicinone in the extract. The most satisfactory procedure for the isolation of alkaloids from crude mixtures involved partition on a Supercell–phosphoric acid column. Water-saturated chloroform eluted desoxyvasicinone and vasicinone in one fraction whilst vasicine and harmine were quantitatively retained. The latter two bases could then be removed with ammonia-saturated chloroform. Fraction one was further chromatographed on alumina, yielding pure desoxyvasicinone and vasicinone which were crystallized and/or sublimed to

[6] D. GROGER and K. MOTHES, *Arch. Pharm.* **293**, 1049 (1960).
[7] D. GROGER, S. JOHNE and K. MOTHES, *Experientia* **21**, 13 (1965).
[8] D. GROGER, S. JOHNE and K. MOTHES, *Abhandl. Deut. Akad. Wiss. Berlin Kl. Chem. Geol. Biol.* 581 (1966).
[9] E. LEETE, in *Biogenesis of Natural Compounds* (edited by P. BERNFELD). Pergamon Press (1963).
[10] D. R. MEHTA, J. S. NARAVANE and R. M. DESAI, *J. Org. Chem.* **28**, 445 (1963).
[11] L. SKURSKY, *Collection Czech. Chem. Commun.* **30**, 2080 (1965).

constant specific radioactivity. The mixture of vasicine and harmine was then oxidized and the resulting vasicinone separated from harmine on Supercell as before. Thus all specific activities of vasicine were measured after conversion to vasicinone.

Feeding of Labelled Precursors

Mature plants were fed sodium carbonate-^{14}C through excised stems to establish that alkaloid synthesis was occurring in the above-ground portions of the plant. After 3 hr the tracer appeared in the alkaloid fraction as shown in Fig. 1.

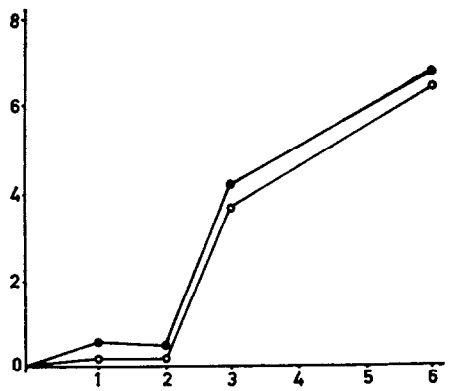


FIG. 1. SPECIFIC ACTIVITY OF ALKALOIDS IN PLANT TOPS AFTER FEEDING SODIUM CARBONATE-^{14}C TO EXCISED STEMS OF *P. harmala*

Ordinate: dpm/mM $\times 10^{-3}$.
Abscissa: time (hr).
Legend: ● = vasicine; ○ = vasicinone + desoxyvasicinone.

The following precursors were administered to intact plants to determine the origin of the skeletal atoms of the pyrroloquinazoline ring system. The feeding of tryptophan [benzene ring-^{14}C(U)] led to the isolation of radioactive products (Table 1). Incorporation of activity

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

Precursor	Activity fed (μc)	Specific activity (dpm/mM $\times 10^{-5}$) % incorporation in brackets			
		Vasicine	Vasicinone	Desoxy-vasicinone	Harmine
Tryptophan [benzene ring-^{14}C(U)] (roots)	100	0·62 (0·017)	0·06 (0·001)	0·07 (0·003)	2·13* (0·02)
		—	0·23 (0·0008)	—	1·00 (0·12)
Ornithine-2-^{14}C	100	10·9 (1·6)	8·38 (0·07)	7·90 (0·25)	—
Ornithine-5-^{14}C	100	8·39 (0·39)	1·92 (0·03)	10·3 (0·33)	—
Putrescine-1,4-^{14}C	100	14·4 (1·2)	9·98 (0·04)	69·1 (0·59)	—
Tritiated vasicine (roots)	103	1500 (56·4)	800 (6·6)	2·69 (0·05)	—
		298 (0·89)	143 (1·05)	—	0·78 (0·10)

* After addition of cold carrier.

