

INDOLE METABOLISM IN *PIPTADENIA PEREGRINA*

LINDA E. FELLOWS* and E. A. BELL†

Department of Biochemistry, King's College, Strand, London, W.C. 2

(Received 30 September 1970)

Abstract—An unusual enzymatic reaction: the direct hydroxylation of tryptamine to 5-hydroxytryptamine (5-HT) has been shown to occur in tissues of *Piptadenia peregrina*. 5-HT, but not *N,N*-dimethyltryptamine, was found to be a good precursor of bufotenine and it is suggested that the intermediates in the synthesis of bufotenine from tryptophan in this plant are tryptamine, 5-HT and *N*-methyl-5-HT. The presence of a number of unidentified indoles in the dormant and germinating seeds is reported.

INTRODUCTION

INDOLEAMINES occur in several families of higher plants including the Gramineae,¹ Rutaceae,² Aceraceae² and Leguminosae³ and are also found in toxic fungi belonging to the genera *Paneolus*⁴ and *Amanita*.⁵ There is no common pattern of indole metabolism in these plants however and different species may show marked differences in the types of compound which they synthesize. L-5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), and 5-hydroxyindole-3-acetic acid (5OH-IAA) have been identified in species of *Paneolus*⁶ indicating that one major pathway of tryptophan metabolism in this genus may be the tryptophan, 5-HT and 5OH-IAA route which is well-known in mammalian biochemistry.⁷ In contrast, species of *Amanita* are reported⁵ to contain 5-HT, *N*-methyl-5-hydroxytryptamine (NM-5HT), *N,N*-dimethyl-5-hydroxytryptamine (bufotenine), bufotenine *N*-oxide, *N,N*-dimethyltryptamine (DMT), and 5-methoxy-*N,N*-dimethyltryptamine suggesting a pattern of indole metabolism more like that of the toad *Bufo vulgaris*⁸ than of mammals or *Paneolus*. Wide variations in the concentrations of indoleamines in plants have also been reported; the fruit pulp of *Musa sapientum* (banana) for example contains 28 µg/g of 5-HT⁹ while leaves of *Griffonia simplicifolia* at certain stages of the plant's life cycle accumulate as much as 250 µg/g fr. wt. of the same compound.¹⁰

Another legume which is particularly rich in indoleamines is *Piptadenia peregrina*. The seeds of this tree, which are used by Indian tribes of the Caribbean islands and South America to make an intoxicating and hallucinogenic snuff,¹¹ were shown by Stromberg¹² to

* Present address: Department of Biochemistry, Imperial College, London.

† Present address: Department of Botany, The University of Texas at Austin, Texas 78712, U.S.A.

¹ S. WILKINS, *J. Chem. Soc.* 2079 (1958).

² I. J. PATCHER, D. E. ZACHARIAS and O. RIBEIRO, *J. Org. Chem.* **24**, 1285 (1959).

³ S. GHOSAL and B. MUKHERJEE, *Chem. & Ind.* **48**, 1800 (1964).

⁴ V. E. TYLER, *Science*, **128**, 718 (1958).

⁵ V. E. TYLER and D. GROEGER, *Planta Med.* **12**, 334 (1964). *ibid.* **12**, 397 (1964).

⁶ V. E. TYLER and A. H. SMITH, *Abhandl. Dent. Akad. Vis. Berlin*, p. 45 (1963).

⁷ S. UDENFRIEND, H. WEISSBACH and D. F. BOGDANSKI, *J. Biol. Chem.* **224**, 803 (1957).

⁸ H. WIELAND, W. KONZ and H. MITTASCH, *Annalen* **513**, 1 (1934).

⁹ T. P. WAALKES, A. SJOERDSMA, C. R. GROVELING, H. WEISSBACH and S. UDENFRIEND, *Science* **127**, 648 (1958).

¹⁰ L. E. FELLOWS and E. A. BELL, *Phytochem.* **9**, 2389 (1970).

¹¹ J. M. COOPER, in *Handbook of South American Indians*, U.S. Bureau of American Ethnology Bulletin 143 (edited by J. H. STEWARD), Vol. 5, p. 536 (1949).