Values refer to alkaloids from stems and leaves unless otherwise stated. Percentage incorporation (brackets) = 100 × total dpm isolated/total dpm fed.

into harmine was expected as tryptophan is a known precursor of this[12] and other indole alkaloids.[13] However, the only report of a non-indole alkaloid arising from tryptophan involves the biosynthesis of the furoquinoline skimmianine.[14] In this example as in the formation of the quinazoline alkaloids,[6,7] anthranilic acid has been shown to be a more immediate precursor as judged from a higher rate of incorporation. Apparently in *Skimmia japonica* and *P. harmala* there is an enzyme with similar action to tryptophan pyrrolase cleaving the 2,3-indolyl bond and leading to anthranilic acid as outlined in Scheme 1.

The second component of the biogenetic hypothesis proposed for the pyrroloquinazoline alkaloids was proline.[4] Ornithine and putrescine (VII) are metabolically closely linked to proline, and have been shown to act as precursors for the pyrrolidine ring of nicotine and hyoscyamine.[13] Ornithine-2-^{14}C, ornithine-5-^{14}C and putrescine-1,4-^{14}C were fed to *P. harmala*, and the alkaloids isolated showed in general a high rate of incorporation (Table 1). This agreed with the results of Groger *et al.*[8] who showed putrescine to be a precursor of vasicine in *Adhatoda vasica*. The differences in rates of incorporation in experiments involving ornithine-2-^{14}C and ornithine-5-^{14}C are not surprising, and are most probably due to differences between plants used. As shown in Table 2 the relative amounts of the three quinazoline alkaloids isolated from each feeding experiment varies markedly.

TABLE 2. VARIATION IN QUANTITY OF INDIVIDUAL ALKALOIDS FROM PRECURSOR FEEDING EXPERIMENTS

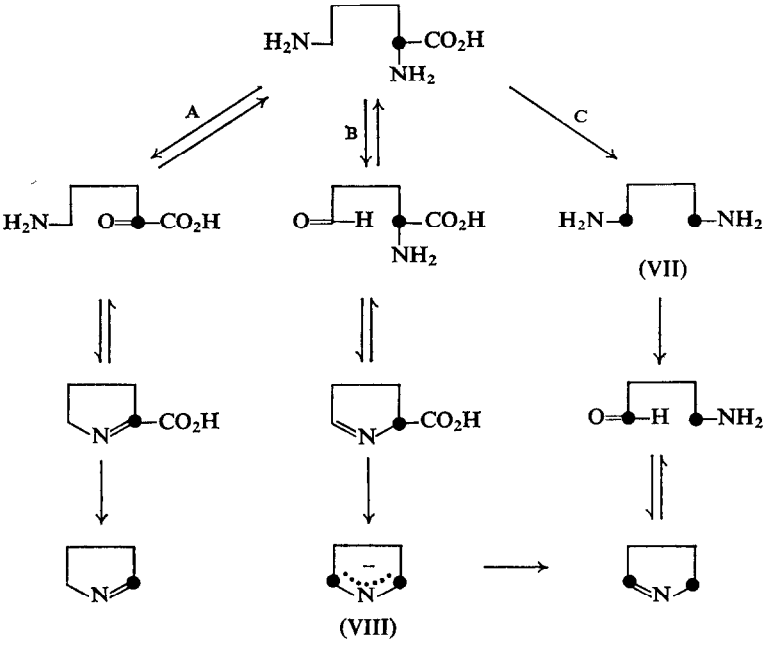
Precursor	Alkaloid isolated (mg)			
	Vasicine	Vasicinone	Desoxyvasicinone	Harmine
Tryptophan [benzene ring-^{14}C(U)]	117	76	157	47*
(roots)	—	15	—	567
Ornithine-2-^{14}C	603	36	129	—
Ornithine-5-^{14}C	193	59	132	—
Putrescine-1,4-^{14}C	344	19	35	—
Tritiated vasicine	162	38	79	—
(roots)	13	34	—	610

* 43 mg carrier harmine added.