¹² V. L. STROMBERG, *J. Am. Chem. Soc.* **76**, 1707 (1954).

contain bufotenine. Fish *et al.*¹³ found that *P. peregrina* and *P. macrocarpa* also contained the *N*-oxides of both bufotenine and DMT in their seeds and free DMT in their pods; while Iacobucci and Ruveda¹⁴ reported the presence of tryptamine bases in a third species (*P. excelsus*) and their absence from *P. rigida*, *P. vidiflora* and *P. paraguayensis*.

The presence of DMT in *P. peregrina* led to the proposal¹³ that bufotenine might be formed in this plant by the peroxidic oxidation of DMT. No experimental evidence for such an oxidation was given however.

While studying the distribution of indoles in a variety of plants we observed that chromatograms prepared from seed extracts of *P. peregrina* showed high concentrations of an Ehrlich-reacting compound with *R_f* values corresponding to those of commercially obtained bufotenine, but the same chromatograms showed no 'spots' corresponding to DMT or to the *N*-oxides of DMT and bufotenine prepared by the method of Fish *et al.*¹³ It seemed possible that the concentrations of these compounds were too low to be detected on the chromatograms, but repeated analyses using partially purified and highly concentrated extracts confirmed the original finding. These experiments with concentrated solutions led however to the detection of no fewer than 12 other Ehrlich-reacting compounds which occur together with high concentrations of bufotenine either in the dormant seeds or in seeds which had been allowed to germinate for 3 days in the dark.

The difference in indole content between the seeds used in the present investigations and those studied by Fish *et al.*¹³ may be due either to environmental factors or to genetic changes which have led to the establishment of distinct 'chemical races' of this species. Whichever of these explanations is correct, the discovery of bufotenine-rich plants which did not contain complimentary concentrations of DMT cast doubt on the hypothesis that DMT is the precursor of bufotenine.

In the present paper we describe a series of experiments in which possible precursors of bufotenine, labelled with ¹⁴C or ³H, were incubated with tissues of *P. peregrina* and the incorporation of radioactivity from these compounds into bufotenine determined. We have also recorded the *R_f* values, ionic mobilities and colour reactions of the more important unidentified indoles accompanying bufotenine in the seeds.

RESULTS

Indoles in Seeds

Indoles in dormant seeds and in seeds which had been germinated for 3 days in the dark were extracted with methanol and partially resolved by elution from columns of alumina with increasingly dilute solutions of ethanol. The fractions of eluate were concentrated and analysed by paper chromatography and high-voltage electrophoresis.

Bufotenine was the major indole found in both the dormant and germinated seeds. Lower concentrations of nine other Ehrlich-reacting compounds were detected in the dormant seeds, and three of these gave a sky-blue colour with the reagent rather than the violet given by most indole derivatives. The *R_f*s and ionic mobilities of these minor components did not correspond with those of any known indole including DMT and the *N*-oxides of DMT and bufotenine which were used as 'markers' for chromatography and electrophoresis.

After 3 days of germination three additional Ehrlich-reacting compounds were found

¹³ M. S. FISH, N. M. JOHNSON and E. C. HORNING, *J. Am. Chem. Soc.* **77**, 5892 (1955).

¹⁴ G. A. IACOBUCCI and E. A. RUVEDA, *Phytochem.* **3**, 465 (1964).

in the extracts while one of the minor components present in the dormant seeds was no longer detectable.

The composition of the fractions eluted from the alumina columns and the R_f s, ionic mobilities and colour reactions of individual Ehrlich-reacting compounds which have been designated by letters are given in Figs. 1 and 2 and Tables 1–3. Lack of seed material

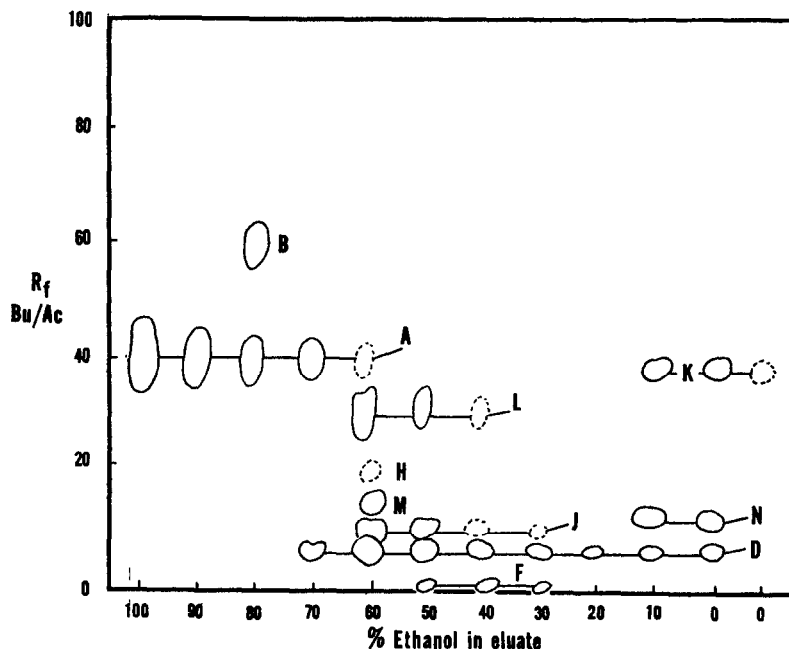


FIG. 1. A DIAGRAMMATIC REPRESENTATION OF THE SEPARATION OF EHRlich-REACTING COMPOUNDS FROM THE SEEDS OF *P. peregrina* WHICH WAS ACHIEVED BY ELUTION FROM AN ALUMINA COLUMN WITH INCREASINGLY DILUTE ETHANOL FOLLOWED BY CHROMATOGRAPHY ON PAPER USING BUTANOL ACETIC ACID AS SOLVENT

A is bufotenine. The remaining compounds designated by letters are 'unknowns'.

TABLE 1. R_f s OF PRINCIPAL INDOLES OF *Piptadenia peregrina*

Indoles	$R_f (\times 100)$ in solvent systems*				
	1	2	3	4	5
A	43	83	53	100	100
B	63	38	63	27	53
C	10	16	30	100	100
D	6.5	40	29	53	79
J	11	62	34	76	87
K	40	13	55	19	51
L	28	60	48	100	100

* Details of solvents given in Experimental. A is bufotenine, B–L are unidentified Ehrlich-reacting compounds.

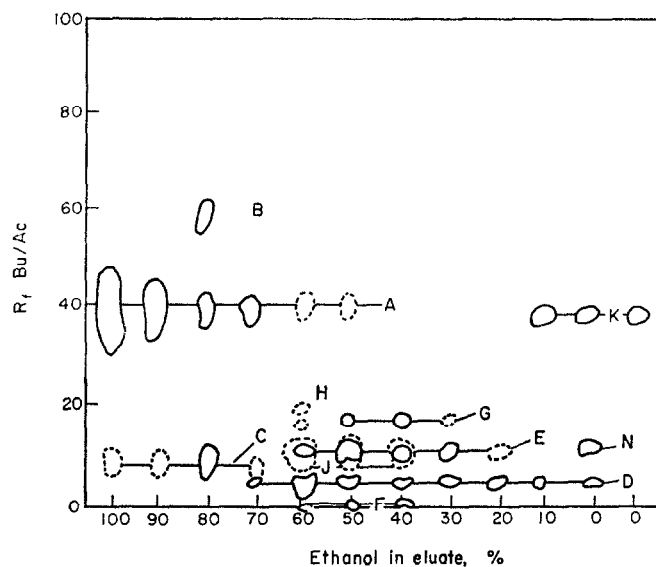


FIG. 2. A DIAGRAMMATIC REPRESENTATION OF THE SEPARATION OF EHRlich-REACTING COMPOU FROM GERMINATING SEEDS OF *P. peregrina* (METHODS AS IN FIG. 1). A is bufotenine. The remaining compounds designated by letters are 'unknowns'.

TABLE 2. IONIC MOBILITY* OF PRINCIPAL INDOLES OF *Piptadenia peregrina* SEEDS AT pH 1.9

A	B	C	C	J	K	L
0.99	1.08	1.0	0.56	0.84	0.6	0.9

* The mobility of 5-HT at this pH taken as unity. A is bufotenine, B-L are unidentified Ehrlich-reacting compounds.

TABLE 3. COLOUR REACTIONS OF INDOLES IN THE SEEDS OF *Piptadenia peregrina*

Indole	Ehrlich reagent	Potassium persulphate	Pauly reagent
A	Blue	n.r.	Wine red
B	Pink-mauve to violet	n.r.	n.r.
C	Blue-grey (fades)	n.r.	n.r.
D	Sky blue	Sky Blue (slow)	n.r.
E	Sky blue	Sky Blue (slow)	n.r.
F	Sky blue	n.r.	n.r.
G	Grey (fades)	n.r.	n.r.
H	Blue	n.r.	n.r.
J	Purple-blue grey	n.r.	n.r.
K	Pinkish-purple	n.r.	n.r.
L	Immediate	n.r.	n.r.