There are several mechanisms by which ornithine may be incorporated into the vasicine alkaloids and some possible intermediates involved are shown in Scheme 2. Different pathways are followed during the biosynthesis of nicotine and hyoscyamine from this amino acid. Nicotine isolated from *Nicotiana tabacum* fed with ornithine-2-^{14}C has activity equally distributed between C-2 and C-5 of the pyrrolidine ring[15] which suggests that a symmetrical intermediate (e.g. VIII) is involved. However, the incorporation of ornithine-2-^{14}C into hyoscyamine is stereospecific[16] leading to retention of the label on one carbon atom of the alkaloid.

The pathway operating in *P. harmala* will be resolved finally by chemical degradation of the labelled alkaloids. The question is partly answered however by the results of experiments from the feeding of α- and δ-^{15}N-labelled ornithine (Table 3). This equal incorporation of

[12] D. GROGER and H. SIMON, *Abhandl. Deut. Akad. Wiss. Berlin, Kl. Chem. Geol Biol.* 343 (1963)
[13] E. LEETE, *Science* **147**, 1000 (1965).
[14] M. MATSUO and Y. KASIDA, *Chem. Pharm. Bull. (Tokyo)* **14**, 1108 (1966).
[15] E. LEETE, *J. Am. Chem. Soc.* **80**, 2162 (1958).
[16] E. LEETE, *J. Am. Chem. Soc.* **84**, 55 (1962); *Tetrahedron Letters* 1619 (1964).



SCHEME 2. POSSIBLE INTERMEDIATES IN THE METABOLISM OF ORNITHINE.

label can be explained in one of two ways. Firstly, it may be that in the plant there are enzymes that specifically oxidize ornithine at the α- and δ-positions (Scheme 2, A and B). If these and subsequent reactions occurred at the same rate, the observed equal incorporation of ^{15}N would result. A more likely interpretation is that the pathway from ornithine proceeds (Scheme 2, C) via the symmetrical putrescine (VII). No distinction between the amino groups would then be exhibited by amine oxidase during further modification of the molecule. This is supported by the similar rates of incorporation of ^{14}C-labelled ornithine and putrescine (Table 1).

TABLE 3. ENRICHMENT OF ALKALOIDS FOLLOWING FEEDING OF ^{15}N-LABELLED ORNITHINES TO *Peganum harmala*

Precursor*	Excess ^{15}N (at. %) in		
	Vasicine	Vasicinone	Desoxyvasicinone
Ornithine-α-^{15}N	0·23	0·10	1·21
Ornithine-δ-^{15}N	0·28	0·12	1·21

* Excess ^{15}N, at. % 97 for both compounds.

These results must be compared with those[8] from the feeding of γ-hydroxyglutamic acid-α-^{14}C to *A. vasica*. Groger *et al.* considered that introduction of the hydroxyl group (which ultimately appears at the 3-position of vasicine) may occur before construction of the pyrroloquinazoline system and not after as would be expected if ornithine and putrescine are

immediate precursors. Modification of γ-hydroxyglutamic acid leads to α-hydroxy-γ-aminobutyraldehyde, which has been shown[17] to condense with *o*-aminobenzaldehyde to give vasicine. The validity of this hypothesis has been partly shown[8] by the isolation of labelled vasicine after feeding of γ-hydroxyglutamic acid-α-^{14}C. However, similar incorporations have been obtained[8] from non-hydroxylated precursors proline and putrescine in the same system.

From these results and those reported in this paper it would seem that hydroxylation before or after completion of the vasicine ring system is possible. However, the feeding of desoxyvasicine-1,2,3,10-^{14}C to *A. vasica* led to a non-specific incorporation into vasicine.[8] This result suggests that γ-hydroxyglutamic acid is a more immediate precursor than putrescine in *A. vasica*. However, it is impossible to reconcile this information with the present results from ornithine-α- and δ-^{15}N feeding experiments. Although there is no known metabolic route from putrescine or ornithine to γ-hydroxyglutamic acid, any conversion would involve the loss of the δ-amino group and the observed equal incorporation of ^{15}N from the α- and δ-positions into vasicine would then not occur.