Key: n.r. = no reaction; A is bufotenine, B-L are unidentified compounds. None of the compounds reacted with ninhydrin.

prevented characterization of the minor components but it was possible to elute sufficient of D and J (the two unknowns occurring in highest concentration) from chromatograms to show that their UV and fluorescence spectra were characteristically those of indoles. When heated in acid solution both these compounds were destroyed; when heated in alkaline solutions under an atmosphere of nitrogen J was unaffected, while D was converted to a compound which could not be distinguished chromatographically from L.

Indoles in Seedlings

Seedlings were germinated and grown in the dark. For 7 successive days following germination shoots were removed from representative seedlings, pooled, and analysed for Ehrlich-reacting compounds in the same manner as the seeds. During the first 3 days of growth bufotenine was the only compound detected. Subsequently however 5-HT, NM-5-HT and, after 7 days, tryptophan itself were found in the extracts (Table 4).

TABLE 4. APPEARANCE OF INDOLES IN SEEDLINGS
Piptadenia peregrina DURING FIRST WEEK OF
GERMINATION

Day	1	2	3	4	5	6	7
Bufotenine	+	++	++	++	++	++	++
5-HT	.	.	.	+	++	++	++
NM-5HT	.	.	.	.	+	+	++
Try	.	.	.	.	.	.	+

+ = positive reaction with Ehrlich's reagent.

++ = strong reaction with Ehrlich's reagent.

Metabolism of Possible Bufotenine Precursors (Labelled)

In a series of experiments, radioactively labelled L-tryptophan D-tryptophan, 5-HT, L-5-HTP, D-5-HTP, tryptamine and DMT were incubated at 25° for 15 hr with etiolated stem tissue under uniform conditions. After this period, 80 per cent of the label in 5-HT had been incorporated into bufotenine and the remaining 20 per cent was present as unmetabolized 5-HT. Approximately 20 per cent of the label from both L- and D-tryptophan was incorporated into bufotenine and somewhat less into an unidentified indole which ran behind tryptophan in butanol-acetic acid but was distinct from 5-HT. An interesting difference in the metabolism of the two isomers was apparent in the location of the remaining label. In the experiment using L-tryptophan 95 per cent of this was found in the original unmetabolized amino acid and 5 per cent in a third indole identified (by co-chromatography and electrophoresis) as indole-3-acetylserpic acid (IAAA). In the experiment using labelled D-tryptophan the situation was reversed, the great majority of the remaining label was in the IAAA and only a trace in the D-tryptophan. When labelled L-tryptophan was incubated under the same conditions but for varying periods of time (1.5–22 hr) no accumulation of the label in possible intermediates (5-HT, 5-HTP nor tryptamine) was detected. Incorporation of label into the slow moving unidentified indole detected in the previous experiments appeared to parallel incorporation into bufotenine however.

Label from both the L- and D-isomers of 5-HTP was incorporated primarily into 5-HT and to a lesser extent into bufotenine and a further unidentified metabolite with a very low

R_f in butanol-acetic acid. Although the pattern of incorporation was similar, the L-isomer was metabolized faster than the D-isomer. The incorporation into bufotenine from both L- and D-5-HTP was less than from tryptophan or tryptamine.

Incubation with U- ^3H -tryptamine led to the labelling of 5-HT, NMT and bufotenine, no label was detected in DMT, 5-HTP, IAA or IAAA. Tritiated DMT was not metabolized by the stem segments.

Metabolism of Possible Bufotenine Precursors (Unlabelled)

When higher concentrations (10^{-3} M) of unlabelled tryptophan and 5-HTP were incubated with etiolated stem segments no changes in the indole content of the stem material could be detected on chromatograms or electrophoresis papers. Incubation with the same concentration of 5-HT however produced elevated levels of NM-5-HT. When 10^{-3} M tryptamine was used in the experiments an additional indole not previously found in stem material was detected. This compound was identified (by co-chromatography and electrophoresis) as *N*-methyl tryptamine (NMT).