It is important therefore to examine any interconversions between the three quinazoline alkaloids in *P. harmala*. A preliminary result is reported (Table 1). Tritiated vasicine was prepared by heating the hydrochloride in tritiated water with a platinum catalyst, a method that favours exchange of the aromatic hydrogen atoms.[18] Administration of the purified product to the plants led to some conversion to vasicinone and desoxyvasicinone (Table 1). Breakdown of the ring system is apparent as only 65 per cent of the original activity was recovered, and it is significant that this includes some radioactive harmine. Further feeding experiments using labelled vasicinone and desoxyvasicinone are in progress.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage apparatus and u.v. absorption spectra in a Unicam SP800 spectrophotometer.

Source of Materials

Sodium carbonate-^{14}C, 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; DL-ornithine-5-^{14}C (9·8 mc/mM) from Commissariat à l'Energie Atomique, France, and DL-ornithine-2-^{14}C (3·27 mc/mM) from Baird Atomic Inc., U.S.A. Samples of DL-ornithine-α-^{15}N and δ-^{15}N were generously provided by Professors E. Leete and P. Boulanger.

Physical Constants of Alkaloids Isolated from Peganum harmala

Above-ground portions of *P. harmala* L. were collected in the Taylorville area of South Australia. Extraction and purification of the contained alkaloids was essentially by the method of Koretskaya.[3] Thus DL-vasicine had m.p. 197–199° (decomp.) and 210–212° (*in vacuo*), $[\alpha]_D^{18}$ 0° (c=1·0; $CHCl_3$); DL-vasicinone m.p. 211–212°, $[\alpha]_D^{18}$ 0° (c=0·5; $CHCl_3$); desoxyvasicinone m.p. 109–110° and harmine m.p. 256°. These samples were used as spectral and chromatographic standards during the isolation of radioactive alkaloids from feeding experiments.

U.v. spectra, determined in 95 per cent ethanol, were as follows: Vasicine λ_{max} 302 nm, log ε 3·90; vasicinone λ_{max} 268, 302, 314 nm, log ε 3·90, 3·59, 3·52, λ_{min} 295, 309 nm, log ε 3·48, 3·45; desoxyvasicinone λ_{max} 266, 271 (sh) 303, 315 nm, log ε 3·84, 3·81, 3·52, 3·45, λ_{min} 286, 310 nm, log ε 3·32, 3·40; harmine λ_{max} 240, 300, 325 (sh) nm, log ε 4·68, 4·26, 3·76, λ_{min} 271 nm, log ε 3·45.

Paper chromatography was carried out on Whatman No. 1 chromatography paper using the solvents *n*-butanol saturated with 5 per cent acetic acid (upper phase) (system 1) and ethanol–0 880 ammonia (99:1)

[17] N. J. LEONARD and M. J. MARTELL, *Tetrahedron Letters* 44 (1960).
[18] E. A. EVANS, *Tritium and its Compounds*, p. 275. Butterworth, London (1966).

(system 2). Sample areas were detected by taking contact photographs under u.v. irradiation.[19] The following R_f values were found (system 1, system 2): vasicine 0·40, 0·62; vasicinone 0·73, 0·57; desoxyvasicinone 0·85, 0·69; harmine 0·54, 0·74.

Administration of Labelled Compounds to Peganum harmala *L. Plants*

Attempts to raise suitable plants from seed proved unsatisfactory. Established plants were collected whilst dormant in the winter months, planted in large pots and grown in the open. Feeding experiments were performed 12–15 weeks after new spring growth, and labelled compounds administered by the wick-feeding method. Two plants were used in each experiment. A double strand of unmercerized cotton was threaded through each stem of the plant and the ends dipped into solutions of the labelled compound contained in glass vials. After the initial absorption, the vials were repeatedly refilled with distilled water. At the end of a growing period of 5–6 days, the above-ground portions were harvested and the cotton wicks and vials washed with water and residual activity determined. In no case did this exceed 1 per cent of that administered.