DISCUSSION

Bufotenine is the principal indole found in the seeds of *P. peregrina*. Earlier workers¹³ also found the seeds to contain *N,N*-dimethyltryptamine (DMT) and the *N*-oxides of both bases but we have been unable to detect these three compounds in the seeds which we have analysed. We did however find other Ehrlich-reacting compounds which have not yet been identified. The reason for this difference (whether genetic or environmental) between the earlier findings and our own remains to be determined. The absence of the unidentified indoles from the vegetative parts of the plant suggests that these compounds may be conjugated forms of bufotenine and/or its precursors and derivatives which only accumulate in the storage tissues of the seed and are metabolized on germination.

During the first 3 days of germination the shoots of etiolated seedlings were found to contain only bufotenine, subsequently however 5-HT, NM-5-HT and tryptophan were detected in increasing concentrations.

When radioactively labelled possible precursors of bufotenine were incubated with tissue taken from etiolated stems it was found that incorporation of label into bufotenine was most rapid and complete (80% in 15 hr) from ^{14}C -5-HT. Complimentary experiments using higher concentrations of unlabelled 5-HT led to a rise in the level of NM-5-HT in the tissue. This finding is consistent with the observation that 5-HT and NM-5-HT were present in seedlings after 5 days of germination and with the following representation of the final stages of bufotenine synthesis: 5-HT $\rightarrow$ NM-5-HT $\rightarrow$ bufotenine. Theoretically tryptophan can be metabolized to bufotenine by four different routes. These are represented in their simplest possible forms in Fig. 3.

The ready incorporation of label from tryptamine into 5-HT, NMT and bufotenine and the finding that incorporation of label into bufotenine from 5-HTP is slower than from

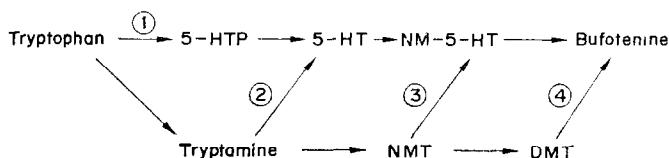


FIG. 3. FOUR ROUTES BY WHICH TRYPTOPHAN COULD BE METABOLIZED TO BUFOTENINE.

tryptophan suggested that routes ② and ③ were the most likely. The accumulation of NMT but not NM-5-HT in tissues incubated with unlabelled tryptamine and the total lack of incorporation from DMT all suggest that the principal pathway of bufotenine synthesis in this plant is route ②.

The formation of 5-HT by the direct oxidation of tryptamine is unexpected, as 5-HT is formed in mammals and other plants such as *Griffonia simplicifolia* by the decarboxylation of 5-HTP. This finding indicates that the 5-hydroxylase system of *P. peregrina* is very different in enzyme specificity from those of mammalian brain and *G. simplicifolia* neither of which is able to use tryptamine as a substrate.¹⁰ The incorporation of label from 5-HTP into bufotenine suggests that an enzyme system capable of this decarboxylation also occurs in *P. peregrina*. The relatively low rate of incorporation from 5-HTP when compared with the rate of incorporation from L-tryptophan itself suggests that 5-HTP is not the normal precursor of bufotenine in this plant and indeed the decarboxylation of 5-HTP may merely reflect a lack of absolute specificity in the decarboxylase for which L-tryptophan is the normal substrate.

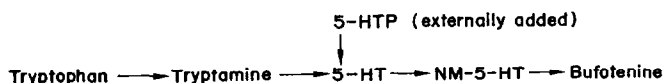


FIG. 4. POSSIBLE ROUTE OF BUFOTENINE BIOSYNTHESIS IN *P. peregrina*

The high concentration of bufotenine in both seeds and vegetative parts of *P. peregrina* suggests that it plays a significant role in the plant's economy. While this role may not be central to the plant's basic biochemistry, it may nevertheless be vital to the plant's survival providing perhaps, protection from animal or insect predators in a manner which has been discussed recently in connection with the accumulation of physiologically active amino acids in legumes.¹⁵

EXPERIMENTAL

Plant Material

Seeds of *P. peregrina* were kindly supplied by Dr. M. H. Gaskins of the U.S.D.A. Agricultural Research Division in Puerto Rico.

Paper Chromatography

One dimensional chromatograms, ascending and descending, were prepared on Whatman No. 1 or 3 mm paper. Extracts were spotted on the papers and dried in a stream of cold air. Papers were equilibrated for 2 hr in an atmosphere saturated with the appropriate solvent before running. Solvents used^{16,17} were: (1) *n*-BuOH-HOAc-H₂O, 12:3:5, (2) *iso*-PrOH-NH₄OH (0.88 spec. gr.-H₂O, 20:1:2, (3) *t*-BuOH-HCO₂H-H₂O, 207:6:86, (4) C₆H₆-EtOH-MeNH₂ (30%, v/v); 22:7:1, (5) *n*-BuOH-MeNH₂ (30%, v/v); 80:30, all by volume. Two dimensional chromatograms were run in solvents 1 and 2. When indoles were to be eluted from the papers for spectroscopic analysis all solvents were redistilled before use and the papers washed with de-ionized water.

High-voltage Electrophoresis

Electrophoresis was carried out on Whatman 3 mm paper using a Shandon flat-plate watercooled apparatus and a potential difference of 60 V/cm for a period of 30 min. Buffer solutions of pH 1.9, 3.6, 6.5,¹⁸ and 12.0¹⁹ were used.

¹⁵ E. A. BELL and D. H. JANZEN, *Nature* **229**, 136 (1971).

¹⁶ I. SMITH, *Chromatographic and Electrophoretic Techniques*, Vol. 1, p. 185, Heinemann, London (1960).

¹⁷ B. B. STOWE and K. V. THIMANN, *Arch. Biochem.* **51**, 499 (1954).

¹⁸ E. A. BELL and A. S. L. TIRIMANNA, *Biochem. J.* **97**, 104 (1965).

¹⁹ D. G. GRAHAME-SMITH, *Biochim. Biophys. Acta* **86**, 176 (1964).

Preparation and Chromatography of Seed Extracts

Dormant seeds of *P. peregrina* (5.0 g) were ground with 20 ml of light petroleum (b.p. 40–60°). The suspension of seed was filtered and washed with a further 20 ml of petroleum. The defatted seed was then extracted with methanol (2×50 ml) and the combined extracts concentrated to 4 ml in a rotary evaporator at 25°.

The concentrated extract was then applied to a column (20×1.7 cm) of alumina and the Ehrlich-reacting compounds eluted by passing successive 200 ml volumes of 100, 90, 80, 70, 60, 50, 40, 30, 20 and 10% ethanol followed by 400 ml of water through the column. The eluate was collected in 200 ml fractions each of which was taken to dryness at 25° under reduced pressure. Each residue was redissolved in methanol (0.5 ml) and aliquots (50 μ l) were chromatographed on paper using butanol-acetic acid as solvent. After drying, the papers were developed with Ehrlich's reagent (Fig. 1).

The same procedure was applied to seed cotyledons which were excised from seeds which had been germinated in the dark for 3 days (Fig. 2).

Further aliquots of these concentrated eluates were used for chromatography in other solvent systems and for electrophoresis. The R_f s and ionic mobilities at pH 1.9 of the indoles which occurred in more than trace amounts are given in Tables 1 and 2

Location Reagents

The following reagents, prepared according to the methods of Smith,¹⁵ were used for the location and identification of indoleamino acids, indoleamines and their derivatives on chromatograms and electrophoresis papers: (1) Ninhydrin for amino acids, (2) Ninhydrin acidified with acetic acid for unsubstituted tryptamines, (3) Ehrlich's reagent for indoles, (4) Pauly reagent for 4, 5 and 6-hydroxyindoles, (5) Potassium persulphate for IAA and compounds giving IAA on hydrolysis and for indolylacetyl derivatives of amino acids.

Elution of Indoles from Chromatograms

The area of paper containing the indole was cut out, cut up into small pieces, covered with methanol (redistilled from ferrous sulphate²⁰) and allowed to stand for 2 hr in the dark at 4°. The paper was then removed by filtration, washed with redistilled methanol and the combined filtrate and washings used for spectroscopic analyses.

Fluorimetry

Fluorimetric determinations were made with an Aminco-Bowman fluorimeter.

Determination of Radioactivity

Autoradiographs were prepared on Kodirex X-ray film (Kodak Ltd.). Chromatograms were scanned (after fixing to T.L. plates with adhesive tape) in a Panax Thin-Layer Scanner RTLS.