Isolation of Alkaloids

The stems and leaves or roots were finely ground with methanol in a mortar and soaked in fresh portions of solvent until the solution was free from alkaloid. In a representative experiment 70 g wet weight of plant material was extracted over 24 hr with 1 l. of methanol. The solvent was removed under reduced pressure and the green residue dissolved in 5 per cent acetic acid (100 ml). After extraction with ether (6 × 100 ml) the solution was basified (NH_3) and extracted with $CHCl_3$ (6 × 100 ml). To minimize oxidation of vasicine to vasicinone, each $CHCl_3$ extract was evaporated to dryness immediately after separation, yielding a total of 0·401 g mixed alkaloids.

Control extractions of pure vasicine from acetic acid solutions by this method showed that less than 2 per cent was oxidized to vasicinone.

Isolation of individual alkaloids from the mixture was achieved by combination of the methods below, and yields are shown in Table 2.

Separation of Mixed Alkaloids

(*a*) *Stability of vasicine in aqueous solution.* Vasicine (5 mg) was dissolved in 0·2 M sodium acetate (5 ml), pH 6·0, and 0·05 M tris-acetate (5 ml), pH 8·3. A stream of air was passed through each solution illuminated by a 500 W lamp. Aliquots were removed at intervals over 5 days, but no change was apparent in the u.v. spectra, nor was any vasicinone detected by paper chromatography.

(*b*) *Chromatography on Dowex* 50; *identification of vasicinone as an alkaloid.* Dried above-ground portions of *P. harmala* (30 g) were extracted as above and the methanol solution evaporated to dryness. The residue was dissolved in HCl (0·1 N, 90 ml), extracted with ether, and introduced to the top of a column of Dowex 50 (H^+) (1 × 10 cm column). After washing with 0·1 N HCl (200 ml) elution was continued with 800 ml of a linear gradient from water to 2 N HCl, 10-ml fractions being collected. U.v. spectra and paper chromatography against authentic compounds showed fractions 48–56 to contain a mixture of vasicinone and desoxyvasicinone and 61–72 to contain vasicine.

(*c*) *Chromatography on cellulose.* A sample of crude mixed alkaloids (124 mg) dissolved in 10 ml *n*-butanol saturated with 5 per cent acetic acid was added to the top of a 19 × 3·5 cm column of Whatman standard grade cellulose powder packed in *n*-butanol saturated with 5 per cent acetic acid. Elution was continued with this solvent and 5-ml fractions taken. Fractions 21–25 contained 61 mg of a mixture of vasicinone and desoxyvasicinone; fractions 27–33 contained 53 mg of vasicine. A suitable control showed that no auto-oxidation of vasicine occurred in this solvent system.

(*d*) *Chromatography on alumina.* A solution of mixed alkaloids (0·116 g) containing added pure harmine (10·8 mg) in the minimum amount of 1:1 benzene–$CHCl_3$ was added to a 30 × 2 cm alumina column (Woelm neutral, activity 1) packed in 1:1 benzene–$CHCl_3$. The column was eluted with the following solvents, and 15-ml fractions taken: 1:1 benzene–$CHCl_3$ (500 ml), $CHCl_3$ (250 ml), 5 per cent methanol in $CHCl_3$ (250 ml), 10 per cent methanol in $CHCl_3$ (300 ml). Fractions 17–19 contained desoxyvasicinone (36·6 mg), 39–47 harmine (11·8 mg), 48–57 vasicinone with a trace of harmine (7·6 mg), 58–72 vasicinone (17·5 mg), 73–75 a mixture of vasicinone and vasicine (5 mg) and 76–84 vasicine (21·9 mg).

(*e*) *Separation of vasicinone from harmine.* A column of Supercell (1 g) mixed with 10 per cent phosphoric acid (0·5 ml) was packed in water-saturated $CHCl_3$ (Cf. Ref. 10). A mixture of vasicinone (4 mg) and harmine (3 mg) was applied and the column eluted with the same solvent, taking 5-ml fractions. All the vasicinone appeared in fractions 2 and 3. After a further three fractions $CHCl_3$ saturated with 10 per cent ammonia was applied and harmine was eluted in fraction 9.