Metabolism of 3^{14}C -L-Tryptophan and 3^{14}C -D-Tryptophan and 2^{14}C -5-HT

Shoots of etiolated seedlings were sliced vertically and then horizontally into sections 0.5 cm long. Approximately 0.5 g (wet wt.) of pooled sections were added to separate beakers containing respectively 2 μ c of 3^{14}C -L-tryptophan, 2 μ c of 3^{14}C -D-tryptophan and 2 μ c of 2^{14}C -5-HT, each solution having been adjusted to a concentration of 4×10^{-5} M with the appropriate unlabelled compound, and protected against contamination by the addition of penicillin G (10 units/ml) and streptomycin sulphate (100 μ g/ml); antibiotics which did not interfere with the enzymic reactions under investigation. Duplicate preparations using boiled stem material served as controls. The solutions were covered and incubated for 15 hr at 25° in the dark. The stem segments were then removed, rinsed with water and homogenized in methanol (1 ml). After 2 hr at 4° the homogenates were filtered and aliquots (50 μ l) of the filtrates used for the preparation of chromatograms and autoradiograms

Metabolism of 3^{14}C -L-5-HTP and 3^{14}C -D-5-HTP

The isomers of 3^{14}C -DL-5-HTP were separated by paper chromatography using MeOH-pyridine-H₂O, (20:5:1, by vol.) as solvent²¹ and 2 μ c of each were incubated with etiolated stem material as described in the previous experiments and autoradiographs prepared.

Metabolism of U- ^3H -Tryptamine

A solution (2 ml) containing 20 μ c of U- ^3H -tryptamine was incubated with etiolated stem segments as before. Aliquots of filtrate from the homogenized stem material were chromatographed on paper together with suitable 'markers' using butanol-acetic acid as solvent. After drying the markers were developed with

²⁰ D. BURNETT and L. J. AUDUS, *Phytochem.* **3**, 395 (1964).

²¹ E. A. BELL and L. E. FELLOWS, *Nature* **210**, 529 (1966).

Ehrlich's reagent and the chromatograms of stem extract scanned for radioactive components. Three radioactive zones were detected on the chromatogram and these corresponded in position to 5-HT, bufotenine and a diffuse spot on the marker strip (caused by the incomplete resolution of tryptamine, NMT and DMT 'markers'). The three radioactive zones were eluted with methanol, concentrated and rechromatographed on ion-exchange paper (Whatman P8I cellulose phosphate) using isopropanol-0.02 M ammonium acetate, 1:2, by vol. The first two radioactive zones again gave single peaks corresponding to 5-HT and bufotenine while the more diffuse peak of the first chromatogram was resolved into two sharp peaks corresponding to tryptamine and NMT. Identification of the labelled compounds was confirmed by eluting the compounds from the ion-exchange paper and re-chromatographing the concentrated eluates together with 'markers' on paper in a third solvent (isopropanol-ammonia).

Preparation and Metabolism of Tritiated DMT

Tritiated DMT was prepared by refluxing U-³H-tryptamine (500 µg, specific activity 262 mc/m-mole) in acetone (2 ml) containing methyl iodide (40 µl). The reaction mixture was evaporated at 25° under reduced pressure and the residue redissolved in a few drops of methanol. The methanolic solution was subjected to two dimensional chromatography on paper and the area corresponding to DMT eluted with methanol. A small portion of the eluate was used to confirm the identity of the derivative by co-chromatography with authentic DMT in two other solvent systems. The remainder of eluate was taken by dryness at 25° under reduced pressure and the residue redissolved in the minimum volume of 10⁻⁴ N HCl. The acid solution was diluted to 4 ml with water and the pH adjusted to 7. Incubation was carried out as previously described, 2 ml of the solution being used for the control.

Time Course of ³¹⁴C-L-Tryptophan Metabolism

A series of experiments was arranged in which different solutions of ³¹⁴C-L-tryptophan were incubated for periods of 1.5, 2.5, 17 and 22 hr before the preparation of chromatograms and autoradiographs.

Incubation of Unlabelled Indoles with Stem Segments

Etiolated stem segments were incubated as before with higher concentrations (10⁻³ M) of unlabelled tryptamine hydrochloride, L-tryptophan and 5-HT creatinine sulphate complex.

Acknowledgements—The authors thank the Science Research Council for financial support.