[19] R. J. Block, E. L. Durrum and G. Zweig, *A Manual of Paper Chromatography and Paper Electrophoresis*, p. 66. Academic Press, New York (1958).

Feeding of Sodium Carbonate-^{14}C

Four stems were cut from one plant and the ends dipped into a vial containing sodium carbonate-^{14}C (100 μc) in water (2 ml). Most of this was adsorbed in 1 hr and distilled water was added at times to maintain the volume at *ca.* 2 ml. Stems were removed after 1, 2, 3 and 6 hr, ground to a fine powder with liquid N_2, and extracted with boiling methanol (3 × 30 ml). The methanol was evaporated under reduced pressure and the mixed alkaloids isolated in the usual way. Vasicine was separated from vasicinone and desoxyvasicinone in each case by chromatography on cellulose and allowed to auto-oxidize to vasicinone. Individual samples were then sublimed, chromatographed on Dowex 50, resublimed and dissolved in 95 per cent ethanol. Alkaloid concentrations were determined by u.v. absorption and aliquots plated and radioactivity determined.

TABLE 4. ALKALOIDS FROM SODIUM CARBONATE-^{14}C FEEDING TO EXCISED STEMS OF *P. harmala*

Time of sampling (hr)	Mixed alkaloids (mg)	Alkaloid mg/g stem wet wt.	Vasicine (mg)	Vasicinone + desoxyvasicinone (mg)
1	21	3·5	9·9	3·8
2	18	3·0	6·8	4·5
3	21	3·1	10·6	3·8
6	24	3·3	10·6	5·3

Feeding of ^{15}N-Labelled Ornithine

In two separate experiments ornithine-α-^{15}N and ornithine-δ-^{15}N (40 mg) were administered to two plants. After a growing period of 6 days the above-ground portions were harvested, and the quinazoline alkaloids extracted and purified. Enrichment with ^{15}N (Table 3) was determined by oxidation to nitrogen by the semi-micro Kjeldahl technique and analysis in an A.E.I. MS2 mass spectrometer.

Tritiation of Vasicine

Pt_2O (25 mg) and vasicine (99 mg) were mixed in a tube with tritiated H_2O (0·5 ml, 0·2 c/ml) and 3 drops of conc. HCl. The catalyst was reduced with H_2, the tube flushed with N_2 and sealed. After heating at 120° for 40 hr, the tube was opened, the mixture filtered and the residue washed with dil. HCl. The solvent was removed from the filtrate by freeze drying and the residue chromatographed on cellulose. Tritiated vasicine so obtained was not contaminated with vasicinone and had specific activity 1·54 mc/mM. It was stored as the HCl-ide to prevent oxidation.

A sample of the product with specific activity $3{\cdot}25 \times 10^9$ dpm/mM was oxidized to vasicinone by H_2O_2 in acetone, using the method of Mehta *et al.*[10] The vasicinone isolated after chromatography on Supercell–phosphoric acid had specific activity $2{\cdot}56 \times 10^9$ dpm/mM.

Assay of Radioactivity

Aliquots from solutions in 95 per cent ethanol were plated and dried on 2-in. stainless-steel planchets. Radioactivity was determined on a Beckman Lowbeta II gas flow counter with a background of 2 counts/min and 23 per cent efficiency for ^{14}C. Samples containing tritium were dissolved in 95 per cent ethanol (2 ml) and added to a dioxan scintillation fluid containing naphthalene (100 g), 2,5-diphenyloxazole (PPO, 10 g) and 1,4-bis-2(5-phenyloxazolyl)benzene (POPOP, 0·25 g) per l. Radioactivity was assayed on a Packard Tricarb liquid scintillation spectrometer model 3375.

Acknowledgements—I am indebted to Professors E. Leete and P. Boulanger for generous gifts of α- and δ-^{15}N-ornithine, to Dr. S. Siddiqui for alkaloid samples and to Mr. D. E. Symon for positive identification of plant material. Mr. C. J. Weyland rendered able technical assistance and Mr. D. H. Hein performed the ^{15}N determinations.